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

TOPICAL REPORT: PLANT 1 (SOUTHEAST)

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ABSTRACT

TERESA (Toxicological Evaluation of Realistic Emissions of Source Aerosols) involves exposing laboratory rats to realistic coal-fired power plant and mobile source emissions to help determine the relative toxicity of these PM sources. There are three coal-fired power plants in the TERESA program; this report describes the results of fieldwork conducted at the second plant, located in the Southeastern United States.

The project was technically challenging by virtue of its novel design and requirement for the development of new techniques. By examining aged, atmospherically transformed aerosol derived from power plant stack emissions, we were able to evaluate the toxicity of PM derived from coal combustion in a manner that more accurately reflects the exposure of concern than existing methodologies. TERESA also involves assessment of actual plant emissions in a field setting – an important strength since it reduces the question of representativeness of emissions.

Seven sets of animal exposures were carried out from March-September 2005 to a number of simulated atmospheric scenarios. Toxicological endpoints included (1) pulmonary function and breathing pattern; (2) bronchoalveolar lavage fluid cytological and biochemical analyses; (3) blood cytological analyses; (4) *in vivo* oxidative stress in heart and lung tissue; (5) heart and lung histopathology; and (6) cardiac function via telemetry and electrocardiogram data collection.

Continuous exposure data collected included RH, temperature, PM mass (TEOM), ozone, NO, NO_2, SO_2, and particle count. Particle number concentrations were lowest (910 cm^{-3}) for the primary particle scenario (P) and highest (40,811 cm^{-3}) for the most complex neutralized scenario (PONS). Mass concentrations ranged from 13.9 $\mu g/m^3$ for the P scenario to 385 $\mu g/m^3$ for one of the oxidized emissions + SOA scenarios (POS). Substantial day-to-day variability was observed in $PM_{2.5}$ mass concentrations, likely due to the inherent variation in the power plant operation. Concentrations of ozone, NO_x and SO_2 were below 50 ppb. Integrated measurements indicated that sulfate concentrations ranged from 82 to 175 $\mu g/m^3$, while nitrate was low in all scenarios except the neutralized scenario (PONS). Ammonium was similarly low in all scenarios except PONS. Higher-than-expected EC and OC concentrations are likely to be an artifact due to the use of filtered room air for flushing the denuders. Elemental data suggest substantial day-to-day variability in concentrations. All elements had low concentrations except for sulfur. Prominent among these were: Si, Br, Ca, K, La and Cu. Few other elements were found to be present during specific exposure rounds.

Pulmonary function data suggest subtle changes in some respiratory parameters in some scenarios. The *in vivo* chemiluminescence (CL) dataset for Plant 1 suggests that both lung and heart oxidative stress occur in response to several scenarios. No changes in histology, bronchoalveolar lavage fluid, or blood cytology were evident. Stage II assessments conducted for the PONS scenario at Plant 1 suggest no apparent effect on heart rate or on several measures of heart rate variability. However, this scenario resulted in an increase in cardiac arrhythmias (premature ventricular beats; PVBs) in exposed animals compared to sham/control animals.

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EXECUTIVE SUMMARY

Much of the research on the health effects of power plant emissions has used coal fly ash or a pilot combustor, neither of which accurately reflects exposure to the secondary particulate matter (PM) formed through atmospheric oxidation of power plant emissions. The TERESA project involves exposing laboratory rats via inhalation to realistic coal-fired power plant and mobile source emissions to help determine the relative toxicity of these PM sources. The emissions are then extensively characterized to provide insight into the gas- and particle-phase components contributing to toxicity. Multiple pulmonary and cardiovascular toxicological endpoints are evaluated. The primary objective of the project is to increase understanding of the PM sources and components responsible for adverse health effects, specifically as these relate to coal combustion and mobile source emissions. There are three coal-fired power plants in the TERESA program; this report describes the results of fieldwork conducted at the second plant, located in the Southeast.

The project was technically challenging by virtue of its novel design and requirement for the development of new techniques. Previous studies have either involved instillation of collected coal fly ash, or have carried out inhalation exposures to emissions from lab-scale combustors. Neither of these approaches accurately simulates population exposures to atmospheric PM derived from coal combustion, largely because with the widespread introduction of particulate controls on power plants, primary PM emissions are very low. It is the secondary particulate matter formed from SO_2 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, the subject project has been able to evaluate the toxicity of coal combustion emissions in a manner that more accurately reflects the exposure of concern. The subject study also involves assessment of actual plant emissions in a field setting – an important strength since it reduces the question of representativeness of emissions.

Initially, a sampling system consisting of a venturi orifice and aspirator was assembled to draw emissions from the stack. However, after testing the equipment at the plant, it was suspected that primary particle losses may have been occurring in the sampler, and the sampling system was redesigned. The modified system resulted in no substantial increase in particle concentration in the emissions. This observation, coupled with stack sampling conducted according to standard EPA protocol, led us to the conclusion that the sampled emissions are representative of those exiting the stack into the atmosphere.

Two mobile laboratories were outfitted for the study: (1) chemical laboratory in which the atmospheric aging was conducted and which housed the bulk of the analytical equipment; and (2) toxicological laboratory, which contained animal caging and the exposure apparatus.

Animal exposures began in March 2005, and were carried out as follows:

- March 21-24: oxidized emissions + secondary organic aerosol (SOA)
- May 3-6: oxidized emissions + SOA (repeated)
- May 9-12: oxidized emissions
- May 31-June 3: oxidized emissions + ammonia + SOA
- June 6-9: primary emissions
- July 8 and 13: oxidized emissions + SOA (MI rats)
- September 9 and 9: oxidized emissions + SOA (MI rats) (repeated)

Toxicological endpoints included (1) pulmonary function and breathing pattern; (2) bronchoalveolar lavage fluid cytological and biochemical analyses; (3) blood cytological analyses; (4) *in vivo* oxidative stress in heart and lung tissue; and (5) heart and lung histopathology.

Pulmonary function data suggest subtle changes in some respiratory parameters in some scenarios. The *in vivo* chemiluminescence (CL) dataset for Plant 1 suggests that both lung and heart oxidative stress occur in response to several scenarios. No changes in histology, bronchoalveolar lavage fluid, or blood cytology were evident. Stage II assessments conducted at Plant 1 for the PONS scenario (primary + oxidized + NH_3 + SOA) suggest no apparent effect of any of the scenarios on heart rate or on several measures of heart rate variability. However, this scenario resulted in an increase in cardiac arrhythmias (premature ventricular beats; PVBs) in exposed animals compared to sham/control animals.

1.0 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 study 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 (gasoline and/or diesel engine) emissions and their oxidative 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 emissions, 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 emissions to human population exposure is unclear, since primary PM emissions are now very low with the widespread introduction of particulate controls on power plants. It is the <u>secondary</u> particulate matter formed from SO_2 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 enabling the determination of 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. The fact that TERESA involves assessment of actual plant emissions in a field setting is an important strength of the study, since it directly addresses the 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 to simulate oxidative atmospheric chemistry, and both primary and secondary materials are extensively characterized, including NO_2, SO_2, ozone, NH_3, hydrocarbons, particle number and mass (including ultrafines), sulfate, nitrate, elemental/organic carbon (EC/OC), ammonium, and metals. Test atmospheres containing depleted emissions and emission oxidative products are utilized in two toxicological assessment steps, the first utilizing normal laboratory rats, and the second consisting of a comprehensive toxicological evaluation in a rat model of susceptible individuals. This last step includes telemetric methods for the assessment of cardiac function.

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 ambient particles; (2) provide insight into the effects of

atmospheric conditions on the formation and toxicity of secondary particles from coal combustion and mobile source emissions through the simulation of multiple atmospheric conditions; (3) provide information on the impact of coal type and pollution control technologies on emissions toxicity; and (4) provide insight into toxicological mechanisms of PM-induced effects, particularly as they relate to susceptible subpopulations. The study findings will help to answer questions regarding which constituents of PM are responsible for the negative health outcomes observed, the likely sources of these constituents, and the degree to which further regulation of PM will improve human health.

There are three coal-fired power plants in the TERESA program. This topical report presents results from the second plant, located in the Southeast.

2.0 EXPERIMENTAL METHODS

2.1 Emissions Sampling System

The emissions sampling system is described in detail in a manuscript currently in preparation (Ruiz et al., 2005a), and was also described in the topical report prepared for the first plant. Therefore, the details are not repeated here. The final dilution sampling scheme employed is shown in Figure 1.

