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Toxicological evaluation of realistic emission source aerosols (TERESA): summary and conclusions

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Abstract

The toxicological evaluation of realistic emissions of source aerosols (TERESA) study seeks to delineate health effects of aerosols formed from emissions of particulate matter sources. This series of papers reports the findings of experiments using coal-fired power plants as the source of emissions and this paper summarizes the findings and knowledge acquired from these studies. Emissions were drawn directly from the stacks of three coal-fired power plants in the US, and photochemically aged in a mobile laboratory to simulate downwind power plant plume processing. The power plants used different sources of coal and had different emission controls. Exposure scenarios included primary particles, secondary particles and mixtures of these with common atmospheric constituents (α-pinene and ammonia). Extensive exposure characterization was carried out, and toxicological outcomes were evaluated in Sprague-Dawley rats exposed to different emission scenarios. Breathing pattern, pulmonary inflammatory responses, *in vivo* pulmonary and cardiac chemiluminescence and cardiac response in a model of acute myocardial infarction were assessed. The results showed no response or relatively mild responses to the inhaled aerosols studied; complex scenarios which included oxidized emissions and α-pinene to simulate biogenic secondary organic aerosol tended to induce more statistically significant responses than scenarios of oxidized and non-oxidized emissions alone. Relating adverse effects to specific components did not consistently identify a toxic constituent. These findings are consistent with most of the previously published studies using pure compounds to model secondary power plant emissions, but importantly add substantial complexity and thus have considerable merit in defining toxicological responses.

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Declaration of interest

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Keywords

Multi-pollutant aerosols; particulate matter; ambient particles; power plant emissions; pollution sources; pulmonary inflammation; pulmonary function; *in vivo* chemiluminescence; myocardial infarction model

Introduction

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 extent of NO_2) and a small amount of primary particulate matter (PM). This primary 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 coal 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 is, 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 ammonium sulfate and nitrate particles, administered as pure compounds, cause health effects only at very high levels of exposure, although sulfate has been linked to health effects in several epidemiological studies. It is difficult to disentangle the toxicity of total PM mass and sulfate. This is because their mass concentrations are often highly correlated because, sulfate is the one of 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 included: (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.

summary of findings

Technological advances

The toxicological evaluation of realistic emissions of source aerosols (TERESA) study involved withdrawal of emissions directly from the stacks of three coal-fired power plants. The emissions were aged and photochemically transformed to simulate downwind power plant plume processing. In order to carry out these studies in the field at the power plants, mobile laboratories were constructed which included reaction chambers, instrumentation for characterization, and animal exposure chambers. In addition, systems were developed to aspirate the emissions from the stacks of the power plants and deliver them to the mobile

laboratories. Within the mobile laboratories, reaction chambers were constructed with lamps to simulate sunlight, and a design which could be easily used in the field (Ruiz et al., 2007a and 2007b). After aging the aerosol, removal of excess gases was a critical feature of the design. This was accomplished with a counter current diffusion denuder developed specifically for these studies (Ruiz et al., 2006). Exposure characterization included gasphase and particulate species and is described in detail in previously published papers by Ruiz et al. (2007b) and in this series by Kang et al. (2011).

Toxicological evaluations were carried out in rats exposed to different emissions scenarios. The design of the mobile toxicology laboratory met the lighting, temperature and air flow requirements of the NIH standards for care and housing of laboratory animals. This mobile laboratory included animal housing, a suitable platform for exposure, use the exposure chambers as whole body plethysmographs to obtain breathing pattern and air flow data, space for biological analyses including broncho-alveolar lavage, cell counting, *in vivo* chemiluminescence, animal dissection, and rat thoracic surgery to produce the rat model of myocardial infarction, as well as refrigerated and frozen storage space to temporarily store collected biological samples to be sent back to our laboratory in Boston. All of this was designed for use at an industrial field site. The design of the toxicological laboratory is described in detail in the papers by Godleski et al. (2011b) and Diaz et al. (2011). In this field study, the level of technological advance in the design, engineering and use of these facilities can be appreciated in this series of papers by the quality of the exposure and biological data obtained.

