San Joaquin Valley Health Effects Research Center

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San Joaquin Valley Aerosol Health Effects Research Center
University of California, Davis
Three-Year Progress Report Overview

The overall mission of the San Joaquin Valley Aerosol Health Effects Research Center (SAHERC) is to provide a mechanistic link between ambient particles and the health effects that they elicit. This entails two goals: (1) Understanding the metabolic response of tissue and organs when they are exposed to particulate pollutants and (2) understanding the characteristics of the particulate pollutants and their gaseous co-pollutants that elicit these responses. To forward these goals, the center is divided into five complementary and cooperating projects, supported by a like number of cores. The projects explore metabolic response of pulmonary and cardiovascular tissues to pollutant exposure, whole animal effects of exposure, transport of particles from the airways to other organs and tissues, and disruption of juvenile airway development in response to particle exposure. Thus, the projects take top down approaches, identifying particle characteristics that elicit health responses, and bottom up approaches, examining the health effects that the particles elicit. Our field studies take place in the San Joaquin Valley of California, one of the most polluted airsheds in the country. SAHERC activities also extend beyond the direct exploration of health effects to sponsoring conferences and outreach to the community, and educating graduate students and post-doctoral fellows.

SAHERC has sponsored scientific conferences that forward air quality research and complement our mission, including an annual meeting on aviation and the environment (300 attendees), an annual meeting on agricultural emissions of air pollutants (130 attendees), a biennial meeting on photochemical mechanisms in air quality models (100 attendees) and a biennial meeting on modeling aerosol dynamics (100 attendees). SAHERC has sponsored an outreach program whereby female air quality faculty and research staff mentor Girl Scouts on science careers. SAHERC investigators also work together on a training grant that educates PhD students in atmospheric aerosols and health from science, engineering, sociological, economic and policy viewpoints.

SAHERC is in its first funding cycle, so initial efforts were focused more on methods, infrastructure development and screening studies, although substantial research results have also been obtained.

Project 1: Pulmonary Metabolic Response. This project is motivated by elevated susceptibility to polycyclic aromatic hydrocarbons (PAHs) in the young as well as national increases in juvenile and adult asthma. We hypothesize that exposure to PAHs on PM during lung development leads to oxidant stress and increased airway inflammation, and that repeated cycles of injury and repair predispose the lung to a more responsive, asthmatic phenotype. We further hypothesize that these alterations and responses will differ in fundamental ways between neonates and adults. To explore these hypotheses, we have developed novel exposure strategies using custom laboratory generated PM +/- PAHs in collaboration with the center's PM generation core. We are applying site-specific approaches, developed at UC Davis, to study airway level specific responses to PM.

Our results show that particles with and without PAHs have unique cytokine signatures in the lung with particles containing the PAH 1-nitronaphthalene (1-NN) causing the greatest increase in a number of cytokines associated with inflammation. We have also found that neonatal and adult rat airways metabolize 1-NN differently leading to age-dependent adduction of different cellular proteins. Exposure to low PAH flame generated soot causes the temporal pattern of increases in circulating and migratory PMNs to differ between neonates and adults and increases cytokine expression and elevates markers of oxidant stress including hemeoxygenase-1 and enzymes involved in GSH synthesis. These responses occur in the absence of frank cellular toxicity or morphologic changes.

Project 2: Cardiovascular Metabolic Response. This project investigates mechanisms whereby particulate matter elicits acute cardiovascular effects. The focus is on endothelial cell responses to particulates and the interaction between pulmonary inflammation and systemic vascular responses. We use cultured human endothelium and airway epithelium to determine the gene, protein and signaling responses to collected ambient particulate matter. We use gene array analysis to identify patterns of altered transcription in response to PM collected from differing sites and seasons in collaboration with the field studies managed by Project 3. Results of these studies drive mechanistic evaluation of PM specific signaling pathways. To extend these results in vivo, we use mice exposed as part of Project 3's field studies to determine whether pulmonary and systemic endothelial activation occurs and whether this leads to alterations in circulating markers of inflammation or coagulation.

We have determined that collected ambient PM stimulates transcription of xenobiotoic metabolizing enzymes in both endothelium and airway epithelium. We demonstrate PM induced signaling by the aryl hydrocarbon receptor system that leads to up regulation of several of these enzymes. Additional gene pathways emphasize inflammatory activities including e-selectin. We have developed antibody probes for e-selectin in fixed tissues and are evaluating lungs from field exposures. Our field exposure evaluations had demonstrated activation of pro-inflammatory, myelopoetic and pro-coagulant activites in exposed mice. We demonstrated that an increase in the number of blood platelets in exposed animals was correlated with an increase in platelet aggregation and secretion suggesting platelet activation could be an important contributor to PM induced cardiovascular effects.

Project 3: Inhalation Exposure Assessment of San Joaquin Valley Aerosol. This project examines the health effects elicited in mice due to real-time exposure to ambient particles present in urban and rural locations of the San Joaquin Valley during summer and winter seasons. Animals are examined to determine respiratory and cardiovascular responses and their correlation to particle concentration, size and composition for each location and season. The hypothesis is that particle size and composition, differentiated by location and season, elicit different health effects.

Both location and season significantly affect particle mass, size and composition. Our urban site in Fresno has demonstrated higher particle mass concentrations than present in our rural Westside location. The summer season in both urban and rural sites typically have higher particle mass concentrations compared with the winter season, but this may also be due to periods of rain that reduced particle mass during the winter season. To date the most significant

biological effects of particle exposure have been observed at our rural site, compared with our urban site for both summer and winter seasons. Significant increases in the proportion and number of neutrophils recovered from the lungs by bronchoalveolar lavage have been observed, as well as alterations in systemic measures of platelet activation conducted under Project 2. Also noted have been significant increases in the levels of several circulating pro-inflammatory cytokines during the winter season in rural Westside. Heart rate variability studies at the Fresno urban site have yielded inconclusive findings, due to the presence of arrhythmias in both control and particle-exposed mice. However, a significant reduction in heart rate variability has been noted following exposure to concentrated ambient particles in Davis, CA.

Project 4: Transport and Fate of Particles. This project uses a combined in vitro and in vivo approach to determining the nature and extent of transport of inhaled PM. Our hypothesis is that inhaled fine and ultrafine particles translocate to the circulation and localize to other tissues, mediating the adverse health effects observed outside of the respiratory system. We use size specific synthetic particles with either fluorescent, electron dense or radioisotopic labeling to study mechanisms of transport across cellular barriers in vitro with fluorescence and confocal and electron microscopy. Our in vivo studies use positron emission tomography (PET) to study transport from the lung to other organs after intratracheal instillation of radioisotope labeled (^{64}Cu) PM in live mice.

Our in vitro findings suggest that transport of ultrafine PM across endothelial barriers occurs through vesiculocaveolar transport. Cultured bronchial epithelial cells create a barrier that is relatively impermeable to ultrafine PM. Our in vivo results demonstrate that PET is a powerful tool for visualizing the deposition pattern and subsequent migration of PM. Studies in normal animals show increased levels of PM in the heart after deposition. Studies in an animal model with pre-existing disease show accumulation of PM in the vessel wall at sites of atherosclerosis.

Project 5: Architecture Development and Particle Deposition. This project is motivated by national increases in juvenile and adult asthma. We hypothesize that exposure to PM during lung development leads to altered airway architecture and function, and concomitant particle deposition. To explore this hypothesis, we developed a method for identifying the entire airway tree down to the terminal bronchioles, and along with Project 1 and the PM generation core, we have developed a flame generated soot exposure facility. Methods are also under development for measuring bolus dispersion and airway-by-airway particle deposition in the rat.

Four publications have resulted from this work to date. The first presents a model of airway architecture development that successfully reproduces the human airway tree from a few simple reproduction rules. The second demonstrates that evolution optimizes airway architecture to maximize peak performance. The third describes the method for automatically obtaining airway architecture in rats and other mammals with similar thoracic volumes and the fourth uses this method to characterize airway architecture and its variability in 6 normal rats. We have exposed rats to ozone during development, finding insignificant alterations in their architecture, but significant alterations in their lung capacity and compliance. The first PM exposures have been completed and are being analyzed.

Date of Report: July 31, 2008

EPA Agreement Number: R832414-010

Center: San Joaquin Valley Aerosol Health Effects Research Center (SAHERC)

Project Title: Project 1 **--** Pulmonary Metabolic Response

Investigator(s): Laura Van Winkle, Michelle Fanucchi, Alan Buckpitt

Institution(s) of PI(s): University of California Davis

Research Category: Airborne Particulate Matter

Project Period: October 1, 2005 to September 30, 2010

Project Period Covered by this Report: July 1, 2007 through June 30, 2008

OBJECTIVE OF RESEARCH:

To determine whether the increased pulmonary vulnerability to polycyclic aromatic hydrocarbons (PAHs) in neonates is exacerbated when the PAH is adsorbed to particulate matter. By compromising detoxification mechanisms, particles of mixed composition, such as carbon and a PAH, carbon and a transition metal, or carbon with both a transitional metal and a PAH, will result in more injury than particles composed of only one component.

PROGRESS SUMMARY AND ACCOMPLISHMENTS:

1. Insufflation with 3 different carbon particles +/- 1-NN: 1-Nitronaphthalene (1-NN) is a compound that is not very volatile, making it difficult to create a consistent atmosphere for dose-response studies. In addition, 1-nitronapththalene is also water-insoluble, making it difficult to administer it by instillation. However, significant amounts of 1-NN are associated with particulate air pollution. During this period of the grant, we focused on completing the analysis of the insufflation studies using a dry powder insufflator (PennCentury Model DP-4 Dry Powder Insufflator) which we used to resuspend soot particles with minimal impact on their original size and distribution. Rats were exposed to 2.5 mg of particles and necropsied at 24hrs after exposure. We compared carbon black, acetylene soot, ethylene soot and 1-NN (40 ug/mg) coated ethylene soot and 1NN coated acetylene soot. Sample were taken for high resolution histopathology of particle effects and of the particles themselves as well as cytokine analysis in the airway tissue. We found that equivalent doses of “clean” carbon black and flame generated soots illicit different cytokine responses and that the addition of 1-NN induced changes in epithelial cytokine profiles are unique to each carbon particle. Further the source and coating of the particle had an influence on cytotoxicity. “Clean” carbon black is cleared from the airways by 24 hours and shows little evidence of cytotoxicity. “Clean” flame generated soots do not clear from the airways by 24 hours and airway epithelium shows evidence of cytotoxicity (very thin epithelium, change in epithelial cell composition). Ethylene generated soot translocates into the

subepithelial interstitium. 1-Nitronaphthalene-doped carbon black doesn't clear completely from airways and results in changes in epithelial cell composition (mucous cells). Further, 1-Nitronaphthalene-doped flame generated soots do not clear from the airways and both result in extensive epithelial sloughing and cytotoxicity. One of the more interesting findings of these studies is the possibility of a cytokine "signature" for the different pollutants (Figure 1).