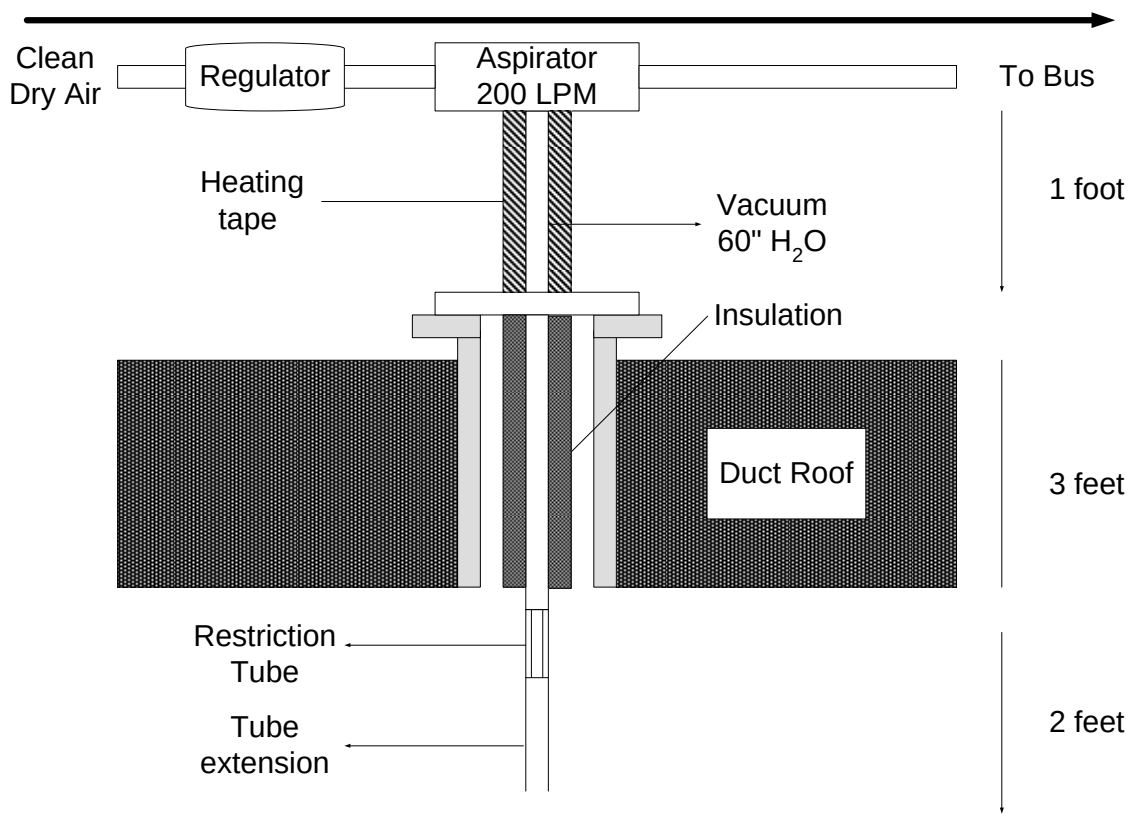


Figure 1. Final configuration of emissions sampling system.

2.2 Atmospheric Reaction Simulation System

The atmospheric reaction simulation system is described in detail in a manuscript currently under review (Ruiz et al., 2005b), and was also described in the topical report for the first plant. In brief, the system consists of dual chambers (Figure 2). 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 -- produced from the reaction of water vapor with $O(^1D)$ from the photolysis of ozone by UV light -- to form H_2SO_4. Relatively high intensity 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.

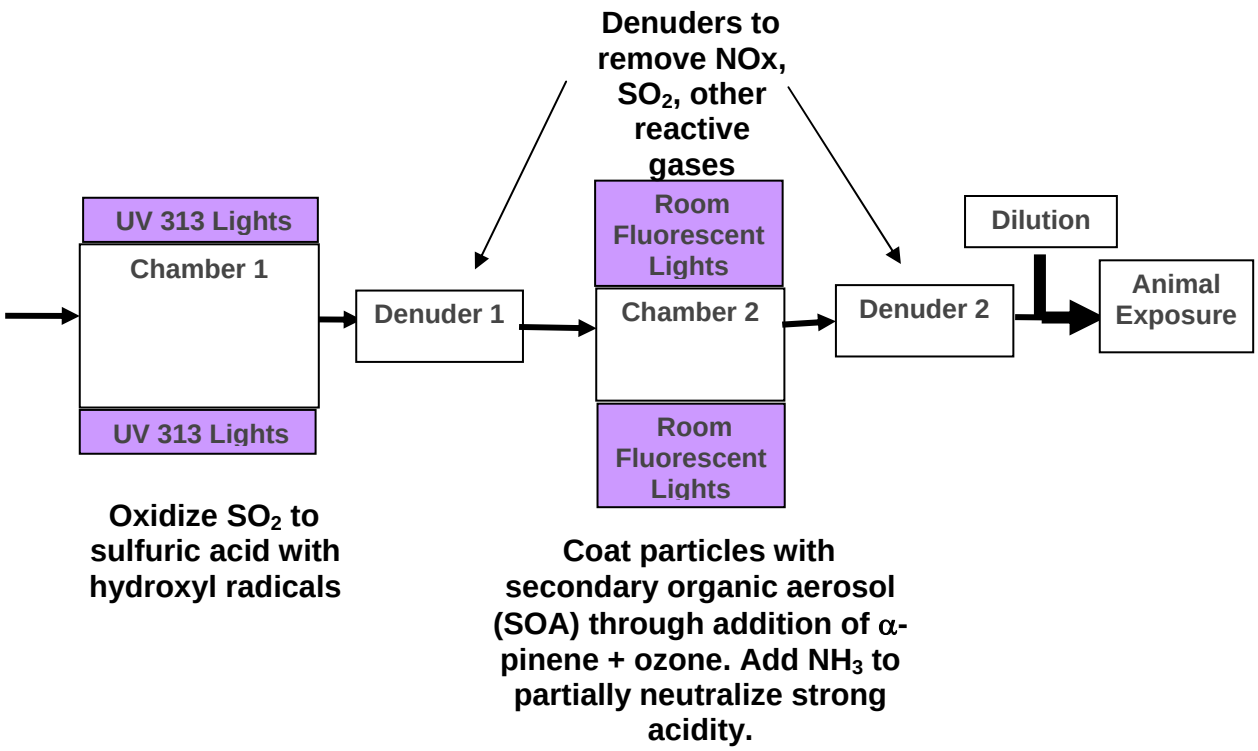


Figure 2. Dual chamber system for atmospheric simulations.

Removal of Excess Reactive Gases

Excess reactive gases are removed from the first stage reaction mixture (while keeping the secondary particles suspended in air) using denuders. The denuder system is described in detail in Ruiz et al. (2006); additional detail is provided in the topical report for the first plant. The reaction mixture that is drawn out of the first chamber passes through a counter-current diffusion denuder that removes 80-90% of the SO_2, NOx, 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 SO_2 and NOx concentrations prior to animal exposures.

2.3 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 (Figure 3). For the purposes of this report, the measurements at the animal exposure chambers are of greatest interest. At this sampling port, the following measurements were carried out:

<u>Continuous Measurements</u>

- $PM_{2.5}$ mass, using an R&P Tapered Element Oscillating Microbalance (TEOM)
- Particle number, using a condensation particle counter (CPC TSI 3022)
- SO_2 (pulsed fluorescence method)
- NO_x (chemiluminescence method)
- O_3 (UV absorbance method)
- Temperature
- Relative humidity (RH)

<u>Integrated Measurements</u>

- $PM_{2.5}$ 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)

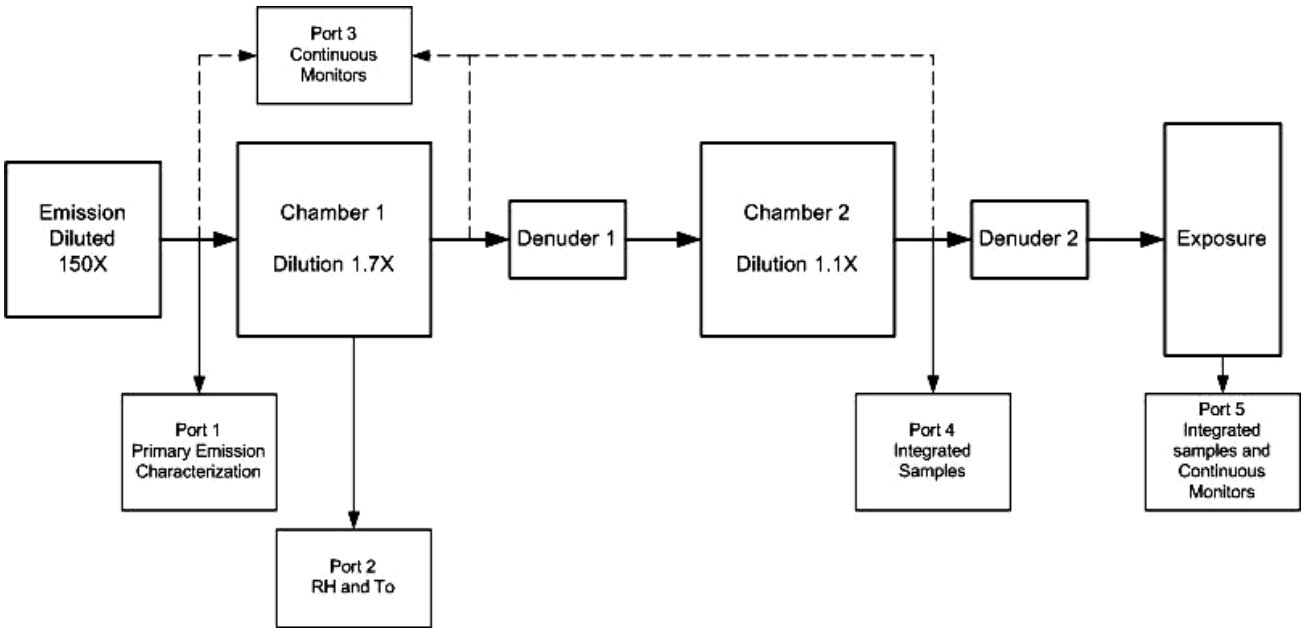


Figure 3. Location of sampling ports.

