

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

Semi-Annual Technical Progress Report

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ABSTRACT

This report documents progress made on the subject project during the period of September 1, 2005 through February 28, 2006. The TERESA Study is designed to investigate 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 study involves on-site sampling, dilution, and aging of coal combustion emissions at three coal-fired power plants, as well as mobile source emissions, followed by animal exposures incorporating a number of toxicological endpoints. The DOE-EPRI Cooperative Agreement (henceforth referred to as “the Agreement”) for which this technical progress report has been prepared covers the performance and analysis of field experiments at the first TERESA plant, located in the Upper Midwest and henceforth referred to as Plant 0, and at two additional coal-fired power plants (Plants 1 and 2) utilizing different coal types and with different plant configurations.

During this reporting period, data processing and analyses were completed for exposure and toxicological data collected during the field campaign at Plant 1, located in the Southeast. To recap from the previous progress report, Stage I toxicological assessments were carried out in normal Sprague-Dawley rats, and Stage II assessments were carried out in a compromised model (myocardial infarction—MI—model). Normal rats were exposed to the following atmospheric scenarios: (1) primary particles; (2) oxidized emissions; (3) oxidized emissions + SOA – this scenario was repeated; and (4) oxidized emissions + ammonia + SOA. Compromised animals were exposed to oxidized emissions + SOA (this scenario was also conducted in replicate).

Mass concentrations in exposure atmospheres ranged from 13.9 $\mu\text{g}/\text{m}^3$ for the primary particle scenario (P) to 385 $\mu\text{g}/\text{m}^3$ for one of the oxidized emissions + SOA scenarios (POS). There was a fair amount of day-to-day variation in mass concentration, even within a given exposure round; this is likely due to the inherent variation in the power plant operation. Concentrations of ozone, NO_x and SO_2 , and carbonyls were below 50 ppb. Total sulfate concentration ranged from 82 to 175 $\mu\text{g}/\text{m}^3$. Elemental data suggest substantial day-to-day variations which again provide insight about the inherent variations attributed to plant operation. All elements were present at low concentrations except for sulfur. Other prominent elements were: Si, Br, Ca, K, La and Cu. SOA was speciated using GC-MS, with typical α -pinene oxidation products being observed.

Toxicological results obtained to date from Plant 1 indicate some biological responses to some exposure scenarios. We observed pulmonary function changes, increased oxidative stress, and increases in cardiac arrhythmias in response to certain scenarios. For the oxidative stress endpoint, an increase in chemiluminescence occurred only in those scenarios including SOA. More detailed statistical modeling also points to the importance of organic material in these scenarios; additional analyses are currently underway to better understand this finding.

Fieldwork for Plant 2, located in the Midwest, is scheduled for June-September 2006, and logistical planning is now underway.

During the next reporting period, we will complete fieldwork at Plant 2. A draft topical report for Plant 0 was submitted to DOE-NETL in December 2005, with the final report to be submitted in April, 2006. We will also complete a topical report for Plant 1 by June 30, 2006.

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1.0 INTRODUCTION

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 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 will enable 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 will allow 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 NO₂, SO₂, ozone, NH₃, 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.

The DOE-EPRI Cooperative Agreement for which this technical progress report has been prepared involves the analysis and interpretation of the field data collected at the first power plant (henceforth referred to as Plant 0, located in the Upper Midwest), followed by the performance and analysis of similar field experiments at two additional coal-fired power plants (Plants 1 and 2) utilizing different coal types and with different plant configurations. The Agreement also includes a comparison of the toxicity of coal power plant emissions, mobile source emissions and concentrated ambient particles (CAPs). Animal exposure experiments to evaluate the toxicity of mobile source emissions and CAPs are also part of the overall TERESA program, but will be performed by the project team independently of the Agreement.

2.0 EXECUTIVE SUMMARY

Activities conducted during this reporting period (September 1, 2005 through February 28, 2006) focused on completing data analysis and processing for exposure characterization and toxicological data collected during Plant 1 fieldwork. Methods development, laboratory outfitting, and stack sampling at Plant 1 have been previously reported and these results are therefore not included here.

