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, 2004 through August 31, 2004. 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 analysis and interpretation of the field data collected at the first power plant (henceforth referred to as Plant 0, and 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.

Significant progress was made on the Project during this reporting period, with field work being initiated at Plant 0. Initial testing of the stack sampling system and reaction apparatus revealed that primary particle concentrations were lower than expected in the emissions entering the mobile chemical laboratory. Initial animal exposures to primary emissions were carried out (Scenario 1) to ensure successful implementation of all study methodologies and toxicological assessments. Results indicated no significant toxicological effects in response to primary emissions exposures.

Exposures were then carried out to diluted, oxidized, neutralized emissions with the addition of secondary organic aerosol (Scenario 5), both during the day and also at night when primary particle concentrations in the sampled stack emissions tended to be slightly higher. Exposure concentrations were about 249 $\mu g/m^3$ PM, of which 87 $\mu g/m^3$ was sulfate and approximately 110 $\mu g/m^3$ was secondary organic material (~44%). Results indicated subtle differences in breathing pattern between exposed and control (sham) animals, but no differences in other endpoints (*in vivo* chemiluminescence, blood cytology, bronchoalveolar lavage fluid analysis).

It was suspected that primary particle losses may have been occurring in the venturi aspirator/orifice sampler; therefore, the stack sampling system was redesigned. The modified system resulted in no substantial increase in particle concentration in the emissions, leading us to conclude that the electrostatic precipitator at the power plant has high efficiency, and that the sampled emissions are representative of those exiting the stack into the atmosphere. This is important, since the objective of the Project is to carry out exposures to realistic coal combustion-derived secondary PM arising from power plants.

During the next reporting period, we will document and describe the remainder of the fieldwork at Plant 0, which we expect to be complete by mid-November 2004. This report will include detailed Phase I toxicological findings for all scenarios run, and Phase II toxicological findings for one selected scenario. Depending upon the outcome of the ongoing fieldwork at Plant 0 (i.e. the biological effects observed), not all the proposed scenarios may be evaluated. The next report is also expected to include preliminary field data for Plant 1, located in the Southeast.

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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.

EXECUTIVE SUMMARY

Activities conducted during the second reporting period (March 1, 2004 through August 31, 2004) primarily focused on initiating the field work at Plant 0 in the Upper Midwest. Methods development and laboratory outfitting activities were described in detail in the last semiannual report. Important accomplishments during this second reporting period include:

Technical Advisory Committee Activities:

- An interim teleconference of the TERESA Technical Advisory Committee was held on June 23, 2004.

EPRI and DOE-NETL Site Visits:

- Annette Rohr (EPRI) visited the Upper Midwest plant on May 13, 2004.
- Annette Rohr and Bill Aljoe (NETL) visited the Upper Midwest plant on June 29, 2004.

Field Work at Plant 0:

- Field work was initiated at the plant in early May, 2004.
- Animal exposures to the first exposure scenario (primary emissions) were carried out on May 10, 11, 12, and 13, 2004.
- Potential particle loss issues were hypothesized based on the low mass and number concentration of primary particles in the stack samples entering the mobile laboratory.
- A revised stack sampling system was designed and implemented.
- Additional sets of animal exposures were carried out on June 22, 23, 25, and 26 (daytime exposures), and again on June 28, 29, 20, and July 1 (nighttime exposures). Both of these sets of exposures were to the most complex atmospheric scenario (oxidized, neutralized emissions + secondary organic aerosol).
- Exposure characterization data were generated.
- Toxicological data for the pulmonary function/breathing pattern, *in vivo* chemiluminesence, and bronchoalveolar lavage endpoints were processed and interpreted.

Planning for Remaining Host Plants:

- Annette Rohr and Steve Ferguson (Harvard) visited an additional plant in the Upper Midwest (Plant 2) on September 9, 2004. The plant appears to be appropriate for study, and stack access was established. Permission has already been granted and planning is underway for the use of Plant 1, located in the Southeast.

Results of the toxicological testing completed in June/July 2004 indicate very subtle pulmonary function differences between exposed and control animals. No differences in the other biological endpoints (*in vivo* chemiluminescence, blood cytology, and bronchoalveolar lavage fluid analysis) were observed. These are data for one scenario only.

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

Technical Progress - 12 Months

Task #	Description	Planned % completed	Actual % completed
1	Complete Study at Upper Midwest Power Plant	100%	65%
2	Field Study at Power Plant #1	0%	0%
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	0%	0%
6	Project management and reporting	35%	35%

Priorities for the next reporting period (September 1, 2004 - February 28, 2005) include:

- Completion of fieldwork at Plant 0, including detailed interpretation of findings.
- Presentation of findings at a minimum of one scientific conference.
- 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.
- Preliminary interpretation of Plant 1 toxicological data.
- Identification of 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.

EXPERIMENTAL

This section describes the revised stack sampling system and presents results related to its development and performance. The section does not repeat the detailed description of the experimental setup and methods development as these were covered in the previous semiannual report dated March 31, 2004.

Revision of the Stack Sampling System

During initial testing at the plant in May, low and highly variable particle number and mass concentrations were measured in the primary emissions. Primary PM concentrations during the first animal exposures in May ranged from 0.5 –1 μg/m^3, and particle counts were approximately 1000 cm^{-3}.

