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

Semi-Annual Technical Progress Report

Reporting Period Start Date: September 1, 2004
Reporting Period End Date: February 28, 2005

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Report Date: March 31, 2005

DOE Award Number: DE-FC26-03NT41902

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ABSTRACT

This report documents progress made on the subject project during the period of September 1, 2004 through February 28, 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, all fieldwork at Plant 0 was completed. Stack sampling was conducted in October to determine if there were significant differences between the in-stack PM concentrations and the diluted concentrations used for the animal exposures. Results indicated no significant differences and therefore confidence that the revised stack sampling methodology described in the previous semiannual report is appropriate for use in the Project.

Animal exposures to three atmospheric scenarios were carried out. From October 4-7, we conducted exposures to oxidized emissions with the addition of secondary organic aerosol (SOA). Later in October, exposures to the most complex scenario (oxidized, neutralized emissions plus SOA) were repeated to ensure comparability with the results of the June/July exposures where a different stack sampling setup was employed. In November, exposures to oxidized emissions were performed. Stage I toxicological assessments were carried out in Sprague-Dawley rats. Biological endpoints included breathing pattern/pulmonary function; *in vivo* chemiluminescence (an indicator of oxidative stress); blood cytology; bronchoalveolar lavage (BAL) fluid analysis; and histopathology. No significant differences between exposed animals and sham animals (exposed to filtered air) were observed for any of the endpoints; histopathological results are pending and will be reported in the next semiannual report.

The scenarios evaluated during this reporting period were slightly modified from those originally proposed. We substituted a new scenario, secondary aerosol + SOA, to investigate the effects of a strongly acidic aerosol with a biogenic component. Since we did not observe any biological response to this scenario, the neutralized secondary aerosol scenario (i.e., oxidized emissions + ammonia) was deemed unnecessary. Moreover, in light of the lack of response observed in the Stage I assessment, it was decided that a Stage II assessment (evaluation of cardiac function in a compromised rat model) was unlikely to provide useful information. However, this model will be employed at Plant 1 and/or 2.

During this reporting period, significant progress was made in planning for fieldwork at Plant 1. Stack sampling was carried out at the plant in mid-December to determine the concentration of primary particles. It was found that $PM_{2.5}$ mass concentrations were approximately three times higher than those observed at Plant 0. In mid-February, installation and setup for the mobile laboratories began. Animal exposures are scheduled to begin at this plant on March 21, 2005.

During the next reporting period, we will initiate fieldwork at Plant 1. At either or both Plants 1 and 2, a detailed Stage II assessment will be performed, even if no significant findings are observed in Stage I. The next seminannual report is expected to include a detailed description of the fieldwork at Plant 1, including toxicological findings and interpretation.

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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 eliminates any question of representativeness of emissions.

The study involves on-site sampling and dilution of coal combustion emissions at three coal-fired power plants, as well as mobile source emissions. Emissions are introduced into a reaction chamber to simulate oxidative atmospheric chemistry, and both primary and secondary materials are extensively characterized, including CO, 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 (September 1, 2004 through February 28, 2005) focused on completing the second round of fieldwork at Plant 0 in the Upper Midwest. Methods development, laboratory outfitting, and results from the first round of fieldwork at this plant were described in detail in the semiannual report covering the period March 1 – August 30, 2004. Important accomplishments during the current reporting period include:

Technical Advisory Committee Activities:

- A meeting of the TERESA Technical Advisory Committee was held on December 5, 2004 at the Harvard School of Public Health, Boston, MA. All TAC members were present.

Fieldwork at Plant 0:

- Fieldwork was completed.
- Stack sampling was carried out on October 19-21 to determine in-stack $PM_{2.5}$ concentrations.
- Exposures to oxidized emissions + secondary organic aerosol were carried out on October 4-7, 2004.
- Exposures to oxidized and neutralized emissions + SOA (most complex scenario) were carried out on October 11-14, 2004.
- Exposures to oxidized emissions only were carried out on November 3-5, 2004.
- Complete exposure characterization datasets were developed.
- Toxicological data for the pulmonary function/breathing pattern, *in vivo* chemiluminesence, bronchoalveolar lavage, and blood cytology endpoints were processed and interpreted.

Planning for Fieldwork at Plant 1:

- Stack sampling was carried out on December 13-14, 2004 to determine in-stack $PM_{2.5}$ concentrations.
- Installation of sampling ports and preparation for fieldwork began on February 14, 2005.
- Animal exposures at Plant 1 began on March 21, 2005.

Results of the toxicological testing completed in October/November indicate no significant differences in any endpoint between exposed and sham animals exposed to air only. These data reflect three different atmospheric scenarios.

Because of the lack of biological response observed in the Stage I toxicological assessment, it was decided that a Stage II assessment at Plant 0 using a compromised animal model was not necessary. This is based primarily on the fact that the *in vivo* chemiluminescence endpoint is very sensitive, and if it was not indicative of even subtle effects, it was deemed not worth pursuing more complex experimental work. We will, however, conduct such an assessment at either or both of the remaining two TERESA plants.

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

Technical Progress - 18 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%	5%
3	Field Study at Power Plant #2	0%	0%
4	Relative Toxicity of Coal Plant Emissions, Mobile Sources, and CAPs	0%	0%
5	Preparation of Peer-Reviewed Journal Articles	25%	25%
6	Project management and reporting	56%	56%

Priorities for the next reporting period (March 1, 2005 – August 31, 2005) include:

- Processing and interpretation of the histopathological data for Plant 0.
- As required under the Agreement, completion of a topical report for the Plant 0 findings.
- Completion of fieldwork at Plant 1, located in the Southeast.
- Interpretation of Plant 1 toxicological data.
- Preparation for fieldwork at Plant 2, located in the Midwest.
- Initiation of planning for an appropriate approach for the mobile source emissions component of TERESA. This component is not funded by NETL, but as part of the Project will be reported.

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 and December 2, 2004.

Three scenarios (and three sets of exposures) were carried out during this reporting period:

- October 4-7, 2004: Secondary particles (oxidized emissions) plus secondary organic aerosol (SOA).
- October 11-14, 2004: Secondary particles (oxidized emissions) plus ammonia (to neutralize strong acidity) and SOA.
- November 3-5, 2004: Secondary particles (oxidized emissions).

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

<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 (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)

All measurements were conducted as proposed, with the following modifications:

1. CO was not measured because it was expected to be extremely low after the dilution and denuder steps.
2. The elemental streaker was not used due to technical problems; however, elemental concentrations on 6-hour integrated samples were determined using XRF.

