STATEMENT OF PROJECT OBJECTIVES

DE-FC26-05NT42303

Cardiopulmonary Toxicity Induced by Ambient Particulate Matter

A. OBJECTIVES

This project is designed to investigate the sources and components of fine particulate matter ($PM_{2.5}$) responsible for adverse health effects, with an emphasis on coal-fired power plant-derived PM. The project is highly innovative in design by virtue of the use of a mobile ambient particle concentrator coupled with a mobile toxicological laboratory to evaluate the health effects of PM dominated by different sources. The concentrator/laboratory will be stationed in three locations for field experiments: one dominated by mobile source-related PM (both diesel and gasoline emissions), one dominated by both coal combustion-derived PM and local industrial sources, and one heavily dominated by coal combustion-derived PM. Importantly, the coal-related PM represents a realistic exposure, since it is secondary PM formed by atmospheric conversion of SO_2 emitted by power plants. *In vivo* inhalation toxicology experiments will be conducted using spontaneously hypertensive (SH) rats. By exposing this susceptible rat model to concentrated ambient particles (CAPs) from these different locations, the influence of different PM sources and components on cardiopulmonary toxicity will be ascertained. Such information is needed to inform the regulatory process regarding $PM_{2.5}$. Cardiopulmonary health endpoints have been selected for study based on the preponderance of epidemiological and toxicological findings linking PM exposure to cardiopulmonary effects. A key feature of the study is the synergy with a large epidemiology/exposure assessment study being conducted concurrently in Detroit, the location of one of the project study sites. The project will generate animal data that will be directly comparable to exposures in humans, thus resulting in a truly integrative study incorporating the three health research disciplines of toxicology, epidemiology, and exposure assessment.

Thus, the primary objective of the project is to evaluate the potential for adverse cardiopulmonary effects from ambient exposure to realistic (environmentally relevant) coal-fired power plant and traffic-related PM. Secondary objectives of the study are to (1) provide insight into toxicological mechanisms of PM-induced cardiopulmonary effects, particularly as they relate to susceptible subpopulations; and (2) generate toxicological data to directly correspond to epidemiology and exposure assessment data from concurrent studies being conducted at one of the project locations, providing a rich dataset of human and animal data exploring the associations between PM sources and components and health.

B. SCOPE OF WORK

The project consists of a multiple-site field study to investigate the toxicity of secondary $PM_{2.5}$ derived from coal-fired power plants and other sources, including mobile sources. The project utilizes a portable ambient particle concentrator coupled with a mobile toxicological laboratory to enable the assessment of the health effects of concentrated ambient particles (CAPs) in regions dominated by different PM sources. The project includes three study locations, each to be evaluated during both winter and summer seasons because the variations in the ambient concentrations and composition of PM are typically greatest between summer and winter at the selected study sites. The first study location is located near the Ambassador Bridge in Detroit, MI, and is heavily influenced by both idling diesel truck traffic and gasoline-fueled commuter traffic.

This site also corresponds to the location of an EPRI-funded cardiovascular epidemiology study. The second site is located in Steubenville, OH, an area dominated by both regional power plant-derived PM as well as local industrial sources. The third site is located in Maurice K. Goddard State Park in Northwest Pennsylvania, an area also heavily influenced by power plant emissions, but lacking urban or industrial influences. The selection of sites and seasons is based on achieving the highest degree of variability in PM composition and contribution from different sources. Spontaneously hypertensive (SH) rats will be exposed to CAPs from these locations and assessed for a wide suite of cardiopulmonary endpoints. The rats will be implanted with telemeters and evaluated for pulmonary, systemic, and cardiovascular effects. Rats will be exposed to CAPs for 8 hours/day for 13 consecutive days, and simultaneous comprehensive exposure characterization will be carried out to enable linking of adverse health impacts with PM composition. Also, importantly, source apportionment will be carried out to enable attribution of toxicological effects to specific PM sources. At least 7 manuscripts will be submitted to peer-reviewed journals describing the toxicological results and source attribution work, and linking the Detroit toxicological results to the concurrent epidemiology and exposure assessment studies. Key project team members are EPRI, Michigan State University, and the University of Michigan. Figure 1 shows the overall project tasks and performance schedule, based on a nominal start date of July 1, 2005.

