TOXICOLOGICAL EVALUATION OF REALISTIC EMISSIONS OF SOURCE AEROSOLS (TERESA): APPLICATION TO POWER PLANT-DERIVED $PM_{2.5}$

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ABSTRACT

Determining the health impacts of different sources and components of fine particulate matter ($PM_{2.5}$) is an important scientific goal, because PM is a complex mixture of both inorganic and organic constituents that likely differ in their potential to cause adverse health outcomes. The TERESA (Toxicological Evaluation of Realistic Emissions of Source Aerosols) study focused on two PM sources – coal-fired power plants and mobile sources – and sought to investigate the toxicological effects of exposure to realistic emissions from these sources. The DOE-EPRI Cooperative Agreement covered the performance and analysis of field experiments at three power plants. The mobile source component consisted of experiments conducted at a traffic tunnel in Boston; these activities were funded through the Harvard-EPA Particulate Matter Research Center and will be reported separately in the peer-reviewed literature.

TERESA attempted to delineate health effects of primary particles, secondary (aged) particles, and mixtures of these with common atmospheric constituents. The study involved withdrawal of emissions directly from power plant stacks, followed by aging and atmospheric transformation of emissions in a mobile laboratory in a manner that simulated downwind power plant plume processing. Secondary organic aerosol (SOA) derived from the biogenic volatile organic compound α-pinene was added in some experiments, and in others ammonia was added to neutralize strong acidity. Specifically, four scenarios were studied at each plant: primary particles (P); secondary (oxidized) particles (PO); oxidized particles + secondary organic aerosol (SOA) (POS); and oxidized and neutralized particles + SOA (PONS). Extensive exposure characterization was carried out, including gas-phase and particulate species. Male Sprague Dawley rats were exposed for 6 hours to filtered air or different atmospheric mixtures. Toxicological endpoints included (1) breathing pattern; (2) bronchoalveolar lavage (BAL) fluid cytology and biochemistry; (3) blood cytology; (4) *in vivo* oxidative stress in heart and lung tissue; and (5) heart and lung histopathology. In addition, at one plant, cardiac arrhythmias and heart rate variability (HRV) were evaluated in a rat model of myocardial infarction. Statistical analyses included analyses of variance (ANOVA) to determine differences between exposed and control animals in response to different scenario/plant combinations; univariate analyses to link individual scenario components to responses; and multivariate analyses (Random Forest analyses) to evaluate component effects in a multipollutant setting.

Results from the power plant studies indicated some biological responses to some plant/scenario combinations. A number of significant breathing pattern changes were observed; however, significant clinical changes such as specific irritant effects were not readily apparent, and effects tended to be isolated changes in certain respiratory parameters. Some individual exposure scenario components appeared to be more strongly and consistently related to respiratory parameter changes; however, the specific scenario investigated remained a better predictor of response than individual components of that scenario. Bronchoalveolar lavage indicated some

changes in cellularity of BAL fluid in response to the POS and PONS scenarios; these responses were considered toxicologically mild in magnitude. No changes in blood cytology were observed at any plant or scenario. Lung oxidative stress was increased with the POS scenario at one plant, and cardiac oxidative stress was increased with the PONS scenario also at one plant, suggesting limited oxidative stress in response to power plant emissions with added atmospheric constituents. There were some mild histological findings in lung tissue in response to the P and PONS scenarios. Finally, the MI model experiments indicated that premature ventricular beat frequency was increased at the plant studied, while no changes in heart rate, HRV, or electrocardiographic intervals were observed. Overall, the TERESA results should be interpreted as indicating toxicologically mild adverse responses to some scenarios. The varied responses among the three plants indicate heterogeneity in emissions. Ongoing studies using the TERESA approach to evaluate the toxicity of traffic-related pollution will yield valuable data for comparative toxicity assessment and will give us a better understanding of the contribution of different sources to the morbidity and mortality associated with exposure to air pollution.

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EXECUTIVE SUMMARY

Emissions from coal-fired power plants are primarily comprised of sulfur dioxide (SO_2), nitrogen oxides (NO_x, consisting mostly of NO and to a lesser extend of NO_2), and a small amount of primary particulate matter (PM). This PM is generally composed of metal oxides and sulfates. Downwind of the power plant, a portion of the SO_2 is oxidized to secondary acidic sulfate, while simultaneously ambient ammonia neutralizes this strong acidity. In addition, secondary organic aerosol is formed from the oxidation of ambient volatile organic compounds (VOCs). In TERESA, we attempted to simulate these major atmospheric processes to more realistically evaluate the toxicity of power plant emissions. The majority of previous research on power plant-derived PM has used primary PM collected from electrostatic precipitators (coal fly ash); however, because of the universal use of primary PM controls on power plants in the United States and the subsequent reduction in PM emissions of 99% or more, the relevance of coal fly ash to human population exposures is unclear. That said, while power plants in the US contribute minimal primary mass to ambient PM, they do contribute secondary mass in the form of sulfate (and nitrate, to some degree). Toxicological evidence suggests that both sulfate and nitrate, administered as pure compounds, cause health effects only at high levels of exposure, although sulfate has been statistically linked to health effects in several epidemiological studies. It is difficult to disentangle the contribution of total PM mass and sulfate; the two are often highly correlated because sulfate is the largest contributor to mass in many areas. To date, no toxicological studies examining the potency of secondary particles formed downwind from actual power plants have been conducted, and efforts to model or simulate actual atmospheric conditions have been sparse.

The primary objective of the study was to evaluate the potential for adverse health effects from ambient exposure to realistic coal-fired power plant emissions. Secondary objectives were to: (1) evaluate the relative toxicity of coal combustion emission secondary products in comparison to ambient particles; (2) provide insight into the effects of atmospheric conditions on the formation and toxicity of secondary particles from coal combustion emissions through the simulation of multiple atmospheric conditions; (3) provide information on the impact of coal type and pollution control technologies on emissions toxicity; and (4) provide insight into toxicological mechanisms of PM-induced effects, particularly as they relate to susceptible subpopulations.

Three power plants were selected for study, each utilizing a different coal type and with varying emissions controls. All three plants operated under similar boiler temperatures (~1500 ºC). Plant 1, located in the Upper Midwest, burned a low sulfur (~0.2% S) sub-bituminous coal. Electrostatic precipitators (ESPs) were used to control particulate emissions. Plant 2 was located in the Southeast and burned relatively low-to-medium sulfur (~1.0% S) bituminous coal. It used an ESP and selective catalytic reduction (SCR) for NO_x control. Plant 3, in the Midwest, burned

a high sulfur (~3.0% S) bituminous coal and employed a forced oxidation wet flue gas desulfurization (FGD) scrubber to reduce SO_2 emissions, along with an ESP and SCR.

Stack emissions were extracted from a port on the duct passing into the stack downstream of the emissions controls, and then diluted with compressed dry, filtered air with dilution factors of 75-150. Because the wet FGD at Plant 3 resulted in highly humid stack emissions, the extraction system used at the other two plants was modified to prevent water condensation. Diluted stack exhaust was drawn into the first of two reaction chambers in a mobile laboratory, where it was exposed to atmospheric oxidants (i.e., hydroxyl radicals) to convert SO_2 and NO_x to sulfuric acid and nitric acid. In the second chamber, either gas-phase NH_3 was added to neutralize most of the H_2SO_4 aerosol and/or α-pinene and O_3 were added to produce secondary organic aerosol (SOA). A nonselective countercurrent parallel plate membrane diffusion denuder to remove excess gaseous co-pollutants during transfer from the 1st chamber to the 2nd chamber was developed in order to avoid known toxicological effects of high concentrations of ozone, SO_2, and NO_2.

Four atmospheric scenarios were selected for study, along with three control scenarios: (1) primary emissions only ("P"); (2) the oxidation of flue gas SO_2 to form H_2SO_4 aerosol, along with primary particles ("PO"); (3) the oxidation of SO_2 plus the reaction of α-pinene with ozone to form SOA (to simulate the plume mixing with biogenic emissions), along with primary particles ("POS"); (4) the neutralization of H_2SO_4 aerosol by NH_3, along with primary particles and SOA ("PONS"); (5) an oxidized SO_2 scenario that included primary gases but excluded primary particles (control scenario "O"); (6) the "O" control scenario with added SOA (control scenario "OS"), and; (7) SOA alone, produced using particle-free ambient air, with no primary particles or gases (control scenario "S"). The control scenarios (O, OS, and S) were conducted only at Plant 3, as the composition of these exposures was likely to be very similar among plants; the "S" scenario in particular did not include power plant emissions at all. Exposures were comprehensively characterized, with measurement of particle mass, count, and size distribution, sulfate, nitrate, and ammonium ion, particle strong acidity, organic carbon, organic speciation of particle-phase pinene oxidation products, trace elements,, and gaseous copollutants (NO, NO_2, SO_2, and O_3).

Male Sprague-Dawley rats were exposed to filtered air or the various power plant scenarios for 6 hours in individual whole-body chambers. A number of toxicological outcomes were assessed, including (1) breathing pattern; (2) bronchoalveolar lavage (BAL) fluid cytology and biochemistry; (3) blood cytology; (4) *in vivo* oxidative stress in heart and lung tissue; and (5) heart and lung histopathology. In all assessments, comparisons between scenario-specific aerosol exposures and filtered air (sham) control exposures were made. In addition, at Plant 2, cardiac arrhythmias and heart rate variability (HRV) were evaluated in a rat model of myocardial infarction in response to the POS scenario. Sample sizes were not consistent for all experiments investigating the same endpoint, nor were they the same for each plant because some scenarios were repeated while some were not. This causes tests of an effect to be more powerful for some scenarios than for others. Therefore, in interpreting the results of our statistical analyses, we focused on not only the strength (statistical significance) of the effects but also on the magnitude of these estimated effects. Statistical analyses were multi-layered. In the first level, ANOVA techniques were employed to assess whether differences between exposed and filtered air responses varied by exposure scenario. In the second level, univariate associations between mass

levels or exposure composition and health outcomes were determined. Single-component analyses were carried out in which separate regression models were fitted using differences between exposed and filtered air responses as the outcome and either mass, particle number, or measured component concentration as the exposure metric. We used the resulting *p*-values from these models to rank the strength of associations between each component and the particular biological response. In the third level, to further explore univariate associations, the concept of variable importance in a random forest analysis was introduced to investigate joint effects of multiple pollution components.

The aged particle mass concentrations varied significantly from 44 to 257 $\mu g/m^3$ with respect to scenario and power plant. The highest mass concentration was present when oxidized aerosols were neutralized by gas-phase NH_3 with added SOA (PONS). This scenario is considered to be the most representative of urban areas downwind of coal-fired power plants. Our results indicate that the secondary sulfate (as well as aged particle mass) concentrations depend on the ratio of SO_2 and NO emissions, which, in turn, depend on the type of coal and emissions control devices. At all three coal-fired power plants, sulfate and organic carbon (OC) were major components of the aged particles, whereas the contributions of trace elements (derived from primary particles) were very low as compared to urban ambient particles. This is not unexpected, given the use of primary particle controls (primarily ESPs) on US power plants.

Toxicological results indicated some biological responses to some plant/scenario combinations, as summarized below:

Breathing Pattern: Of the 12 respiratory outcomes assessed, each showed statistically significant changes at some plants and in response to some of the four scenarios; however, significant clinical changes such as specific irritant effects were not readily apparent. The most robust outcomes were found with exposure to the PO scenario (increased respiratory frequency with decreases in inspiratory and expiratory time) and the PONS scenario (decreased peak expiratory flow and expiratory flow at 50% [EF50]). PONS findings were most strongly associated with ammonium, neutralized sulfate, and elemental carbon (EC) in univariate analyses, but only with EC in multivariate analyses. When we examined the more robustly significant associations in the univariate analyses and compared them to the top random forest variables for a given exposure variable, relatively few exposure parameters were exactly the same. The components for which results were consistent included:

- Frequency was significantly associated with Al (negative) and NO (positive), and in random forest analyses these two components were ranked 2 and 4, respectively;
- Inspiratory time (Ti) was marginally associated with pinene (positive), and this parameter was ranked 3 by random forest;
- Expiratory time (Te) was associated with NO (negative), which was ranked 3 by random forest;
- Tidal volume (TV) was associated with nickel and magnesium (increase), which were ranked 5 and 6 respectively in random forest analyses;
- Minute ventilation (MV) was significantly associated with EC and NO_2 (negative) and these parameters were ranked 1 and 4, respectively, by random forest, as well as Na (positive), which was ranked 5 by random forest;

- Peak inspiratory flow (PIF) was associated with EC (negative), which was ranked 1, as well as Na and Mg (positive) which were ranked 3 and 4 respectively;
- Peak expiratory flow (PEF) was associated with EC and NH_4 (negative), which were ranked 1 and 3 in random forest analyses; and
- Expiratory flow at 50% (EF50) was associated with EC and NH_4 (negative), which were ranked 1 and 6 by random forest.

Adjusted R^2 analyses showed that scenario was a better predictor of respiratory responses than individual components, suggesting that the complex atmospheric mixture was contributing to the effects.

*Bronchoalveolar Lavage, Blood Cytology, and Histopathology***:** Exposure to the PONS and POS scenarios produced significant increases in BAL total cells and macrophage numbers at two plants. The PONS and P scenarios were associated with significant increases in BAL neutrophils and the presence of occasional neutrophils and increased macrophages in the airways and alveoli of exposed animals. Univariate analyses and random forest analyses showed that increases in total cell count and macrophage cell count were significantly associated with neutralized sulfate and several correlated measurements. Increases in neutrophils in BAL were associated with zinc. There were no significant differences in complete blood count (CBC) parameters or blood vessel wall thickness by histopathology. The association between neutrophils and zinc raises the possibility that metals play a role in this response. The BAL changes are considered mild toxicological responses, and these were observed only in scenarios with added SOA and the most complex atmospheric reactions.

In Vivo Oxidative Stress: Exposure to P or PO aerosols led to no changes compared to filtered air in lung or heart CL at any individual plant or when all data were combined. POS caused significant increases in lung CL and TBARS at only one plant, and not in combined data from all plants; PONS resulted in increased lung CL only when data from all plants were combined. Heart CL was also significantly increased with exposure to POS only when data from all plants were combined. PONS increased heart CL significantly in one plant with TBARS accumulation, but not in combined data. Exposure to O, OS, and S had no CL effects. Univariate analyses of individual measured components of the exposure atmospheres did not identify any component associated with increased CL.

Arrhythmias and HRV: Exposure of the MI model to the POS scenario at Plant 2 caused an increase in premature ventricular beats, but no change in heart rate, HRV, or electrocardiographic intervals.

Based on the TERESA results, the role of exposure components can be summarized as follows:

Role of sulfate**:** As expected, sulfur compound concentrations dominated most scenarios. Our data from the breathing pattern and pulmonary inflammation studies are generally consistent with previously reported findings of lack of toxicological effects with partially to totally neutralized sulfate in laboratory studies. Neutral sulfate was associated with increases in BAL cells due to increases in macrophages, but not significant inflammation as assessed by neutrophils or BAL biochemical parameters. We did not observe consistency in findings with varied statistical methods; nor did the findings of varying pulmonary endpoints consistently

compare. Acid sulfate produced increased breathing rate in multivariate analysis, but overall did not appear to play a significant role in pulmonary responses in the TERESA study. In vivo CL measurements were not influenced by any form of sulfate.

Role of organic components**:** Elemental carbon (EC) figured prominently in many of the analyses. EC concentrations in this study were abnormally high in many cases. These elevations were considered artifactual and were attributed at least in part to pyrolyzed OC erroneously reported as EC, since power plants emit very low concentrations of EC. Furthermore, the associations with EC that we observed are likely not due to EC itself as this material is generally inert. It is more likely that any effects observed were due to adsorbed organic materials that were able to more effectively reach pulmonary regions. We also observed several interesting findings with respect to the measured pinene oxidation products: formaldehyde, acetaldehyde, acetone, and total aldehydes. In addition, the addition of SOA appeared to be linked with the inflammatory responses observed, although from examination of control scenarios and organic carbon univariate results, it was not clear why SOA was important in the BAL responses.

Role of metals**:** Metals did not appear to play a large role in the TERESA respiratory responses, although there were some consistent results. Aluminum, silicon, lead, magnesium, nickel, and sodium were all significantly associated with some changes in breathing parameters. In the BAL study, increased neutrophils were associated with zinc; however, exposure distributions and concentrations did not make this a convincing result.

Role of gases**:** In univariate analyses of breathing pattern responses, we observed several strong associations with gases, including NO and NO_2. By design, gaseous copollutant concentrations in the exposure scenarios were low through the use of denuders (maxima for ozone, NO_2, and SO_2 over all scenarios at all plants were 29 ppb, 18 ppb, and 73 ppb, respectively). Therefore, any associations observed with gases were likely not reflective of a true biological association, but rather, because gases were common to all scenarios, they may have served as tracers for scenarios. Gases did not play a role in the inflammatory or chemiluminescence responses.

Overall, the TERESA results should be interpreted as indicating toxicologically mild adverse responses to some scenarios. The varied responses among the three plants indicate heterogeneity in emissions. Ongoing studies using the TERESA approach to evaluate the toxicity of traffic-related pollution will yield valuable data for comparative toxicity assessment and will give us a better understanding of the contribution of different sources to the morbidity and mortality associated with exposure to air pollution.

1
INTRODUCTION AND BACKGROUND

In an effort to enhance our understanding of the sources and components of air pollution, specifically fine particulate matter ($PM_{2.5}$), associated with adverse health effects, many controlled toxicological studies have investigated the health effects of emissions from specific air pollution sources, e.g., engine exhaust, power plants, wood-burning fireplaces, etc. (Jaspers et al., 2005; Mills et al., 2007; Smith et al., 2006; Chen et al., 1990; Hsu and Kou, 2001; Ho and Kou, 2002). However, a limitation of many of these investigations has been lack of consideration of the secondary pollutants formed through photochemical reactions during the transport of sources in the atmosphere. People are rarely exposed to "fresh" power plant emissions. Accordingly, a critical goal of the TERESA power plant studies was to better simulate population exposures to coal combustion emissions, taking into account the aging that occurs in the atmosphere.

Emissions from coal-fired power plants are primarily comprised of sulfur dioxide (SO_2), nitrogen oxides (NO_x, consisting mostly of NO and to a lesser extend of NO_2), and a small amount of primary PM. This PM is generally composed of metal oxides and sulfates. Downwind of the power plant, a portion of the SO_2 is oxidized to secondary acidic sulfate, while simultaneously ambient ammonia neutralizes this strong acidity. In addition, secondary organic aerosol is formed from the oxidation of ambient volatile organic compounds (VOCs). Thus, in TERESA, we attempted to simulate these major atmospheric processes to more realistically evaluate the toxicity of power plant emissions.

There is a relatively large literature on the toxicity of components of coal combustion emissions or related atmospheres. Much of this work has used primary PM, typically collected from electrostatic precipitators (in the case of full-scale plants), or generated using a lab-scale combustor. This material, called coal fly ash, has been reported to cause some effects in toxicological studies, although generally at high concentrations (Gilmour et al., 2004; Alarie et al., 1975; Schreider et al., 1985; Fisher et al., 1983).

However, the relevance of coal fly ash to human population exposures is unclear for several reasons. First, primary PM emissions from coal-fired power plants are very low in the United States, because all plants have particulate controls such as electrostatic precipitators or baghouses. Most plants are thus able to achieve upward of 99% reduction in primary PM (Department of Energy, 2007). The contribution of power plant-derived primary PM to ambient $PM_{2.5}$ is correspondingly low; Marmur et al. (2006) estimated that primary $PM_{2.5}$ from coal combustion represented <4% of all primary $PM_{2.5}$ (significantly lower for total $PM_{2.5}$). Second, collected coal fly ash used in laboratory studies represents the material that is retained in the particulate control equipment (e.g., ESP), and thus is not the material that is exiting the stack into the ambient environment. Furthermore, such studies with collected fly ash typically utilize this material in *in vitro* or intratracheal instillation studies, neither of which are optimal modes of

delivery of PM due to the likelihood of extremely high tissue doses and overload and possible alteration of the physicochemical properties of the material while in storage. Finally, while pilot-scale combustors do generate whole emissions, there is concern that they may not accurately mimic stack emissions due to differences in surface-to-volume ratios and thus time-temperature histories. In addition, they include co-exposures to unrealistically high concentrations of gaseous pollutants such as NO_x and SO_2 that would not occur in the ambient environment.

While power plants in the United States contribute minimal primary mass to ambient PM, they do contribute secondary mass. This mass is formed from SO_2 and NO_x in stack emissions as these compounds are oxidized to form sulfate and nitrate, respectively, the latter mostly in the form of nitrate salts. Martello et al. (2008) used source apportionment techniques to estimate that secondary transported material (dominated by ammonium sulfate) comprised 47% of total $PM_{2.5}$ mass in Pittsburgh, while in the Southeastern US secondary sulfate comprised 30% of total fine mass (Zheng et al., 2002). Sulfate concentrations are highest in the Eastern and Midwestern US, and lowest in the Western US, in part because of the significantly lower prevalence of coal-fired power plants.

Toxicological evidence suggests that both sulfate and nitrate, administered as pure compounds, cause health effects only at high levels of exposure (see Schlesinger and Cassee, 2003 and Schlesinger, 2007 for reviews of this topic). Toxicological studies in Boston, MA using concentrated ambient particles (CAPs) involved high sulfate exposures, and associations were found with sulfate as well as many other CAPs constituents for some parameters of inflammation (Saldiva et al., 2002), but no associations were found between sulfate concentrations and toxicological outcomes in several other studies using CAPs at the same location (Batalha et al., 2002, Gurgiera et al., 2003, Wellenius et al., 2003). To date, no toxicological studies examining the potency of secondary particles formed downwind from actual power plants have been conducted, and efforts to model or simulate actual atmospheric conditions have been sparse (McDonald et al., 2009).

Although coal fly ash, sulfate, and nitrate have yielded few effects in toxicological studies, sulfate has been linked to health effects in several epidemiological studies (Dockery et al., 1993; Pope et al., 2002, Franklin et al., 2006). It is also worth noting that other studies do not show such a positive association between sulfate and health effects (e.g., Lipfert et al., 2006; Tolbert et al., 2007). However, it is difficult to disentangle the contribution of total PM mass and sulfate; the two are often highly correlated because sulfate is the largest contributor to mass (Reiss et al., 2007; Schlesinger, 2007). It has been hypothesized that effects are observed in human populations exposed to the complex atmospheric mixture because of the interactions occurring among pollutants, e.g., sulfate/organics, sulfate/metals. Thus, a toxicological approach was required that would allow the examination of a realistic human exposure atmosphere, including different atmospheric conditions and the formation of sulfate particles, in the context of a complex environmental mixture.

As noted above, ambient particle concentrators have also been used to determine PM toxic components. Repeated animal (and to a lesser extent, human) CAPs exposures in conjunction with comprehensive particle characterization have made it possible to conduct “mini time-series studies”, where PM constituent concentrations can be correlated with an array of health

endpoints over a period of days or weeks. These CAPs investigations have used factor analysis to determine the contribution of source factors. To date, factors related to sulfate and other products of coal combustion have not been identified among the most toxic factors (Clarke et al., 2000; Kodavanti et al., 2000; Wellenius et al., 2003; Saldiva et al., 2002). The CAPs approach differs from the TERESA approach in that CAPs represent the full and complex ambient environment; these particles contain contributions from a variety of pollutant sources. Thus, the challenge lies with the utilization of statistical techniques to link specific sources and/or components with observed health impacts. In contrast, the TERESA approach begins with an identifiable "source", i.e., power plants, and simulates the complex environment by manipulating the mixtures of atmospheric components. An overall schematic illustrating the TERESA approach is shown in Figure 1.1.

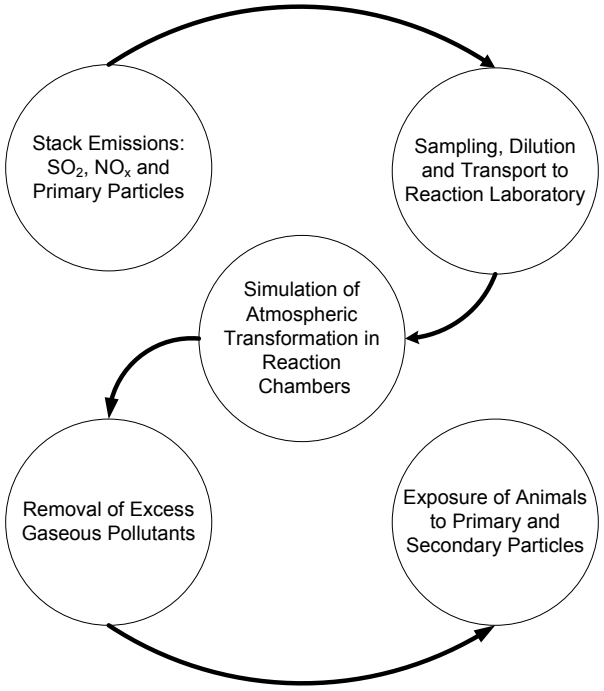


Figure 1-1
Overview of the TERESA approach

2
OBJECTIVES

The overall objective of the TERESA program is to investigate and clarify the impact of the sources and components of fine particulate matter ($PM_{2.5}$) on human health via a set of realistic animal exposure experiments. The DOE-sponsored portion of the TERESA program, covered by a Cooperative Agreement between DOE and EPRI, is designed to assess the toxicity of coal combustion emissions by exposing laboratory animals to actual plant emissions that have been "aged" and converted to reaction products in a manner that simulates the conversion experienced by coal power plant plumes in the atmosphere en route to ambient receptor sites. Thus, the primary objective of the DOE-EPRI Cooperative Agreement is to evaluate the potential for adverse health effects from ambient exposure to realistic coal-fired power plant emissions. Secondary objectives of the study include: (1) evaluate the relative toxicity of coal combustion emissions and mobile source emissions, their secondary products, and ambient particles; (2) provide insight into the effects of atmospheric conditions on the formation and toxicity of secondary particles from coal combustion and mobile source emissions through the simulation of multiple atmospheric conditions; (3) provide information on the impact of coal type and pollution control technologies on emissions toxicity; and (4) provide insight into toxicological mechanisms of PM-induced effects, particularly as they relate to susceptible subpopulations.

3
EXPERIMENTAL METHODS

The design of the study focused on exposing laboratory rats to simulated atmospheric scenarios (Figure 1-1). In order to carry out these technically and logistically challenging toxicology studies in a field setting, it was necessary to develop a number of innovative methodological advancements, which are described in detail in a series of publications (Ruiz et al., 2006; 2007a; 2007b). The following sections detail (1) power plant selection; (2) emissions sampling; (3) atmospheric simulation; (4) exposure scenarios; (5) mobile laboratory; (6) exposure characterization; (7) animal exposures; and (8) toxicological assessments. Note that more comprehensive details can be found in the companion papers to this overview document.

3.1 Power Plant Selection

Three power plants were selected for study, with the motivation to include plants utilizing different coal types, combustion conditions, and air pollution control devices (APCDs). These factors clearly influence the composition of stack emissions; in particular, they can affect inorganic ash, sulfuric acid (H_2SO_4), soot, and condensable organic compounds (Lighty et al., 2000). All three plants operated under similar boiler temperatures (~1500 ºC). Access to each of the plants was arranged through the Electric Power Research Institute in Palo Alto, CA. Table 3-1 shows key characteristics of these power plants and the coals fired in them.

The first plant, located in the Upper Midwest (Plant 1) was described previously (Ruiz et al., 2007b). Briefly, this plant burned a low sulfur (~0.2% S) sub-bituminous coal from the Wyoming Powder River Basin and had two units with a generating capacity of 600 MW each. Electrostatic precipitators (ESPs) were used to control particulate emissions from each unit. The second plant was located in the Southeast (Plant 2), and burned relatively low-to-medium sulfur (~1.0% S) bituminous coals from various regions, such as Kentucky, West Virginia, and South America. The plant consisted of a single unit with an electric power capacity of 650 MW. It used an ESP for particulate control and selective catalytic reduction (SCR) for NO_x control. The third plant, in the Midwest (Plant 3) burned a high sulfur (~3.0% S) bituminous coal from Indiana mines and was composed of two units with an electric power capacity of 500 MW each. At Plant 3, each unit had a forced oxidation wet flue gas desulfurization (FGD) scrubber to reduce SO_2 emissions, along with an ESP and SCR. The wet FGD scrubber uses limestone as alkaline slurry to absorb SO_2 from the flue gas and produce calcium-sulfur compounds, with the primary product calcium sulfate. The FGD process produced highly humid stack emissions with a relatively low temperature compared to the first two plants.

Because of the length of time spent at each of the three power plant facilities, it was not possible to collect accurate representative coal samples for the study period; however, limited compositional information was available for the general time frame over which the studies were conducted (Table 3-1). There should be no direct comparison between the plant emissions as

reported in the set of TERESA papers and the coal composition data provided; this information should be considered from a qualitative perspective only.

Table 3-1
Characteristics of power plants and coal types

Parameter	Plant 1	Plant 2	Plant 3
Characteristics of power plants			
Max. Capacity (MW)	600	650	500
NO_x (ppm)	250	30	40
SO_2 (ppm)	350	460	80-120
Temperature at Sampling Port (°C)	149	143	54
Coal Type	Wyoming Powder River Basin sub-bituminous coal	Kentucky, West-Virginia and South American Region bituminous coal	Indiana bituminous coal
APCDs[a]	ESP	ESP + SCR	ESP + SCR + FGD
Coal composition (as-fired)[b]			
% Moisture	30	6	5
% Carbon	49	74	69
% Hydrogen	3	5	5
% Nitrogen	1	1.6	1.4
% Sulfur	0.3	0.7	3
% Ash	5.4	7	9
% Oxygen	11	5	7
HHV[c](Btu/lb)	8,500	13,000	13,000

[a]Air pollution control devices: ESP: Electrostatic Precipitator for particle removal; SCR: Selective Catalytic Reduction for NO_x control; FGD: Flue Gas Desulfurization Scrubber for SO_2 removal
[b]Coal composition data are obtained from the proximate/ultimate analyses and are provided for general information only as the data do not correspond exactly with the time period of the study. Data provided for Plant 2 represent Kentucky coal.
[c] Higher heating value

3.2 Emissions Sampling

The custom-designed sampling system used at Plants 1 and 2 was described in detail previously (Ruiz et al., 2007b). Briefly, stack emissions were extracted from a port on the duct passing into the stack downstream of the APCDs, and then diluted with compressed dry, filtered air with dilution factors of 75-150. Sampling was conducted downstream of the APCDs where the exhaust had cooled enough that much of the condensable mass would have been in the particle phase. A venturi aspirator was used for dilution; the aspirator accelerated a flow of 150 LPM of

compressed, particle-free ambient air through a narrow constriction. Thus, by the Bernouli principle, a vacuum was created in a side arm perpendicular to the constriction, which drew the stack gas through the sampling probe and simultaneously diluted it with ambient air. Validation and testing data for the system were presented by Ruiz et al. (2007b). The dilution factors were intended to be sufficient to avoid water condensation when the stack emissions were cooled from stack temperature to ambient temperature, as well as to attain SO_2 and NO_x concentrations suitable for our experiments.

Plant 3 used a wet FGD scrubber to capture SO_2, along with an ESP and a SCR unit. Because the wet scrubber resulted in highly humid stack emissions, the extraction system used at the other two plants was modified to prevent water condensation. The new extraction probe consisted of concentric sampling tubes: an outer tube with a flow of dry, warm (about 100°C) filtered air for initial dilution of the wet stack emissions, and a narrow inner tube used to pass the initially diluted stack emissions into the aspirator. The inner tube was heated using an electric heating coil to prevent potential clogging due to water condensation. The temperature was set just warm enough to prevent condensation of water vapor, but not hot enough to result in significant volatilization of semivolatile elements. Consequently, the stack sampling rate could be adjusted by (1) changing the flow rate of hot, dry filtered air; and (2) changing the amount of vacuum by controlling the total flow of dry filtered air into the aspirator. Validation and testing data for this system are presented by Kang et al., 2011.

3.3 Atmospheric Simulation

A critical requirement of TERESA was the ability to successfully simulate a variety of atmospheric conditions. The specific scenarios and the rationale for their selection are discussed later in this paper, but we herewith describe the reaction laboratory and the features thereof that enabled atmospheric simulation. Additional details can be found in Ruiz et al. (2007a).

The reaction laboratory contained two reaction chambers. In the first reaction chamber, diluted stack exhaust was exposed to atmospheric oxidants (i.e., hydroxyl radicals, •OH) to convert SO_2 and NO_x in the stack exhaust to sulfuric acid and nitric acid. The second chamber was used to neutralize H_2SO_4 aerosol and/or to form secondary organic aerosol (SOA). Diluted stack emissions were transported to the 1st reaction chamber through a 25-30 m stainless steel tube. This reaction chamber was a 630-L well-mixed flow reactor with Teflon film walls (Ruiz et al., 2007a). Under ultraviolet (UV) irradiation, the chamber produced high concentrations of hydroxyl radical by mixing the diluted emissions with O_3 and added water vapor. Enough O_3 was added to completely oxidize the entire NO to NO_2, with a sufficient excess supplied to form hydroxyl radical with the uv irradiation. The hydroxyl radical oxidized SO_2 to produce secondary H_2SO_4 aerosol. The chamber was designed to oxidize 30-50% of SO_2 to sulfuric acid within an approximately 20-minute residence time. This target conversion proportion represents a reasonable atmospheric scenario, taking into account transport, deposition, and typical rates of oxidation. The conversion rate for SO_2 to sulfuric acid is on the order of 2-4% hr^{-1} (Luria et al., 1983; Seinfeld and Pandis, 1986). By converting a similar fraction in the chamber we maintained an environmentally relevant ratio of metals to sulfate in the exposure chamber, representative of atmospheres downwind of power plants. In fact, the sulfur/Se ratio from this study (1979 ± 1675; Plant 3 only, n=15) is roughly similar to the same ratio of $PM_{2.5}$ collected in Boston, MA from

2003-2008 (2152 ± 3613; n=1193). This demonstrates that the relative proportion of primary and secondary particles in TERESA is broadly consistent with that of ambient northeastern PM.

The 2nd chamber (110-L) was made of polycarbonate plastic, with internal surfaces covered with Teflon film. In this chamber, either gas-phase NH_3 was added to neutralize most of the H_2SO_4 aerosol and/or α-pinene and O_3 were added to produce secondary organic aerosol (SOA).

The development of a nonselective countercurrent parallel plate membrane diffusion denuder to remove excess gaseous co-pollutants during transfer from the 1st chamber to the 2nd chamber was an important technological advance (Ruiz et al., 2006). Low concentrations of NO_x, SO_2, and O_3 were important to avoid known toxicological effects of these gases. The denuder made designs with a relatively small chamber and relatively short residence times possible, which was key to operating within a mobile laboratory. A second diffusion denuder was employed downstream of the 2nd chamber. The experiments conducted to verify the performance of the denuder system have been described in detail (Ruiz et al., 2006).

3.4 Exposure Scenarios

The fundamental rationale behind the selection of exposure scenarios was to investigate the importance of photochemical processes by differentiating the toxicity of primary and secondary particles. Because realistic exposures were the goal of the TERESA studies, we sought to simulate the most common conditions within a power plant plume entering the ambient environment (Table 3-2). The following specific scenarios were selected for study: (1) primary emissions only ("P"); (2) the oxidation of flue gas SO_2 to form H_2SO_4 aerosol, along with primary particles ("PO"); (3) the oxidation of SO_2 plus the reaction of α-pinene with ozone to form SOA (to simulate the plume mixing with biogenic emissions), along with primary particles ("POS"); (4) the neutralization of H_2SO_4 aerosol by NH_3, along with primary particles and SOA ("PONS"); (5) an oxidized SO_2 scenario that included primary gases but excluded primary particles (control scenario "O"); (6) the "O" control scenario with added SOA (control scenario "OS"), and; (7) SOA alone, produced using particle-free ambient air, with no primary particles or gases (control scenario "S"). The control scenarios (O, OS, and S) were conducted only at Plant 3, as the composition of these exposures was likely to be very similar among plants; the "S" scenario in particular did not include power plant emissions at all.

Table 3-2
Exposure scenarios, composition, and simulated atmospheric conditions.

Code	Scenario	Composition	Simulated Atmospheric Condition
P	Primary	Primary (un-aged) diluted emissions	Primary stack emissions
PO	Primary + oxidized	Primary emissions + •OH	Aged plume, oxidized stack emissions, sulfate aerosol formation
POS	Primary + oxidized + SOA	Primary emissions + •OH + α-pinene/ozone	Aged plume, unneutralized acidity, secondary organic aerosol (SOA) derived from biogenic emissions
PONS	Primary + oxidized + neutralized + SOA	Primary emissions + •OH + NH_3 + α-pinene/ozone	Aged plume, mixture of neutralized sulfate and SOA
O	Oxidized	Primary emissions + •OH, no primary PM	Control scenario
S	SOA	α-pinene/ozone only	Control scenario
OS	Oxidized + SOA	Primary emissions + •OH + α-pinene/ozone, no primary PM	Control scenario

The initial "aging" of the primary flue gas was carried out to simulate long-range transport of the power plant plume. Achieving an SO_2 oxidation rate close to the target of 30-50% was desirable, because it would maintain the ratio of metals to sulfate consistent with atmospheric processes. Typically in the atmosphere, approximately 50% of the SO_2 emitted will be lost by dry deposition and the remaining 50% will ultimately be oxidized to sulfate over a period of days (Luria et al., 1983; Seinfeld and Pandis, 1986). We sought to achieve particle size in the accumulation mode, thereby accurately reflecting population exposures to aged ambient particles. α-Pinene was selected for use as a model biogenic VOC because it is known as the most important terpene emitted on a global scale (Kanakidou et al., 2005), and it represents a well-defined system for which particle formation (Zhang et al., 1992; Jang and Kamens, 1999), and to some extent, toxicity (Wolkoff et al., 2000; Rohr et al., 2002), has been established.

