

ASSESSMENT OF THE HEALTH EFFECTS OF COAL COMBUSTION EMISSIONS: PRELIMINARY RESULTS FROM THE TERESA STUDY

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ABSTRACT

The Toxicological Evaluation of Realistic Emissions of Source Aerosols (TERESA) study is a comprehensive effort to evaluate the toxicity of secondary particulate matter (PM) derived from coal-fired power plants. To date, the toxicity of coal combustion emissions has been examined only in terms of primary particles, but these emissions may not reflect population exposures because of atmospheric chemistry. An approach was therefore needed that would evaluate the health effects of coal combustion emissions in a realistic manner that included the simulation of various atmospheric conditions. In TERESA, emissions are drawn from the stack at three separate power plants into a mobile laboratory, where oxidants are added to convert SO₂ to sulfate, and other atmospheric constituents are added (e.g., NH₃, secondary organic aerosol [SOA]). Laboratory rats are then exposed to the resulting atmospheric mixtures, with PM present at reasonably environmentally relevant concentrations, and cardiovascular and pulmonary effects are evaluated. In each exposure scenario, 42 rats are exposed (13/day for 4 days) to the emissions atmosphere, and 42 are exposed to filtered air (control animals). Exposures are 4 hours in duration. We recently completed fieldwork at the first TERESA study plant in the Upper Midwest. We carried out exposures to 4 scenarios: (1) primary particles only; (2) secondary particles; (3) secondary particles + SOA; and (4) neutralized secondary particles + SOA. PM_{2.5} concentrations were in the vicinity of 200-300 µg/m³. In scenario (2), strong acidity was about 50 µg/m³ H₂SO₄. Outcome parameters studied included pulmonary function and breathing pattern, bronchoalveolar lavage fluid analysis (to evaluate possible lung inflammation), heart and lung chemiluminescence (a very sensitive measure of oxidative stress in these organs), blood analysis, and lung tissue histopathology. Findings indicate no adverse effects from any of the exposures evaluated. There were no differences in the measured parameters between exposed and control animals. We have also completed exposures at Plant 2, located in the Southeast; data are currently being analyzed. Following the power plant assessment, we will carry out the same assessment using mobile source emissions. The ultimate goal of TERESA is to compare the toxicity of secondary coal combustion and mobile source emissions to better understand the components of PM responsible for adverse health effects. The TERESA study is the first to investigate the toxicity of actual power plant emissions using mobile laboratories, and the first to incorporate secondary atmospheric chemistry to ensure that exposures are realistic and representative of human exposures.

INTRODUCTION

In the face of further regulation of particulate matter (PM), there is a critical need for increased knowledge regarding the PM sources and components responsible for the health effects observed in epidemiological and toxicological studies. Currently, PM is regulated as if it and its constituents were toxicologically identical, regardless of contributing sources, using a mass-based standard. Recent findings from a large epidemiological study in Atlanta, GA (ARIES) point to the importance of the carbon-containing fraction of PM, which may be derived from mobile, biogenic, and other sources (e.g., fireplaces, agricultural burning) (Klemm et al., 2005; Metzger et al., 2004; Peel et al., 2005; Sinclair and Tolsma, 2005).

The TERESA project investigates the role played by specific emissions sources and components in the induction of adverse health effects by examining the relative toxicity of coal combustion and mobile source emissions and their oxidized products. The work is a significant improvement over previous studies to investigate the toxicity of coal combustion-derived particulate matter by virtue of several highly innovative and unique design features. First, all toxicological studies of coal combustion emissions to date (some of which have shown biological effects) have used primary PM, ie. coal fly ash (e.g. MacFarland et al., 1971; Alarie et al., 1975; Raabe et al., 1982; Schreider et al., 1985). The relevance of primary PM to human population exposure is unclear, since emissions of primary PM are now very low with the widespread introduction of particulate controls on power plants. It is the secondary particulate matter formed from SO₂ and NO_x in stack emissions as well as any residual primary PM that is of interest. No efforts to consider and account for secondary atmospheric chemistry have been made to date. By examining aged, atmospherically transformed aerosol derived from stack emissions, TERESA is able to determine the toxicity of emissions sources in a manner that more accurately reflects the exposure of concern. In addition, the atmospheric simulation component of the project allows the investigation of the effect of different atmospheric conditions on the formation and toxicity of secondary PM. Second, the primary PM used in the studies to date has typically been generated through the use of pilot combustors in a laboratory setting. There is concern that pilot combustors may not accurately mimic stack emissions due to differences in surface to volume ratios and thus time-temperature histories. TERESA involves assessment of actual plant emissions in a field setting – an important strength of the study since it minimizes any question of representativeness of emissions.

The study involves on-site sampling and dilution of coal combustion emissions at three coal-fired power plants, as well as mobile source emissions. Emissions are introduced into a reaction chamber that simulates oxidative atmospheric chemistry, and both primary and secondary materials are extensively characterized, including NO₂, SO₂, ozone, NH₃, hydrocarbons, particle number and mass (including ultrafines), sulfate, nitrate, elemental/organic carbon (EC/OC), ammonium, and metals. Test atmospheres containing diluted emissions and oxidized emission products are utilized in a comprehensive toxicological assessment in laboratory rats.

The primary objective of the project is to evaluate the potential for adverse health effects from ambient exposure to realistic coal-fired power plant emissions. Secondary objectives of the study are to: (1) evaluate the relative toxicity of coal combustion emissions and mobile source emissions, their secondary products, and concentrated ambient particles from Boston; (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.

The overall study design is shown in Figure 1. It should be noted that due to differences in emission composition as well as study setup, the mobile source assessment may not incorporate the identical atmospheric aging approach as the power plant emissions.