The study generated exposure data having multiple levels of resolution: (1) whether an animal was exposed to emissions or filtered air; (2) the specific exposure scenario corresponding to a given day; (3) and the chemical composition of a particular exposure scenario. For statistical analyses, a multi-layered approach was developed to analyze the associations between exposure and health that utilized all three levels of exposure characterization. The technological advances in the statistical analyses used in these studies are described in detail in this series by Coull et al. (2011). For a given health outcome, we began by fitting standard analysis of variance (ANOVA) models that treated exposure as a categorical variable. A first ANOVA model assessed differences in exposure effects across scenarios, separately for each power plant. Then, an ANOVA model assessed overall differences between exposed and filtered air responses, pooled across the three plants. Second, to assess unadjusted associations between pollutant concentrations and health, single-constituent (univariate) analyses were conducted using the difference between the response means under exposed and filtered air conditions and a single-constituent concentration as the predictor. Third, a novel multivariate analysis of exposure composition and health was used based on random forests®, a recent extension of classification and regression trees that were applied to the same mean differences used in univariate analyses. For each constituent concentration, this approach yielded a non-parametric measure of the importance of that constituent in predicting differences in response on a given day, controlling for the other measured constituent concentrations in the model. An R^2 analysis compared the relative importance of exposure scenario, plant and constituent concentrations on each health outcome. A very important aspect of the TERESA study design is that control animals were assessed for each exposure occasion. Therefore, responses of exposed animals are always compared to those of the corresponding controls. The number of animals assessed varied both by exposure scenario and plant (Godleski et al., 2011b) which affects the precision of the effect estimates, but does not produce bias in these estimates.

Exposure and exposure assessment

Details of the exposure system and exposure assessment are provided in the paper by Kang et al. (2011) in this series. The power plants used different sources of coal and had different

emission control devices in place. The exposure system simulated chemical reactions that power plant emissions undergo in a plume during transport from the stack to receptor areas. Several types of test atmospheres were used to form secondary particles for toxicological testing of animals. In order to simulate atmospheric transformations that coal power plant emissions undergo in a plume, the following scenarios were chosen: (1) primary emissions only (P); (2) the oxidation of 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 secondary organic aerosol (SOA), along with primary particles (POS); (4) the neutralization of H_2SO_4 aerosol by NH_3, along with primary particles and SOA (PONS); (5) three control scenarios excluding primary particles from the stack (O, OS, and S). The primary particle mass ranged from 1.0 to 43.2 $\mu g/m^3$, and aged particle mass concentrations varied significantly from 46.0 to 257.1 $\mu g/m^3$ with respect to scenarios and power plant emissions. The highest concentrations were found when oxidized aerosols were neutralized by gas-phase NH_3 (neutralized scenario) with added SOA. The mass concentration depended primarily on the ratio of SO_2 to NOx (particularly, NO) emissions, which was determined mainly by the coal composition and the emission control devices. Particulate sulfate (H_2SO_4 + neutralized sulfate) and organic carbon (OC) were major components in the aged particles, while trace elements were present at very low concentrations and the fractions of trace element to the aged particle mass were lower than those observed in typical urban environments. Finally, the physical and chemical properties of aged particles depended primarily on coal types, emission control devices and scenarios used for the coal-fired power plant.

Breathing pattern and air flow

Male Sprague-Dawley rats were exposed for 6 h to either filtered air or different atmospheric mixtures derived from the power plant emissions. Chemical reaction exposure scenarios studied at each of the three power plants included: P, PO, POS and PONS scenarios, and the three control scenarios O, OS and S at one plant. Continuous respiratory data were obtained during these exposures from the animals using whole body plethysmography chambers connected to a Buxco data acquisition system.