It is important to note that we would not expect the TERESA exposure atmospheres to be similar to the ambient environment. While the TERESA scenarios simulated atmospheric reactions of coal-fired power plant emissions, ambient particles are comprised of constituents from multiple sources, including mobile sources, oil-fired power plants and a variety of industrial emissions. In addition, both biogenically- and anthropogenically-derived SOA is not limited to α-pinene as a VOC precursor in ambient air, unlike in the TERESA scenarios. That said, PONS scenario was deemed the most representative of human exposure. This is because it contains oxidized SO_2, partially neutralized sulfate, and secondary organic aerosol that would be expected to associate with particles formed downwind of the plant. The neutralization is important, because acidity in ambient air is generally low (Koutrakis et al., 1988; Dockery et al., 1992; Peel et al.,

2005). For comparison between PONS and concentrated ambient particles (CAPs) from different regions of the US, the reader is directed to Kang et al., 2011 where this comparison is discussed. Similarly, direct comparisons between outcomes for the PONS scenario and CAPs can be found in Lemos et al., 2011.

3.5 Mobile Laboratory

A mobile laboratory was designed and constructed specifically for this project. The mobile lab was comprised of two separate structures: (1) Bus with the photochemical chambers, pre-exposure sampling equipment, and denuders to remove excess pollutant gases (Ruiz et al., 2006; Ruiz et al., 2007a; Ruiz et al., 2007b); and (2) Exposure trailer, custom-built as a laboratory and conditioned to meet the National Institutes of Health standards for the care and housing of animals for research.

The mobile toxicological laboratory had a Thoren Maxi-Miser Caging System (Hazelton, PA) rat housing facility for 72 animals. This caging system was equipped with a self-contained HEPA filtering unit and provided filtered air and air exhaust individually to each cage in the system. A filter cover on each cage further protected the animal when the cage was removed from the unit. All of the air provided to the animals in the unit was drawn from filtered room air from the trailer. Therefore, within the unit, the animals received double filtered air, and during transfer to exposure units the animals were protected by the filter system of the trailer and their individual cage filtering units. The air exhausted from the housing units was directed to the outside at the farthest possible point from the air intake to the mobile lab. The mobile lab was positioned so that the air intake was upwind of the animal exhaust port in relationship to the usual prevailing ground level winds at the site. An alarm system was connected to the caging system to ensure that air was going to the cages; this alarm system was also wired to a main alarm and monitoring system, which allowed for remote monitoring and included sensors for CO, temperature, relative humidity (RH), CO_2, motion detection, intrusion, and airflow.

A ductless fume hood enclosure was installed in the mobile toxicology laboratory within which the surgery associated with the myocardial infarction model studies was conducted. The enclosure had charcoal and HEPA filters as well as electrostatically charged pre-filters to remove dust particles. Bench working areas, storage areas and exposure site sampling equipment were also included in the mobile toxicology laboratory.

Interior and exterior photos of these mobile facilities are presented by Kang et al. (2011).

3.6 Exposure Characterization

The exposure sampling system and characterization have been described in detail for Plant 1 (Ruiz et al., 2007b), and for all three plants (Kang et al., 2011). Briefly, sampling ports were installed upstream of the 1st reaction chamber, downstream of the 2nd reaction chamber, and in the toxicology laboratory to characterize primary emissions, aged particles, and gases, during and following atmospheric simulation.

Particle mass was monitored both continuously and as an integrated, gravimetric measurement. Particle count (serving as a proxy for ultrafine particles) was continuously monitored using a condensation particle counter (CPC), and particle size distribution was determined semi-continuously using a scanning mobility particle sizer (SMPS). Sulfate, nitrate, and ammonium ion were measured by ion chromatography and particle strong acidity by pH analysis. Organic carbon was measured by the thermal optical reflectance (TOR) method, and organic speciation of particle-phase pinene oxidation products was conducted by gas chromatography. Trace elements were quantified by X-ray fluorescence (XRF). Continuous measurement of gaseous pollutants was carried out: NO and NO_2 by chemiluminescence; SO_2 by pulsed fluorescence; and O_3 by UV photometry.

3.7 Animal Exposures

All exposures were conducted in temperature- and humidity-controlled exposure chambers located in the mobile toxicological laboratory. These individual whole body chambers also served as plethysmographs for the collection of respiratory data, and are described in detail by Diaz et al. (2011). Aerosol from the reaction chamber was drawn through the exposure chambers (1.5 LPM each) in parallel. All exposures were 6 hours in duration unless otherwise stated, during which an equal number of animals exposed to plant emissions and control animals exposed to filtered air were simultaneously studied. All protocols were approved by the Harvard Medical Area Standing Committee on Animals.

3.8 Toxicological Assessments

Toxicological endpoints were selected to provide a comprehensive, multi-system assessment of the impacts of exposure to the different scenarios. It should be noted that no endpoint should be considered more significant than any other; all toxicological data are considered in a holistic manner to reach conclusions about the effects of exposures. In addition, all of the endpoints evaluated were used in previous work in our laboratory with concentrated ambient particles (CAPs), thereby providing a basis for comparison between Boston CAPs and power plant-derived particles.

Normal male Sprague Dawley rats were used as the animal model for most of the experiments. In addition, at two of the power plants an additional scenario was investigated using a susceptible myocardial infarction (MI) rat model used previously by our research group (Wellenius et al., 2002 and 2004).

The toxicological endpoints included those related to the respiratory and cardiovascular systems, as described below. In all assessments, comparisons between scenario-specific aerosol exposures and filtered air (sham) control exposures were made.

Breathing pattern and respiratory air flow were evaluated continuously during exposures using the BUXCO system (Buxco Biosystem 2.9). This is a sensitive indicator of effects in the lung, and can be used to assess changes throughout the entire exposure period. Parameters of interest included peak expiratory flow (PEF), tidal volume (TV), respiratory frequency (*f*), and minute

ventilation (MV) (Clarke et al., 1999; Nikolov et al., 2008; Clougherty et al,. 2010). Methodological details and results are described by Diaz et al. (2011).

Cellular and biochemical changes in the lungs and blood were evaluated by conducting bronchoalveolar lavage (BAL), histopathological techniques, and collection of blood samples. BAL fluid was analyzed for cellular content (cell viability, total cell counts, cell type) and biochemical markers of pulmonary injury (lactate dehydrogenase (LDH), β-n-acetyl glucosaminidase (βNAG), and total BAL protein) using standard methodologies. Pulmonary histopathology was evaluated by fixing lungs and heart tissues, slicing these tissues into uniform transverse sections 2 mm thick, numbering each slice, and then randomly selecting three slices of lung and three of heart for processing by paraffin histology techniques. Blood cytology (total white blood cell counts and differential profiles) was evaluated 24 hours following the last day of exposure. Detailed information on these methods and results can be found in Godleski et al. (2011).

In vivo oxidative stress of heart and lung tissue was conducted by evaluating *in vivo* organ chemiluminescence, a novel method that refers to the ultra-weak light emission produced by biological systems due to the de-excitation of high-energy byproducts of the chain reaction of lipid peroxidation (Boveris and Cadenas, 2000; Boveris et al., 1980). This method has been successfully used in models of oxidative injury in the lung (Gurgueira et al., 2002; Evelson et al., 2000) and the heart (Rhoden et al., 2004, Ghelfi et al., 2008). Details of the methods and results are described by Lemos et al. (2011).

Most of the biologic outcomes were assessed under each of the experimental scenarios and at all 3 power plants. However, due to the complexity of the acute MI animal model mentioned above, we chose *a priori* to conduct these experiments only under the exposure scenario producing the greatest effects in normal rats. The decision of which scenario to investigate needed to be made rapidly after the initial exposures to each scenario were completed, because time was needed for refurbishment and implantation of telemeters. Preliminary analyses of chemiluminescence data from Plant 1 suggested no cardiac oxidative stress under any scenario, so experiments using the MI model were not carried out. Preliminary analyses of data from Plant 2 suggested increases in cardiac chemiluminescence in the same range with the POS and PONS scenarios. We empirically selected the POS scenario for the MI model experiments; the same scenario was chosen for experiments at Plant 3 for consistency. Exposures took place 12 hours after surgery to induce the infarction, as this is the period of greatest vulnerability to cardiac arrhythmias (Wellenius et al., 2002). In these animals, cardiac function was assessed by electrocardiography (ECG), with endpoints of interest including heart rate, heart rate variability (standard deviation of the normal beat-to-beat intervals; SDNN), and arrhythmias. Methodological details and results of studies using the MI model are described by Wellenius et al (2011).

3.9 Experimental Design

Animals were exposed to either aged aerosol or filtered air by inhalation in individual whole body chambers as described above. For any given exposure scenario run, 6-hour exposures were run on usually 4 consecutive days, with each set having 5 animals exposed to the scenario aerosol and 5 animals exposed to filtered air as controls. Of these 5 animals per group, 2 had in

vivo oxidative stress assessments and the remaining 3 were either used for BAL or histopathology. Typically, for animals assessed by BAL, their exposure took place on days 1 and 3 in the sequence, whereas animals assessed by histopathology were exposed on days 2 and 4 in the sequence. The total number of animals used and analyzed for BAL per scenario run was 12 (6 aerosol exposed, 6 filtered air controls), and the total number used for histology per scenario run was 12 (6 aerosol exposed, 6 filtered air controls). Blood for complete blood count was collected at the time of sacrifice for the BAL and histopathology so that the total number of animals for this assessment per scenario run was 24 (12 aerosol exposed, 12 filtered air controls). A total of 78 exposure days were included in the study, including "control" scenarios (O, S, OS); Table 3-3 shows the dates on which experiments were conducted at each of the three plants.

Table 3-3
Dates for toxicological experiments, Plants 1-3.

Code	Dates (2004) Plant 1	Dates (2005) Plant 2	Dates (2006) Plant 3	Animals Studied
P	May 10-13	June 6-9	August 8-13	Normal
PO	November 13-15	May 9-12	September 19-22	Normal
POS	October 4-7	March 21-24 (no SCR) May 3-6 (SCR)	July 19-22 August 14-15	Normal
PONS	June 22-26 June 27-30 October 11-14	May 31-June 3	July 25-28	Normal
POS	-	July 8, 13 September 8, 9	August 16-17	Compromised
OS	-	-	August 28-31	Normal
O	-	-	September 1-4	Normal
S	-	-	September 6-9	Normal

As can be seen in Table 3-4, which shows the total number of animals used in the study, broken down by plant, scenario, and endpoint, the sample sizes were not consistent for all experiments investigating the same endpoint, nor were they the same for each plant. For example, for the in vivo oxidative stress experiments at Plant 1, the sample size ranged from 12 to 48, depending on the scenario. At Plant 2, the sample size for the same biological endpoint ranged from 16 to 32, again depending on scenario. These differences occurred because some scenarios were repeated; rather than 4 exposure days, there might have been 8 days, or even 12 days for the PONS scenario at Plant 1. Since this was the first plant studied, we repeated scenarios in some cases to be certain outcomes were optimized and not influenced by the field setting. Since we had no reason to reject these collected data, they were included in the final analyses. Because filtered air exposures were conducted on each exposure day, this imbalance in sample size does not bias our scenario-specific estimates of exposure, but does cause tests of an effect to be more powerful for some scenarios than for others. Therefore, in interpreting the results of our statistical analyses, we focus on not only the strength (statistical significance) of the effects but also on the magnitude of these estimated effects

Table 3-4
Number of animals used by toxicological endpoint, scenario, and plant (exposed/control). All animals were normal male Sprague-Dawley rats, except where otherwise specified. Control scenarios (O, S, and OS) are not included.

Toxicological Endpoint	Plant 1				Plant 2					Plant 3			
	P	PO	POS	PONS[2]	P	PO	POS[3]	POS[4]	PONS	P	PO	POS	PONS
Breathing Pattern	20/20	15/15	20/20	60/60	20/20	20/20	20/20	20/20	20/20	20/20	20/20	20/20	20/20
Bronchoalveolar Lavage	-	5/5	6/6	18/18	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
In Vivo Oxidative Stress	8/8	6/6	8/8	24/24	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Pulmonary Histopathology	6/6	5/5	6/6	18/18	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
Blood Cytology	12/12	10/10	12/12	36/36	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12
Arrhythmias and Heart Rate Variability[1]	-	-	-	-	-	-	15/14	-		-	-	8/7[5]	-

[1] This endpoint employed the myocardial infarction model developed by Wellenius et al. (2004).
[2] 3 repetitions were done at different times of the day. No individual differences found between runs. All data analyzed together.
[3] SCR on
[4] SCR off
[5] 6/2 usable for analysis see Wellenius et al. (2011)
- Endpoint not evaluated

3.10 Data Analysis

The statistical approaches used in the TERESA study are described in detail by Coull et al. (2011). The statistical analyses employed a multi-layered approach, whereby multiple analyses were conducted using exposure metrics of increasing sensitivity. In the first level, ANOVA techniques were employed to assess whether differences between exposed and filtered air responses (i.e. a binary exposure covariate) varied by exposure scenario. In the second level, univariate associations between mass levels or exposure composition and health outcomes were determined. Single-component analyses were carried out in which separate regression models were fitted using differences between exposed and filtered air responses as the outcome and either mass, particle number, or measured component concentration as the exposure metric. We used the resulting *p*-values from these models to rank the strength of associations between each component and the particular biological response. In the third level, to further explore univariate associations, the concept of variable importance in a random forest analysis was introduced to investigate joint effects of multiple pollution components.

In addition to the complexity of the TERESA exposures, statistical analyses of the data must also account for the fact that the endpoints are recorded at different time resolutions. For example, BAL, blood, and chemiluminescence parameters are recorded once for each animal exposure, whereas for the respiratory parameters, readings are recorded continuously and averaged in epochs of 10 minutes. Thus, the strategy of increasing the sensitivity of the exposure metric is nested within regression extensions that respect the design of the study and the correlation structure of the data. Another design issue is the fact that the study design exposes animals to both pollution exposures and filtered air across multiple days, which are nested within weeks, which are in turn nested within three power plants. Thus, efficient estimation of health effects required that we control for nuisance day-to-day variability in a biologic outcome among the filtered air animals.

4
RESULTS: EXPOSURE CHARACTERIZATION

4.1 Diluted Stack Emissions

Figure 4-1 shows on-site sampling, dilution and aging of coal combustion emissions conducted using a mobile reaction laboratory (the front bus) and animal exposure using a mobile toxicology laboratory (the back trailer). Figure 4-2 is a schematic diagram showing the path from the diluted stack emissions to the exposure atmosphere, with additional dilutions at different locations in this path, and sampling ports. At Plants 1 and 2, the stack sampling flow was controlled using a stainless-steel restriction tube placed inside the stack (Figure 4-3a). Consequently, the dilution factor was about 130 at Plant 1 and about 150 at Plant 2. Plant 3 used a wet FGD scrubber to reduce SO_2 emissions, along with an ESP and a SCR unit. Because the wet scrubber resulted in highly humid stack emissions, the extraction system used at the other two plants had to be modified to prevent water condensation. The new extraction probe (Figure 4-3b) consisted of concentric sampling tubes: an outer tube (183 cm long and 7.63 cm ID) with a flow of dry, warm (about 100°C) filtered air for initial dilution of the wet stack emissions, and a thin inner tube (244 cm long and 1.27 cm ID) used to pass the initially diluted stack emissions into the aspirator. The inner tube was heated using an electric heating coil to prevent potential clogging due to water condensation. The stack sampling rate could be adjusted by changing the flow rate of hot, dry filtered air and by changing the amount of vacuum by controlling the total flow of dry filtered air into the aspirator. As a result, the dilution factor of 75 was used at Plant 3.

Figure 4-1
Mobile laboratory

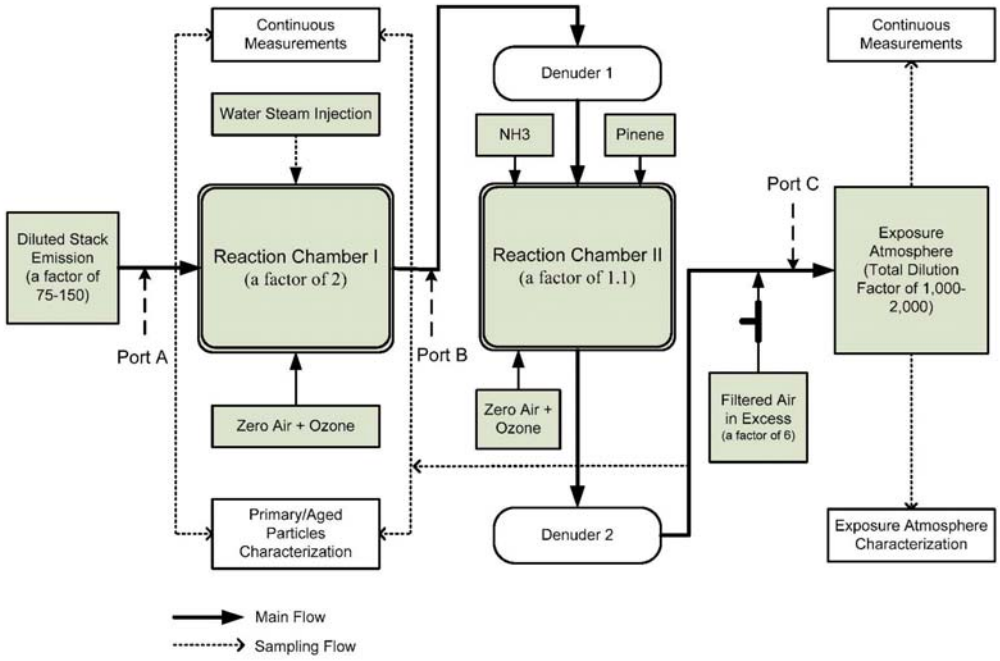


Figure 4-2
Overview of the dilution and sampling scheme: Total dilution factors were approximately 1700, 2000 and 1000 at Plants 1 though 3, respectively.

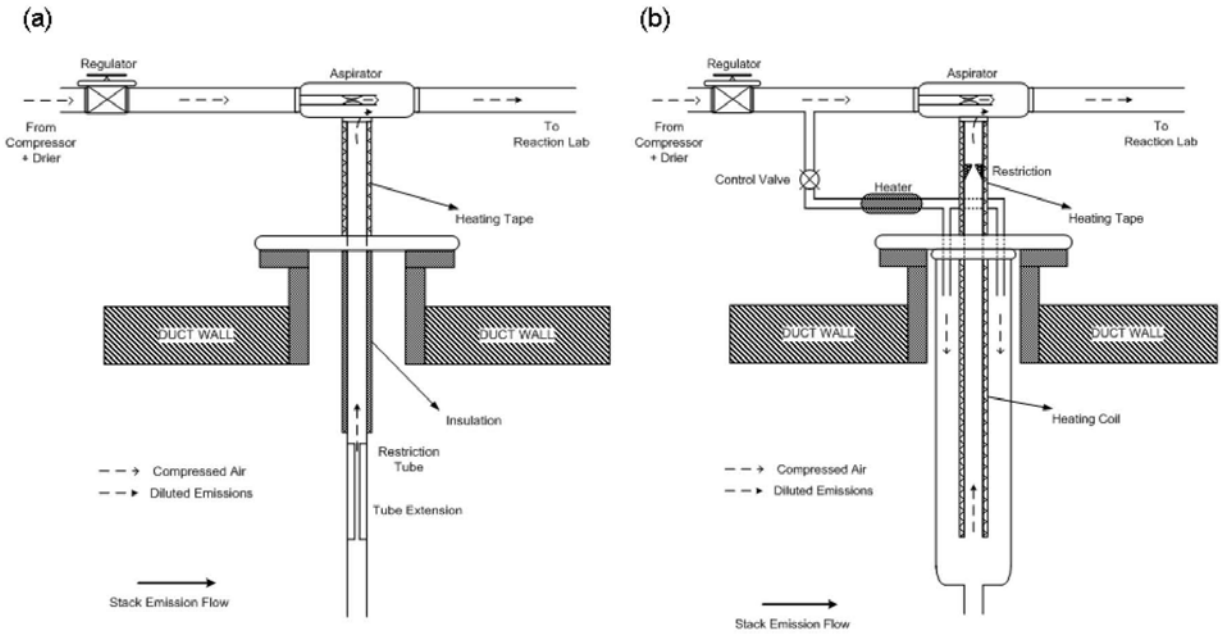


Figure 4-3
Schematic diagram of the stack extraction systems: (a) Plants 1 and 2; (b) Plant 3

Stack emissions were diluted initially by a factor of 75 to 150 in the stack extraction system on the duct. Particle size distribution, particle mass concentration, and concentrations of gaseous species were monitored at Port A, prior to the 1st reaction chamber to characterize the diluted stack emissions. Concentrations of gases and primary particles entering the 1st reaction chamber are summarized in Table 4-1.

Table 4-1
Diluted stack emissions in the stack extraction system

	SO_2 (ppb)	NO (ppb)	NO_2 (ppb)	SO_2/NOx ratio	Primary Particles ($\mu g/m^3$)
Plant 1[1]					
P (n=4)[2]	2009 (692-2621)[3]	1875 (379-2197)	25 (2-232)	1.1	17 (5-30)
PO (n=1)	838 (351-1188)	657 (406-836)	4 (2-29)	1.1	2
POS (n=4)	2860 (2733-3080)	2279 (2014-2478)	34 (6-82)	1.3	5 (3-6)
PONS (n=10)	1979 (1001-3298)	1681 (998-3301)	36 (3-186)	1.2	3 (2-6)
Plant 2[1]					
P (n=4)	4373 (3603-5264)	169 (129-203)	8 (2-25)	22.0	1 (0-1)
PO (n=4)	3369 (2292-6360)	104 (74-154)	12 (4-27)	34.0	9 (0-31)
POS(All, n=12)	3942 (2386-5756)	257 (60-521)	9 (1-32)	13.0	6(0-28)
POS(Non-SCR, n=4)	4659 (3640-5416)	470 (401-521)	8 (1-19)	9.4	2 (1-3)
POS(SCR, n=8)	3534 (2386-5756)	136 (60-304)	10 (4-27)	35.0	8 (0-28)
PONS (n=4)	4547 (2819-6123)	165 (99-302)	14 (8-32)	22.5	2 (0-3)
Plant 3[1]					
P (n=4)	1824 (747-6407)	760 (275-1602)	11 (1-51)	2.3	968 (520-1417)
PO (n=4)	1335 (792-1873)	591 (330-1025)	10 (2-45)	2.2	173 (98-255)
POS (n=8)	1418 (485-3937)	434 (128-1603)	11 (2-75)	3.5	790 (527-1116)
PONS (n=4)	1277 (613-2586)	335 (148-800)	18 (2-63)	4.3	467(344-708)
OS (n=4)	1027 (142-5074)	368 (116-1602)	10 (2-46)	2.5	-[4]
O (n=4)	1092 (439-1824)	398 (148-615)	11 (1-60)	2.8	-
S (n=4)	55 (19-160)	2 (2-16)	7 (3-27)	22.2	-

[1]Initial dilution factors on the stack: about 130 times at Plant 1, about 150 times at Plant 2 and about 75 times at Plant 3
[2]Number of primary particle samples measured at Part A; For SO_2 and NOx, continuous measurements were used for data summary.
[3]All values are an average and range
[4]Primary particles for OS and O scenarios were zero because a HEPA filter was used to remove primary particles upstream of the 1st reaction chamber

Gaseous Species: For all species and for all three plants, there was a fair amount of day-to-day variation in primary emissions, both within and between exposure days. This variation was most likely due to the inherent variation in power plant operations because a relatively constant factor was used to dilute stack emissions at each plant during the respective study periods. The concentrations of gaseous species are likely to depend on coal type and the emissions controls employed at each coal-fired power plant.

At Plant 1, equipped with an ESP, a higher NO concentration was observed in diluted stack emissions compared to the other plants with an SCR unit for NOx ($NO+NO_2$) emission control. This led to a lower ratio of SO_2 to NOx concentrations, which is expected to decrease the formation rate of secondary sulfate particles. At Plant 2, a higher ratio of SO_2 to NO was found in diluted stack emissions compared to the other plants because the plant used low-to-medium sulfur (~1.0% S) bituminous coal with an SCR. Note that the SCR unit was operational at Plant 2 during "ozone season" (May - September, 2005). The results for the POS scenario thus can be compared for the SCR-off period in March (the first 4 days) and for the SCR-on period during the period of May to September (the last 8 days). NO emissions during the SCR-on period were about 25% of those during the SCR-off period.

Diluted stack emissions at Plant 3 used a higher sulfur bituminous coal, had a lower SO_2 concentration (due to the use of a wet FGD scrubber), as compared to the other plants. Lower SO_2 stack emission required less stack dilution to produce comparable sulfate in the 1st reaction chamber. In contrast, high water content of stack emissions produced by the scrubber required a higher dilution to prevent water condensation in the transmission tube. In order to solve this eilema, a new stack extraction probe was designed, which made it possible to run at a lower initial dilution. A validation test of new stack extraction system was carried out during a period of relatively constant particle emissions at Plant 3. Primary particle mass concentration was measured downstream of the extraction system with different dilution factors. Dilution factors used were between about 20 and 70. Sampling time varied from 2 to 15 hours at a sampling flow of 10 L/min. During the test, diluted primary particle mass concentration ranged from 104 to 2,544 $\mu g/m^3$, with an average of 1,468 $\mu g/m^3$. A consistent relationship (slope=-47, R^2=0.98) was found between primary particle mass concentrations and dilution factors. This strong correlation indicates that the new extraction system prevented the clogging due to water condensation in the sampling probe that had been observed for this plant when using the original extraction system.

At Plant 3, diluted stack emissions had a large variability. For example, SO_2 concentration diluted at a factor of 75 varied from 142 ppb to 5,074 ppb during the period of the OS scenario. The most likely explanation for this instability was the occasionally unstable operation of the scrubber; two or three times during the entire study period, the scrubber was shut down. However, the shutdowns did not occur during animal exposures. The instability may also have been influenced by varying sulfur content in the coal.

Primary Particles: As shown in Table 4-1, much lower concentrations of primary particles were observed at Plant 1 (an average of 6.2 $\mu g/m^3$, n=19) and Plant 2 (an average of 4.7 $\mu g/m^3$, n=24) compared to Plant 3 (an average of 637.8 $\mu g/m^3$, n=20). When the concentrations were determined for direct emissions (downstream of the ESP at Plants 1 and 2, and downstream of FGD scrubber at Plant 3), taking into account dilution in the extraction system, the mean direct emissions of primary particles were 0.8 mg/m^3 at Plant 1, 0.7 mg/m^3 at Plant 2 and 47.8 mg/m^3 at Plant 3. The difference in primary particle emissions likely results mainly from different emissions control configurations, rather than different coal types (see discussion below).

SO_2 Conversion Ratio: Diluted SO_2 was converted to H_2SO_4 in the 1st chamber, under conditions of UV irradiation, O_3 and water vapor. The SO_2 conversion ratio was affected by the ratio of SO_2 to NOx concentrations, when keeping the other parameters constant (total added ozone, humidity and intensity of UV irradiation). This is because the SO_2 to H_2SO_4 conversion is restricted in the presence of NOx. The NOx-OH reaction competes with, and is faster than the SO_2-OH reaction (Luria et al., 1983; Seinfeld and Pandis, 1986). Consequently, with lower NOx concentration there is greater excess of ozone (as a source of OH radical, under sufficient UV irradiation and water vapor), which resulted in a higher reaction rate of SO_2. The SO_2 conversion ratio was calculated as follows:

SO_2 Conversion Ratio = $(F_{sulfate} - P_{sulfate})/P_{SO2}$

Where, $F_{sulfate}$ is the sulfate concentration ($\mu g/m^3$) downstream of the chamber; P_{SO2} is the SO_2 concentration ($\mu g/m^3$ sulfate equivalent) transmitted into the chamber; and $P_{sulfate}$ is primary sulfate concentration ($\mu g/m^3$) emitted from the stack. On average, the SO_2 conversion ratio was 16.7% at Plant1 (Ruiz et al., 2007b). At Plant 2, a higher ratio of SO_2 to NO was found in diluted stack emissions compared to the other plants because the plant used higher sulfur coals with an SCR. The lower NO concentration resulted in a higher excess of ozone (for a fixed amount of ozone added) which made it possible to obtain a higher conversion ratio. For instance, NO emissions during the SCR-off period were four times higher than during the SCR-on period. On average, the SO_2 conversion ratio was 7.7% during the SCR-off period, while the ratio was 19.0% during the SCR-on period.

Diluted stack emissions at Plant 3 had relatively lower SO_2 and higher NO concentrations than those at Plant 2. Because sulfate was found to be a major component of primary particles, the primary sulfate concentration was deducted from the measured sum ($F_{sulfate}$) of primary plus secondary sulfate to calculate the SO_2 conversion ratio. On average, a higher conversion ratio (22.2%) was observed at Plant 3, which was most likely due to lower SO_2 concentrations entering the 1st chamber as compared to Plant 2.

4.2 Exposure Atmospheres

Diluted stack emissions were additionally diluted about 12 times to be used for animal exposures. As a result, total dilution factors were approximately 1700, 2000 and 1000 at Plants 1, 2 and 3, respectively. Gaseous co-pollutants and particulate species measured at the sampling port (Port C) in the toxicological laboratory were characterized for all exposure atmospheres. Gaseous co-pollutants, temperature and relative humidity are summarized for each scenario in Tables 4-2, 4-3, and 4.4, for each power plant, respectively. Particle mass and its constituents are also summarized by scenario in Tables 4-5, 4-6, and 4-7. Note that the fieldwork at Plant 3 differed in two ways from the other two plants: 1) Control scenarios (OS, O and S) excluding primary particles were tested to evaluate the effects of primary particles and of atmospheric constituents; 2) because the TEOM, SMPS and APS were not operational (as mentioned above), DustTrak Aerosol Monitors were used to measure continuous particle mass concentration**.**

Gaseous Co-Pollutants: Diluted stack emissions underwent photochemical reactions to simulate aging of coal combustion emissions in the reaction chambers. After these reactions, there were significant amounts of gaseous co-pollutants, such as SO_2, NO, NO_2, O_3 and volatile organic compounds (VOCs), together with the primary and secondary particles. Two parallel plate diffusion denuders were used to decrease concentrations of these gases to prevent unwanted toxic effects for animal exposures.

VOC concentrations (Tables 4-2 through 4-7) are much more likely to have come from the photochemical products formed in the reaction chambers than from the primary emissions. Only relatively small amounts, if any, of VOCs are expected to be formed during coal combustion. Because these VOCs would then have been diluted with particle-free ambient air with a total factor of 1000 to 2000 by power plants, and then mostly removed by passing through the two

denuders, only insignificant concentrations from combustion sources were expected to be in the exposure atmospheres.

Available toxicological information suggests that no significant toxic effects would be expected for any of the co-pollutant gases at concentrations below 100ppb (see Ruiz et al., 2007a). To be conservative, our target value for maximum concentrations of gaseous co-pollutants was chosen to be 50ppb. All gaseous co-pollutant concentrations for all plants were below this target value, except for one day for SO_2 (272 ppb) during the POS scenario at Plant 3 when the FGD scrubber malfunctioned. At Plant 3, organic gases were not measured because no significant amounts of these species were detected at the other plants for which the same conditions were used to form SOA. Temperature and relative humidity depended on those of ambient air and were similar at all three power plants.

Table 4-2
Gaseous species concentrations and metrological parameters for each scenario at Plant 1

Species	P (n=4)[2]	PO (n=3)	POS (n=4)	PONS (n=12)
O_3 (ppb)	1.0 ± 1.2[3]	26.9 ± 1.0	26.8 ± 6.9	26.6 ± 9.3
NO (ppb)	5.9 ± 3.7	3.9 ± 0.5	3.5 ± 2.9	3.7 ± 1.6
NO_2 (ppb)	6.7 ± 1.7	8.4 ± 1.8	17.5 ± 6.6	16.9 ± 7.0
SO_2 (ppb)	NM[4]	31.7 ± 4.3	38.9 ± 8.3	34.5 ± 5.9
HNO_3 (ppb)	0.7 ± 0.4	0.6 ± 0.1	1.6 ± 0.3	2.4 ± 0.5
HONO (ppb)	2.7 ± 2.4	5.0 ±1.0	11.2 ± 5.1	8.3 ± 2.5
NH_3 (ppb)	26.0 ± 26.5	9.9 ± 6.2	20.8 ± 3.8	5.9 ± 8.3
Formaldehyde (ppb)	NM	NM	13.4 ± 3.0	17.6 ± 4.3
Acetaldehyde (ppb)	NM	NM	2.9 ± 0.6	3.0 ± 0.7
Acetone (ppb)	NM	NM	6.7 ± 2.2	8.8 ± 5.2
α-pinene (ppb)	NM	NM	0.1 ± 0.0	0.2 ± 0.1
Temp. (°C)	23.4 ± 0.5	23.9 ± 0.1	24.4 ± 0.4	24.4 ± 1.1
RH (%)	38.7 ± 7.6	18.4 ± 1.9	21.6 ± 5.5	37.7 ± 10.8

[1]VOCs were not measured for the non-SOA scenarios, P and PO, but the concentrations were expected to have been close to typical ambient levels, resulting from relatively high dilution with particle-free ambient air, required to supply the exposure atmospheres and characterization measurements
[2]Number of days
[3]Average ± standard deviation.
[4]Not measured

Table 4-3
Gaseous species concentration and metrological parameters for each scenario at Plant 2

Species	P (n=4)[2]	PO (n=4)	POS			PONS (n=4)
			Non-SCR (n=4)	SCR (n=8)	All (n=12)	
O_3 (ppb)	0.0 ± 0.0[3]	13.5 ± 1.9	29.6 ± 9.8	17.5 ± 13.8	21.5 ± 13.5	19.0 ± 2.1
NO (ppb)	5.5 ± 1.8	8.4 ± 3.0	1.3 ± 0.4	5.5 ± 2.4	4.1 ± 2.8	6.5 ± 1.0
NO_2 (ppb)	2.1 ± 0.5	2.2 ± 4.3	0.4 ± 0.5	0.7 ± 1.2	0.6 ± 1.0	0.1 ± 0.1
SO_2 (ppb)	34.3 ± 12.0	37.0 ± 3.5	36.0 ± 3.0	30.8 ± 7.3	32.5 ± 6.5	25.7 ± 2.2
HNO_3 (ppb)	0.2 ± 0.1	0.9 ± 0.5	1.2 ± 0.1	0.4 ± 0.1	0.6 ± 0.4	1.1 ± 0.5
HONO (ppb)	2.9 ± 2.2	1.9 ± 0.6	4.5 ± 0.9	2.1 ± 1.2	2.9 ± 1.6	2.2 ± 1.2
NH_3 (ppb)	0.1 ± 0.2	0.0 ± 0.0	3.2 ± 3.3	15.4 ± 23.3	11.3 ± 19.6	1.9 ± 2.2
Formaldehyde (ppb)	NM[4]	NM	20.7 ± 2.8	11.8 ± 6.7	14.7 ± 7.1	10.7 ± 7.3
Acetaldehyde (ppb)	NM	NM	6.8 ± 1.1	4.2 ± 1.5	5.0 ± 1.9	5.7 ± 1.9
Acetone (ppb)	NM	NM	22.6 ± 2.9	10.0 ± 7.1	14.2 ± 8.6	20.5 ± 4.5
α-pinene (ppb)	NM	NM	7.8 ± 8.0	6.2 ± 4.2	6.8 ± 5.4	6.0 ± 3.4
Temp. (°C)	24.2 ± 0.1	22.7 ± 1.2	23.3 ± 1.1	22.7 ± 0.9	22.9 ± 0.9	23.1 ± 0.7
RH (%)	58.2 ± 1.8	37.7 ± 4.4	70.4 ± 34.6	52.4 ± 20.7	58.4 ± 26.1	50.7 ± 2.4

[1]VOCs were not measured for the non-SOA scenarios, P and PO, but the concentrations were expected to have been close to typical ambient levels, resulting from relatively high dilution with particle-free ambient air, required to supply the exposure atmospheres and characterization measurements
[2]Number of days
[3]Average ± standard deviation

[4]Not measured

Table 4-4
Gaseous species concentrations and metrological parameters for each scenario at Plant 3

Species	P (n=4)[2]	PO (n=4)	POS (n=8)	PONS (n=4)	OS (n=4)	O (n=4)	S (n=4)
O_3 (ppb)	8.9 ± 3.3[3]	28.8 ± 8.8	29.2 ± 10.0	15.2 ± 6.6	19.9 ± 3.3	18.3 ± 4.2	21.0 ± 2.4
NO (ppb)	7.5 ± 2.4	9.4 ± 2.2	7.2 ± 1.5	6.3 ± 0.3	7.5 ± 3.8	5.8 ± 0.1	8.4 ± 0.7
NO_2 (ppb)	4.4 ± 0.8	3.7 ± 0.7	4.7 ± 1.2	3.7 ± 2.2	2.0 ± 0.4	2.5 ± 0.7	3.8 ± 1.0
SO_2 (ppb)	38.9 ± 15.7	34.7 ± 7.1	72.8 ± 83.2	23.1 ± 6.9	15.1 ± 6.6	24.4 ± 14.5	22.1 ± 8.1
HNO_3 (ppb)	0.0 ± 0.0	0.0 ± 0.1	0.1 ± 0.2	0.8 ± 1.0	0.3 ± 0.4	0.0 ± 0.0	0.1 ± 0.1
HONO (ppb)	3.5 ± 0.5	0.3 ± 0.6	2.9 ± 1.4	3.8 ± 3.1	1.2 ± 1.8	0.2 ± 0.4	1.0 ± 1.0
NH_3 (ppb)	0.3 ± 0.4	2.2 ± 4.3	2.3 ± 3.2	4.6 ± 4.4	8.3 ± 9.9	0.3 ± 0.6	3.3 ± 2.6
α-pinene (ppb)	NM[4]	NM	0.4 ± 0.6	5.2 ± 5.6	2.3 ± 1.0	NM	3.0 ± 0.5
Temp. (°C)	23.3 ± 1.0	23.4 ± 1.2	23.8 ± 1.1	21.9 ± 0.3	24.5 ± 1.5	23.5 ± 0.0	23.9 ± 0.3
RH (%)	56.1 ± 17.6	26.5 ± 11.6	53.3 ± 19.6	53.0 ± 17.1	47.5 ± 10.2	38.7 ± 10.3	36.0 ± 2.0

[1]Aldehydes and acetone were not measured for this power plant because these concentrations were expected to be similar to the other two plants
[2]Number of days
[3]Average ± standard deviation
[4]Not measured

Particle Mass Concentration: At Plants 1 and 2, filter-based gravimetric and continuous TEOM measurements were conducted to determine particle mass concentration. As shown in Figure 4.4a, the results from the two methods are highly correlated (R^2=0.93), but continuous TEOM concentrations are about 35% less than those obtained from the gravimetric method. This is due to the loss of semi-volatile compounds and nitrate in particles from the heated (50 °C) filter in the TEOM monitor. In addition, calculated mass concentration was estimated from the sum of specific components analyzed, including NO_3^-, SO_4^{2-}, H^+, NH_4^+, OC, EC and elements (excluding sulfur). The calculated mass concentrations are well-correlated with gravimetric mass (R^2=0.90) and continuous TEOM mass (R^2=0.89), respectively, but the slopes of less than unity indicate lower values for the calculated mass. Several studies (Chow et al., 2004; Watson et al., 2001; Lee 2001) that reported $PM_{2.5}$ mass concentrations emitted from coal-fired power plants also found that the sum of analyzed species (water-soluble ions, OC, EC and elements) accounted only for 35%-60% of the total $PM_{2.5}$ mass. This might be due to the particle-bound water in addition to unmeasured species. The calculated mass may be the best indicator of particle mass concentration to investigate correlations between health effects and particle mass exposures. Thus this paper reports the calculated mass for particle mass concentration, which is slightly different from the concentrations reported previously at Plant 1 (Ruiz et al., 2007a).