4.0 RESULTS AND DISCUSSION

This section describes the results of (1) stack sampling at Plants 0 and 1; (2) exposure characterization for the remaining animal exposures at Plant 0; (3) the remaining Stage I toxicological assessments at Plant 0; and (4) planning/preparation for field activities at Plant 1.

4.1 Stack Sampling at Plant 0 and Plant 1

The objective of the stack sampling was to evaluate possible differences between in-stack primary $PM_{2.5}$ concentrations and the diluted concentrations used in the animal exposures. On October 19-21, in-stack sampling was carried out at Plant 0. A $PM_{2.5}$ cyclone with a filter holder was placed inside the duct (Figure 1). 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).

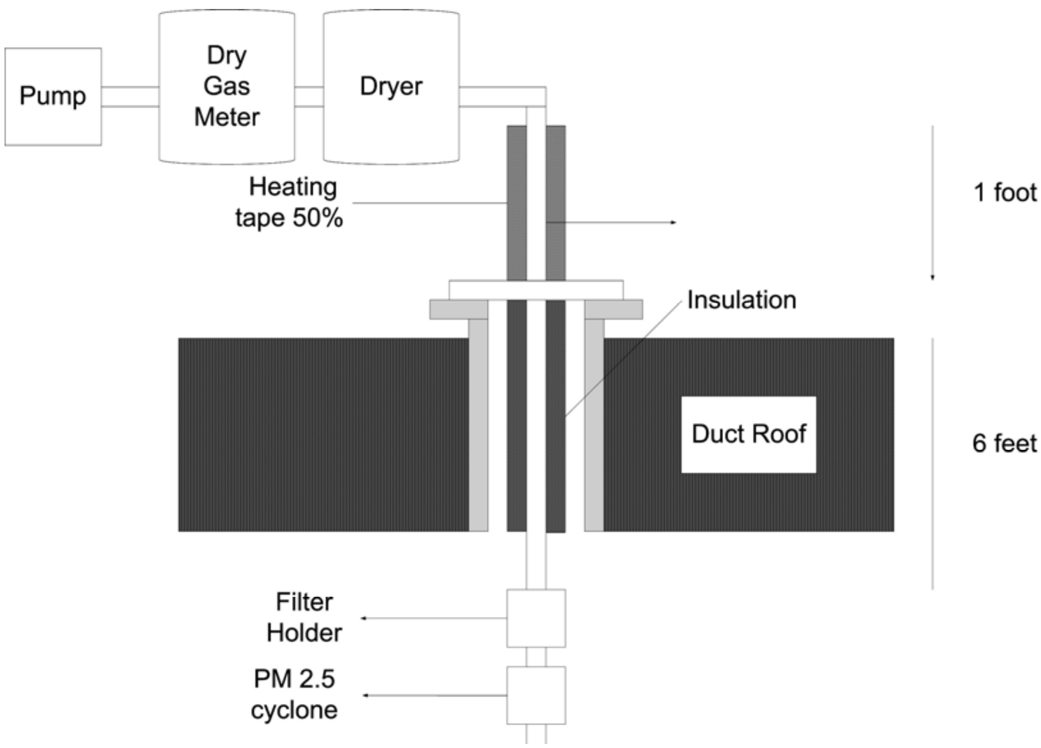

Figure 1. In-stack sampling setup employed at Plants 0 and 1.

After the sampling had been carried out, it was determined that the system had been operating with a lower cutpoint of approximately 1.7 μm, making the comparison between dilution and in-stack measurements difficult to interpret. In addition, a limitation of this type of testing is the fragility of the quartz filters. This led to high variability in the gravimetric blank samples, which prevented the accurate determination of mass concentration. However, we have more confidence in the elemental measurements performed using XRF (Table 1). The table shows elemental concentrations measured in-stack and in the diluted primary emissions. The table also includes the ratio between both measurements (i.e., in-stack concentration divided by dilution system concentration). If a dramatic difference existed, we would have expected to observe a very high ratio for most elements. On the contrary, ratios were usually close to one and more often slightly less than 1 (e.g., S, Ca, Fe). At Plant 0, it is not important to determine the exact uncertainties in the ratios since the overall results show no significant difference between measurements made in-stack and post-dilution. We therefore conclude that the revised stack sampling scheme (discussed in the previous semiannual report) is appropriate for use in the TERESA study.

Table 1. Elemental concentrations ($\mu g/m^3$) in stack exhaust and diluted primary emissions.

Element	Dilution	In-Stack	Ratio	Element	Dilution	In-Stack	Ratio
Al	33.22	7.54	0.2	**Ni**	0.06	0.13	2.2
P	11.65	0.00	0.0	**Cu**	0.20	0.06	0.3
S	33.99	23.62	0.7	**Zn**	0.06	0.05	0.9
K	1.02	0.31	0.3	**Se**	0.04	0.88	22.3
Ca	121.96	71.21	0.6	**Sr**	3.49	1.81	0.5
Ti	4.27	1.91	0.4	**Mo**	0.02	0.02	1.2
V	0.09	0.03	0.3	**Pd**	0.05	0.04	0.8
Cr	0.04	0.33	7.3	**Sn**	0.00	0.04	Div 0
Mn	0.14	0.24	1.7	**Ba**	6.46	5.22	0.8
Fe	17.99	22.00	1.2	**Pb**	0.00	0.06	Div 0

Stack sampling was then conducted at Plant 1 (Southeast) on December 13-16, 2005 to evaluate $PM_{2.5}$ mass concentration and to obtain information on elemental composition. Five samples (3 hour integration period) were collected directly from the stack using an in-stack sampling system with a $PM_{2.5}$ cut-off cyclone (again based on EPA Conditional Test Method 040) . Samples were collected 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.

Based on gravimetric measurements (Table 2), the average mass concentration (± standard deviation) was 1735 ± 1318 $\mu g/m^3$, ranging from 464 to 3900 $\mu g/m^3$. In-stack fine particle mass at Plant 1 therefore appears to be approximately 3 times higher than that mass at Plant 0 (~250-500 $\mu g/m^3$). In addition, the mass concentration at Plant 1 seems to be more variable than Plant 0. In-stack sampling at both plants were carried out under similar conditions, such as flow rate and sampling duration.

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

Sample No.	Plant 0			Plant 1		
	Gravimetric Mass	Estimated* Mass	Ratio	Gravimetric Mass	Estimated* Mass	Ratio
1	Not available	202	-	464	302	0.65
2	Not available	227	-	1626	785	0.48
3	Not available	155	-	3900	2644	0.68
4	246	251	1.02	1749	733	0.42
5				937	417	0.45
Mean	246	209	1.02	1735	976	0.54
S.D.	-	41	-	1318	954	0.12

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

Based on the XRF elemental data, the estimated mass concentration (mean ± standard error) of major oxides and trace elements, excluding ionic and carbonaceous species, was approximately 976 ± 954 $\mu g/m^3$, ranging from 302 $\mu g/m^3$ to 2644 $\mu g/m^3$ (Table 3). The mean ratio of estimated to gravimetric mass was about 0.54. The remainder of the total mass (~ 40%) 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.

