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 March 1, 2005 through August 31, 2005. 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, fieldwork was completed at Plant 1, located in the Southeast. 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 + secondary organic aerosol (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).

Stage I assessment endpoints included breathing pattern/pulmonary function; *in vivo* chemiluminescence (an indicator of oxidative stress); blood cytology; bronchoalveolar lavage (BAL) fluid analysis; and histopathology. Stage II assessments included continuous ECG monitoring via implanted telemeters and blood chemistry (complete blood count, circulating cytokines (interleukins-1 and –6), C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), and endothelin-1).

Only a subset of exposure data was available at the time of preparation of this report. Continuous $PM_{2.5}$ mass (TEOM) results indicate a mass concentration of 14 $\mu g/m^3$ for the primary particle scenario, and a range of 151 to 385 $\mu g/m^3$ for the oxidized emissions scenarios.

Toxicological results obtained to date from Plant 1 indicate subtle biological responses to some of the exposure scenarios. We observed statistically significant changes in several breathing pattern parameters, including tidal volume and frequency. For one scenario (oxidized emissions + SOA), we observed a significant increase in Enhanced Pause (Penh), a parameter that may reflect airflow restriction. However, the respiratory changes are very subtle and do not present a clear picture of a particular respiratory effect (e.g., airway restriction, sensory irritation, or pulmonary irritation). A significant increase in lung chemiluminescence (a marker of oxidative stress in lung tissue) in exposed animals (vs. air-exposed controls) was observed in animals exposed to oxidized emissions + SOA. No changes were observed in heart tissue, nor in any other scenario.

Stage II assessments were conducted to the secondary + SOA scenario; ECG and blood analysis data are pending.

Planning was initiated for Plant 2, located in the Midwest. Because of the requirement for both the FGD and the SCR to be concurrently operational for appropriate reaction conditions, fieldwork at Plant 2 is scheduled for Summer 2006.

During the next reporting period, we will complete all remaining exposure and toxicological analyses for Plant 1, and the next semiannual report will include a detailed description of these data and their interpretation. We are also in the process of preparing a topical report for Plant 0.

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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 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 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 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.

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 (March 1, 2005 through August 31, 2005) focused on completing fieldwork at Plant 1 in the Southeast. Methods development, laboratory outfitting, and results from Plant 0 were described in detail in previous progress reports. Stack sampling at Plant 1 was also completed during the previous reporting period and these results are therefore not included here. The histopathology results for Plant 0 were not available at the time of the last progress report; no differences between exposed and control animals were observed.

Seven sets of animal exposures were carried out at Plant 1 according to Table 1 below. 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.

.Table 1. Experimental runs at Plant 1, March-September 2005.

Round	Dates	Exposure Scenario	SCR[1] Operation	Animal Model
1	March 21 -- 24, 2005	Unneutralized, oxidized emissions + secondary organic aerosol (SOA)	No	Normal
2	May 3 -- 6, 2005	Unneutralized, oxidized emissions + secondary organic aerosol (SOA)	Yes	Normal
3	May 9 -- 12, 2005	Unneutralized, oxidized emissions	Yes	Normal
4	May 31 -- June 3, 2005	Neutralized, oxidized emissions + SOA	Yes	Normal
5	June 6 -- 9, 2005	Primary particles only	Yes	Normal
6	July 8 and July 13, 2005	Unneutralized, oxidized emissions + SOA	Yes	MI
7	September 8 -- 9, 2005	Unneutralized, oxidized emissions + SOA	Yes	MI

[1] SCR: Selective catalytic reduction

As of the time of preparation of this report, only a subset of the exposure data was available. Continuous $PM_{2.5}$ mass (TEOM) results indicate a mass concentration of 14 $\mu g/m^3$ for the primary particle scenario, and a range of 151 to 385 $\mu g/m^3$ for the oxidized emissions scenarios.

Stage I toxicological assessment data are available for the pulmonary function and *in vivo* chemiluminescence endpoints. In contrast to Plant 0, where we observed no biological responses to any of the scenarios investigated, some subtle effects were noted at Plant 1. Statistically significant changes in some breathing pattern parameters were evident in some of the experimental scenarios; however, the changes do not consistently indicate any particular type of respiratory effects (e.g. airway restriction, sensory irritation, or pulmonary irritation). In one scenario there was increased oxidative stress in lung tissue from exposed animals compared with control animals. Taken together, these findings indicate subtle effects in response to some exposure scenarios at Plant 1, with additional Stage I assessment results pending.