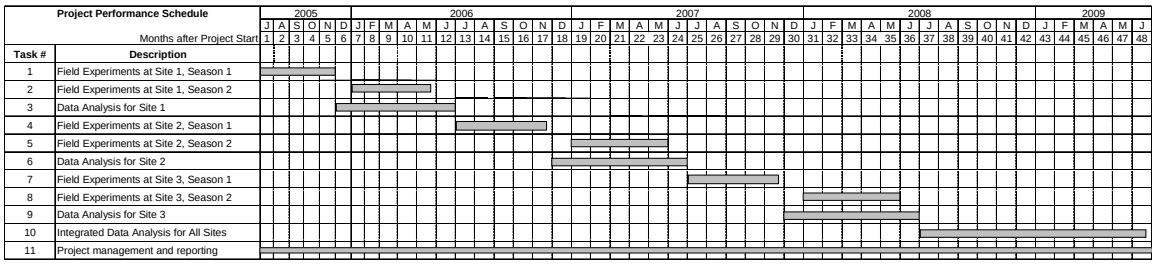

Figure 1 - Project Tasks and Performance Schedule

C. TASKS TO BE PERFORMED

Task 1 - Field Experiments at Site 1, Season 1

Subtask 1.1: Mobile Concentrator/Laboratory Setup. This subtask will be carried out by teams from Michigan State University and the University of Michigan.

A state-of-the-art mobile concentrator/laboratory ("AirCARE 1," Figure 2) will be used to generate the CAPs for animal exposures and serve as the location of the toxicological assessments. AirCARE 1 consists of a specialized 53-ft long transport trailer providing more than 400 sq. ft of laboratory floor space, and housing a mobile ambient particle concentrator. Prior to transportation of AirCARE 1 to the exposure site, a performance check of the concentrator and exposure chambers will be conducted at the Engineering Research Laboratory at Michigan State University (MSU).

AirCARE1 will be transported to each monitoring site by a licensed truck driver. Prior to the trailer arriving at the site arrangements will be made for a power drop and meter to be placed in a safe and secure location, typically located within a fenced-in area. AirCARE1 has its own transformer that is transported inside the trailer to the site, and an electrician will connect the power to the trailer. A smaller pull-behind trailer houses the large pumps that drive the particle concentrator, and the electrical and vacuum lines are attached to the underside of AirCARE1. The

telescoping 30-foot meteorological tower will be removed from its storage bin under the trailer and mounted on the side of the trailer.

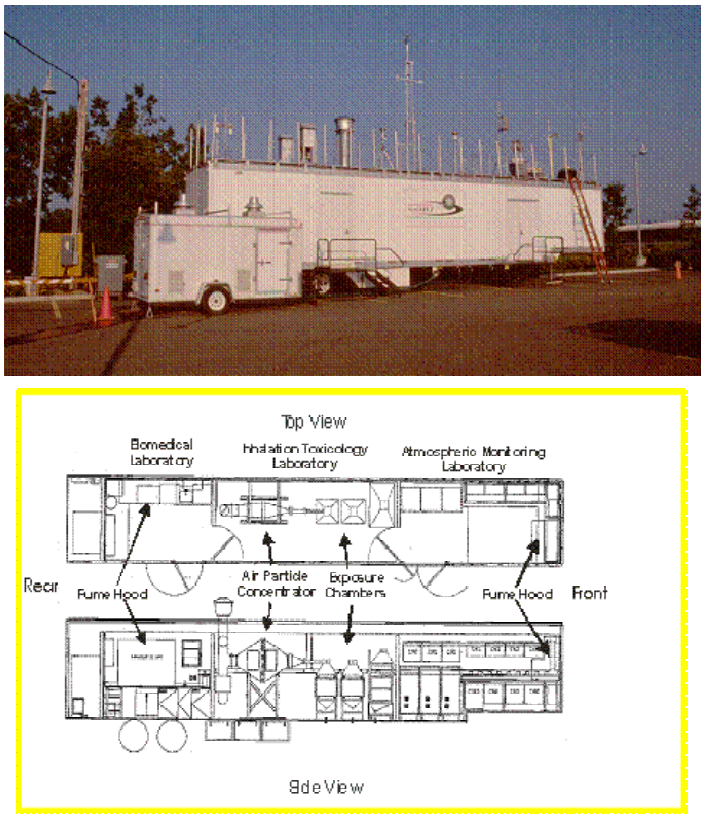

Figure 2 - AirCARE 1 Mobile Particle Concentrator and Animal Exposure Laboratory

After the power and concentrator connections have been completed, the instrumentation required for exposure assessment will be unpacked and set up in the front laboratory of the mobile facility. All sampling equipment will be set up, tested, calibrated, and operational one to two days before the actual animal exposures are started. While the work inside AirCARE1 is progressing, members of the team will mount the meteorological sensors on the tower and mount and secure all of the ambient sampling instruments and inlets. All samplers will undergo a rigorous checkout prior to the exposure period. Data acquisition systems will be synchronized, and final preparation of the concentrator and sampling systems will be verified prior to each exposure day. Electrocardiogram (ECG) monitoring hardware will be installed, which involves wiring of telemetry cables into exposure chambers, and placement of data modules and a desktop computer. Animals will be moved into the laboratory 2 days prior to exposures to acclimatize to the laboratory environment