Contribution of major oxides (Al_2O_3, SO_3, K_2O, CaO, TiO_2, Mn_3O_4, Fe_2O_3, SrO, BaO) and trace elements to PM mass concentrations at Plants 0 and 1 are shown in Table 3 and Figure 2. Most of the elements at Plant 1 were present at higher concentrations than at Plant 0. The major oxides were present at both plants at similar concentrations, except for CaO, which was much lower at Plant 1 than at Plant 0.

Table 3. Mass contribution (%) of elements/major oxides to estimated mass at Plants 0 and 1.

Major Oxides (Wt%)	Plant 0	Plant 1
Al	3.08	12.18
S	11.62	11.25
Cl	0.00	0.25
K	0.13	4.10
Ca	33.87	4.32
Ti	0.88	2.24
V	0.01	0.33
Cr	0.16	5.20
Mn	0.12	0.06
Fe	11.04	16.27
Ni	0.07	1.87
Cu	0.03	0.52
Zn	0.03	0.16
Ga	0.00	0.03
Ge	0.00	0.05
As	0.00	0.11
Se	0.45	0.41
Br	0.00	0.01
Rb	0.00	0.02
Sr	0.86	0.39
Y	0.00	0.04
Zr	0.04	0.06
Mo	0.01	0.14
Ba	2.54	0.44
Hg	0.00	0.06
Pb	0.03	0.09

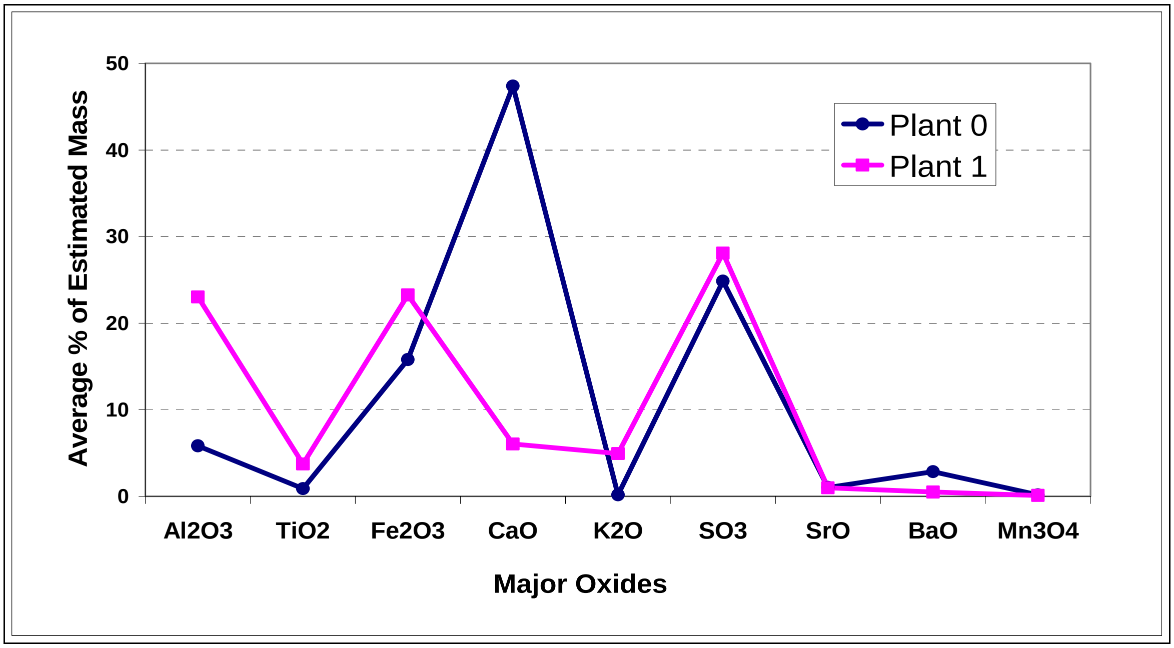

Figure 2. Mass contribution of major oxides to in-stack $PM_{2.5}$ at Plants 0 and 1.

4.2 Exposure Characterization

The results discussed in this report cover the last three sampling rounds, which were carried out in October and November, 2004. All exposure data, including those that were not yet available for the June-July sampling rounds discussed in the previous progress report, are presented for the October-November runs. Updated/augmented data for the earlier sampling rounds are not presented for simplicity; however, the complete set of exposure characterization data for all rounds will be reported in the Plant 0 topical report currently in preparation. Also, note that due to the short turnaround time between completion of laboratory analyses and the preparation of this report, there has been insufficient time to develop a detailed understanding of the differences in measured values for the different scenarios investigated.

Selection of Exposure Scenarios

Three scenarios were investigated:

- Round 1: October 4-7, un-neutralized, oxidized emissions + SOA
- Round 2: October 11-14, neutralized, oxidized emissions + SOA
- Round 3: November 3-5, un-neutralized, oxidized emissions

The Round 1 scenario was not originally included in the study plan; however, we decided that this might be the most likely to induce biological effects, given that it would be highly acidic and contain SOA. Since we had not previously observed biological effects, it was deemed a useful scenario to investigate. We also repeated the most complex scenario (Round 2) since there had been a change in the sampling scheme from the June-July exposures. Round 3 was carried out to secondary aerosol with no added components given that this material would also be highly acidic. We did not evaluate the neutralized, oxidized scenario because it is likely to be the least biologically potent scenario.

Integrated Measurements

Integrated measurement data for the October and November experimental runs are provided in Table 4 below. As described below, several puzzling results were observed, which will be explored further and interpreted in the topical report for Plant 0.

During the first round (October 4-7), acidity was high, as expected, as was OC, whereas ammonium was very low. The sum of sulfate and OC approximated the total $PM_{2.5}$ mass.

During the second round (October 11-14), there were no qualitative or quantitative changes in the composition of PM compared with the previous rounds (June/July). Acidity was low and OC was high, and the sum of sulfate and OC approximated the total $PM_{2.5}$ mass. OC was not as high as for the earlier run in October. It is possible that the OC values for both runs could be biased because the quartz fiber filters can adsorb VOCs. There was also an unexplained decrease in the total secondary aerosol and sulfate generated, and an increase in nitrate.

During the third round (November 3-5), PM mass was also lower than expected. It is unclear why OC is elevated in this scenario without secondary organic aerosol. Again, as with Round 2, sulfate was lower than expected.