To recap, seven sets of animal exposures were carried out at Plant 1. Normal Sprague-Dawley rats were exposed to four different scenarios (with one repeated), while MI (compromised) rats were exposed to one scenario, which was conducted in replicate.

Continuous exposure data collected included RH, temperature, PM mass (TEOM), ozone, NO, NO₂, SO₂, 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 µg/m³ for the primary particle scenario (P) to 385 µg/m³ 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. In general, RH remained around 53% and temperature was steadily maintained at an average value of 23°C. Concentrations of ozone, NO_x and SO₂ were below 50 ppb.

Analysis and processing of the integrated measurements was also completed. Sulfate concentrations ranged from 82 to 175 µg/m³. 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). 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. SOA was speciated to evaluate the contribution of known α-pinene oxidation products. The sum of the identified SOA components contribute about 46% and 57% of the corresponding OC mass concentrations for the PONS and POS scenarios, respectively.

Pulmonary function data were presented in the previous progress report. The now-complete *in vivo* chemiluminescence (CL) dataset for Plant 1 suggests that both lung and heart oxidative stress occur in response to several scenarios. Using Generalized Linear Models (GLM), we found that both the POS and PONS scenarios resulted in increases in CL in heart and lung tissue at Plant 1. As both of these scenarios include organic compounds, 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 an(other) component(s) of the mixture. More detailed analyses currently underway, along with additional scenarios at Plant 2, are expected to shed light on this issue.

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 PONS scenario resulted in an increase in cardiac arrhythmias (premature ventricular beats; PVBs) in exposed animals compared to sham/control animals.

Overall progress on the Project tasks is shown in the Table below. Note that the scheduled completion date for the Project has been extended due to delays in methods development and logistical issues related to power plant access. We now anticipate completion of the project by December 31, 2008.

Technical Progress - 30 months

Task #	Description	Planned % completed	Actual % completed
1	Complete Study at Upper Midwest Power Plant	100%	100%
2	Field Study at Power Plant #1	100%	100%
3	Field Study at Power Plant #2	100%	0%
4	Relative Toxicity of Coal Plant Emissions, Mobile Sources, and CAPs	75%	0%
5	Preparation of Peer-Reviewed Journal Articles	82%	40%
6	Project management and reporting	88%	47%

Priorities for the next reporting period (March 1, 2006 – September 30, 2006) include:

- Completion of a topical report for the Plant 0 findings
- Completion of a topical report for the Plant 1 findings (by June 30, 2006)
- Completion of fieldwork at Plant 2, located in the Midwest

3.0 EXPERIMENTAL

A detailed description of the experimental setup and methods development is not provided in this report as these topics were covered extensively in prior semiannual reports. Table 1 provides a summary of the scenarios/exposures carried out at Plant 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

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

The following measurements were conducted at the exposure chamber for all tested scenarios.

Continuous Measurements

- PM_{2.5} mass, using an R&P Tapered Element Oscillating Microbalance (TEOM)
- Particle number, using a portable condensation particle counter (CPC TSI)
- 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 (ion chromatography; Teflon filters)
- Particle nitrate (ion chromatography; Teflon filters)
- Particle strong acidity (pH analysis; Teflon filters)

- Particle ammonium (ion chromatography; Teflon filters)
- Particle elements (X-ray fluorescence)
- EC/OC (thermal optical reflectance [TOR] method; quartz fiber filters)
- Sulfur dioxide (diffusion denuder , ion chromatography)
- Nitric acid vapor (diffusion denuder, ion chromatography)
- Nitrous acid vapor (diffusion denuder, ion chromatography)
- Ammonia (diffusion denuder technique with ion chromatographic analysis)
- Ketones and aldehydes (DNPH cartridges)
- α -pinene (Tenax tubes)
- SOA (secondary organic aerosol) analysis for representative filters (GC-MS, Teflon filters)

4.0 RESULTS AND DISCUSSION

4.1 Exposure Characterization

Continuous Measurements

Available continuous data are provided in Table 2. Exposure parameters measured included RH, temperature, PM mass (TEOM), ozone, NO, NO₂, SO₂, 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 µg/m³ for the primary particle scenario (P) to 385 µg/m³ 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 µg/m³). 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 SO₂ vs. NO_x in the first reaction chamber, and less sulfate (Table 3), 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 1 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 SO₂ were kept below 50 ppb (Table 2).