Originally, an aethalometer was to have been used to measure elemental/black carbon; however, because of the extremely low elemental carbon concentrations expected in the coal combustion emission scenarios, this was not employed. Similarly, CO monitoring, although originally proposed, was not carried out because it was expected to be extremely low after the dilution and denuder steps. Finally, the proposed elemental streaker was not used due to technical problems; however, elemental concentrations on 6-hour integrated samples were determined using XRF.

2.4 Animal Exposure Laboratory

From the reaction chamber, aged emissions enter 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. Because a higher ventilation rate was needed, the trailer had to have a second electric service added to handle the larger heating requirement. This additional electrical capacity also provided more flexibility in the use of auxiliary equipment. The Harvard Animal Resource Committee (ARC) inspected the facility and approved it for use in field studies using animals.

2.5 Toxicological Methods

A two-stage toxicological assessment was conducted. In Stage I, overall cardiac and pulmonary toxicity was determined in normal laboratory rats, followed by a more comprehensive and cardiac-focused Stage II assessment in a compromised rat model. The Stage II assessment was conducted for one specific scenario in which biological effects were observed in Stage I.

All exposures were carried out in male Sprague-Dawley rats. 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 8 rats in the oxidative stress group and 12 rats in

which pulmonary function, BAL, and blood cytology are assessed. For the Stage II assessment, four animals were exposed simultaneously. Animals were placed into modified whole-body plethysmographs during exposure. Exposures were 5 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.

In the Stage I toxicological assessment, pulmonary, cardiac, and systemic effects in normal rats were evaluated via bronchoalveoloar lavage (BAL), histopathology, pulmonary function, *in vivo* oxidative stress, and blood cytology.

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. A rejection algorithm is automatically included in the breath-by-breath analysis. 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 using endotoxin-free Dulbecco's phosphate-buffered saline. 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 re-suspended 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, two markers of pulmonary injury were tested: (1) a lysosomal enzyme, β-n-acetyl glucosaminidase (βNAG), as a marker of phagocyte activation and lysing; and (2) total BAL protein as a marker of pulmonary inflammation and vasculature permeability. Total protein was measured using a standard kit from Pierce (Product #23235; Rockford, IL). Determination of βNAG was done by the methods of Selliger et al. (1960) and Pesce et al. (1964), respectively. The βNAG kit was obtained from Diazyme Laboratories (San Diego, CA), βNAG kit results were read using a spectrophotometer (Beckman Instruments, Fullerton, CA) BAL fluid samples were frozen and stored for possible future analysis of cytokines or other inflammatory mediators (e.g., lactate dehydrogenase; LDH).

Histopathology

At autopsy, lungs were fixed with 2.5% glutaraldehyde via the airways at 20 cm of H_2O. 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 measures the steady-state concentration of singlet oxygen ($^1O_2$) and follows the square of the intracellular concentration of H_2O_2. The latter constitutes a unique experimental advantage of the technique, since small variations in H_2O_2 are exponentially reflected in the values of CL. 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). Of particular relevance to this project, Gurgueira et al. used this method to assess heart and lung oxidative stress in rats after exposure to concentrated ambient particles (CAPs) and residual oil fly ash (ROFA).

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 photon counter; the experimental setup is shown in Figure 4. Chemiluminescence from the surface of the tissues was measured for 10 seconds and expressed as counts per second per square centimeter (cps/cm^2). After the measurements of CL, the animal was sacrificed by exsanguination, and the heart and the lungs were frozen and shipped to the laboratory for future TBARs analysis.

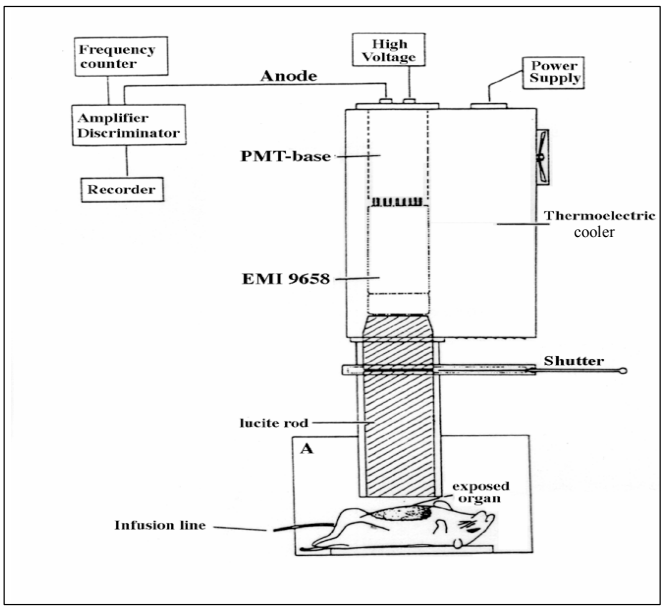


Figure 4. Single-photon counting apparatus used in chemiluminescence assay (from Boveris et al., 1980)

Blood Cytology

Blood cytology was evaluated 24 hours following the 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 at a commercial Veterinary Diagnostic Laboratory (Idexx Preclinical Research Services. North Grafton, MA).

Telemetry and Electrocardiogram Analysis

A myocardial infarction (MI) rat model (Wellenius et al, 2002) was used for the cardiac function analyses. To produce the MI model, the fine tip electrode of a portable high-temperature thermocautery unit was briefly and repeatedly applied to one or more branches of the left coronary artery. Visible discoloration of the affected region indicates that blood flow has been successfully interrupted. Telemeters for electrocardiogram monitoring were surgically implanted in Male Sprague-Dawley rats, heart rhythm was monitored throughout exposure. Blood chemistry and pulmonary function were also evaluated. For the MI exposures, 4 animals were exposed at a time. One exposure scenario (oxidized + SOA; "POS") was assessed (along with sham animals exposed to filtered room air only). The POS scenario was repeated 2 times, for a total sample size of 15 exposed and14 sham animals.

Cardiac function was assessed by electrocardiography (ECG), with endpoints of interest including heart rate, heart rate variability (standard deviation of the normal beat-to-beat intervals; SDNN), and arrhythmias. Pulmonary function was assessed using the BUXCO method as described earlier.

2.6 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 $p < 0.05$. Additional details of specific statistical techniques used for certain biological endpoints are included in the relevant sections below.

3.0 RESULTS AND DISCUSSION

Animal exposures were carried out between March and September, 2005, as summarized below in Table 1. Note the following naming convention introduced to succinctly describe the scenarios:

- P = primary PM
- PO = primary PM + oxidized emissions
- POS = primary PM + oxidized emissions + SOA
- PONS = primary PM + oxidized, neutralized emissions + SOA

Also note that the first POS scenario was completed while the SCR (selective catalytic reduction) for NOx removal was not operational, while the other POS scenario was carried out while the SCR was running. There were no material differences in exposure or toxicology results between these two scenarios.

Table 1. Summary of Plant 1 exposure scenarios and experiments.

Exposure Round	Code	Scenario	Dates	Animal Model
1	POS	Oxidized + SOA (non SCR period)	March 21 – 24, 2005	Normal Rats
2	POS	Oxidized + SOA	May 3 – 6, 2005	Normal Rats
3	PO	Oxidized	May 9 – 12, 2005	Normal Rats
4	PONS	Oxidized + Neutralized + SOA	May 31 – June 3, 2005	Normal Rats
5	P	Primary	June 6 – 9, 2005	Normal Rats
6	POS	Oxidized + SOA	July 8 and 13, 2005	MI Rats
7	POS	Oxidized + SOA	September 8 and 9, 2005	MI Rats

3.1 Stack Sampling Results

Stack sampling was then conducted at Plant 1 from December 13-16, 2004 to evaluate $PM_{2.5}$ mass concentration (Table 2) and to evaluate possible differences between in stack primary $PM_{2.5}$ concentrations and the diluted concentrations used in the animal exposures. 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 PM_{10} and $PM_{2.5}$ Emissions*, www.**epa**.gov/ttn/emc/ctm/ctm-**040**.pdf). Additional discussion of the stack sampling methodology and approach are provided in the topical report for the first plant.