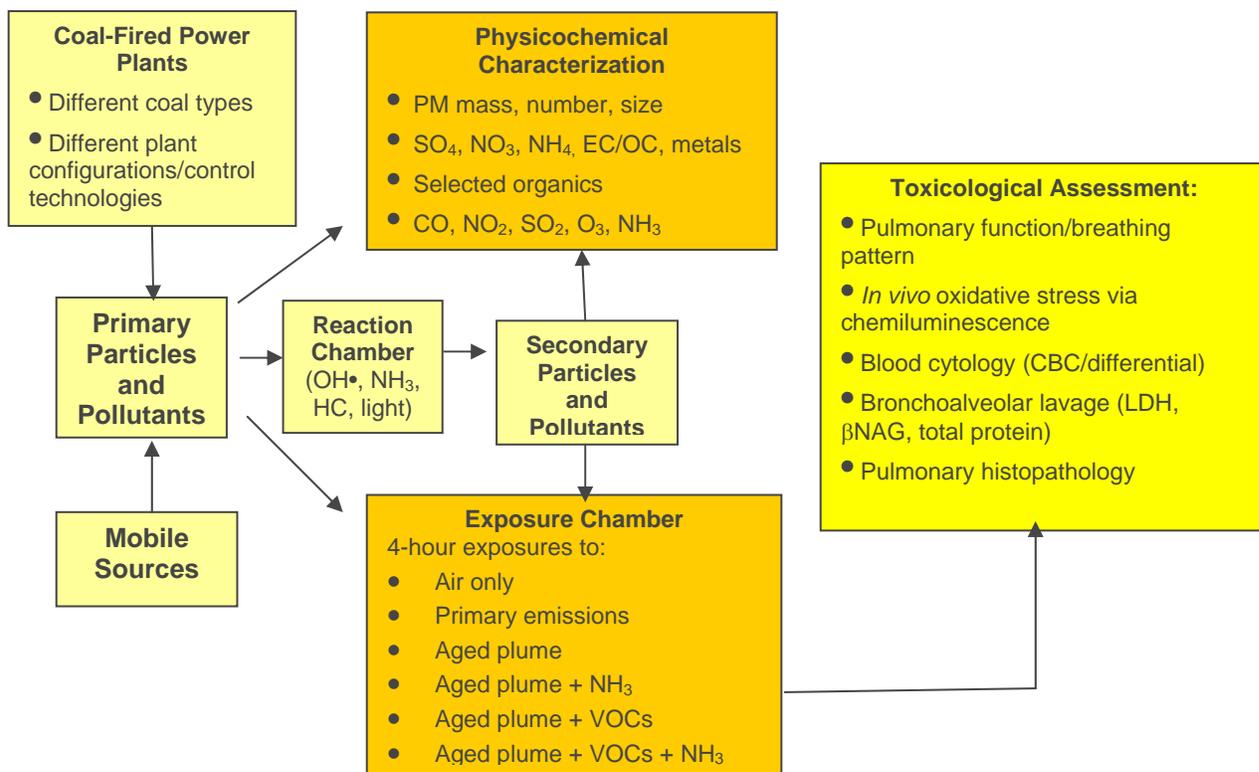


Figure 1. TERESA study design.

EXPERIMENTAL

Plant selection. The TERESA program includes three coal-fired power plants. Plant 1 is located in the Upper Midwest and utilizes Powder River Basin (Wyoming) coal, with very low sulfur and low ash. Plant 2, in the Southeast, burns low sulfur (<1%) eastern bituminous coal and has a selective catalytic reduction (SCR) unit for NO_x removal. Plant 3 is located in the Midwest and uses medium-to-high sulfur (>2-3%) eastern bituminous coal. This unit employs both an SCR as well as wet scrubber for post-combustion SO₂ removal.

Exposure Scenarios. Exposure scenarios and their corresponding simulated atmospheric conditions are shown in Table 1.

Table 1. Exposure scenarios and corresponding atmospheric conditions.

Scenario	Composition	Simulated Atmospheric Condition
1	Gas- and particle-free air	Sham exposure
2	Primary (un-aged) emissions diluted using clean air (same dilution as for 3, 4, and 5 below)	Primary stack emissions
3	Primary emissions + hydroxyl radicals	Aged plume, oxidized stack emissions, sulfate aerosol formation from nucleation
4	Primary emissions + hydroxyl radicals + ammonia	Aged plume, sulfate aerosol partially neutralized by ammonia
5	Primary emissions + hydroxyl radicals + VOCs	Aged plume plus secondary organic aerosol derived from biogenic emissions
6	Primary emissions + hydroxyl radicals + ammonia + VOCs	Aged plume, mixture of neutralized sulfate and secondary organic aerosol derived from biogenic emissions

Emissions Sampling System. The initial design and final modifications of the emissions sampling system represented a technical challenge, with significant care being taken to avoid particle losses in the system. A continuous sample passed through a stainless steel tube running from the pre-stack duct to a mobile chemical laboratory on the ground. The sampling tube had a size selective inlet to remove particles nominally larger than 2.5 μm and ports for the addition of filtered (dry) air. Dilution air cooled the exhaust to normal ambient temperature and dried the exhaust to prevent condensation of water in the sampling line. It also reduced gas concentrations to appropriate levels for the reaction chamber and to result in target secondary particle exposure concentrations of 200-300 $\mu\text{g}/\text{m}^3$. Sampling flow rate, dilution airflow, and tubing dimensions were optimized to minimize losses of ultrafine particles, SO_2 , and acidic sulfate particles.

Several iterations of the design of the stack sampling system were tested; the final design is shown in Figure 2.