Complete description of the findings of these studies can be found in the report of Diaz et al. (2011). Breathing pattern and air flow are sensitive indicators of toxicological responses. In the design of this study, more animals were tested for these outcomes than any other, and thus, respiratory outcomes had the greatest potential to identify significant changes. Although a number of statistically significant findings were observed, the data do not show consistently robust adverse pathophysiological responses. The PO scenario produced significant respiratory pattern changes consisting of an increase in respiratory frequency (f) with corresponding decreases in inspiratory time (Ti) and expiratory time (Te) at two of the three plants (Plants 2 and 3). Random forest analyses as a multivariate approach found acidic sulfate to be the best individual predictor of observed exposure related increases in f and decreases in Ti and Te, but this relationship was not confirmed by univariate analysis. The PO scenario did not significantly reduce minute volume, and only marginally significantly reduced tidal volume, peak expiratory flow (PEF),and expiratory flow at 50% (EF50) at one plant (Plant 2). In the combined data, none of these were significantly decreased by the PO scenario. Thus, the PO changes are not of great pathophysiological significance. However, PEF and EF50 decreased significantly with the PONS scenario in two of the three plants and when all plants were combined ($p<0.0005$). These changes were most strongly associated with neutral sulfate, ammonium concentrations, nitrate and elemental carbon in univariate analyses, but the random forest analysis only consistently identified elemental carbon. Marginally significantly, the decrease of minute volume by the PONS scenario in the combined data was associated with ammonium concentrations, elemental carbon, and five other components in univariate analyses, and again only

elemental carbon in random forest analysis. Adjusted R^2 analyses to determine the relative strength of associations between plant/scenario versus individual components showed that plant/scenario explained most of the variances for all respiratory parameters, and thus plant/scenario was the dominant factor. Thus, the data suggest that the test scenarios that we designed for these experiments were equal or better predictors of respiratory effects than any particularly measured component by itself. This finding suggests that it is the mixture as a whole that was responsible for the observed effects.

Pulmonary and systemic inflammation

Twenty-four hours after exposure, pulmonary cellular and biochemical responses to the inhaled aerosol were assessed by broncho-alveolar lavage (BAL); complete blood count was used as a screen for systemic responses; and lung and cardiac histopathology assessed the presence of inflammation as well as changes in lung and cardiac vessels. Details of this study are reported by Godleski et al. (2011a) in this series. Compared to filtered air controls, the PONS and POS scenarios produced significant increases in BAL total cell count and lung macrophage numbers at two of the three plants studied as well as when data from all three plants were combined. The PONS and P scenarios were associated with significant increases in BAL polymorphonuclear neutrophils (PMNs) at one of the three plants (Plant 3), and these increases remained when all plant data were combined. These findings were confirmed by histology with increases in macrophages and the presence of occasional PMNs in the airways and alveoli of animals exposed to the PONS scenario at Plant 3. No changes in BAL total cell and macrophage counts occurred with the P and PO scenarios at any plant, and no changes in BAL lymphocytes, protein, nor β-n-acetyl glucosaminidase were found with any scenario exposure at any plant. Univariate analyses and random forest analyses, used as a multivariate screen to identify components associated with the cellular changes, showed that increases in total cell count and macrophage cell count were associated with neutralized sulfate and several correlated measurements. Increases in PMNs in BAL were associated with zinc, whereas decreases in PMNs were related to elemental carbon. There were no significant differences from control in any complete blood count parameter with any exposure scenario. Lung and cardiac blood vessel wall thickness had no differences from control with any exposure scenario. The statistically significant changes in total cell count and macrophages by BAL are considered mild toxicological responses, and these were found only in scenarios with added atmospheric constituents and the most complex atmospheric reactions. Increases in neutrophils in BAL without increases in BAL protein, enzymes, or circulating neutrophils are considered mild responses. The significant association of increase in neutrophil with zinc, raises the possibility that metals may play a role in this response.