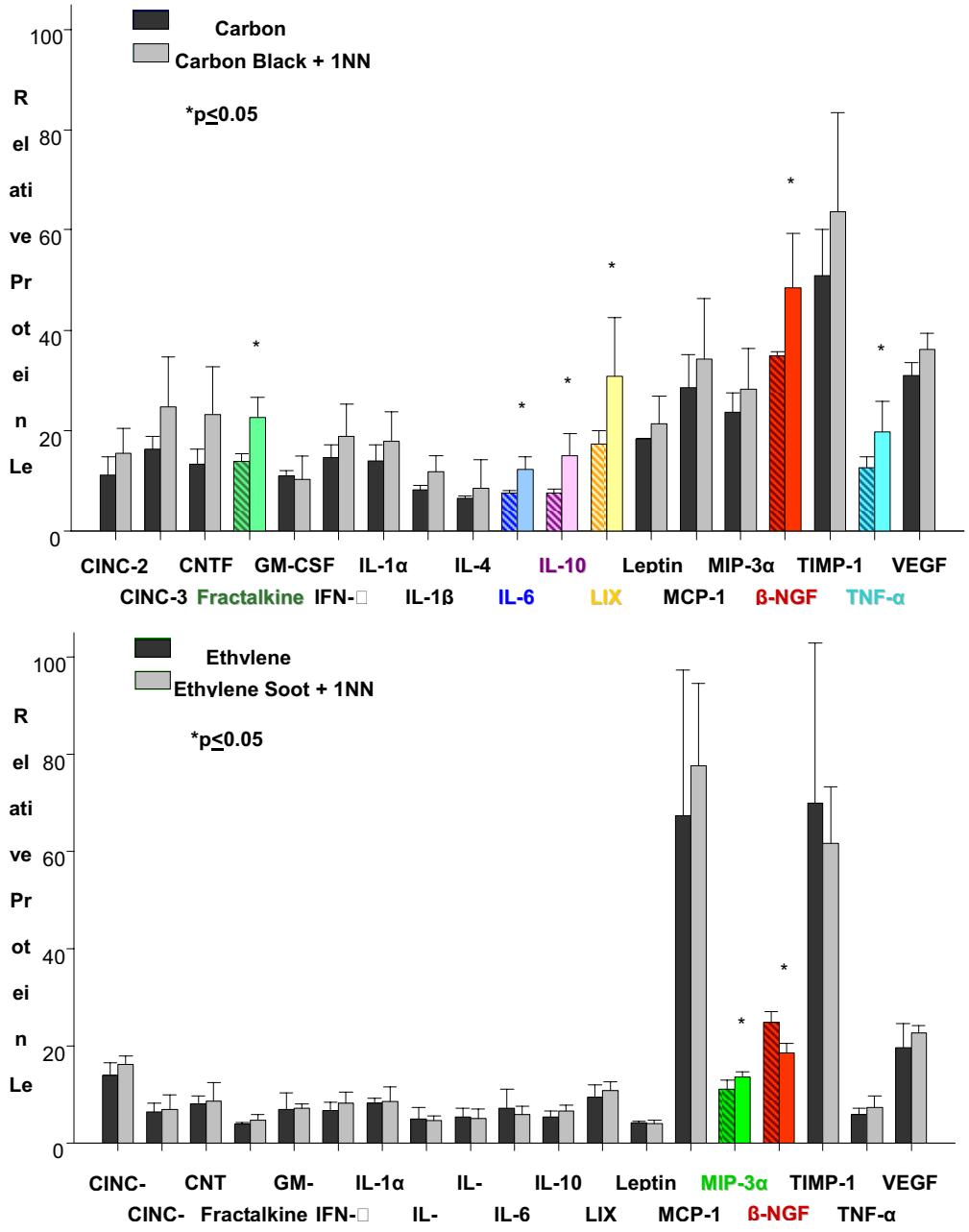


Figure 1: Comparison of the cytokine profile within the airway tissue for 4 different particles in the airways of adult rats.

Paper in preparation: Acute Airway Epithelial Injury from Three Different Carbon Particles With and Without Adsorbed 1-Nitronapthalene (Fanucchi lead author)

2. Analysis of soluble metabolites and protein bound l-NN adducts in neonatal and adult rats. *1*-nitronaphthalene cytotoxicity in the conducting airways of postnatal mice and rats cannot solely be attributed to site-specific bioactivation of the PAH (neonates have less) or baseline levels of GSH (neonates have more). Yet neonates are more susceptible- one hypothesis is that adduction of a critical protein for cellular function occurs in neonatal cells but not in adults. To address this hypothesis we incubated microdissected airways from adult and postnatal rats with 2 hrs with14C-1NN, 100 mM 1.1 E6 DPM/nmole. Then the samples were examined for total covalent binding to proteins, the types of proteins covalently adducted (analyzed by 2Dgel electrophoresis) and the types of metabolites formed (separated by HPLC and to be analyzed using MS). We made significant progress on this aim and initial analysis indicates that the some of the proteins adducted in the neonatal animals (susceptible) are different that in the adult animals (less susceptible).

Papers in preparation:

Age-Specific Pulmonary Metabolism of 1-Nitronaphthalene (Fanucchi lead author)

Alterations in Extracellular Nasal and Pulmonary Glutathione Pools in Adult and Postnatal Rat Following 1-Nitronapthalene Exposure (Fanucchi lead author)

Formation of 1-NN adducts in airways of neonatal and adult rats (Van Winkle, Buckpitt lead authors)

3. Acute exposure to diffusion flame PM in neonatal and adult rats in vivo and in vitro

The goal with these studies is to develop a reproducible exposure system for low PAH and high PAH particles which we can then subsequently modify to generate “custom” particles containing various PAHs of interest. We have completed (in collaboration with Project 5) assembly and testing of a diffusion flame generated soot chamber exposure system (low PAH) and have exposed both neonatal and adult rats to diffusion flame soot (130-170 ug/m3) acutely for 6 hours. We examined timepoints at 2 and at 24 hours after exposure so that we could gauge temporal responses. Endpoints included peripheral blood CBC and cytokines, Cell counts in lavage and lavage cytokines, immunohistochemistry of key antioxidant genes, RT-PCR/microarray and histopathology. With the exception of the array, all the endpoints have received preliminary analysis. We did not detect airway cytotoxicity in the airways of exposed rats. Although diffusion flame particles do NOT cause frank cellular toxicity that is visible morphologically, they do cause a release of cytokines and stimulate neutrophil responses that differ by age and compartment with the adults having more of a peripheral blood increase at 24 hours and the

neonates having an increase in lavage at 2 hrs. Further, there is upregulation of key antioxidant proteins: HO-1 and gamma GCS.

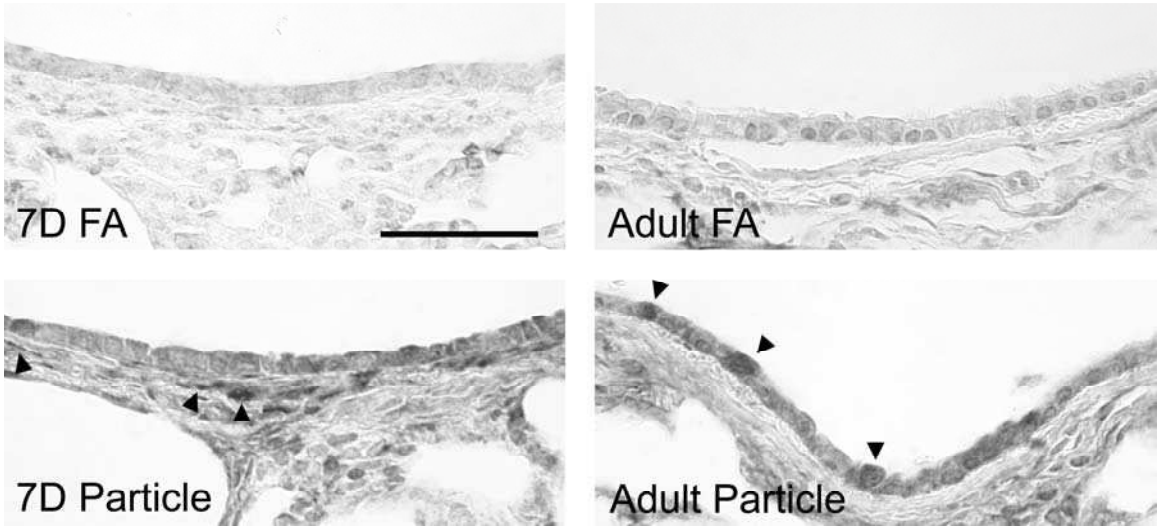


Figure 2: Up regulation of the key enzyme in glutathione synthesis, gamma gluamyl cysteine synthetase is apparent in the PM exposed groups only (dark staining).

The in vitro aspects of these studies have been shifted to incorporate field particulates as well as laboratory generated particulates in microdissected airway explaints. We are using airway explants because they are metabolically competent and many cell lines are not. Endpoints include cytotoxicity and cytokine release.

FUTURE ACTIVITIES

The need to address all the aims in the time allotted means that Project 1 needs to choose an exposure paradigm and stick with it to facilitate comparisons between the biological responses to different particle compositions. For the in vivo exposures to laboratory generated particles, we will continue whole body chamber exposures. Therefore, Project one will emphasize acute exposure of a single day duration with additional timepoints to take some advantage of the most recent SAC recommendation regarding injury/proliferation responses. Following the SAC panel's recommendation we will monitor cell proliferation, but we will use PCNA labeling instead of BRdU so that we don't have to inject a nucleotide reagent (the BrdU) which may compromise our RT-PCR endpoints. We have considerable experience with this sort of staining and feel that this will give us important information regarding wound healing responses at all of the timepoints we are sampling with minimal need to regenerate samples to add this information. We are going to add pulmonary function tests immediately following the acute exposures in both the very young and the mature rats, but only for the diffusion flame exposures. The rationale is that if the most innocuous regimen of laboratory generated particles can cause responses, addition

of other particles, many of which contain more factors that may contribute to hyperreponsiveness, is unlikely to be informative until we have a good handle on the dose response. Planned exposures in the next year include a repeat of the diffusion flame exposures to add endpoints and the completion of the high PAH particle exposures using the pre-mixed flame apparatus which is now operational. Then we will start testing the effect of the addition of ozone into the exposure paradigm.

For the in vitro studies, we will take the panels recommendation to put the particles in the agarose of the airway explants. We will continue to monitor PAH content and to determine total mass of all our particles but will also use surface area as a normalizing parameter. Project 1 will test the particles from the field studies but will also test extracted fractions from the same particles to measure soluble PAHs from the particles and their effects on cells in culture.

PUBLICATIONS AND PRESENTATIONS

Publications:

Day KC, Plopper CG, Fanucchi MV. Age-specific pulmonary cytochrome P-450 3A1 expression in postnatal and adult rats. *Am. J. Physiol. Lung Cell Mol. Physiol*. 2006 Jul;291(1):L75-83.

Kennedy,I.M., 2007. The health effects of combustion-generated aerosols, Proceedings of the Combustion Institute 31, pp. 2757-2770.

D Lee, MV Fanucchi, CG Plopper, J Fung and AS Wexler Pulmonary architecture in the conducting regions of six rats.Anat Rec (Hoboken). 2008 Aug;291(8):916-26.

D Lee, AS Wexler, MV Fanucchi and CG PlopperExpiration rate drives human airway design.J Theor Biol. 2008 Jul 21;253(2):381-7. Epub 2008 Mar 14.

D Lee, SS Park, GA Ban-Weiss, MV Fanucchi, CG Plopper and AS Wexler Bifurcation model for characterization of pulmonary architecture. Anat Rec (Hoboken). 2008 Apr;291(4):379-89.

Abstracts:

2008 KM Sutherland, PC Edwards, LS Van Winkle. Mechanisms of Pulmonary Tolerance in Female Mice to the Cytotoxicant Naphthalene *FASEB J*. 22: 918.4

2008 LS Van Winkle and JR Harkema. Oxidant Air Pollution and Childhood Asthma. *The Toxicologist* Vol 102, S-1, p117 A568.

2008 M.V. Fanucchi, B.M. Kumfer, E.D. Wallis, I.M. Kennedy.Acute Airway Epithelial Injury from Three Different Carbon Particles with and without Adsorbed 1-Nitronapthalene, *AJRCCM* Vol 177 pA425

Presentations:

LS Van Winkle: “Oxidant air pollution and Childhood asthma” Society of Toxicology Seattle, March 2008 Symposium chair and organizer

LS Van Winkle, invited talk “Ozone and the Lung: Biological Effects during Development” and Human Health Effects panelist “Sierra Ozone Summit” Grass Valley June 2008

LS Van Winkle present invited talk ““Naphthalene Toxicity and Metabolism in the mEH Knockout Mouse (B6;129-Ephx1)”, lead roundtable discussion of Research Directions for Naphthalene “2008 Naphthalene Research Meeting “ Storrs CT June 2008

SUPPLEMENTAL KEYWORDS: None.

Date of Report: July 31, 2008

EPA Agreement Number: R832414-010

Center: San Joaquin Valley Aerosol Health Effects Research Center (SAHERC)

Project Title: Project 2 **--** Endothelial Cell Responses to PM -- in vitro and in vivo

Investigator(s): Dennis Wilson and Jack Rutledge

Institution(s) of PI(s): University of California Davis

Research Category: Airborne Particulate Matter

Project Period: October 1, 2005 to September 30, 2010

Project Period Covered by this Report: July 1, 2007 through June 30, 2008

OBJECTIVE OF RESEARCH

The overall goal of this project is to determine the relationship between vascular disease and systemic effects of particulate matter.