It is surprising that elemental carbon was observed in any of the scenarios since it is present at low concentrations in emissions from coal-fired power plants and at even lower concentrations in the diluted stack emissions used in this study. None of the chemical reactions are expected to produce EC.

Table 4. Integrated measurements for October-November 2004 experimental runs. October 4-7: oxidized emissions + SOA; October 11-14: oxidized and neutralized emissions + SOA; November 3-5: oxidized emissions only. Values expressed as mean ± SD.

	October 4-7 n=4	**October 11-14** n=4	**November 3-5** n=3
Mass ($\mu g/m^3$)	193 (73)	141 (16)	69 (10.4)
SO_4 ($\mu g/m^3$)	57.1 (24)	38.7 (11)	31.8 (1.3)
NO_3 ($\mu g/m^3$)	1 (0.4)	37.7 (6.2)	1.1 (1.2)
NH_4^+ ($\mu g/m^3$)	3.1 (1.2)	14.7 (4.1)	3.3 (1.7)
H^+ (nmoles/m^3)	1003 (463)	33 (36)	459 (82.3)
Acidity ($\mu g/m^3$ H_2SO_4)	49.1 (22.7)	1.6 (1.7)	22.5 (4)
SO_2 (ppb)	17.5 (4.4)	16 (3)	9.3 (3.5)
HNO_3 (ppb)	1.6 (0.3)	2.3 (0.6)	0.6 (0.1)
HONO (ppb)	11.2 (5.1)	7.8 (1.5)	5 (1)
NH_3 (ppb)	20.8 (3.8)	16.1 (6.2)	9.9 (6.2)
OC ($\mu g/m^3$)	130.7 (7.1)	100.6 (6.6)	54.9 (6.9)
EC ($\mu g/m^3$)	12.1 (9.4)	4.3 (0.7)	2.8 (1.6)
TC ($\mu g/m^3$)	142.8 (8.2)	104.8 (7.3)	57.6 (8)
Formaldehyde ($\mu g/m^3$)	16.1 (3.6)	18.1 (3.9)	N/A
Acetaldehyde ($\mu g/m^3$)	5.2 (1)	4.8 (0.6)	N/A
Acetone ($\mu g/m^3$)	15.5 (5.2)	13 (2.9)	N/A
Total Carbonyls ($\mu g/m^3$)	36.8 (9.2)	35.9 (5.3)	N/A
Pinene ($\mu g/m^3$)	0.6 (0.1)	0.8 (0.3)	N/A

TC = EC + OC

Total Carbonyls = Formaldehyde + Acteladehyde + Acetone

N/A applies to species in the November round because no pinene was added

Continuous Measurements

Results for the continuous analyses are shown in Table 5. The TEOM values are higher than the integrated, gravimetric mass measurements, which is surprising given that for atmospheric measurements the heated filter for the TEOM causes losses of some semi-volatile species, usually resulting in concentrations lower than the gravimetric mass values. We do not have an explanation for the observed higher TEOM values.

As mentioned above, there has not been sufficient time to comprehensively review all the continuous measurements and develop a detailed understanding of the measured values.

Table 5. Continuous measurements during October-November 2004 experimental runs. October 4-7: oxidized emissions + SOA; October 11-14: oxidized and neutralized emissions + SOA; November 3-5: oxidized emissions only. Values expressed as mean ± SD.

	October 4-7 n=4	**October 11-14** n=4	**November 3-5** n=3
TEOM ($\mu g/m^3$)	138.2 (53.4)	116.8 (25.2)	58.2 (5.8)
CPC ($\#/cm^3$)	16924 (4495)	66445 (8913)	6723 (3550)
O_3 (ppb)	26.8 (6.9)	15.6 (6)	26.9 (1)
SO_2 (ppb)	38.9 (8.3)	40.8 (3.8)	31.7 (4.3)
NO (ppb)	3.5 (2.9)	4.6 (1.2)	3.9 (0.5)
NO_2 (ppb)	17.5 (6.6)	10.1 (4.2)	8.4 (1.8)
RH (%)	11.1 (12.6)	27.2 (2.4)	13.5 (8.7)
T (ºC)	24.4 (0.4)	24.8 (1.3)	23.9 (0.1)

Elemental Measurements

Integrated elemental measurements are provided in Table 6. The results are bold for those values 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. Note also that each sample has a different set of uncertainty values because with XRF, the uncertainty is related to corrections for interference by masking of elements higher than any given element, and the distribution of element magnitudes is different for each sample.

All elements were present at low concentrations, with the exception of sulfur, which was present in all samples at 10 – 29 $\mu g/m^3$. Silicon, calcium, bromine, and lanthanum were commonly detected in multiple samples. Less commonly observed elements included Mg, Al, Cl, K, Cr, Fe, Ni, Cu, Zn, Se, Ba, and Hg.

Differences in metal concentrations between scenarios may be a result of variations in stack gas mass concentrations reflecting differences in the size distribution of particles. The operation of the electrostatic precipitator may be sufficiently variable to allow such differences.

Table 6. Elemental concentrations during October-November 2004 experimental runs. October 4-7: oxidized emissions + SOA; October 11-14: oxidized and neutralized emissions + SOA; November 13-15: oxidized emissions only.