Reactive oxygen species in the lung and heart

In vivo chemiluminescence (CL) studies have shown increases in cardiac and pulmonary reactive oxygen species (ROS) after exposure to concentrated ambient particles (CAPs) (Gurgueira et al 2002). In this study, we used CL to assess the pulmonary and cardiac responses to aerosols derived from the emissions of coal-fired power plants and mixtures comprised of these emissions with other atmospheric constituents. Details of these studies are reported by Lemos et al. (2011). Immediately after 6 h of exposure, CL in the lung and heart was measured. Tissues were then excised, frozen and later assayed for thiobarbituric acid reactive substances (TBARS). Exposure to the P or PO scenarios led to no changes in lung or heart CL at any individual plant or when all data were combined. The POS scenario caused significant increases in lung CL and TBARS at one plant, but not others. The PONS scenario exposure resulted in increases in lung CL when all plants were combined but none were individually significant. Heart CL was increased in rats exposed to the POS scenario at two plants, but these were not individually significant. Exposure to the PONS scenario also

increased heart CL in two of the three plants and one of these was individually significant and was also reflected in TBARS accumulation. When all data from the plants were considered together, the increase in heart CL was statistically significant only with exposure to POS, and the increase in lung CL was statistically significant only with exposure to PONS. Univariate analyses of individual measured components of the exposure atmospheres did not identify (a) specific component(s) associated with these increases. These data suggest that only atmospheres comprised of coal-fired power plant emissions combined with other atmospheric constituents can produce significant pulmonary and cardiac oxidative stress in normal animals.

The CL measurements of this study represent the most directly comparable data to published data with exposure to CAPs because, the exposure dose is in the same range for most of the scenarios, the exposure duration is the same as in many CAPs studies, and the equipment and the study protocols are exactly the same. Increases in CL are associated with increased oxidative damage in the tissue under study and a treated/control ratio of 1.4 is considered the threshold between oxidative stress and damage (González-Flecha et al., 1991). With inhalation exposure to Boston CAPs, the treated/control ratio for both lung and heart CL with exposures of 3 h or longer showed values ranging 1.9-3.8 for the heart and about 1.7 for the lung (Gurgueira et al., 2002).The exposed/sham ratios found for the different exposure scenarios tested here range 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. However, of the 24 plant/scenario/organ (lung or heart) combinations investigated, only two exceeded that treated/control ratio of 1.4. 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 is analogous to those reported to produce significant electrophysiological alterations when using CAPs (Ghelfi et al., 2008). However, the fact that only one of three plants produced these comparable responses suggests that unique features of the power plant-derived aerosol may play a role in this response. Since no significant positive univariate associations were found, it does not appear that a specific component is causing this outcome. 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 presented in Diaz et al. (2011).

Cardiovascular findings in a model of acute myocardial infarction

In a previously described susceptible animal model (Wellenius et al., 2002, 2004), the effect of aged stack emissions on cardiac electrophysiology and respiratory function was evaluated under exposure conditions intended to simulate an aged plume with unneutralized sulfate and secondary organic aerosols (POS scenario). These studies are described in detail by Wellenius et al. (2011). Rats with acute myocardial infarction were exposed to either stack emissions ($n = 15$) or filtered air ($n = 14$) for 5 hours at a single power plant (plant 2) within the first 24 h after the infarction, which is the most vulnerable period for the development of arrhythmias. Respiration and electrocardiograms were continuously monitored via telemetry and heart rate, heart rate variability (HRV), premature ventricular beat (PVB) frequency, electrocardiographic intervals, and respiratory intervals and volumes were evaluated. Logistics of telemeter implantation necessitated that these experiments should be run early in the course of all studies and at the very end of the study period at each plant. Similar experiments at another power plant (plant 3) were planned and attempted. At Plant 3, half of the planned exposures had to be aborted after creating the infarct because of unplanned plant shutdowns. Because these occurred at the end of the study period when the plant was about

to do a planned change-over of its emissions control equipment, the resultant inadequate number of test animals could not be increased. This problem, plus a slightly higher number of data quality problems with the animals that were studied, made the data of the plant 3 studies uninterpretable.