Integrated Measurements

Integrated measurements obtained are shown in Table 3. Total sulfate concentration ranged from 82 to 175 µg/m³. 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, and these positive artifacts may have been introduced into the system via the clean room air used for flushing the series of 2 denuders (refer to experimental section of previous reports). Filtered room air may have a condensable OC fraction and part of OC could have been misclassified as EC during the TOR analysis method. Also, gas concentrations and carbonyl concentrations were kept below 50 ppb (Table 4).

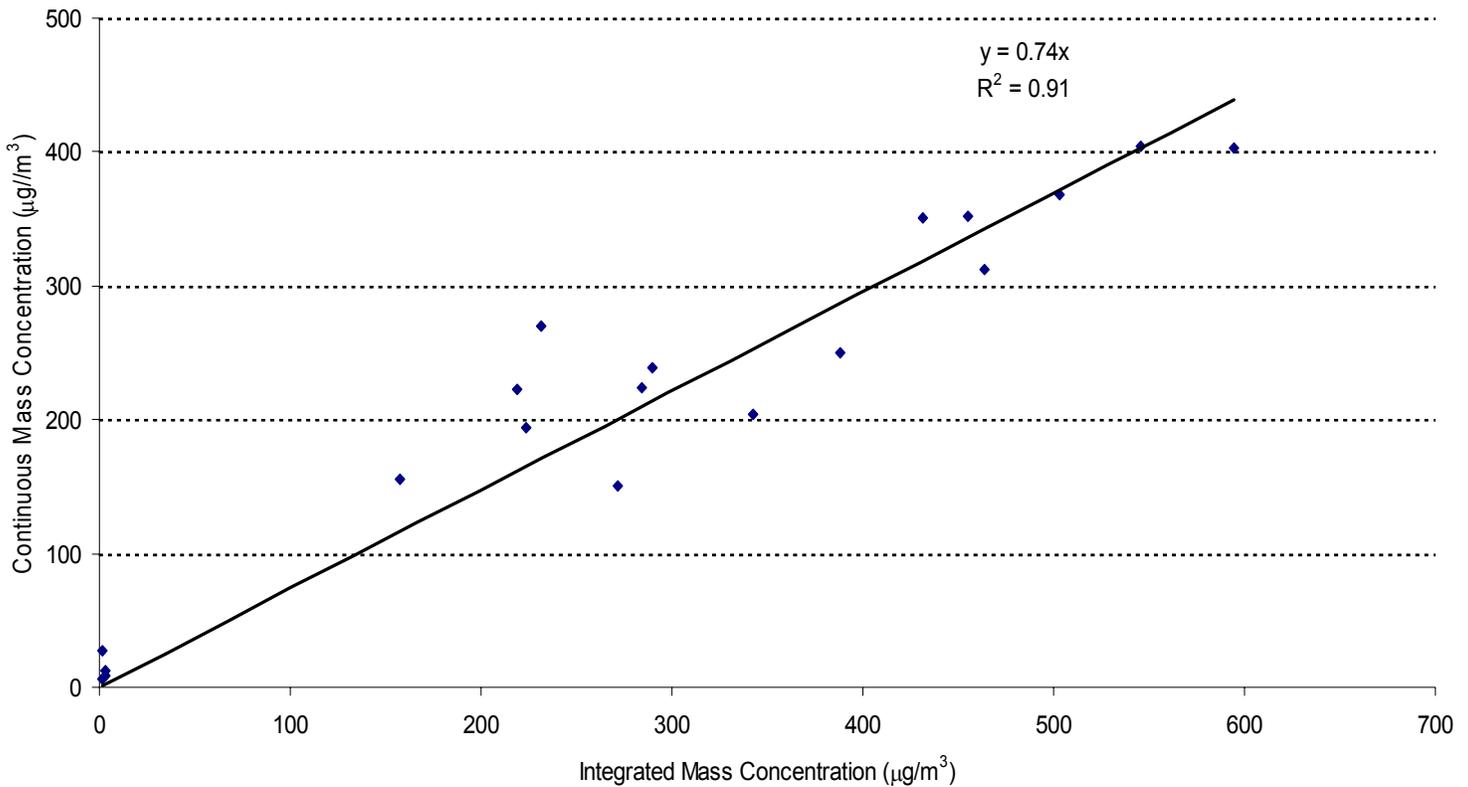


Figure 1. Comparison between mass concentrations measured via continuous and integrated methods.