PROGRESS SUMMARY AND ACCOMPLISHMENTS

Our previous work demonstrated that collected PM 2.5 stimulates pro-inflammatory and PAH metabolizing genes in cultured human endothelial cells. We further examined PAH response elements and demonstrated that HAEC respond to PM2.5 by activating AHR signaling. Finally, we examined the hypothesis that TGFβ family signaling was elicited by PM as it is by several other endothelial cell stimuli. We determined that PM2.5 does not stimulate TGFβ family second messenger responses. In the current year progress, we extended the gene response studies to cultured human bronchiolar cells and determined that similar proinflammatory and PAH response genes were upregulated but that signaling activites were less evident in epithelial cells. Overall, both cell types had only modest gene responses compared with treatments with endothelial cell toxins or lipolysis products of blood lipids. Based on evidence that napthoquinone (NQ) is a photo-oxidation product of vehicular emissions, we evaluated its ROS generating capacity as free compound or bound to proteins. We found a modest ROS generation in cultured cells that was markedly enhanced by pre-binding NQ to a sulfydryl containing protein. These results suggest that protein binding by reactive intermediates of PAH metabolism are not necessarily detoxifying reactions and that bound intermediates can retain ROS generating activity. This also implies that binding of reactive intermediates in organic fractions of airborne particulates may exert a stabilizing effect that enhances their toxicity.

In collaboration with project 3, we asked whether systemic inflammatory and pro-coagulant responses occurred in CAPs exposed mice. We found increased platelet numbers and an increased proportion of activated platelets in mice exposed for 2 weeks to CAPs. These findings were correlated with a modest upregulation of circulating cytokines involved with immune response regulation and bone marrow stimulation. We next asked whether PM interaction with monocytes might contribute to systemic cytokine secretion. We found upregulation of genes for several cytokine activities in human blood monocytes treated with collected PM2.5. Finally, we asked whether pulmonary expression of pro-inflammatory adhesion molecules could be detected by immunohistochemistry in CAPs exposed mice. While we demonstrated enhanced staining in postive control mice from ETS experiments, no significant differences were

found in CAPs exposed mice. These findings extend the understanding of potential mechanisms of cardiovascular injury to the concept that pro-inflammatory activation of endothelium and moncyte-platelet-endothelial interactions could initiate thrombotic events in susceptible regions of arteries such as atherosclerotic plaque.

Specific aim 1: To characterize human endothelial cell culture responses to direct concentrated ambient PM 2.5 exposure.

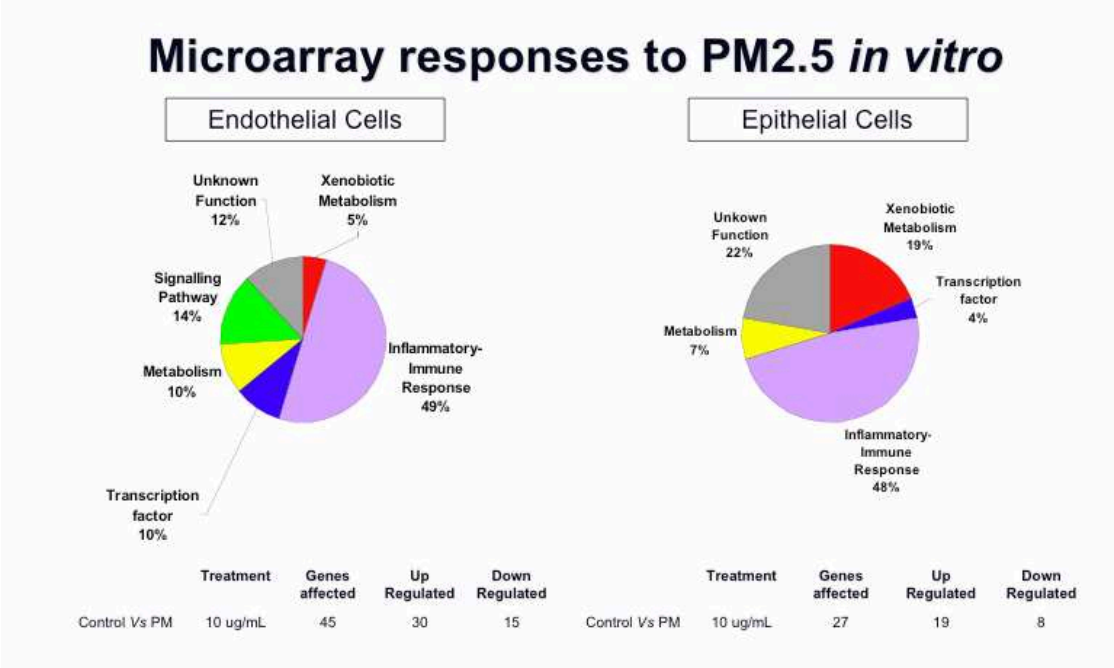
Microarray gene analysis in cultured human aortic endothelial cells (HAEC) exposed to PM 2.5.

Characterize PAH receptor signaling in response to PM 2.5

Evaluate ROS generation and associated Nrf2 signaling in PM 2.5 treated HAEC

We extended our previous microarray gene response studies to cultured human bronchiolar cells and determined that similar pro-inflammatory and PAH response genes were upregulated to that previously found with endothelial cells but that signaling activites were less evident in epithelial cells (Figure 1). Overall, both cell types had only modest gene responses compared with treatments with endothelial cell toxins or lipolysis products of blood lipids.

Figure 1; Comparison of gene responses to collected ambient PM 2.5 (Winter Urban 2007). HAEC or HBEC were treated with 10 ul/ml for 4 hours.



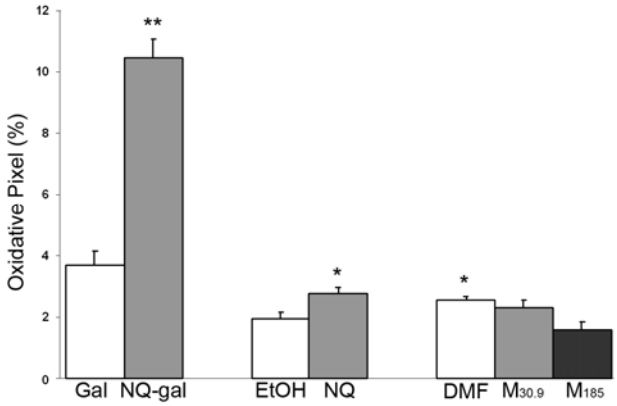
	Treatment	Genes affected	Up Regulated	Down Regulated
Control *Vs* PM	10 ug/mL	45	30	15

	Treatment	Genes affected	Up Regulated	Down Regulated
Control *Vs* PM	10 ug/mL	27	19	8

Our studies with PAH receptor signaling are complete and currently in manuscript preparation. We are currently performing ROS assays and evaluating Nrf2 nuclear translocation in cultured aortic endothelial cells exposed to collected ambient PM 2.5. A related project evaluated the effects of napthoquinone, an apparent product of photo-oxidation of vehicular napthalene release, on ROS generation and Nrf2 signaling in cultured human pulmonary endothelial cells (HPAEC). This study examined the

potential that protein binding of reactive intermediates such as NQ was a detoxifying event. We found instead that while NQ alone elicited a modest ROS response and stimulated Nrf2 translocation to the nucleus, tranfection of cells with NQ bound to a glutathione residue rich protein elicited a markedly increased ROS response (Figure 2).

Figure 2: ROS generation based on CM-H_2DCFDA fluorescence in HPAEC treated with NQ, Galectin adducted with NQ or Monocrotaline (M).



Specific aim 2: To determine the effects of direct PM exposure on permeability and pro-coagulant activity in endothelium

Monolayer permeability in endothelial cell cultures
Platelet activation and Systemic markers of coagulation and inflammation

We have accomplished studies of platelet activation and systemic markers of coagulation and inflammation in CAPs exposures to mice. We performed preliminary studies with Summer Rural exposures in 2007 and found evidence of platelet activation. We did a more thorough study with the Winter Rural study in 2008 that included both platelet studies and multiplex immunoassays for systemic cytokines. Using a panel of 32 mouse specific bead associated antibodies for cytokines, we found 7 upregulated in exposed mice (Figure 3). Of these, three are general markers of inflammation, two markers of TH-2 responses, one TH-1 associated and one associated with bone marrow stimulation. Mice exposed to CAPs for 2 weeks had increases in blood platelet counts. Platelets from exposed mice had significantly more aggregates than control mice. More platelets bound fibrinogen in response to thrombin and a significantly increased percentage expressed a marker of platelet lysosomal secretion, LAMP-1 (Figure 4).

We have yet to fully evaluate effects of PM 2.5 on endothelial cell barrier permeability. We presently are performing experiments evaluating effects of PM on HAEC actin cytoskeleton and have developed preliminary data with a high throughput real time electrical resistance based permeability system that will allow us to compare effects of multiple synthetic and environmental source PM samples.

Figure 3: Significantly altered cytokine responses in blood of mice exposed to Winter Rural source CAPs for 2 weeks.

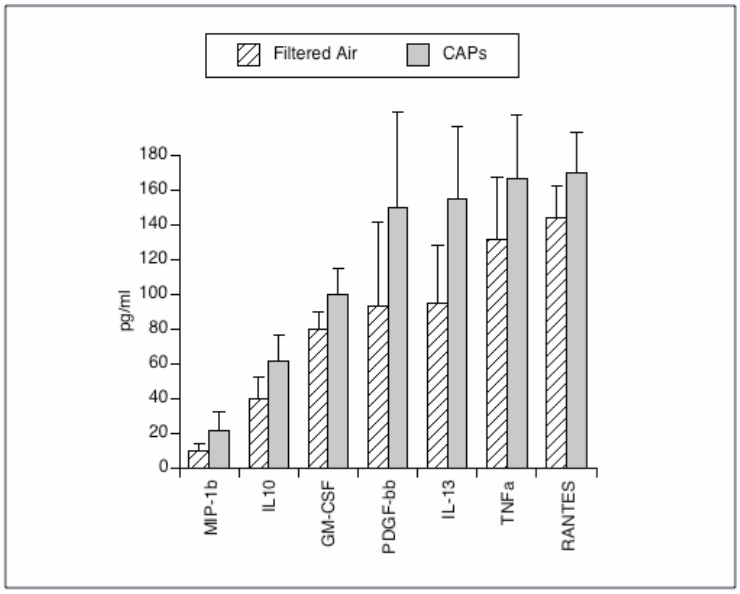
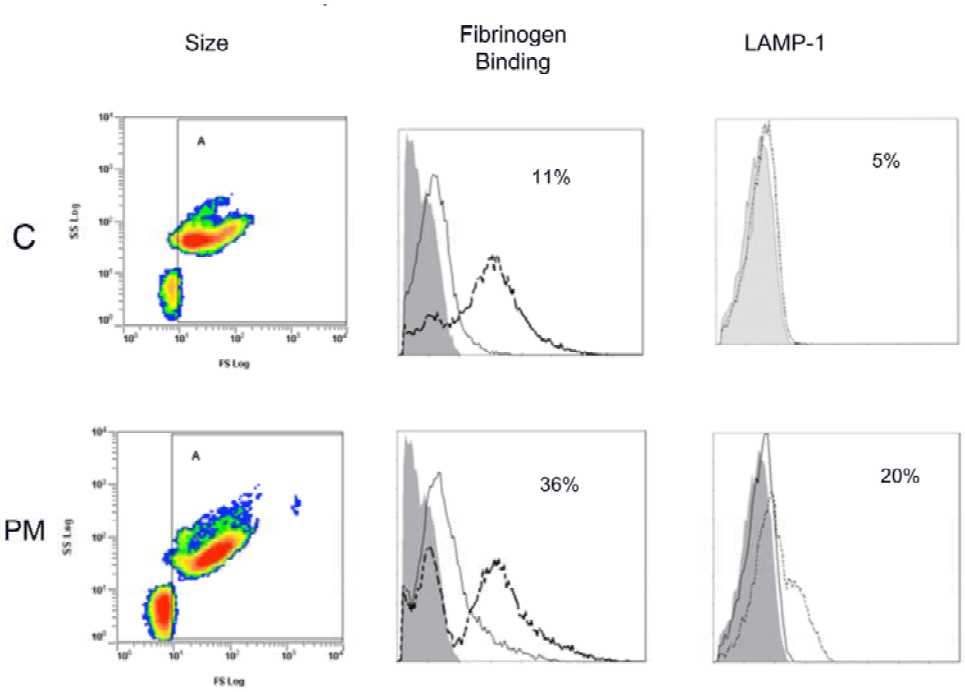


Figure 4: Platelet activation in blood of mice exposed to Winter Rural source CAPs for 2 weeks. Platelet from exposued mice had an aggregated population that was absent in control mice and significant increases in fibrinogen binding activity as well as lysosomal secretion (degranulation) as determined by surface expression of Lysosomal Associated Membrane protein 1 (LAMP-1).