We speculated that the original sampling system (venturi aspirator and orifice) may be producing a sampling artifact by artificially increasing particle losses, and a new sampling system was designed. This system operates on the simple principle of flow balance. A pulling pump was placed at ground level, drawing approximately 202 LPM, while a clean air flow of 200 LPM was adjusted at the sampling port. A T connecting the clean air flow, the sampling port, and the pump at ground level was installed, allowing a stack sample to be automatically collected and diluted. A valve at ground level allowed us to control the amount of flow pulled by the pump, thereby controlling the dilution ratio. Surprisingly, results from this system did not show any improvement over the previous sampling system. On the contrary, this system raised serious issues related to flow control and dilution ratios. In addition, it created a large pressure drop in the chemical laboratory that is not suitable for the particle measurement instruments. We concluded that this system was not suitable for our purposes.

We then proceeded with further investigation of the sampling port using the aspirator and critical orifice technique. We realized that the temperature at which the sampling tube connecting the interior of the stack to the aspirator was extremely important. Using thermocouples, we were able to measure the temperature in the tube. Using the past configuration used for the previous animal exposures (in May), the temperature in the tube was about 60 C. Under these conditions, we observed large variability in particle size distribution. The most uncertain element was a mode of small particles ranging from 10 to 50 nm, which changed dramatically during the day with respect to particle number, size distribution, and mass. An example is shown in Figure 1.

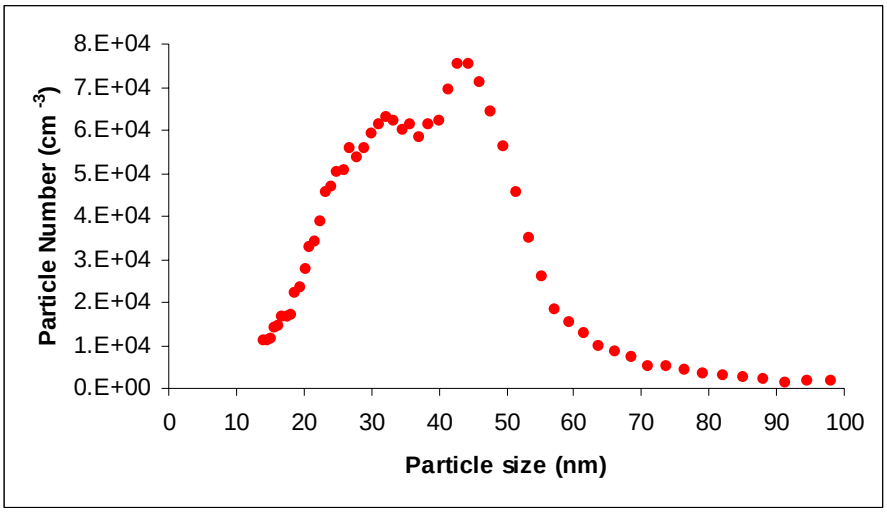

Figure 1. Example ultrafine size distribution during experimental run on August 22, 2004, with low sampling tube temperature (~60 C). Particle number is adjusted for dilution ratio and therefore represents in-stack concentrations.

In contrast, when the tube temperature was adjusted to an optimal 100 C, the emissions stabilized in a constant and repeatable size distribution. Particle number appeared to be lower, but particles were larger, centered around 100 to 150 nm. We believe that this is representative of the true emissions, and that the particles observed in the figure above, for example (when the temperature is low or not optimal), represents particle condensation occurring in the sample tube. We believe that it is the sulfite (SO_3) present in the emissions that condenses with water to form new H_2SO_4 particles. Figure 2 shows an example of the stable size distribution obtained after controlling the sample temperature (again representing in-stack concentrations). The size distribution with the temperature adjusted was stable for both SMPS and APS readings.

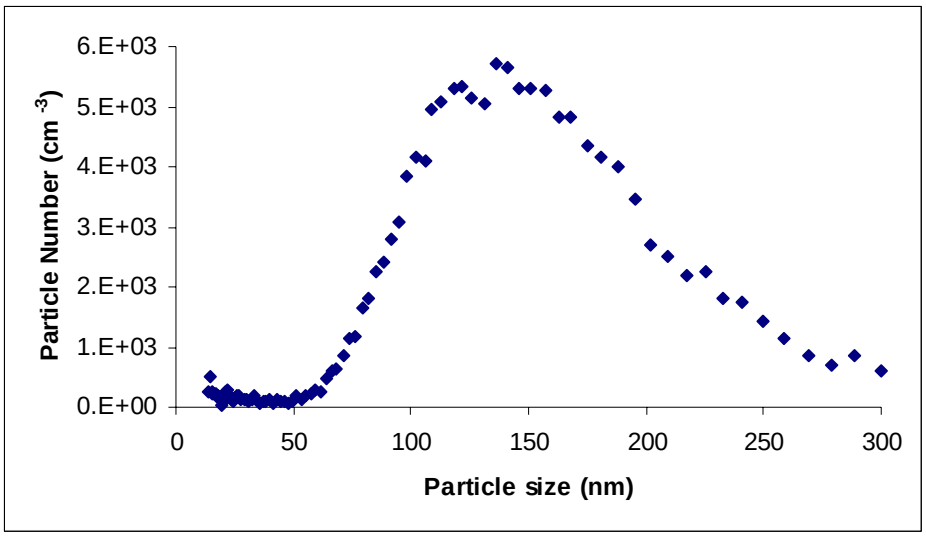

Figure 2. Example ultrafine size distribution during experimental run on August 26, 2004 with higher sampling tube temperature (~100 C). Particle number is adjusted for dilution ratio and therefore represents in-stack concentrations.

Power plant emissions followed a daily trend related to plant performance. Typically at night and during the weekends, the plant decreases its loading. This decrease in loading is associated with a decrease in NO emissions. We found that also the total particle mass as measured by both the APS and SMPS followed this pattern. This behavior was repetitive and predictable. However, performing experiments at night or on weekends was impractical, primarily because of plant security issues. An example of the correlation of NO and PM is shown in Figure 3; concentrations are not dilution-adjusted, so they represent a dilution ratio of about 100:1.