Elemental Measurements

Elemental data obtained from integrated measurements performed at Plant 1 are presented in Table 4. 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. Few other elements were found to be present during specific exposure rounds. For instance, Fe, Ni and Se were present at significant concentrations during round 1 (non-SCR period) but for the rest of the exposure rounds their concentrations were considerably lower.

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 5 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 2. 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 \pm SD.

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

Table 3. 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 \pm SD.

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

*NA: not applicable

Table 4: Elemental concentrations ($\mu\text{g}/\text{m}^3$) 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
<i>Mean</i>	<i>0.269</i>	<i>0.102</i>	<i>0.028</i>	<i>1.557</i>	<i>34.492</i>	<i>0.038</i>	<i>0.015</i>	<i>0.037</i>	<i>0.006</i>	<i>0.004</i>	<i>0.018</i>	<i>0.002</i>	<i>0.002</i>
<i>SD</i>	<i>0.496</i>	<i>0.115</i>	<i>0.056</i>	<i>1.609</i>	<i>22.469</i>	<i>0.083</i>	<i>0.017</i>	<i>0.054</i>	<i>0.006</i>	<i>0.004</i>	<i>0.039</i>	<i>0.005</i>	<i>0.003</i>

Table 4 (contd.): Elemental concentrations ($\mu\text{g}/\text{m}^3$) 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 5: SOA speciation for 2 representative filters from exposures at Plant 1; concentrations expressed in ng/m³.

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

4.2 Toxicological Assessments

Pulmonary function, bronchoalveolar lavage (BAL), and blood cytology data for Plant 1 were presented in the previous progress report. Some *in vivo* chemiluminescence data were also presented; however, the current report provides an update with data not available/processed in September 2005.

In Vivo Chemiluminescence

Evidence of heart and lung oxidative stress was observed in the POS and PONS scenarios (Figures 2, 3, and 4). 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 (Figures 5 and 6).

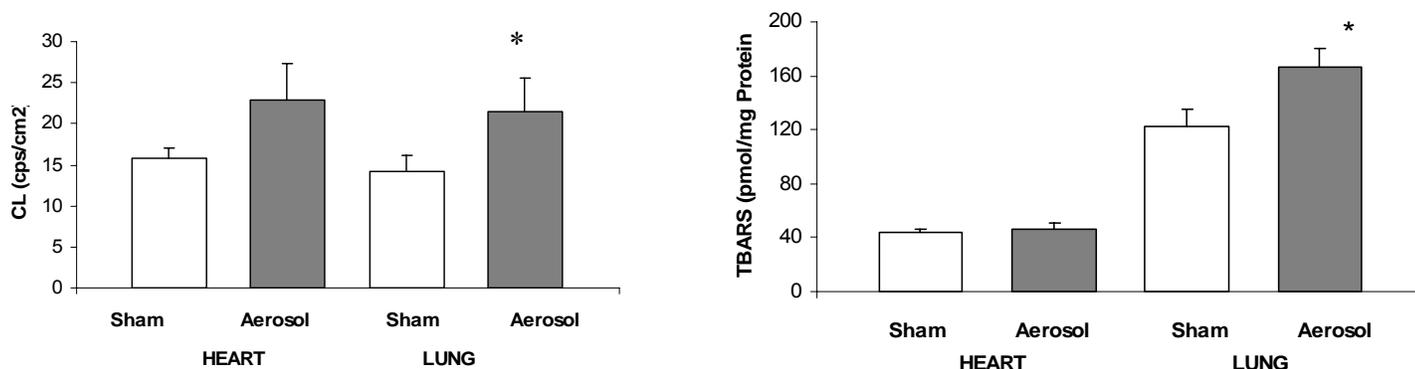


Figure 2. Oxidative stress, as measured by CL and TBARS in Sprague-Dawley rats exposed to oxidized emissions and secondary organic aerosol (POS), Plant 1, March 2005. * indicates statistically significant.