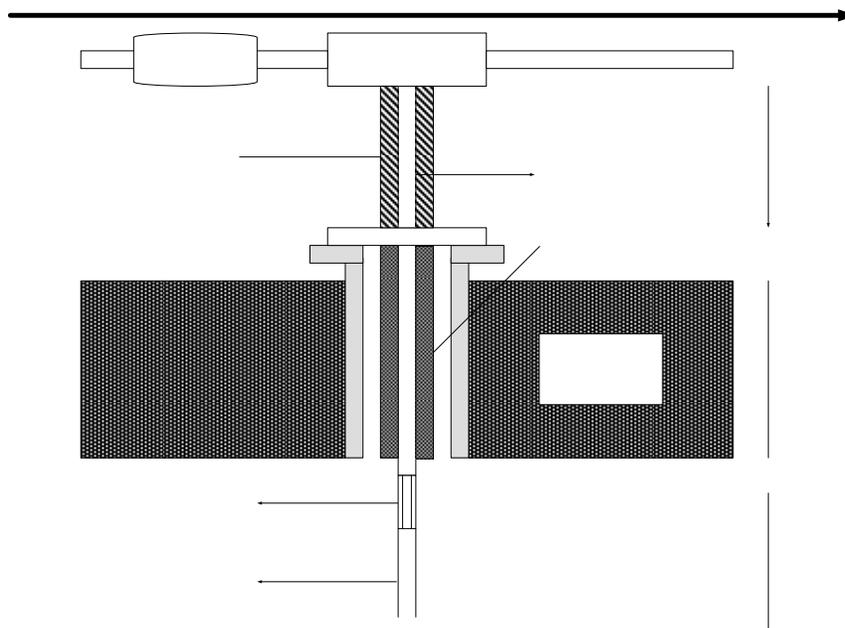


Figure 2. Final configuration of emissions sampling system.

Atmospheric Reaction Simulation System. The atmospheric reaction simulation system consists of dual chambers (Figure 3). This dual-chamber conceptual model and physical configuration assumes that the oxidation of SO_2 to form H_2SO_4 takes place primarily in the plume that is formed from the initial dispersion of the emitted stack gas. In the first chamber, SO_2 reacts with hydroxyl radicals (generated via the photolysis of ozone) to form H_2SO_4 . Relatively high energy UV light was used to produce sufficient hydroxyl radical concentrations to oxidize the SO_2 . The second stage occurs when the H_2SO_4 mixes with and is neutralized by ammonia introduced to the chamber to simulate that from ground level sources, and where the neutralized or acidic sulfate particles also mix, independently, with introduced VOCs to simulate those from both anthropogenic and natural sources, and particle-phase organics are formed. Thus, in the TERESA system, in the second reaction chamber, the acidic aerosol can be neutralized with ammonia, and/or α -pinene (as a representative biogenic VOC) can be reacted with ozone to produce organic particulate matter, depending on the scenario desired.

The first stage reaction chamber is 152 x 122 x 30 cm, with a total volume of approximately 500L. The side (152 x 30 cm) and end (122 x 30 cm) surfaces of the chamber are made of opaque PTFE Teflon sheet. The larger 152 x 122 cm top and bottom surfaces are made of transparent PTFE Teflon film (in order to transmit UV irradiation). The chamber was designed to attach and detach the Teflon film easily, allowing periodic sheet replacement. Also, the chamber has wheels that facilitate its movement into and out of an enclosure that holds an array of UV lamps that face the two transparent Teflon film surfaces of the chamber. The second stage reaction chamber has Lexan (polycarbonate) walls lined with FEP Teflon film to minimize wall reactions. The dimensions are 60 x 50 x 30 cm with a total volume of 90 L. At 5 LPM the residence time in the chamber is 18 minutes.

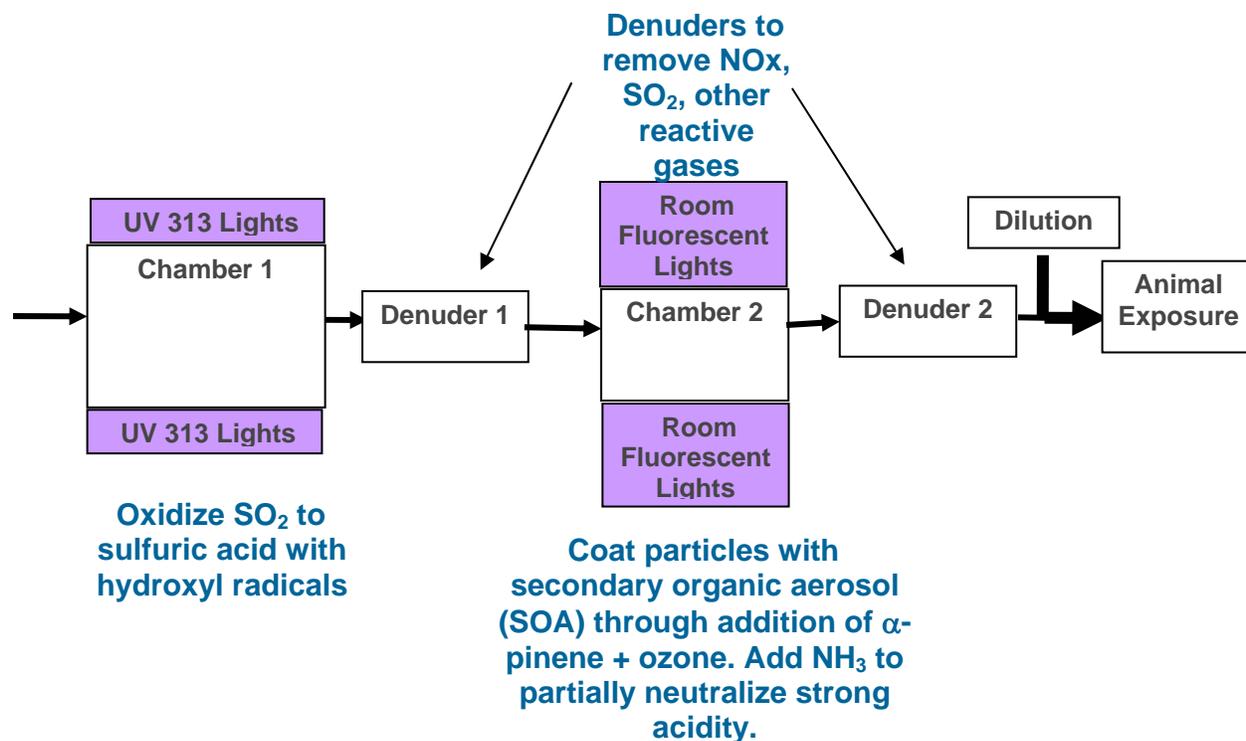


Figure 3. Dual chamber system for atmospheric simulations.