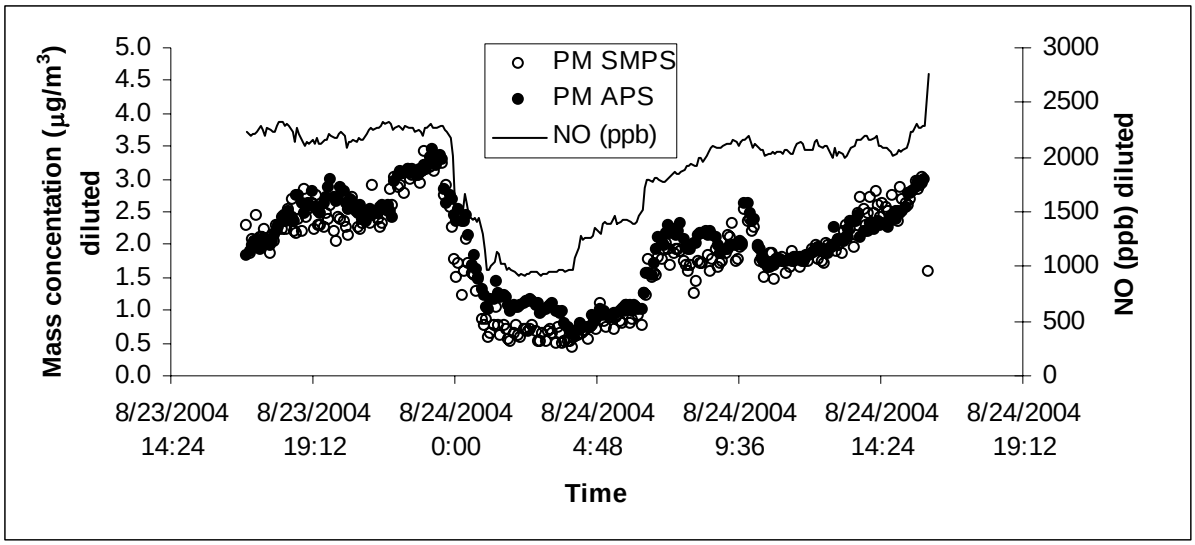

Figure 3. PM mass, as measured by the SMPS and APS, and NO in sampled stack emissions on August 23, 2004.

A modification of the sampling system was tested. In this test the critical orifice was replaced by a stainless steel tube of about 1 meter long and 0.12" ID. This tube allowed us to control the sample flow. Since the flow through this tube will depend on the pressure drop applied, the dilution rate was easily controlled by changing the pressure drop applied to the aspirator. We found that changing the backpressure applied by the system at ground level changes the pressure drop applied by the aspirator. This system allowed us to test different dilution ratios.

Using this technique, we determined if changing the flow through the sampling tube affected the quality of the aerosol sampled. We hypothesized that if there were any loss mechanism in the tube, the losses should be decreased if the residence time is decreased in the tube. This proved not to be the case for small particles. Measurements made with the SMPS showed that the size distribution was not changed by changing dilution ratio (residence time in the tube). Total particle counts were also unchanged (Figure 4a), with particle concentration corrected for dilution and representing in-stack concentrations. For larger particles measured using the APS, we observed that a lower dilution ratio improved particle collection (Figure 4b). This may be the effect of the residence time, but we also believe that it may be the effect of removing the back pressure at ground level, which improves the particle collection efficiency for big particles.

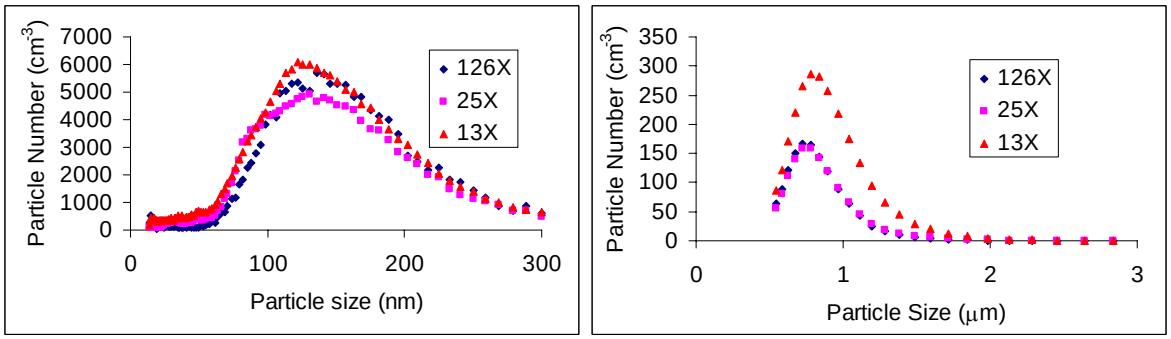

Figure 4. Effect of dilution ratio on size distribution pf (a) ultrafine particles (measured using an SMPS); and (b) particles 0.5 – 3 µm (measured using an APS) in sampled emissions.

Particle elemental composition was determined in samples taken during the first (May) and second (June-July) field efforts, and compared to samples taken with our current improved sampling system. As shown in Table 1, the current sampling system showed a marginal improvement in particle elemental concentration compared to the samples collected in previous attempts. Table 1 also shows that primary particle emissions from Plant 0, as compared to previous published studies using concentrated ambient particles (CAPs) collected in the Boston area, contain substantially lower elemental concentrations than CAPs. We are currently conducting stack sampling tests to confirm with certainty that the primary particles entering the reaction chamber are wholly representative of those in the stack, with respect to both concentration and composition.