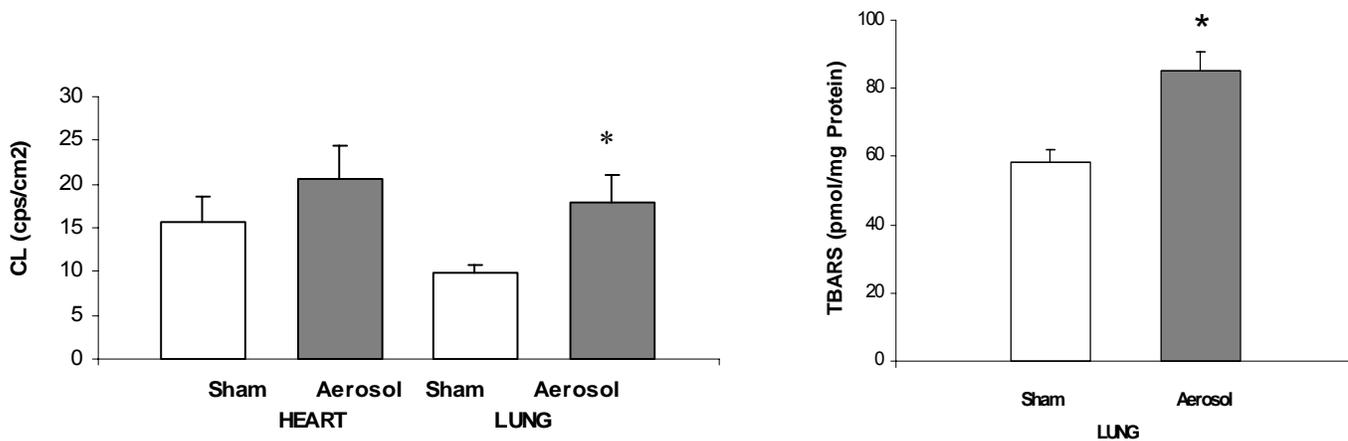


Figure 3. Oxidative stress, as measured by CL and TBARS in Sprague-Dawley rats exposed to oxidized emissions and secondary organic aerosol (POS), Plant 1, May 2005. * indicates statistically significant.

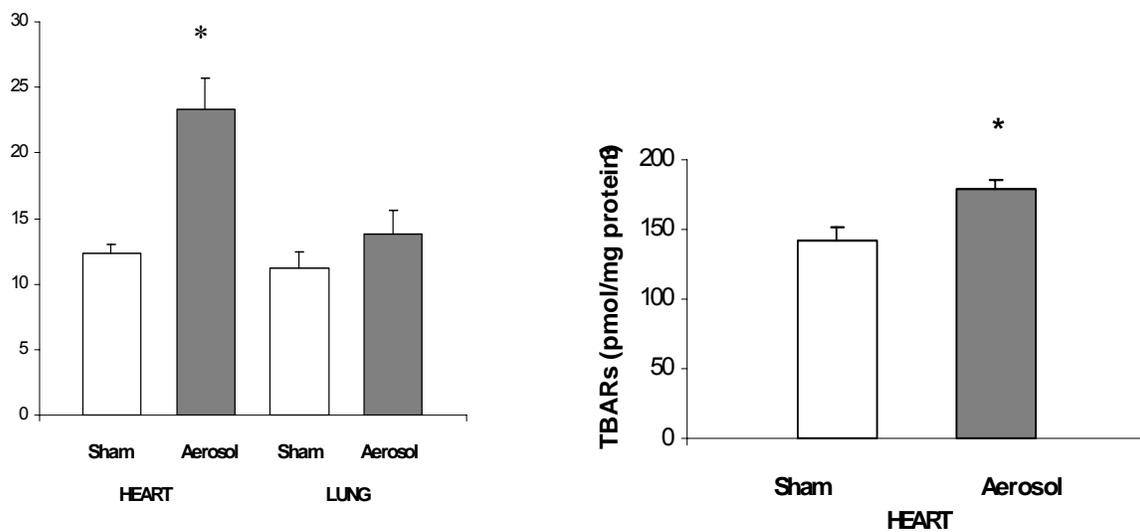


Figure 4. Oxidative stress, as measured by CL and TBARS in Sprague-Dawley rats exposed to oxidized, neutralized emissions and SOA (PONS), Plant 1, May-June 2005. * indicates statistically significant.