At Plant 1, the average aged particle mass concentrations (i.e. calculated particle mass concentrations) ranged from the lowest of 46.0 μg/m^3 for the oxidized scenario (PO) to the highest of 154.9 μg/m^3 for the most complex neutralized scenario (PONS). Primary particles (1.0 μg/m^3, for the P scenario) accounted for <1% of particle mass for the aged scenarios. Day-to-day and within-day variation in SO_2 and NO_x emissions resulted in substantial variation of the amount of secondary particle formation, as mentioned above. At Plant 2, the pattern of particle mass by scenarios was the same as at Plant 1. The aged particle mass concentrations ranged from 115.5 μg/m^3 for the PO scenario to 257.1 μg/m^3 for the PONS scenario. Primary particles (1.7 μg/m^3) accounted for <1% of total mass for the aged scenarios. During the SCR-off period higher NO concentrations might have resulted in the observed lower ratio of SO_2 to NO_x concentrations and thus less formation of secondary sulfate and aged particles, compared to the SCR-on period. On the other hand, particle number and carbonaceous species showed similar levels regardless of SCR use.

Figure 4-4b shows relationships between gravimetric, calculated and continuous DustTrak mass concentrations at Plant 3. As for the other plants, the calculated mass well-correlated with the gravimetric mass (R^2=0.74), and was lower than gravimetric mass. The continuous values from the DustTrak were expected to be a good surrogate of particle mass measurement. However, since light scattering is not a direct measurement of particle mass concentration, the DustTrak values must be corrected using direct particle mass values. The correction factor was determined as the ratio of the calculated particle mass to the averaged DustTrak values. Because the correction factor was expected to vary with particle composition which varied by scenario, it was determined from the ratios for each scenario. The experimentally determined factors were; 1.7, 3.9, 6.6, 7.1, 6.8, 3.9, and 7.3 for the P, PO, POS, PONS, OS, O and S scenarios, respectively.

Table 4-5
Particle component concentrations at Plant 1.

Species	P (n=4)[1]	PO (n=3)	POS (n=4)	PONS (n=12)
Particle Mass ($\mu g/m^3$)[2]	1.0 ± 0.9[3]	46.0 ± 12.6	123.3 ± 28.4	154.9 ± 41.7
Gravimetric Mass ($\mu g/m^3$)	2.3 ± 2.6	69.5 ± 10.4	192.6 ± 73.3	212.6 ± 59.7
Continuous Mass ($\mu g/m^3$)[4]	0.0 ± 3.3	58.2 ± 5.8	138.2 ± 53.4	173.6 ± 56.8
Number (particles/cm^3)	1726 ± 1277	6723 ± 3550	16 924 ± 4494	52 109 ± 11951
Total Sulfate ($\mu g/m^3$)	0.2 ± 0.3	36.1 ± 7.7	55.8 ± 22.8	68.2 ± 28.8
Acidic Sulfate ($\mu g/m^3$)[5]	2.3 ± 0.4	27.6 ± 9.5	50.2 ± 21.6	14.7 ± 13.6
Neutralized Sulfate ($\mu g/m^3$)[6]	0.0 ± 0.0	8.4 ± 2.6	5.6 ± 3.4	53.6 ± 16.8
Nitrate ($\mu g/m^3$)	0.1 ± 0.2	0.7 ± 1.2	0.3 ± 0.4	30.4 ± 8.2
Ammonium ($\mu g/m^3$)	0.0 ± 0.0	3.2 ± 1.5	2.5 ± 1.1	20.7 ± 6.0
OC ($\mu g/m^3$)	0.0 ± 0.0	2.6 ± 4.5	51.6 ± 3.4	53.6 ± 16.8
EC ($\mu g/m^3$)	0.7 ± 0.8	3.1 ± 1.7	11.9 ± 9.1	5.0 ± 2.9
Na (ng/m^3)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mg (ng/m^3)	0.2 ± 0.4	0.0 ± 0.0	2.2 ± 2.7	1.3 ± 2.7
Al (ng/m^3)	2.2 ± 1.6	9.2 ± 6.6	13.9 ± 5.9	4.1 ± 5.3
Si (ng/m^3)	1.9 ± 1.3	20.2 ± 16.3	10.9 ± 7.9	4.2 ± 4.9
P (ng/m^3)	0.5 ± 0.4	2.3 ± 3.9	2.7 ± 1.8	0.6 ± 0.9
S ($\mu g/m^3$)	0.1 ± 0.1	12.0 ± 2.6	18.6 ± 7.6	22.7 ± 9.6
K (ng/m^3)	0.2 ± 0.2	0.0 ± 0.1	0.2 ± 0.2	0.3 ± 0.2
Ca (ng/m^3)	7.0 ± 1.7	12.4 ± 7.5	49.8 ± 22.2	16.2 ± 17.4
Ti (ng/m^3)	0.2 ± 0.3	0.5 ± 0.6	2.1 ± 1.1	0.7 ± 0.8
Cr (ng/m^3)	3.0 ± 1.1	14.3 ± 24.8	0.0 ± 0.1	0.0 ± 0.0
Fe (ng/m^3)	8.4 ± 4.2	60.0 ± 101.2	7.4 ± 4.2	2.8 ± 2.9
Ni (ng/m^3)	0.6 ± 0.2	7.6 ± 13.0	0.0 ± 0.0	0.0 ± 0.1
Zn (ng/m^3)	0.0 ± 0.0	0.4 ± 0.5	0.0 ± 0.0	0.0 ± 0.1
Se (ng/m^3)	0.5 ± 0.4	0.6 ± 0.6	0.5 ± 0.5	0.3 ± 0.4
Pb (ng/m^3)	0.4 ± 0.4	0.1 ± 0.2	0.0 ± 0.0	0.1 ± 0.2

[1]Number of days
[2]Particle mass was calculated from the sum of analyzed components
[3]All values are average ± standard deviation
[4]Continuous mass was determined using a TEOM monitor
[5]H_2SO_4 equivalent
[6]Total Sulfate – H_2SO_4

Table 4-6
Particle component concentrations at Plant 2

Species	P (n=4)[a]	PO (n=4)	POS			PONS (n=4)
			Non-SCR (n=4)	SCR (n=8)	All (n=12)	
Particle Mass (μg/m^3)[b]	1.7±1.8[c]	115.5±18.5	213.6±60.2	212.1±39.7	212.7±45.1	257.1±10.0
Gravimetric Mass (μg/m^3)	1.9±1.3	224.3±53.3	377.2±100.0	432.5±95.0	414.1±96.0	477.7±51.6
Continuous Mass (μg/m^3)[d]	13.9±9.5	202.9±36.7	201.3±49.8	308.1±59.7	279.0±74.0	354.8±37.7
Number (particles/cm^3)	910±964	4281±1911	22 500±19 085	11 473±3774	14 481±10 457	40 811±2179
Total Sulfate (μg/m^3)	0.0±0.0	100.3±16.3	81.5±29.0	146.0±36.7	122.6±46.1	154.8±12.4
Acidic Sulfate (μg/m^3)[e]	0.0±0.0	71.6±17.0	68.8±22.0	107.9±31.7	93.7±33.7	15.7±3.8
Neutralized Sulfate (μg/m^3)[f]	0.0±0.0	28.8±1.3	12.9±10.7	38.1±12.0	28.9±16.8	139.1±15.5
Nitrate (μg/m^3)	0.0±0.0	0.2±0.2	0.8±0.2	0.3±0.3	0.5±0.3	6.4±1.7
Ammonium (μg/m^3)	0.0±0.0	6.1±0.4	4.7±1.2	9.1±3.4	7.5±3.5	50.3±5.3
OC (μg/m^3)	0.0±0.0	0.0±0.0	114.3±71.6	59.0±20.2	81.2±52.5	35.1±10.1
EC (μg/m^3)	1.7±1.8	7.3±3.2	10.8±3.9	12.5±6.9	11.9±6.0	10.2±3.9
Na (ng/m^3)	0.0±0.0	2.5±4.9	1.3±1.9	0.0±0.0	0.3±0.8	0.0±0.0
Mg (ng/m^3)	2.1±2.4	0.8±1.6	2.3±3.2	0.0±0.0	0.5±1.4	0.1±0.1
Al (ng/m^3)	0.2±0.5	2.8±2.8	2.7±1.6	2.2±2.4	2.3±2.2	0.2±0.4
Si (ng/m^3)	1.5±1.5	8.1±14.3	4.2±0.7	2.5±2.4	2.9±2.2	1.2±0.5
P (ng/m^3)	0.0±0.0	4.3±8.5	0.1±0.2	0.7±1.6	0.6±1.4	0.0±0.0
S (μg/m^3)	0.0±0.0	33.4±5.4	27.2±9.7	48.7±12.2	40.9±15.4	51.6±4.1
K (ng/m^3)	0.2±0.4	0.1±0.2	0.3±0.2	0.0±0.1	0.1±0.1	0.2±0.2
Ca (ng/m^3)	0.1±0.2	0.3±0.4	0.4±0.5	0.7±1.2	0.6±1.0	0.2±0.3
Ti (ng/m^3)	0.1±0.2	0.1±0.1	0.2±0.2	0.2±0.1	0.2±0.1	0.1±0.1
Cr (ng/m^3)	0.1±0.2	0.0±0.0	0.0±0.0	21.2±33.7	17.0±31.0	2.2±3.6
Fe (ng/m^3)	3.4±3.9	0.2±0.3	0.0±0.0	175.3±270.4	140.2±249.6	16.8±30.1
Ni (ng/m^3)	0.0±0.1	0.0±0.0	0.2±0.1	17.9±28.1	14.3±25.8	1.8±3.5
Zn (ng/m^3)	0.0±0.1	0.1±0.1	0.0±0.0	0.1±0.2	0.1±0.2	0.0±0.0
Se (ng/m^3)	0.1±0.3	0.4±0.5	0.0±0.0	0.1±0.2	0.1±0.2	0.2±0.3
Pb (ng/m^3)	0.5±1.1	0.1±0.1	0.0±0.0	0.1±0.2	0.1±0.2	0.6±0.7

[1]Number of days
[2]Particle mass was estimated from the sum of analyzed components
[3]All values are average ± standard deviation
[4]Continuous mass was determined using a TEOM monitor
[5]H_2SO_4 equivalent
[6]Total Sulfate – H_2SO_4

Table 4-7
Particle component concentrations at Plant 3.

Species	P (n=4)[1]	PO (n=4)	POS (n=8)	PONS (n=4)	OS (n=4)	O (n=4)	S (n=4)
Particle Mass (μg/m^3)[2]	43.2±14.6[3]	82.3±15.6	144.4±31.6	173.5±20.9	137.8±9.3	43.8±3.5	61.4±6.6
Gravimetric Mass (μg/m^3)	73.8±28.0	193.1±3.5	269.2±53.1	244.4±10.2	205.1±47.9	84.9±22.0	62.2±18.4
Continuous Mass (μg/m^3)[4]	45.1±25.8	81.3±21.2	162.6±38.0	167.8±25.3	137.9±12.1	43.8±7.4	61.0±5.2
Number (particles/cm^3)	55 947±11 769	69 372±8 523	40 446±6657	38 483±3651	35 959±6290	29 294±2392	7574±1598
Total Sulfate (μg/m^3)	34.0±13.3	77.9±14.5	83.4±21.3	85.0±12.9	47.2±14.6	40.6±3.8	1.3±0.4
Acidic Sulfate (μg/m^3)[5]	12.8±7.1	66.6±16.8	68.9±18.2	2.5±2.0	30.3±11.6	31.7±5.8	1.0±1.3
Neutralized Sulfate (μg/m^3)[6]	21.2±9.2	10.3±2.8	14.5±7.1	82.5±13.5	16.9±11.6	8.9±2.3	0.7±0.5
Nitrate (μg/m^3)	0.0±0.0	0.0±0.0	0.1±0.2	7.3±2.2	0.1±0.1	0.0±0.0	0.0±0.0
Ammonium (μg/m^3)	6.7±2.6	3.1±1.4	4.5±1.6	28.9±4.8	6.3±4.2	2.6±0.7	0.3±0.2
OC (μg/m^3)	1.9±3.8	0.0±0.0	54.7±27.5	52.0±23.0	83.6±9.6	0.0±0.0	59.7±6.1
EC (μg/m^3)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Na (ng/m^3)	113.6±40.2	86.2±64.2	138.3±108.0	92.7±34.7	NM[7]	NM	NM
Mg (ng/m^3)	30.6±5.0	19.6±9.7	29.9±18.9	13.4±8.4	NM	NM	NM
Al (ng/m^3)	21.3±4.4	14.0±5.6	17.1±10.3	10.2±2.4	NM	NM	NM
Si (ng/m^3)	5.9±6.1	9.8±6.6	5.2±3.9	1.3±1.5	NM	NM	NM
P (ng/m^3)	79.5±14.3	46.9±15.9	84.1±65.8	58.0±5.6	NM	NM	NM
S (μg/m^3)	11.3±4.4	26.0±4.8	27.8±7.1	28.3±4.3	NM	NM	NM
K (ng/m^3)	0.8±0.1	1.6±0.5	0.9±0.9	0.9±0.7	NM	NM	NM
Ca (ng/m^3)	7.4±4.4	14.8±2.0	7.1±5.3	6.8±1.6	NM	NM	NM
Ti (ng/m^3)	0.5±0.4	0.9±0.3	0.4±0.4	0.6±0.7	NM	NM	NM
Cr (ng/m^3)	5.0±2.7	1.5±0.4	6.5±5.0	7.3±1.5	NM	NM	NM
Fe (ng/m^3)	33.7±17.9	26.2±5.9	39.3±26.9	44.0±8.2	NM	NM	NM
Ni (ng/m^3)	2.0±1.2	0.8±0.4	2.9±2.1	2.8±0.7	NM	NM	NM
Zn (ng/m^3)	1.5±0.6	0.5±0.2	1.4±0.9	4.3±2.7	NM	NM	NM
Se (ng/m^3)	43.8±18.5	7.3±3.0	30.0±8.5	19.3±5.9	NM	NM	NM
Pb (ng/m^3)	0.4±0.8	0.2±0.3	0.4±0.9	0.1±0.1	NM	NM	NM

[1]Number of days
[2]Particle mass was calculated from the sum of analyzed components
[3]All values are average ± standard deviation
[4]Continuous mass was determined using a DustTrak aerosol monitor (with adjustment of measured values, as described above)
[5]H_2SO_4 equivalent
[6]Total Sulfate – H_2SO_4
[7]Not measured; Primary particles were removed by a HEPA filter

The relationship between the calculated mass and corrected DustTrak mass concentrations for all scenario days is also shown in Figure 4-4b. The slope was close to unity and the correlation coefficient (R^2=0.90) was reasonable.

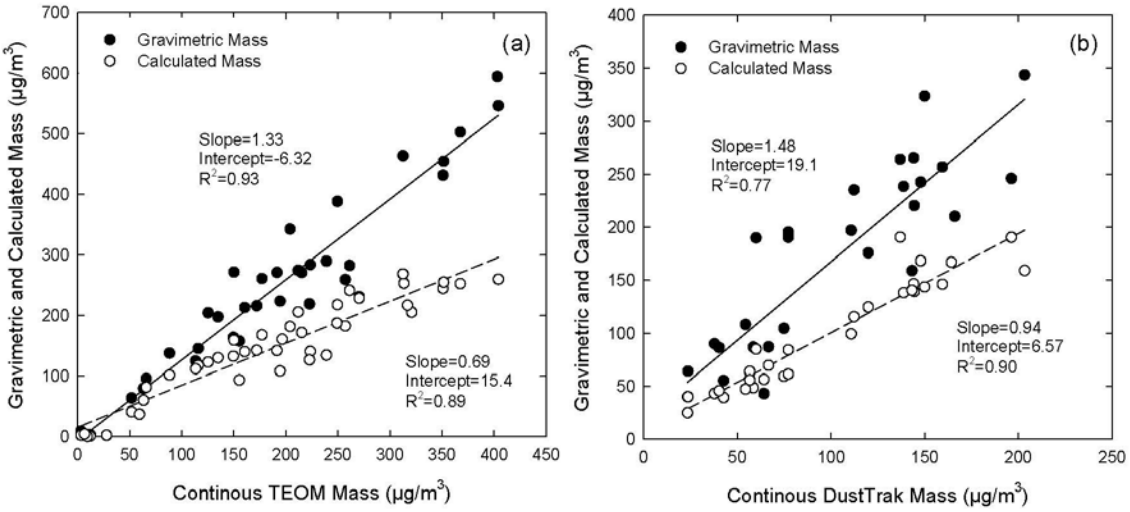


Figure 4-4
Comparison of gravimetric, calculated and continuous mass concentrations: (a) TEOM measurement was used at Plants 1 and 2; (b) DustTrak measurement was used at Plant 3; The DustTrak mass concentrations were adjusted with the calculated mass by scenario.

As for Plants 1 and 2, there was a fair amount of day-to-day variation in the aged particle mass concentration at Plant 3. Note, however, that with prior 6-hr chamber stabilization, aged particle mass concentrations in exposure atmospheres at all power plants were relatively constant throughout the exposure period on any given day even though there was substantial variation in SO_2 and NO_x emissions. While primary particles contributed a small fraction to the aged particle mass concentration at Plants 1 and 2, at Plant 3 primary particle emissions were considerably higher, due possibly to the FGD scrubber (discussed below), as well as to a lower dilution. The aged particle mass concentration averaged by scenario ranged from 43.8 µg/m^3 for the control scenario (O) to 173.5 µg/m^3 for the PONS scenario. The relative magnitudes for different scenarios at Plant 3 showed the same pattern as at the other two plants. However, at Plant 3 the P scenario contained a relatively higher particle mass concentration (43.2 µg/m^3), with a higher number concentration (55,947 particles/cm^3), compared to the other plants. Primary particles were composed mainly of sulfate, which was composed maily of H_2SO_4 and neutralized sulfate, accounting for about 79% of the total mass. The ionic ratio of $(H^+ + NH_4^+)/(2SO_4^{2-} + NO_3^-)$ ranged from 0.85 to 0.97, with an average of 0.92, which is similar to typical ambient particles with minimal amounts of other salts such as sodium and calcium. The H^+/SO_4^{2-} ratio indicating relative amount of neutralization was about 0.72, which means that about 36% of primary sulfate was acidic at this plant. The mass contribution of primary particles to the aged particles was calculated from dilution factors (~12 times, Port A through Port C), primary particle mass concentrations (Port A) and the aged particle mass concentration (Port C) during exposure runs. As a result, primary particles contributed about 8-24% to the total aged particle mass

concentrations for the PO, POS, and PONS scenarios, and 115% for the P scenario. An overestimate for the P scenario is likely due to relatively small particle losses that occurred in the pathway to the exposure system.

Particle Number and Size Distribution: It is also worthwhile to compare particle number concentration of the aged particles in the different scenarios. At Plant 1, the highest average was 52,109 particles/cm^3 for the neutralized scenario (PONS, which also had the highest aged particle mass concentration), followed by the POS, PO, and P scenarios, with averages of 16,924, 6,723, and 1,726 particles/cm^3, respectively. This pattern was also observed at Plant 2, showing the highest (40,811 particles/cm^3) for the PONS scenario, followed by the POS, PO, and P scenarios, with averages of 14,481, 4,281, and 910 particles/cm^3, respectively. These results suggest that particle number concentration increases with the complexity of scenario, due possibly to increasing particle nucleation.

In addition to primary particle mass concentration, Plant 3 also had a significantly different pattern in particle number concentration. More specifically, the P scenario had a particle number concentration of 55,947 particles/cm^3, compared to the first two plants (1,726 and 910 particles/cm^3 at Plant 1 and 2, respectively). The highest number (69,372 particles/cm^3) was for the PO scenario, while the lowest (38,483 particles/cm^3) was for the neutralized scenario (PONS). Except for the PO scenario (carried out during the last period at this plant), particle number concentration overall decreased inversely with increased scenario complexity, and this could have been a result of increasing particle coagulation with increasing aerosol complexity.

Figure 4-5 shows the particle size distribution of a typical example of primary particles and aged particles for the PONS scenario at Plants 1 and 2. For primary particles, particle size distribution was monitored using an SMPS plus an APS upstream of the 1st reaction chamber. The particle size distribution for the aged particles was monitored downstream of the 2nd reaction chamber. The particle size distributions were more or less identical for these two plants. Primary particles were mostly ultra-fine particles and had a unimodal distribution with a peak of about 20 nm, while the aged particles had a unimodal distribution with a peak of about 100 nm. Overall, the result clearly indicated that the particles grow in size during the aging process.

Ionic Species: Particulate (total) sulfate for exposure atmospheres was composed mainly of secondary sulfate formed in the 1st reaction chamber; primary sulfate was negligible at Plants 1 and 2. Total sulfate concentration includes both acidic and neutralized sulfate. Acidic sulfate was calculated from strong acidity (pH) measurement as the equivalent of H_2SO_4 aerosol (Koutrakis et al., 1988). Neutralized sulfate was determined by subtracting acidic sulfate from total sulfate. As expected, all three power plants had similar proportions of the two sulfate species for the same scenario. Day-to-day variation of sulfate within a given scenario contributed significantly to the variation observed in overall aged particle mass concentration.

At Plant 1, the highest total sulfate average was observed for the neutralized scenario (68.2 µg/m3, accounting for 44.0% of the total mass), followed, in order, by the POS and PO scenarios, with averages of 55.8 and 36.1 µg/m3, respectively. The highest acidic sulfate value was 50.2 µg/m3 for the POS scenario, followed by the PO and PONS scenarios, with averages of 27.6 and 14.7 µg/m3, respectively. As expected, neutralized sulfate was present at a higher

concentration (53.6 µg/m3) for the neutralized scenario as compared to the unneutralized scenarios (0-8.4 µg/m3). At Plant 2, the highest total sulfate average (154.8 µg/m3, accounting for 62.2% of the total mass) was observed for the neutralized scenario (PONS), followed by the SCR-on POS, PO, and SCR-off POS, with averages of 146.0, 100.3, and 81.5 µg/m3, respectively. The highest acidity sulfate was 107.9 µg/m3 for the SCR-on POS scenario, followed by the PO, SCR-off POS, and PONS scenarios, with averages of 71.6, 68.8, and 15.7 µg/m3, respectively. Acidic sulfate concentrations were higher with lower NOx emission during the SCR-on period than with higher NOx emission during the SCR-off period. As expected, neutralized sulfate concentration also showed the highest concentration for the neutralized scenario, 139.1 µg/m3, followed by the SCR-on POS, PO, and SCR-off POS scenarios, with averages of 38.1, 28.8, and 12.9 µg/m3, respectively.

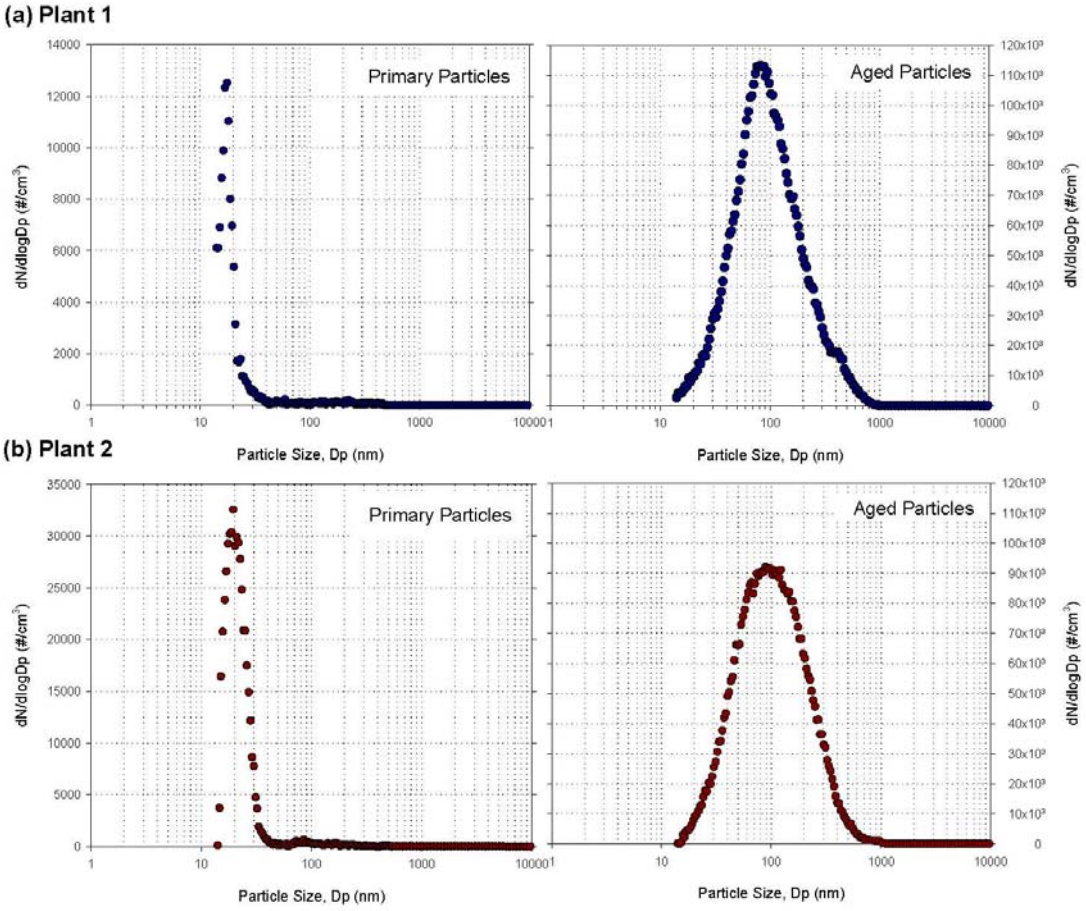


Figure 4-5
Particle size distributions for primary and aged particles at Plants 1 and 2.

As for the first two plants, at Plant 3 total sulfate concentration for the PONS scenario was the highest (85.0 µg/m^3) accounting for 49.0% of the total mass, followed by the POS and PO scenarios, with averages of 83.4 and 77.9 µg/m^3, respectively. As expected, the lowest total sulfate concentration was found for the S scenario using particle-free ambient air, instead of

stack emissions. For the P scenario, total and neutralized sulfate accounted for 78.7 and 49.1% of the total mass, respectively. Higher concentrations of acidic sulfate were measured for the un-neutralized POS (68.9 $\mu g/m^3$) and PO (66.6 $\mu g/m^3$) scenarios, which accounted for 47.7 and 80.9% of the total mass, respectively. In addition, lower concentrations of acidic sulfate were observed for the neutralized scenario at this plant compared with Plants 1 and 2 (PONS, 2.5 $\mu g/m^3$), which demonstrates that most of the H_2SO_4 aerosol was neutralized by the addition of gas-phase NH_3. At all three power plants, addition of gas-phase NH_3 to neutralize H_2SO_4 aerosol for the PONS scenario not only resulted in over five times higher NH_4^+ concentrations than for the un-neutralized scenarios, but also increased particle NO_3^- concentrations.

Carbonaceous Species and Secondary Organic Aerosol: Abnormally high contributions of OC to total particle mass were observed for the primary emissions scenario (P) at all three power plants. These high contributions might have been due to different filter media (Quartz and Teflon filters). A fair amount of artifact OC most likely originated from gas-phase adsorption of VOCs on quartz filters (Mcdow and Huntzicker, 1990; Turpin et al., 1994). While the source of this VOC is uncertain, it is possible that it may have been due in part to VOCs in ambient air. These could have entered through the purge channel of the parallel plate diffusion denuders and from the addition of particle-free ambient dilution air. A valid statistical comparison of particle OC concentrations with toxicological endpoints requires adjustment for the artifact OC. Toxicological effects are determined from the difference in responses between exposed animals and sham animals (exposed only to particle-free air). It was thus decided to subtract a background OC level to correct the measured OC concentrations for all scenarios. At Plants 1 and 2, the OC concentrations for the P scenario were chosen as the most suitable background value because the total mass (1.0 and 1.7 $\mu g/m^3$, respectively) was negligible. At Plant 3, the OC concentration for the control oxidized scenario (O) was chosen as the background OC value because it was the lowest value of all scenarios at this plant. The OC concentrations presented in Tables 4-5 through 4-7 are net values reflecting subtraction of this background OC level. These values are different from the unadjusted values reported previously.

At all three power plants, the amount of OC formed was determined by the type of scenario. The S code represents adding α-pinene (and ozone) into the 2nd reaction chamber to produce SOA. At Plant 1, after adjustment for background OC, the POS and PONS scenarios had significant amounts of OC, with averages of 51.6 and 53.6 $\mu g/m^3$, respectively. At Plant 2, inadvertently more than the routine amount of α-pinene was used for the first two days of the SCR-off POS scenario, which led to abnormally high OC, particle number and mass concentrations. As for Plant 1, the SCR-on POS and PONS scenarios showed significant amounts of OC, with averages of 59.0 and 35.1 $\mu g/m^3$, respectively. At Plant 3, particulate OC concentrations for the POS, PONS, OS and S scenarios ranged from 52.0 to 83.6 $\mu g/m^3$, which are substantially higher than those for the P, PO and O scenarios (0-1.9 $\mu g/m^3$), all of which lack SOA.

Particulate EC concentrations at the first two power plants were low, with a high variability; 0-11.9 $\mu g/m^3$ at Plant 1 and 1.7-12.5 $\mu g/m^3$ at Plant 2. Concentrations were lowest in the P scenarios (Tables 4-5 and 4-6), but with aging and addition of SOA, EC increased, suggesting that a portion of this is likely artifactual and representative of pyrolyzed OC erroneously reported as EC (Birth, 1998; Yu et al., 2002). The variability is also likely due to a fair amount of day-to-day variation in primary emissions. In McDonald et al. (2009), EC comprised 10% of the ash,

which in turn comprised 1% of the total mass of the lab-based exposure atmosphere. This corresponded to less than 1 $\mu g/m^3$, a value substantially lower than at these plants. On the other hand, EC at Plant 3 was not detected for any of the scenarios. It has been reported that a wet FGD scrubber can remove some of the primary particles that penetrated through the ESP, and the scrubber itself is not a source for EC emissions (Meij, 1994; U.S. DOE, 2003). Thus, the absence of EC at Plant 3 is likely due to a high collection efficiency of primary particles by the combined ESP and FGD scrubber.

A subset of particulate SOA species collected on Teflon filters was measured using GC/MS method. Several representative filters were selected; two and one each from the POS and PONS scenarios at Plants 1 and 2, respectively. This analysis was not conducted at Plant 3 as the pattern was expected to be similar to the previous two plants. Three field blanks were also analyzed to correct for background. Seven SOA species were identified (Table 4-8). At Plant 1, cis-pinic acid was the dominant species for both scenarios, accounting for about 90% of total SOA species. At Plant 2, the dominant species were c*is*-pinic acid (about 70% of total SOA species) and pinolic acid (about 20% of total SOA species). On the other hand, pinonaldehyde, *cis*-nor-pinic acid, pinalic acid, *trans*-nor-pinic acid, *cis*-pinic acid, and *trans*-pinic acid were minor species at both power plants and scenarios. All these species are typical SOA products of α-pinene (Kanakidou et al., 2005). The sum of the identified SOA species varied considerably and accounted for 50-107% of the corresponding OC concentrations measured by TOR analytical method. The difference might have resulted from analytical or sampling errors due to different filter media (Quartz and Teflon filters) or from different environmental conditions (e.g. relative humidity and temperature). However, the composition of the identified SOA species was relatively similar for the two power plants at which they were measured.

Table 4-8
Selected SOA species at Plants 1 and 2.

Species	Plant 1[1]		Plant 2	
	POS (n=2)[2]	PONS (n=2)	POS (n=1)	PONS (n=1)
Pinonaldehyde	298 (1.0)[3]	590 (3.5)	1218 (3.5)	792 (2.6)
Pinalic acid	137 (0.5)	42 (0.2)	1009 (2.9)	242 (0.8)
Trans-nor-pinic acid	560 (1.9)	404 (2.4)	453 (1.3)	515 (1.7)
Cis-pinonic acid	98 (0.3)	110 (0.6)	808 (2.3)	888 (2.9)
Cis-pinic acid	27 102 (89.8)	15 448 (90.7)	23 099 (66.4)	21 414 (70.9)
Trans-pinic acid	376 (1.2)	212 (1.2)	241 (0.7)	155 (0.5)
Pinolic acid	1600 (5.3)	232 (1.4)	7964 (22.9)	6196 (20.5)
Total SOA species	30 171	17 038	34 792	30 202
Organic Carbon	60 934	16 903	32 441	37 185
Total %[4]	50 %	101 %	107 %	81 %

[1]Ruiz et al., 2007a
[2]Number of samples
[3]Concentration is ng/m^3 with % fraction of species to total SOA in parentheses
[4]Total % of SOA species to corresponding OC concentration

Elemental Composition: Elemental concentrations are summarized by scenario in Tables 4-5, 4-6, and 4-7. Two times the uncertainty of each XRF measurement was used as the method detection limit (MDL) for each element. Initially, XRF analysis was performed with filter samples collected from the exposure atmospheres. However, these results showed very few elements above their respective MDLs. This was due to the high dilution ratios (a total factor of 1000-2000) between the stack emissions and the exposure atmospheres, coupled with relatively low elemental concentrations in the stack emissions. To overcome this limitation, XRF analysis was subsequently performed for primary particles collected upstream of the 1st reaction chamber (Port A). The elemental composition of aged particles used for exposure atmospheres was estimated using the dilution factors of Port A through Port C. Sulfur concentrations were independently determined from sulfate concentrations by IC because sulfur is expected to be dominated by secondary sulfate formed and IC sulfate is more reliable than XRF sulfur.

From Table 4-5 (Plant 1) and Table 4-6 (Plant 2), a total of 15 elements were identified. Except for sulfur, they were all present at low concentrations. On average, the sum of elements except sulfur account for about 0.1% of the aged particle mass concentration. Elemental concentrations also varied significantly. At Plant 2, in particular, substantially higher concentrations of Cr, Fe and Ni were observed for 3 days (July 13, September 7 and 8, 2005) of the SCR-on POS scenario days, compared to the other scenarios. A slight elevation in these elements was also observed for one day at Plant 1 (November 5, 2004). On these days primary particle concentrations also increased by a factor of 17, compared to the other days. The episode of high Cr, Fe and Ni concentrations are discussed in detail below. Selenium (Se), an element as a tracer for coal-fired power plant emissions in source apportionment studies (e.g, Thurston and Spengler, 1985), was present at concentrations less than detection limits, due to very low primary particle mass concentrations (1-2 μg/m^3) present.