Table 1. Elemental exposure concentrations ($\mu g/m^3$) for May 2004 and June/July 2004 field efforts. Elemental concentrations in published concentrated ambient particle (CAPs) studies shown for comparison.

Element	May 04 concentration (μg/m3)	June/July 04 concentration (μg/m3)	August 04 concentration (μg/m3)	CAPs (Clarke et al., 2000)	CAPs (Batalha et al., 2002)	August 04:CAPs ratio[1]
Na	NM	0.003	NM	0.569	NM	
Mg	0.001	-	0.009	NM	NM	
Al	0.003	0.001	0.045	0.680	0.560	0.07
Si	0.006	0.003	0.031	2.760	3.370	0.01
P	-	-	0.016	-	NM	
S	0.027	0.007	0.046	19.100	22.010	0.002
Cl	-	-	-	0.445	0.000	
K	0.004	-	0.001	1.121	0.990	0.001
Ca	0.017	0.018	0.167	1.711	1.190	0.11
Ti	0.001	0.001	0.006	0.359	0.130	0.02
V	-	-	0.0001	0.105	0.030	0.002
Cr	0.006	0.0002	0.0001	0.007	0.010	0.01
Mn	-	-	0.0002	0.075	0.060	0.003
Fe	0.018	0.002	0.025	2.934	2.600	0.01
Ni	0.002	-	0.0001	0.070	0.040	0.001
Cu	-	-	0.0003	0.095	0.090	0.003
Zn	0.001	0.001	0.0001	0.335	0.220	0.0003
As	-	0.0004	0.0001	0.010	0.010	0.01
Se	0.0004	0.0002	0.0001	0.017	0.010	0.004
Br	0.00004	-	-	0.054	0.060	
Rb	-	-	0.0001	-	NM	
Sr	-	-	0.005	-	NM	
Cd	-	0.002	-	0.019	0.010	
Sn	0.002	0.001	-	-	NM	
Sb	-	-	0.0004	-	NM	
Ba	-	-	0.009	0.604	0.730	0.01
Hg	-	0.0003	0.00001	-	NM	
Pb	0.0001	0.0002	-	0.123	0.110	

[1] CAPs concentration used for calculation is average of two studies presented.

- Indicates less than detection limit

NM = not measured

RESULTS AND DISCUSSION

This section describes the following Stage I toxicological assessments:

1. Exposure to primary emissions only on May 10, 11, 12, and 13;
2. Exposure to aged, neutralized emissions + secondary organic aerosol on June 22, 23, 25, and 26 (daytime exposures); and
3. Exposure to aged, neutralized emissions + secondary organic aerosol on June 28, 29, 20, and July 1 (nighttime exposures).

As stated in the Experimental section above, there was concern that sampling artifacts produced non-representative primary particle (and therefore elemental) concentrations during the May exposures. The first animal exposures carried out using the re-designed sampling system were to the most complex particle production and processing scenario (aged, neutralized emissions + SOA) because we wanted to maximize the likelihood of observing health effects.

The section is divided into exposure characterization and toxicological results. Because of the pilot nature of the initial (May) exposures, toxicological results are not presented for this field campaign.

Exposure Characterization

As described in the Cooperative Agreement, the following measurements were conducted at the exposure chamber during both the May and June/July field campaigns.

Continuous Measurements

- PM mass, using a Tapered Element Oscillating Microbalance (TEOM)
- Particle number, using a condensation particle counter (CPC)
- SO_2 (pulsed fluorescence method)
- NO_x (chemiluminescence method)
- O_3 (UV absorbance method)
- Temperature
- Relative humidity (RH)

Integrated Measurements

- PM mass (gravimetric analysis; teflon filters)
- Sulfate (ion chromatography; teflon filters)
- Nitrate (ion chromatography; teflon filters)
- Particle strong acidity (pH analysis; teflon filters)
- Ammonium (ion chromatography; teflon filters)
- EC/OC (thermal optical reflectance [TOR] method; quartz filters)
- Ammonia (diffusion denuder technique with ion chromatographic analysis)

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

1. Because of the extremely low elemental carbon concentrations expected in the coal combustion emission scenarios, an aethalometer was not employed. Moreover, selected organic analysis was not conducted because of the very low expected concentrations of these materials. However, enhanced/augmented organic analysis will be a critical component of the mobile source emissions scenarios.
2. CO was not measured because it was expected to be extremely low after the dilution and denuder steps.
3. The elemental streaker was not used due to technical problems; however, elemental concentrations on 6-hour integrated samples were determined using XRF.
4. EC/OC data were not yet available at the time of preparation of this report, but will be reported in the next semiannual report.

May 2004 Exposures

Exposure data for the exposures carried out from May 11-13 are provided below in Table 2. This exposure was to primary (un-aged) emissions. All parameters were very low.

Table 2. Exposure concentrations for animal exposures conducted in May, 2004.