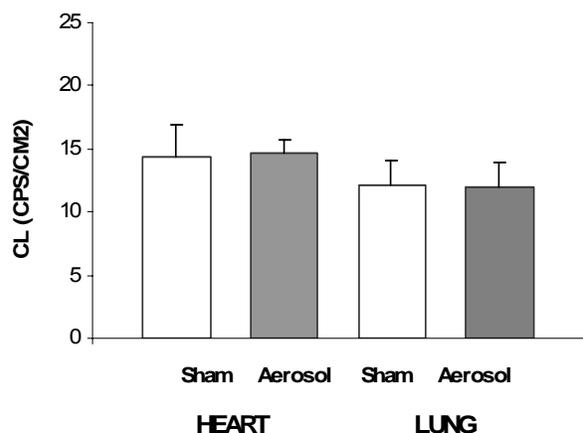


Figure 5. Oxidative stress, as measured by CL and TBARS in Sprague-Dawley rats exposed to primary particles (P), Plant 1, June 2005.

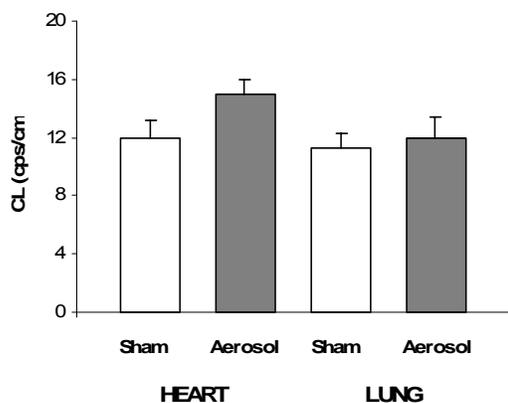


Figure 6. Oxidative stress, as measured by CL in Sprague-Dawley rats exposed to oxidized emissions (PO), Plant 1, May 2005.

We used Generalized Linear Models (GLM) using heart or lung chemiluminescence as the dependant variable and site or scenario as the independent variable; results are shown in Table 6. 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 resulted in 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 an(other) component(s) of the mixture. More detailed analyses, along with additional scenarios, at Plant 2 are expected to shed light on this issue.

Table 6. 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 performed 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 were 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 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 7), SDNN (Figure 8), or rMSSD (Figure 9), although the HR changes approached significance ($p=0.06$; see Table 7).

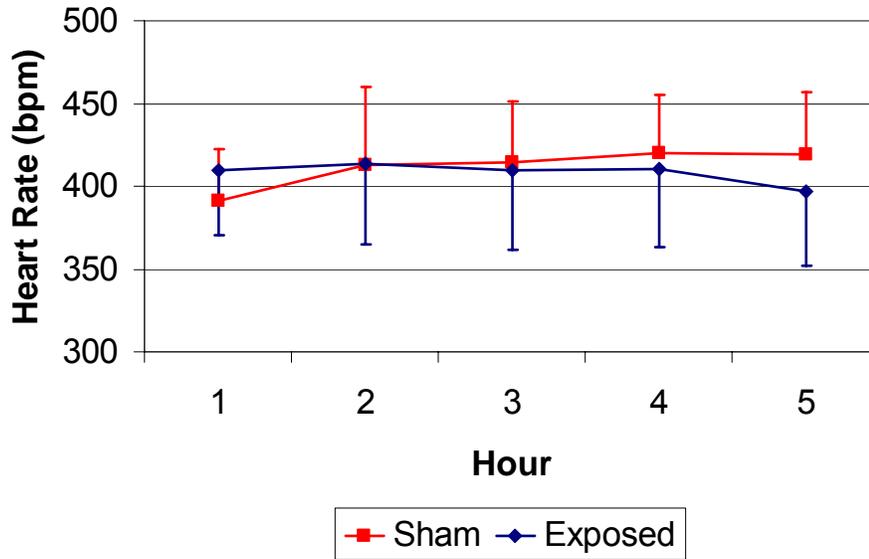


Figure 7. Heart rate in control and exposed rats by exposure hour.