At plant 2, POS exposure was associated with increased PVB frequency and decreased respiratory expiratory time and end-inspiratory pause, but not with changes in heart rate, HRV, or electrocardiographic intervals. From the studies at Plant 2, we conclude that short-term exposure to primary and secondary particulate matter formed from aged emissions from a single coal-fired power plant, as simulated by the POS scenario, may be associated with increased risk of ventricular arrhythmias in susceptible animals.

Integrated summary of role of major exposure components

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 minimal toxicological effects with partially to totally neutralized sulfate in laboratory studies. We did not observe consistency in findings with varied statistical methods; nor did the findings of varying pulmonary end points consistently concur Acidic sulfate produced increased breathing rate by multivariate analyses, but overall did not appear to play a significant role in pulmonary responses in the TERESA study. Neutralized 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. *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 artifacts 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 may not be 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 α-pinene oxidation products: formaldehyde, acetaldehyde, acetone and total aldehydes. Furthermore, the addition of SOA appeared to be linked with the inflammatory responses observed, although, from examination of control scenarios and organic carbon univariate results, there was not a clear evidence showing 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, concentrations were exceedingly low.

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 co pollutant concentrations in the exposure scenarios were low due to 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 they may have served as tracers for scenarios. Gases did not play a role in the inflammatory or chemiluminescence responses.

Responses by power plant—Although differences in plant emission controls 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 at outcomes in individual plants controlled for scenario. Although differences in measured component concentrations from plant-to-plant and between scenarios complicated the interpretation of such analyses, certain trends are worthy of mention in this summary. All scenarios at plant 3 showed non-significant increases in inspiratory flow whereas at plants 1 and 2, all scenarios showed decreases in inspiratory flow. Thus, comparison of 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 non-significant 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 among 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 emissions 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.

study strengths and limitations

The most important strength of this study was the technological developments needed to carry out these studies. The diffusion denuder system, dynamic reaction chamber system, exposure system and mobile laboratory and statistical approaches developed specifically for these studies all have the potential to make long term contributions to toxicological studies of multi-pollutant atmospheres. Another 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.

Differences in the number of animals used per scenario and per plant influenced the statistical power to detect an effect in ANOVA analyses. 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. Thus, there was greater power to detect some changes within some scenarios. Although the experiments were planned using all the same numbers, we repeated some scenarios to be certain that outcome results were consistent within multiple repetitions of experiments. Since there was no reason to select certain runs to maintain the same numbers of animals and there were no reasons to not use all data, all of these data were included in analyses. 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 toxicological assessments used in the TERESA studies were those that have been used successfully in defining the responses to inhalation of CAPs. The TERESA studies relied primarily on pulmonary outcomes, but also included systemic and cardiovascular responses

in normal animals and in a susceptible animal model of cardiovascular disease. The results of the TERESA studies show no response or relatively mild responses to the inhaled aerosols studied. These findings are consistent with most of the previously published toxicological studies using pure compounds to model secondary power plant emissions, but importantly add substantial levels of complexity to those studies and thus have considerable merit in defining these toxicological responses.

One important issue with these studies that may be considered both a strength and limitation is that we did not test primary and secondary particles from coal-fired power plants in the presence of particles from many other sources. Urban particles have many different components which may interact with or act as catalysts in the formation of secondary particles. Whether and in what way these other particles might contribute to the toxicity of power plant emissions was not tested in these studies, and thus limits the extent of knowledge gained. Alternatively, the focus on power plant primary particles and gases as the sole source of potential reactants and catalysts for secondary pollutant formation using a light source of defined wavelengths and the addition of a small number of additional materials (α-pinene, ammonia, ozone, and water) resulted in more controllable experiments and therefore may be also considered a strength of these studies. In the future, the current approach may be refined to test the interaction of the emission components of a single source with a realistic mixture that represents other sources of an urban aerosol.