Continuous Measurements	Units	**5/10/2004**	**5/11/2004**	**5/12/2004**	**5/13/2004**	**Mean**
$PM_{2.5}$ (TEOM) [1]	$\mu g/m^3$	3.2	-0.4	-3.4	242.6	60.5
Number (CPC)	cm^{-3}	1966.0	3429.5	604.1	906.0	1726.4
SO_2 [2]	ppb	-13.0	-13.0	-12.9	-12.9	-13.0
NO_2	ppb	6.9	5.7	5.2	9.1	6.7
NO	ppb	0.5	7.7	7.1	8.4	5.9
O_3	ppb	1.2	2.5	-0.4	0.8	1.0
Temperature	C	23.6	23.9	22.7	23.5	23.4
Relative Humidity	%	30.0	0.0	41.9	44.1	29.0
Integrated Measurements	Units	**5/10/2004**	**5/11/2004**	**5/12/2004**	**5/13/2004**	**Mean**
$PM_{2.5}$	$\mu g/m^3$	4.9	4.2	-2.6	-1.8	1.2
SO_4	$\mu g/m^3$	0.5	1.4	0.8	0.0	0.7
NO_3	$\mu g/m^3$	0.4	1.1	0.0	0.8	0.6
NH_4	$\mu g/m^3$	0.4	0.6	0.0	0.0	0.3
pH	$nmoles/m^3$	0.5	0.6	0.7	0.6	0.6
SO_2	ppb	4.5	1.4	n/a	10.0	5.3
HNO_3	ppb	1.0	0.4	1.2	0.4	0.8
HONO	ppb	3.3	1.8	5.7	0.0	2.7
NH_3	ppb	2.8	30.1	61.7	9.5	26.0

[1] Negative values were considered to be due to instrument noise; the values were very low and likely to be below the limit of detection. A disconnected line may have accounted for the greater, positive result during Exposure 4 (May 13).

[2] The SO_2 measurements during this period reflect equipment malfunction.

June/July 2004 Exposures

Exposure data for the daytime and nighttime exposures are provided below in Tables 3 and 4, respectively. The average PM and sulfate concentrations for the 8 exposure days were 249 and 87 $\mu g/m^3$, respectively. At the time of preparation of this report, EC/OC data were not yet available; however, we can roughly estimate the SOA contribution as the difference of PM mass and the sum of sulfate, nitrate, and ammonium. Tables 3 and 4 indicate an SOA concentration of 108 $\mu g/m^3$, or a mass contribution of 44%. Acidity was 20 $\mu g/m^3$ H_2SO_4, corresponding to 77% neutralization. We had aimed for 30% SOA and 85-90% neutralization of acidity; therefore, these parameters were in the range of those expected.

Table 3. Exposure concentrations for daytime animal exposures conducted on June 22-23 and 25-26, 2004.

Continuous Measurements	Units	**6/22/2004**	**6/23/2004**	**6/25/2004**	**6/26/2004**	**Mean**
$PM_{2.5}$ (TEOM)	$\mu g/m^3$	257	172	261	212	226
Number (CPC)	cm^{-3}	48366	49611	41095	48496	46892
SO_2	ppb	31.9	31.3	32.1	30.9	31.6
NO_2	ppb	18.7	20.6	22.6	17.6	19.9
NO	ppb	3.5	4.0	3.1	1.2	3.0
O_3	ppb	33.0	30.9	38.0	37.8	34.9
Temperature	C	25.6	24.6	25.7	23.4	24.8
Relative Humidity	%	30.3	46.6	-	34.8	37.2
Integrated Measurements	Units	**6/22/2004**	**6/23/2004**	**6/25/2004**	**6/26/2004**	**Mean**
$PM_{2.5}$	$\mu g/m^3$	258	217	278	270	256
SO_4	$\mu g/m^3$	94	74	118	98	96
NO_3	$\mu g/m^3$	21.9	28.4	22.8	26.7	25.0
NH_4	$\mu g/m^3$	26.3	22.9	28.1	26	25.8
Acidity	$\mu g/m^3$	25.6	11.3	44.6	27.8	27.3
SO_2	ppb	11.8	11.1	11.8	11.4	11.5
HNO_3	ppb	2.9	2.6	2.6	2.5	2.7
HONO	ppb	7.4	9.4	7.2	5.5	7.4
NH_3	ppb	0.9	0.75	0.5	0.46	0.7
$SO_4 + NO_3 + NH_4$	$\mu g/m^3$	142.2	125.3	168.9	150.7	146.8
Estimated SOA	$\mu g/m^3$	115.8	91.7	109.1	119.3	109.0
Estimated % SOA		44.9%	42.3%	39.2%	44.2%	42.6%

Table 4. Exposure concentrations for nighttime animal exposures conducted on June 28-July 1, 2004.

Continuous Measurements	Units	**6/28/2004**	**6/29/2004**	**6/30/2004**	**7/1/2004**	**Mean**
$PM_{2.5}$ (TEOM)	$\mu g/m^3$	215.2	196.5	125.4	177	178.5
Number (CPC)	cm^{-3}	38908	43390	44674	44992	42991
SO_2	ppb	23.5	30.7	33.3	37.6	31.3
NO_2	ppb	12.5	19.7	19.4	31.4	20.8
NO	ppb	1.7	2.9	3.3	6.6	3.6
O_3	ppb	27.6	29.9	27.9	32	29.3
Temperature	C	23.8	24	23.8	23	23.6
Relative Humidity	%	51.4	41.5	47.6	54.3	48.7
Integrated Measurements	Units	**6/28/2004**	**6/29/2004**	**6/30/2004**	**7/1/2004**	**Mean**
$PM_{2.5}$	$\mu g/m^3$	266	-	201	257	241
SO_4	$\mu g/m^3$	101	87	45	74	77
NO_3	$\mu g/m^3$	22	28.2	40.3	38.2	32.2
NH_4	$\mu g/m^3$	29.8	26.2	16.7	25.2	24.5
Acidity	$\mu g/m^3$	16.8	18.1	1.1	11.6	11.9
SO_2	ppb	4.8	9.5	9.8	14.8	9.7
HNO_3	ppb	1.9	2.1	2.1	2.8	2.2
HONO	ppb	5.9	7.5	13.5	12.5	9.9
NH_3	ppb	0.5	0.5	1.4	0.6	0.8
$SO_4 + NO_3 + NH_4$	$\mu g/m^3$	152.8	141.4	102.0	137.4	133.4
Estimated SOA	$\mu g/m^3$	113.2	-	99.0	119.6	107.9
Estimated % SOA		42.6%	-	49.3%	46.5%	44.7%

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.