	October 4-7				October 11-14				November 3-5			Blanks
Exposure Day	1	2	3	4	1	2	3	4	1	2	3	1
Date	10/4/04	10/5/04	10/6/04	10/7/04	10/11/04	10/12/04	10/13/04	10/14/04	11/3/04	11/4/04	11/5/04	10/8/04
PM (μg/m^3)	270	196	211	94	144	136	123	161	62	77	N/A	-3.1
Mg	0.0276	0.0000	0.0712	0.0000	0.0568	**0.2478**	0.1214	0.1612	0.0000	**0.1914**	0.0802	0.0000
Al	0.0439	0.0312	0.0568	0.0000	0.0259	0.0337	**0.0749**	0.0612	0.0534	0.0457	0.0677	0.0000
Si	0.0000	0.0000	0.0000	**0.0281**	**0.0444**	**1.5734**	**0.0601**	**0.0643**	**0.0406**	**0.1216**	**0.1694**	0.0000
P	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
S	**25.2556**	**16.8394**	**29.3006**	**11.6510**	**15.1108**	**12.9898**	**18.3342**	**23.1029**	**12.4818**	**11.1993**	**10.4914**	0.0126
Cl	0.0164	0.0000	0.0000	**0.0488**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
K	0.0079	**0.0299**	0.0091	**0.0119**	**0.0117**	**0.0267**	**0.0223**	0.0077	0.0010	0.0026	0.0000	0.0000
Ca	**0.0380**	**0.0382**	0.0131	**0.0188**	**0.0321**	**0.0477**	**0.0396**	0.0025	**0.0149**	**0.0185**	**0.0155**	0.0000
Ti	0.0000	0.0000	0.0052	0.0000	0.0000	0.0000	0.0037	0.0000	0.0000	0.0047	0.0009	0.0000
Cr	0.0013	0.0051	0.0046	0.0000	0.0000	0.0057	**0.0068**	0.0000	0.0005	0.0021	**0.0090**	0.0000
Mn	0.0013	0.0000	0.0000	0.0019	0.0000	0.0000	0.0068	0.0000	0.0000	0.0000	0.0030	0.0000
Fe	0.0105	0.0025	0.0000	0.0025	0.0062	0.0127	0.0081	0.0000	0.0000	0.0000	**0.0358**	0.0000
Ni	0.0046	0.0083	0.0059	0.0044	0.0062	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0086**	0.0006
Cu	0.0026	0.0000	0.0000	0.0038	0.0000	**0.0133**	0.0000	0.0006	0.0000	0.0000	0.0034	0.0000
Zn	0.0046	0.0000	0.0000	0.0000	0.0000	**0.3539**	0.0068	**0.0089**	0.0000	0.0000	0.0013	**0.0063**
Se	**0.0137**	**0.0127**	0.0020	0.0038	0.0013	0.0000	**0.0093**	0.0013	0.0000	**0.0090**	0.0065	0.0006
Br	**0.0295**	**0.0178**	**0.0065**	**0.0131**	**0.0086**	**0.0108**	**0.0075**	0.0051	0.0000	**0.0077**	0.0030	0.0000
Sr	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0025	0.0019	0.0015	0.0000	0.0000	0.0000
Ba	**0.0445**	**0.0401**	0.0117	**0.0300**	0.0185	0.0146	0.0248	0.0000	**0.0216**	0.0034	0.0000	0.0163
La	**0.0387**	0.0159	0.0026	0.0156	0.0074	**0.0305**	**0.0322**	0.0236	**0.0329**	**0.0185**	0.0004	**0.0232**
Hg	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0192**	0.0000	0.0000	0.0060	0.0004	0.0000

4.3 Toxicological Assessments

The Stage I toxicological 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 toxicological results for all experiments at Plant 0 (including the June-July exposures which were previously reported) are presented below. In the case of the most complex scenario, which was carried out in triplicate, all animals were combined. The total number of animals for each scenario is shown in Table 7.

Table 7. Number of experimental animals per scenario.

SCENARIO	RESPIRATORY PARAMETERS		BAL PARAMETERS		BLOOD PARAMETERS	
	Control	Exposed	Control	Exposed	Control	Exposed
Primary	20	20	0	0	12	12
Secondary	15	15	5	5	9	9
Secondary + SOA	60	60	18	18	36	36
Secondary + NH_3 + SOA	20	20	6	6	12	12

Pulmonary Function

As with the June-July scenarios reported in the previous progress report, we observed no differences between exposed and control animals for any of the pulmonary function/breathing pattern parameters examined (Figures 3, 4, and 5).

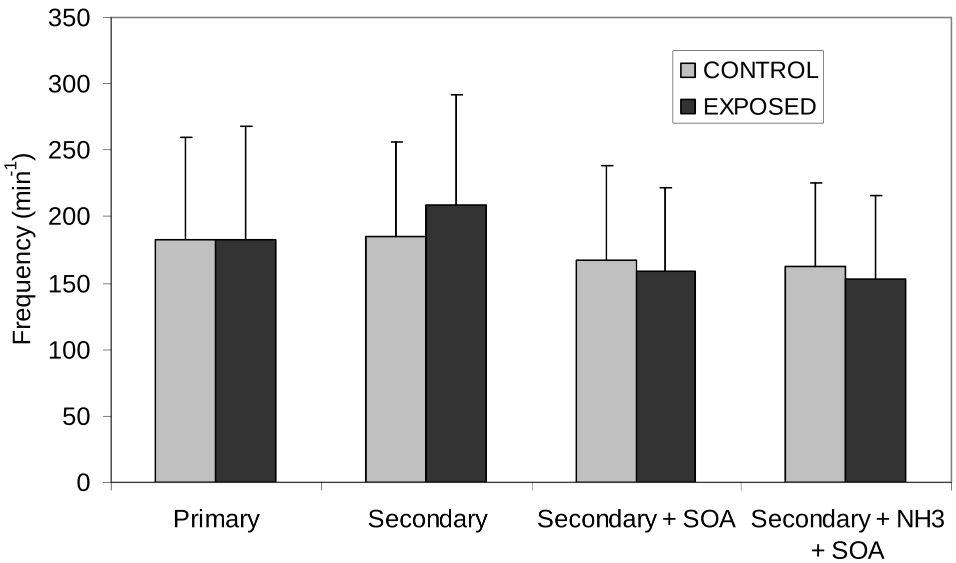

Figure 3. Respiratory frequency in Sprague-Dawley rats exposed to different power plant emission scenarios, Plant 0, June/July and October/November, 2004.

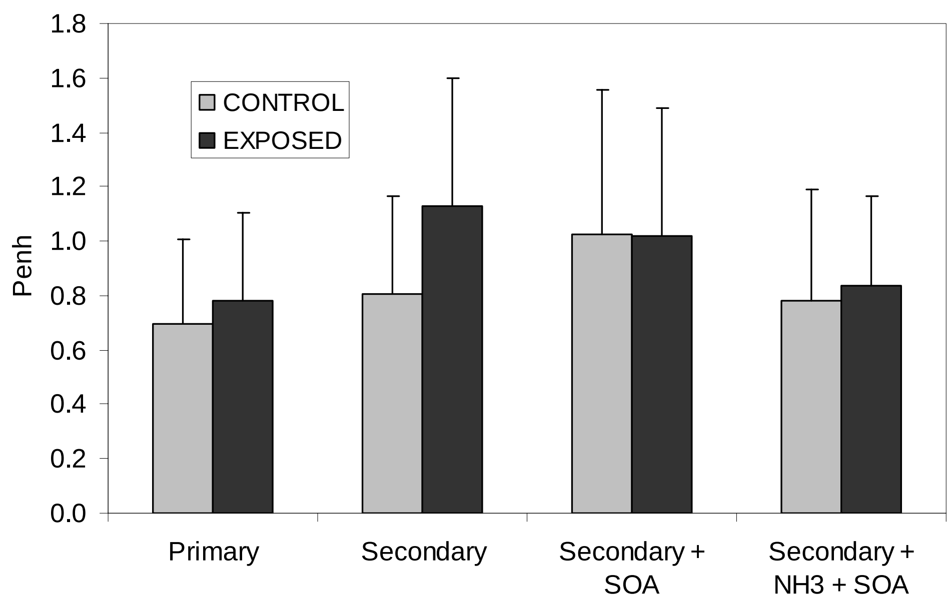

Figure 4. Enhanced Pause (Penh) as a measure of bronchoconstriction in Sprague-Dawley rats exposed to different power plant emission scenarios, Plant 0, June/July and October/November, 2004.