Five samples (3-hour integration period) were collected directly from the stack on quartz fiber filters and subjected to gravimetric and XRF analysis. In contrast to Plant 0, there were no filter and/or particle losses during sampling and shipping at Plant 1, and this is evident from the three field blanks that show a good agreement between on- and off-weights. Sampling and weighing error were within 1% of measurements.

Table 2. Comparisons of gravimetrically-determined and estimated in-stack $PM_{2.5}$. All concentrations in $\mu g/m^3$.

Sample No.	Plant 1		
	Gravimetric Mass	Estimated* Mass	Ratio
1	464	300	0.65
2	1626	780	0.48
3	3900	2637	0.68
4	1749	729	0.42
5	937	414	0.44
Mean	1735	972	0.53
S.D.	1318	953	0.12

* based on the sum of major oxides and trace elements.

The estimated mass concentrations were based on the XRF elemental data using assumed major oxides plus trace elements. These values do not include silicon (since the filters are made of quartz fiber) and some ionic and carbonaceous species. For Plant 1, the estimated mass mean ± standard deviation value was 972 ± 953, and the mean measured gravimetric mass value was 1735 (± 1318) $\mu g/m^3$, resulting in a ratio of estimated to gravimetric mass of 0.53. The remainder of the total mass (~ 47%) can be explained as unanalyzed components such as Si (which cannot be determined since the collection is on quartz fiber filters), ionic species, and carbonaceous species.

3.2 Exposure Characterization Results

Continuous Measurements

Continuous data are provided in Table 3. Exposure parameters measured included RH, temperature, PM mass (TEOM), ozone, NO, NO_2, SO_2, and particle count. Particle number concentrations were lowest (910 cm^{-3}) for the primary particle scenario (P) and highest (40,811 cm^{-3}) for the most complex neutralized scenario (PONS). Mass concentrations ranged from 13.9 $\mu g/m^3$ for the primary particle scenario (P) to 385 $\mu g/m^3$ for one of the oxidized emissions + SOA scenarios (POS). The four exposure rounds conducted for the oxidized emissions + SOA scenario (POS) showed a wide range of mass concentrations (201, 282, 385, and 283 $\mu g/m^3$). Among these four exposure rounds, the first exposure round was conducted when the SCR was not operational. This in turn resulted in lower ratios for SO_2 vs. NO_x in the first reaction chamber, and less sulfate (Table 4), as compared to the subsequent exposure rounds which were operated when the SCR was operational. Higher sulfate production in the later rounds can therefore explain part of the variation observed in mass concentrations. It is important to note that there is a fair amount of day-to-day variation in mass concentration (both continuous and integrated), even within a given exposure round. This is likely due to the inherent variation in the power plant operation.

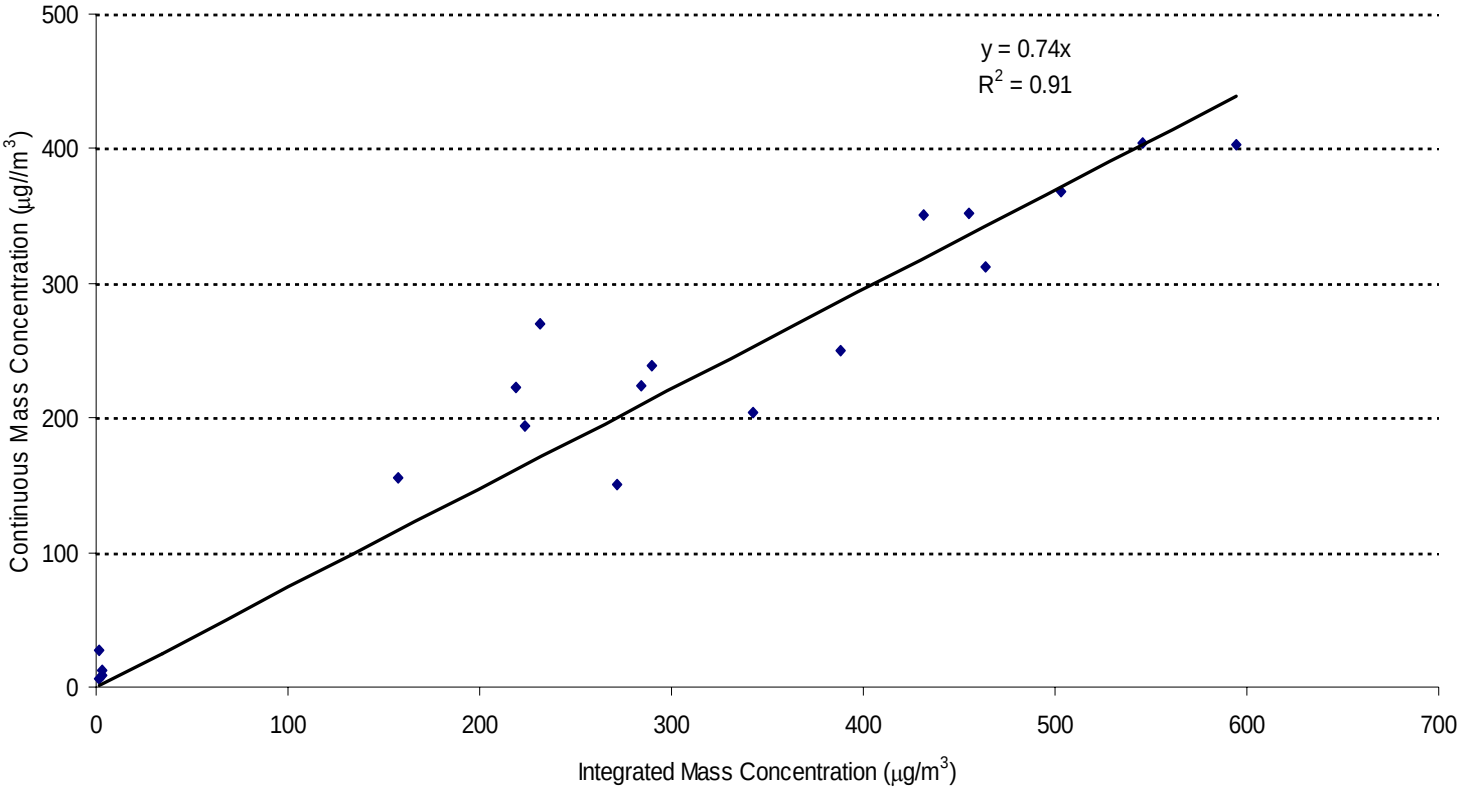


Figure 5. Comparison between mass concentrations measured via continuous and integrated methods.

Figure 5 provides a comparison between mass concentration measured via continuous and integrated methods. It is evident that there is good agreement, and the TEOM (continuous) measures on average 74% of the integrated particulate mass. At the exposure end, barring rounds 1 and 3, RH in general remained around 53% and temperature was steadily maintained at an average value of 23°C. Also, as specifically required for the toxicological tests, the gas concentrations for ozone, NO_x and SO_2 were kept below 50 ppb (Table 3).

Integrated Measurements

Integrated measurements obtained are shown in Table 4. Total sulfate concentration ranged from 82 to 175 µg/m^3. Nitrate was low in all scenarios, but highest in the neutralized scenario (PONS). Ammonium was similarly low in all scenarios except the neutralized run (PONS). The extraordinarily high EC and OC concentrations seem incorrect. We are unable to explain these anomalies at present. Gas concentrations, including carbonyls, were kept below 50 ppb (Table 3).

Elemental Measurements

Elemental data obtained from integrated measurements performed at Plant 1 are presented in Table 5. The complete dataset is presented instead of summary statistics to clearly depict substantial day-to-day variations recorded for the elemental concentrations which again provide insight about the inherent variations attributed to plant operation. The values are bold for those that are at least twice the uncertainty values. However, there may be some usefulness for values

less than twice the uncertainty, so they are also included in the table. Also, note that each sample has a different set of uncertainty values because with XRF, the uncertainty for each element is related to corrections for interference by a different set of elements, and the distribution of element magnitudes is different for each sample. All elements had low concentrations except for sulfur and the most prominent of these were: Si, Br, Ca, K, La and Cu.

SOA Speciation

SOA analysis of PM collected on Teflon filters was performed using GC-MS. Only 2 representative filters were selected, one each from the POS and PONS scenarios. In addition, one field blank was analyzed to correct for background. Table 6 shows the results for concentrations of SOA components. Typical products of α-pinene oxidation were observed for both scenarios, with cis-pinic acid being the most prominent species. The sum of the identified SOA components contributed about 46% and 57% of the corresponding OC mass concentrations for the PONS and POS scenarios, respectively.

Table 3. Continuous measurements during experimental runs at Plant 1, March – September, 2005. Rounds 1-5 were four days in duration; Rounds 6 and 7 were two days in duration. Values expressed as mean ± SD.