- Histopathological analysis of lung tissue by fixing lungs and randomly selecting three slices for processing by paraffin histology techniques.
- Blood cytology (total white blood cell counts and differential profiles), evaluated 24 hours following the last day of exposure.

Each scenario includes 3 exposures, each with 5 rats (2 for *in vivo* oxidative stress and 3 for the other biological endpoints). Thus, for each scenario there are 6 rats in the oxidative stress group and 9 rats in which pulmonary function, BAL, and blood cytology are assessed.

The toxicological results for the June/July exposures are presented below. Due to the pilot nature of the May exposures, results are not described; however, no differences were observed between exposed and control (sham) animals.

June/July 2004 Exposures

The results of the Buxco, chemiluminescence, and BAL cytology analyses are available and are reported below. The histopathological and BAL biochemical analyses have not yet been completed but will be reported in the next progress report.

Pulmonary Function

For frequency (*f*), a gradual reduction over the first hour was observed in both exposed and sham animals as they acclimatized to the whole-body chambers (Figure 5).

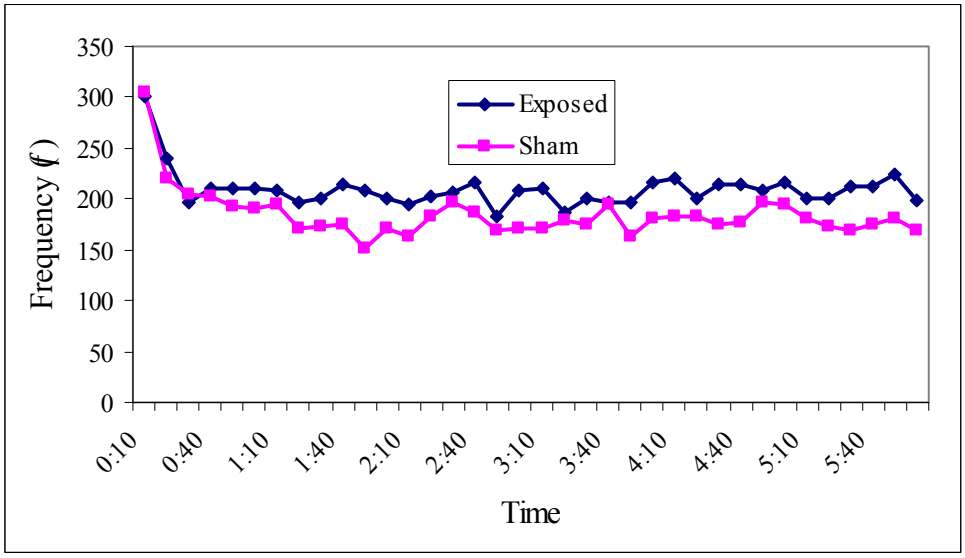

Figure 5. Temporal pattern of respiratory frequency in exposed and sham animals. Each point represents the average of 40 animals (5 animals/day for 8 days). Standard errors not shown for figure simplicity.

Mean responses for each respiratory parameter over the entire 8-day experimental period are shown in Figure 6. Using a simple 2-tailed t-test assuming equal variances in the two samples, inter-group differences were noted for all parameters. However, it is important to recognize that while these differences are statistically significant ($p<0.05$), they may not be biologically significant. For example, the enhanced pause parameter (Penh) provides a measure of airway

restriction. Typically when animals are experiencing bronchoconstriction, this parameter increases dramatically. Our results indicate only very subtle differences in Penh between exposed and sham groups (0.70 vs. 0.78 for exposed and sham, respectively).

