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Toxicological Evaluation of Realistic Emission Source Aerosols (TERESA): Introduction and overview

John J. Godleski¹, Annette C. Rohr², Choong M. Kang¹, Edgar A. Diaz¹, Pablo A. Ruiz³, and Petros Koutrakis¹

¹Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA

²Electric Power Research Institute, Palo Alto, CA, USA

³School of Public Health, Faculty of Medicine, University of Chile, Santiago, Chile

Abstract

Determining the health impacts of sources and components of fine particulate matter (PM_{2.5}) is an important scientific goal. PM_{2.5} is a complex mixture of inorganic and organic constituents that are likely to differ in their potential to cause adverse health outcomes. The Toxicological Evaluation of Realistic Emissions of Source Aerosols (TERESA) study focused on two PM sources—coal-fired power plants and mobile sources—and sought to investigate the toxicological effects of exposure to emissions from these sources. The set of papers published here document the power plant experiments. TERESA attempted to delineate health effects of primary particles, secondary (aged) particles, and mixtures of these with common atmospheric constituents. TERESA involved withdrawal of emissions from the stacks of three coal-fired power plants in the United States. The emissions were aged and atmospherically transformed in a mobile laboratory simulating downwind power plant plume processing. Toxicological evaluations were carried out in laboratory rats exposed to different emission scenarios with extensive exposure characterization. The approach employed in TERESA was ambitious and innovative. Technical challenges included the development of stack sampling technology that prevented condensation of water vapor from the power plant exhaust during sampling and transfer, while minimizing losses of primary particles; development and optimization of a photochemical chamber to provide an aged aerosol for animal exposures; development and evaluation of a denuder system to remove excess gaseous components; and development of a mobile toxicology laboratory. This paper provides an overview of the conceptual framework, design, and methods employed in the study.

Keywords

Coal power plant; emissions; fine particulate matter; secondary particles; toxicology

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Address for Correspondence: Dr. John J. Godleski, MD, Department of Environmental Health, Harvard School of Public Health, 665 Huntington Ave, II-227, Boston, MA 02115, USA. Phone: 617-432-1252; jgodlesk@hsph.harvard.edu.

Declaration of interest

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Introduction

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. (Chen et al., 1990; Hsu & Kou, 2001; Ho & Kou, 2002; Jaspers et al., 2005; Smith et al., 2006; Mills et al., 2005). 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 (SO₂), nitrogen oxides (NO_x, consisting mostly of NO and to a lesser extent of NO₂), 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 SO₂ is oxidized to secondary acidic sulfate, while simultaneously ambient ammonia neutralizes this strong acidity. In addition, secondary organic aerosol (SOA) 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 (ESPs) (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 (Alarie et al., 1975; Fisher et al., 1983; Schreider et al., 1985; Gilmour et al., 2004).

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 ESPs 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 SO₂ 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 SO₂ 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 & 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, Gurgueira et al., 2002, 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., 2011).

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., 2007). 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; Saldiva et al., 2002; Wellenius et al., 2003). 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.

TERESA objectives

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: (i) evaluate the relative toxicity of coal combustion emission secondary products in comparison to ambient particles; (ii) 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; (iii) provide information on the impact of coal type and pollution control technologies on emissions toxicity; and (iv) provide insight into toxicological mechanisms of PM-induced effects, particularly as they relate to susceptible subpopulations.

TERESA design

The design of the study focused on exposing laboratory rats to simulated atmospheric scenarios (Figure 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 (i) power plant selection; (ii) emissions sampling; (iii) atmospheric simulation; (iv) exposure scenarios; (v) mobile laboratory; (vi) exposure characterization; (vii) animal exposures; and (viii) toxicological assessments. Note that more comprehensive details can be found in the companion papers to this overview document.

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 ($\sim 1500^\circ\text{C}$). Access to each of the plants was arranged through the Electric Power Research Institute in Palo Alto, CA. Table 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 ($\sim 0.2\%$ S) sub-bituminous coal from the Wyoming Powder River Basin and had two units with a generating capacity of 600 MW each. 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 ($\sim 1.0\%$ 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 ($\sim 3.0\%$ 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 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.

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 Bernoulli 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 SO₂ and NO_x concentrations suitable for our experiments.

Plant 3 used a wet FGD scrubber to capture SO₂, along with an ESP and an 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 (i) changing the flow rate of hot, dry filtered air; and (ii) 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).

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 SO₂ and NO_x in the stack exhaust to sulfuric acid and nitric acid. The second chamber was used to neutralize H₂SO₄ aerosol and/or to form 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 O₃ and added water vapor. Enough O₃ was added to completely oxidize the entire NO to NO₂, with a sufficient excess supplied to form hydroxyl radical with the UV irradiation. The hydroxyl radical oxidized SO₂ to produce secondary H₂SO₄ aerosol. The chamber was designed to oxidize 30–50% of SO₂ to sulfuric acid within an approximately 20-min 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 SO₂ to sulfuric acid is on the order of 2–4% h⁻¹ (Luria et al., 1983; Seinfeld & 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 NH₃ was added to neutralize most of the H₂SO₄ aerosol and/or α -pinene and O₃ were added to produce 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, SO₂, and O₃ 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 a 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).