Subtask 1.2: Particle Concentration and Animal Exposures. This subtask will be carried out primarily by the Michigan State University team with assistance from Dr. Annette Rohr of EPRI, the Project Manager.

Exposure aerosol will be extracted from ambient air outside the mobile lab using a Harvard/EPA fine ambient air particulate concentrator (Sioutas et al., 1995). This concentrator consists of a series of three virtual impactors to concentrate ambient fine particles ($PM_{2.5}$), and has an output flow from the third stage of ~50 liters per minute (LPM), of which 15 LPM is used for

characterization measurements and the remaining 35 LPM of flow is administered to the animal exposure chambers. The concentrator enriches particles in the size range of 0.1 to 2.5 μm approximately 30-fold. Therefore, when the ambient concentration of $PM_{2.5}$ is 25 μg/m^3, the chamber concentration will be approximately 750 μg/m^3 with 7-8 changes per hour. Exposure CAPs concentrations will depend on ambient $PM_{2.5}$ concentrations at the three study locations, but exposure concentrations are expected to be in the range of 300-700 μg/m^3. These are concentrations that are environmentally reasonable, but also sufficiently high to observe measurable outcomes.

In vivo inhalation toxicology experiments will be conducted using spontaneously hypertensive (SH) rats, a compromised rat model. Two to three weeks before exposures, rats will be surgically implanted with telemetry transmitters for continuous ECG recording and exposed in two stainless steel Hinners-type whole body inhalation chambers located within AirCARE 1. Continuous ECG readings will be recorded before, during and after exposures. Rats will be returned to their individual cages overnight. Male SH rats , 11-12 weeks old, will be used for the exposures. Sixteen SH rats will be exposed in each chamber at the same time. Exposures will be 8 hours in duration, for 13 consecutive days.

Animals will be maintained and studied in accordance with the National Institutes of Health guidelines for the care and use of animals in research, and all protocols will be approved by the All University Committee on Animal Use and Care at Michigan State University.

Subtask 1.3: Exposure Characterization. This subtask will be carried out by the University of Michigan team.

Concurrent with the animal inhalation exposures, intensive characterization of the CAPs will be conducted using state-of-the-art monitoring methods. The mobile exposure assessment trailer is equipped with a suite of continuous and time-integrated measurement instruments. Ambient samples will be collected atop AirCARE 1. The 53' by 8' monitoring platform has ample space for all ambient monitoring instrumentation and meteorological sensors. Analysis of CAPs will be carried out for both organic and inorganic constituents, including acidity, sulfate, nitrate and ammonium ion, elemental carbon (EC), organic carbon (OC), and trace elements (total and water soluble). A novel semicontinuous slurry sampler will be used to obtain finer time resolution for trace metals and EC/OC. Standard Operating Procedures (SOPs) for all of the measurements described below will be precisely followed by experienced laboratory staff. Ambient criteria gases (CO, SO_2, NO_X, and ozone) and meteorological parameters will also be measured.

Table 1. Particle Measurements and Analysis.

Measurement	PM Property	Sampling Media	Sample Duration (hr)	Analytical Method
TEOM	Mass	-	Continuous	
APS 3320	Size (0.5-20 μm)	-	Continuous	
Aethelometer	Black carbon	-	Continuous	
SMPS 3936	Size (0.01-.6 μm)	-	Continuous	-
MOI	Size (10 stages)/Mass & Trace elements	Teflon	8	Gravimetric/ICP-MS
Filter ($PM_{2.5}$)	Trace elements	Teflon	8	Gravimetric/ICP-MS

Filter ($PM_{2.5}$)	Soluble trace elements	Teflon	8	Gravimetric/ICP-MS
$PM_{2.5}$	Trace elements		Semi-continuous	Slurry sampler
Annular Denuder-Filter Pack System	Acid gases & aerosols and major ions	Teflon/Glass /denuders	8	IC/pH
Filter	Elemental & organic carbon	Quartz	8	TOA

Subtask 1.4: Toxicological Assessments. This subtask will be carried out primarily by the Michigan State University team, with assistance from Dr. Rohr of EPRI.