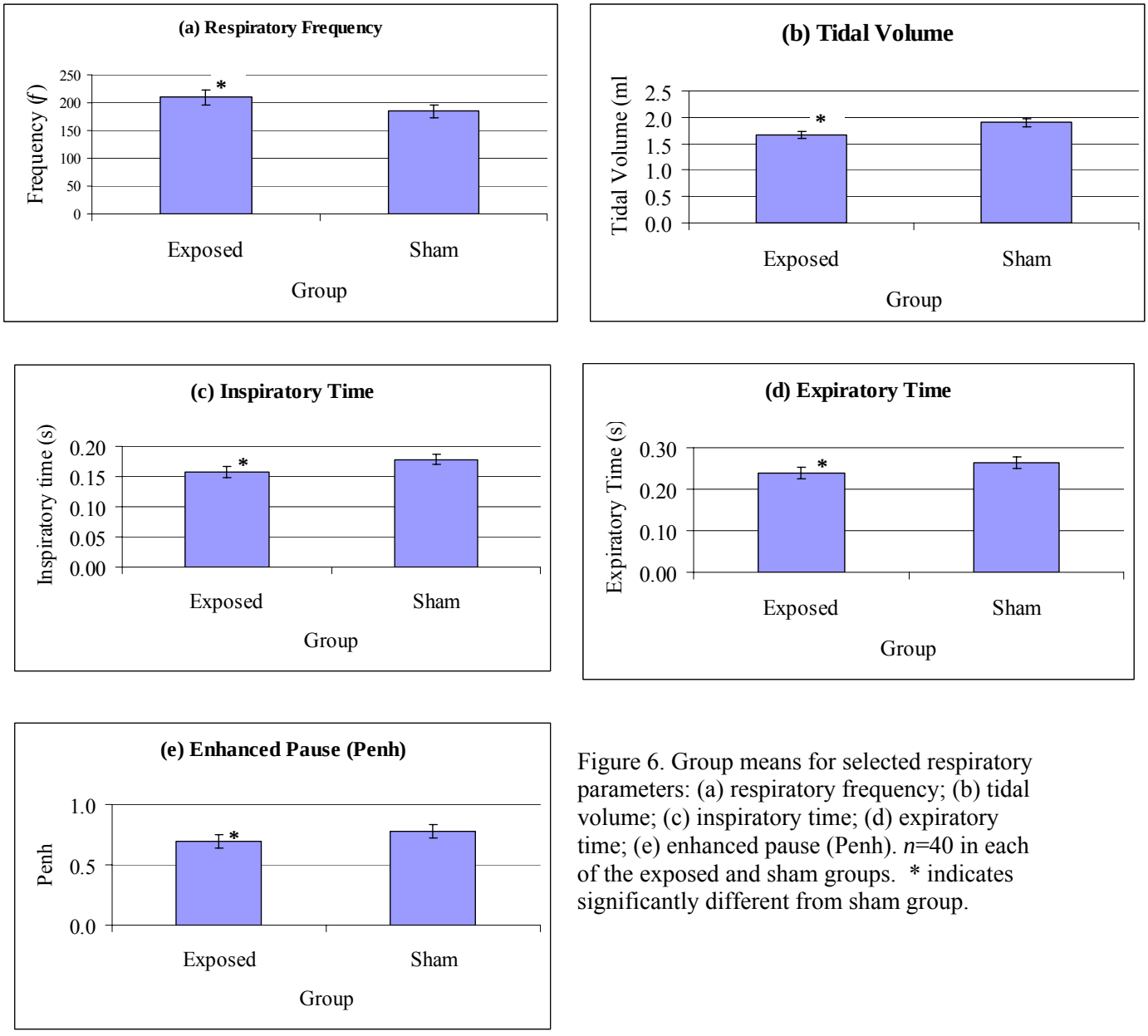

Figure 6. Group means for selected respiratory parameters: (a) respiratory frequency; (b) tidal volume; (c) inspiratory time; (d) expiratory time; (e) enhanced pause (Penh). *n*=40 in each of the exposed and sham groups. * indicates significantly different from sham group.

In Vivo Chemiluminescence

Two animals were evaluated each day of exposure, for a total of 16 animals. No significant differences between exposed and sham animals were observed (Figure 7).

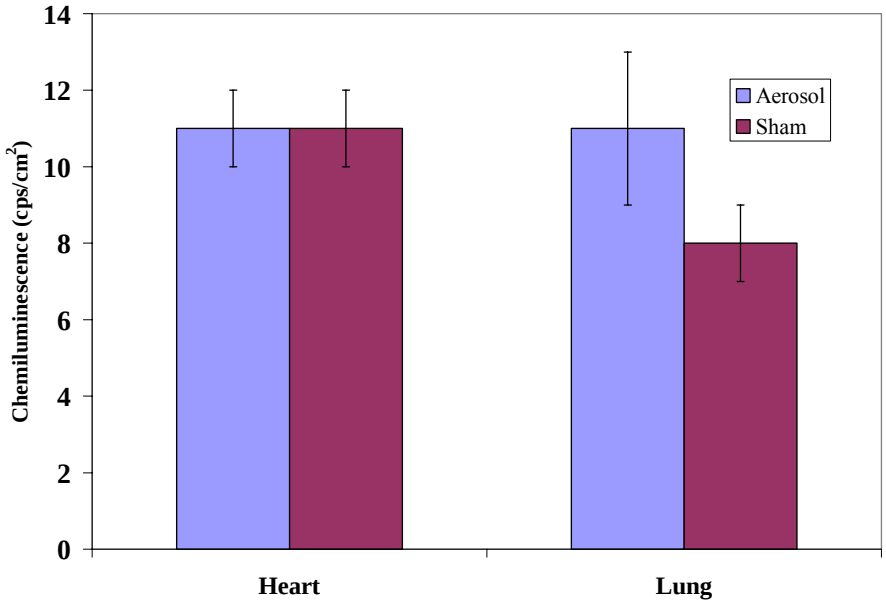

Figure 7. *In vivo* chemiluminescence, June/July 2004 (*n*=15 for exposed and sham groups).

Bronchoalveolar Lavage Fluid Analysis

Selected results of the BAL fluid analyses are shown in Figure 8. No significant differences between exposed and sham animals were observed. Results for the biochemical markers (LDH, βNAG, and total protein) are not yet available.