Exposure Parameter	Round 1 (POS) Oxidized + SOA	Round 2 (POS) Oxidized + SOA	Round 3 (PO) Oxidized	Round 4 (PONS) Oxidized + NH3+ SOA	Round 5 (P) Primary	Round 6 (POS) Oxidized + SOA	Round 7 (POS) Oxidized + SOA
RH (%)	70.4 ± 2.7	53.6 ± 4.4	37.7 ± 4	50.7 ± 1.6	58.2 ± 0.2	52.4 ± 0.6	49.8 ± 0.5
Temperature (°C)	23.3 ± 0.2	22.5 ± 2.8	22.7 ± 3.6	23.1 ± 0.2	24.2 ± 0.1	23.4 ± 0	22.4 ± 0
Mass (µg m^{-3})	201.3 ± 49.8	282 ± 52.5	202.9 ± 31.2	354.8 ± 25.1	13.9 ± 11.2	385.4 ± 1	282.9 ± 51.3
O_3(ppb)	29.6 ± 7.4	30.2 ± 1.6	13.5 ± 1.7	19 ± 1.5	0 ± 0	5.8 ± 0.8	3.8 ± 0.1
NO (ppb)	1.3 ± 1	7.2 ± 2.2	8.4 ± 2.2	6.5 ± 0.6	5.5 ± 0.6	4 ± 0.2	3.7 ± 0.2
NO_2 (ppb)	0.4 ± 1	0.6 ± 5.7	2.2 ± 15	0.1 ± 0.1	2.1 ± 1.4	1.5 ± 0.3	0.1 ± 0
SO_2 (ppb)	36 ± 1.5	35.4 ± 2.1	37 ± 4.7	25.7 ± 0.7	34.3 ± 1.8	28.4 ± 0.3	24.1 ± 0
PM Count (# cm^{-3})	16875 ± 11213	11274 ± 667	4281 ± 2203	40811 ± 1939	910 ± 531	14959 ± 634	8383 ± 43

Table 4 Integrated measurements during experimental runs at Plant 1, March – September, 2005. Rounds 1-5 were four days in duration; Rounds 6 and 7 were two days in duration. Values expressed as mean ± SD.

Exposure Parameter	Round 1 (POS) Oxidized + SOA	Round 2 (POS) Oxidized + SOA	Round 3 (PO) Oxidized	Round 4 (PONS) Oxidized + NH3+ SOA	Round 5 (P) Primary	Round 6 (POS) Oxidized + SOA	Round 7 (POS) Oxidized + SOA
Mass (μg m^{-3})	378.2 ± 100.1	257.7 ± 37.2	222.6 ± 53.9	474.1 ± 49.8	2.5 ± 0.9	548.6 ± 64.8	394.7 ± 93.7
Total Sulfate (μg m^{-3})	82.3 ± 29.0	127.0 ± 35.7	101.1 ± 16.4	155.7 ± 12.4	0.4 ± 0.5	171.4	175.1 ± 22.9
Neutral Sulfate (μg m^{-3})	13.3 ± 11.0	37.3 ± 15.6	29.4 ± 1.2	139.7 ± 15.4	0.4 ± 0.5	43.4	39.6 ± 9.8
Acid Sulfate (μg m^{-3})	69.1 ± 22.0	89.7 ± 29.7	71.8 ± 17.0	16.0 ± 3.8	0.0	128.0	135.6 ± 13.1
Nitrate (μg m^{-3})	0.9 ± 0.3	0.4 ± 0.3	0.2 ± 0.2	6.4 ± 1.7	0.0	0.5 ± 0.0	0.0
Ammonium (μg m^{-3})	5.0 ± 1.2	8.6 ± 4.4	6.0 ± 0.3	47.7 ± 5.0	0.1 ± 0.2	4.8 ± 6.3	10.0 ± 0.8
OC (μg m^{-3})	143.4 ± 71.6	92.2 ± 24.8	17.9 ± 8.4	64.2 ± 10.1	42.0 ± 50.8	79.9 ± 0.1	7.5 ± 7.4
EC (μg m^{-3})	10.8 ± 3.9	6.6 ± 1.5	7.4 ± 3.2	10.2 ± 3.9	1.7 ± 1.8	21.0 ± 3.8	15.9 ± 3.0
SO2 (ppb)	27.8 ± 5.0	26.2 ± 10.5	24.4 ± 2.7	8.6 ± 6.6	91.4 ± 112.8	15.7 ± 19.5	0.0
HNO3 (ppb)	1.2 ± 0.1	0.4 ± 0.1	0.9 ± 0.5	1.1 ± 0.5	0.2 ± 0.1	0.2 ± 0.0	4.3 ± 5.4
HONO (ppb)	4.4 ± 0.9	1.4 ± 0.3	1.9 ± 0.6	2.2 ± 1.2	2.8 ± 2.2	2.0 ± 1.9	0.0
NH3 (ppb)	3.4 ± 3.5	4.2 ± 6.8	0.0	2.0 ± 2.3	0.1 ±0.2	14.5 ± 11.7	0.0
Total Carbonyls (μg m^{-3})	50.1 ± 4.4	23.1 ± 11.5	NA*	36.9 ± 4.8	NA*	33.7 ± 12.2	23.8 ± 6.2
Formaldehyde	20.7 ± 2.8	6.9 ± 4.5	NA*	10.7 ± 7.3	NA*	20.6 ± 1.4	12.7 ± 1.7
Acetaldehyde (μg m^{-3})	6.8 ± 1.1	4.4 ± 1.8	NA*	5.7 ± 1.9	NA*	4.8 ± 1.6	3.0 ± 0.2
Acetone (μg m^{-3})	22.6 ± 2.9	11.8 ± 7.9	NA*	20.5 ± 4.5	NA*	8.4 ± 9.2	8.0 ± 7.7
α-Pinene (μg m^{-3})	7.8 ± 8.0	4.4 ± 1.4	NA*	6.0 ± 3.4	NA*	8.7 ± 9.1	7.5 ± 2.1

**NA: not applicable*

Table 5: Elemental concentrations (μg/m^3) for each exposure day at Plant 1.

Round	Na	Mg	Al	Si	S	Cl	K	Ca	Ti	Mn	Fe	Ni	Cu
1(POS)	**1.856**	**0.277**	0.021	**0.306**	**24.655**	0.000	**0.066**	**0.225**	**0.013**	0.002	**0.031**	**0.006**	**0.002**
1(POS)	**0.592**	0.000	0.000	**0.310**	**23.950**	0.000	**0.031**	**0.028**	**0.010**	0.002	**0.010**	**0.005**	0.000
1(POS)	**1.552**	0.195	**0.105**	**0.236**	**22.242**	0.000	**0.026**	**0.022**	**0.010**	0.000	**0.007**	**0.003**	0.000
1(POS)	0.000	**0.356**	**0.232**	**0.344**	**25.192**	0.000	**0.030**	**0.047**	**0.008**	0.002	**0.016**	**0.003**	0.000
2(POS)	0.077	0.069	**0.052**	**0.664**	**21.921**	0.000	0.000	0.004	0.000	0.006	0.000	0.000	**0.001**
2(POS)	0.000	0.133	0.017	**1.102**	**52.879**	0.000	**0.006**	0.012	0.003	0.001	0.000	0.000	**0.004**
2(POS)	**0.641**	**0.289**	0.000	**1.019**	**46.902**	0.000	0.002	**0.026**	**0.011**	0.000	0.000	0.000	0.000
2(POS)	0.000	0.000	0.000	**0.931**	**40.775**	0.000	0.001	0.012	0.001	0.007	0.000	0.000	**0.004**
3(PO)	0.000	0.014	0.000	**2.133**	**28.844**	0.000	0.000	**0.015**	0.001	**0.008**	0.000	0.001	0.000
3(PO)	0.000	0.000	0.000	**2.419**	**31.605**	0.000	**0.017**	**0.023**	0.006	**0.009**	0.000	0.000	0.000
3(PO)	0.000	0.091	0.000	**4.096**	**23.074**	0.000	**0.030**	**0.161**	**0.020**	0.001	**0.074**	0.000	**0.004**
3(PO)	0.000	0.053	0.000	**1.973**	**26.683**	0.000	**0.019**	**0.056**	0.000	**0.009**	0.001	0.000	**0.004**
4(PONS)	0.000	**0.246**	**0.136**	**2.794**	**81.378**	0.000	0.000	**0.067**	**0.020**	0.000	0.003	0.001	**0.009**
4(PONS)	**0.572**	**0.240**	0.031	**2.008**	**61.688**	0.034	**0.039**	**0.054**	0.006	**0.009**	**0.037**	**0.003**	**0.004**
4(PONS)	0.000	0.119	0.047	**1.644**	**62.063**	**0.244**	0.000	0.003	0.000	**0.011**	0.005	0.000	**0.002**
4(PONS)	0.000	0.000	0.000	**1.461**	**68.465**	**0.316**	**0.013**	**0.045**	**0.011**	**0.012**	0.000	0.000	**0.002**
5(P)	0.000	0.007	0.014	**0.038**	**0.029**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5(P)	0.000	0.000	0.000	0.000	0.000	**0.039**	**0.006**	0.000	0.004	0.000	0.000	0.000	0.000
5(P)	0.024	0.000	0.000	0.000	0.000	**0.046**	0.000	0.001	0.000	0.000	0.000	0.000	0.000
5(P)	0.005	0.000	0.000	**0.154**	**0.012**	**0.089**	**0.025**	0.004	**0.011**	0.000	**0.068**	0.000	0.000
6(POS)	**0.179**	0.014	0.000	**6.354**	**52.683**	**0.141**	**0.006**	0.000	**0.010**	0.000	0.000	0.000	0.000
6(POS)	0.075	0.000	0.000	**4.676**	**46.750**	0.000	**0.008**	0.000	0.007	0.003	**0.170**	**0.022**	0.000
7(POS)	**0.545**	**0.221**	0.000	**1.131**	**40.591**	0.000	**0.030**	**0.015**	0.000	0.001	**0.021**	0.002	**0.011**
7(POS)	**0.330**	**0.124**	0.006	**1.580**	**45.420**	0.000	0.000	**0.061**	0.005	0.007	0.000	0.000	**0.010**
Mean	*0.269*	*0.102*	*0.028*	*1.557*	*34.492*	*0.038*	*0.015*	*0.037*	*0.006*	*0.004*	*0.018*	*0.002*	*0.002*
SD	*0.496*	*0.115*	*0.056*	*1.609*	*22.469*	*0.083*	*0.017*	*0.054*	*0.006*	*0.004*	*0.039*	*0.005*	*0.003*