Specific aim 3: To compare the nature and location of endothelial cell responses in pulmonary and cardiac tissue from CAPs exposed mice.

Immunohistochemistry
Cytokine secretion in BAL (Collaboration with project 3)

We have developed immunofluorescent probes for VCAM-1 and E-selectin and verified their use in lungs of mice exposed to ETS. Figure 5 demonstrates positive responses for E-selectin expression in arterioles of mice exposed for 2 weeks to ETS. Figure 6 shows a relative lack of responses in the lungs of mice exposed for 2 weeks to CAPs. An apparent upreguation of VCAM-1in airway epithelium of urban CAPs exposed mice from Winter 2007 (Figure 7) was not replicable in exposures from rural exposures in Winter 2008 (data not shown).

Figure 5 and 6: Expression of E-Selectin in lungs of mice exposed to either ETS or CAPs for 2 weeks.

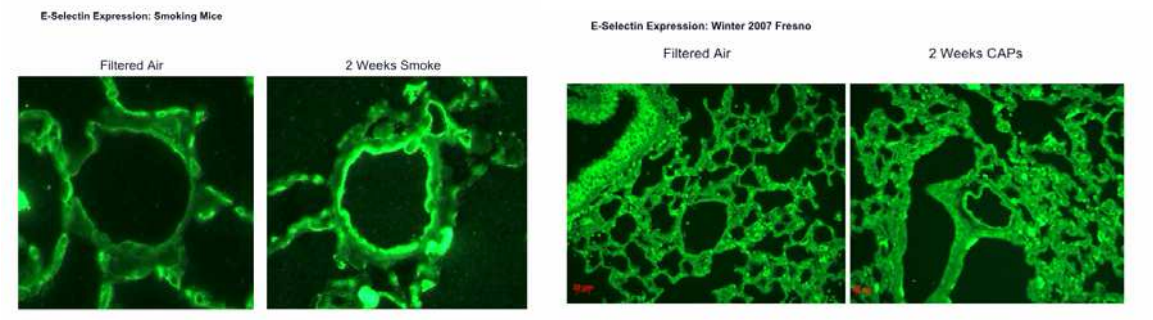
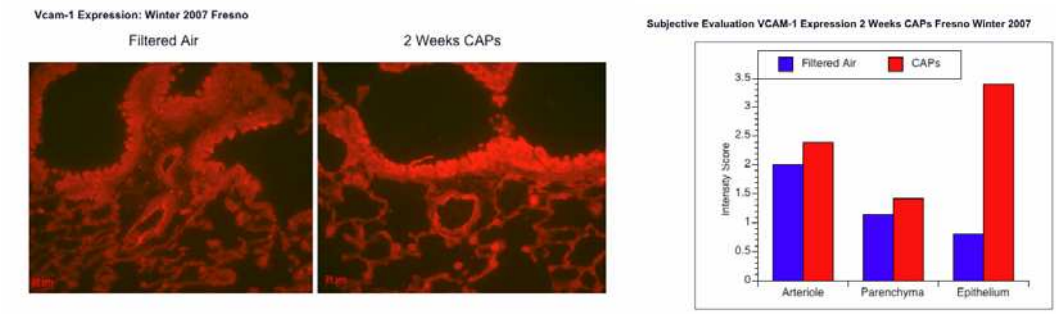


Figure 7: Expression of VCAM-1 in airway epithelium in response to a 2 week CAPs exposure: A) Immunohistochemistry, B) Subjective evaluation of VCAM and E-Selectin Expression.



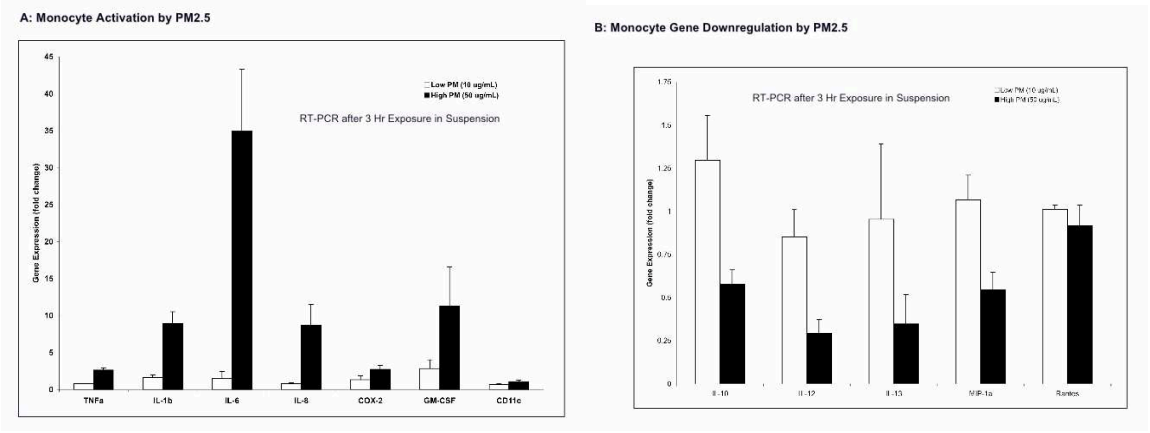
BAL cytokine assays were performed with material from mice exposed to Rural CAPs in Winter 2008. These assays did not detect significant levels of any of the cytokines in the panel in either control or treated mice. Future studies will require concentration methods to increase sensitivity.
Ongoing studies in specific aim 3 will compare immunohistochemical responses with HO-1 based on results with synthetic PM exposures in Project 1. Similarly, VCAM and E Selectin expression will be evaluated in cardiac vasculature of CAPs exposed mice.

Specific aim 4: To determine the effects of CAPs exposure on the progression of preexisting vascular disease in ApoE -/- mice.
We propose to significantly alter this aim with the intention to focus more on interaction of blood lipids, platelets and endothelium relative to PM and metabolic syndrome. In our revised aim, we will evaluate:

Monocyte upregulation of cytokines in response to collected PM 2.5
Phagocytosis and ROS production in monocytes exposed to collected PM 2.5
PM induced platelet-endothelial interaction in vitro.

We have completed evaluation of a variety of monocyte source cytokine activities by measuring gene expression in isolated human blood monocytes by RT-PCR. We find a mixture of upregulated and downregulated activities (Figure 8a and b). Ongoing studies are evaluating whether these responses are associated with PM phagocytosis using synthetic fluorescently labeled PM and Raman spectroscopic evaluation of monocytes exposed to collected PM 2.5. Preliminary fluorescence studies suggest synthetic silica particles are adherent but not phagocytosed during the time course of gene upregulation.

Figure 8a and b: cytokine gene responses to collected PM 2.5 (Winter Rural 2007) in isolated human blood monocytes treated with 50 ug/ml for 1 hour. A) Upregulated Activities. B) Downregulated activities



Proposed Studies Year 4:

Systemic Coagulation markers after intratracheal instillation of collected ambient PM 2.5: Supported also by CARB (Tablin & Wilson)
Evaluation of Monocyte Platelet and Endothelial cell interface in systemic coagulation *in vitro*
Comparison of ROS generating capacity of differing source collected ambient PM 2.5 (Anastasio) vs. ROS generation and Nrf2signaling in cultured cells.
More focused evaluation of pro-coagulant mechanisms and activities in plasma in field exposure studies

IL-11
Thrombopoetin
Tissue Factor
Thrombospondin

Evaluate cytokines in BAL in collaboration with Project 3 and inflammatory mediator expression in airway epithelium and vasculature. Extend these studies to markers of ROS response elements.

Multiplex assay of endothelial cell barrier functional responses to synthetic and collected PM.

Abstracts and publications in preparation;

Feliz, A.M. M.W. Lame, B. Kumfer, I. Kennedy, D.W. Wilson Interaction of Ultra Fine Particles and Transcytosis in Human Aortic Endothelial Cells. **ATS** 2007, Manuscript in preparation

L. Nakayama, M.W. Lame, A.D. Jones, and D.W. Wilson Intracellular oxidation and the translocation of Nrf2 in human pulmonary artery endothelial cells exposed to monocrotaline pyrrole, 1,4-naphthoquinone, and naphthoquinone adducted galectin-1. **ATS** 2007, Manuscript in preparation

Aung, H.H, M.W. Lamé, K. Gohil1, J. C. Rutledge, D. W. Wilson Particulate Matter-Induced Expression of CYP1A1gene through the AhR pathway in Human Aortic Endothelial Cells **ATS** 2008. Manuscript in preparation.

Aung, H-H, F. Tablin K. Pinkerton, L. Plummer, M.W. Lame, and DW Wilson Platelet and serum cytokine activation in CAPs exposed mice. Manuscript in Preparation

Endothelial cell responses to environmental particulate matter
Lewis, K.; Nakayama-Wong, L.; Lamé, M., Wilson, D. W. Merck Meriel Summer Scholars Annual Conference 2008.

In vitro effects of particulate matter on human endothelial adhesion molecule expression
Blum, A.; Lamè, M.; Tablin, F.; Wilson, D. W. Merck Meriel Summer Scholars Annual Conference 2008.

Date of Report: July 31, 2008
EPA Agreement Number: R832414-010
Center: San Joaquin Valley Aerosol Health Effects Research Center (SAHERC)
Project Title: Project 3 **--** Inhalation Exposure Assessment of San Joaquin Valley Aerosol
Investigator(s): Kent Pinkerton, Mike Kleeman
Institution(s) of PI(s): University of California Davis
Research Category: Airborne Particulate Matter
Project Period: October 1, 2005 to September 30, 2010
Project Period Covered by this Report: July 1, 2007 through June 30, 2008

OBJECTIVE OF RESEARCH:

Epidemiological evidence suggests that the association between cardiac mortality and PM 10 concentrations changes between the summer and winter months in the San Joaquin Valley (SJV). This shift is likely caused by seasonal variation in the size and composition distribution of airborne particles. This project will perform inhalation exposure and particle characterization studies at rural and urban locations in different seasons to quantify the features of the airborne particles that are associated with adverse health effects.

PROGRESS SUMMARY AND ACCOMPLISHMENTS:

Progress by Specific Aim

1. ***Differences in particle concentration, size distribution, and composition that occur as a function of season and location in the San Joaquin Valley (SJV) result in different health outcomes; these outcomes can be detected during inhalation exposure experiments.***

We have now completed four field measurement and exposure studies in the San Joaquin Valley. These include an urban site located in Fresno, CA (500 East Shaw Avenue) for the summer and winter seasons, as well as a rural site located at Westside, CA also studied during the summer and winter seasons. The field studies in Fresno were conducted September 5-16, 2006, and February 13-24, 2007. The field studies in Westside were conducted August 14-25, 2007 and February 6-17, 2008. In each study male C57/BL6 mice were exposed to concentrated ambient particles (CAPs) for 6 hours/day, 5 days/week for two weeks. Fine/ultrafine ambient particles were collected and concentrated onsite using a Versatile Aerosol Concentrator Enhancement System (VACES). CAPs samples collected during each exposure were analyzed for chemical composition. The results of these exposure studies are ongoing, but are reported in part in this

progress report.

2. ***The increased toxicity of airborne particles during the winter season in the SJV is associated with increased concentrations of ultrafine carbon particles.***

A multi-pronged approach is being applied to examine the cardiopulmonary consequences of particle exposure, i.e., using both whole body to 1) evaluate cellular and inflammatory indicators in the lungs by bronchopulmonary lavage and cytokine measurements, 2) measure vascular components in platelets and other blood elements, 3) examine heart tissues directly for histopathology and 4) measure neurological changes in gene expression for pro-inflammatory cytokines.

3. The increased toxicity of airborne particles in the SJV during the winter season is associated with increased concentrations of accumulation mode ammonium/nitrate/sulfate particles.

Ambient and CAPs concentrations for the summer (2006) and winter (2007) Fresno and the summer (2007) and winter (2008) Westside Concentrated Ambient Particles (CAPs) exposures have been determined. These values are shown in the table below. RAAS represents the average ambient concentration of particles, while CAPs represents the enhanced concentration of particles to which the mice were exposed. The enhancement factor of ambient particles using the Virtual Aerosol Concentrator Exposure System (VACES) is also shown in the table below.