Since we observed biological responses at Plant 1, we proceeded with the Stage II toxicological assessment, consisting of monitoring electrocardiograms (ECGs) using implanted telemeters, and carrying out more extensive blood biochemical analysis. We selected the unneutralized, oxidized emissions + SOA scenario for study since that scenario appeared to induce the largest biological response in the Stage I assessment. The ECG results and blood analyses are pending and will be reported in the next semiannual report.

Overall progress on the Project tasks is shown in the Table below.

Technical Progress - 24 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%	75%
3	Field Study at Power Plant #2	71%	0%
4	Relative Toxicity of Coal Plant Emissions, Mobile Sources, and CAPs	0%	0%
5	Preparation of Peer-Reviewed Journal Articles	55%	25%
6	Project management and reporting	71%	71%

Priorities for the next reporting period (September 1, 2005 – February 28, 2006) include:

- Completion of a topical report for the Plant 0 findings.
- Completion of exposure data analyses and toxicological data evaluation from Plant 1 fieldwork.
- Preparation for fieldwork at Plant 2, located in the Midwest.

Note that the mobile source emissions work will be funded through the Harvard/EPA PM Research Center and is not scheduled to begin until at least 2007. This means that Task 4 (Determination of relative toxicity of coal plant emissions, mobile sources, and CAPs) will also not be completed until that time frame. The end date for the Project, therefore, will need to be adjusted accordingly; this date will be specified once we have a firmer idea of the timing of the mobile source experiments. Comparison of the coal combustion atmospheres with ambient Boston particles (vis-a-vis concentrated ambient particles – CAPs) will begin sooner using existing datasets.

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 dated March 31, 2004, December 2, 2004, and March 31, 2005.

Four scenarios (and seven sets of exposures) were carried out during this reporting period:

- March 21-24: Oxidized emissions (secondary particles) + 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 particles
- July 8 and July 13: Oxidized emissions + SOA
- September 8 and 9: Oxidized emissions + SOA (repeated)

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 condensation particle counter (CPC TSI 3022)
- SO_2 (pulsed fluorescence method)
- NO_x (chemiluminescence method)
- O_3 (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)

4.0 RESULTS AND DISCUSSION

4.1 Exposure Characterization

Due to the short turnaround time between the completion of fieldwork at Plant 1 and the preparation of this report, only a subset of exposure data were available for reporting.

Selection of Exposure Scenarios

Four 4-day experimental runs were carried out in normal laboratory rats (Stage I), with one scenario repeated:

- Round 1: March 21-24, oxidized emissions + SOA
- Round 2: May 3-6, oxidized emissions + SOA (repeated)
- Round 3: May 9-12, oxidized emissions
- Round 4: May 31-June 3, oxidized emissions + ammonia + SOA
- Round 5: June 6-9, primary particles only

Round 1 was carried out without the SCR operating, while the remainder of the runs were conducted with the SCR in operation. We selected the scenario that had yielded the most noticeable biological effects for the Stage II assessment in compromised rats. Thus, the oxidized, unneutralized + SOA scenario was investigated in two 2-day experimental runs as follows:

- Round 6: July 8 and July 13, oxidized emissions + SOA
- Round 7: September 8 and 9, oxidized emissions + SOA

Continuous Measurements

Available continuous data are provided in Table 2. Available parameters included RH, temperature, PM mass (TEOM), ozone, NO, NO_2, SO_2, and particle count. Mass ranged from 13.9 $\mu g/m^3$ for the primary particle scenario to 385 $\mu g/m^3$ for one of the oxidized emissions + SOA scenarios. Among the four scenarios investigating oxidized emissions + SOA, there was a wide range of mass concentrations (151, 282, 385, and 283 $\mu g/m^3$). The reasons for this wide variation are unclear, although the lowest mass concentration occurred when the SCR was not operational. The integrated mass concentration for Round 1 (see next section and Table 3) was substantially larger than the TEOM value, likely due to trapping of water by sulfuric acid particles which was then volatilized by the TEOM.

Concentrations of all analyzed gases were low.