Table 5 (contd.): Elemental concentrations (μg/m^3) for each exposure day at Plant 1.

Round	Zn	Se	Br	Sr	Mo	Pd	Cd	Sn	Ba	La
1(POS)	0.014	0.002	0.016	0.008	0.000	0.002	0.000	0.000	0.000	0.000
1(POS)	0.003	0.007	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1(POS)	0.005	0.008	0.010	0.009	0.000	0.000	0.000	0.000	0.000	0.000
1(POS)	0.011	0.009	0.012	0.000	0.005	0.011	0.000	0.010	0.000	0.000
2(POS)	0.000	0.006	0.009	0.006	0.001	0.000	0.007	0.000	0.004	0.000
2(POS)	0.000	0.001	0.014	0.004	0.000	0.000	0.000	0.045	0.000	0.011
2(POS)	0.000	0.001	0.011	0.000	0.025	0.024	0.000	0.001	0.000	0.023
2(POS)	0.000	0.004	0.011	0.000	0.004	0.009	0.000	0.000	0.004	0.015
3(PO)	0.000	0.003	0.011	0.000	0.007	0.000	0.017	0.026	0.003	0.019
3(PO)	0.000	0.004	0.010	0.003	0.011	0.000	0.022	0.000	0.000	0.000
3(PO)	0.000	0.013	0.008	0.000	0.012	0.000	0.036	0.035	0.021	0.021
3(PO)	0.000	0.008	0.008	0.002	0.000	0.015	0.006	0.038	0.005	0.006
4(PONS)	0.000	0.003	0.012	0.003	0.002	0.000	0.000	0.000	0.000	0.028
4(PONS)	0.000	0.002	0.014	0.014	0.000	0.000	0.006	0.000	0.013	0.018
4(PONS)	0.000	0.005	0.012	0.006	0.000	0.025	0.004	0.052	0.003	0.033
4(PONS)	0.000	0.005	0.015	0.008	0.005	0.015	0.000	0.000	0.000	0.028
5(P)	0.000	0.003	0.000	0.001	0.000	0.000	0.003	0.000	0.000	0.000
5(P)	0.006	0.001	0.000	0.000	0.008	0.000	0.000	0.000	0.009	0.011
5(P)	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.004
5(P)	0.062	0.005	0.004	0.011	0.010	0.000	0.034	0.000	0.002	0.001
6(POS)	0.099	0.001	0.112	0.004	0.000	0.020	0.013	0.000	0.006	0.007
6(POS)	0.013	0.003	0.085	0.000	0.000	0.000	0.000	0.054	0.016	0.030
7(POS)	0.000	0.004	0.055	0.006	0.024	0.000	0.000	0.092	0.040	0.050
7(POS)	0.000	0.002	0.058	0.017	0.000	0.000	0.000	0.013	0.013	0.036
Mean	0.009	0.004	0.021	0.004	0.005	0.005	0.006	0.015	0.006	0.014
SD	0.023	0.003	0.028	0.005	0.007	0.008	0.011	0.025	0.010	0.014

Table 6: SOA speciation for 2 representative filters from exposures at Plant 1; concentrations expressed in ng/m^3.

SOA component	**Oxidized+SOA+NH_3 (PONS)**	**Oxidized+SOA (POS)**
Pinonaldehyde	791.5	1217.8
Cis-norpinic acid	43.5	61.4
Pinalic acid	242.1	1009.4
Trans-norpinic acid	514.9	452.9
Cis-pinonic acid	887.6	808.3
Cis-pinic acid	21413.6	23099.4
Trans-pinic acid	155.0	241.1
Pinolic acid	6195.8	7964.0
OC (from TOR)	66300.0	61500.0
Percent of OC as SOA	**46**	**57**

3.3 Toxicological Results

The toxicological results for all experiments are presented below. In the case of scenarios conducted in replicate, animals were combined. The total number of animals for each scenario is shown in Table 5.

Table 7. Number of experimental animals per scenario.

Scenario	Exposed	Sham	Buxco	Ox. Stress	Hist	BAL	Blood	Stage II (ECG)
Oxidized + SOA (no SCR)	20	20	40	16	12	12	24	-
Oxidized + SOA	20	20	40	16	12	12	24	-
Oxidized	20	20	40	16	12	12	24	-
Oxidized + NH_3 + SOA	20	20	40	16	12	12	24	-
Primary Particles	20	20	40	16	12	12	24	-
Oxidized + SOA (MI)	7	7	14	-	13	-	13	13
Oxidized + SOA (MI)	8	8	16	-	16	-	16	16
TOTAL	**115**	**115**	**230**	**80**	**89**	**60**	**149**	**29**

Pulmonary Function and Breathing Pattern

Some changes in breathing pattern were noted in animals exposed at Plant 1. Two examples are shown in Figures 6 and 7, whereby MI rats exposed to oxidized emissions demonstrated significant increases in respiratory frequency and reductions in tidal volume, compared with control (sham) animals.

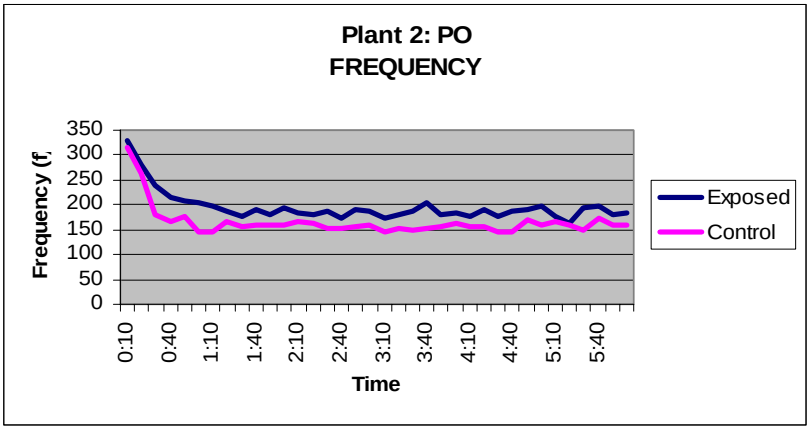


Figure 6. Respiratory frequency in Sprague-Dawley rats (n=48) exposed to oxidized emissions at Plant 1, 2005.

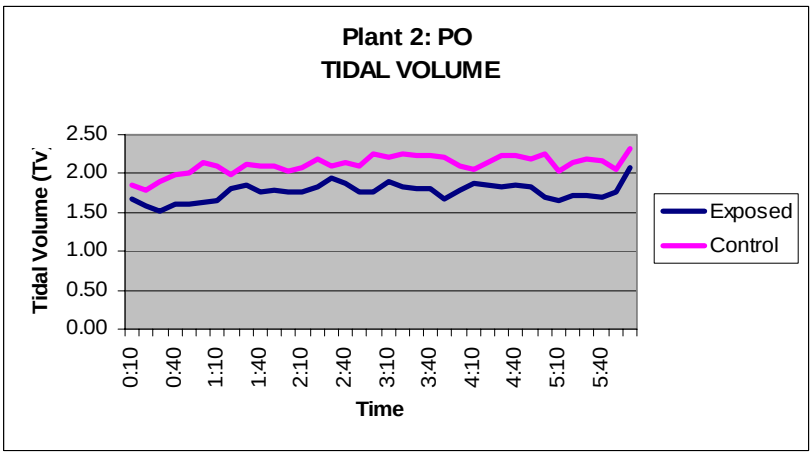


Figure 7. Tidal volume in Sprague-Dawley rats (n=48) exposed to oxidized emissions at Plant 1, 2005.