	Fresno				**West Side**			
	Summer		**Winter**		**Summer**		**Winter**	
	Week 1	Week 2	Week 1	Week 2	Week 1	Week 2	Week 1	Week 2
RAAS (ug/m3)	18.5	21.2	23.7	11.0	7.54	11.5	15.4	14.7
CAPS (ug/m3)	207	361	130	97.2	106	145	88.9	82.4
Concentration Factor	11.2	17.0	5.48	8.85	14.1	12.6	5.78	5.59

Andersen Volume	18	m3
Concentrator Volume	1.08	m3

The chemical speciation for the Fresno and Westside summer and winter studies is also complete for nitrates, sulfates, bromine, ammonium, sodium, chloride, elemental carbon and organic carbon. The measurement for a number of trace elements has also been completed. These elements include potassium, vanadium, iron, lead, arsenic, calcium and barium.

4. The health effects of San Joaquin Valley aerosol can be directly related to the emissions source of the fine and ultrafine particles.

We have completed the lung cytokine analysis of the summer (2006) and winter (2007) Fresno and the summer (2007) and winter (2008) Westside Concentrated Ambient Particles (CAPs) exposures. Bronchoalveolar lavage analysis for these same locations and seasons demonstrated no significant changes in total cells recovered from the lungs, but a statistically significant increase in neutrophil counts for both summer and winter seasons in Westside. For the lung cytokine assays, the following patterns were observed:

Fresno summer: Significant decreases in IL-1β, IL-6 IL-10, INF-gamma, and MCP-1 were ob served following CAPs exposure in lung homogenates.
Fresno winter: No significant changes were noted in the levels of cytokines in lung homogenates following CAPs exposure compared with controls.
Westside summer: Demonstrated no statistically significant changes in cytokines following C APs exposure.
Westside winter: Demonstrated significant increases in cytokine levels in lung homogenates f or TNFa, IL-1a, IL-1b, IL-6, IL-12, GM-CSF, INF-gamma, MCP-1 and MIP-1a following CAPs exposure.

Supernatant from BAL is being analyzed for protein content and the presence of cytokines. In collaboration with the Wilson laboratory, optimal conditions for cytokine measurement are under way.

Lung tissues have been reoriented and embedded to enhance 1) airway analysis of central and distal airways. Staining is complete to examine for the presence and frequency of alcian blue/periodic acid Schiff (AB/PAS) positive airway epitheial cells.

With the completion of the first four CAPs studies for summer and winter seasons in both an urban and rural area of the San Joaquin Valley, we are now in the process of establishing

potential relationships between biological responses observed and the emission sources for fine and ultrafine particles. Size-resolved particulate matter samples collected during each exposure experiment will be analyzed for organic molecular markers by extracting them with organic solvents followed by analysis using gas chromatography – mass spectrometry (GC-MS). The information obtained from this analysis will be combined with size-resolved source profiles to determine size-resolved source contributions to airborne particulate matter using the chemical mass balance (CMB) receptor model. The smallest size fraction analyzed will be 0.056-0.1 μm particle diameter (fully in the ultrafine size range).

Summary of Particle Size and Composition Fall and Winter Exposure Experiments in Fresno

Samples of airborne particulate matter were collected during September 4-9 and September 12-16, 2006 (late summer season) and again on February 13-17 and February 20-24, 2007 (winter season) using six Micro Orifice Uniform Deposit Cascade Impactors (MOUDIs) and three Reference Ambient Air Sampler (RAAS). Samples were collected for 6hrs each day during the animal exposure periods. Three of the MOUDIs were loaded with Teflon collection substrates (used for gravimetric, water soluble ions, and trace metals analysis) while the other three MOUDIs were loaded with Aluminum Foil substrates (used for gravimetric and carbon analysis). Upstream of each MOUDI a PM1.8 cyclone was used to remove coarse particles that might otherwise bounce off collection substrates. Six size fractions below 1.8 μm aerodynamic particle diameter were resolved with the MOUDI operated in this configuration. The RAAS sampler was equipped with multiple channels that employed Teflon filters and Quartz filters to characterize PM1.8 mass.

The PM1.8 mass collected during the Fresno summer event was 15.8 μg m^{-3} during September 4-9, 2006 and 18.8 μg m^{-3} during September 12-16, 2007. The PM1.8 mass collected during the Fresno winter event was 23.7 μg m^{-3} during February 13-17, 2007 and 11.0 μg m^{-3} during February 20-24, 2007. These concentrations are significantly lower than concentrations experienced during typical air pollution events in the SJV, when PM1.8 concentrations can increase to values greater than 100 μg m^{-3}. Ultrafine ($PM_{0.1}$) mass concentrations were measured to be 0.3-0.4 μg m^{-3} during all Fresno summer sampling events (summer and winter). $PM_{0.1}$ concentrations greater than 2.0 μg m^{-3} have been measured during previous winter pollution events in the San Joaquin Valley. The low concentrations during the current study period are attributed to the weather conditions (atmosphere was well mixed during all days; rain was recorded at times).

The particle concentrator system can compensate for low ambient concentrations by increasing the exposure concentration by a factor of approximately 13. This will yield representative results during exposure experiments if the composition of the ambient particles is similar to the composition of particles during a true stagnation event. This assumes that the size and composition distribution of particles during the clean and polluted events are similar, but the absolute concentrations are lower during the clean event. The source apportionment of ultrafine particles during these periods of low concentrations will be very challenging and may not be possible if insufficient mass is available for analysis.

Figures 1 and 2 illustrate the size and composition distribution of particles collected during at Fresno during the summer and winter experiments, respectively. Organic carbon, elemental carbon, and water soluble ions (sulfate, nitrate, etc) make up the majority of the particle size distribution during summer months. The Fresno sampling site was located in relatively close proximity to busy surface streets and highways in Fresno. It is expected that the majority of these particles are derived from tailpipe exhaust emissions and / or road dust sources. Nitrate contributions are strongly evident during winter months, when colder temperatures favor the partitioning of ammonium nitrate to the particle phase. Elemental analysis of these samples using ICPMS has been completed and the data is being reviewed for QA/QC.

Summary of Particle Size and Composition Fall and Winter Exposure Experiments in Westside

The PM1.8 mass concentrations measured at Westside were 9.4 μg m^{-3} during the summer experiment and 16.7 μg m^{-3} during the winter experiment. The PM0.1 concentrations measured at Westside were 0.5 μg m^{-3} during the summer experiment and 0.4 μg m^{-3} during the winter experiment. These concentrations are similar to those measured in the Fresno experiment, but since Westside is much further away from any direct emissions sources, it is expected that particles collected at this location have undergone significantly more atmospheric aging than particles collected at Fresno.

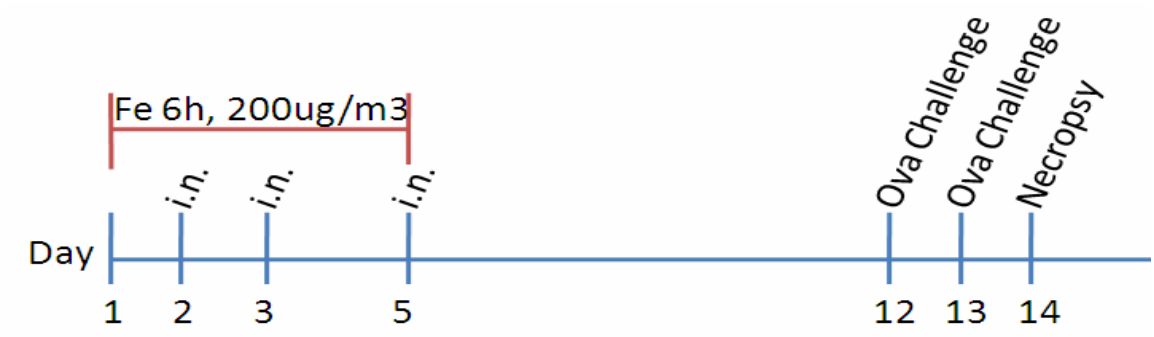
Figures 3 and 4 illustrate the size and composition distribution of particles collected at Westside during the summer and winter experiments, respectively. Trends are qualitatively similar to those observed at Fresno. Carbonaceous aerosol dominates during the summer months, with the addition of ammonium nitrate during winter months. Source apportionment analysis will be used to quantify source contributions to primary carbonaceous aerosol.

FUTURE ACTIVITIES

For the next reporting period, our plan are to (1) repeat the 10 day (2 week) CAPs study in Fresno for the summer and winter months, 2) create an allergic mouse model for incorporation into future CAPs studies, including the upcoming Fresno studies for the summer and winter seasons, and 3) acquire and configure a new larger trailer to allow more subjects in the field studies.

Background, rationale and plans for the ovalbumin allergic mouse model: Ovalbumin is a protein allergen that has been widely used in murine animals as a model of IgE-mediated allergic airway disease. Commonly the animals are sensitized to the allergen via intraperitoneal injection in the presence of an adjuvant, such as alum, and later challenged with an inhalation exposure of the allergen. Studies have revealed that local sensitization in the lung can be achieved through utilizing multiple intranasal instillations of the allergen. Further it has recently been illustrated that while fewer instillations of the allergen will not sensitize the animals, with instillate containing low levels of ultrafine particulate matter, an adjuvant effect is witnessed resulting in a sensitized animal. The PM Center is currently conducting research to validate these recent claims and to further investigate the adjuvant effect and its mechanism. Studies are being carried out which investigate the adjuvant effect through concomitant inhalation exposure to particulate matter with instilled allergen and instilled PM-enriched allergen to attempt to elucidate the mechanism of the particulate matter's adjuvant effect and determine its applicability to human exposures. Further research studies utilizing CAPs via the VACES exposure system will investigate the effects of atmospheric particles on both the sensitization and exacerbation of the allergic model in California's worst region of air pollution and asthma prevalence.

A pilot study to examine the adjuvant effects of particles on the ovalbumin model is underway using an iron/soot aerosol as a surrogate to San Joaquin Valley particles. The following exposure scenario has been implemented:



Work to acquire a second trailer for field studies is underway. This trailer will be larger in size compared with the current trailer to accommodate greater room for housing of animals, improve the environmental and housing conditions of the animals, facilitate working with the equipment and VACES system in the trailer, along with better lighting. Delivery is scheduled for September 17, 2008.

Cytokine analysis of BAL and lung tissues for Westside summer and winter CAPs studies will continue under the direction of Laurel Plummer. Further training is planning for Julian Recendez in filter preparation, instrument preparation and particle analysis for the upcoming summer 2008 Fresno CAPs study. Tentative dates for this study are August 18 to September 11.

PUBLICATIONS AND PRESENTATIONS

Smith, K.R., J.M. Veranth, U.P. Kodavanti, A.E. Aust, and K.E. Pinkerton. Acute pulmonary and systemic effects of inhaled coal fly ash in rats: comparison to ambient environmental particles. *Toxicol. Sci.* 93(2):390-399, 2006.

Donaldson, K., P.J.A. Borm, G. Oberdorster, K.E. Pinkerton, V. Stone, and C.L. Tran. Concordance between *in vitro* and *in vivo* dosimetry in the proinflammatory effects of low-toxicity, low-solubility particles: the key role of the proximal alveolar region. *Inhal. Toxicol.* 20:53-62, 2008. doi:10.1080/08958370701758742

Riddle, S.G., M.A. Robert, C.A. Jakober, M.P. Hannigan, and M.J. Kleeman. Size-Resolved Source Apportionment of Airborne Particle Mass in a Roadside Environment. Environ. Sci. Technol. In press, 2008.

Kleeman, M.J., S.G. Riddle, and C.A. Jakober. Size Distribution of Particle-phase Molecular Markers during a Severe Winter Pollution Episode. Environ. Sci. Technol. In press, 2008.

SUPPLEMENTAL KEYWORDS: None.