Banks of UV-313 lamps are present on the sides of Chamber 1, about 10 cm from the Teflon film walls. Cellulose acetate film (CA, supplied by McMaster-Carr, New Brunswick, NJ) used as a light filter for wavelengths below 295 nm (McLeod, 1997; Holmes, 2002). Wavelengths below 295nm are not present in the ground level solar spectra and therefore these wavelengths were removed to ensure that they did not catalyze any type of reaction that we were not aware of and that does not occur in the troposphere.

Excess reactive gases are removed from the first stage reaction mixture (while keeping the secondary particles suspended in air) using a denuder. The reaction mixture that is drawn out of the first chamber passes through a counter-current diffusion denuder that removes 80-90% of the SO₂, NO_x, and ozone. A second denuder system is employed downstream of the second chamber to remove excess gas-phase organics and ozone, as well as to further reduce SO₂ and NO_x concentrations prior to animal exposures.

Exposure Measurements. Analytical measurement of the exposure atmospheres was extensive, and sampling was carried out at a number of locations in the chamber/denuder system. At the animal exposure chambers, the following measurements were carried out:

Continuous Measurements

- PM_{2.5} mass, using an R&P Tapered Element Oscillating Microbalance (TEOM)
- Particle number, using a condensation particle counter (CPC TSI 3022)
- SO₂ (pulsed fluorescence method)
- NO_x (chemiluminescence method)
- O₃ (UV absorbance method)
- Temperature
- Relative humidity (RH)

Integrated Measurements

- PM_{2.5} mass (gravimetric analysis; Teflon filters)
- Particle sulfate (denuder/filter pack system, ion chromatography)
- Particle nitrate (denuder/filter pack system, ion chromatography)
- Particle strong acidity (denuder/filter pack system, pH Analysis)
- Particle ammonium (denuder/filter pack system, ion chromatography)
- Particle elements (X-ray fluorescence)
- EC/OC (thermal optical reflectance [TOR] method; quartz fiber filters)
- Sulfur dioxide (denuder/filter pack system, ion chromatography)
- Nitric acid vapor (denuder/filter pack system, ion chromatography)
- Nitrous acid vapor (denuder/filter pack system, ion chromatography)
- Ammonia (denuder/filter pack system, ion chromatography)
- Ketones and aldehydes (DNPH cartridges)
- α -pinene (Tenax tubes)

Animal Exposure Laboratory. From the reaction chamber, aged emissions entered a temperature- and humidity-controlled exposure chamber located in the mobile toxicological laboratory. This laboratory is comprised of a trailer outfitted with alarm systems and added

electrical systems. The Harvard Animal Resource Committee (ARC) inspected the facility and approved it for use in field studies using animals.

Toxicological Methods. Pulmonary, cardiac, and systemic effects in female Sprague-Dawley rats were evaluated via bronchoalveolar lavage (BAL), histopathology, pulmonary function, *in vivo* oxidative stress, and blood cytology. Each scenario included 4 days of exposures, each with 5 rats (2 for *in vivo* oxidative stress and 3 for the other biological endpoints). Thus, for each scenario there were 6 rats in the oxidative stress group and 9 rats in which pulmonary function, BAL, and blood cytology are assessed. Animals were placed into modified whole-body plethysmographs during exposure. Exposures were 6 hours in duration. Animals were maintained and studied in accordance with the National Institutes of Health guidelines for the care and use of animals in research. All protocols were approved by the Harvard Medical Area Standing Committee on Animals.

Pulmonary Function and Breathing Pattern. Pulmonary function and breathing pattern were assessed using an automated software system (Buxco Biosystem 1.5.3A, Buxco Electronics, Sharon, CT), which calculates a number of respiratory parameters from flow changes in a pressure transducer connected to the plethysmograph. Markers of interest include peak expiratory flow (PEF), tidal volume (TV), respiratory frequency (*f*), and minute ventilation (MV).

Bronchoalveolar Lavage. BAL was performed through a tracheal incision. The first lavage was 4 ml; subsequent lavages were ~5 ml, based on the body weight of the animals. Cell viability (> 95%) and total cell count were determined by hemacytometer counts of small aliquots of the resuspended BAL fluid diluted in trypan blue solution. Cell type was determined from modified Wright-Giemsa-stained cytocentrifuge preparations; 200 cells were counted per sample. Within the acellular BAL supernatant, three markers of pulmonary injury were tested: (1) lactate dehydrogenase (LDH) as an indicator of cytotoxicity; (2) a lysosomal enzyme, β -n-acetyl glucosaminidase (β NAG), as a marker of phagocyte activation and lysing; and (3) total BAL protein as a marker of pulmonary inflammation and vasculature permeability. BAL fluid samples were frozen and stored for possible future analysis of cytokines or other inflammatory mediators.

Histopathology. At autopsy, lungs were fixed with 2.5% glutaraldehyde via the airways at 20 cm of H₂O. Total lung volumes were determined by displacement, and the lungs were cut horizontally into 2 mm numbered sections. Three slices were randomly selected for processing by paraffin histology techniques.