Temporal patters of tidal volume (TV) changes are shown in Figure 5 as a mean of all animals in each scenario. Although in some instances there were differences in the mean between groups from the beginning to the end of exposure, the important distinction here is that the same trends are seen in both groups indicating that there was no effect of aerosol exposure on the parameter. Since the purpose of these graphs is to view trends, error bars are not shown. In all instances, error bars completely overlap for each group. There was a suggestion of a slight reduction in TV over the course of the 6-hour exposure period for both the control (sham) and exposed animals. There were no significant differences between control (sham) and exposed animals, however.

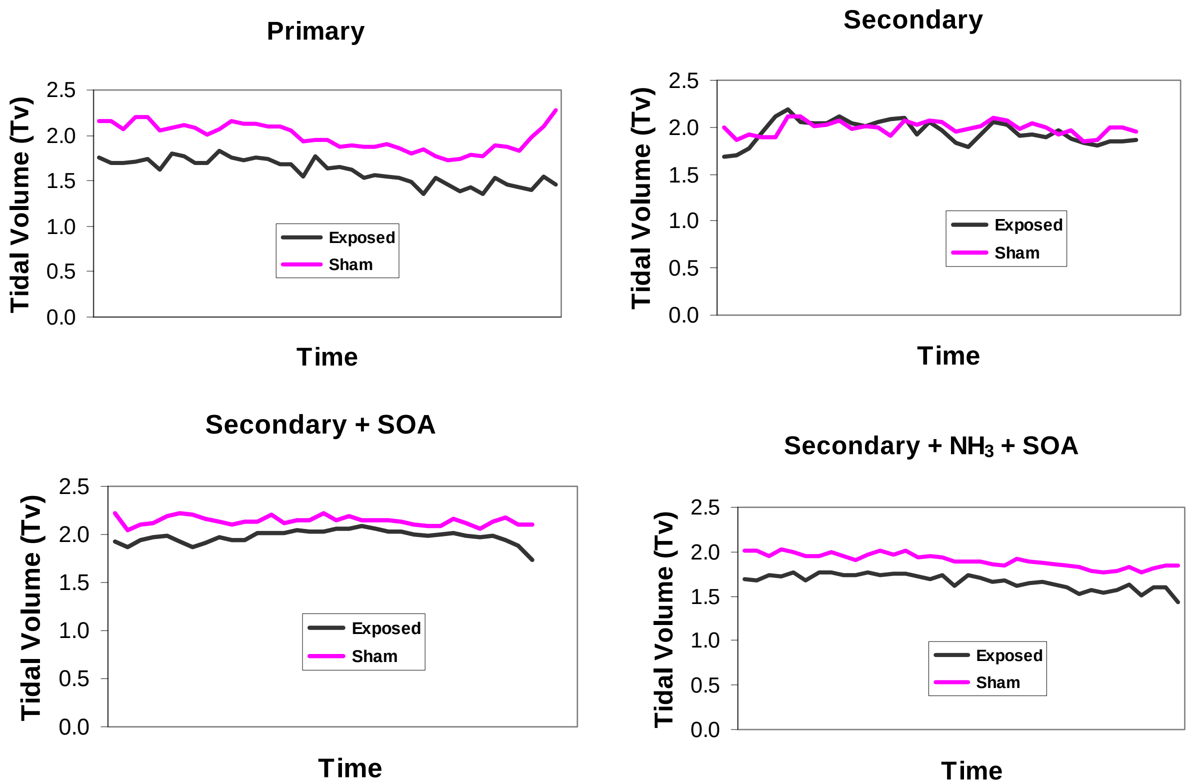

Figure 5. Tidal volume changes over the exposure period in Sprague-Dawley rats exposed to different power plant emission scenarios, Plant 0, June/July and October/November, 2004. The x-axis represents the entire 6-hour exposure period.

Bronchoalveolar Lavage Parameters

Selected results of the BAL fluid analyses are shown in Figures 6 and 7. No significant differences between exposed and control animals were observed for cytological parameters (total cell count, polymorphonuclear neutrophils). Results for biochemical markers (LDH, βNAG, and total protein) also showed no significant differences between groups (data not shown).

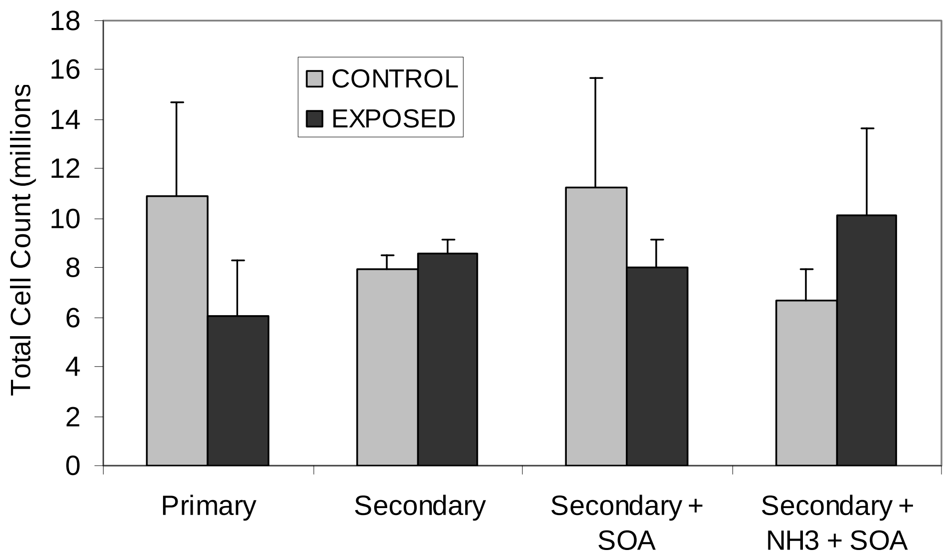

Figure 6. Total cell count in BAL fluid from Sprague Dawley rats after exposure to different power plant emission scenarios, Plant 0, June/July and October/November, 2004.