Integrated Measurements

Integrated measurements obtained to date are shown in Table 3. Sulfate ranged from 82 to 156 $\mu g/m^3$ in Rounds 1-4. Nitrate was low in all scenarios, but highest in the neutralized scenario (Round 4). Ammonium was similarly low in all scenarios except the neutralized run.

Elemental Measurements

Elemental data for Plant 1 are not yet available.

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 ± SD.

	Round 1 Oxidized + SOA	Round 2 Oxidized + SOA	Round 3 Oxidized	Round 4 Oxidized + NH_3 + SOA	Round 5 Primary	Round 6 Oxidized + SOA	Round 7 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 (°)	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})	150.9 ± 34.8	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 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 ± SD.

	Round 1 Oxidized + SOA	Round 2 Oxidized + SOA	Round 3 Oxidized	Round 4 Oxidized + NH_3 + SOA	Round 5 Primary	Round 6 Oxidized + SOA	Round 7 Oxidized + SOA
Mass (μg m^{-3})	378.2 ± 100.1	-	-	-	-	-	
Sulfate (μg m^{-3})	82.3 ± 29.0	127.0 ± 35.7	101.1 ± 16.4	155.7 ± 12.4	0.4 ± 0.5	-	-
Nitrate (μg m^{-3})	0.9 ± 0.3	0.4 ± 0.3	0.2 ± 0.2	6.4 ± 1.7	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	-	-
OC (μg m^{-3})	161.4 ± 71.7	-	-	-	-	-	-
EC (μg m^{-3})	11.1 ± 3.9	-	-	-	-	-	-
α-Pinene (μg m^{-3})	7.8 ± 8.0	4.4 ± 1.4	NA*	6.0 ± 3.4	-	-	-

- not available
N/A: not applicable

4.2 Toxicological Assessments

Both Stage I and Stage II toxicological assessments were conducted at Plant 1; numbers of animals used for each endpoint are presented in Table 4.

The Stage I assessment consists of the following endpoints/procedures, evaluated in female Sprague-Dawley rats:

- Measurement of pulmonary function using the Buxco system (Buxco Biosystem 1.5.3A). Parameters of interest include frequency, tidal volume, inspiratory time, expiratory time, peak expiratory flow, and enhanced pause (Penh).
- *In vivo* chemiluminescence to measure oxidative stress in heart and lung tissue, conducted via organ chemiluminescence, a novel method that refers to the ultra-weak light emission produced by biological systems due to the de-excitation of high-energy byproducts of the chain reaction of lipid peroxidation (Boveris and Cadenas, 2000; Boveris et al., 1980). This method has been successfully used in models of oxidative injury in the lung (Gurgueira et al., 2002; Evelson et al., 2000; Turrens et al., 1988; Barnard et al., 1993).
- Bronchoalveolar lavage (BAL) to assess pulmonary inflammation. BAL fluid was analyzed for cellular content (cell viability, total cell counts, cell type) and biochemical markers of pulmonary injury (lactate dehydrogenase (LDH), β-n-acetyl glucosaminidase (βNAG), and total BAL protein) using standard methodologies.
- Blood cytology (total white blood cell counts and differential profiles), evaluated 24 hours following the last day of exposure.
- Histopathological analysis of lung and cardiac tissue by fixing tissue and randomly selecting three slices for processing by paraffin histology techniques.

The Stage II assessment is carried out in a myocardial infarction (MI) rat model (Wellenius et al, 2002). To produce the MI model, the fine tip electrode of a portable high-temperature thermocautery unit is 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 are then surgically implanted in Male Sprague-Dawley rats, and monitoring of cardiac function via electrocardiography (ECG) is carried out throughout exposure. The Stage II assessment thus includes:

- ECG data collection, including heart rate, heart rate variability (standard deviation of the normal beat-to-beat intervals; SDNN), and arrhythmias.
- Blood chemistry (complete blood count, interleukins-1 and –6, C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), and endothelin-1).

At the time of preparation of this report, only the pulmonary function and chemiluminescnece data were available; other analyses, including ECG analyses, will be reported in the next progress report.

Table 4. Number of experimental animals per scenario.

Scenario	Exposed	Sham	Buxco	Ox. Stress	Hist	BAL	Blood	Stage II (ECG and Blood Chemistry)
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

Some changes in breathing pattern were noted in animals exposed at Plant 1. One example is shown in Figure 1, whereby MI rats exposed to oxidized emissions + SOA demonstrated a significant increase in Enhanced Pause, a marker of airflow restriction, compared with control (sham) animals. In contrast, Figure 2 shows a nonsignificant change in Penh in normal animals exposed to the same exposure scenario.