In Vivo Oxidative Stress. Organ chemiluminescence (CL) refers to the ultra-weak light emission produced by biological systems due to the de-excitation of high-energy by-products of the chain reaction of lipid peroxidation (Boveris and Cadenas, 1999; Boveris et al., 1980). Organ CL has been successfully used in models of oxidative injury in the intact lung (Gurgueira et al., 2002; Evelson et al., 2000; Turrens et al., 1988) as well as in the perfused lung *in vitro* (Barnard et al., 1993). After the exposure, the animal was anesthetized with pentobarbital (0.25mg/kg). A surgical procedure was performed to expose the heart and/or lungs to the counter of intrinsic chemiluminescence. In a dark field, the counter measured the chemiluminescence for 10 seconds, which was then sent to an amplifier to the computer, where the calculations were performed and expressed as counts per second per square centimeter (cps/cm²).

Blood Cytology. Blood cytology was evaluated 24 hours following the last day of exposure. Rats were euthanized with an overdose of sodium pentobarbital (65 mg i.p.). Blood was obtained

by cardiac puncture. A 1 ml aliquot of whole blood was collected in a 1.5 ml EDTA-treated collection tube to prevent clotting. Total white blood cell counts (WBCC) and differential profiles were assessed.

Statistical Analysis. For each biological endpoint, analysis of variance (ANOVA) tests were employed with SAS computer software to compare intra-animal alterations in physiological parameters due to exposure. Two-way ANOVA determinations were employed to determine if intra-group differences were significant. Differences are considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

At Plant 1, animal exposures were carried out between May and November, 2004, as summarized below in Table 2.

Table 2. Schedule of completion of fieldwork. Note that the neutralized secondary particle scenario with secondary organic aerosol was repeated 3 times.

Exposure Round	Scenario	Dates
1	Primary	May 10, 11, 12 and 13
2	Secondary + NH ₃ + SOA (run 1)	June 22, 23, 25 and 26
3	Secondary + NH ₃ + SOA (run 2)	June 27, 28, 29 and 30
4	Secondary + SOA	October 4, 5, 6 and 7
5	Secondary + NH ₃ + SOA (run 3)	October 11, 12, 13 and 14
6	Secondary	November 13, 14 and 15

Stack Sampling Results. The objective of the stack sampling was to evaluate possible differences between in-stack primary PM_{2.5} concentrations and the diluted concentrations used in the animal exposures. Low primary particle concentrations were found in the exposure scenarios conducted in May, based on the measurements of diluted samples determined by multiplying the measured concentrations in the diluted samples entering the reaction chamber by the dilution factor (about 150). Therefore, it was important to assess whether particle losses were occurring during dilution, and how the composition of the two types of samples (direct stack sampling and dilution sampling for animal exposures) might differ. In-stack sampling was carried out using a PM_{2.5} cyclone with a filter holder placed inside the duct. Samples were collected on quartz fiber filters for periods of up to 4 hours (USEPA Conditional Test Method 040, December 3, 2002, *Method for the Determination of PM₁₀ and PM_{2.5} Emissions*, www.epa.gov/ttn/emc/ctm/ctm-040.pdf). Results indicated no significant differences between the in-stack measurements and the dilution samples, leading us to the conclusion that the sampled emissions are representative of those exiting the stack into the atmosphere.

Exposure Characterization Results. Summary results for PM-related measurements and gases are provided in Tables 3 and 4, respectively, for all six experimental rounds at Plant 1.

Table 3. PM species concentrations, May-November, 2004. Mean and SD shown for each sampling round.

Exposure Parameter/Units	Concentrations					
	Round					
	1	2	3	4	5	6
	Primary	Secondary + NH ₃ + SOA	Secondary + NH ₃ + SOA	Secondary + SOA	Secondary + NH ₃ + SOA	Secondary
Mass ¹ µg/m ³	2.3 ± 2.6	255.6 ± 27	241.1 ± 35.2	192.6 ± 73.3	141.2 ± 15.9	69.5 ± 10.4
Mass ² µg/m ³	-0.2 ± 3.3	225.6 ± 42.1	178.5 ± 38.7	138.2 ± 53.4	116.8 ± 25.2	58.2 ± 5.8
GMD nm	380.3 ± 109.1	391.1 ± 14.5	389.4 ± 28.1	442.3 ± 37.5	373.1 ± 25.6	419.4 ± 45.7
Number Concentration #/cm ³	1726 ± 1277	46892 ± 3905	42991 ± 2809	16924 ± 4495	66445 ± 8913	6723 ± 3550
Total Sulfate µg/m ³	0.7 ± 0.6	96 ± 18	76.9 ± 23.8	57.1 ± 24	38.7 ± 11	31.8 ± 1.3
Acid Sulfate µg/m ³	1.2 ± 0.2	27.3 ± 13.6	11.9 ± 7.7	49.1 ± 22.7	1.6 ± 1.7	22.5 ± 4
Nitrate µg/m ³	0.6 ± 0.5	24.9 ± 3.1	32.2 ± 8.6	1 ± 0.4	37.7 ± 6.2	1.1 ± 1.2
Ammonium µg/m ³	0.3 ± 0.3	25.8 ± 2.1	24.5 ± 5.6	3.1 ± 1.2	14.7 ± 4.1	3.3 ± 1.7
OC µg/m ³	24.6 ± 12.2	83.6 ± 24.4	62.9 ± 5.7	86.7 ± 7.1	57.6 ± 6.5	23.2 ± 7.4
EC µg/m ³	-1.4 ± 19.3	3.1 ± 38.6	3.8 ± 9	9.7 ± 11.2	1.9 ± 10.3	1 ± 11.6

¹ Continuous

² Integrated

Table 4. Gas concentrations, May-November, 2004. Mean and SD shown for each sampling round.