Extensive toxicological evaluation will be conducted, focusing on cardiopulmonary endpoints. Continuous ECG recording from telemetered animals will begin 24 hours before animals are placed in exposure chambers and will last for the duration of the study (i.e., 15 days). Animals will be sacrificed 24 hours after the last exposure for collection of tissues for biochemical, molecular, and pathological analyses.

ECG analysis will be carried out through subjective ECG waveform review, automated ECG analysis, and calculation of heart rate variability, including time-domain measures of SDNN (standard deviation of the normal-to-normal intervals), RMSSD (square root of the mean squared differences of successive normal-to-normal intervals), and SDANN (standard deviation of the average normal-to-normal intervals calculated over short periods). Data collected by these methods will allow for analysis of multiple ECG endpoints including heart rate, waveform morphology, rhythm analysis, QT and QTc, and heart rate variability. Tissue histological analysis will be carried out on heart and airway tissues from lung and nose. Standard morphometric, histochemical, immunochemical, and molecular techniques will be used to identify exposure-related alterations in cardiac and pulmonary tissues. Bronchoalveolar lavage fluid (BALF) will be analyzed for cellularity and soluble markers of inflammation and injury (TNF-α). Blood analysis will be performed for a complete blood count (CBC). Plasma analysis will be performed for soluble markers of inflammation and cardiac injury, which may include cardiac troponin, C-reactive protein, myeloperoxidase, and asymmetric dimethylarginine [ADMA].

Task 2 - Field Experiments at Site 1, Season 2

The subtask structure is identical to that described under Task 1 above, except that the exposures will be conducted in the season "opposite" to the season in which Task 1 is performed. It is anticipated that Task 1 will be performed in the summer and Task 2 will be performed in the winter.

Task 3 - Data Analysis for Site 1

Source apportionment analyses will be conducted on the exposure CAPs in order to enable linking of specific PM sources to biological effects. This work will be carried out using multivariate receptor modeling techniques, including Positive Matrix Factorization (PMF) and the UNMIX model. HYSPLIT4 (Hybrid Single-Particle Lagrangian Integrated Trajectory) Model will be used to calculate backward air mass trajectories from each of the exposure sites during each study period to allow the investigation of sources and the source areas impacting the site. Specific chemical tracers that will be examined and used in the source attribution analyses include S, Se, K, Ca, and Fe (coal), Fe, Zn, Mn, Cu, Pb, EC, and OC (motor vehicles), and Sb,

Pb, Zn, and Hg (waste incinerators). The source apportionment component of the data analysis will be completed by the University of Michigan team.

Toxicological data will be expressed as the mean group value ± the standard error of the mean (n = 8/group). Analyses of variance (ANOVA) will be performed using the factors of exposure (air vs. CAPs) and strain (SH vs. WKY) for the field site. Correlations of CAPs physicochemical characteristics with ECG endpoints will compare mean 30-minute data points within rat strains. Correlations of physicochemical characteristics of CAPs with toxicological endpoints will be made by Pearson Product Moment analysis within each strain, using CAPs mean values for entire 13-day exposures at the site. A time series analysis will be performed to detect potential lag effects of CAPs exposure metrics with ECG abnormalities using SPSS 10.0 statistical software. Upon completion of this analysis, the source contributions calculated from the 30-minute averaged gaseous and PM composition data will be merged with the ECG data to investigate specific source impacts. The toxicological data analyses will be conducted by the Michigan State University with assistance from Dr. Rohr of EPRI.

Following completion of the data analysis for Site 1, a topical report for this study location (Tasks 1-3) will be prepared and submitted to DOE.

Task 4 - Field Experiments at Site 2, Season 1

The subtask structure is identical to Task 1, except that the work will be carried out at the second field site.

Task 5 - Field Experiments at Site 2, Season 2

The subtask structure is identical to that described under Task 1 above, except that the exposures will be conducted in the season "opposite" to the season in which Task 4 is performed. It is anticipated that Task 4 will be performed in the summer and Task 5 will be performed in the winter.

Task 6 - Data Analysis for Site 2

This task is identical to Task 3, except that the data for Site 2 will be the subject of the analysis. Following completion of the data analysis for Site 2, a topical report for this study location will be prepared and submitted to DOE.