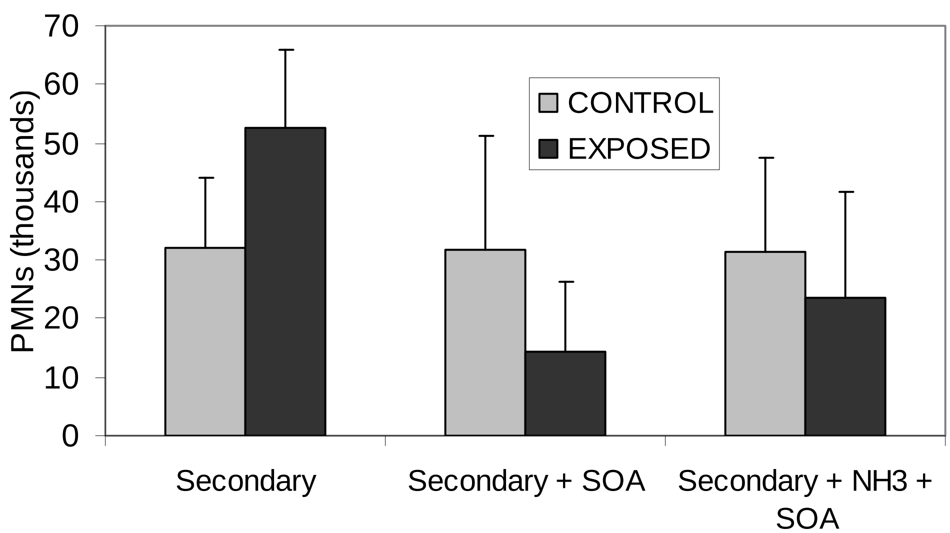

Figure 7. Polymorphonuclear neutrophils (PMNs) in BAL fluid from Sprague Dawley rats after exposure to different power plant emission scenarios, Plant 0, June/July and October/November, 2004.

Blood Cytology

Results of selected blood cytological analyses are provided in Figures 8 and 9 below. No significant differences between exposed and sham animals were observed.

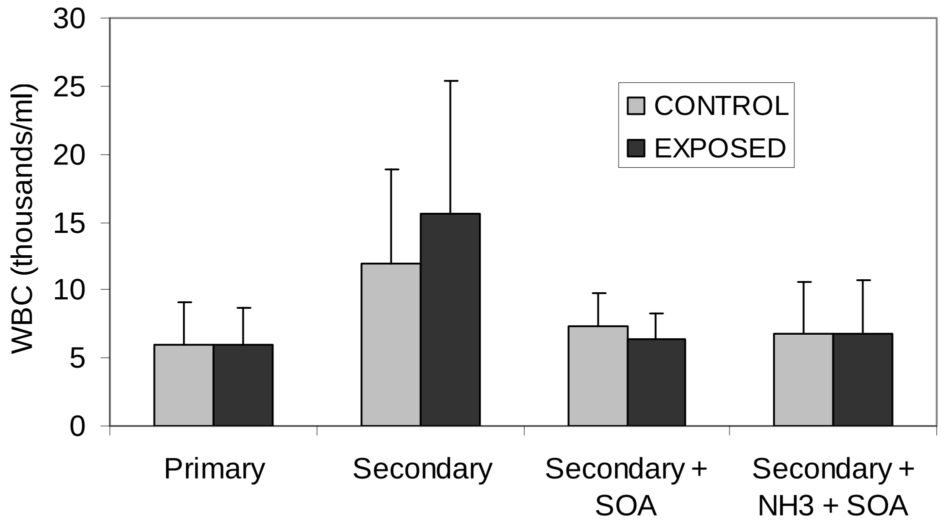

Figure 8. White blood cell counts, Sprague-Dawley rats after exposure to different power plant emission scenarios, Plant 0, June/July and October/November, 2004.

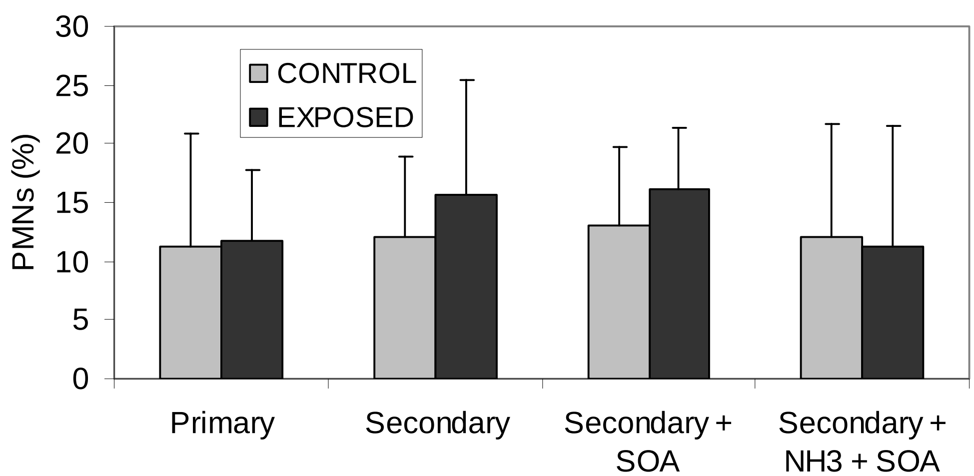

Figure 9. Blood polymorphonuclear neutrophils in blood from Sprague-Dawley rats after exposure to different power plant emission scenarios, Plant 0, June/July and October/November, 2004.

In Vivo Chemiluminescence

To confirm the chemiluminescence findings, the TBARS (thiobarbituric acid reactive substances) assay was also carried out for the two scenarios completed in October. Only TBARS was employed in the November sampling round. For the October 4-7 exposures (Figure 10), no significant differences between exposed and sham animals were observed.

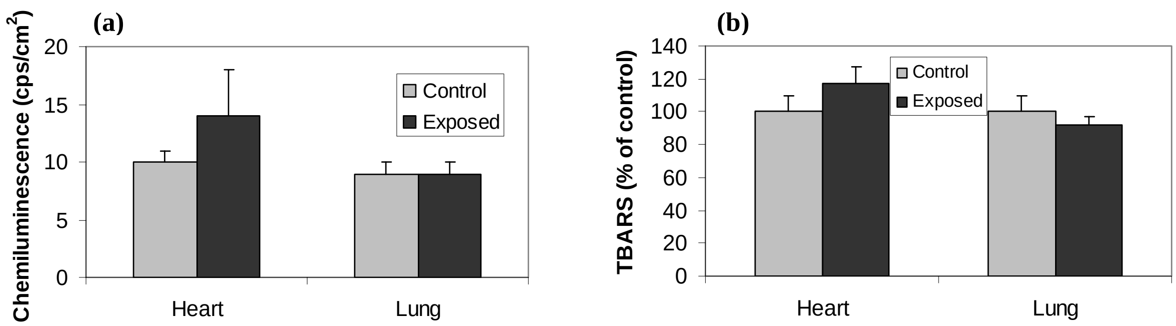

Figure 10. Oxidative stress in Sprague-Dawley rats exposed to oxidized emissions and secondary organic aerosol, Plant 0, October 4-7, 2004. (a) Chemiluminesence, *n*= 6 for control, heart; 7 for exposed, heart; 7 for control, lung; and 8 for exposed, lung. (b) TBARS, *n*=8 for all groups.