Compound	Concentrations					
	Round					
	1	2	3	4	5	6
	Primary	Secondary + NH ₃ + SOA	Secondary + NH ₃ + SOA	Secondary + SOA	Secondary + NH ₃ + SOA	Secondary
SO ₂ (ppb) ¹	5.3 ± 4.4	11.5 ± 0.3	9.7 ± 4.1	17.5 ± 4.4	16 ± 3	9.3 ± 3.5
SO ₂ (ppb) ²	- ³	31.5 ± 0.6	31.3 ± 5.9	38.9 ± 8.3	40.8 ± 3.8	31.7 ± 4.3
HNO ₃ (ppb)	0.7 ± 0.4	2.6 ± 0.2	2.2 ± 0.4	1.6 ± 0.3	2.3 ± 0.7	0.6 ± 0.1
HONO (ppb)	2.7 ± 2.4	7.4 ± 1.6	9.8 ± 3.7	11.2 ± 5.1	7.8 ± 1.5	5 ± 1
NH ₃ (ppb)	26 ± 26.5	0.7 ± 0.2	0.8 ± 0.4	20.8 ± 3.8	16.1 ± 6.2	9.9 ± 6.2
NO(ppb)	5.9 ± 3.7	3 ± 1.2	3.6 ± 2.1	3.5 ± 2.9	4.6 ± 1.2	3.9 ± 0.5
NO ₂ (ppb)	6.7 ± 1.7	19.9 ± 2.2	20.8 ± 7.8	17.5 ± 6.6	10.1 ± 4.2	8.4 ± 1.8
O ₃ (ppb)	1 ± 1.2	34.9 ± 3.5	29.3 ± 2	26.8 ± 6.9	15.6 ± 6	26.9 ± 1
Formaldehyde (µg m ⁻³)	-	24.9 ± 5.9	20.3 ± 4.1	16.1 ± 3.6	18.1 ± 3.9	-
Acetaldehyde(µg m ⁻³)	-	6.4 ± 1.5	4.6 ± 0.2	5.2 ± 1	4.8 ± 0.6	-
Acetone (µg m ⁻³)	-	16.6 ± 1.9	31.8 ± 16.2	15.5 ± 5.2	13 ± 2.9	-
Total carbonyls (µg m ⁻³)	-	47.8 ± 7.7	56.7 ± 14.6	36.8 ± 9.2	35.9 ± 5.3	-
α-Pinene (µg m ⁻³)	-	0.6 ± 0.1	1.2 ± 0.9	0.6 ± 0.1	0.8 ± 0.3	-

¹ Continuous

² Integrated

³ Monitor not working

In general, concentrations of PM measures were in the range of expected values (Table 3). PM_{2.5} mass was variable across the different scenarios investigated, with values ranging from 2.3 µg/m³ to 256 µg/m³. The low value for the primary particle scenario is not surprising (Round 1), given the known high efficiency of the electrostatic precipitator. In Rounds 2 and 3 (neutralized secondary particles + SOA), acidity was low and OC was high, as expected. The sum of sulfate and OC approximated the total PM_{2.5} mass. In Round 4 (unneutralized secondary particles + SOA), acidity was high, as expected, as was OC, whereas ammonium was very low. During Round 5 (neutralized secondary particles + SOA), there were no qualitative or quantitative changes in the composition of PM compared with Rounds 2 and 3 conducted in June/July. Acidity was low and OC was high, and the sum of sulfate and OC approximated the total PM_{2.5} mass. OC was not as high as for the earlier run in October. There was also an unexplained decrease in the total secondary aerosol and sulfate generated, and an increase in nitrate. During Round 6 (unneutralized secondary particles), PM mass was also lower than expected. It is unclear why OC is elevated in this scenario without secondary organic aerosol. It may be that VOCs or SVOCs adsorbed onto chamber walls and other surfaces in the earlier tests volatilized subsequently and were collected on the quartz filters. Again, as with Round 5, sulfate was lower

than expected. The measured elemental carbon that was observed is not likely to have originated in the stack gas (given that the overall dilution from stack to exposure chamber is 1500-2000 times) and we were also informed by the plant operators that with the ESPs used, no measurable EC is emitted. However, although ESPs do remove EC-containing particles, they do so with less efficiency than for non-conductive particles, so some EC particles do penetrate. Also, the plant operator's method of measuring EC is much less sensitive than the TOR method. Moreover, none of the chemical reactions are expected to produce EC. However, the measured OC values are quite a bit higher than the EC values. It is quite reasonable to assume that the EC values result from the uncertainty in the thermal optical reflectance method. As the OC is heated, some of it forms a char containing elemental carbon. The method is supposed to correct for this by measuring the change in optical reflectance. If the change in optical reflectance due to charring underestimates the amount of OC that charred, then the remaining char will give a positive artifact EC value.

Concentrations of pollutant gases were low in all six experimental runs (Table 4). This is important given the effects of ozone, NO₂, and SO₂ on respiratory endpoints.

All elements were present at low concentrations, with the exception of sulfur, which was present in oxidized emissions samples at 10 – 43 µg/m³ (data not shown). Silicon, calcium, and bromine were commonly detected in multiple samples. Less commonly observed elements included Mg, Al, Cl, K, Cr, Fe, Ni, Cu, Zn, Se, Ba, and Hg.

Toxicological Results. The toxicological results for all experiments are presented below. In the case of the most complex scenario, which was carried out in triplicate, all animals were combined. The total number of animals for each scenario is shown in Table 5.

Table 5. Number of experimental animals per scenario.