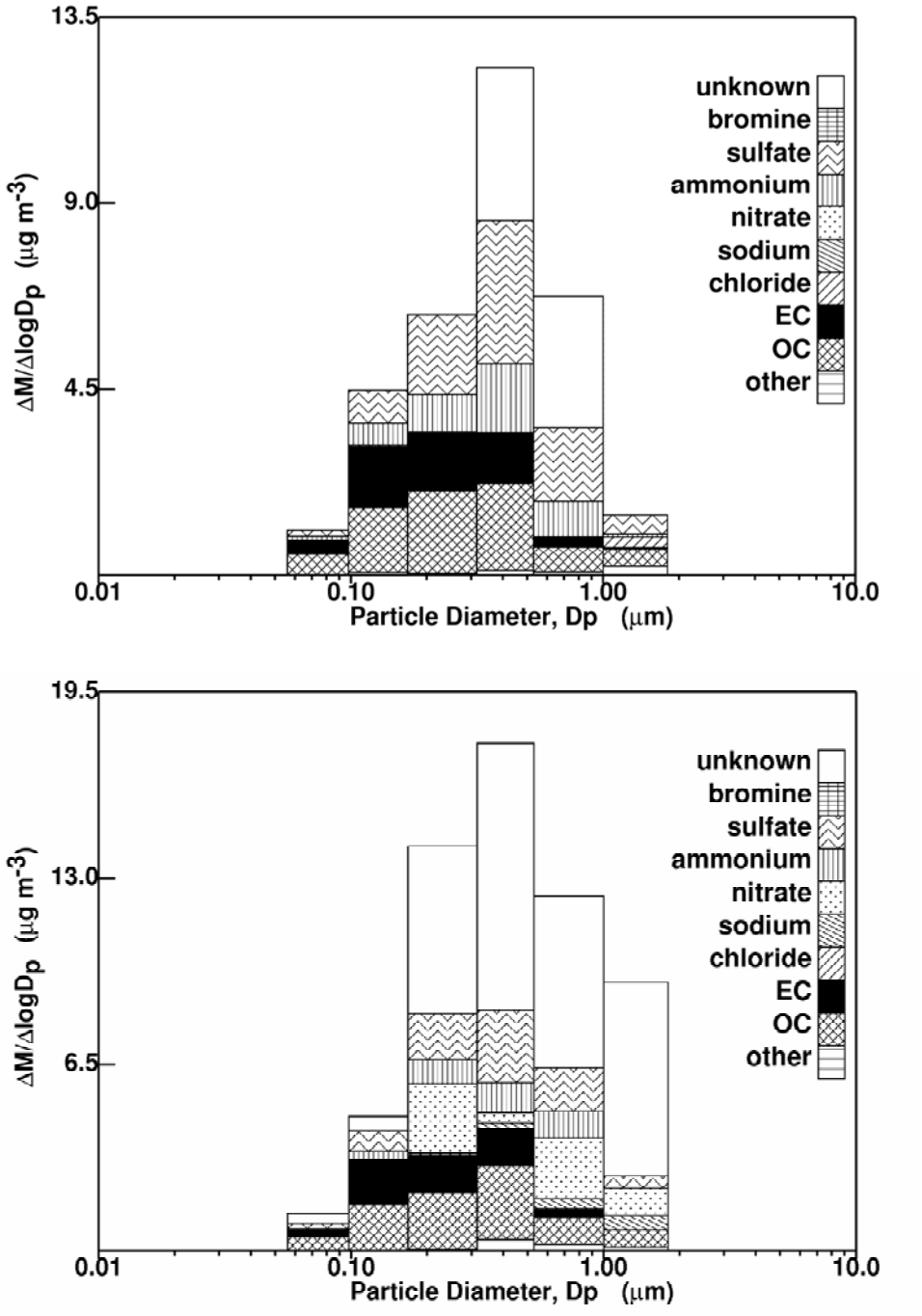


Figure 1: Size and composition distribution of airborne particulate matter measured at Fresno during Sept 4-9, 2006 (week 1) and Sept 12-16, 2006 (week 2). The majority of the "unknown" material is likely common crustal components such as Si, Al, and Fe.

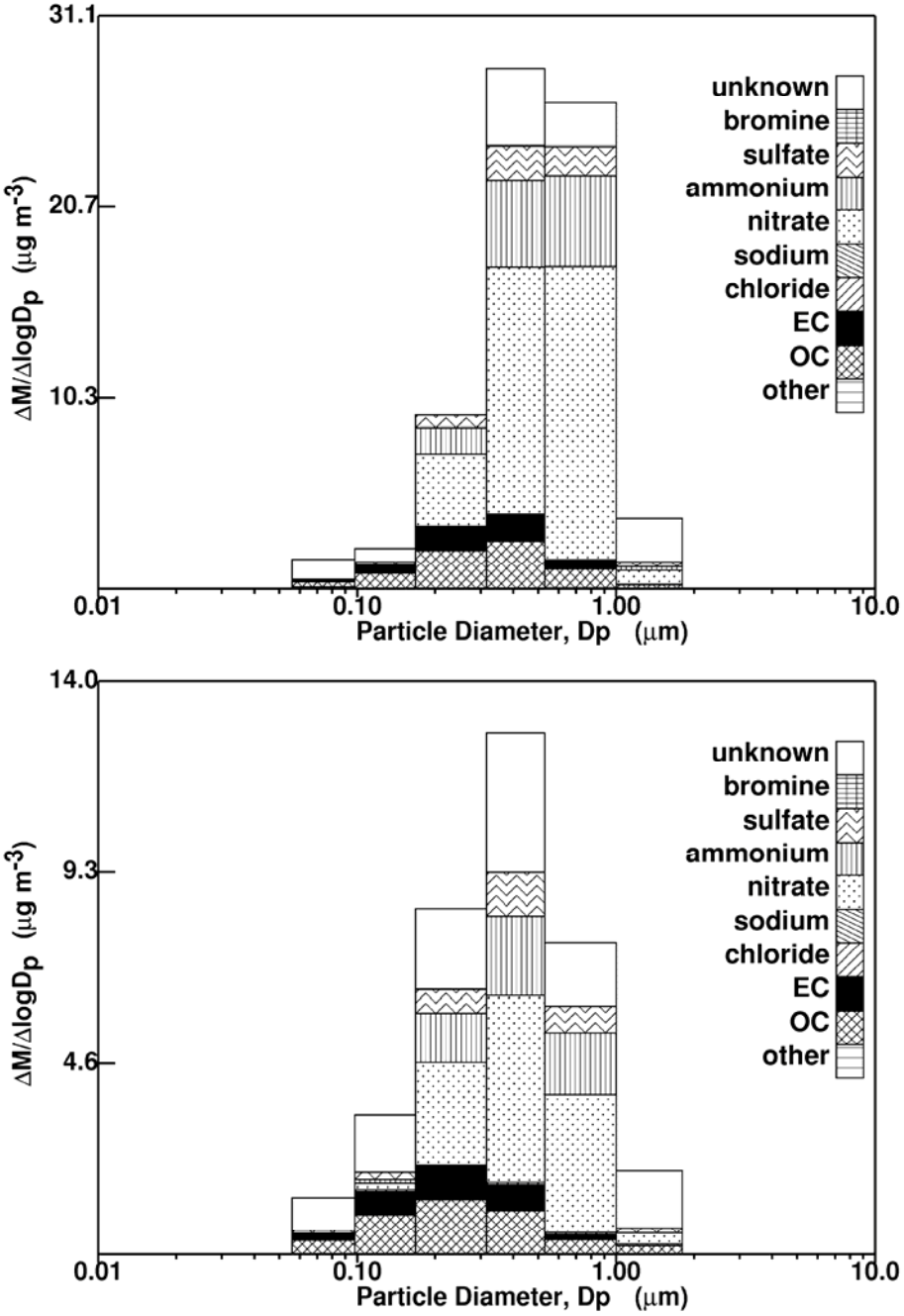


Figure 2: Size and composition distribution of airborne particulate matter measured at Fresno during Feb 13-17, 2007 (week 1) and Feb 20-24, 2007 (week 2).

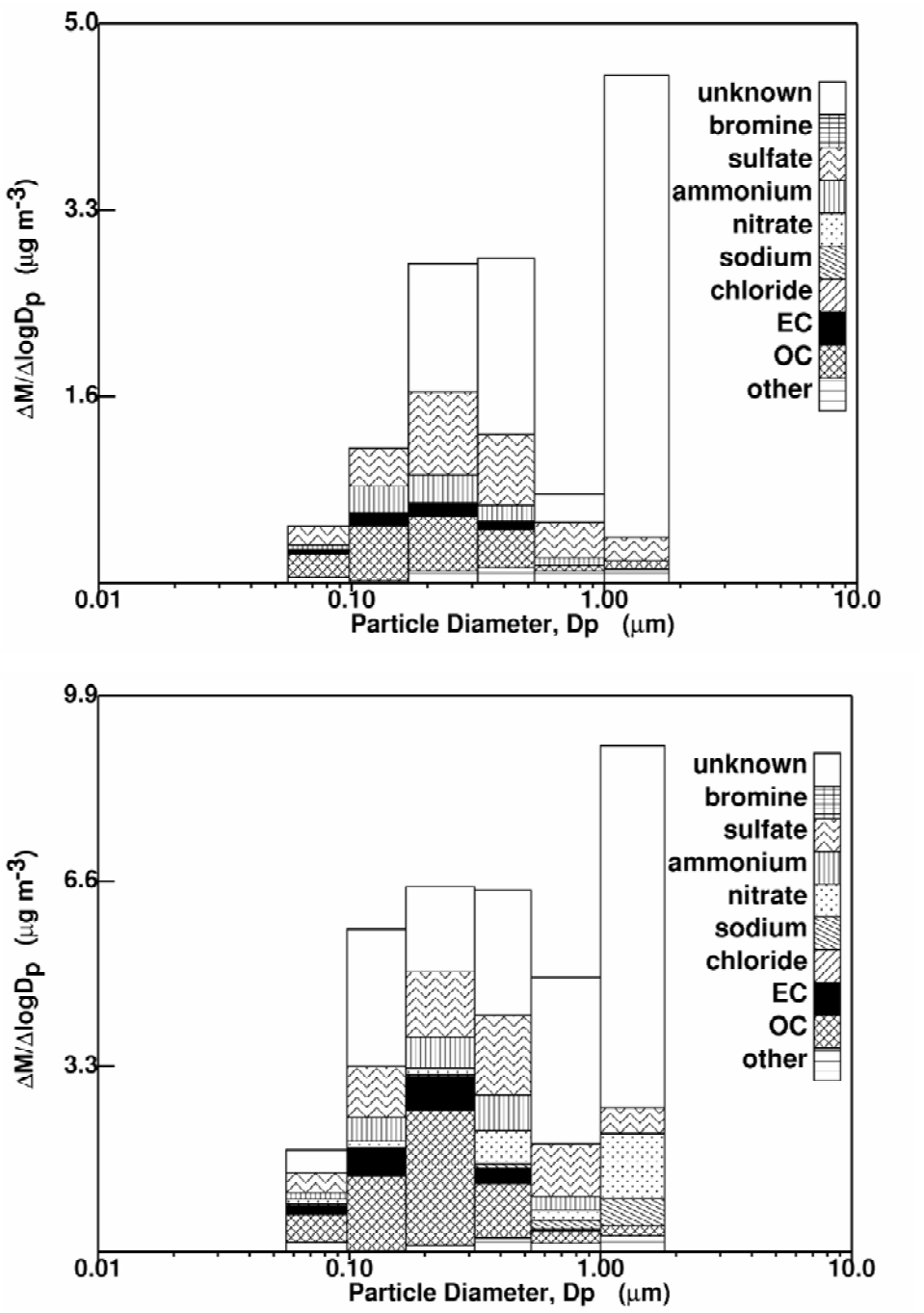


Figure 3: Size and composition distribution of airborne particulate matter measured at Westside summer during week 1 and week 2. The majority of the "unknown" material is likely common crustal components such as Si, Al, and Fe.