For each scenario, pulmonary function/breathing pattern data were analyzed using statistical modeling to assess the size and strength of association between exposure and each outcome. Additive mixed models were applied to 10-minute averaged data collected from all exposed and sham animals exposed during that scenario. A form of repeated measures model for longitudinal data -- additive mixed models (Coull et al., 2001; Ruppert et al., 2003) -- represent an extension of linear regression models that allows one to (1) estimate potentially non-linear effects of independent variables; and (2) include random effects as independent variables in order to account for clustering of observations that results from repeated measurements being taken on the same animal during the exposure period. For each outcome, additive mixed models were fit using as independent variables (1) a general nonlinear mean trend for sham animals over the exposure period; (2) a exposure indicator, which implies a constant shift in the mean trend due to test exposure; and (3) random animal effects reflecting animal-to-animal heterogeneity that results in correlation among 10-minute averages taken on the same animal over time. All models were fit using the gamm() function in the R software (R Development Core Team. 2004).

Finally, a more general model that relaxed the assumption of a constant shift due to test exposure was also fitted to the data. This model specified distinct mean trends over the exposure period for the sham and exposed animals, again including random animal effects to account for the repeated measurements taken on each animal. The difference between these estimated trends represents the time-varying effect of the test exposure over the exposure period.

The following breathing pattern parameters were examined: frequency, tidal volume, minute ventilation, inspiratory time, expiratory time, peak inspiratory flow, peak expiratory flow, enhanced pause, end inspiratory pause, and end expiratory pause. Parameters showing significant differences over time between exposed and sham animals are summarized in Table 8, which describes the directional trends and the level of significance of the changing trend.

Table 8. Summary of respiratory changes in normal and compromised rats at Plant 1. NC = no change; ns = not significant. Significant results bolded.

Scenario	Respiratory Frequency	Tidal Volume	Inspiratory Time	Expiratory Time	Enhanced Pause (Penh)
POS (#1)	↑ ns	**↓ p=0.003**	NC…..ns	NC…..ns	↓ ns
POS (#2)	↑ ns	NC…..ns	NC…..ns	NC…..ns	**↓ p=0.001**
PO	↑ p=0.06	**↓ p=0.04**	**↓ p=0.02**	↓…p=0.06	**↓ p=0.01**
POS (MI model)	**↑p=0.024**	NC…..ns	NC…..ns	**↓ p=0.005**	**↑…p=0.03**
PONS	↓ ns	**↓ p=0.002**	NC…..ns	NC…..ns	**↓ p=0.001**
P	↓ ns	**↓ p=0.001**	NC…..ns	NC…..ns	**↓ p=0.003**

In examining respiratory pattern data such as these, we can look for three types of effects:

1. Sensory irritation: characterized by a reduction in respiratory frequency and the appearance of a pause after inspiration.
2. Pulmonary irritation: characterized by an increase in respiratory frequency, a decrease in tidal volume, and a decrease in both inspiratory and expiratory time.
3. Airflow restriction: characterized by an increase in Penh, an increase in expiratory time, and a decrease in expiratory flow rate.

In looking at the data in Table 8, sensory irritation does not appear to be evident, based on the fact that no significant decreases in respiratory frequency were observed. Pulmonary irritation could play a role in some of the responses, with a significant increase in frequency observed in the MI model, and a decrease in tidal volume observed in several scenarios. However, the picture is not clear, given the lack of consistency, even within the same scenario (e.g., oxidized emissions + SOA in normal rats) and the lack of change in inspiratory and expiratory time. Airflow limitation may have occurred in the MI model, as evidenced by the significant increase in Penh; however, again, this is not clear in light of the concurrent reduction in expiratory time and increase in frequency. Taken together, we can conclude that subtle changes appear to occur, without a strong indication of any particular type of adverse effect.

Bronchoalveolar Lavage Parameters

No significant differences in BAL parameters were observed between exposed and control animals (data not shown).

Blood Cytology

No significant differences in blood cytological parameters were observed between exposed and control animals (data not shown).

In Vivo Oxidative Stress

Evidence of heart and lung oxidative stress was observed in the POS and PONS scenarios (Figures 8, 9, and 10). The chemiluminescence findings were confirmed using the TBARS (thiobarbituric acid reactive substances) assay, also shown in these figures. No evidence of oxidative stress was observed in the P or PO scenarios (data not shown).

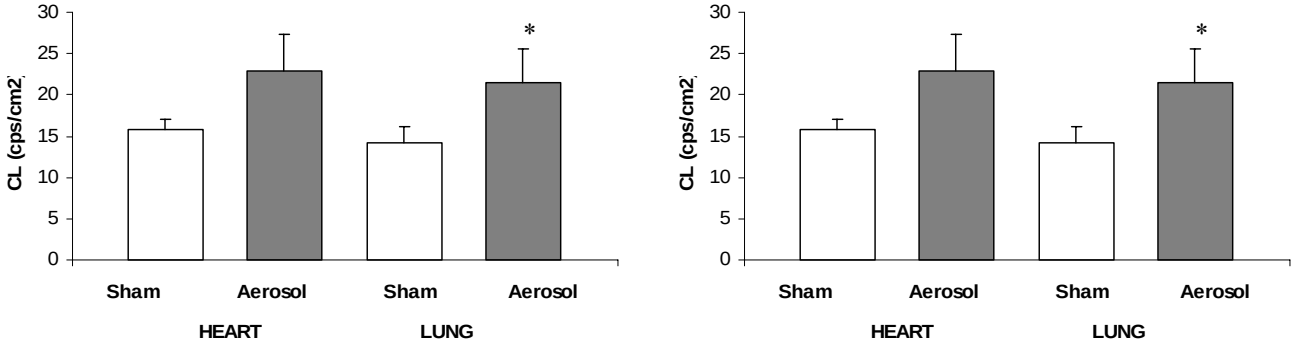


Figure 8. Oxidative stress, as measured by CL, and lipid peroxidation, determined as accumulation of TBARS in Sprague-Dawley rats exposed to oxidized emissions and secondary organic aerosol (POS), Plant 1, March 2005. * indicates statistically significant

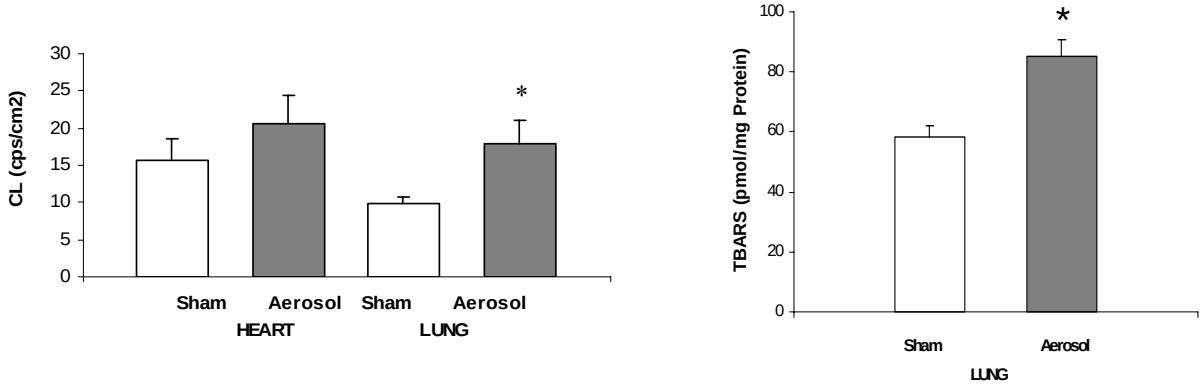
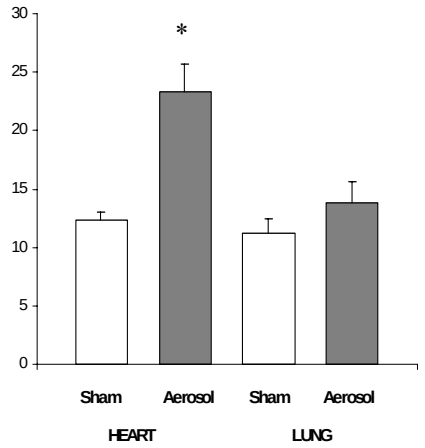


Figure 9. Oxidative stress in Sprague-Dawley rats exposed to oxidized emissions and secondary organic aerosol (POS), Plant 1, May 2005. * indicates statistically significant.