Overall, Al, Si, S, Ca and Fe were the most abundant elements, relative to other elements, at Plant 1. At Plant 2, Al, Si, and S were the most abundant elements present in the exposure

atmospheres, except for the three days with higher levels of Cr, Fe and Ni. The former three elements are common elements resulting from coal combustion process. Furthermore, the relatively higher amount of Ca at Plant 1 is most likely due to the use of Ca-rich subbituminous coal. In general, coals contain significant amounts of Al, Si, Fe, Ca and Mg in the form of primarily quartz (SiO_2), kaolin ($Al_2Si_2O_5[OH]_4$), pyrite (FeS_2), calcite ($CaCO_3$) or dolomite ($MgCa[CO_3]_2$). In addition, trace elements in coal undergo complex physical and chemical transformations in the boiler in the process of forming particles. They are vaporized in a high temperature boiler, and as the temperature decreases upstream of the ESP, the elements can nucleate and/or condense onto existing particles (Quann et al., 1990; U.S. DOE, 2003).

At Plant 3 (Table 4-7), the relative variability of the concentrations was significantly lower than that observed for the other two plants. Most elemental concentrations present in the table were above the MDL values. The abundant elements included Na, Mg, Al, P, S, Fe and Se for this plant, which was generally different from those for Plants 1 and 2. In addition, the sum of elements (excluding S) corresponded on average to 0.4% of the aged particles, which was higher than that at Plants 1 and 2.

The following may help to explain why the primary particles had a different elemental composition at Plant 3. While the wet FGD scrubber is expected to remove some of primary particles including trace elements, it is also expected to be a source of stack emission particles (Meij , 1994; U.S. DOE, 2003; Srivastava et al., 2004). Meij (1994) reported that fly dust downstream of wet FGD scrubbers were composed of 40% fly ash, 10% gypsum ($CaSO_4 \cdot 2H_2O$), and 50% evaporated droplets saturated with gypsum. However, primary particle composition at Plant 3 was somewhat different from the compositions reported previously. This is most likely because of different particle sizes between two studies; primary particles at Plant 3 are expected to be the smaller sizes (presumably, submicron size). Srivastava et al. (2004) also described the formation and emissions of submicron H_2SO_4 aerosol by wet FGD scrubbing in coal-fired power plants.

The removal efficiency of ESPs increases with particle size. While the overall removal efficiency of the ESP is about 99% (of overall particle mass), it is considerably less efficient for submicron particles (0.1-1.0 μm) which are more highly enriched in condensed trace elements (Quann et al., 1990; U.S. EPA, 1993; Meij, 1994). At Plant 3, the overall removal efficiency through the combination of an ESP and a wet FGD scrubber would likely be greater than by an ESP alone because the wet FGD scrubber is expected to remove some particles. On the other hand, the wet scrubbing process is likely to be a source of particles. The finding that a major component of the primary particles downstream of the scrubber was sulfate (composed mainly of H_2SO_4 and NH_3-nutralized sulfate), accounting for 79% of the total mass, supports this hypothesis. This composition also suggests that primary particles at Plant 3 fell primarily in the submicron size range because sulfate is typically found in this size range in both ambient air (Koutrakis and Kelly, 1993; Hazi et al., 2003) and power plant emissions (Srivastava et al., 2004). Consequently, most of the primary particles at Plant 3 were likely derived from the wet scrubbing rather than from coal combustion.

The wet FGD scrubber was an inhibited oxidation system which means that thiosulfate (or elemental S) was added to prevent the formation of CaSO4. 2H2O (predominately; CaSO3

½H2O) in a co-current absorber tower. A small amount of sodium formate (NaCOOH) was also added to buffer the pH drop through the absorber and enhance SO2 removal. Thus it is possible that Na, the most abundant element after sulfur, results from aerosolization of sodium salts in the wet scrubber. Furthermore, selenium (Se) was present at relatively high concentrations at the plant. As Se is a volatile element, it is typically present in vapor phase in stack emissions and its behavior is complex in power plant emissions (EPRI, 2008). It is also found in both fly ash and flue gas, and a substantial amount of Se is not removed by the ESP.

Even though limestone particles containing substantial amounts of Ca are used by the wet FGD scrubber, relatively low concentrations of Ca (6.7-14.8 ng/m^3) were found in the exposure atmosphere. It is likely that the particle sizes which passed through the stack extraction system at Plant 3 were within the submicron range, as for the other two plants. On the other hand, calcium sulfate formed in the FGD scrubbing process is an abrasive, sticky, and compressible material and is composed primarily of finely divided crystals ranging from 1-250 µm. When FGD scrubbers using limestone were tested with both low sulfur and high sulfur coals, less Ca was found in the flue gas downstream of the scrubber for high sulfur coal combustion (U.S. DOE, 2003). These processes could explain the relatively low amounts of Ca found in the scrubbed stack gas, and consequently, the Ca in the exposure atmospheres could have been mostly from the smaller particles (relatively inefficiently removed by the ESP) that were present in the flue gas due to the combustion of coal, rather than from the limestone.

High Cr, Fe and Ni Episodes: It is important to address the high content of these elements mentioned above. Coals contain significant amounts of Al, Si, Fe, Ca and Mg; however, Cr and Ni are typically found in only trace amounts. Relatively high amounts of Cr and Ni in relation to primary elemental constituents might have been due to: 1) a foreign, non-coal source, such as corrosion, and; 2) coal with a much higher-than-normal Cr and Ni content. The latter might be supported by the fact that Plant 2 used bituminous coals derived from various regions.

High Cr and Ni concentrations were also observed from in-stack measurements performed prior to animal exposure experiments at Plant 2. As part of the study, preliminary in-stack tests were conducted to investigate primary particle mass and elemental composition at both Plants 1 and 2, during October 19-24, 2004 and December 13-14, 2004, respectively. Several fine particle samples were collected for 20 minutes through 3 hours on quartz filters with a 2.5µm cut-off cyclone inside the stack without dilution, and then analyzed for gravimetric mass and elemental concentrations. This test was conducted in accordance with the EPA Conditional Method 040 (US EPA, 2002). Figure 4-6 shows mass contributions of selected elements to in-stack particle mass concentrations at Plants 1 and 2. In this figure, elements associated with coal combustion (Al, S, Ca, Ti and Se) are shown in addition to Cr, Fe and Ni. Si was not available because of the quartz filter media used.

At Plant 1, the selected elements clearly varied together; moreover, there was no elevation of Cr and Ni. Ca was the most abundant element accounting for about 20% of the total mass, which was consistent with exposure atmospheres at this power plant. On the other hand, at Plant 2 some of the in-stack samples indicated elevated Cr, Fe, and Ni concentrations consistent with those observed on the 3 “high” days during the exposure runs. Averaged mass contributions of Cr, Fe and Ni to the in-stack particle mass were 3.4%, 8.9% and 1.2%, respectively, with two

samples (Samples # 1 and #3) having substantially higher Cr and Ni concentrations. In Sample #1, the mass contributions of all elements were elevated altogether, while in Sample #3 only Cr and Ni fractions were increased without the concomitant elevation of Fe. Because contamination by corrosion is expected to result in elevations of Cr, Fe, and Ni together, regardless of other elements, this result suggests that the elevated Cr and Ni in these samples is not likely to have been influenced by corrosion.

It is also expected that any oxidized corrosion particles that may have been aerosolized by mechanical processes would predominantly exist in greater-than-submicron size ranges. At the same time, only relatively much smaller particles pass through the ESP with reasonable efficiency, although some larger particles may escape collection. In order to identify single particle size and composition, Scanning Electron Microscope (SEM) analysis was performed for the primary particles collected at Port A for days with both high and low elemental concentrations. An uncoated slice of each of the eight collected filters was placed on a 25 mm Zeiss SEM stub and then examined with a LEO 1450VP Scanning Electron Microscope (Carl Zeiss SMT, Inc., Thornwood, NY) at 15 KeV using a magnification of 2000 times. The X-ray analysis was performed with an Inca300/SEM Si (Li) X-ray detector (Oxford Instruments, Concord, MA). From the SEM analyses, the particle sizes were generally submicron particles with a spherical shape. Particles that had substantially higher Cr, Fe and Ni content were in a similar size (submicron) range to those that contained less of these elements. These elements were also observed generally together on a single particle. This result suggests that the spherical, submicron particles with high Cr, Fe and Ni content are more likely due to the combustion process, rather than a result of corrosion. However, we are unable to resolve this issue with certainty because of somewhat conflicting information regarding the source of the elevated Cr, Fe and Ni on certain days.

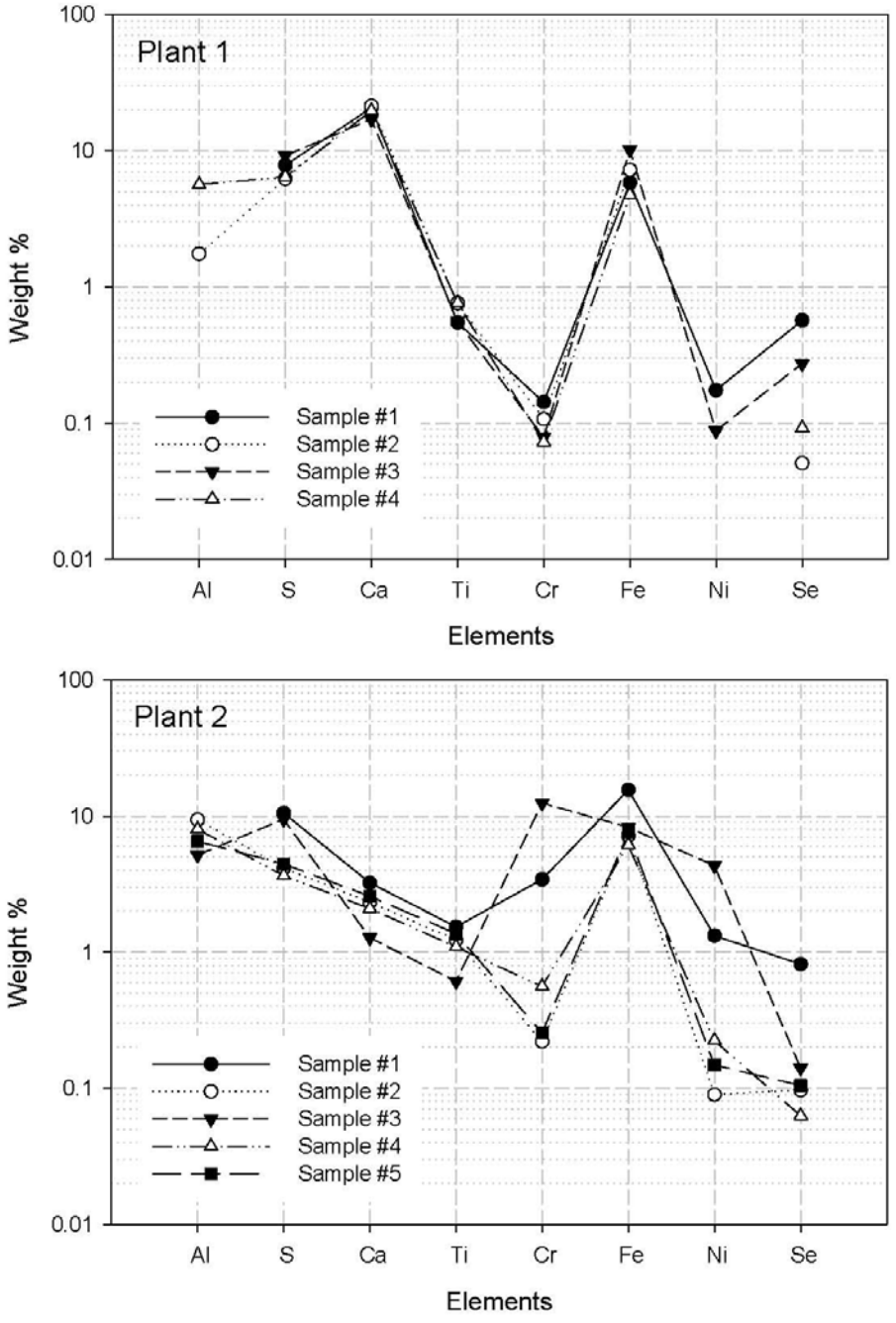


Figure 4-6
Weight % of selected elements to in-stack particle mass concentrations at Plants 1 and 2.

4.3 Comparison of the Results Among Coal-fired Power Plants

In this section, we compare the physical and chemical properties of the aged particles used for toxicological studies from all three power plants. Particle mass, number, total sulfate, acidic sulfate, neutralized sulfate and organic carbon are compared in Figure 4-7.

Of the plants studied, primary particles at Plant 3 contributed to the aged particle mass more than primary particles at either of the other two plants. Figure 4-7 also shows that the primary particles consisted mostly of H_2SO_4 and NH_3-neutralized sulfate; this is likely a result of emissions from the wet FGD scrubber. On the other hand, at Plants 1 and 2 the primary particle mass concentrations were too low to estimate their composition. For all power plants, the aged particle mass concentrations increased with the complexity of scenario, with the highest values for the most complex, neutralized scenario (PONS), followed by POS, PO and P scenarios. Furthermore, particle number concentrations at the first two power plants increased with scenario complexity, which suggests that nucleation of H_2SO_4 and SOA was a dominant mechanism of particle formation. In contrast, at Plant 3 particle number concentration decreased somewhat with scenario complexity, which suggests that coagulation was a dominant mechanism of particle formation.

The major components of the aged particles are total sulfate composed of acidic (H_2SO_4) and neutralized sulfate (ammonium sulfate, letovicite or ammonium bisulfate). Of the three plants studied, Plant 2 had the highest particle mass and sulfate concentrations in the aging scenarios. It was most likely due to the higher ratio of SO_2 to NOx emissions, due to the use of a SCR for NO_x emission control without SO_2 scrubbing. This was also confirmed with higher sulfate during the SCR-on period than during the SCR-off period. Overall, total sulfate accounted for between 38-95% of the aged particle concentrations for the three power plants: H_2SO_4 aerosol accounted for about 1-81% and neutralized sulfate accounted for about 5-54% of the aged particles.

OC concentrations exhibited similar patterns at all three power plants. The OC level depended primarily on whether α-pinene was added to simulate atmospheric production of SOA (scenarios with S code). The POS and PONS scenarios thus had more OC, as well as higher particle mass concentrations, than the other scenarios. Overall, OC accounted for about 14-54% of the aged particle mass concentrations. Previous studies have reported that organic compounds represent about 20-50% of the ambient fine particle mass at continental mid-latitudes (Saxena and Hildemann, 1996; Putaud et al., 2004). In contrast to major components, the contribution of trace elements to the aged particle mass concentrations was very low because of the use of ESPs at all three coal-fired power plants.

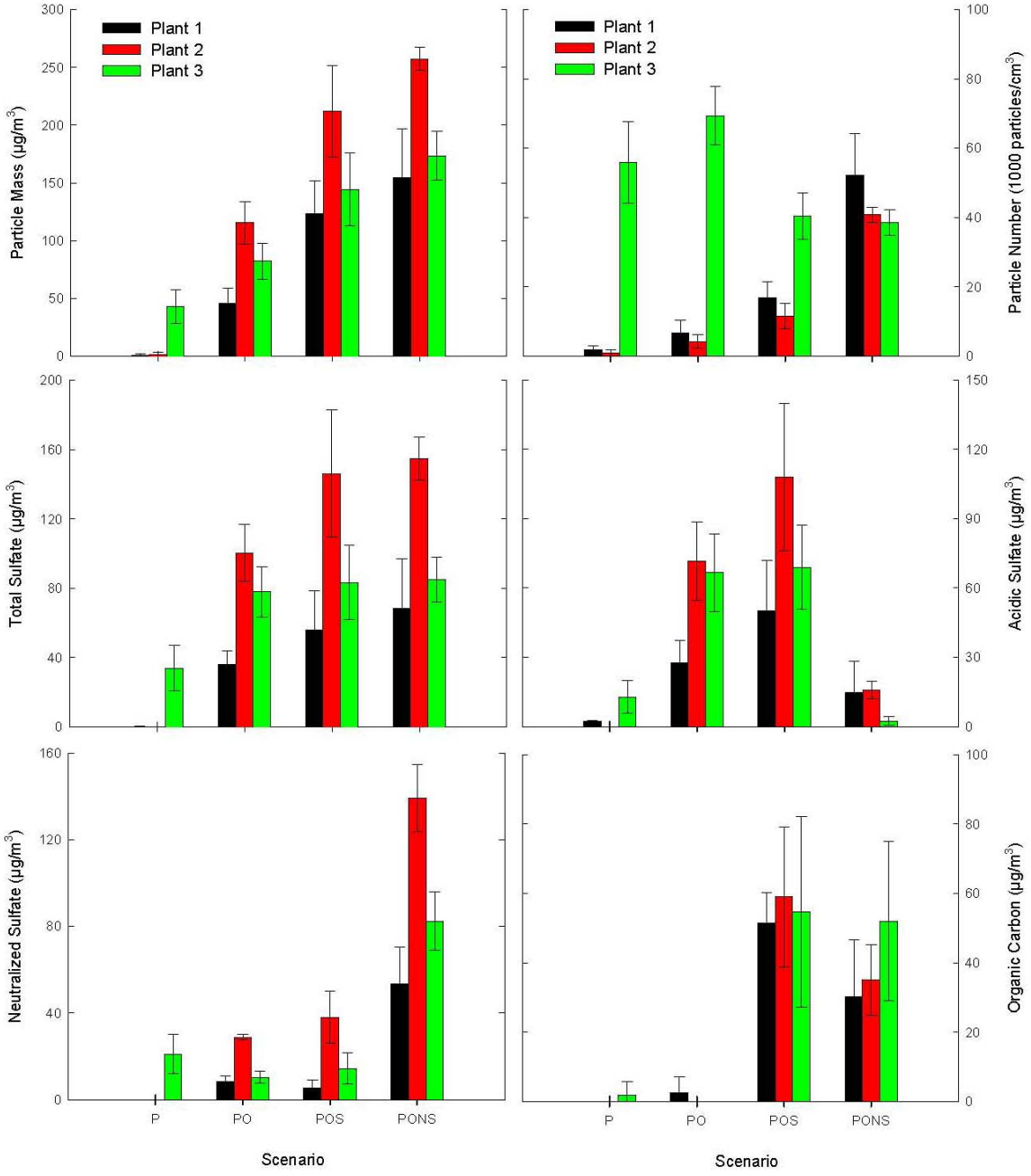


Figure 4-7
Particle component concentrations at the three power plants: The averages with standard deviations (error bars) are shown for each scenario from each of the different plants

4.4 Comparison with Ambient Environments

In order to provide additional context for the toxicological effects documented in the companion TERESA papers, it was of interest to compare the TERESA exposure atmospheres with ambient particles. In particular, our research group has conducted many studies of concentrated ambient particles (CAPs) collected in Boston, and has a rich dataset of CAPs composition and response. It is important to note that we would not expect the TERESA exposure atmospheres to be similar to the ambient environment. While the TERESA scenarios simulated atmospheric reactions of coal-fired power plant emissions, ambient particles are comprised of constituents from multiple sources, including mobile sources, oil-fired power plants and a variety of industrial emissions. In addition, both biogenically- and anthropogenically-derived SOA is not limited to α-pinene as a VOC precursor in ambient air, unlike in the TERESA scenarios. However, it is of interest to determine how closely (or disparately) the TERESA atmospheres compare to typical ambient environments.

For the purposes of this comparison, we selected the PONS scenario, as it was expected to be most representative of human exposure. This is because it contains oxidized SO2, partially neutralized sulfate and secondary organic aerosol that would be expected to associate with particles formed downwind of the plant. The TERESA PONS exposures were compared to those reported in several CAPs studies, primarily in Boston (Clarke et al., 2000; Batalha et al., 2002; Gurgueira et al., 2002; Wellenius et al., 2003; Wellenius et al., 2004), but also in Chapel Hill, NC (Kodavanti et al., 2005) and Fresno, CA (Smith et al., 2003). The concentrations of major particle constituents at each of the three power plants are compared to CAPs in Table 4-9.

Particle mass and OC concentrations were lower in our study compared to CAPs, while sulfate concentrations were generally comparable or higher, with the exception of Chapel Hill CAPs, which were very high in sulfate. Note that sulfate in our study simulated not only primary sulfate emissions from the power plants but also secondary sulfate formation from SO2 emitted from coal-fired power plants during transport. Particle number concentrations were roughly similar in our study compared with CAPs, except for Fresno CAPs, which were present in significantly higher numbers. Elemental carbon was present at lower concentrations in TERESA than in CAPs, which would be expected given the low EC emissions from power plants and the significant contribution of mobile sources to CAPs in urban areas. All of the individual elemental concentrations in our exposure scenarios were substantially lower than those reported in CAPs studies, with the exception of Cr, which at Plant 3 was about 35% of the Cr in Boston CAPs, and Se, which at Plant 3 was 76% of that in Boston CAPs. As a result, the total contribution of measured elements to mass was much lower in TERESA (0.01 – 0.2%) than in CAPs studies (0.2 – 7.9%). The comparison with ambient CAPs suggests that sulfate derived from coal-fired power plant emissions contributes significantly to ambient particles, while the elemental contribution is substantially less.

Acidic sulfate in TERESA atmosphere was compared to that in ambient particles because the acidity was not available in CAPs studies. Acidic sulfate concentrations in the TERESA PONS atmospheres, 14.7 $\mu g/m^3$ at Plant 1, 15.7 $\mu g/m^3$ at Plant 2, and 2.5 $\mu g/m^3$ at Plant 3, were

substantially higher than in ambient particles, 0.48 μg/m^3 and 1.77 μg/m^3 in St. Louis, MO and Eastern Tennessee, respectively (Dockery et al., 1992) and 0.8̲ μg/m^3 in Atlanta, GA (Peel et al., 2005), which are both affected by regionally transported power plant emissions. However, on a total mass basis, acidic sulfate in the TERESA PONS atmospheres contributed 9.5%, 6.1%, and 1.4% at Plants 1, 2, and 3, respectively, which are comparable to 2.7% in St. Louis, 8.4% in Eastern Tennessee, and 4.6% in Atlanta.

Table 4-9
Comparison with CAPs composition.

Parameter	Plant 1 (PONS, n=12[1])	Plant 2 (PONS, n=4)	Plant 3 (PONS, n=4)	Boston CAPs Studies[2]	Fresno CAPs Study[3]	Chapel Hill CAPs Study[4]
Particle Mass (μg/m^3)	155	257	174	333	475	1172
Sulfate (μg/m^3)	68 (44%)	155 (60%)	85 (49%)	71 (21%)	29 (6.1%)	338 (29%)
OC (μg/m^3)	30 (19%)	35 (14%)	52 (30%)	75 (23%)	95 (20%)	287 (24%)
EC (μg/m^3)	5 (3.2%)	10 (3.9%)	0 (0%)	18 (5.4%)	17 (4%)	17 (1.5%)
Particle Number (particles/cm^3)	52 100	40 800	38 500	44 000	110 000	-[5]
Sum of Elements[6] (μg/m^3)	0.03 (0.02%)	0.02 (0.01%)	0.3 (0.2%)	26 (7.8%)	20 (4.2%)	2.8 (0.2%)
Na (μg/m^3)	0.000	0.000	0.093	5.0	-	-
Mg (μg/m^3)	0.001	0.000	0.013	-	-	-
Al (μg/m^3)	0.004	0.000	0.010	1.8	3.0	1.6
Si (μg/m^3)	0.004	0.001	0.001	6.5	8.3	-
P (μg/m^3)	0.001	0.000	0.058	-	-	-
S (μg/m^3)	23	52	28	32	6.7	-
K (μg/m^3)	0.000	0.000	0.001	2.0	2.2	-
Ca (μg/m^3)	0.016	0.000	0.007	3.4	2.4	-
Ti (μg/m^3)	0.001	0.000	0.001	0.4	0.3	-
Cr (μg/m^3)	0.000	0.002	0.007	0.02	-	-
Fe (μg/m^3)	0.003	0.017	0.044	6.0	3.3	-
Ni (μg/m^3)	0.000	0.002	0.003	0.1	0.01	0.03
Zn (μg/m^3)	0.000	0.000	0.004	0.6	0.5	1.0
Se (μg/m^3)	0.000	0.000	0.019	0.03	0.01	-
Pb (μg/m^3)	0.000	0.001	0.000	0.3	0.1	0.2

[1] Number of samples
[2] Averaged concentrations reported by Clarke et al., 2000; Batalha et al., 2002; Gurgueira et al., 2002; Wellenius et al., 2003; Wellenius et al., 2004
[3] Average concentrations reported by Smith et al., 2003
[4] Average concentrations reported by Kodavanti et al., 2005
[5] Not available
[6] Sum of elemental concentration excluding sulfur

5
RESULTS: BREATHING PATTERN

Continuous respiratory data were obtained during exposures using whole body plethysmography chambers. Details of the respiratory studies are published in the paper by Diaz et al (2011). Of the 12 respiratory outcomes assessed, each showed statistically significant changes at some plants and in response to some of the four scenarios. The most robust outcomes were found with exposure to the PO scenario (increased respiratory frequency with decreases in inspiratory and expiratory time); and the PONS scenario (decreased peak expiratory flow and expiratory flow at 50% [EF50]). PONS findings were most strongly associated with ammonium, neutralized sulfate, and elemental carbon (EC) in univariate analyses, but only with EC in multivariate analyses. Control scenario O (oxidized without primary particles) showed similar changes to PO. Adjusted R^2 analyses showed that scenario was a better predictor of respiratory responses than individual components, suggesting that the complex atmospheric mixture was responsible for respiratory effects.

All respiratory data were analyzed as 10 minute averages over the 6 hour exposure time. Figures 5-1a and 5-1b illustrate this time-based approach showing EF50 in the PO scenario at Plant 2. Figure 5-1a illustrates the average of all exposed animals (dark line) compared to the average of the control animals (light line) over the six hours of exposure. After the initial period of acclimation in which EF50 decreases in both the exposed and filtered air groups, there was lesser flow in the exposed group compared to control; Figure 5-1b illustrates the average difference between individual exposed animals and the daily average for the corresponding control animals over time (solid line) with the standard errors (dotted line). For subsequent analyses, all respiratory data were assessed as differences between specific parameters in individual exposed animals and the average control (filtered air sham exposures) for any given day. Thus, all biological parameter outcomes were assessed statistically as differences from control. These differences from control were assessed by power plant by scenario, and then using pooled data from all plants (for a given scenario) to provide an overall picture of the response. Individual exposure measurements using univariate analyses and random forest approaches used combined data, but specify plant and scenario.

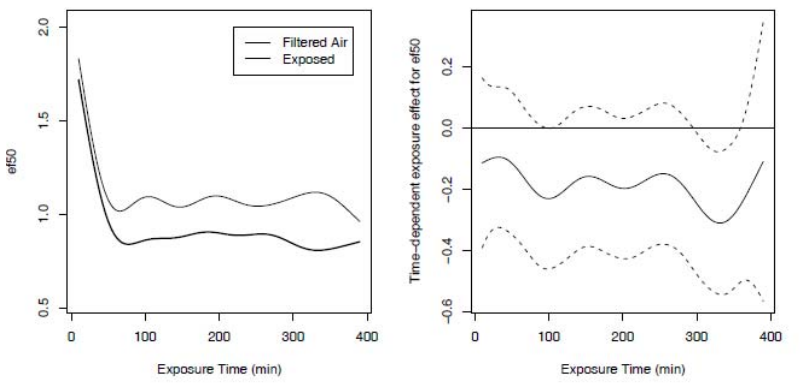


Figure 5-1
Trend over time of exposure for EF50 in the PO scenario at Plant 2. Figure 5-1a illustrates the average of all exposed animals (dark line) compared to the average of the control animals (light line) over time; this difference was marginally significant by ANOVA analyses. Figure 5-1b illustrates the difference between individual exposed animals and the daily average for the corresponding control animals over time (solid line) with the standard errors (dotted line).

5.1 Results by Power Plant/Scenario

Figure 5-2 shows the difference between exposed and control animals in respiratory parameters at the individual power plants. Changes from control with exposure having p-values <0.007 are considered robust based on the Bonferoni correction, whereas those with p<0.05 are considered marginally significant (Coull et al., 2011).

Changes in frequency and times of inspiration and expiration were observed at Plants 2 and 3, while changes in expiratory flow parameters in the PONS scenario were robust at Plant 2 and in the P scenario at Plant 1. Plant 2 showed the most respiratory effects overall. Based on the Bonferroni corrections for multiple comparisons, p<0.007 was considered as strongly significant, and all p-values at p<0.005 exceeded this criterion. The p-values for plant to plant differences controlling for scenario are indicated in the box on each respiratory parameter graph.

Significant increases in frequency and decreases in Ti and Te were observed at Plant 3, with less significant changes at Plant 2 and no significant changes at Plant 1. Since there were no instances where p-values fell between 0.007 and 0.005, we show all robust p-values as p<0.005. Decreases in PEF, PIF, and EF50 were exhibited most strongly at Plant 2, and were supported to a lesser extent in Plant 1, whereas at Plant 3 there were no significant changes in any flow parameters. Decreases in TV were observed at Plants 1 and 2, but not at Plant 3 where it increased. Statistically significant decreases in Enhanced Pause (Penh) were observed at all power plants. There was little change in End Expiratory Pause; however, End Inspiratory Pause

was significantly reduced at Plants 1 and 2, but not at Plant 3 (increased). There were decreases in respiratory pause in all power plants, but they only reached a significant level at Plant 2, the PO scenario at Plant 1 and the PONS scenario at Plant 3. Since there are plant-to-plant differences in responses, we assessed the differences in outcomes between plants controlling for scenario. Of the twelve respiratory parameters, three had strongly significant differences when comparing among plants. These were TV, EIP and Pause. Ti, PIF, EEP and Penh were marginally significant, and the remaining parameters were not significant.

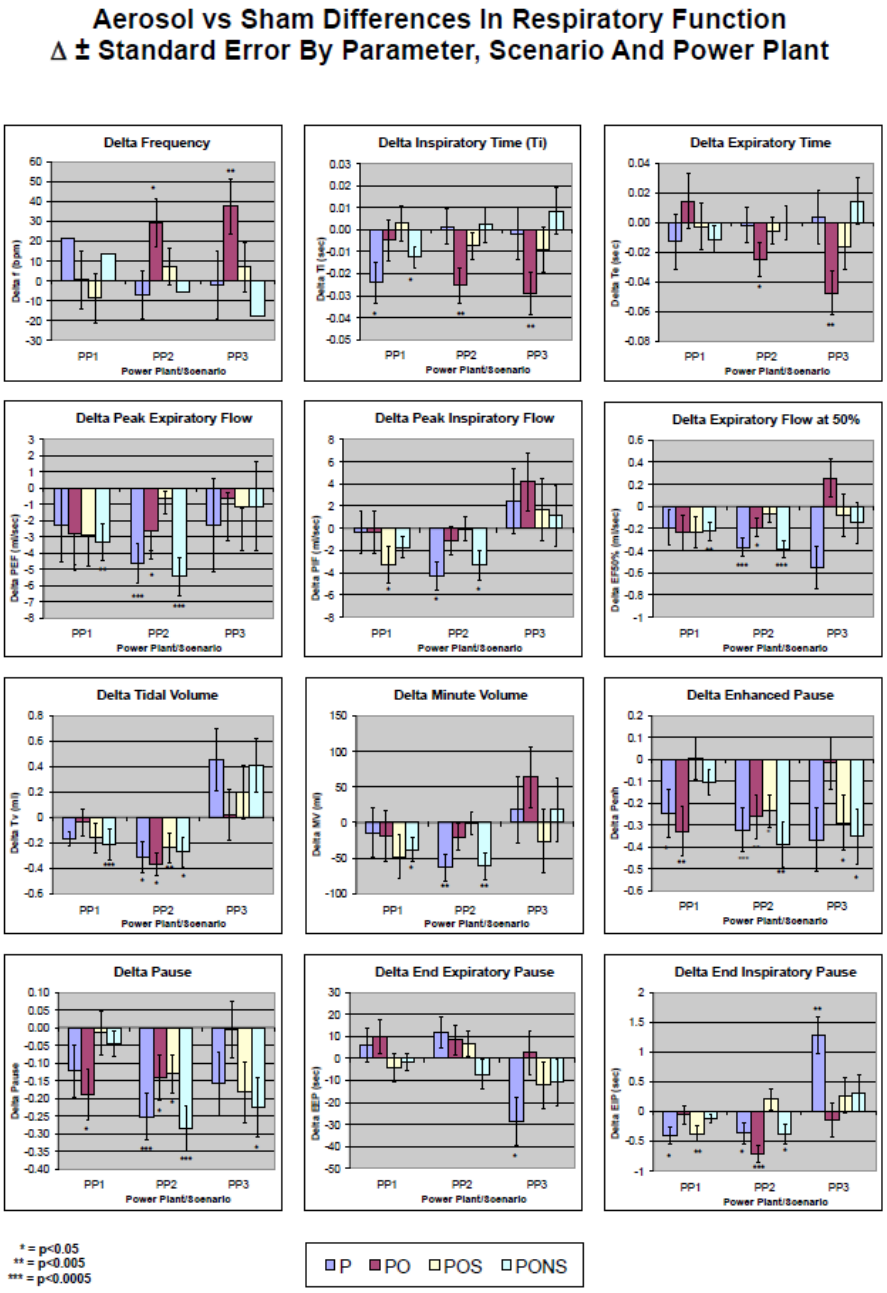


Figure 5-2
Differences in respiratory parameters by scenario and power plant.

5.2 Results by Scenario (All Power Plants Combined)

Since there were only 3 parameters exhibiting robust plant-to-plant differences, combining data from all plants for ANOVA analyses as well as to explore univariate and multivariate analysis in relationship to compositional components was done. Figure 5-3 shows the data for all plants combined by scenario. Robust decreases in PEF and EF50 were observed with the PONS scenario. Reduced EIP was also observed in response to the PONS scenario ($p<0.0005$). Pause and Penh had significant reductions with all scenarios. Marginally significant changes in frequency, Ti, Te, and PIF were also observed with exposure to various scenarios.

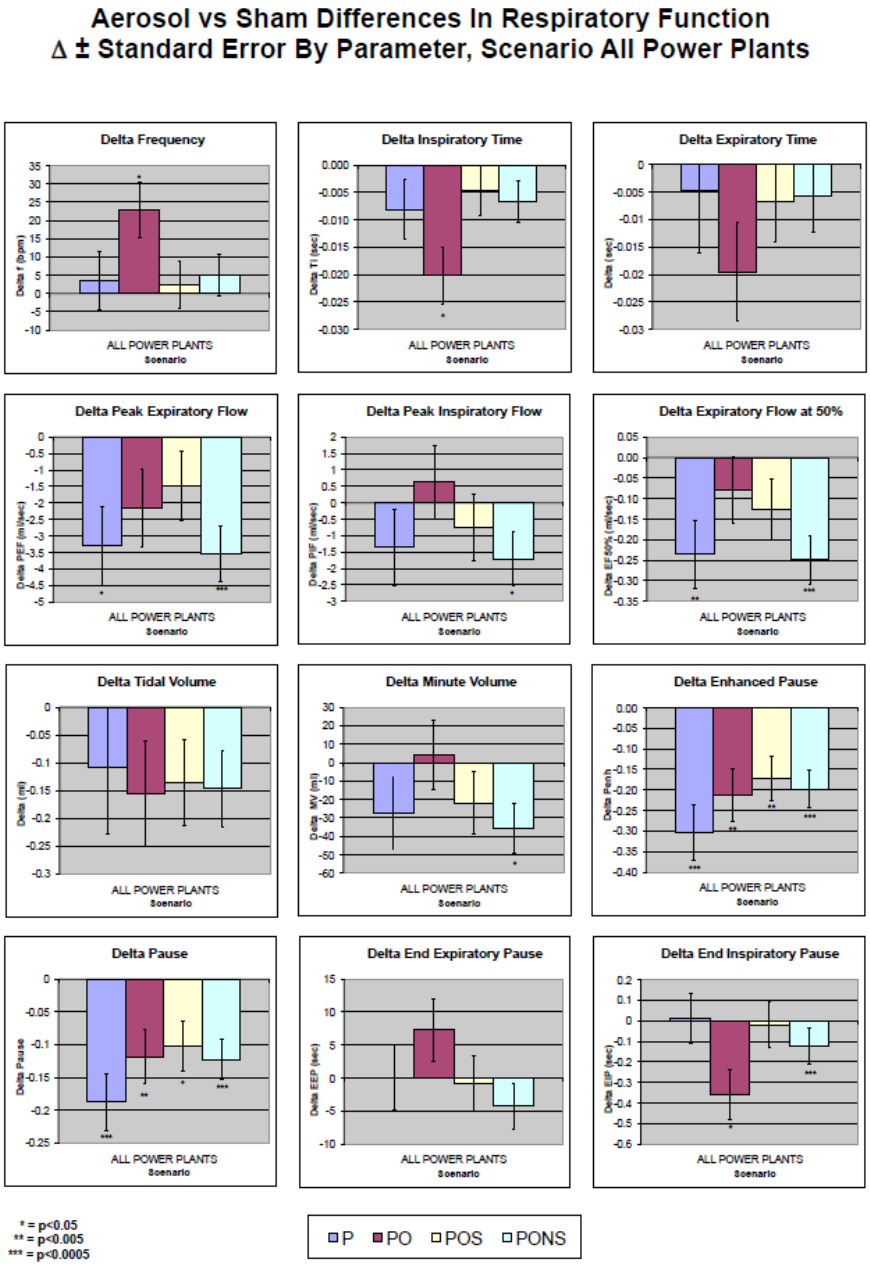


Figure 5-3
Differences in respiratory parameters by scenario: Data from all power plants combined,

graphics show the difference between filtered air control and TERESA aerosol-exposed animals by scenario in all power plants combined. Robustly significant findings included reduced PEF and EF50 with the PONS scenario, and reduced Penh in all scenarios.