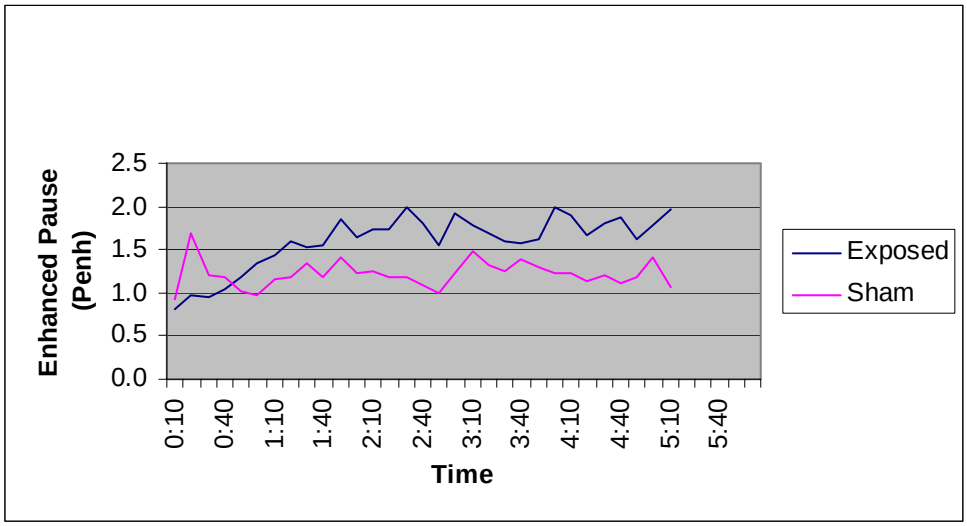

Figure 1. Changes in Enhanced Pause (Penh) in MI rats exposed to unneutralized, oxidized emissions + secondary organic aerosol.

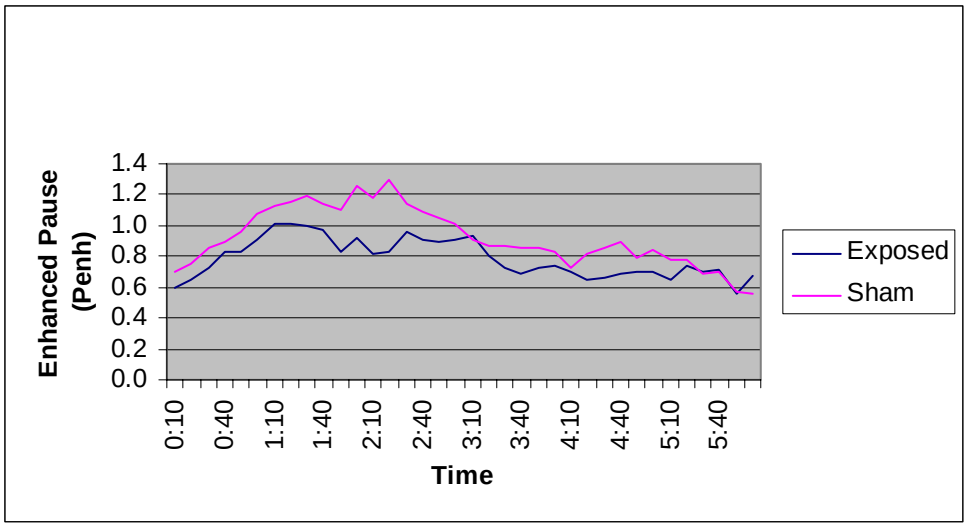

Figure 2. Changes in Enhanced Pause (Penh) in normal Sprague-Dawley rats exposed to unneutralized, oxidized emissions + secondary organic aerosol.

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 5, which describes the directional trends and the level of significance of the changing trend.