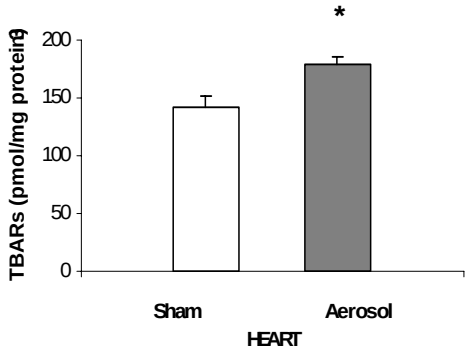


Figure 10. Oxidative stress in Sprague-Dawley rats exposed to oxidized, neutralized emissions and SOA (PONS), Plant 1, May-June 2005. * indicates statistically significant.

We used Generalized Linear Models (GLM) using heart or lung chemiluminescence as the dependent variable and site or scenario as the independent variable; results are shown in Table 9. In separate analyses of Plants 0 and 1, no significant associations were observed for Plant 0, while at Plant 1, the POS scenario resulted in increases in lung and heart CL. In the combined analysis, both the POS and the PONS scenarios caused significant increases in heart and lung CL. Recall that these two scenarios include organics; these results suggest that there may be something in the SOA scenario that could account for the biological responses observed. We do not know whether this is the SOA itself, a product formed from the organics and the remainder of the mixture, or a synergistic effect of the SOA with other component(s) of the mixture.

Table 9. GLM output for Plants 0 and 1 alone and combined. NS=not significant; *p*-values provided for significant findings, along with direction of change.

Scenario	P		PO		POS		PONS	
Plant	Plant 0	Plant 1	Plant 0	Plant 1	Plant 0	Plant 1	Plant 0	Plant 1
CL Lung	NS	NS	NS	NS	NS	↑**0.005**	NS	NS
CL Heart	NS	NS	NS	NS	NS	↑**0.006**	NS	↑**0.07**
Plants 0 and 1 Combined	P		PO		POS		PONS	
CL Lung	NS		NS		↑ **0.05**		↑ **0.012**	
CL Heart	NS		NS		↑ **0.002**		↑ **0.03**	

Histopathology

Generally, histopathology mirrors bronchoalveolar lavage (BAL) findings if the BAL is peformed at the optimal time. If BAL is performed too early or too late, histology findings can be another parameter that can be quantified to define specific histopathological findings to indicate that the lack of BAL findings were due to insufficient time for development or that the response had passed. At Plant 1, there is little to suggest that BAL parameters have any consistent findings. Therefore, a qualitative review of the histology was performed to determine if there was a disconnect between the BAL findings and the histology; there was none. Next, heart and lung histology was evaluated to determine if there were any morphological changes that could be assessed quantitatively; there were none.

ECG Analyses (Stage II)

ECG data were available from 29 MI (compromised model) rats, of which 15 were exposed to the POS scenario, and 14 were exposed to filtered room air only (sham). Beats were automatically labeled and verified by the investigator. Heart rate variability (HRV) was calculated over 3 minutes at the start of each hour for the duration of exposure. Parameters measured included heart rate (HR), the standard deviation of the R-R interval (SDNN), and the root mean square of the difference of successive R-R intervals (rMSSD).

Additive mixed models were applied to heart rate variability (HRV) data to assess trends over time in comparison to sham exposure (repeated measures model for longitudinal data). There were no significant differences between exposed and sham animals for HR (Figure 11), SDNN (Figure 12), or rMSSD (Figure 13), although the HR changes approached significance (p=0.06; see Table 10).

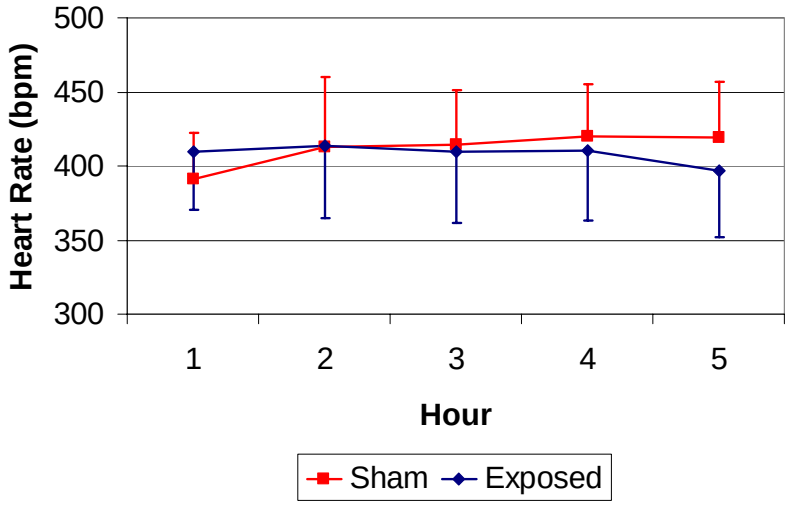


Figure 11. Heart rate in control and exposed rats by exposure hour.

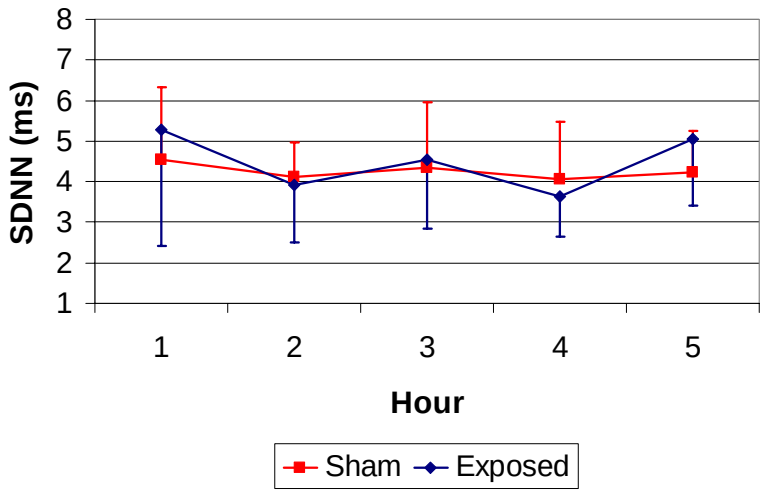


Figure 12. SDNN in control and exposed rats by exposure hour.

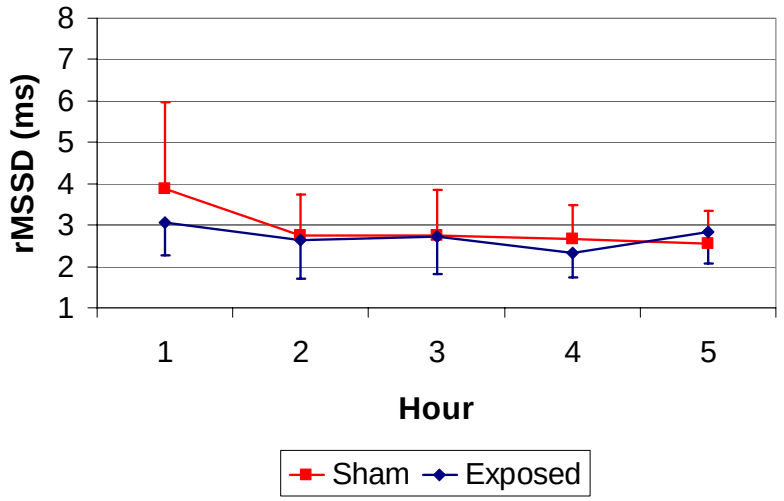


Figure 13. rMSSD in control and exposed rats by exposure hour.

Table 10. Average change in outcome (per hour) in sham and exposed groups.

	Sham	Exposed	*p*-value
HR	+5.34 bpm/hr	-2.98 bpm/hr	0.058
SDNN	-0.96 %/hr	0.37 %/hr	0.75
rMSSD	-7.41 %/hr	-2.41 %/hr	0.17

For arrhythmias, Poisson regression was used to estimate the effect of treatment during each hour, accounting for within-subject correlation. For these analyses, an unstructured covariance matrix was assumed. Results indicate that arrhythmias decreased in sham animals over time, but

increased in exposed animals (Table 11). The overall increase was 87% (p=0.05). Comparing time points, the 4-hour time point was significantly different in the exposed vs. sham group.

Table 11. Mean number of premature ventricular beats (PVBs) per hour, by hour and exposure group.

	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
Sham	3.15	2.57	2.00	2.09	1.09
Exposed	3.71	4.20	3.30	6.44	3.22

4.0 CONCLUSIONS

We investigated four exposure scenarios at a power plant in the Southeast burning low sulfur Eastern bituminous coal, and some biological effects were observed in animals exposed to some scenarios. Specifically, pulmonary function data suggest subtle changes in some respiratory parameters in some scenarios. The *in vivo* chemiluminescence (CL) dataset suggests that both lung and heart oxidative stress occur in response to several scenarios. No changes in histology, bronchoalveolar lavage fluid, or blood cytology were evident. Stage II assessments conducted at Plant 1 suggest no apparent effect of any of the scenarios on heart rate or on several measures of heart rate variability. However, the POS scenario resulted in an increase in cardiac arrhythmias (premature ventricular beats; PVBs) in exposed animals compared to sham/control animals.

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