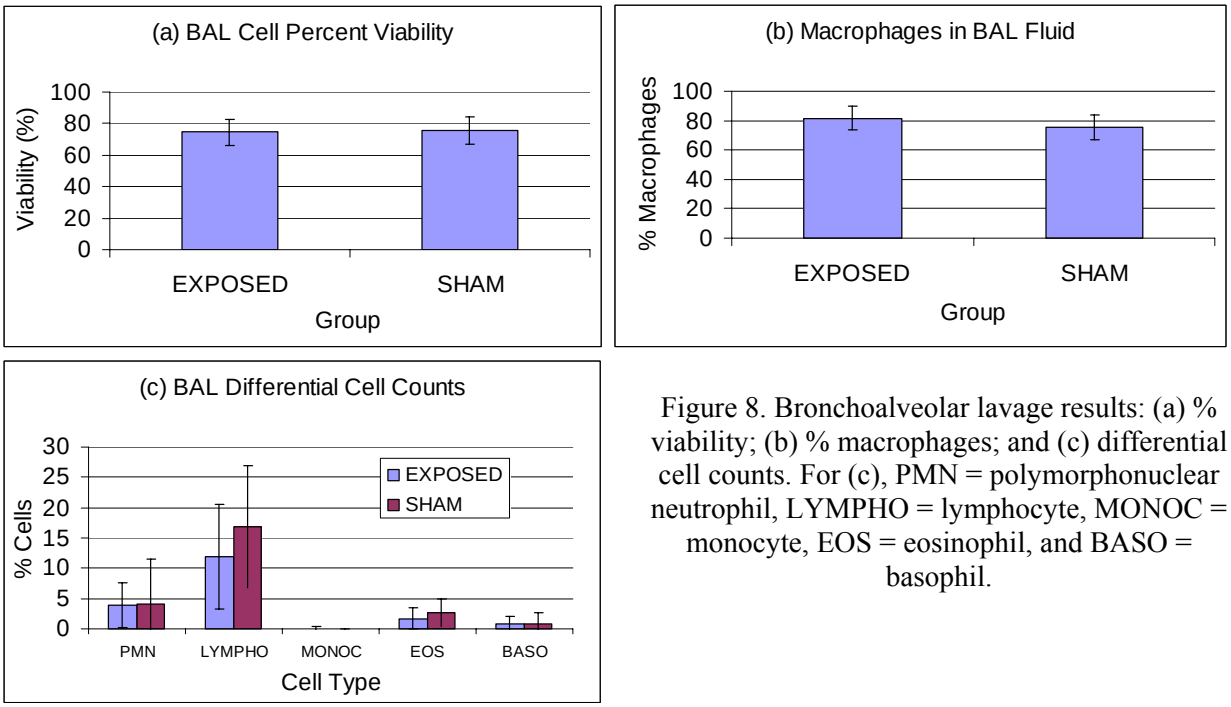

Figure 8. Bronchoalveolar lavage results: (a) % viability; (b) % macrophages; and (c) differential cell counts. For (c), PMN = polymorphonuclear neutrophil, LYMPHO = lymphocyte, MONOC = monocyte, EOS = eosinophil, and BASO = basophil.

Blood Cytology

Results of the blood cytological analyses are provided in Table 5 below. No significant differences between exposed and sham animals were observed.

Table 5. Blood cytology results, June/July 2004 exposures.

	Exposed		Sham		
Parameter	**Mean**	**SD**	**Mean**	**SD**	**Normal Range**
WBC (Thous./uL)	5.98	3.06	5.99	2.70	5-17
RBC (Million/uL)	6.40	0.41	6.35	0.41	6-10
HGB (g/dl)	13.10	0.61	12.98	0.84	11-18
HCT (%)	43.35	2.27	43.13	3.03	36-48
MCV (fL)	67.90	3.11	68.00	2.65	
MCH (pg)	20.50	0.86	20.45	0.78	
MCHC (g/dL)	30.22	0.70	30.11	0.79	
NRCB (/100 WBC)	0.14	0.48	0.00	0.00	
Neutrophil Seg (%)	11.24	9.56	11.81	6.01	9-34
Neutrophil Band (%)	0.00	0.00	0.00	0.00	
Lymphocyte (%)	85.57	10.00	84.38	5.88	65-85
Monocyte (%)	2.81	1.91	3.24	2.36	0-5
Eosinophil (%)	0.33	0.73	0.57	0.75	0-6
Basophil (%)	0.05	0.22	0.00	0.00	0-1.5
Platelet Estimate	Adequate	N/A	Adequate	N/A	500-1300
Polychromasia	N/A	N/A	N/A	N/A	
Absolute Neutrophil Seg (/uL)	638.62	589.71	718.60	510.77	
Absolute Neutrphil Band (/uL)	0.00	0.00	0.00	0.00	
Absolute Lymphocyte (/uL)	5161.14	2613.74	5032.00	2312.79	
Absolute Monocyte (/uL)	152.33	108.44	207.65	166.62	
Absolute Eosinophil (/uL)	21.57	62.61	31.75	45.93	
Absolute Basophil (/uL)	2.52	11.57	0.00	0.00	

CONCLUSIONS

Significant progress was made on the Project during the second reporting period. We finalized all methodologies and successfully employed them in the field setting at Plant 0. Importantly, we believe that the sampled primary particles are in fact representative of those being emitted from the stack. We plan to further document and verify this, since the applicability of the results depends greatly on the extent to which the experimental atmospheres reflect actual population exposures.

We began the toxicological assessments with Scenario 1, the primary (un-aged) emissions, as originally proposed. However, due to low primary PM in the system and the observation of no health effects in the Stage I assessment, we moved to the most complex scenario (Scenario 5: aged, neutralized emissions with SOA) in order to increase the likelihood of biological effects. If effects were observed, we could then eliminate atmospheric components in a stepwise manner to learn more about the components responsible. However, only very subtle health responses have

been observed in Scenario 5. We are currently repeating Scenario 5 with the redesigned stack sampling system and conducting Stage I assessments.

During the next reporting period, we will document and describe the remainder of the fieldwork at Plant 0, which we expect to be complete by mid-November 2004. This report will include detailed Stage I toxicological findings for all scenarios run, and Stage II toxicological findings for one selected scenario. Depending upon the outcome of the ongoing fieldwork at Plant 1 (i.e. the biological effects observed), not all the proposed scenarios may be evaluated. The next report is also expected to include preliminary field data for Plant 1, located in the Southeast.

Thus, priorities for the next reporting period (September 1, 2004 - February 28, 2005) include:

- Completion of fieldwork at Plant 0, including detailed interpretation of findings.
- Presentation of findings at a minimum of one scientific conference.
- 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.
- Preliminary interpretation of Plant 1 toxicological data.
- Identification of 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.

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