5.3 Control Scenarios

Figure 5-4 shows control scenarios (without primary particles) conducted at Plant 3. The only strongly significant findings were reductions in Ti, Te, and EIP in response to the O scenario. There were marginally significant increases in f with the O and S scenarios.

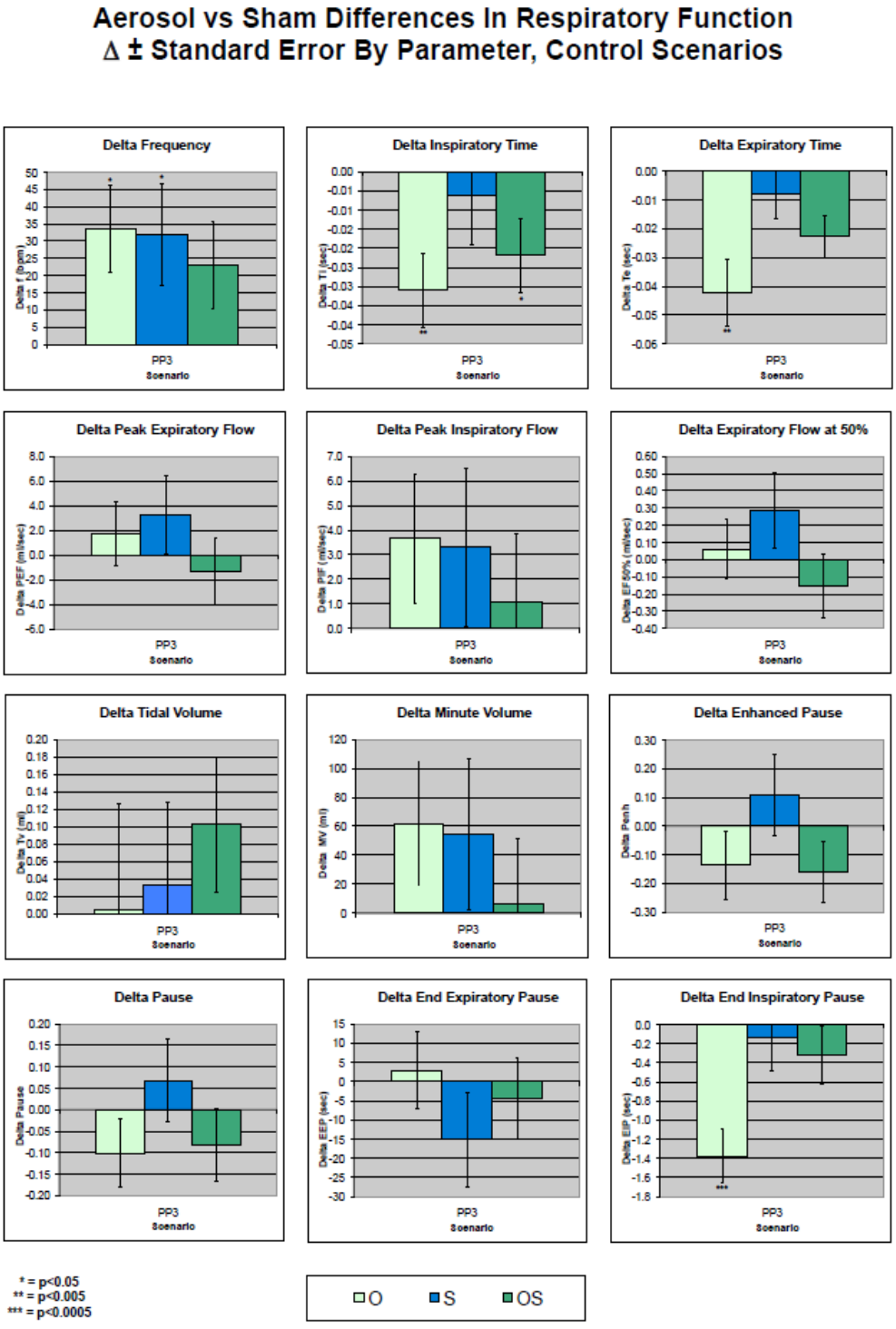


Figure 5-4
Differences in respiratory parameters in control scenarios at PP3: The O scenario produced a strongly significant reduction in Ti, Te, and EIP. No flow parameters were affected in the control scenarios.

5.4 Univariate Analyses

Univariate analyses assessed dose response relationships for various exposure metrics and specific respiratory parameter outcomes. Table 5-1 shows the standardized respiratory parameter changes vs. components of the aerosol using all data from all power plants.

Table 5-1
Univariate analyses: Standardized respiratory parameter changes vs. aerosol composition. Changes in outcome parameter (TERESA aerosol – filtered air sham) per SD of the concentration ± SE. Shaded boxes show statistically significant associations between the parameter change and components.

UNIVARIATE ANALYSIS STANDARDIZED RESPIRATORY PARAMETERS CHANGES Vs. AEROSOL COMPOSITION

	f	Ti	Te	Tv	Mv	PIF	PEF	EF50	PAU	EIP	EEP	Penh
	Coef ± SE	Coef ± SE	Coef ± SE	Coef ± SE	Coef ± SE	Coef ± SE	Coef ± SE	Coef ± SE	Coef ± SE	Coef ± SE	Coef ± SE	Coef ± SE
Mass	-3.20 ± 2.59	0.002 ± 0.002	0.000 ± 0.003	-0.04 ± 0.04	-10.71 ± 6.45	-0.60 ± 0.40	-0.41 ± 0.33	-0.04 ± 0.03	-0.01 ± 0.02	0.07 ± 0.10	-0.81 ± 1.91	-0.02 ± 0.02
PM	0.10 ± 2.66	-0.001 ± 0.002	-0.003 ± 0.003	0.07 ± 0.04	6.48 ± 6.66	0.46 ± 0.40	-0.29 ± 0.34	0.02 ± 0.03	0.02 ± 0.02	0.15 ± 0.10	-4.68 ± 1.96 b	0.02 ± 0.02
Total SO4	-0.40 ± 2.62	0.000 ± 0.002	-0.004 ± 0.003	-0.03 ± 0.04	-6.25 ± 6.54	-0.12 ± 0.40	-0.32 ± 0.33	-0.02 ± 0.03	-0.03 ± 0.01	0.08 ± 0.10	-0.41 ± 1.92	-0.04 ± 0.02
Acidic SO4	4.49 ± 2.58	-0.004 ± 0.002	-0.006 ± 0.003	-0.03 ± 0.04	5.10 ± 6.56	0.59 ± 0.40	0.59 ± 0.33	0.06 ± 0.03 b	0.00 ± 0.02	0.02 ± 0.10	2.65 ± 1.92	0.00 ± 0.02
Neutralized SO4	-4.39 ± 2.58	0.003 ± 0.002	0.001 ± 0.003	-0.01 ± 0.04	-12.39 ± 6.43	-0.66 ± 0.40	-0.88 ± 0.33 *	-0.06 ± 0.03 *	-0.03 ± 0.01 b	0.08 ± 0.10	-2.72 ± 1.92	-0.04 ± 0.02 b
NH4	-5.86 ± 2.56 b	0.004 ± 0.002	0.002 ± 0.003	-0.02 ± 0.04	-13.89 ± 6.35 b	-0.80 ± 0.39 b	-0.94 ± 0.33 *	-0.07 ± 0.03 *	-0.03 ± 0.01 b	0.08 ± 0.10	-2.39 ± 1.89	-0.04 ± 0.02
S	-0.40 ± 2.62	0.000 ± 0.002	-0.004 ± 0.003	-0.03 ± 0.04	-6.25 ± 6.54	-0.12 ± 0.40	-0.32 ± 0.33	-0.02 ± 0.03	-0.03 ± 0.01	0.08 ± 0.10	-0.41 ± 1.92	-0.04 ± 0.02
OC	-2.95 ± 2.60	0.003 ± 0.002	0.004 ± 0.003	-0.01 ± 0.04 **	-2.43 ± 6.56	-0.37 ± 0.40	0.21 ± 0.33	-0.01 ± 0.03	0.01 ± 0.02	0.08 ± 0.10	-0.55 ± 1.92	0.02 ± 0.02
EC	-4.42 ± 2.57	0.003 ± 0.002	0.001 ± 0.003	-0.12 ± 0.04	-15.29 ± 6.19 *	-1.37 ± 0.37 **	-0.77 ± 0.32 b	-0.07 ± 0.03 *	0.00 ± 0.02	-0.05 ± 0.10	2.78 ± 1.89	-0.01 ± 0.02
TC	-3.36 ± 2.59	0.003 ± 0.002	0.004 ± 0.003	-0.03 ± 0.04 **	-6.81 ± 6.56	-0.51 ± 0.40	0.11 ± 0.34	-0.02 ± 0.03	0.01 ± 0.02	0.05 ± 0.10	-0.18 ± 1.92	0.02 ± 0.02
Al	-6.70 ± 2.56 *	0.004 ± 0.002	0.006 ± 0.003	0.12 ± 0.04	7.54 ± 6.70	0.46 ± 0.41	-0.29 ± 0.34	0.00 ± 0.03	-0.02 ± 0.02	0.21 ± 0.10 **	-4.03 ± 1.90 b	-0.04 ± 0.02
Si	-3.89 ± 2.66	0.004 ± 0.002	0.003 ± 0.003	-0.02 ± 0.04 *	-7.69 ± 6.70	-0.54 ± 0.41	-0.53 ± 0.34	-0.03 ± 0.03	0.00 ± 0.02	0.04 ± 0.10	1.97 ± 1.95	0.00 ± 0.02
Fe	-6.84 ± 2.60 b	0.004 ± 0.002	0.003 ± 0.003	0.10 ± 0.04	6.48 ± 6.74	0.31 ± 0.41	-0.09 ± 0.34	0.00 ± 0.03	-0.03 ± 0.01 b	0.18 ± 0.10	-3.35 ± 1.92	-0.06 ± 0.02 b
Ni	-4.76 ± 2.63	0.003 ± 0.002	0.004 ± 0.003	0.07 ± 0.04 **	1.65 ± 6.76	0.09 ± 0.41	-0.10 ± 0.34	-0.01 ± 0.03	-0.03 ± 0.01 b	0.09 ± 0.10	-1.94 ± 1.95	-0.06 ± 0.02 b
Zn	-6.3 ± 2.63 b	0.004 ± 0.002	0.004 ± 0.003	0.14 ± 0.04	9.03 ± 6.79	0.64 ± 0.41	0.39 ± 0.35	0.02 ± 0.03	-0.01 ± 0.02	0.18 ± 0.10	-0.53 ± 1.94	-0.03 ± 0.02
Pb	-0.52 ± 2.70	0.000 ± 0.002	-0.001 ± 0.003	-0.02 ± 0.04 **	-13.5 ± 6.8 b	-0.12 ± 0.41	-0.24 ± 0.34	-0.01 ± 0.03	-0.01 ± 0.02	-0.04 ± 0.10	0.28 ± 1.97	-0.03 ± 0.02
Mg	-3.87 ± 2.66	0.002 ± 0.002	-0.004 ± 0.003	0.14 ± 0.04 **	15.15 ± 6.49 b	0.87 ± 0.40 b	-0.22 ± 0.34	0.03 ± 0.03	-0.02 ± 0.02	0.29 ± 0.10 *	-0.22 ± 1.91 b	-0.04 ± 0.02 b
Na	-3.73 ± 2.66	0.001 ± 0.002	-0.005 ± 0.003	0.13 ± 0.04	17.71 ± 6.29 *	0.81 ± 0.40 b	-0.06 ± 0.34	0.02 ± 0.03	-0.03 ± 0.02 b	0.29 ± 0.10 *	-4.29 ± 1.29 b	-0.06 ± 0.02 *
S3	3.10 ± 2.60	-0.002 ± 0.002	-0.004 ± 0.003	-0.01 ± 0.04	9.48 ± 6.48	0.39 ± 0.40	0.37 ± 0.33	0.04 ± 0.03	0.03 ± 0.01 b	0.10 ± 0.10	0.34 ± 1.92	0.04 ± 0.02
NO	6.44 ± 2.58 *	-0.005 ± 0.002 b	-0.005 ± 0.003 *	0.04 ± 0.04	11.81 ± 6.42	0.78 ± 0.39 b	0.34 ± 0.33	0.05 ± 0.03	0.00 ± 0.02	-0.07 ± 0.10	-1.51 ± 1.91	0.01 ± 0.02
NO2	0.60 ± 2.62	0.000 ± 0.002	-0.004 ± 0.003	-0.04 ± 0.04	-12.07 ± 6.49 b	-0.77 ± 0.39 b	-0.50 ± 0.33	-0.03 ± 0.03	0.06 ± 0.01 *	0.02 ± 0.10	-1.02 ± 1.91	0.07 ± 0.03 **
NO3	-2.09 ± 2.61	0.001 ± 0.002	0.001 ± 0.003	-0.05 ± 0.04	-14.13 ± 6.35 b	-0.82 ± 0.39 b	-0.75 ± 0.33 b	-0.06 ± 0.03 b	0.02 ± 0.01	0.02 ± 0.10	-0.48 ± 1.92	0.03 ± 0.02
SO3	0.00 ± 2.62	0.000 ± 0.002	-0.005 ± 0.003	0.03 ± 0.04	-0.12 ± 6.56	0.20 ± 0.40	0.25 ± 0.33	0.02 ± 0.03	0.01 ± 0.02	0.02 ± 0.10	-0.52 ± 1.97	0.01 ± 0.02
Pinene	-4.41 ± 2.61	0.004 ± 0.002 b	-0.005 ± 0.003	-0.02 ± 0.04	-4.50 ± 6.56	-0.46 ± 0.40	-0.05 ± 0.34	-0.02 ± 0.03	-0.02 ± 0.02	0.08 ± 0.10	-1.51 ± 1.94	-0.02 ± 0.02
Formaldehyde	1.59 ± 3.02	0.000 ± 0.003	-0.005 ± 0.003	-0.03 ± 0.03	-3.37 ± 5.31	-0.02 ± 0.38	0.09 ± 0.32	0.03 ± 0.02	0.07 ± 0.01 *	0.14 ± 0.07 b	-4.25 ± 1.84 *	0.09 ± 0.02 **
Acetaldehyde	-0.89 ± 3.02	0.002 ± 0.003	-0.005 ± 0.003	-0.01 ± 0.03	-2.73 ± 5.32	-0.15 ± 0.38	0.10 ± 0.32	0.02 ± 0.02	0.04 ± 0.02 *	0.14 ± 0.07 b	-4.03 ± 1.85 b	0.06 ± 0.02 *
Acetone	-1.51 ± 3.02	0.002 ± 0.003	-0.005 ± 0.003	0.00 ± 0.03	-2.71 ± 5.32	-0.16 ± 0.38	0.11 ± 0.32	0.02 ± 0.02	0.04 ± 0.02 *	0.13 ± 0.07	-3.28 ± 1.79 b	0.07 ± 0.02 *
Total Aldehyde	-0.19 ± 3.03	0.001 ± 0.003	0.004 ± 0.003	-0.01 ± 0.03	-3.28 ± 5.31	-0.11 ± 0.38	0.10 ± 0.32	0.02 ± 0.02	0.06 ± 0.01 *	0.15 ± 0.07 b	-4.29 ± 1.85 *	0.08 ± 0.02 **

b = p<0.05
* = p<0.01
** = p<0.001

Coef = Change in Δ per SD of the concentration

There were 33 relationships with $p<0.01$ out of 336 assessments. Of these, 13 had $p<0.001$. Increases in frequency were associated with NO ($p<0.01$), while decreases were associated with Al ($p<0.01$). Decreases in f were associated with Fe, Zn, and NH_4 ($p<0.05$). NO was also associated with reductions in Ti ($p<0.05$) and Te ($p<0.01$) that typically accompany increases in f. An increase in Ti was observed for pinene ($p<0.05$). Statistically significant decreases in TV

were associated with OC, TC, Si, and Pb, while significant increases were observed with Ni and Mg. Only EC was associated with a robust decrease in MV, while Na was associated with a significant increase in this parameter. Decreases ($p<0.05$) in MV were observed with NH_4, Pb, NO_2, and NO_3, while increases were associated with Mg. A significant reduction in PIF was associated with EC, with marginally significant decreases observed with NH_4, NO_2, and NO_3. Marginally significant increases in PIF were observed with Mg, Na, and NO.

PEF and EF50 are important flow parameters, reductions in which may be indicative of bronchoconstrictive effects. Significant reductions in PEF and/or EF50 were associated with neutralized sulfate and NH_4 (both $p<0.01$). Reductions with EC, were PEF ($p<0.05$) and EF50 ($p<0.01$), while marginally significant associations were observed for NO_3 (decrease) and acidic sulfate (increase). Significant increases in Enhanced Pause (Penh), which can also be indicative of airway restriction, were associated with NO_2, formaldehyde, acetaldehyde, acetone, and total aldehydes. Reductions in Penh were associated with Na, neutralized SO_4, Fe, and Ni. EIP, an increase of which can be indicative of a sensory irritant effect if coupled with a reduction in frequency, significantly increased in relation to Al, Mg, and Na, and marginally increased in relationship to formaldehyde, acetaldehyde, and total aldehydes. Increases in EEP, which can be indicative of pulmonary irritation, were not observed in association with any pollutants; however, decreases in EEP were strongly associated with formaldehyde and total aldehydes.

5.5 Multivariate Analyses (Random Forest)

The Random Forest statistical approach was used as a form of multivariate analysis to rank the importance of each measured exposure component in predicting differences between exposed and control animals. Table 5-2 shows all the random forest ratings for components and all outcomes.

Table 5-2
Random forest ranking of exposure components for each respiratory function outcome. Relative importance ranking of each measured component in predicting differences between exposed and control animals.

Random Forest Ranking of Exposure Components
for Each Respiratory Functional Outcome

	F	TI	Te	Tv	Mv	PIF	PEF	EF50	PAU	EIP	EEP	Penh
Mass	18	17	16	16	6	15	6	4	4	5	10	3
PN	16	18	10	9	9	12	5	7	6	4	2	5
Total SO4	13	11	5	12	12	20	15	11	2	6	14	2
Acidic SO4	1	1	1	13	14	18	8	12	22	7	16	20
Neutralized SO4	11	12	6	10	7	13	9	8	11	8	4	11
NH4	14	13	4	7	8	10	3	6	10	9	6	6
OC	8	7	17	11	11	8	20	18	13	17	7	16
EC	10	10	9	2	1	1	1	1	9	13	20	14
TC	7	6	12	14	2	2	12	2	14	15	8	13
Al	2	4	2	18	21	19	17	19	16	2	9	18
Si	6	5	11	23	17	17	21	21	20	23	18	21
Fe	3	2	14	8	20	6	19	15	3	16	12	12
Ni	9	9	18	5	22	11	14	13	8	18	13	8
Zn	20	20	22	3	16	16	16	16	19	11	17	9
Pb	21	21	19	22	18	22	2	22	23	21	23	22
Mg	19	19	20	6	19	4	11	10	18	1	21	19
Na	23	23	21	1	5	3	13	17	17	3	5	15
O3	15	8	7	4	3	5	2	3	12	12	1	4
NO	4	15	3	15	10	9	18	20	5	22	22	7
NO2	12	16	13	20	4	7	4	5	1	14	11	1
NO3	17	22	15	19	15	14	10	14	15	20	15	17
SO2	22	14	23	21	23	23	23	23	21	19	19	23
Pinene	5	3	8	17	13	21	7	9	7	10	3	10

A typical random forest plot is shown in Figure 5-5. This analysis was supplemented by plotting raw mean differences for each adverse outcome versus the components identified in the univariate and random forest analyses.

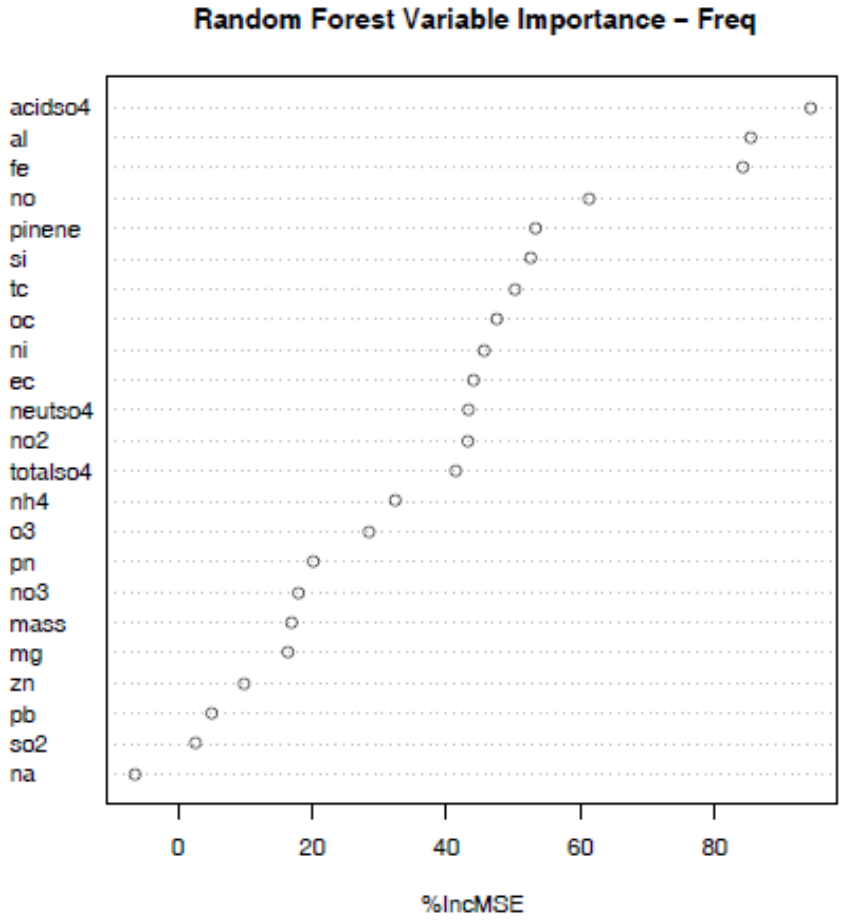


Figure 5-5
Random forest variable importance for frequency: Changes in f were best predicted by acid sulfate, Al, and Fe.

Figure 5-5 shows the random forest for frequency; acidic sulfate was first on this list and aluminum and iron were second and third, respectively. These three pollutants are the most important variables, as evidenced by their location at the far right-hand side of the plot. Univariate analyses (Figure 5-6) showed a positive (but not significant) association with acidic sulfate, a marginally significant positive association with Fe, and a strongly significant negative association with aluminum. Figures 5-6a and 5-6b show scatter plots for the top eight random forest ranked components with identification of data points in terms of scenario and power plant. Acid sulfate was first in the random forest ranking; however, as mentioned, the univariate analysis was not significant, as illustrated in the scatter plot, which shows a wide distribution for both outcome and concentration data. Aluminum, ranked second, had a negative slope driven by the P and POS scenarios. Fe ranked third and its negative slope appeared to be driven largely by the POS and PONS scenarios at Plant 3. NO ranked fourth, had a wide distribution for both outcome and concentration data, and had a positive slope with the PO and OS scenarios mostly above the slope. Pinene, ranked 5th, and Si, ranked 6th were associated with a decrease in frequency. OC, ranked 8th, and total sulfate, ranked 13th, are shown to illustrate their lack of significant associations in both the univariate and random forest analyses.

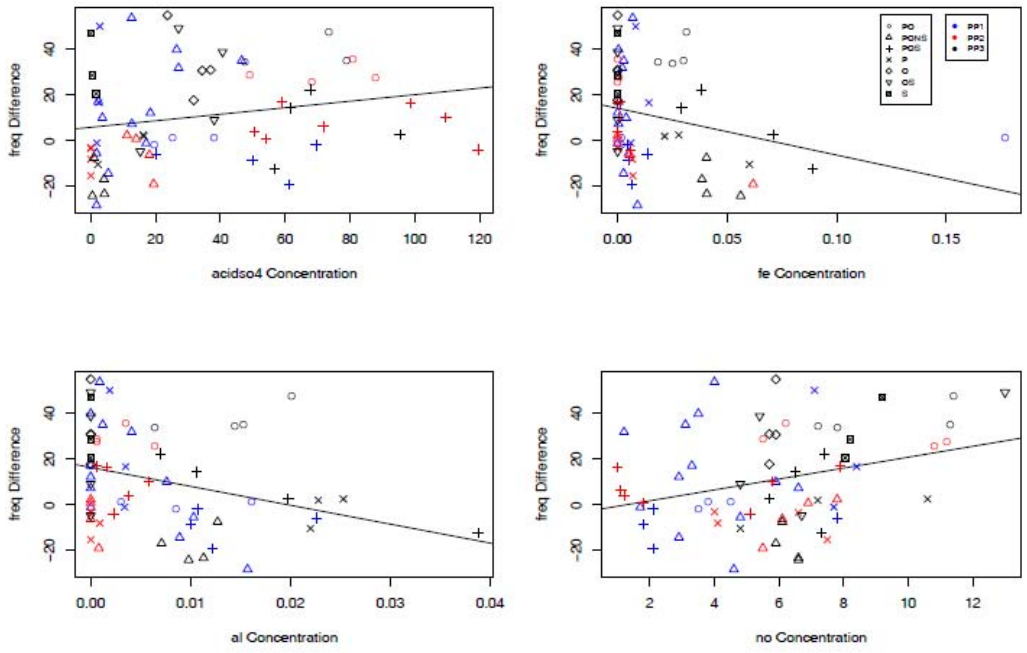
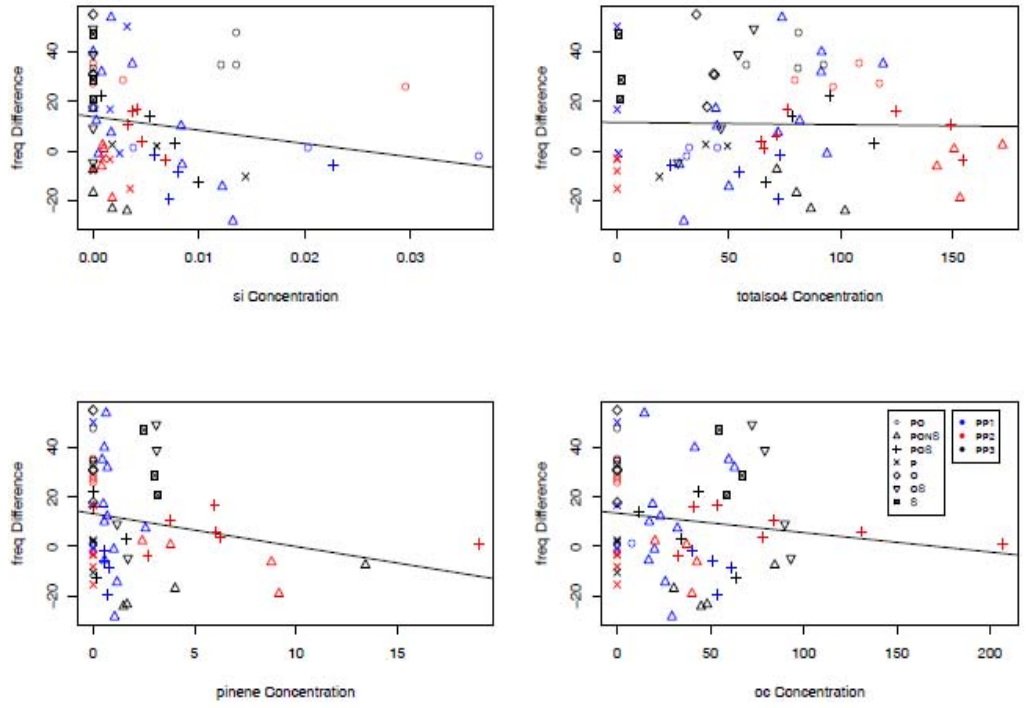


Figure 5-6
Figure 5-6a (above) and 5-6b (below). Scatter plots for top 8 ranked elements in random forest for frequency. Increases in frequency were most strongly related to NO, while decreases were related to Al.



5.6 R^2 Analysis

In an attempt to determine the relative importance of plant/scenario versus individual measured component impact on the respiratory outcomes, we calculated the adjusted R^2 for plant/scenario ANOVA models and the adjusted R^2 for the component regression models for all outcomes (Table 5-3). While plant/scenario models were able to explain 0.2 to 0.5 (median = 0.4) of the variance in the outcomes, the single components in univariate analysis were only able to explain 0.001 to 0.14 (median = 0.015) of the variance in the outcomes. We also calculated the predicted R^2 for the random forest as a multivariate analysis, and found that for this approach individual components explained less than 0.1 of the variance. There were no major differences between plant/scenario ANOVA and random forest analyses. As an example, predicted R^2 values for frequency were 0.36 and 0.32; for EF50 0.20 and 0.18 and for PEF 0.1 and 0.09, for ANOVA and random forest analyses, respectively.

Table 5-3
Comparison of adjusted R^2: The plant/scenario approach vastly outperforms any single component in predicting any given outcome of respiratory function.

Comparison of adjusted R^2 for Plant/Scenario ANOVA models and various exposure metrics identified in univariate regression models

Parameter	Scenario /Plant	Mass	Neutral Sulfate	Amonium ion	Total Sulfate	Acidic Sulfate	Elemental Carbon
f	0.4580	0.0080	0.0280	0.0410	-0.0150	0.0300	0.0290
Ti	0.3283	0.0050	0.0210	0.0310	-0.0150	0.0340	0.0130
Te	0.2566	-0.0060	-0.0020	0.0080	-0.0110	0.0320	0.0160
PEF	0.4301	0.0080	0.0880	0.1060	-0.0010	0.0290	0.0660
PIF	0.4339	0.0190	0.0260	0.0460	-0.0140	0.0180	0.1380
EF50	0.4979	0.0190	0.0730	0.0950	-0.0100	0.0390	0.0840
Tv	0.4132	0.0040	-0.0140	-0.0120	-0.0060	-0.0070	0.1450
MV	0.3948	0.0260	0.0390	0.0540	-0.0010	-0.0060	0.1030
Penh	0.4297	-0.0070	0.0440	0.0320	0.0280	-0.0150	-0.0140
PAU	0.3999	-0.0070	0.0660	0.0520	0.0310	-0.0150	-0.0140
EIP	0.2210	-0.0070	-0.0100	-0.0100	-0.0100	-0.0150	-0.0110
EEP	0.2383	-0.0120	0.0150	0.0090	-0.0140	0.0140	0.0170

*Adjusted R^2 may have negative values because adjustment for model size.

5.7 Possible Physiological Mechanisms

Examination of the breathing pattern data can provide insight into the possible physiological mechanism(s) at play. For example, a reduction in frequency coupled with an increase in EIP is indicative of a sensory irritant effect, mediated by the trigeminal nerve (Alarie, 1966). A reduction in frequency coupled with an increase in EEP is suggestive of pulmonary irritation, as is a rapid, shallow breathing pattern (increased f concomitant with reduced Ti and Te, and decreased TV) (Vijayaraghavan et al., 1993). Finally, as discussed earlier, changes in flow parameters such as PEF and EF50 can be indicative of a bronchoconstrictive effect, particularly when occurring simultaneously with elongated Te and reduced TV (Vijayaraghavan et al., 1993). Our data indicate the occurrence of pathophysiological effects, with the PO scenario at PP2, where we observed a significant increase in f (with reductions in Ti and Te) and decrease in TV.

As mentioned earlier, this rapid, shallow breathing pattern has been well documented with exposures to strong acids. In this scenario, acidic sulfate was 108 $\mu g/m^3$, the highest of all scenario/plant combinations, and roughly two orders of magnitude higher than typical environmental concentrations (Dockery et al., 1992). While there was significantly increased EIP in the P scenario at PP3, this was not accompanied by a reduction in f, so sensory irritation was not evident. Reductions in flow parameters (PEF and EF50) did not co-occur with increased Te; therefore, we have less confidence in the occurrence of a frank bronchoconstrictive effect. However, with the PONS scenario at PP1 and PP2, PEF, EF50, TV and MV were all decreased at levels of significance ranging from $p<0.05$ in one instance (TV at PP2) to $p<0.005$ in three instances (PEF and EF50 at PP1, and MV at PP2) to $p<0.0005$ in the remaining 4 measurements. These decreases cannot be ignored even though prolonged Te was not observed.

Measurements of change in breathing pattern, air flow in the breathing cycle, and changes in pause parameters have been used in many studies to assess respiratory toxicology (Glaab et al., 2006, Glaab et al., 2002, Hoymann, 2006, Hoymann, 2007, Lundblad et al., 2002, Mitzner and Tankersley, 2003, Saldiva et al., 1985). In this study, we assessed large numbers of animals during exposure to determine these changes, their magnitude, and correlation with parameters of exposure. We used step-wise exposures of increasing complexity to simulate changes that might occur in a power plant plume as it mixes with other atmospheric constituents and travels to distant sites. We used step-wise statistical analyses of increasing complexity to evaluate exposures at individual plants with specific combinations of coal types and emissions controls. After the plant-specific evaluation, we combined data from all plants in an attempt to delineate any specific patterns or components of the exposure linked with respiratory changes. Overall, we observed a number of significant breathing pattern changes in response to exposure to power plant-derived particles and added constituents. Significant clinical changes in breathing pattern, such as specific irritant effects, were difficult to dissect from the results because we tended to observe a number of isolated changes in certain respiratory parameters. Some individual exposure scenario components appeared to be more strongly and consistently related to respiratory parameter changes; however, the specific scenario investigated remained a better predictor of response than individual components, suggesting that it is the chemical reactions with other compounds (from different sources) in the atmosphere that may increase the toxicity of emitted gasses and primary particles from coal fired power plants.

6
RESULTS: IN VIVO OXIDATIVE STRESS

In vivo chemiluminescence (CL) is a measure of reactive oxygen species in tissues. CL was used to assess pulmonary and cardiac responses to inhaled aerosols derived from aged emissions of three coal-fired power plants. Details of these studies are reported by Lemos et al. (2011). Tissues were also assayed for thiobarbituric acid reactive substances (TBARS). Exposure to P or PO aerosols led to no changes compared to filtered air in lung or heart CL at any individual plant or when all data were combined (Figure 6-1). POS caused significant increases in lung CL and TBARS at only one plant, and not in combined data from all plants; PONS resulted in increased lung CL only when data from all plants were combined. Heart CL was also significantly increased with exposure to POS only when data from all plants were combined. PONS increased heart CL significantly in one plant with TBARS accumulation, but not in combined data. Exposure to O, OS, and S had no CL effects. Univariate analyses of individual measured components of the exposure atmospheres did not identify any component associated with increased CL.

6.1 Results by Power Plant/Scenario

Comparisons of the exposed/sham ratios for heart and lung CL for each scenario including all three power plants showed a significant increase in heart CL in rats exposed to POS ($p<0.0006$). Lung CL for the PONS scenario ($p<0.04$) was marginally significant. A trend of increase was also seen for lung CL in the POS scenario ($p<0.06$). The same analysis performed on normalized (log) ratios yielded p values of 0.046 for exposure to PONS in the lung and 0.009 for exposure to POS in the heart. No other significant differences were observed. The p values for the interaction between mean values by scenario were 0.6 for the lung and 0.02 for the heart for the raw data and 0.7 for the lung and 0.1 for the heart for the normalized data.

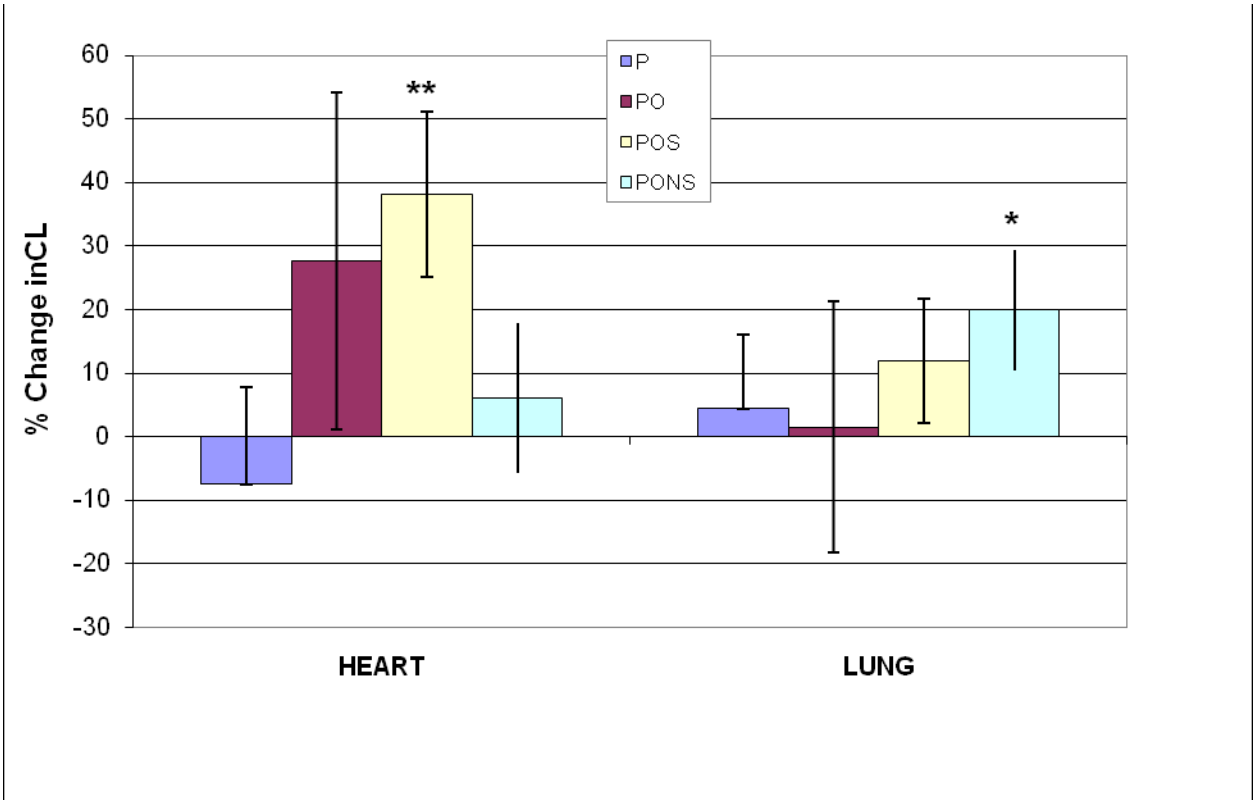


Figure 6-1
Changes in heart and lung CL for different exposure scenarios, all power plants combined. The bars represent average percentages of change in heart and lung CL for each scenario at all three power plants ± SEM. * p< 0.05, ** p< 0.01.