Scenario	Respiratory Parameters		BAL Parameters		Blood Parameters	
	Control	Exposed	Control	Exposed	Control	Exposed
Primary	20	20	0	0	12	12
Secondary	15	15	5	5	9	9
Secondary + SOA	60	60	18	18	36	36
Secondary + NH ₃ + SOA	20	20	6	6	12	12

Pulmonary Function and Breathing Pattern

No differences between exposed and control animals were observed for any of the pulmonary function/breathing pattern parameters examined. Figure 4 shows results for Enhanced Pause.

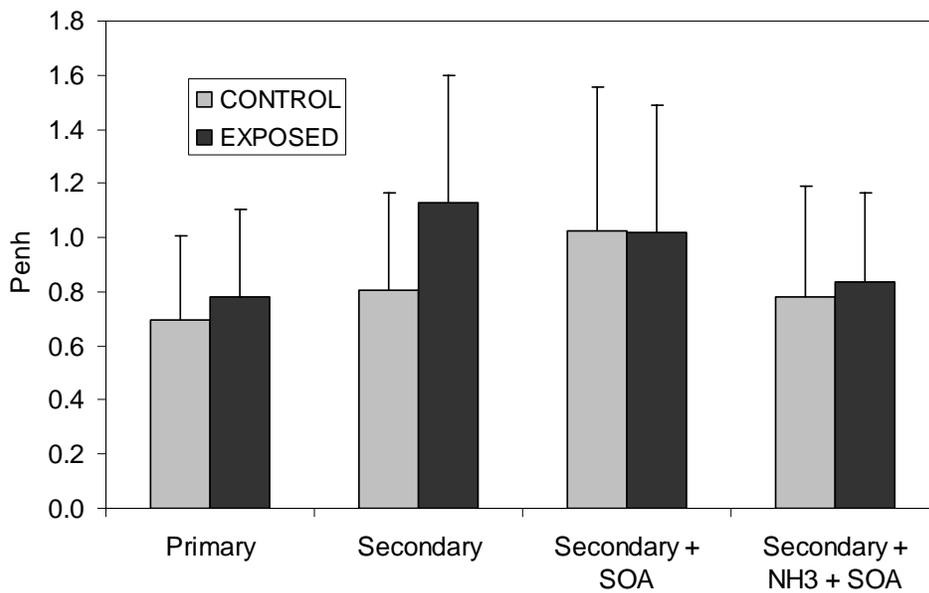


Figure 4. Enhanced Pause (Penh) as a measure of bronchoconstriction in Sprague-Dawley rats exposed to different power plant emission scenarios, May-November, 2004.

Bronchoalveolar Lavage

Selected results of the BAL fluid analyses are shown in Figure 5. No significant differences between exposed and control animals were observed for cytological parameters (total cell count, neutrophils, macrophages, lymphocytes, eosinophils, epithelial cells) or biochemical markers (LDH, β NAG, and total protein).

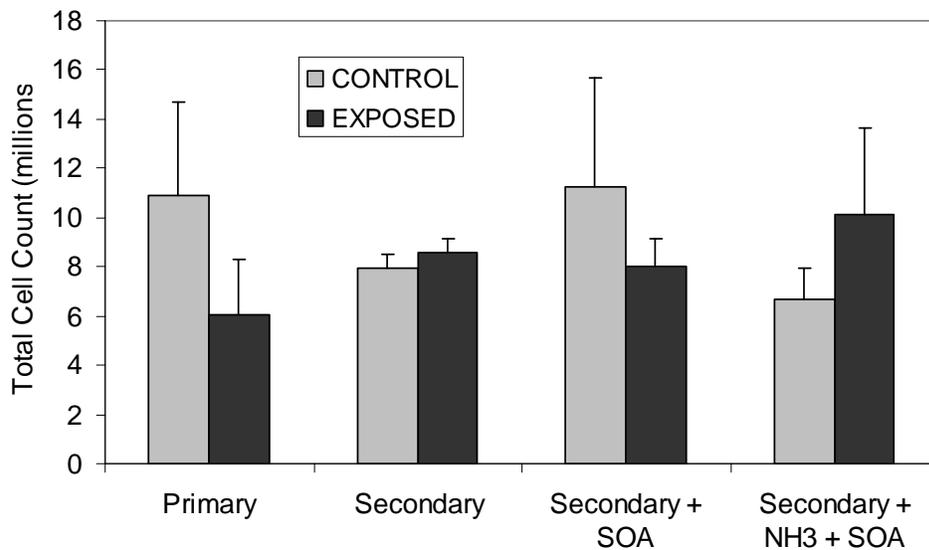


Figure 5. Total cell count in BAL fluid from Sprague Dawley rats after exposure to different power plant emission scenarios, May-November, 2004.

Blood Cytology

Results of selected blood cytological analyses are provided in Figure 6 below. No significant differences between exposed and sham animals were observed for Hgb & Hct, platelet count, white blood cell count, neutrophils, lymphocytes, monocytes, or eosinophils.

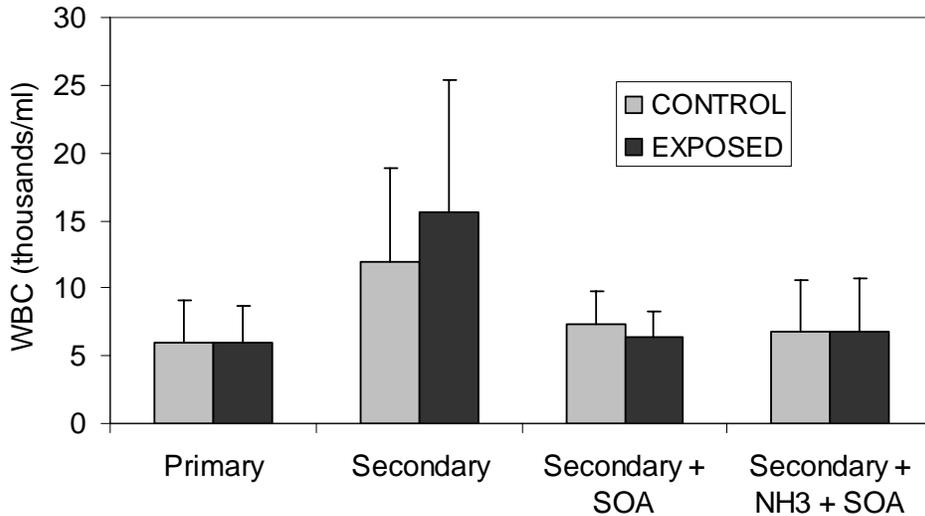


Figure 6. White blood cell counts, Sprague-Dawley rats after exposure to different power plant emission scenarios, May-November, 2004.