Task 7 - Field Experiments at Site 3, Season 1

The subtask structure is identical to Task 1, except that the work will be carried out at the third field site.

Task 8 - Field Experiments at Site 3, Season 2

The subtask structure is identical to that described under Task 1 above, except that the exposures will be conducted in the season "opposite" to the season in which Task 7 is performed. It is anticipated that Task 7 will be performed in the summer and Task 8 will be performed in the winter.

Task 9 - Data Analysis for Site 3

This task is identical to Task 3, except that the data for Site 3 will be the subject of the analysis. Following completion of the data analysis for Site 3, a topical report for this study location will be prepared and submitted to DOE.

Task 10 – Integrated Data Analysis for All Sites

The analytical methods used in Tasks 3, 6, and 9 will be employed to make comparisons across sites and within treatment groups by ANOVA using factors of exposure, strain and site. Correlations of physicochemical characteristics of CAPs with toxicological endpoints will be made by Pearson Product Moment analysis within each strain, using CAPs mean values for entire 13-day exposures at all sites.

Task 11 – Project Management and Reporting

EPRI will coordinate all field, laboratory, data management, and data analysis activities of the subcontractors, and will be responsible for the deliverables/briefings specified in Sections D and E below.

Dr. Annette Rohr is the Project Manager, and will have overall responsibility for the technical, schedule and financial success of the project. She will be the primary point of contact for liaison with the DOE Project Manager, and will be responsible for assuring DOE's satisfaction with the process and end product of the project. In order to assure project success, Dr. Rohr will employ the proven project management actions of planning, assessing actual performance against planned performance, and applying corrective action when necessary. The structured approach for defining, implementing and managing the project will use a work breakdown structure, resource requirements and responsibilities plan, and milestone/schedule/cost performance tracking. Dr. Rohr will be responsible for assuring that sufficient programmatic and technical project discussions take place to correctly communicate expectations and resolve issues. We anticipate that quarterly project team meetings will be held during the first year of the project and semiannual meetings for the remainder of the period of performance, in addition to frequent conference calls.

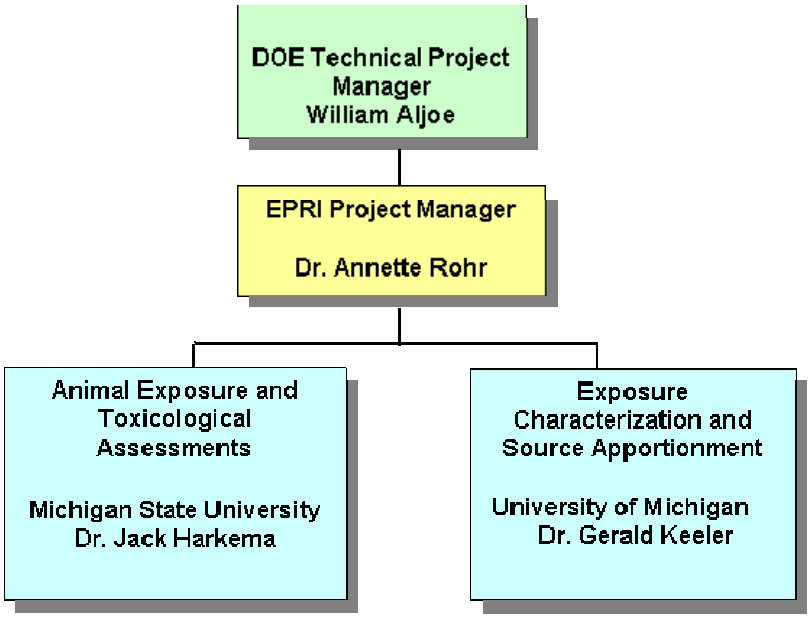

Figure 3 - Project Organization

Reporting will be carried out in accordance with the Federal Assistance Reporting Checklist contained in the model financial assistance agreement and the instructions accompanying the checklist. EPRI will be responsible for submission of the periodic progress, topical, and final reports.

EPRI will also oversee and be responsible for the preparation and submission of approximately seven peer-reviewed journal articles:

1. Description of the toxicological results for the Detroit site.
2. Description of the toxicological results for the Steubenville site.
3. Description of the toxicological results for the Goddard State Park site.
4. Integration of the toxicological results across the three sites for the cardiac function endpoints (i.e., ECG data).
5. Integration of the toxicological results across the three sites for the histopathological data.
6. Integration of the toxicological results across the three sites for the blood and plasma analyses.
7. Integration of the overall Project results with the Detroit exposure and epidemiological study results.