6.2 Univariate Analyses

Univariate regression analyses show significant associations of increasing lung CL with decreasing Al and Mg concentrations (Table 6-1). No significant associations were seen for heart CL. Given these findings, multivariate analyses as described by Coull et al. (2011) were not conducted with the data from this study.

Table 6-1
Univariate regression of lung CL and concentrations of specific components

Component	Coefficient/ SEM	
	Heart	Lung
O_3	-0.0035	0.0662
NO	0.1002	0.0298
NO_2	-0.0911	0.0118
SO_2	-0.0159	-0.0697
Formaldehyde	0.0191	0.0349

Acetaldehyde	0.0380	0.0640
Acetone	0.0658	0.0405
Total Aldehydes	0.0504	0.0443
Pinene	0.0619	0.0267
Mass	0.0728	0.0293
NC	-0.0862	-0.0028
SO_4	0.0936	0.0298
NO_3	-0.0443	0.0546
NH_4	0.0307	0.0299
Acidic SO_4	0.0538	-0.0075
Neutralized SO_4	0.0436	0.0360
OC	0.0334	-0.0204
EC	-0.0001	0.0416
TC	0.0312	-0.0130
Al	-0.0672	**-0.0973**
Si	0.0408	0.0497
Mg	-0.3737	**-0.3980**
Na	-0.0103	-0.0162
S	0.0936	0.0298
Fe	-0.0366	-0.0688
Ni	0.0008	-0.0634
Zn	-0.0580	-0.0602
Pb	0.0234	-0.0046

Values in bold denote significance at $p < 0.05$

This study was aimed at determining whether exposure to primary or aged PM from power plants, plus common atmospheric constituents, leads to cardiac and/or pulmonary oxidant stress. Our results show that: 1) emissions from different power plants subjected to the same photochemical aging triggered different oxidant responses in the heart and lung of rats, 2) in the power plant where responses were observed, different scenarios produced different effects on either the heart or the lung, 3) neither primary particles alone nor oxidized particles (P and PO) produced any changes in heart or lung CL whereas oxidized particles with organics and oxidized neutralized particles with organics (POS and PONS) showed tissue-specific responses in one power plant, and 4) the pulmonary responses showed specific, but inverse, associations with Al and Mg concentrations.

Organ chemiluminescence has been successfully used to detect increases in the steady-state concentrations of ROS in several experimental models in animals (Barnard et al. 1993; Boveris and Cadenas 1999; Evelson and González-Flecha 2000; Ghelfi et al. 2008; Gurgueira et al. 2002; Lores Arnaiz and Llesuy 1993; Rhoden et al. 2005; Turrens et al. 1988) and humans (Ferreira et al. 1989; Ferreira et al. 1988; González-Flecha et al. 1991) (Table 6-2). Increases in CL are associated with increased oxidative damage in the tissue under study and a ratio treated / control of 1.4 is considered the threshold between oxidative stress and damage (González-Flecha et al. 1991). Consistently, the treated/control ratio was reported to be 2.1 and 1.8 in the lung of animals exposed to paraquat and 85% O_2, respectively, and 10 and 1.7 for the heart of rats exposed to adryamicin and 85% O_2 (Table 6-2). In a model of inhalation exposure to Boston CAPs, the treated/control ratio for both lung and heart CL was found to be dependent on the length of inhalation exposure and on the composition of the CAPs aerosol (Gurgueira et al 2002). Exposures of 3 hours or longer showed significant increases in lung and heart CL, with values for the exposed/control ratio ranging 1.9-3.8 for the heart and ~ 1.7 for the lung. As expected,

increases in the treated/control ratio in heart CL were prevented by pretreatment with antioxidants such as mannitol or NAC (Rhoden et al 2004) (Table 6-2)

Table 6-2
Treated/control ratio for lung and heart CL in different experimental models.

Species/ Tissue	Model		Treated/Control CL		Reference
Rat Lung	Paraquat	30 mg/Kg	2.1		(Turrens et al. 1988)
	85% Oxygen	1 day	1.8		(Evelson and González-Flecha 2000)
	Boston CAPs	1 hour	1.0		(Gurgueira et al. 2002)
	3 hours		1.2		
	5 hours		1.7		
	Power Plant Emissions		Plant 2	All Plants	Present study
	P		1.0	1.0	
	PO		1.1	1.0	
	POS		1.8	1.1	
	PONS		1.3	1.2	
Mice Heart					
	Doxorubicin	15 mg/Kg	10.0		(Lores Arnaiz and Llesuy 1993)
Rat Heart	85% Oxygen	1 day	1.7		(Evelson and González-Flecha 2000)
	Boston CAPs	1 hour	0.9		(Gurgueira et al. 2002)
	3 hours		1.5		
	5 hours		1.9		
	Boston CAPs	5 hours	2.0-3.8		(Rhoden et al. 2005)
	+ NAC		1.0		
	Boston CAPs	5 hours	1.9-2.8		(Ghelfi et al. 2008)
	Power Plant Emissions		Plant 2	All Plants	Present study
	P		1.1	0.9	
	PO		1.2	1.3	
	POS		1.3	1.4	
	PONS		1.9	1.1	

The exposed/sham ratios found for the different exposure scenarios tested here ranged from 1.0-1.8 for the lung and 0.9-1.9 for the heart, indicating that some of the aged aerosols used in this study have an oxidant effect above 1.4. Interestingly, the effects of POS and PONS aerosols in the heart and lung are comparable to those observed for some Boston CAPs. For example, the 1.9 exposed/sham ratio found in the heart of rats exposed to PONS aerosols at Plant 2 are

analogous to those reported to produce significant electrophysiological alterations when using the CAPs inhalation model (Ghelfi *et al.* 2008). Similarly, increases in lung CL of 1.8 were associated with significant lung inflammation in a model of instillation exposure to PM from Washington DC (Rhoden *et al.* 2008). The PONS scenario, where lung CL was increased non-significantly in all plants, produced significant decreases in expiratory air flow (Diaz et al., 2011) in the same animals at Plants 1 and 2, but not at Plant 3. When data from all plants were combined, change in lung CL increased only with the PONS scenario, and it was the PONS scenario that was most strongly associated with decreases in expiratory air flow when all data were combined (Diaz et al., 2011). The PONS scenario had the highest mass concentrations among all the scenarios studied, and the lack of any positive univariate associations raises the possibility that the CL effect with the PONS scenario in the lung combining all data could represent a response to inhalation of particulate mass.

Indeed, the lack of positive univariate associations for measured components with the effects observed is most surprising in our study because the number of exposure days in our study and in the study of respiratory assessments (Diaz et al., 2011) were the same and positive univariate associations were found in that study. At the same time, positive univariate associations were found with cellular responses and components of the PONS scenario (Godleski et al 2011a) and in that study the number of exposure days for bronchoalveolar lavage outcomes was approximately half. The numbers of animals assessed in the respiratory studies was about two times more than in this study, but the numbers of animals assessed for the cellular response by bronchoalveolar lavage was slightly less than in our study. Therefore, neither the differences in exposure days nor differences in numbers of animals can account for finding a lack of positive univariate associations. The finding of significant negative univariate associations with measurements of aluminum and magnesium in the exposure aerosol are explained by the fact that Plant 3 in all scenarios had at least 5 fold higher levels of these elements compared to Plants 1 and 2 (Kang et al., 2011), and Plant 3 had no significant increases in lung CL in any scenario (Table 6-2).

The POS scenario had strongest effects on lung CL at Plant 2, yet the POS scenario had no respiratory or inflammatory cellular effects at Plant 2 (Diaz et al., 2011; Godleski et al., 2011a). When all data for lung CL were combined, the POS scenario had no significant effect. Heart CL was nonsignificantly increased at Plants 2 and 3 with the POS scenario. When all data across the plants were combined, heart CL significantly increased with the POS scenario. Again there were no significant univariate associations with measured components and heart CL. An empiric choice to use the POS scenario with the MI model studies was made, and at Plant 2, increased frequency of ventricular arrhythmias and decreased heart rate were found (Wellenius et al 2011).

In the CAPs studies, individual sets of exposures within a given study led to different exposed/sham ratios for CL (Table 6-2). Also, the particle components associated with oxidant effects on the lung and the heart were different, with lung CL being associated with the CAPs concentrations of redox-active metals (Fe, Cu, Mn and Zn) and heart CL showing strong associations with metals considered as tracers of crustal components of PM (Al, Si, Fe and Ti) (Gurgueira *et al.* 2002). Recent studies (Ghelfi et al., 2008) have shown that the cardiac CL signal can be blocked by blocking pulmonary vanilloid receptors using inhalation or systemic injection of receptor blockers. Rhoden et al (2005) showed that the cardiac signal can be

generated by agonists of the autonomic nervous system. These findings suggest that the cardiac CL effects are not direct and may be related to neural signals arising from the lung.

In the present study different scenarios gave rise to different responses in the lung and heart, and the same scenario produced different responses across power plants. The lack of association with specific PM components seen for these outcomes may be another instance where the effect on cardiac and pulmonary oxidants is more related to the scenarios rather than a specific component of the exposure as in Diaz et al. (2011).

7
RESULTS: INFLAMATION

Detailed results of the inflammatory responses reported in this study are reported by Godleski et al (2011). Twenty-four hours after exposure to the various scenarios, pulmonary cellular responses were assessed by bronchoalveolar lavage (BAL), complete blood count (CBC), and histopathology. Exposure to the PONS and POS scenarios produced significant increases in BAL total cells and macrophage numbers at two plants. The PONS and P scenarios were associated with significant increases in BAL neutrophils and the presence of occasional neutrophils and increased macrophages in the airways and alveoli of exposed animals. Univariate analyses and random forest analyses showed that increases in total cell count and macrophage cell count were significantly associated with neutralized sulfate and several correlated measurements. Increases in neutrophils in BAL were associated with zinc. There were no significant differences in CBC parameters or blood vessel wall thickness by histopathology. The association between neutrophils increases and zinc raises the possibility that metals play a role in this response.

7.1 Results by Power Plant/Scenario and With All Plants Combined

BAL cellular and fluid parameters are presented in Figures 7-1 and 7-2. Figure 7-1 shows each BAL outcome to each exposure scenario as the difference from filtered air control exposure ± standard error (SE) for each power plant by scenario. In Figure 7-2, these data are shown for all plants combined. In considering these two figures together, it is possible to see the contribution of each power plant to a particular outcome (Figure 7-1) then to view the overall effect of a scenario on that outcome (Figure 7-2). In Figure 7-1, total cell counts significantly increased with the POS and PONS scenarios at two of the three plants ($p<0.001$). This finding persisted when all three plants were considered together (see Figure 7-2), because individually these BAL outcomes increased at all individual plants even though they reached statistical significance individually at only two. Macrophages significantly increased ($p<0.001$) in response to the PONS scenario at Plants 1 and 3, and when all plants were combined. Total cell number and macrophage numbers for the P and PO scenarios showed little change overall and were not statistically significantly different at any individual plants with these scenarios. PMN numbers showed a marginally significant increase for the P and PONS scenario at only one of the three plants (Plant 3); when all plants were combined, the P scenario resulted in higher PMNs. This represents a very modest overall 2.05±1.20 percent change in BAL PMNs, and from Figure 7-1, it can be seen that this result is driven solely by PP3. These significant increases at Plant 3 were sufficient to drive the overall effect even though changes in the P scenario at Plant 2 were but fractionally increased and at Plant 2 were essentially zero. Changes in lymphocyte number, protein, and β-NAG were not significantly different for any scenario at any individual plant, nor overall.

Additional control scenarios were conducted at Plant 3 as described in the Methods section; none showed any significant changes in BAL parameters (lowest p value was 0.32, data not shown).

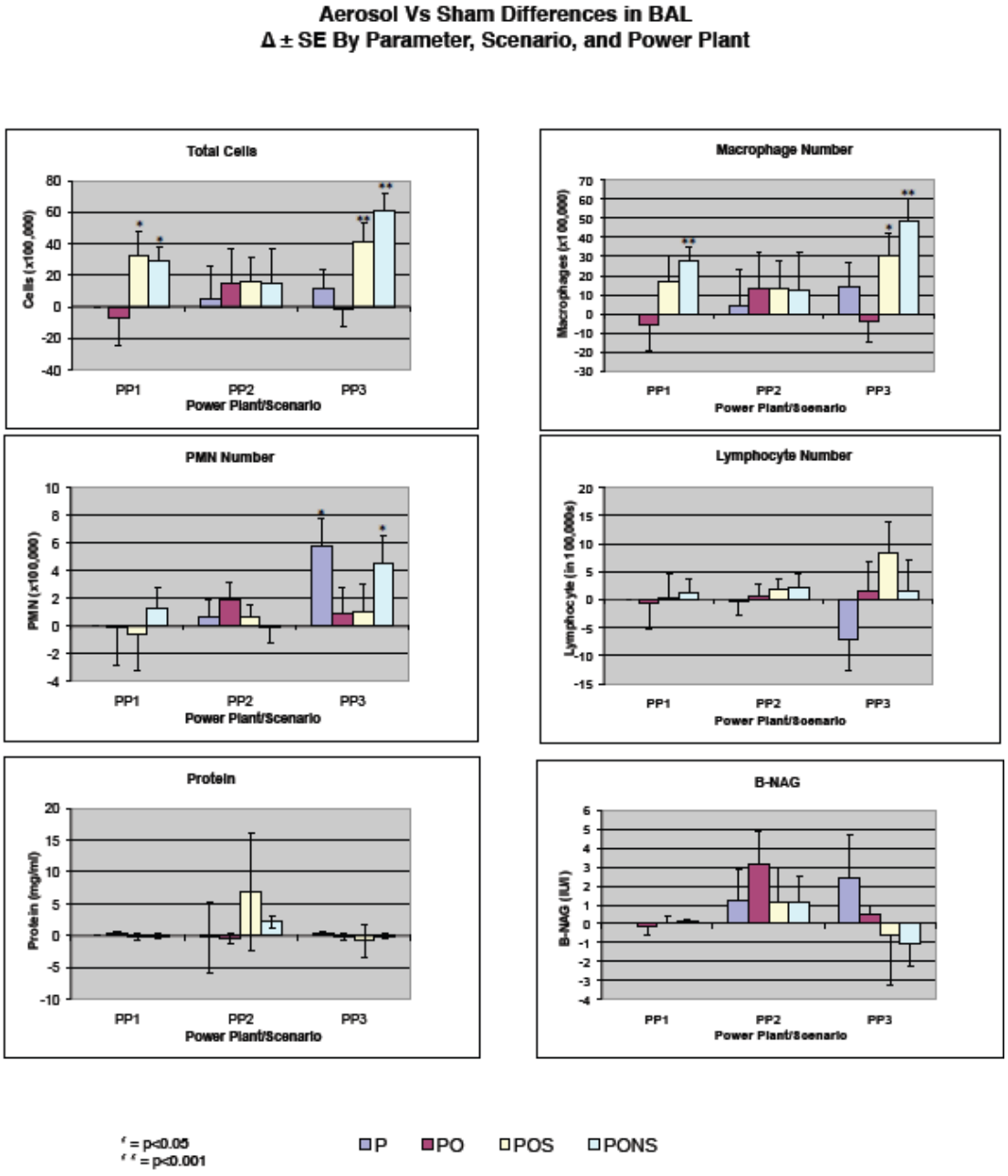


Figure 7-1
Differences in BAL parameters by scenario and power plant: Increases in total cells and macrophages are seen with the POS and PONS scenarios in all power plants, and are significantly increased in 2 of 3 plants. The P and PO scenarios have small insignificant changes in PP1 and PP2. Significantly increased PMNs are found in PP3 in the P and PONS scenarios. Other parameters have minimal variable changes

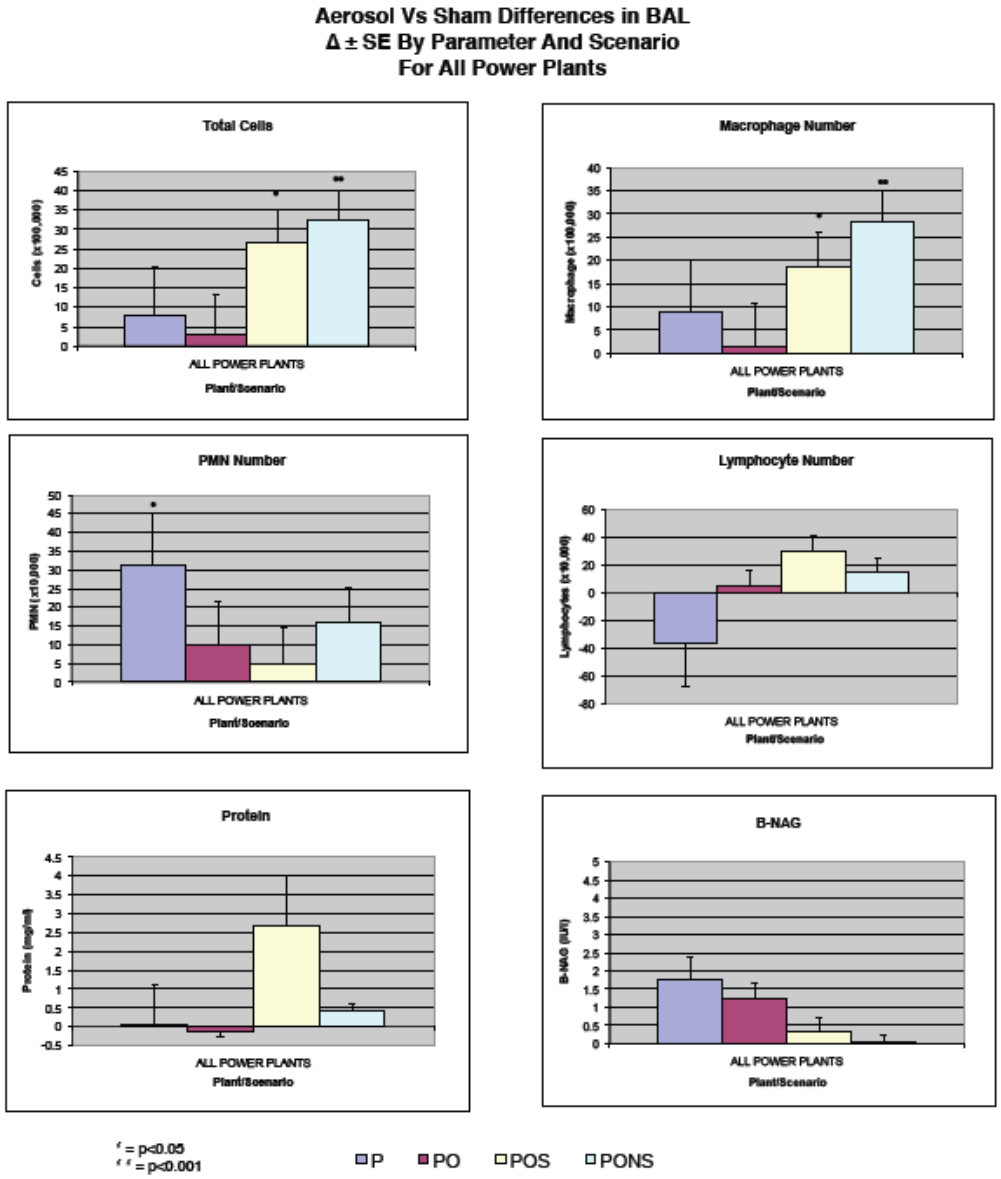


Figure 7-2
Differences in BAL parameters by scenario: Data from all power plants combined show the difference between filtered air control and TERESA aerosol exposed animals by scenario in all power plants combined. The POS and PONS scenarios show significant increases in Total BAL Cells and Macrophage numbers. The P scenario has a significant increase in PMNs

7.2 Univariate Analyses

Univariate analyses for total PM mass, particle number, and twenty-one different measured component concentrations in relationship to BAL total cell count, macrophage number, and PMN number are shown in Table 7-1.

Table 7-1
Univariate Analysis Standardized Bal Parameter Changes vs. Aerosol Composition

UNIVARIATE ANALYSIS
STANDARDIZED BAL PARAMETER CHANGES VS. AEROSOL COMPOSITION

	Total Cell count		Macrophages		PMN	
	Coef ± SE ($x10^3$)	p-value	Coef ± SE ($x10^3$)	p-value	Coef ± SE ($x10^3$)	p-value
Mass	727.21 ± 509.65	0.15	670.98 ± 465.76	0.15	-8.04 ± 35.96	0.82
PN	-159.02 ± 525.34	0.76	-105.77 ± 480.72	0.83	51.35 ± 34.79	0.14
Total SO4	1131.64 ± 485.27	0.02	1005.50 ± 445.91	0.02	4.22 ± 35.99	0.91
Acidic SO4	372.84 ± 536.29	0.49	243.13 ± 490.83	0.62	-41.23 ± 36.28	0.26
Neutralized SO4	1117.68 ± 500.60	0.03	1079.18 ± 451.73	0.02	42.28 ± 36.24	0.24
NH4	1066.75 ± 489.99	0.03	1036.90 ± 443.59	0.02	33.95 ± 35.47	0.34
OC	-234.46 ± 524.43	0.65	-229.06 ± 479.33	0.63	-17.45 ± 35.85	0.63
EC	103.82 ± 525.79	0.84	47.57 ± 481.02	0.92	-70.25 ± 33.71	0.04
TC	-207.11 ± 524.80	0.69	-209.53 ± 479.62	0.66	-25.74 ± 35.69	0.47
Al	164.22 ± 495.60	0.74	-57.76 ± 457.53	0.90	57.07 ± 35.61	0.11
Si	-682.77 ± 480.60	0.16	-548.78 ± 446.55	0.22	-11.08 ± 37.05	0.76
Fe	564.95 ± 485.67	0.24	413.61 ± 451.38	0.36	51.08 ± 35.91	0.15
Ni	571.60 ± 485.41	0.24	447.65 ± 450.29	0.32	29.77 ± 36.71	0.42
Zn	977.71 ± 457.44	0.03	670.52 ± 439.44	0.13	109.06 ± 32.01	0.0007
Pb	195.05 ± 495.22	0.69	219.72 ± 455.89	0.63	-20.44 ± 36.92	0.58
Na	601.76 ± 484.19	0.21	390.82 ± 452.05	0.39	57.63 ± 35.58	0.11
O3	761.77 ± 508.02	0.13	563.73 ± 470.32	0.23	-36.38 ± 35.39	0.30
NO	-493.94 ± 518.59	0.34	-555.43 ± 470.64	0.24	9.89 ± 35.95	0.78
NO2	708.33 ± 510.51	0.17	773.76 ± 460.58	0.09	-1.37 ± 35.99	0.97
NO3	239.58 ± 524.36	0.65	379.38 ± 476.24	0.43	-5.33 ± 35.98	0.88
SO2	-62.03 ± 526.00	0.91	-360.00 ± 476.73	0.45	-21.90 ± 35.77	0.54
Pinene	-392.63 ± 521.37	0.45	-263.08 ± 478.77	0.58	-2.26 ± 35.98	0.95
Total Aldehyde	779.87 ± 743.23	0.29	777.17 ± 668.23	0.24	24.53 ± 31.44	0.44

Shaded = p<0.05 Coef = Change in Δ per SD of the concentration

One strongly significant association was observed between PMNs and zinc (p=0.0007). Associations with p values less than 0.05 for total cell count included increasing concentrations of total sulfate, neutralized sulfate, ammonium ion, and zinc; for macrophage count, associations with p values less than 0.05 also included total sulfate, neutralized sulfate, and ammonium ion; and for PMNs a negative association was found with elemental carbon ($p< 0.05$).

7.3 Multivariate Analyses

Random forest analyses were used in conjunction with univariate analyses results to evaluate component measurements having the greatest influence (Coull et al., 2011). Scatter plots on those identified components then show the directionality and the univariate relationship between each change in outcome with increasing concentration of component. The findings of the random forest analysis (Figures 7-3 and 7-4) and the scatter plots (Figures 7-5 and 7-6) are generally consistent with the scenario and univariate findings. Data for macrophage number and neutrophil number are illustrated. Random forest analyses for macrophage number (Figure 7-3) found the variables with the strongest influence in this outcome were particle mass, total sulfate, neutral sulfate, and ammonium ion.

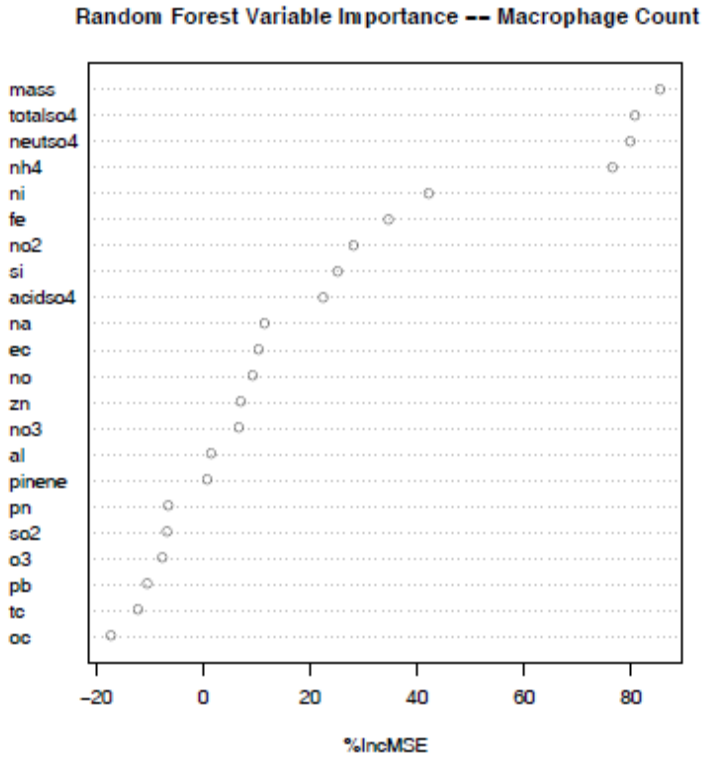


Figure 7-3
Random forest ranking the effect of exposure components in change of BAL total macrophage count: Top ranked and separated components are mass, total sulfate, neutralized SO4, and ammonium ion.

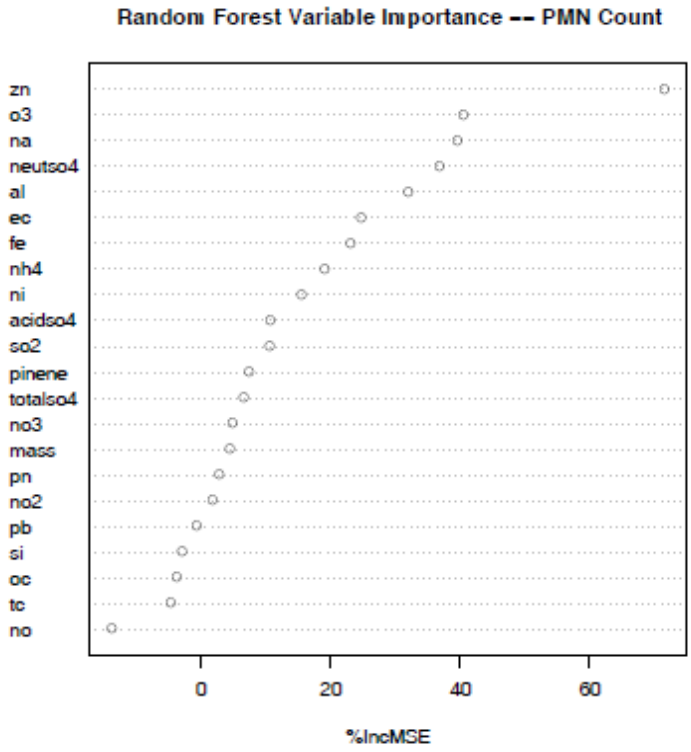


Figure 7-4
Random forest ranking the effect of exposure components in change of BAL PMN count: Top ranked and most separated component is zinc.

A clear break is seen between the top 4 and the remainder of the constituents. Figure 7-5 shows that ammonium ion, neutral sulfate, particle mass, and total sulfate have positive slopes, a distribution across the concentration range, and with POS and PONS scenarios influencing the slopes. Since macrophages dominated the total cell counts, the findings for total cell counts would be expected to be the same. Indeed, for total cell count, the variables with the strongest influence were in order: total sulfate, particle mass, neutral sulfate, and ammonium ion (Data not shown). A clear break in the pattern between ammonium ion and the next constituent was observed as with macrophages. Ammonium ion, neutralized sulfate, mass, and total sulfate all had positive slopes distributed across the concentration range, and with POS and PONS scenarios influencing the slope (data not shown).

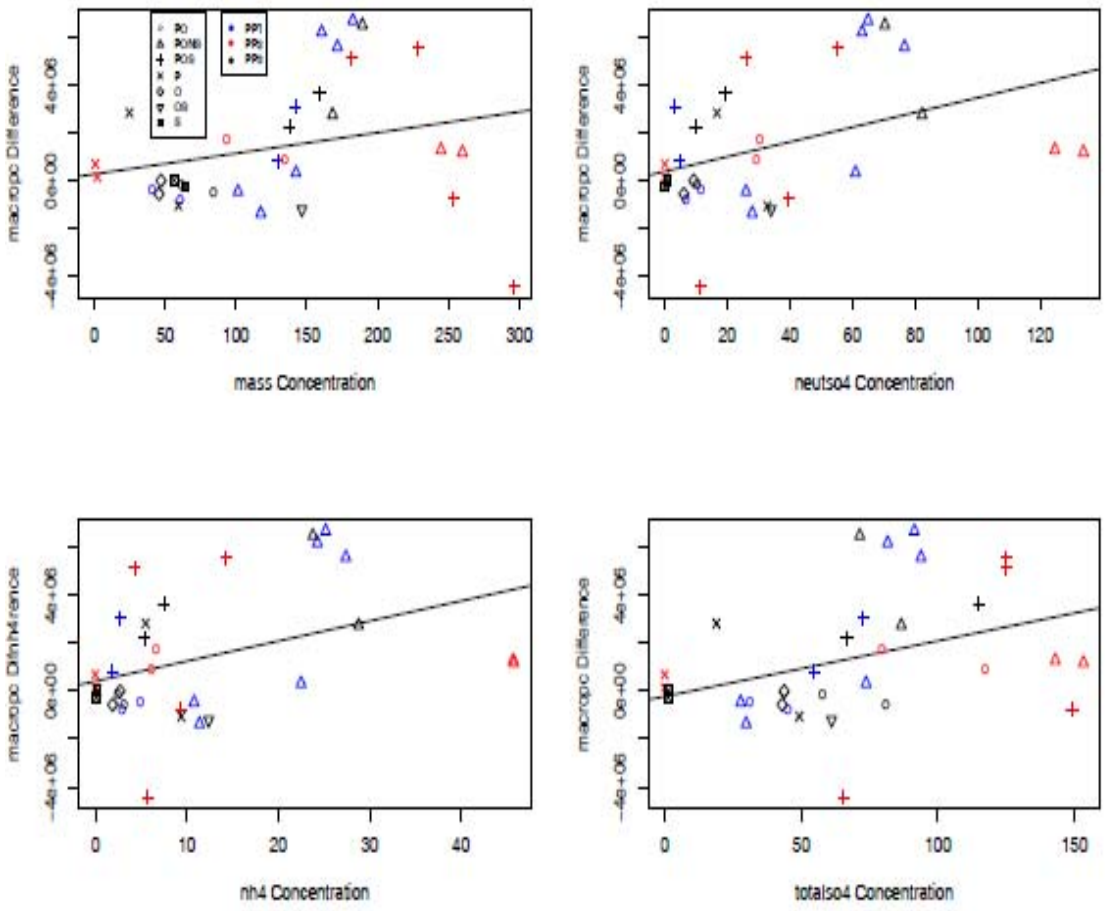


Figure 7-5
Scatter plots for BAL macrophage count: Each point has a color and symbol indicating specific plants and scenarios. Mass, total sulfate, neutral sulfate, and ammonium ion all show positive associations. Total sulfate has a uniform distribution with the POS and PONS scenarios making mostly positive contributions.

Random forest analyses for PMN numbers (Figure 7-4) found the variable with the strongest influence in this outcome was zinc. Zinc, which was strongest in univariate analysis, is atop the random forest listing, and stands alone. Elemental carbon, which was significant in univariate analyses with a negative coefficient, ranks 6th in the random forest listing. Ozone, which had a non-significant negative coefficient in univariate analyses, ranks second but is not close to zinc. The scatter plot for ozone (Figure 7-6) demonstrates the negative slope. However, the scatter plot for PMN numbers with zinc (Figure 7-6) shows that these results are driven largely by four high concentration values all at Plant 3 in the P and PONS scenarios.

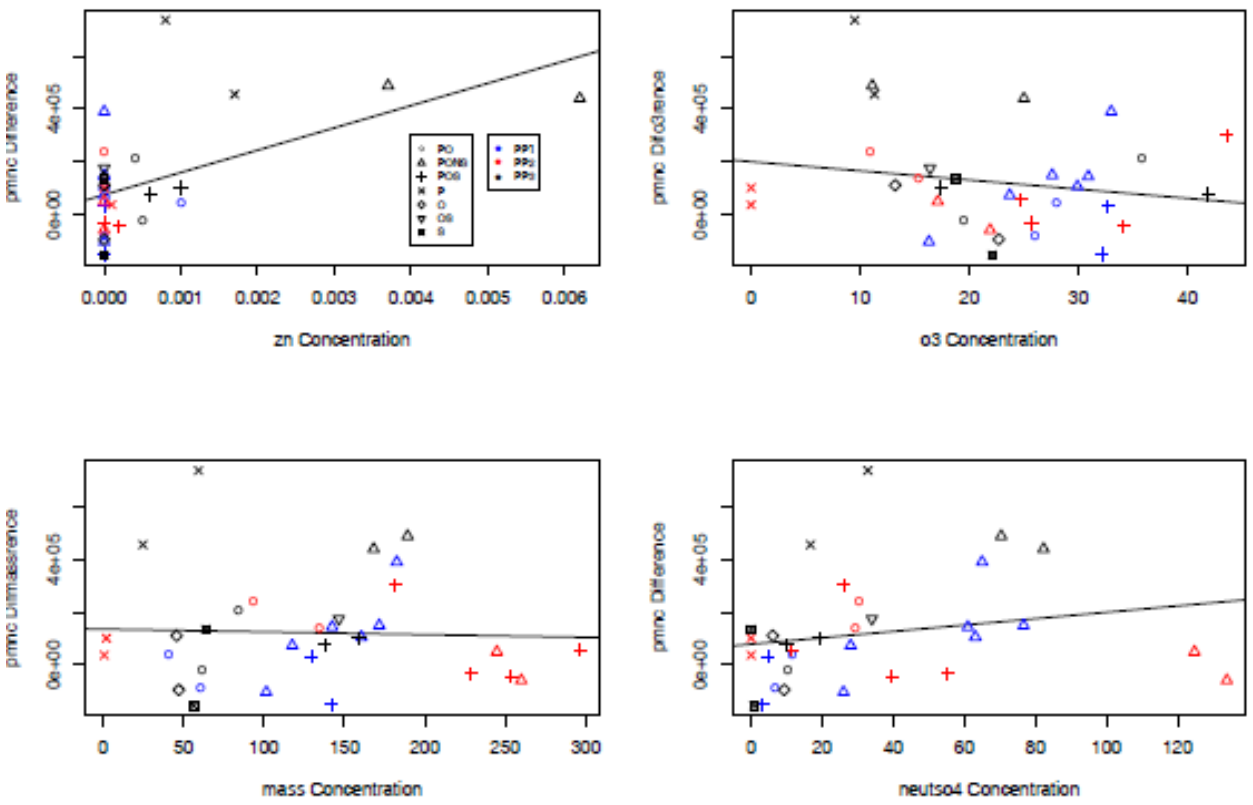


Figure 7-6
Scatter plots for BAL PMN Count: Each point has a color and symbol indicating specific plants and scenarios. The Zinc result appears to be driven by a small number of high concentrations at PP3 in the PONS and P scenarios. PMN count is negatively associated with ozone. Mass has no association, and neutral sulfate has a slight positive slope, but univariate and random forest analyses indicate no significant associations.