In Vivo Oxidative Stress

Oxidative stress was determined using in vivo chemiluminescence of heart and lung tissue. In addition, to confirm the chemiluminescence findings, the TBARS (thiobarbituric acid reactive substances) assay was carried out for the two scenarios completed in October. Results are shown in Figures 7 and 8.

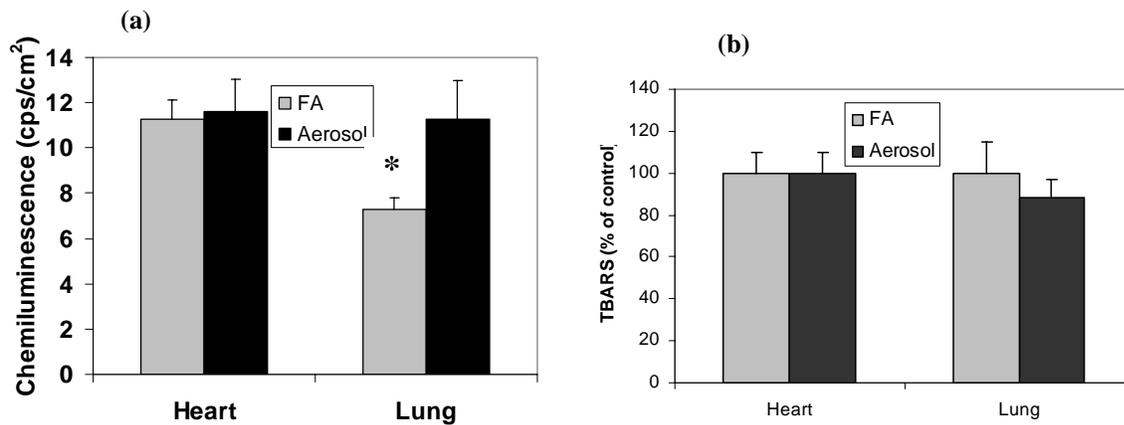


Figure 7. Oxidative stress in Sprague-Dawley rats exposed to oxidized, neutralized emissions and secondary organic aerosol. (a) Chemiluminescence, pooled animals, June and October, 2004. (b) TBARS, October, 2004. * indicates significant difference between sham and exposed animals ($p < 0.05$) using a 2-tailed t-test.

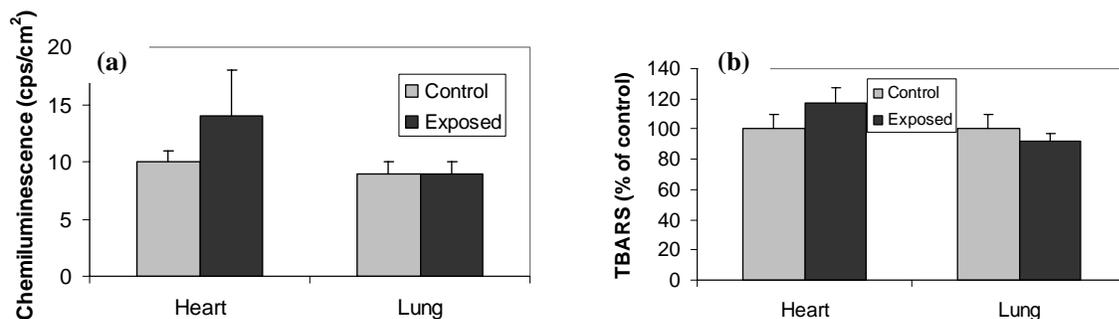


Figure 8. Oxidative stress in Sprague-Dawley rats exposed to oxidized emissions and secondary organic aerosol, October 4-7, 2004. (a) Chemiluminescence. (b) TBARS.

For the combined (pooled) June and October exposures to the most complex scenario (oxidized, neutralized + SOA), a difference in the chemiluminescence lung response was observed in the exposed group (Figure 7). However, although the difference was statistically significant, this difference was primarily driven by the lower chemiluminescence values observed in control animals during the October exposures. When compared with the pooled data for all the control animals, or with the data for control animals exposed to this scenario in June, the aerosol exposed group showed no significant increase in chemiluminescence.

No significant differences between exposed and sham animals were observed following exposure to secondary particles + SOA (Figure 8) or secondary particles alone scenarios.

Histopathology

Histopathological analyses to assess evidence of inflammation in lung airways and parenchyma, and vasoconstriction in lung and cardiac blood vessels, were carried out. Results showed no evidence of such effects.

CONCLUSIONS AND FUTURE DIRECTIONS

We investigated four exposure scenarios at a power plant in the Upper Midwest burning Powder River Basin coal, and no adverse biological effects were observed. Results indicated no differences between exposed and control animals in any of the endpoints examined. Exposure concentrations for the scenarios utilizing secondary particles (oxidized emissions) ranged from 70 - 256 $\mu\text{g}/\text{m}^3$, and some of the atmospheres contained high acidity levels (up to 49 $\mu\text{g}/\text{m}^3$ of equivalent unneutralized H_2SO_4). Fieldwork at Plant 2 in the Southeast is nearing completion and exposure and toxicological data analyses are underway.

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