Table 5. 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)
Oxidized emissions + SOA #1	↑ ns	↓ **p=0.003**	NC…..ns	NC…..ns	↓ ns
Oxidized emissions + SOA #2	↑ ns	NC…..ns	NC…..ns	NC…..ns	↓ **p=0.001**
Oxidized emissions	↑ p=0.06	↓ **p=0.04**	↓ **p=0.02**	↓…p=0.06	↓ **p=0.01**
Oxidized emissions + SOA (MI model)	↑**p=0.024**	NC…..ns	NC…..ns	↓ **p=0.005**	↑**…p=0.03**
Oxidized emissions + NH_3 + SOA	↓ ns	↓ **p=0.002**	NC…..ns	NC…..ns	↓ **p=0.001**
Primary particles	↓ 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 5, 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. More detailed analysis of these data will be carried out to better understand these findings.

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 Chemiluminescence

Evidence of lung oxidative stress was observed in the oxidized emissions + SOA scenario (Figure 3a). The chemiluminescence findings were confirmed using the TBARS (thiobarbituric acid reactive substances) assay (Figure 3b). No evidence of oxidative stress was observed in heart tissue in this scenario, nor in lung or heart tissue in animals exposed to any of the other scenarios.

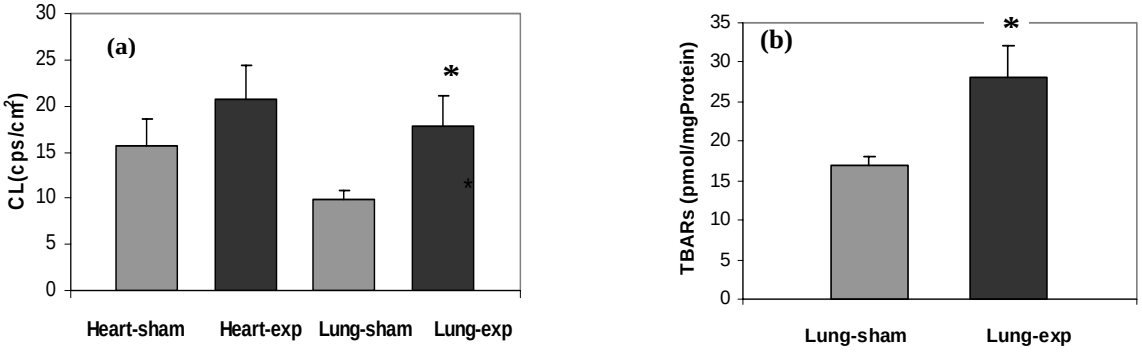

Figure 3. Oxidative stress in Sprague-Dawley rats exposed to oxidized emissions and secondary organic aerosol, Plant 1. (a) Chemiluminesence; (b) TBARS. * indicates statistically significant.

Histopathology

Histopathological analyses are in progress to assess evidence of inflammation in lung airways and parenchyma, and vasoconstriction in lung and cardiac blood vessels. These data collections are not yet completely analyzed; results will be reported in the next semiannual progress report.

ECG Analyses (Stage II)

Cardiac data is currently being analyzed. Arrhythmia data analysis is carried out manually, while some of the heart rate and heart rate variability analyses are automated. Both sets of data are expected to be available by the end of December.

Blood Chemistry (Stage II)

As with the ECG data, blood results will not be available until the end of December.

5.0 CONCLUSIONS

Significant progress was made on the Project during the second reporting period. We completed all fieldwork at Plant 1 and have a subset of the exposure and toxicological data in hand.

We carried out seven sets of exposures for Stage I and II toxicological assessments. Subtle biological effects were observed, including changes in breathing pattern (observed in several

scenarios) and an increase in oxidative stress (observed in one scenario, in lung tissue only). No changes in blood cytological or bronchoalveolar lavage parameters were observed. Stage II endpoints are currently being analyzed. Taken together, we can conclude that subtle changes appear to occur, without a strong indication of any particular type of adverse effect. More detailed analysis of these data will be carried out to better understand these findings.

We are currently completing processing and analysis of the exposure data. Once this is complete, we will be able to compare the exposure composition between plants. Clearly, it is of great interest to understand how exposures may have differed between the two plants in such a way to account for the differential biological responses observed.

Priorities for the next reporting period (September 1, 2005 – February 28, 2006) include:

- As required under the Cooperative Agreement, completion of a topical report for the Plant 0 findings.
- Completion of exposure and toxicological data analyses at Plant 1.
- Exploration of possible reasons for the differential toxicological responses observed at Plants 0 and 1.
- Preparation for fieldwork at Plant 2, located in the Midwest.

6.0 REFERENCES

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