In an attempt to determine the relative importance of plant/scenario versus individual measured component impact on the BAL outcomes, we calculated the adjusted R^2 for plant/scenario ANOVA models and the adjusted R^2 for the component regression models for all outcomes (Table 7-2) (Coull et al 2011). For total cell count and total macrophages, plant/scenario models and single component models explained similar amounts of the variance in the outcomes. For total PMNs, plant/scenario explained 0.5 which was considerably greater than other components. The relative strength was plant/scenario > zinc > mass. Mass explained less than plant/scenario for all outcomes assessed in Table 3, and less than neutral sulfate, ammonium ion, and total sulfate for macrophages and total cells suggesting that the findings of the POS and PONs scenarios were not simply mass effects.

Table 7-2
Comparison of adjusted R^2 for plant/scenario and various exposure metrics identified in univariate and random forest analyses

Comparison of adjusted R^2 for plant/scenario and various exposure metrics identified in univariate and random forest analyses

Parameter	Scenario /Plant	Mass	Neutral Sulfate	Ammonium ion	Total Sulfate	Zinc	Elemental Carbon
Total cell count	-0.074	0.031	0.114	0.105	0.122	0.25	-0.031
Total Macrophages	-0.155	0.033	0.132	0.122	0.113	0.07	-0.032
Total PMNs	0.514	-0.031	0.011	-0.003	-0.032	0.13	0.095

7.4 Histology

On histologic examination, increased numbers of macrophages and/or PMNs were not observed at broncho-alveolar junctions in any scenario at any plant nor were there any visible pulmonary vascular changes. Both of these were the primary histological findings in our published studies of CAPs (Saldiva et al 2002, Batalha et al 2002). Not finding these qualitatively, morphometry was done on at least one scenario from each power plant, and confirmed this no effect observation. In Figure 7-7, the morphological findings in this study are illustrated. Filtered air exposures in all plants with all scenarios showed normal lung and cardiac histology. Figure 7-7a and 7-7b show completely normal alveoli with no evidence of inflammation or cellular increase in filtered air control animals at Plants 2 and 3, respectively. Figure 7-7c shows a representative section from a filtered air control animal at Plant 3 showing a normal small airway, with adjacent vessels in true cross-section with thin walls. Figure 7-7d is a section of the heart showing a true cross-section of a coronary artery in a filtered air control animal. There were no histological changes in the heart or coronary arteries in filtered air controls from any power plant or scenario as illustrated in 7-7d from Plant 2. Nor were there any histological changes in the heart or coronary arteries from TERESA aerosol exposures at any plant or any scenario as illustrated by the example in 7-7hH from Plant 2, POS scenario.

At Plant 3, the lung histology of animals exposed to the P and PONS scenarios showed a slight increase in macrophages and rare neutrophils in alveoli. These findings are illustrated in Figures 7-7e and 7-7f for the PONS scenario at Plant 3. Figure 7-7g illustrates typical findings in the airways of animals exposed to the P and PONS scenario at Plant 3. An increase in macrophages and rare neutrophils are visible on the surface of the ciliated epithelium of the airways. An increase in parenchymal macrophages or PMNs by morphometry (i.e. macrophages and PMNs at the alveolar level morphologically) was not found with CAPs (Saldiva et al 2002), nor was the airway macrophage increase depicted here found with CAPs. On qualitative assessment of macrophages in alveoli and airways at Plant 1 in all scenarios, there was no difference in numbers of macrophages visible in air spaces or airways as compared to filtered air controls; this finding supports BAL findings in the PO and POS scenarios, but not in the PONS scenario. Limited morphometry on the parenchyma in the PONS scenario at Plant 1 showed a very slight increase in macrophages which was not statistically significant (controls = 0.10±0.05; exposed = 0.12±0.05); p=0.3. Animals at Plant 3 had qualitatively more macrophages in the parenchyma in

the PONS scenarios, confirming the BAL findings for that scenario. The histological pictures in Figure 7-7 e, f, and g are representative pictures of the PONS scenario at Plant 3.

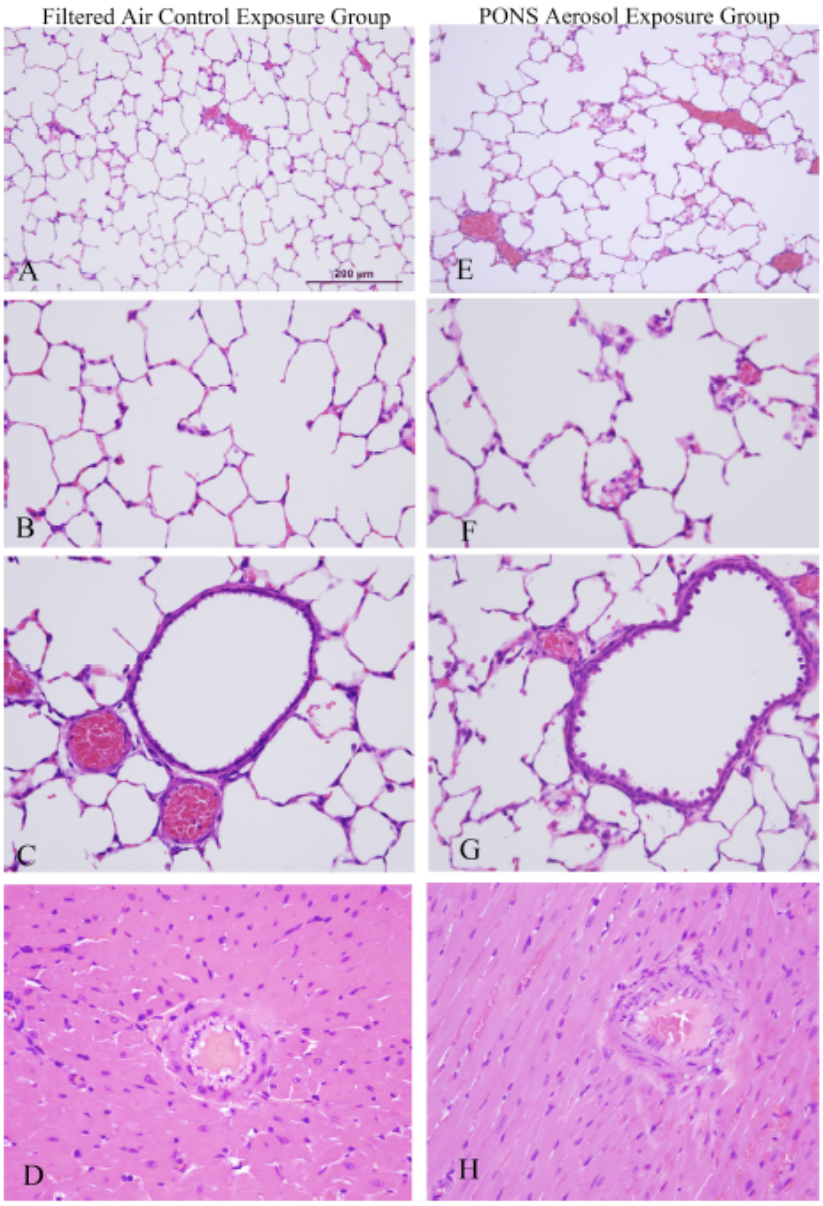


Figure 7-7
Histopathology of lungs and heart in filtered air exposed control animals (A-D) and PONS aerosol exposed animals from PP3 (E-H). No pathological changes are visible in the control animal parenchyma, airways, pulmonary vessels, myocardium or cardiac vessels. In the exposed animals, increases in alveolar macrophages and rare neutrophils are visible in alveoli. The airway in panel G has increased macrophages on the epithelial surface. There are no changes in the myocardium or cardiac vessels in panel H. Bar = 200 µm in panel A, all other original magnifications are 400X.

7.5 Complete Blood Count (CBC)

CBC results for individual plants for all scenarios, as well as combined plant data for all scenarios, are presented in Table 7-3. TERESA aerosol minus sham differences in CBC parameters, including white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), segmented neutrophil count (Neut Seg; a measure of mature neutrophils), band neutrophil count (Neut Band; a measure of immature neutrophils released from the bone marrow as part of an acute inflammatory response), lymphocytes, monocytes, eosinophils, and basophils, are all included. No significant difference in any CBC parameter was found for any scenario at any plant, nor were there significant differences when data from all plants were combined. As can be seen in Table 7-3, differences were extremely small. For example the largest change in WBC count was 1.84±2.79 x 10^3 with the POS scenario at Plant 2. Throughout the data set, in most instances the standard deviation was larger than the mean difference. In this instance, that observation was not an indication of high variability, rather an indication of very little difference between exposed and control means. Of note, there were no changes in red cell parameters, and there was no increase in band forms of neutrophils in any scenario at any plant.

Table 7-3
Aerosol vs Sham Differences In CBC Parameters Δ ± Standard Deviation by Parameter, Scenario and Power Plant

Aerosol vs Sham Differences In CBC Parameters
Δ ± Standard Deviation By Parameter, Scenario And Power Plant

PARAMETER		WBC	RBC	HGB	HCT	Neut Seg	Neut Band	Lymphocyte	Monocyte	Eosinophiles	Basophiles
		Δ ± SD	Δ ± SD	Δ ± SD	Δ ± SD	Δ ± SD	Δ ± SD	Δ ± SD	Δ ± SD	Δ ± SD	Δ ± SD
POWER PLANT 1	PO	0.06 ± 1.35	-0.00 ± 0.00	0.26 ± 0.99	0.52 ± 3.97	451 ± 418	0 ± 0	-325 ± 1217	-28 ± 181	-16 ± 36	0 ± 0
	POS	-1.10 ± 4.43	0.12 ± 0.57	0.13 ± 0.99	-0.83 ± 3.15	-338 ± 392	0 ± 0	-706 ± 1965	-66 ± 151	-10 ± 30	0 ± 0
	PONS	-0.25 ± 3.44	-0.05 ± 0.42	-0.11 ± 0.81	-0.22 ± 2.35	74 ± 800	0 ± 0	-196 ± 2864	50 ± 136	10 ± 62	-4 ± 15
POWER PLANT 2	P	-1.66 ± 1.66	0.29 ± 0.41	0.27 ± 0.64	-0.22 ± 2.79	-327 ± 876	0 ± 0	-1402 ± 1551	-29 ± 108	-2 ± 34	0 ± 0
	PO	0.23 ± 3.60	0.26 ± 0.67	0.28 ± 0.88	1.01 ± 6.11	430 ± 529	0 ± 0	-136 ± 3670	-27 ± 238	-40 ± 103	-12 ± 35
	POS	1.84 ± 2.79	0.30 ± 0.28	0.39 ± 0.46	1.27 ± 2.06	152 ± 527	0 ± 0	2008 ± 1985	-69 ± 644	22 ± 38	0 ± 0
	PONS	-1.27 ± 4.14	0.31 ± 0.37	0.80 ± 0.44	1.87 ± 1.45	-350 ± 979	0 ± 0	-905 ± 3670	-5 ± 300	-7 ± 39	0 ± 0
POWER PLANT 3	P	-0.74 ± 2.51	0.02 ± 0.34	0.02 ± 0.47	-0.36 ± 1.74	-248 ± -248	0 ± 0	-407 ± 1856	-66 ± 130	4 ± 39	0 ± 0
	PO	0.26 ± 2.29	-0.07 ± 0.59	-0.02 ± 1.06	-0.32 ± 3.01	90 ± 90	0 ± 0	145 ± 2043	52 ± 181	-29 ± 96	0 ± 0
	POS	1.42 ± 2.24	0.04 ± 0.36	-0.01 ± 0.58	0.83 ± 3.09	440 ± 440	0 ± 0	1012 ± 2360	-7 ± 76	52 ± 43	0 ± 0
	PONS	0.26 ± 1.26	0.41 ± 1.16	0.61 ± 2.14	-2.83 ± 3.12	69 ± 69	0 ± 0	935 ± 616	-16 ± 16	-2 ± 17	0 ± 0
ALL PLANTS COMBINED	P	-1.05 ± 2.31	0.11 ± 0.37	0.10 ± 0.61	-0.31 ± 2.06	-273 ± 876	0 ± 0	-739 ± 1780	-67 ± 123	2 ± 37	0 ± 0
	PO	0.21 ± 2.57	0.04 ± 0.69	0.15 ± 0.96	0.34 ± 4.39	294 ± 497	0 ± 0	-65 ± 2241	8 ± 184	-28 ± 85	0 ± 0
	POS	0.76 ± 3.46	0.19 ± 0.41	0.22 ± 0.69	0.54 ± 2.76	103 ± 695	0 ± 0	1105 ± 2313	-59 ± 457	22 ± 46	0 ± 0
	PONS	-0.25 ± 3.03	0.17 ± 0.74	0.25 ± 1.31	-0.24 ± 2.66	-2 ± 646	0 ± 0	-59 ± 2611	26 ± 181	5 ± 52	0 ± 0

This study was designed to determine whether primary and secondary particles derived from emissions of coal fired power plants can produce inflammation in the lungs of exposed animals as assessed by BAL and histology, changes in the pulmonary vasculature, and morphological changes in the heart and cardiac vasculature. Results of this study indicate that the POS and PONS scenarios resulted in an increase in total lavaged cells in BAL, and this increase was primarily related to increases in macrophages. Using both univariate and multivariate (random forest) analyses, the BAL increases were most strongly associated with total sulfate, neutral sulfate, and ammonium ion. These are all highly correlated measurements in these experiments; in addition, all are highly correlated with total particle mass, which was also a significant predictor of total cell count in random forest analyses. Increases in PMNs in BAL were found with the P and PONS scenarios at Plant 3, and statistically related to the concentration of zinc by univariate and random forest analyses. Particle mass concentrations in the P scenario were highest at Plant 3, likely because of emissions from the wet FGD scrubber used at this plant. Specifically, small amounts of neutral and acidic sulfate were released by the scrubber itself. Of the three plants, P scenario neutral sulfate and particle number concentrations were highest at Plant 3. The P scenario at Plant 3 had about 40 times the particle mass concentration compared to the same scenario at the other two plants (43.2±14.6 $\mu g/m^3$ compared to 1.0±0.9 and 1.7±1.8 $\mu g/m^3$). However, these observations do not explain the response to the P scenario at Plant 3 since both total mass and neutral sulfate were much higher in other scenarios at other plants where no responses were observed.

The statistically significant changes in total cell count and macrophages by BAL are considered mild toxicological responses, and these were observed only in scenarios with added SOA and the most complex atmospheric reactions. The SOA used in TERESA was derived from a-pinene, a biogenic volatile organic hydrocarbon. It should be noted that the oxidation products of α-pinene have been studied in a number of toxicological experiments, primarily focused on respiratory responses. Rohr et al. (2002a, b) reported a number of airway responses due to exposure to a-pinene oxidation products; these included both gas-phase and aerosol-phase products. Pinene-derived SOA has not been examined for any pulmonary inflammatory effects, although the oxidation products of isoprene, another biogenic terpene, have been used in BAL and nasal lavage studies in mice (Rohr, 2001 [dissertation]). Isoprene oxidation products, including both gas- and aerosol-phase, showed no evidence of inflammatory activity.

Increases in neutrophils in BAL without increases in BAL protein, enzymes, or circulating neutrophils are also viewed as a mild response. The association with the zinc concentration appears to be driven by increased zinc concentrations in the P and PONS scenarios at Plant 3 (Figure 7-6). At Plants 1 and 2 for all scenarios, the zinc concentration was very low and ranged from 0.0-0.4 ng/m^3; at Plant 3, the mean zinc concentrations were 1.5±0.6 for the P scenario, 0.5±0.2 for PO, 1.4±0.9 for POS, and 4.3±2.7 for PONS, which are relatively low, yet all higher than at the other plants (Kang et al 2011). The role of zinc as a metal causing health effects has been controversial because all studies showing significant positive inflammatory effects have been done at very high concentration levels (Chen and Lippmann 2009). Given the distribution of results driving this outcome (Figure 7-6) and the zinc exposure concentrations in this study (noted above), the role of zinc alone in the etiology of PMN inflammation in this study is not convincing. Plant 3 had the wet FGD scrubber and the highest mass concentrations in the P scenario among all plants. The contribution of this scrubber to particle generation is discussed in

detail in Kang et al (2011). However, the P and PONS scenarios at Plant 3 also had substantially higher concentrations of chromium, iron, nickel, as well as zinc compared to these scenarios at Plants 1 and 2 (Kang et al 2011) despite these plants having occasional days with high concentration of these elements. If these concentrations are added together, Plant 3 had 3-4 times higher concentrations of these elements than the other plants. This difference is likely due to the particular coal burned during the time of the experiments, since the FGD scrubber does not emit metals. The possibility of corrosion associated with the sampling lines is discussed and dismissed as unlikely by Kang et al. (2011). Although the measured concentrations of these metals (other than zinc) did not reach significance in univariate analyses, it is possible that zinc may be a tracer for the combined metal effect.

8
RESULTS: MYOCARDIAL INFARCTION MODEL

The results of these studies are reported in detail in Wellenius et al. (2011). We evaluated in susceptible animals the effect of stack emissions on cardiac electrophysiology and respiratory function under exposure conditions intended to simulate an aged plume with unneutralized acidity and secondary organic aerosols (POS exposure scenario). Due to the complexity of the acute MI animal model, we chose *a priori* to conduct these experiments only under the exposure scenario producing the greatest effects in normal rats. Logistical considerations necessitated that the decision of which exposure to use be made shortly after the findings of the initial exposures of normal animals to each scenario were completed. Preliminary analyses of heart chemiluminescence data from Plant 1 suggested no health effects under any scenario, so experiments using the acute MI animal model were not carried out at Plant 1. Preliminary analyses of data from Plant 2 suggested similar increases in heart chemiluminescence with the POS and PONS exposure scenarios, and we chose to use the POS exposure scenario for experiments using the acute MI animal model. The same scenario was chosen for experiments at Plant 3 to be consistent.

At each of Plants 2 and 3 we attempted to expose over 4 experimental days a total of 32 rats with acute myocardial infarction to either stack emissions under the POS scenario or filtered air. At Plant 2, 30 rats survived the MI surgery and were randomized to either filtered air or exposure under the POS scenario (Table 8-1).

Table 8-1
Summary of animal characteristics and sample size

	Power Plant 2		Power Plant 3	
	Filtered Air	POS	Filtered Air	POS
Randomized	15	15	7	8
Died during exposure	0	0	0	2[†]
Histopathology				
Transmural left ventricular infarct	9	13	1	1
Transmural right ventricular infarct	2	1	3	5
Subepicardial left ventricular infarct	4	1	3	0
Electrocardiographic outcomes	14[‡]	15	2[‡]	6
Respiratory outcomes	15	15	5	6

[†]1 animal died for technical problems unrelated to the exposure. [‡]1 animal from Power Plant 2 and 5 animals from Power Plant 3 were excluded from analysis due to poor signal quality.

High quality ECG data were available from 29 (97%) of animals. As expected based on our past experience with this animal model, most rats at Power Plant 2 had transmural left ventricular myocardial infarcts as assessed by histopathology (Figure 8-1).

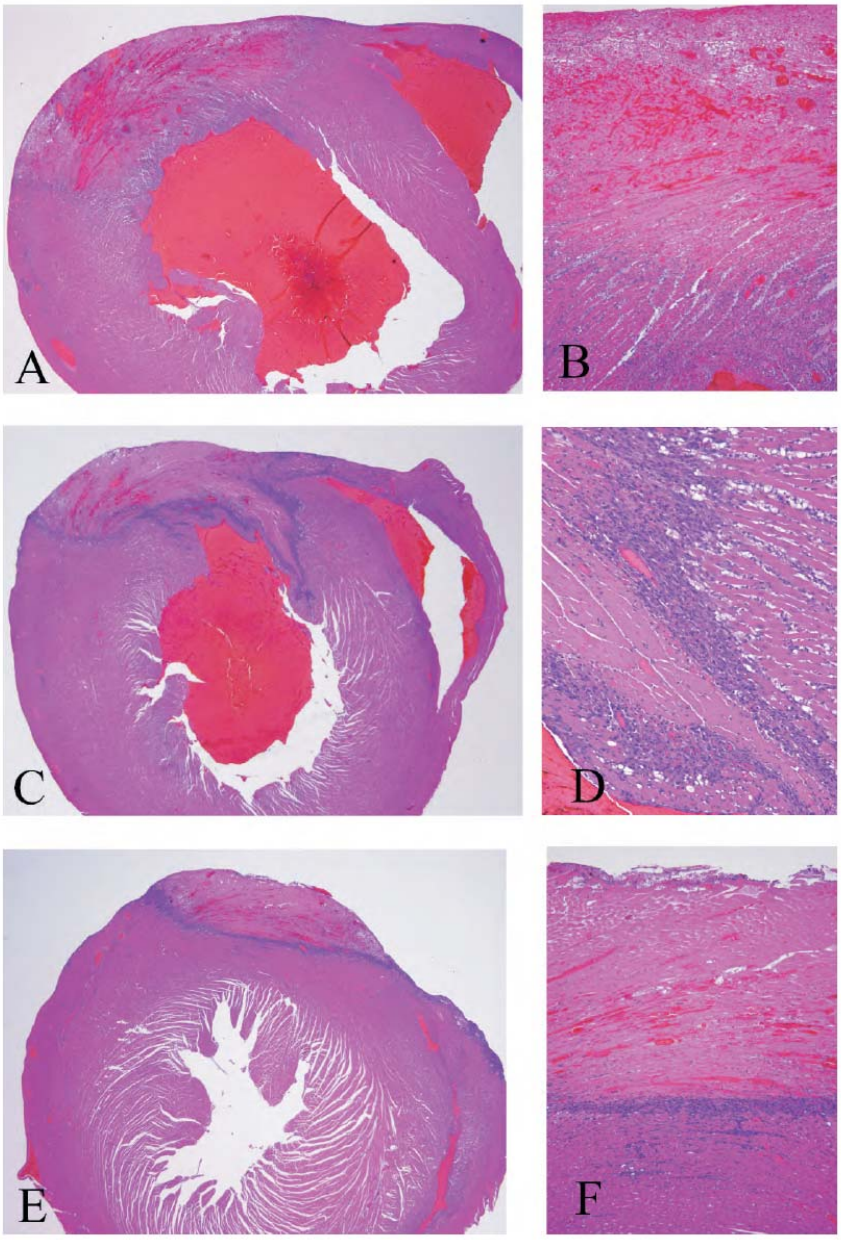


Figure 8-1
H&E-stained heart sections established the presence of MI. These show the infarctions to be in the distribution of the anterior descending coronary, and all have histological characteristics indicating ages of at least 3 days old, but less than one week. (A) Low magnification (20x) picture of a large transmural infarction with extensive cardiac myocyte necrosis surrounded by an influx of inflammatory cells. (B) Same infarction shown at higher magnification (100x). A large area of necrotic myocytes with hemorrhage into the necrotic area and inflammation extending from the epicardium to the subendocardial myocardium is shown. (C) Low magnification (20x) picture of another transmural infarction. The area of this infarction is slightly smaller than that illustrated in A, but has a more dense inflammatory infiltrate surrounding the area of infarction. (D) Same infarction shown at higher magnification (200x). Necrotic myocytes without nuclei and the inflammatory infiltrate of macrophages and neutrophils surrounding the necrotic cells can be seen. Normal myocytes with typical cardiac myocyte nuclei are on the right side of the

inflammatory cells. (E) Low magnification (20x) picture of an infarct that is not transmural. This infarction has the similar histological characteristics as the others illustrated except that it extends from the epicardium to the mid point of the myocardium. The distribution of these subepicardial infactions are the same as those reported previously in the form of two week old infarctions (Wellenius et al 2002). (F) Same infarction at higher magnification (100x) showing the necrosis and inflammatory infiltrate in this non-transmural infaction.

At Plant 3 we experienced a number of unexpected complications. First, due to an unplanned power plant shutdown on 2 of the 4 experimental days, we were only able to expose a total of 15 animals over 2 days. Second, contrary to our past experience with this animal model, many rats used at this power plant had transmural right ventricular myocardial infarcts which could not be explained by apparent differences in surgical technique, surgeon, animal size, or other controllable factors. Third, due to poor signal quality from the telemetry system, high quality ECG data were available from only 8 of the 15 (53%) experimental animals. This high rate of technical complications could also not be explained by identifiable differences in equipment, surgical technique, implantation surgeon, animal size, or other controllable factors. These complications resulted in a small, highly unbalanced dataset obtained from an animal model that differed materially from the intended model and from the animal model used at Plant 2. For this reason, we only report the results from Plant 2.

The POS scenario is intended to simulate an aged plume with unneutralized acidity and secondary organic aerosol derived from biogenic emissions. Exposure data for the experiments at Plant 2 are summarized in Table 8-2.

Table 8-2
Daily mean exposure characteristics under the POS scenario at Plant 2

		Power Plant 2			
Exposure Metric	Units	7/8/2005	7/13/2005	9/7/2005	9/8/2005
O_3	ppb	4.7	6.9	3.4	4.1
NO	ppb	3.6	4.3	4.2	3.2
NO_2	ppb	0.0	3.0	0.1	0.1
SO_2	ppb	24.3	32.5	21.2	26.9
Formaldehyde	$\mu g/m^3$	19.6	21.5	14.0	11.5
Acetaldehyde	$\mu g/m^3$	3.6	5.9	2.9	3.2
Acetone	$\mu g/m^3$	1.9	14.9	2.6	13.4
Total Aldehydes	$\mu g/m^3$	25.1	42.3	19.4	28.1
Pinene	$\mu g/m^3$	15.2	2.3	8.9	6.0
Particle Mass	$\mu g/m^3$	na	252.7	187.4	217.2
Particle Number	$1000/cm^3$	16.1	13.8	3.5	13.3
SO_4^{2-}	$\mu g/m^3$	na	170.5	156.9	190.2
NO_3^-	$\mu g/m^3$	na	0.5	0.0	0.0
NH_4^+	$\mu g/m^3$	na	9.4	9.1	10.0
Acid SO_4^{2-}	$\mu g/m^3$	na	127.8	125.3	144.4
Neutralized SO_4^{2-}	$\mu g/m^3$	na	42.7	31.6	45.7
Organic Carbon	$\mu g/m^3$	50.9	50.8	na	na
Elemental Carbon	$\mu g/m^3$	23.6	18.3	18.0	13.7
Total Carbon	$\mu g/m^3$	74.5	69.1	18.0	13.7
Na	ng/m^3	0.0	0.0	0.0	0.0
Mg	ng/m^3	0.0	0.0	0.0	0.1
Al	ng/m^3	0.0	0.7	2.4	5.7
Si	ng/m^3	0.0	3.8	0.0	1.2
P	ng/m^3	0.0	4.6	0.0	0.0
S	$\mu g/m^3$	na	56.8	52.3	63.4
K	ng/m^3	0.0	0.0	0.0	0.1
Ca	ng/m^3	0.0	0.0	3.3	1.2
Ti	ng/m^3	0.3	0.0	0.1	0.2
Cr	ng/m^3	0.0	45.5	92.1	31.3
Fe	ng/m^3	0.0	446.2	707.0	241.6
Ni	ng/m^3	0.0	67.2	56.5	18.9
Zn	ng/m^3	0.0	0.6	0.0	0.0
Se	ng/m^3	0.0	0.5	0.0	0.0
Pb	ng/m^3	0.0	0.0	0.0	0.0
Temperature	°C	22.7	24.0	22.4	22.4
Relative Humidity	%	52.9	51.8	51.4	48.2

Exposure characteristics were comparable to those observed for other POS scenario studies at the same plant (Kang *et al.*, 2011), with one notable exception. On 3 of the 4 experimental days levels of iron, chromium and nickel were unexpectedly high. PVB frequency was greater among POS-exposed animals versus filtered air controls (Figure 8-2)

23). We used repeated-measures Poisson regression to model the number of PVBs observed ineach hour. When averaged across the entire exposure period PVB frequency was 68.9% (p=0.11) higher in POS-exposed animals versus filtered air controls.

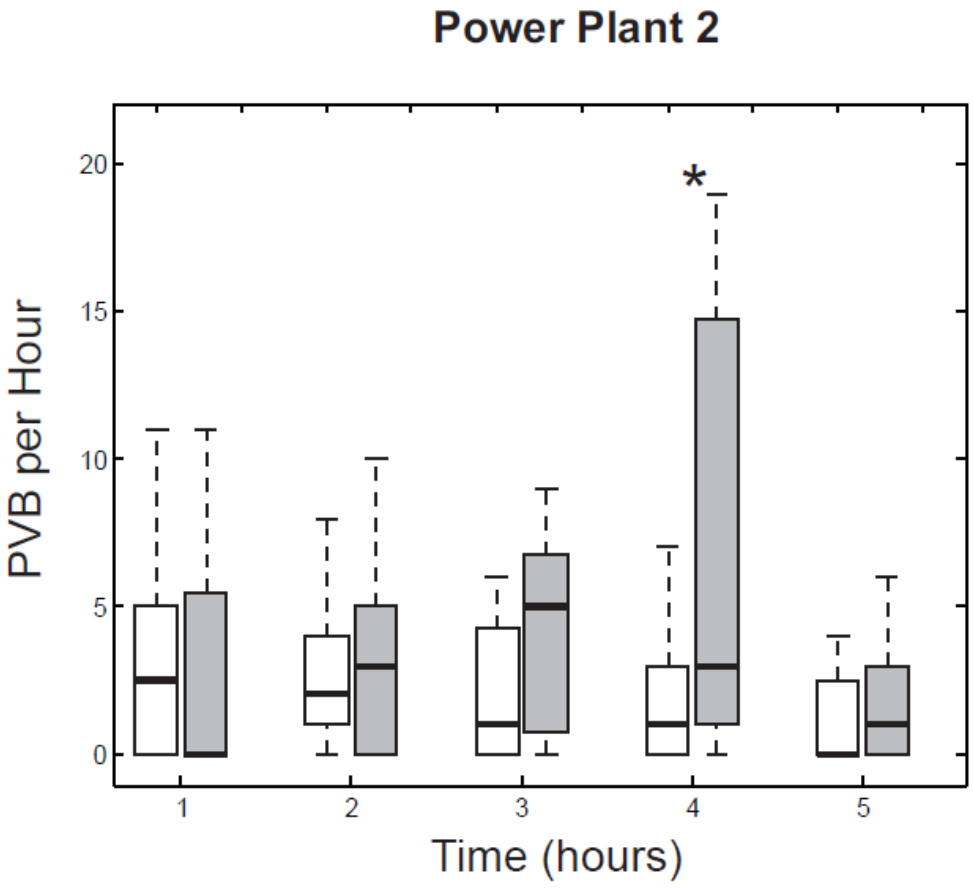


Figure 8-2
Box plots summarizing the number of premature ventricular beats per hour (PVB/h) observed in animals exposed to filtered air (white boxes) or stack emissions under the POS scenario (gray boxes) at Power Plant 2. Each box has lines at the lower quartile, median, and upper quartile values. The whiskers are lines extending from each end of the box to show the extent of the rest of the data (up to 1.5 times the interquartile range). *: p<0.05 comparing the response under the POS scenario versus filtered air controls at the same time point.

At each time point, POS exposure was associated with increased PVB frequency, but this difference was statistically significant only during the 4th exposure hour where PVB frequency was 3.9 times greater among POS exposed animals versus filtered air controls (p=0.045). During the 3rd exposure hour, PVB frequency was 2.6 times greater among the POS-exposed animals, but this difference did not reach statistical significance (p=0.067).

As a sensitivity analysis we excluded from analysis rats exposed on either of the 2 days with the highest levels of chromium, iron, and nickel (2nd and 3rd days of exposure) and found that the effect estimates were of similar magnitude to those in the main analysis, but the standard errors

were larger. As an additional sensitivity analysis, we used repeated-measures logistic regression to model the odds of observing ≥1 PVB in each hour. During the fourth and fifth exposure hours, POS-exposed animals were significantly more likely to have ≥1 PVB versus animals exposed to filtered air (p=0.031 and 0.029 for the fourth and fifth hours, respectively).

We assessed heart rate and time-domain measures of heart rate variability at 0, 60, 120, 180, 240, and 300 min after the start of each exposure (Figure 8-3). Heart rate, SDNN, and RMSSD at the start of exposure were similar in the two exposure groups. POS exposure was not associated with statistically significant changes in heart rate, SDNN or RMSSD, either overall (Table 8-3) or at any given hour (Figure 8-3). Similarly, PR interval and P-wave duration did not differ significantly between the two exposure groups.

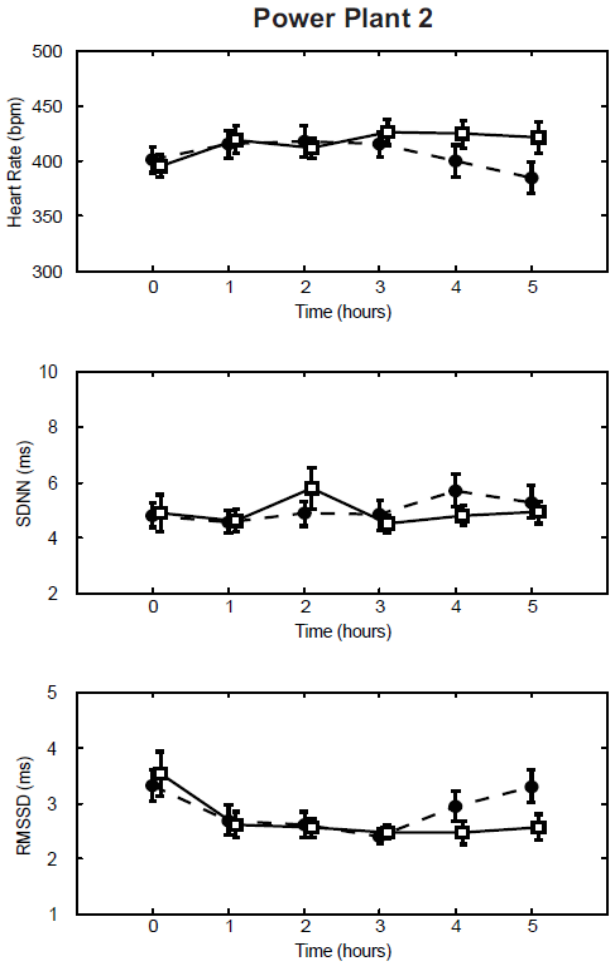


Figure 8-3
Effect of POS exposure on heart rate and measures of heart rate variability at Power Plant 2. The mean heart rate (top panels), SDNN (middle panels), and RMSSD (bottom panels) at different time points after the start of exposure is shown for animals exposed to either filtered air (solid lines) or stack emissions under the POS scenario (dashed lines). Error bars indicate standard errors. *: p<0.05 comparing the response under the POS scenario versus filtered air controls at the same time point.

Table 8-3
Estimated average effect of exposure to POS scenario on electrocardiographic outcomes in rats with acute myocardial infraction.

Outcome	Units	Power Plant 2 (N=29)		
		Estimate	SE	P-Value[†]
Heart Rate Variability				
Heart Rate	bpm	-10.92	12.27	0.38
SDNN	msec	0.09	0.46	0.85
RMSSD	msec	0.17	0.24	0.47
ECG Intervals				
PR Interval	msec	-1.26	1.52	0.41
P-Wave Duration	msec	0.33	0.78	0.67

[†]Values in bold are statistically significant at the α=0.05 level. bpm: beats per minute.

We assessed respiratory outcomes in each rat throughout the exposure. POS exposure was associated with a statistically significant decrease in expiratory time and end inspiratory pause (Table 8-4).

Table 8-4
Estimated average effect to POS scenario on respiratory outcomes in rats with acute myocardial infraction.

Outcome	Units	Power Plant 2 (N=30)			
		Mean Value Among Sham Animals	Estimate	SE	P-Value[†]
Frequency	Breaths/min	176.4	17.8	11.7	0.13
Tidal Volume	ml	2.08	-0.10	0.11	0.37
Inspiratory Time	sec	0.179	-0.021	0.012	0.085
Expiratory Time	sec	0.242	-0.025	0.013	**0.049**
Enhanced Pause	dimensionless	1.34	0.01	0.15	0.97
End Expiratory Pause	msec	51.5	-7.5	6.3	0.23
End Inspiratory Pause	msec	10.2	-1.0	0.5	**0.037**
Minute Volume	ml	333.5	27.8	23.3	0.23
Expiratory Flow at 50%	ml/min	1.28	0.10	0.12	0.40
Pause	dimensionless	1.15	0.08	0.08	0.31
Peak Expiratory Flow	ml/min	20.3	0.5	1.3	0.71
Peak Inspiratory Flow	ml/min	21.6	1.3	1.5	0.38

[†]Values in bold are statistically significant at the α=0.05 level.

The results from Power Plant 2 may suggest that in this susceptible animal model, emissions from coal-fired power plants that are photochemically aged in the presence of pinene, a naturally occurring pollutant, can increase ventricular ectopy. If causal, this effect may be mediated by changes in autonomic function, as suggested by a number of toxicologic and epidemiologic studies of ambient particles (Godleski *et al.*, 2000; Gold *et al.*, 2000; Devlin *et al.*, 2003). However, in this study we did not observe any substantial or statistically significant changes in heart rate or heart rate variability. This could be either because the POS scenario had no effect on autonomic nervous system function or because such changes were difficult to observe in this animal model which already has reduced heart rate variability. Alternatively, the increased arrhythmia frequency could reflect increased oxidative stress. In normal animals, the POS exposure at Plant 2 led to increased cardiac oxidative stress as measured by *in vivo* cardiac chemiluminescence, although this difference did not reach statistical significance (Lemos *et al.*, 2010).