These studies also had several potential limitations which apply to all outcomes. First, to reduce biologic variability, only mature, male, Sprague-Dawley rats were studied. Thus, it is unknown how the effect of exposures to these scenarios might vary by species, gender, or age. Secondly, although matched daily controls were included for all studies and were within the normal ranges established in our laboratory for these parameters, it is unknown whether the ambiance of the industrial setting at the power plant might have in any way influenced the outcome of these studies. Third, exposure concentrations varied from scenario to scenario. Ideally, comparable mass concentrations should have been used to properly compare differences between different scenarios. However, the very low concentrations of primary particles (especially relative to gaseous emissions) which could be obtained from the stack emissions made this impossible. Although we have subsequently developed a higher volume diffusion denuder (Papapostolou, 2011), this technology was not available at the time of these studies. Indeed, the new high volume denuder was developed upon the knowledge acquired from the denuder used in this study. Finally, it should be noted that we selected α-pinene as a representative among biogenic VOCs. It is not known how responses to other biogenic VOCs or anthropogenic VOCs might have differed.

The MI study had several specific potential limitations. 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 that 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 a 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, 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, conclusions from the MI model 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.

Knowledge acquired and lessons learned from these studies

These studies show that statistically significant, but relatively mild, health effects could be produced by inhalation exposure experiments using coal-fired power plants as the source of emissions that were photochemically aged and atmospherically transformed in a mobile laboratory in a manner that simulated downwind power plant plume processing. The observed health effects tended to result from scenarios that had more reactants added and more complex chemical reactions. Sulfate, as expected, was a large component of the exposure atmospheres. Recent reviews (Schlesinger, 2007; Reiss et al., 2007) conclude that there are no serious toxicological health effects from sulfate exposure at moderate exposure levels for toxicological studies. This study largely supports that conclusion. The differences in emissions composition and subsequent biological responses among plants suggests that power plant emissions are heterogeneous and are influenced by a number of factors including coal type, emissions controls, and plant configuration.

As discussed in the limitations section above, there was a need, but impossibility, to have comparable exposure doses from each scenario. We have since improved technology that can make this possible for future studies. Although the primary particle concentrations were exceedingly low, the PO, POS and PONS scenarios were within the same range and comparable to studies using CAPs. Although we investigated four primary and three additional control scenarios, we did not study a scenario comprised of primary particles, secondary oxidized particles, and ammonia without added SOA, or a PON scenario. This scenario might have given us more specific information on atmospherically formed neutralized sulfate exposure. The rationale for not including this scenario was that the addition of SOA typically occurs in nature, so that a PON scenario alone is a very unlikely realistic scenario.

This project defined both the absolute and relative toxicity of secondary particles formed from coal-fired power plants compared to laboratory studies of ambient particles or CAPs. In general, only the most complex scenarios approached, but did not equal or exceed the reported toxicity of inhaled CAPs. The project also modeled and provided insight into the formation of secondary particles formed from the gaseous emissions of the power plants, clearly demonstrating that these transformations could be produced in a field laboratory. Another objective of the TERESA project was to study the type of coal and emissions controls as factors in the response. Although coal from the same source may be used over several weeks at a large power plant, it was not possible to limit the variations in coals used so that, this could be studied in a controlled fashion in this field setting. Among the plants, different coal sources were used, but it was impossible to relate these differences directly to outcomes. An ambitious objective of this study was to gain insight into toxicological mechanisms involved in the responses to the various exposures. Although the relationships between exposures and outcomes provided some new knowledge, specific insights into toxicological mechanisms of response can only be indirectly inferred from the results of these studies.

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. Because of frequent inconsistency between univariate and multivariate analytical results, it was difficult to determine conclusively the components most associated with responses. However, to summarize, 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, most robust among these was a dose-response association of neutrophils on BAL with zinc concentrations which were in an exceedingly low range so that zinc may be acting as a tracer for a combined metal concentration effect. Finally, we observed effects related to gaseous co-pollutants; however, gas concentrations were also very low through the use of denuders and therefore these associations were likely not reflective of a true biological association. Rather, gases may have served as tracers for scenarios. The varied responses among the three plants indicate heterogeneity in emissions.

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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