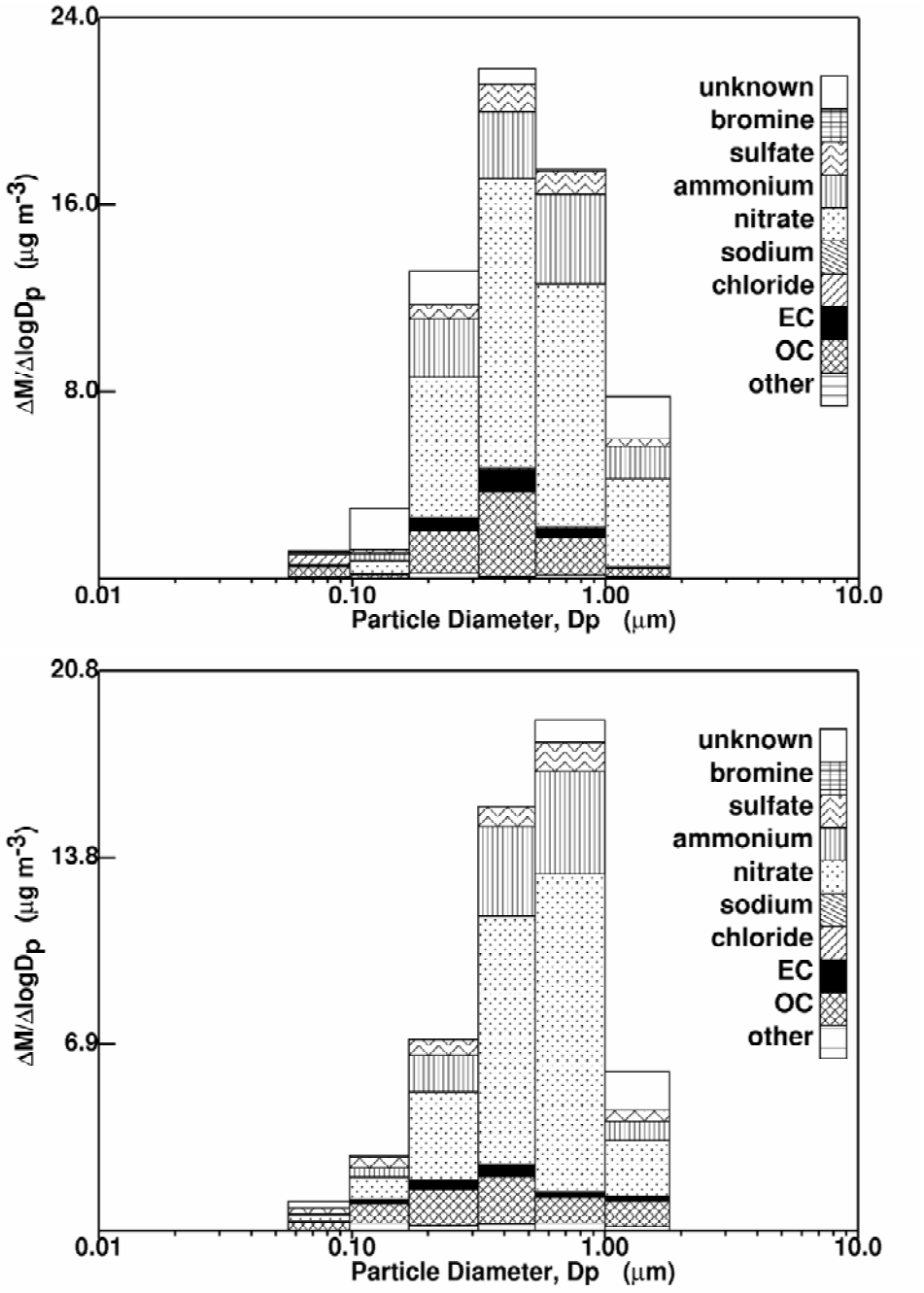


Figure 4: Size and composition distribution of airborne particulate matter measured at Westside winter during week 1 and week 2.

Date of Report: July 31, 2008
EPA Agreement Number: R832414-010
Center: San Joaquin Valley Aerosol Health Effects Research Center (SAHERC)
Project Title: Project 4 – Transport and Fate of Particles
Investigator(s): Dennis Wilson and Angelique Louie
Institution(s) of PI(s): University of California Davis
Research Category: Airborne Particulate Matter
Project Period: October 1, 2005 to September 30, 2010
Project Period Covered by this Report: July 1, 2007 through June 30, 2008

OBJECTIVE OF RESEARCH:

1) To characterize the time course, tissue distribution, and mechanisms of particulate matter (PM) accumulation in the systemic circulation and target organs. 2) To evaluate the effects of size and surface-fixed charge on this process. 3) To determine how altered lung structure affects systemic particle distribution.

PROGRESS SUMMARY AND ACCOMPLISHMENTS:

Specific aim 1: To characterize the time course and distribution of circulating particulates *in vivo.*

Positron Emission Tomography (PET) is currently being used to determine the deposition and translocation of 78nm ^{64}Cu-labeled amine terminated polystyrene beads in the rat model. Figure 1.1 shows translocation of beads to the heart and mucociliary clearance from the lungs into the GI tract in the rat over a 24 hour time period. Figure 1.2 shows a different translocation pattern of free copper in the rat over 24 hours verifying that the translocation and clearance shown in Figure 1.1 is from intact beads and not free copper. Figure 1.3 shows translocation of beads at 0 and 24 hours when beads are administered intraveneously. This was used to compare to Figure 1.1 which showed heart uptake upon instillation. Intraveneous administration shows primarily liver and spleen uptake. Gamma counting is utilized post vivo for bio-distribution (^{64}Cu and ^{111}In labeled beads). Further verification of label stability was determined in Figure 1.4 with beads incubated in both saline at pH 2 and plasma. These solutions were used to test stability in the stomach and blood, respectively. Over the 48 hour time period the labeled beads remain relatively stable, indicating that the signal shown in Figure 1.1 is mainly from intact beads and not free dissociating copper.

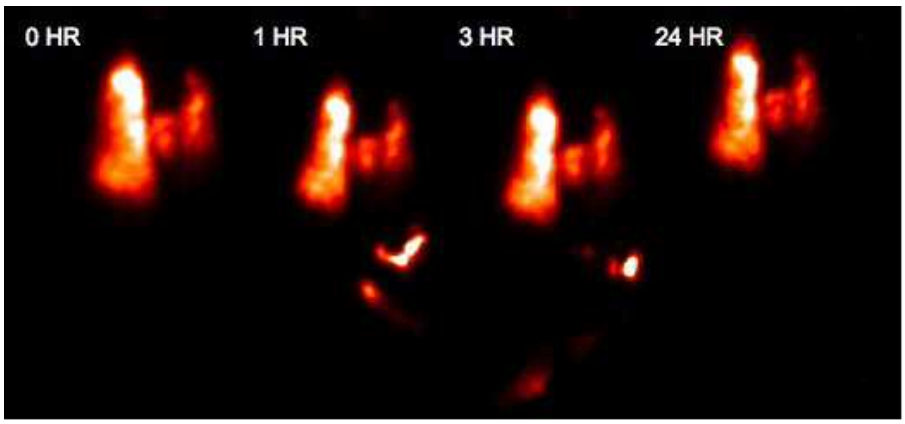


Figure 1.1. ^{64}Cu-labeled amine terminated polystyrene bead translocation in the rat over a 24 hour time period. Beads are shown in lungs, heart and GI tract.

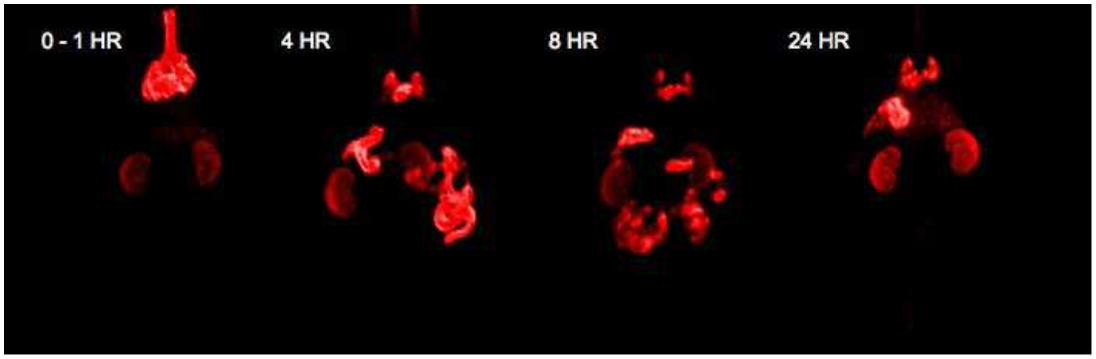


Figure 1.2. Free copper translocation in the rat over a 24 hour time period. Free copper is shown in lungs, kidneys, liver and GI tract.

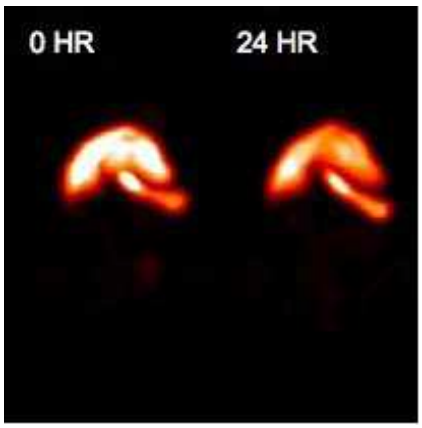


Figure 1.3. Intravenous administration of beads into the mouse model for comparison to lung bio-distribution. Liver and spleen uptake is shown.

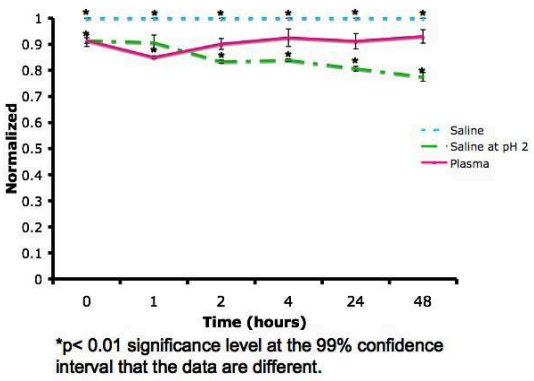


Figure 1.4. Bead stability over 48 hours in saline at pH 2 and plasma.

Future directions for this project include further investigating the mechanism of transport for the heart signal shown in the rat lung instillations. Also, varying surface charge, composition and size of nanoparticles to determine the effects of translocation and overlaying with MRI for anatomical referencing. Blood clearance will be determined for the 78nm beads over the 24 hour time period using gamma counting.

Specific aim 2: To compare the anatomic site of particulate accumulation in tissues with organ distribution as determined by microimaging techniques.

PET imaging has been utilized to determine the deposition pattern for each animal upon instillation. Figure 2.1 shows deposition of polystyrene beads into the lungs vs. stomach. Dynamic imaging has also been implemented to follow translocation of beads over time. Figure 2.2 shows a still image of particles in the trachea and lungs in 2.2a and in lungs in 2.2b. These images were taken over the first hour upon instillation.

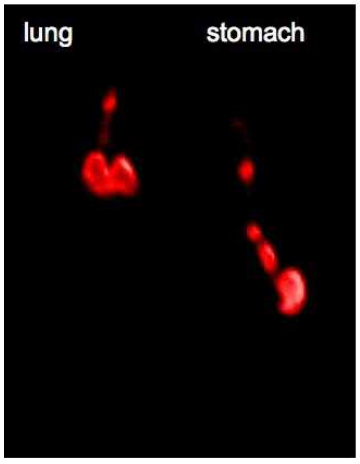


Figure 2.1. Verification of deposition of polystyrene beads in the mouse model with lung deposition on the left and stomach deposition on the right.

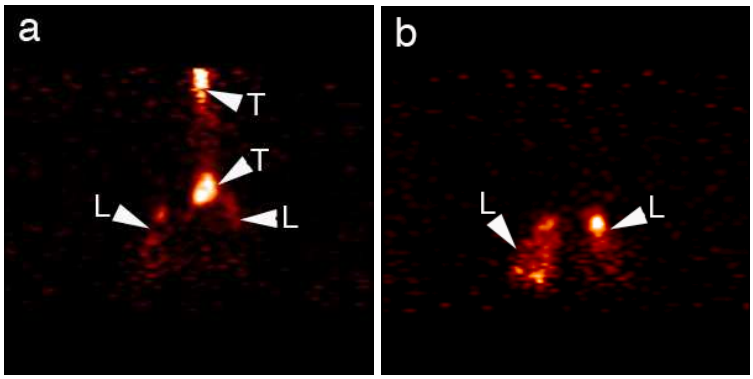


Figure 2.2. Dynamic imaging of beads in the rat model with the instillation during the scan. 2.2a shows a still of movement in the trachea and lungs and 2.2b shows lung region only. L = lungs, T = trachea.

Future directions for this project include utilizing dynamic imaging to follow translocation of nanoparticles over time in the same animal and utilizing PET imaging in conjunction with gamma counting to determine particulate accumulation *in vivo*.

Compromised Cardiovascular Animals: Do animals with pre-existing CV disease accumulate nanoparticles at lesions?

The bio-distribution and accumulation of particulates in compromised animals has begun with an atherosclerosis injury model (ApoE) mouse. The instillation material was comprised of 78nm polystyrene-Cu64 and 35nm fluorescently labeled dextran coated particles. PET imaging of the mouse demonstrated deposition of PS particles into lung and Confocal imaging demonstrated transportation of dextran coated particles to other organs. Fluorescence was seen in the descending thoracic aorta (Figure 2.3) and injured right carotid artery (Figure 2.4).