We have previously used this animal model to evaluate the effects of concentrated ambient particles (CAPs) and found that CAPs exposure was associated with a non-significant 64.2%,

(95% CI: –17.7, 227.6%; $p = 0.16$) increase in arrhythmia frequency during a 1 hr exposure (Wellenius *et al.*, 2004). We have also shown that a 1 hr inhalation exposure to residual oil fly ash significantly increased arrhythmia frequency among animals with preexisting arrhythmias (Wellenius *et al.*, 2002). In the current study, at Plant 2 the frequency of ventricular premature beats peaked during the 4_{th} exposure hour where it was almost three fold higher among animals exposed to POS scenario as compared to filtered air controls. Because of differences in the duration of exposure and experimental design, direct comparison of these results to those of previous studies is not possible.

At Plant 2, the respiratory effects of POS exposure were quite different in normal animals as compared to animals with MI. Specifically, in normal animals POS exposure led to statistically significant decreases in tidal volume, pause, and Penh (Diaz *et al.*, 2011). In contrast, in the current study in animals with MI, POS exposure was associated with decreased expiratory time and end-inspiratory pause. Whether these divergent results are due to differences in the animal models or observed differences in the POS exposure atmosphere is not estimable from the existing data. Alternatively, given the small magnitude of the effects and the large number of respiratory outcomes examined, the changes observed in respiratory parameters in the MI animals may reflect chance findings.

This study has several potential limitations that warrant discussion. First, for unknown reasons we observed unexpectedly high levels of iron, chromium, and nickel at Plant 2 on three of the four exposure days. The source of these trace elements is unclear, but assessment of these particles by single particle analyses using scanning electron microscopy and energy dispersive X-ray analyses suggested they were derived from the emissions of the plant rather than contamination by corrosion of the sampling line from stack (Kang *et al.*, 2011). How (or if) the elevated concentrations may have affected the measured outcomes is unknown, although there is recent evidence to suggest that some of these elements may play a role in cardiovascular effects (Chen and Lippmann, 2009). However, the results were not materially different when we excluded from analyses the 2 days with the highest levels of these metals. A second limitation is that the duration of exposure was limited to 5 h. Thus, it is not known whether a longer exposure would produce similar results. Third, to reduce biologic variability, only mature, male, Sprague-Dawley rats were studied. Thus, it is unknown how the effect of exposure to a POS scenario might vary by gender or age. Fourth, even at Power Plant 2 there were some differences in infarct size and location across the exposure groups. It is unclear how these differences might have affected the results. Fifth, there are important differences between the rat and human heart, including differences in the degree of collateral blood flow, ventricular mass, and electrical properties (Janse *et al.*, 1998) which make extrapolation of these findings to human populations difficult. Finally, our conclusions are based on results from 4 exposure days at a single power plant. Thus, we were unable to evaluate how variations in power plant and coal characteristics may affect these results.

9
DISCUSSION

The primary objective of this study was to evaluate in a more realistic way the health effects of power plant-derived particles, both in the form of primary particles and as secondary particles having undergone chemical transformations in the atmosphere with the addition of other atmospheric constituents. As outlined above, we studied a number of scenarios which were step-wise increases in complexity of the exposures.

9.1 Scenario-Specific Results

The specific scenarios discussed in the succeeding sections are summarized in Table 3-2.

P Scenario: Plants 1 and 2 had the lowest primary particle concentrations in the P scenario (1.0 and 1.7 $\mu g/m^3$, respectively), while Plant 3 had a substantially higher concentration (43.2 $\mu g/m^3$). This difference was due to the operation of the FGD scrubber at Plant 3, which is well-known to both remove and contribute particles to plant emissions (Kang et al., 2011). The contributed particles at Plant 3 tended to be primarily comprised of acidic and neutralized sulfate. Plant 1 showed no robustly significant changes in outcomes with the P scenario, which would be expected with the very low concentration. Plant 3, which had the highest P scenario concentration, showed one robust finding in EIP (decrease); however a reduction in EIP without other respiratory or breathing pattern changes is not indicative of any particular pathophysiological response.

Finding robustly significant respiratory outcomes (decreases in MV, PEF, and EF50, and an increase in Penh) with the P scenario at Plant 2, with its very low particle concentration, was surprising and not easily explained. When all plant data were combined, the strength of the association for EF50 was strongly significant ($p<0.005$) since this parameter in the P scenario at Plants 1 and 3 was also decreased although not individually significant. These findings with the P scenario are difficult to explain based on exposure because this scenario at Plant 2 did not have any measured gaseous or particulate component substantially higher than or outside the range of Plants 1 or 3 (Kang et al., 2011). Although there are inconsistencies in the measurements of continuous, summed, and gravimetric mass (13.9±9.5 $\mu g/m^3$ vs1.7±1.8 $\mu g/m^3$ vs 1.9±1.3 $\mu g/m^3$), all of these measurements are very low for toxicological studies of ambient particle health effects. Particle number was also lower at Plant 2 than at Plants 1 or 3 [910±964 vs 1726±1277 vs 55947±11769 particles/cm^3 respectively] (Kang et al., 2011).

Similarly, there was no consistency between these respiratory findings in the P scenario and the *in vivo* chemiluminescence studies (Lemos et al., 2011), or the studies of inflammation (Godleski et al., 2011a). In both of these studies, the P scenario showed no effects at Plant 2. This point is particularly important because the respiratory measurements are not invasive, and the same

animals are then assessed for the other parameters. For this reason, we expect to see consistency with exposure and response as well as among outcome parameters within the same exposure in order to conclude that an outcome was truly biologically significant. At Plant 3, where EF50 in the P scenario showed the greatest change in magnitude (although not statistically significant), bronchoalveolar lavage studies showed increases in neutrophils, which were associated with the zinc concentration in univariate analyses. However, in the respiratory studies, zinc had no influence on flow parameters in either univariate or random forest analyses.

Penh was also significantly decreased at Plant 2 in the P scenario and in the combined data. Since increased Penh is considered an adverse effect, the biological significance of decreases in this parameter is unclear. Studies comparing two different approaches to whole body plethysmography (flow and pressure) in animals (Lomask, 2006, Lundblad et al., 2002, Mitzner and Tankersley, 2003) have shown that Penh derived from single chamber whole body plethysmography cannot be used as an accurate index of bronchoconstriction. Indeed, Lomask (2006) suggests that Penh is only meaningful when applied to flow-based whole body plethysmography, which measures the difference between the thoracic and nasal flows. Since our system used pressure-based whole body plethysmography without measurement of nasal flows, it may be argued that the finding of changes in Penh in our study has little value. Therefore, although we have reported Penh findings, these results are never emphasized.

PO Scenario: The PO scenario contained the secondary reaction products of power plant emissions alone, i.e., unneutralized sulfuric acid. Although this scenario may be the purest test of the toxicity of secondary particles from power plant emissions, the scenario, with its high concentrations of strong acidity, is never encountered in ambient air due to neutralization by atmospheric ammonia (Kang et al., 2011, Spengler et al., 1990). The changes in breathing pattern evoked by the PO scenario consisted of an increase in respiratory frequency with a corresponding decrease in Ti and Te. These changes were strongest at Plant 2 and to a lesser extent at Plant 3; Plant 1 showed minor changes with this scenario. PEF was nonsignificantly decreased at Plants 1 and 3 and only marginally significantly decreased at Plant 2 along with a marginally significant decrease in TV. When all plants were combined, increased f and decreased Ti and Te were all marginally significant. The PO scenario was characterized by the formation of acid sulfate at all three plants (Plant 1-36.1±7.7 $\mu g/m^3$; Plant 2- 71.6±17.0 $\mu g/m^3$; Plant 3- 68.9±16.8 $\mu g/m^3$) (Kang et al., 2011), and the random forest ranking listed acid sulfate as the component having the strongest association with changes in F, Ti and Te, but these were not significant in univariate analyses.

Data from our control scenarios at Plant 3 are illustrative in evaluating the impact of specific components on respiratory changes. Very few strongly significant findings were observed with the control scenarios, and all were observed with the O scenario: reduced Ti and Te (with a marginally significant increase in f) and reduced EIP. These findings would suggest that the strong acidity in the O scenario increases frequency, a finding that has been widely reported (Amdur, 1989, Amdur et al., 1952a, Amdur et al., 1952b). However, although f increased, tidal volume did not change, and MV, in fact, showed a nonsignificant increase, such that we did not observe a rapid, shallow breathing pattern but simply an increase in frequency. However, this pattern is not entirely reflected in the power plant emissions exposures, with the PO scenarios causing increased f at Plants 2 and 3, with corresponding decreases in Ti and Te, but only Plant 2

had decreases in TV, PEF and EF50 (all $p<0.05$). At the same time, neither the PO scenario nor acid sulfate had any effect on lung inflammation (Godleski et al 2011a) or *in vivo* chemiluminescence of the lung or heart (Lemos et al, 2011). Therefore, lack of consistency across outcomes lessens the impact of this breathing pattern finding.

POS Scenario: The POS scenario, which actually had higher acidity (in the form of acid sulfate) than the PO scenario (Kang et al 2011), did not induce changes in f, Ti or Te, a somewhat puzzling result. Indeed, at individual plants, the POS scenario induced minimal effects. We also observed a marginally significant increase in f with the S control scenario, but a nonsignificant result with the OS scenario.

In comparing POS results from the *in vivo* chemiluminescence studies on the lungs of the same animals (Lemos et al 2011), it can be seen that an increase in lung chemiluminescence was only observed at Plant 2 (where the only strongly significant breathing pattern finding for POS was a decrease in TV). In comparing the POS scenario respiratory results with bronchoalveolar lavage results, also on the lungs of the same animals (Godleski et al., 2011a), increases in total cells and macrophages were observed with the POS scenario at Plants 1 and 3, and these reached marginal significance ($p<0.05$) when all plants were combined. No significant changes in PMNs, generally a more accurate marker of inflammatory processes, were observed. Taken together, despite the fact that the POS scenario induced minimal changes in respiratory parameters, this scenario did result in some other pulmonary changes in the same animals.

The importance of sulfuric acid as a respiratory irritant has been extensively studied in the past (Amdur, 1989, Amdur et al., 1952a, Amdur et al., 1952b). Some studies have reported enhanced effects with sulfuric acid-coated particles (Chen et al., 1992, Chen et al., 1991). EF50 has been suggested as a robust confirmatory marker of bronchoconstriction (Glaab et al., 2006, Glaab et al., 2002). As discussed above, we observed some changes in f, Ti, and Te in the PO scenario, but not in the POS scenario, despite higher acidity (at least at Plants 1 and 2). We also observed no robustly significantly changes in PIF, PEF, or EF50 at any of the plants in response to scenarios containing high sulfuric acid. Coupled with our failure to find strongly significant and consistent associations with acid sulfate itself, our findings suggest that acidity does not play a major role in our respiratory results.

Although most of the studies used normal animals, it is also important to assess the health effects of air pollutants on compromised subjects. Studying compromised subjects in a field setting is a daunting task. We had chosen *a priori* to carry out experiments in compromised animals using a model of acute myocardial infarction (MI) using the exposure scenario that demonstrated the most substantial change in cardiac *in vivo* chemiluminescence in normal animals. This decision was based on studies showing important changes in this measure following exposure to concentrated ambient particles (Gurgueira *et al.*, 2002) that may be abrogated with the anti-oxidant N-acetyl cysteine (Rhoden *et al.*, 2004). Preliminary analyses of data from Plant 2 suggested similar increases in cardiac chemiluminescence in normal animals exposed under the POS and PONS scenarios and we chose to use the POS scenario for the MI experiments. In the final analysis, the change in heart chemiluminescence at Plant 2 was more pronounced for the PONS scenario than for the POS scenario. On the other hand, across all 3 power plants, exposure to the POS scenario increased heart chemiluminescence more than did exposure to the PONS

scenario. Therefore, in retrospect, either the POS or PONS exposure scenarios would have been an acceptable choice for the MI studies.

Our choice to use a complex exposure scenario rather than a scenario involving only primary particles was based on the fact that coal-fired power plant emissions not only oxidize downwind of the plant, but also interact with biogenic and anthropogenic volatile organic compounds (VOCs) in the atmosphere. Although the final products in the atmosphere are not strictly from the power plant, it can be argued that if power plant emissions were not present in that form, different reactions might take place in the environment. Because the reactions that we studied do take place, we believe their use in a study of health effects outcomes is a strength of this study.

The results from Plant 2 may suggest that in a susceptible animal model coal-fired power plant emissions photochemically aged in the presence of pinene, a biogenic VOC, can increase ventricular ectopy. If causal, this effect may be mediated by changes in autonomic function, as suggested by a number of toxicologic and epidemiologic studies of ambient particles (Godleski *et al.*, 2000; Gold *et al.*, 2000; Devlin *et al.*, 2003). However, in this study we did not observe any substantial or statistically significant changes in heart rate or heart rate variability. Alternatively, the increased arrhythmia frequency could reflect increased oxidative stress. In normal animals, the POS exposure at Plant 2 led to increased cardiac oxidative stress as measured by *in vivo* cardiac chemiluminescence, although this difference did not reach statistical significance (Lemos *et al.*, 2011).

We have previously used the MI animal model to evaluate the effects of concentrated ambient particles (CAPs) and found that CAPs exposure was associated with a non-significant 64.2%, (95% CI: –17.7, 227.6%; $p = 0.16$) increase in arrhythmia frequency during a 1 hr exposure (Wellenius *et al.*, 2004). We have also shown that a 1 hr inhalation exposure to residual oil fly ash significantly increased arrhythmia frequency among animals with preexisting arrhythmias (Wellenius *et al.*, 2002). In the current study, at Plant 2 the frequency of ventricular premature beats peaked during the 4^{th} exposure hour where it was almost three fold higher among animals exposed to POS scenario as compared to filtered air controls. Because of differences in the duration of exposure and experimental design, direct comparison of these results to those of previous studies is not possible.

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PONS Scenario: In the PONS scenario, the addition of NH_3 to partially neutralize strong acidity increased mass, dramatically increased neutralized sulfate, and reduced – though did not eliminate - strong acidity. In the breathing pattern study, PONS exposure resulted in a decrease in respiratory airflow parameters (PEF and EF50). These decreases were robust at Plants 1 and

2, and in the combined data, but were not significant at Plant 3. In univariate analyses, the decrease in these outcomes was associated with NH_4, neutralized sulfate, and EC. However, the random forest analysis for both PEF and EF50 failed to rank neutral sulfate in the top 5 variables. However, there was some consistency with other TERESA studies. We observed significant lung *in vivo* chemiluminescence for the PONS scenario at Plant 2, although not at Plant 1, where significant changes in flow parameters were observed (Lemos et al., 2011). We also observed some degree of consistency with the lung inflammatory studies in these animals (Godleski et al., 2011a) in that the PONS scenario produced significant increases in total cells and macrophages at Plants 1 and 3, although not at Plant 2. It should be noted that we did observe consistent results with NH_4 and PEF reductions in both univariate and random forest analyses. Since NH_4 and neutralized sulfate are highly correlated, these results suggest that the PONS scenario may be more responsible for these findings than the individual components, a conclusion confirmed by R^2 analyses in the respiratory studies.

The statistically significant changes in total cell count and macrophages by BAL are considered mild toxicological responses, and these were observed only in scenarios with added SOA and the most complex atmospheric reactions. The SOA used in TERESA was derived from α-pinene, a biogenic volatile organic hydrocarbon. It should be noted that the oxidation products of α-pinene have been studied in a number of toxicological experiments, primarily focused on respiratory responses. Rohr et al. (2002a, b) reported a number of airway responses due to exposure to α-pinene oxidation products; these included both gas-phase and aerosol-phase products. Pinene-derived SOA specifically has not been examined for any pulmonary inflammatory effects, although the oxidation products of isoprene, another biogenic terpene, have been used in BAL and nasal lavage studies in mice (Rohr, 2001 [dissertation]). Isoprene oxidation products, including both gas- and aerosol-phase, showed no evidence of inflammatory activity.

Consistent with the increase in total cells and macrophages with the PONS scenario findings of the inflammation studies (Godleski et al 2011a) is the observation that lung chemiluminescence was significantly increased in the PONS scenario when data from all plants were combined (Lemos et al., 2011). However, the BAL findings were not consistent with those of Lemos et al. at the individual plant/scenario level. Marginally significant increased lung chemiluminescence with the POS scenario was observed only at Plant 2 and not at Plants 1 and 3 whereas total cells and macrophages increased significantly at Plants 1 and 3, but no significant effects were observed at Plant 2. Although lung chemiluminescence increased at all three power plants with the PONS scenario, none of these reached even marginal significance, whereas total cells and macrophages at Plants 1 and 3 were significantly increased but Plant 2 was not. Univariate analyses of lung chemiluminescence and exposure components showed no consistent similarities with any BAL parameters and exposure components.

It is important to compare the results of the studies reported here to studies using concentrated ambient particles (CAPs) in which the same inflammatory endpoints were assessed in order to gain insight into relative response. In CAPs studies in our laboratory in Boston, Clarke et al (1999) found increases in total lavageable cells with inhalation exposure of normal Sprague Dawley rats exposed to CAPs as well as neutrophil and lymphocyte counts significantly elevated by CAPs exposure. Saldiva et al. (2002) also found increases in total cells and neutrophils by BAL with CAPs exposure in normal Sprague Dawley rats exposed for 5 hr/day for 3 days, and

identified neutrophil increases by morphometry in the bronchiole-alveolar junction region of the lungs. Clarke et al. (2000) in dog studies in our laboratory also found Boston CAPs to be associated with altered white and red blood cell counts and hemoglobin concentration. These changes with CAPs were all larger in magnitude than the findings in this study. The exposure mass concentration levels in the CAPs studies (Saldiva et al 2002) were higher, but not appreciably different, to those in this study (Average in CAPs rat studies = 255.5 $\mu g/m^3$; Average PONS concentration, all plants = 195.2 $\mu g/m^3$), suggesting that the CAPs composition, as least in urban Boston, has higher inflammatory potency that the exposures in the present study.

Control Scenarios (O, OS and S): These scenarios lacked primary particles and were used to assess contributions of primary particles to the reaction mixtures and the formation of secondary particles. The findings of the O and OS scenarios were discussed in part in relationship to the PO and POS scenarios above. To further understand the differences in f, Ti, and Te responses between the O/OS and PO/POS scenarios, we can examine the associations between the SOA and its precursors. In univariate analyses and as illustrated in the scatter plots, pinene was associated with a non-significant decrease in frequency and inconsistent changes in Ti and Te, as well as ranking relatively high in random forest analyses. However, pinene reacted in the aging chamber and therefore exposure concentrations were very low (maximum of 8 ppb [Kang et al., 2011]). Useful measures of the reaction products include organic carbon (OC), formaldehyde, acetaldehyde, acetone, and total aldehydes, but none of these parameters was significantly associated with changes in f, Ti, or Te. Previous studies (Rohr et al., 2002) have reported reductions in frequency indicative of sensory irritation in response to pinene oxidation products; however, no such changes were observed with pinene alone. Thus, although it is unclear why the POS scenario appears to produce a lesser response than the PO scenario, it is possible that some of the organic reaction products are having an irritant effect to reduce breathing rate, rather than increasing it as the simpler PO scenario did. It should be noted that we only investigated α-pinene as an SOA precursor, and of course responses could differ significantly with (an)other VOC(s). These control scenarios caused no significant changes in BAL parameters (Godleski et al 2011a) or in vivo chemiluminescence (Lemos et al 2011).

9.2 Responses by Power Plant

Although differences in plant pollution control devices, and differences in the coal used by each plant were part of the design of these studies, analyses of these differences may only be inferred from the differences in outcomes in individual plants controlled for scenario. However, differences in measured component concentrations from plant-to-plant and between scenarios complicated the interpretation of such analyses. Nevertheless, certain trends, which were outlined in the respiratory response section, are worthy of discussion. All scenarios at Plant 3 showed nonsignificant increases in inspiratory flow whereas at Plants 1 and 2 all scenarios showed decreases in inspiratory flow. Thus, comparing these outcomes while controlling for scenario resulted in a significant difference among plants ($p=0.019$). Similarly, Plants 1 and 2 showed decreases in tidal volume with all scenarios, but Plant 3 showed nonsignificant increases in TV in almost all scenarios, and this led to a highly significant difference when controlling for scenario ($p<0.0001$). Differences in outcome trends can also be appreciated for measures of expiratory and inspiratory pause. There were no differences in animals, personnel, mobile laboratory use, or outcome analyses to account for these differences at the three power plants.

The stack extraction system at Plant 3 was different than at Plants 1 and 2 because of the differences in emission controls at Plant 3. Although the outcome trends seen at Plant 3 are in general not adverse outcomes, more research is needed before it can be determined as to whether the pollution control system influenced these outcomes. Although data on inflammatory responses and in vivo chemiluminescence were not analyzed in this way, inspection of the data suggested that the differences among power plants were not as great as with the respiratory data.

9.3 Component-Specific Results

Role of sulfates: Because differences in aerosol component composition existed by virtue of the various scenarios, with additional differences contributed by power plant configuration, it was possible to assess dose response of individual components. As expected, sulfur compound concentrations dominated most scenarios (Kang et al., 2011). In a recent review of sulfate health effects, Schlesinger (2007) emphasized the lack of toxicological effects with partially to totally neutralized sulfate in laboratory toxicological studies. Our data appear to support this conclusion since we did not observe consistency in findings with varied statistical methods; nor did the findings of varying pulmonary endpoints consistently compare. In the associations observed in this study between neutral sulfate and flow parameters (PEF and EF50), neutral sulfate concentrations ranged from 5.6 – 139 $\mu g/m^3$ over all scenarios at all plants. Previous studies evaluating sulfate effects on airflow or bronchoconstrictive effects in humans have reported no effects at 133 and 116 $\mu g/m^3$ (Stacy et al., 1983) or 100 $\mu g/m^3$ (Utell et al., 1983). However, some epidemiological panel studies as well as toxicological studies utilizing concentrated ambient particles (CAPs) have reported associations between sulfate and adverse respiratory effects (Grahame and Schlesinger, 2005; Neas et al., 1996; Raizenne et al., 1996). These studies have found it difficult to determine the extent to which the sulfate associations are due to sulfate itself, to other components that are highly correlated, or to interactions within the complex mixture. In our study, since plant/scenario explained more of the variance than individual components including sulfates, we suggest that the changes observed here are more likely related to the complex mixture than to neutral sulfate. Hypotheses forwarded to explain differing results observed in single-component laboratory sulfate studies versus studies utilizing complex mixtures include the possibility of the formation of organosulfate materials in the atmosphere, or complexation with sulfate leading to an increase in the bioavailability of metals. However, recent work investigating the cardiopulmonary effects of organosulfate compounds has reported little evidence for increased toxicity of these materials (McDonald et al., 2010).

We observed some associations of BAL endpoints with sulfate. In particular, the POS and PONS scenarios resulted in an increase in total lavaged cells in BAL, and this increase was primarily related to increases in macrophages. Using both univariate and multivariate (random forest) analyses, the BAL increases were most strongly associated with total sulfate, neutral sulfate, and ammonium ion. These were all highly correlated measurements in these experiments; in addition, all were highly correlated with total particle mass, which was also a significant predictor of total cell count in random forest analyses. These findings are consistent with some of the literature, including the work of Saldiva et al. (2002), who found increased total BAL cells and neutrophils to be associated with sulfate, among other CAPs components, as well as Clarke et al. (2000), who found a sulfur factor (determined using factor analytical methods) in CAPs to be associated with altered white and red blood cell counts and hemoglobin concentration. However, other

studies have not shown any association with sulfate. Indeed, while a number of CAPs studies in our laboratory and others have shown biological responses (Kodavanti et al 2000; Gurgiera et al 2002; Wellenius et al 2003; Urch et al 2004; Maciejczyk & Chen, 2005), these studies did not find associations between these effects and either measured sulfate or a sulfate factor. Kodavanti et al. (2000) found that the leachable sulfate in CAPs from Research Triangle Park, NC, was not related to inflammatory responses in rats. Increased activation of nuclear factor (NF)-κB in human bronchial epithelial cells did not correlate with sulfate from CAPs from the New York area (where sulfate constituted 65% of the PM mass) (Maciejczyk & Chen, 2005). Another study found that while metals were correlated with the release of cytokines, sulfate was not (Huang et al., 2003). In assessment of cardiovascular outcomes, changes in brachial arterial diameter in human adults were not associated with sulfate in CAPs from Toronto (Urch et al., 2004). Neither increased oxidative stress in rats nor changes in EKG in dogs were associated with a sulfur factor in CAPs from Boston (Gurgueira et al., 2002; Wellenius et al., 2003). Thus, there are also a number of CAPs studies that do not show any effect of sulfate. It is important to note that CAPs have a very different composition than the aerosols in the TERESA study, and we would not necessarily expect to see the same response. The differences between CAPs in various regions of the United States and TERESA aerosols are discussed in detail by Kang et al. (2011). Also, the numbers of animals used in the CAPs studies mentioned above and the numbers of animals used in the TERESA studies were similar. It is unlikely that small numbers of animals can explain the lack of robust inflammatory responses in response to coal-fired power plant emissions.

With respect to sulfate and pulmonary inflammatory responses, the scenario, univariate, and multivariate results lacked robust consistency. Scenario-specific results indicated that POS and PONS generally showed the largest impact, at least at two of the three plants. Univariate analyses showed that neutral sulfate showed one of the stronger associations, while acidic sulfate was uniformly nonsignificant. The POS scenario, which did not contain ammonia, was high in acid sulfate and low in neutral sulfate. Furthermore, the highest neutral sulfate was at Plant 2 (139 $\mu g/m^3$), where no increases in BAL parameters were observed. One hypothesis regarding the acute health effects of sulfate is that the strong acidity sometimes present in this material in the form of sulfuric acid (e.g., Schlesinger, 2007) causes adverse health effects. However, studies with acid sulfate or sulfuric acid have required very high concentrations to elicit biological effects. For example, in human exposure studies with sulfuric acid, concentrations in the range of 1000 $\mu g/m^3$ were needed to find adverse health effects (Frampton et al., 1992; Linn et al,. 1981, 1994). In vivo acid neutralization has been used to explain the need for extremely high levels of acid to induce adverse effects (Larson et al., 1980; Sarangapani and Wexler, 1996). Concentrations of acid sulfate in our study were highest in the POS scenario at Plant 2 (108 $\mu g/m^3$), averaging 37 $\mu g/m^3$ across all scenarios. In addition, we conducted a control exposure to acid sulfate only ("O" scenario at Plant 3), which is directly analogous to the afore-mentioned studies; therefore, our findings are consistent with the lack of effect observed by others. Based on the findings of this study, acid sulfate does not appear to play a significant role in pulmonary inflammatory processes.

Laboratory studies of neutralized sulfates have also been largely negative, or required extremely high concentrations for effects to be observed. In a series of chronic aerosol exposures with acid and neutral sulfur (IV) compound in dogs, no changes in inflammatory parameters were found (Maier et al 1999), but changes in particle clearance parameters were found (Kreyling et al

1999). In other toxicological studies of neutralized sulfates, Cassee et al. (2002) exposed normal rats and others to ammonium bisulfate and ammonium ferrosulfate in different particle size ranges for 4 h/day for 3 consecutive days. Animals were sacrificed 1 day after the last exposure. Exposure concentrations were: ultrafine particles = 70 $\mu g/m^3$; fine particles = 275 $\mu g/m^3$, 344 $\mu g/m^3$, or 410 $\mu g/m^3$. No significant or consistent exposure-related effects were found when assessing some of the same BAL parameters as the study reported here as well as histopathology, and the phagocytic activity to *Escherichia coli* of alveolar macrophages. In another study Cassee et al. (1998) exposed both normal and ovalbumin sensitized mice for 4 h/day for 3 days to ammonium ferrosulfate (0.459μm) at 250$\mu g/m^3$. Animals were sacrificed 1 day after the last exposure, and no significant exposure-related effects on any of the endpoints examined in either group of mice were found, and there was no evidence for any enhanced allergic response due to sulfate. These studies are consistent with the TERESA findings described. Although we did not study an "ON" scenario, which would directly correspond to the single-component studies described here, we studied scenarios with sulfur in an oxidized form (sulfate) and not containing secondary organic aerosol (i.e., PO). We also did not observe any biological effects with these experimental conditions.

Role of organic components: Elemental carbon (EC) figured prominently in many of the analyses. In particular, EC was consistently and strongly associated with reductions in the respiratory parameters MV, PEF, and EF50 in both univariate and random forest analyses. As described in some detail by Kang et al. (2011), EC concentrations in this study were abnormally high in many cases. These elevations were considered artifactual and were attributed at least in part to pyrolyzed OC erroneously reported as EC, since power plants emit very low concentrations of EC. Furthermore, the associations with EC that we observed are likely not due to EC itself as this material is generally inert. It is more likely that any effects observed were due to adsorbed organic materials that were able to more effectively reach pulmonary regions.

We also observed interesting findings with respect to the measured pinene oxidation products: formaldehyde, acetaldehyde, acetone, and total aldehydes. In particular, these four parameters were strongly or marginally associated with increases in Penh, which may be considered physiologically meaningful (see caveat above, however). This is consistent with findings of reductions in airflow in response to pinene oxidation products (Rohr et al., 2002), as well as other reports of specific aldehyde-related bronchoconstrictive effects (Matsuse et al., 2007; Thompson and Grafstrom, 2008). Additional research on the possible role played by these compounds is warranted.

In our study, the addition of secondary organic aerosol appears to be linked with the inflammatory responses observed. Examination of the responses to the control scenarios conducted at Plant 3 provides valuable information to aid interpretation of the data. As mentioned, the effects on BAL cellularity in this study were observed primarily with the POS and PONS scenarios. However, the control scenarios, S and OS, both conducted at Plant 3, showed no significant effects. Organic carbon, the primary indicator of the SOA scenarios, was higher in the control scenarios than in the POS and PONS scenarios at Plant 3. Therefore, we can conclude that the effect does not appear to driven by organic material, an observation supported by the lack of significant results for OC in univariate analyses. This might lead to the hypothesis that the primary particles at Plant 3 are driving the effects on total cells and macrophages;

however, there were no significant increases in either BAL parameter for the P scenario at this plant, suggesting that these particles are not playing a role. Overall, it is unclear why the addition of SOA appears to be important in the BAL responses.

Role of metals: Metals did not appear to play a large role in the TERESA respiratory responses, although there were some consistent results. Aluminum, a component of coal fly ash, was negatively associated with f in both univariate and random forest analyses. This element has been associated with pulmonary inflammatory effects in concentrated ambient particle (CAP) studies (Clarke et al., 2000). Silicon, lead, and magnesium were all strongly associated with a reduction in TV in univariate analyses, but these findings were not confirmed by random forest. Nickel was consistently associated with an increase in TV; however, this change is generally not considered to be of pathophysiological significance. Similarly, sodium was related to increased MV, again not considered an adverse response.

Increases in neutrophils in BAL without increases in BAL protein, enzymes, or circulating neutrophils are also viewed as a mild response. The association with the zinc concentration appears to be driven by increased zinc concentrations in the P and PONS scenarios at Plant 3. At Plants 1 and 2 for all scenarios, the zinc concentration was very low and ranged from 0.0-0.4 ng/m^3; at Plant 3, the mean zinc concentrations were 1.5±0.6 for the P scenario, 0.5±0.2 for PO, 1.4±0.9 for POS, and 4.3±2.7 for PONS, which are relatively low, yet all higher than at the other plants (Kang et al 2010, this series). The role of zinc as a metal causing health effects has been controversial because all studies showing significant positive inflammatory effects have been done at very high concentration levels (Chen and Lippmann, 2009). Given the distribution of results driving this outcome and the zinc exposure concentrations in this study (noted above), the role of zinc alone in the etiology of PMN inflammation in this study is not convincing. Plant 3 had the wet FGD scrubber and the highest mass concentrations in the P scenario among all plants. The contribution of this scrubber to particle generation is discussed in detail in Kang et al. (2011). However, the P and PONS scenarios at Plant 3 also had substantially higher concentrations of chromium, iron, nickel, as well as zinc compared to these scenarios at Plants 1 and 2 despite these plants having occasional days with high concentrations of these elements. If these concentrations are added together, Plant 3 had 3-4 times higher concentrations of these elements than the other plants. This difference is likely due to the particular coal burned during the time of the experiments, since the FGD scrubber does not emit metals. The possibility of corrosion associated with the sampling lines was discussed and dismissed as unlikely by Kang et al. (2011). Although the measured concentrations of these metals (other than zinc) did not reach significance in univariate analyses, it is possible that zinc may be a tracer for the combined metal effect.

Role of Gases: In univariate analyses, we observed several strong associations with gases, including NO with increased frequency and decreased Te, and NO_2 with increased Pause (a parameter not generally reported and of limited physiological meaning) and Penh. Marginally significant associations were observed between ozone and Pause; NO and decreased Ti and increased PIF; and NO_2 and decreased MV and PIF. By design, gaseous copollutant concentrations in the exposure scenarios were low through the use of denuders (Kang et al., 2011). For example, maxima for ozone, NO_2, and SO_2 over all scenarios at all plants were 29 ppb, 18 ppb, and 73 ppb, respectively. Therefore, any associations observed with gases are likely

not reflective of a true biological association, but rather, because gases were common to all scenarios, they may be serving as tracers for scenarios. Gases did not appear to play any role in the inflammatory or chemiluminescence responses.

9.4 Study Strengths and Limitations

A primary strength of this study was the collection of a rich exposure dataset, which allowed us to examine relationships between individual components. However, comparison of the strength of associations between scenario and the components defining the scenario often showed no differences. This indicates that individual components defining a scenario could not be identified as causative for an effect related to a scenario. This may be due either (1) the complexity of the scenarios, and our related inability to fully capture the dynamics of the system; and/or (2) the high correlations between many of the components, particularly within a given scenario.

Due to differences in the number of animals used per scenario and per plant, the statistical power to detect an effect in ANOVA analyses differed. For example, the number of days that each scenario was run ranged from 3-12 at Plant 1, 4-8 at Plant 2, and 4-8 at Plant 3. This imbalance did not affect the univariate or random forest analyses, but the scenario-specific analyses, especially for respiratory studies in the PONS scenario at Plant 1 (with 12 days of exposure, or triple the statistical power of the PO scenario at that plant), should be interpreted accordingly.

The MI study had several potential limitations that warrant discussion. First, for unknown reasons we observed unexpectedly high levels of iron, chromium, and nickel at Plant 2 on three of the four exposure days. The source of these trace elements is unclear, but assessment of these particles by single particle analyses using scanning electron microscopy and energy dispersive X-ray analyses suggested they were derived from the emissions of the plant rather than contamination by corrosion of the sampling line from stack (Kang *et al.*, 2011). How (or if) the elevated concentrations may have affected the measured outcomes is unknown, although there is recent evidence to suggest that some of these elements may play a role in cardiovascular effects (Chen and Lippmann, 2009). However, the results were not materially different when we excluded from analyses the 2 days with the highest levels of these metals. Second, the duration of exposure was limited to 5 h. Thus, it is not known whether a longer exposure would produce similar results. Third, to reduce biologic variability, only mature, male, Sprague-Dawley rats were studied. Thus, it is unknown how the effect of exposure to a POS scenario might vary by gender or age. Fourth, there are important differences between the rat and human heart, including differences in the degree of collateral blood flow, ventricular mass, and electrical properties (Janse *et al.*, 1998) which make extrapolation of these findings to human populations difficult. Finally, our conclusions are based on results from 4 exposure days at a single power plant. Thus, we were unable to evaluate how variations in power plant and coal characteristics may affect these results.

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CONCLUSIONS

The approach employed in TERESA was ambitious and innovative, and as a result numerous technical challenges were encountered and overcome. These included the development of stack sampling technology that prevented condensation of water vapor from the hot, humid power plant exhaust during sampling and transfer, while minimizing losses of primary particles; development and optimization of a photochemical chamber of sufficient capacity to provide an aged aerosol for animal exposures yet small enough to fit into a field laboratory; development and evaluation of a denuder system to remove excess gaseous components; and development of a highly functional mobile toxicology laboratory.

We successfully conducted toxicological studies at three coal-fired power plants during the period of 2004-2006. Rich exposure and outcome datasets were generated, and a multi-layered statistical approach was employed to determine scenario- and plant-specific effects and to evaluate associations with specific exposure components. Overall, we observed toxicologically mild adverse effects in response to some atmospheric scenarios. The varied responses among the three plants, with different fuels and emissions controls, indicate heterogeneity in emissions. Because of frequent inconsistency between univariate and multivariate analytical results, it was difficult to determine conclusively the components most associated with responses, and further analyses suggested that the scenario exposure or the combination of all components in an exposure was, in many instances, a better predictor of response than individual components. The most complex scenarios, which included added SOA, tended to result in the most robust responses. However, to summarize responses to components, neither neutral nor acidic sulfate appeared to play a large role in the biological effects. Elemental carbon, organic carbon, and specific pinene oxidation products were all associated with various responses; however, for a number of reasons these associations were not particularly convincing. Similarly, some trace elements appeared to be linked with responses, but results were also somewhat inconsistent. Finally, we observed effects related to gaseous copollutants; however, gas concentrations were very low through the use of denuders and therefore these associations were likely not reflective of a true biological association. Rather, because gases were common to all scenarios, they may have served as tracers for scenarios.

Overall, the TERESA results should be interpreted as indicating toxicologically mild adverse responses to some scenarios. Ongoing studies are using the TERESA approach to evaluate the toxicity of traffic related pollution; comparison of these data with the findings reported here plus the existing database of toxicological and epidemiological studies will give us a better understanding of the contribution of different sources to the morbidity and mortality associated with exposure to air pollution.

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