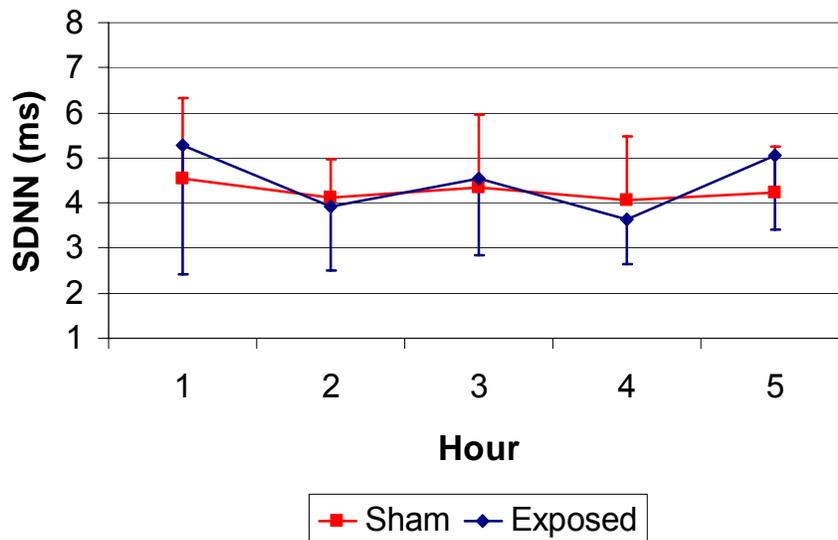


Figure 8. SDNN in control and exposed rats by exposure hour.

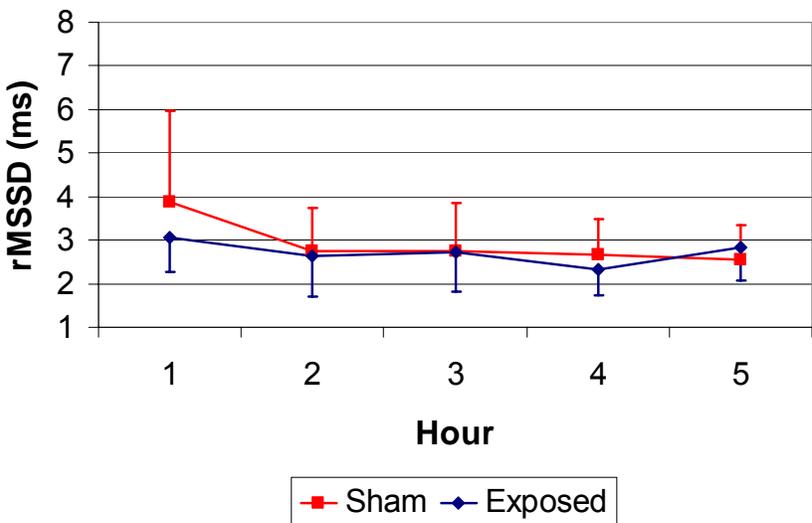


Figure 9. rMSSD in control and exposed rats by exposure hour.

Table 7. Average change in outcome (per hour) in sham and exposed groups.

	Sham	Exposed	<i>p</i> -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 8). 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 8. 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

5.0 CONCLUSIONS

Significant progress was made on the Project during this reporting period. We completed all remaining analyses of exposure and toxicological data for Plant 1 and have initiated data analyses to better understand the contribution of different scenarios and mixture components.

Additional toxicological data not available in the previous progress report demonstrate that some effects on oxidative stress and cardiac function were observed in animals exposed to POS and PONS scenarios. As both of these scenarios include organics, 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 an(other) component(s) of the mixture. More detailed analyses currently underway, along with additional scenarios at Plant 2, are expected to shed light on this issue.

Priorities for the next reporting period (March 1, 2006 – August 31, 2006) include:

- As required under the Cooperative Agreement, completion of a topical report for the Plant 0 findings.
- As required under the Cooperative Agreement, completion of a topical report for the Plant 1 findings (by 6/30/06).
- Completion of fieldwork at Plant 2, located in the Midwest.

6.0 REFERENCES

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