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 2). The following specific scenarios were selected for study: (i) primary emissions only (“P”); (ii) the oxidation of flue gas SO₂ to form H₂SO₄ aerosol, along with primary particles (“PO”); (iii) the oxidation of SO₂ plus the reaction of α -pinene with ozone to form SOA (to simulate the plume mixing with biogenic emissions), along with primary particles (“POS”); (iv) the neutralization of H₂SO₄ aerosol by NH₃, along with primary particles and SOA (“PONS”); (v) an oxidized SO₂ scenario that included primary gases but excluded primary particles (control scenario “O”); (vi) the “O” control scenario with added SOA (control scenario “OS”), and; (vii) 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.

The initial “aging” of the primary flue gas was carried out to simulate long-range transport of the power plant plume. Achieving an SO₂ 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 SO₂ 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 & 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 & 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 being said, PONS scenario was deemed the most representative of human exposure. This is because it contains oxidized SO₂, partially neutralized sulfate, and SOA 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 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).

Mobile laboratory

A mobile laboratory was designed and constructed specifically for this project. The mobile lab was comprised of two separate structures: (i) 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 (ii) 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), CO₂, 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 (MI) 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).

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, and particle size distribution was determined semi-continuously using a scanning mobility particle sizer. 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 method, and organic speciation of particle-phase pinene oxidation products was conducted by gas chromatography. Trace elements were quantified by X-ray fluorescence. Continuous measurement of gaseous pollutants was carried out: NO and NO₂ by chemiluminescence; SO₂ by pulsed fluorescence; and O₃ by UV photometry.

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 h 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.

Toxicological assessments

Toxicological endpoints were selected to provide a comprehensive, multi-system assessment of the impacts of exposure to the different scenarios. In addition, all of the endpoints evaluated were used in previous work in our laboratory with 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 MI rat model used previously by our research group (Wellenius et al., 2002, 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 airflow 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, tidal volume, respiratory frequency (f), and minute ventilation (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 (β -NAG), 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 h 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 et al., 1980; Boveris et al., 1999). This method has been successfully used in models of oxidative injury in lung (Evelson & González-Flecha, 2000; Gurgueira et al., 2002) and 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 h 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, 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).

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-h 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 79 exposure days were included in the study, including “control” scenarios (O, S, OS); Table 3 shows the dates on which experiments were conducted at each of the three plants.

As can be seen in Table 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.

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 min. 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.

Additional papers

Also appearing in this special issue are seven additional publications describing various aspects of the TERESA power plant study, as shown below:

1. Kang et al., “Aged particles derived from emissions of coal-fired power plants: The TERESA field results”: Describes the exposure results, including detailed data on the composition of the multiple atmospheric scenarios.
2. Coull et al., “The Toxicological Evaluation of Realistic Emissions of Source Aerosols study: Statistical methods”: Describes the multi-layered statistical approach employed in the study.
3. Godleski et al., “Toxicological Evaluation of Realistic Emission Source Aerosols (TERESA)–Power plant studies: Assessment of cellular responses”: Describes the BAL results and histopathological analyses.

4. Diaz et al., “Toxicological Evaluation of Realistic Emission Source Aerosols (TERESA)–Power plant studies: Assessment of breathing pattern”: Describes the continuous respiratory responses.
5. Lemos et al., “Cardiac and pulmonary oxidative stress in rats exposed to realistic emissions of source aerosols”: Describes the chemiluminescence (*in vivo* oxidative stress) results in the TERESA study.
6. Wellenius et al., “Electrocardiographic and respiratory responses to coal-fired power plant emissions in a rat model of acute myocardial infarction: Results from the Toxicological Evaluation of Realistic Emissions of Source Aerosols (TERESA) study.”: Describes the use of and results from the MI model at Plants 2 and 3.
7. Godleski et al., “The TERESA study: Summary and integration”: Presents an integrated perspective on the overall results and their implications.

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Figure 1.
Overview of the TERESA approach.

Table 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
<i>Coal composition (as-fired)^b</i>			
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) (%)	8500	13,000	13,000

ESP, Electrostatic precipitator for particle removal; SCR, selective catalytic reduction for NO_x control; FGD, flue gas desulfurization scrubber for SO₂ removal

^a Air pollution control devices.

^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.

Table 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

Table 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 8–10, 13	Normal
PO	November 3–5	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, 23, 25, 26, June 27–30, October 11–14	May 31–June 3	July 25–28	Normal
POS	—	July 8, 13, September 7, 8	August 16–17	Compromised
OS	—	—	August 28–31	Normal
O	—	—	September 1–4	Normal
S	—	—	September 6–9	Normal

Table 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 ^b	P	PO	POS ^c	PONS ^d	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
Bronchoalveolar lavage	—	5/5	6/6	18/18	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
<i>In vivo</i> 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
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
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
Arrhythmias and heart rate variability ^a	—	—	—	—	—	—	15/14	—	—	—	8/7 ^e	—

^aThis endpoint employed the myocardial infarction model developed by Wellenius et al. (2004).

^bThree repetitions were done at different times of the day. No individual differences found between runs. All data analyzed together.

^cSCR on.

^dSCR off.

^e6/2 usable for analysis; see Wellenius et al. (2011)

Endpoint not evaluated.