For the combined (pooled) June and October exposures to the most complex scenario (oxidized, neutralized + SOA), a difference in the chemiluminescence lung response was observed in the exposed group (Figure 11). However, although the difference was statistically significant, this difference was primarily driven by the lower chemiluminescence values observed in control animals during the October exposures. However, when compared with the pooled data for all the control animals run at Plant 0, or with the data for control animals exposed to this scenario in June, the aerosol exposed group showed no significant increase in chemiluminesence.

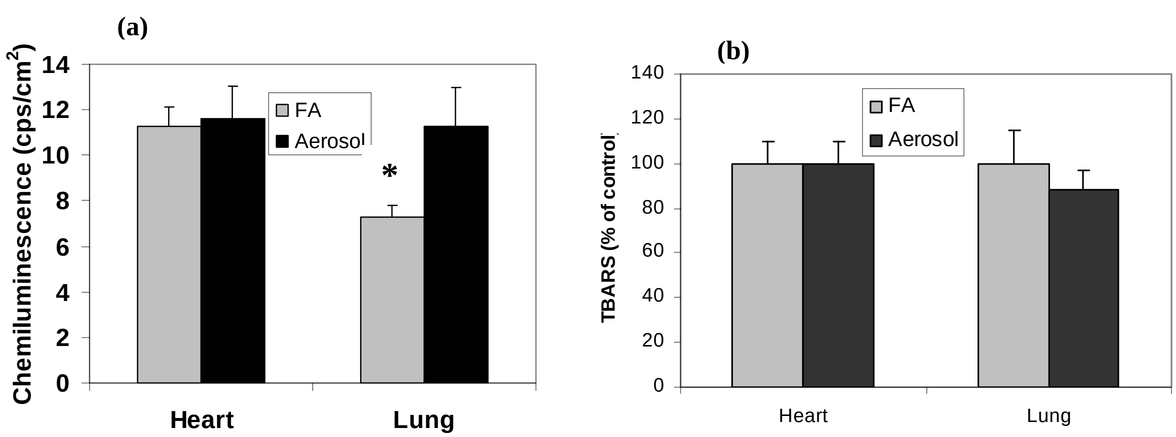

Figure 11. Oxidative stress in Sprague-Dawley rats exposed to oxidized, neutralized emissions and secondary organic aerosol, Plant 0. (a) Chemiluminesence, pooled animals, June and October, 2004. *n*= 22 for control, heart; 21 for exposed, heart; 19 for control, lung; and 17 for exposed, lung. (b) TBARS, October, 2004. *n*=8 for all groups. * indicates significant difference between sham and exposed animals (p<0.05) using a 2-tailed t-test.

For the November exposures (Figure 12), no significant differences between exposed and sham animals were observed.

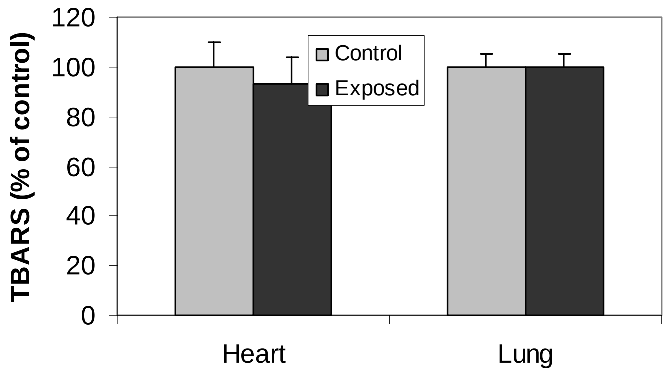

Figure 12. TBARS results for Sprague-Dawley rats exposed to oxidized emissions, Plant 0, November 3-5, 2004. *n*= 8 for all groups.

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.

4.4 Planning and Preparation for Fieldwork at Plant 1

Installation and setup of the mobile chemical and toxicological laboratories at Plant 1 began on February 14, 2005. The primary installation is similar to what had been done previously at Plant 0. A one-inch stainless steel tube (~ 100 ft) is used to deliver diluted stack emissions to the mobile chemical lab. Initial test runs of the use of the mobile lab to produce secondary sulfate from the diluted stack gas are scheduled for the first two weeks of March, with the first set of animal exposures beginning on March 21. Animals will be exposed to the most complex scenario initially; preparation and planning/scheduling for conducting further animal exposures will depend on the results from this initial test run. If biological effects are observed for the most complex exposure scenario, then the complete set of scenarios will be conducted at Plant 1, including the compromised MI rat model (Stage II assessment).

5.0 CONCLUSIONS

Significant progress was made on the Project during the second reporting period. We completed all remaining animal exposure experiments at Plant 0, analyzed all laboratory (exposure characterization) data, and interpreted the toxicological findings. We verified that the sampled primary particles from the stack are in fact representative of those being emitted from the stack.

We carried out three sets of exposures: (1) oxidized emissions + SOA; (2) oxidized and neutralized emissions + SOA; and (3) oxidized emissions. No biological effects were observed in any of the scenarios with the Stage I toxicological assessments.

We have relocated the mobile laboratories to Plant 1, and are currently carrying out testing and characterization work, as well as initiating animal exposures.

During the next reporting period, we will document and describe the fieldwork at Plant 1, which we expect to be complete by mid-summer 2005. This report will include detailed Stage I toxicological findings for all scenarios run, and Stage II toxicological findings for one selected scenario. Again, depending upon the outcome of the fieldwork at Plant 1 (i.e. the biological effects observed), not all the proposed scenarios may be evaluated.

Thus, priorities for the next reporting period (March 1, 2005 – August 31, 2005) include:

- As required under the Cooperative Agreement, completion of a topical report for the Plant 0 findings.
- Completion of fieldwork at Plant 1, located in the Southeast.
- Interpretation of Plant 1 toxicological data.
- Preparation for fieldwork at Plant 2, located in the Midwest.
- Initiation of planning for an appropriate approach for the mobile source emissions component of TERESA. This component is not funded by NETL, but as part of the Project will be reported.

6.0 REFERENCES

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