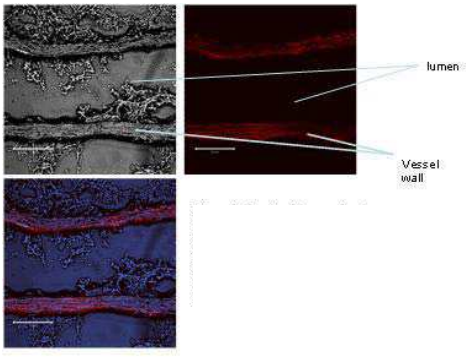


Figure 2.3. Confocal image of aorta of apoE mouse 4h post (lung) instillation of polystyrene (PS-Cu64) and CLIO-Texas Red. Fluorsence in vessel wall is seen. Scale bar = 50 microns. (L=BF, R=fluor, bottom left=overlay).

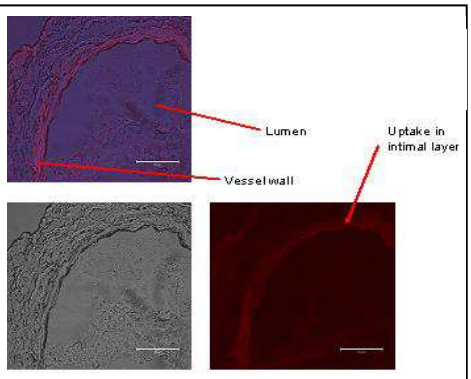


Figure 2.4. Injured carotid artery (clamp followed by iron chloride soak) shows more diffuse uptake in the aorta. Scale bar = 50 microns.

Future directions for this project include synthesizing tri-labeled nanoparticles for PET/MRI/Confocal imaging of a single probe and multimodal imaging (MRI/PET) of particle bio-distribution for both injury and normal animal models.

Specific aim 3: To evaluate potential mechanisms of PM transport across epithelial and endothelial barriers.

Mechanisms of Endothelial and Epithelial transport using synthetic fluorescent and electron dense ultrafine particles.

In previously reported work, we characterized the transport of 30 nm synthetic iron oxide particles across endothelial cell monolayers and demonstrated vesicular transport though caveolar like structures by 4 hours. We further characterized this as vesiculocaveolar transport using fluorescence tagged silica particles with confocal microscopy. We have now extended this work to ask whether similar rates of transport occur in cultured human airway epithelial cells. We found, in contrast to studies with endothelial cells, airway epithelium did not allow transport during a 4 hour incubation period (Figure 3.1 A) compared with extensive transport in endothelium (Figure 3.1B) and furthermore, limited internalization of iron oxide particles occurred despite similar association with cell surfaces (Figure 3.1C). We have recently synthesized Alexafluor 680 conjugated silica particles for use in real time transport studies using deconvolution microscopy. In addition, we have successfully cloned and transfected a GFP caveolin construct that will allow us to co-localize red Alexa 680 labeled PM with green labeled caveoli. Using a new collaboration with the Center for Biophotonics, we propose to follow a time course of particle transport in GFP-caveolin transfected EC using live cells in real time. This technique will then allow inhibitor studies to determine whether this represents an active or passive transport process.

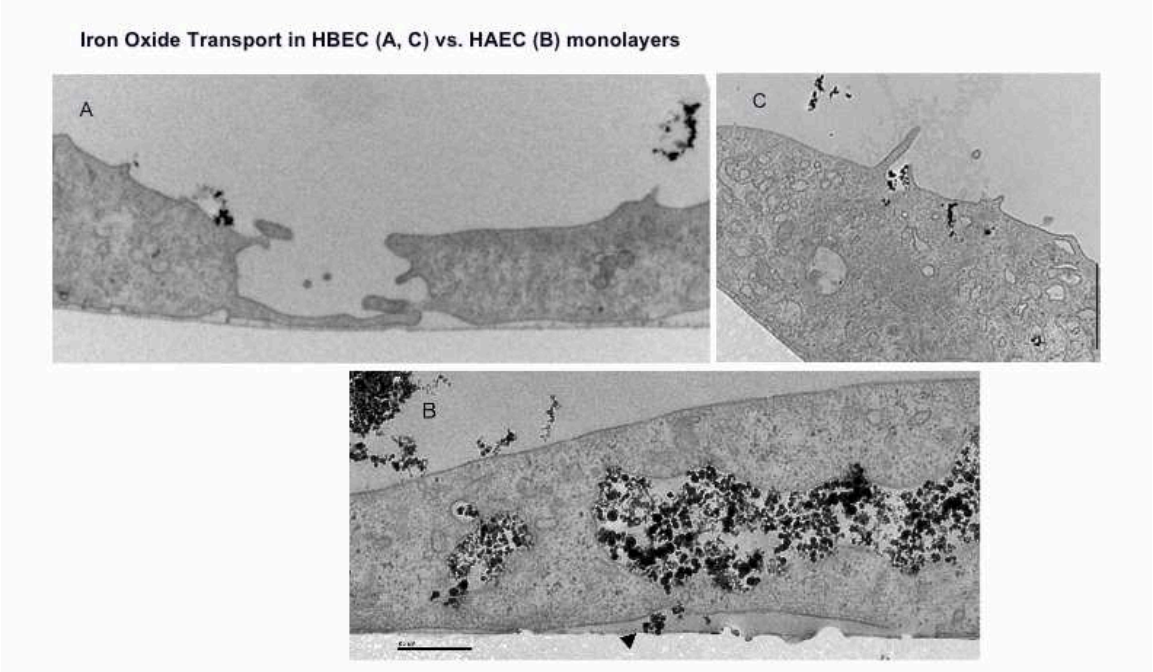


Figure 3.1: Localization of iron oxide particles after 4 hours of incubation with either cultured human airway epithelium (A + C) or aortic endothelium (B). Initial concentration was 10 ug.ml of 30 nm Iron Oxide PM.

Specific aim 4: To characterize the dynamics of interaction between particulates and airways and arterial walls.

We added a new investigator (Dr. Abdul Barakat) to this project to pursue this specific aim. His work has just begun so progress will be reported in the next progress report.

Date of Report: July 31, 2008
EPA Agreement Number: R832414-010
Center: San Joaquin Valley Aerosol Health Effects Research Center (SAHERC)
Project Title: Project 5 **--** Architecture Development and Particle Deposition
Investigator(s): Anthony Wexler and Ed Schelegle
Institution(s) of PI(s): University of California Davis
Research Category: Airborne Particulate Matter
Project Period: October 1, 2005 to September 30, 2010
Project Period Covered by this Report: July 1, 2007 through June 30, 2008

OBJECTIVE OF RESEARCH: Quantify lung architecture, pulmonary function and particle deposition pattern changes due to pollutant exposure during development**.**

PROGRESS SUMMARY AND ACCOMPLISHMENTS: Three current activities are highlighted below: (1) characterization of normal rat lung architecture, (2) lung architecture and function changes due to ozone exposure during development, and (3) development of a combustion particle generator for exposing animals to PM during development.

1. Characterization of normal rat lung architecture

We analyzed the airway architecture of six normal rats using an algorithm to characterize model airway architecture that we developed previously (Lee et al. 2008). On the whole, inter-subject variability was small both in generation- and diameter-based analysis. Variation of airway size with generation number (Figure 1) indicates that global features of airway architecture are similar between subjects. Variations of asymmetry, branching angle and rotation angle shows that more detailed features are similar between subjects (Figure 2). Furthermore, distribution functions of asymmetry and rotation angle were very similar between subjects (Figure 3). Overall, these patterns in airway architecture indicate that models based on only several rats can be representative if a sufficient number of airways are analyzed.

Current study also showed that the mean value and standard deviation of the geometric parameters are insufficient to characterize airway architecture in the lung. For example, we found that the twist angle, i.e., the angle between successive bifurcations, is far from normally distributed. Thus, the typical distribution of the values of airway geometry must be taken into account to quantitatively describe pulmonary architecture.

2. Lung architecture and function changes due to ozone exposure during development

To test the effects of ozone exposure during lung development period, Sprague-Dawley rat pups (20 pups for each group) were exposed to filtered air or to 0.2 or 0.5 ppm ozone 8hrs/night, 5days/week from 7 days to 28 days after birth. At 56th days (after 28 days recovery), lung

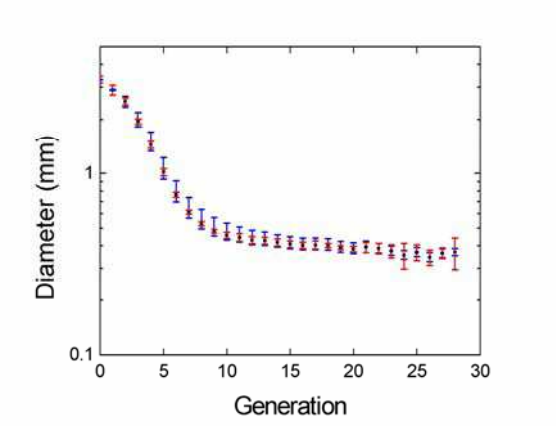


Figure 1. Airway diameter vs Generation

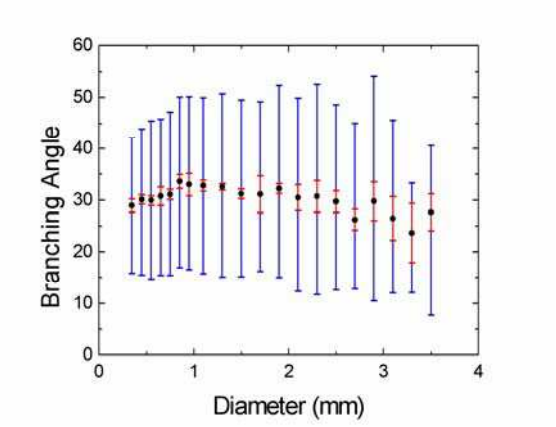


Figure 2. Branching angle vs Diameter

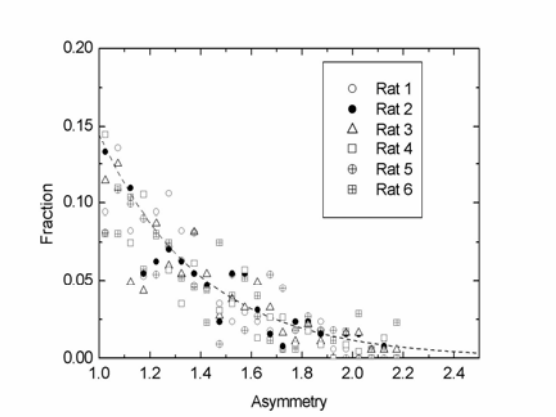


Figure 3. Distribution of asymmetry between subjects for the, $0.6 \leq d_p \leq 0.7$ mm interval

mechanics were evaluated for half of them and half of them were casted to analyze lung architecture. For lung architecture, diameter, length rotation angle, branching angle, asymmetry based on generation or airway diameter were calculated and fractal analysis were conducted. There were no significant difference between control and exposures in lung architectures. For lung function, resistance, compliance and TLC were evaluated using a forced oscillation technique. While baseline airway resistance did not change, elastance increased and TLC decreased especially for 0.5 ppm exposure (Figure 4, 5).

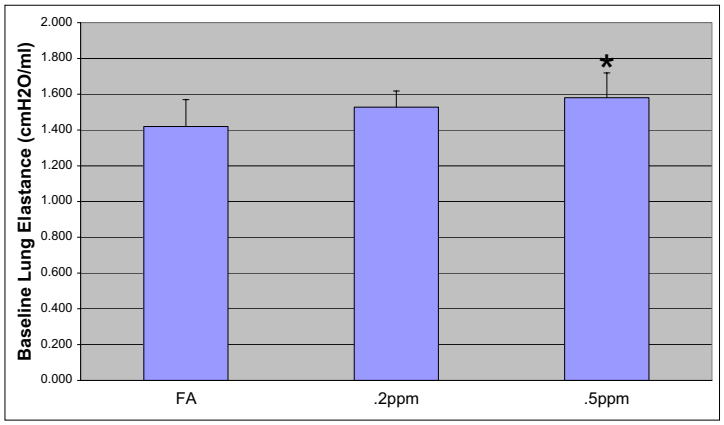


Figure 4. Lung Elastance

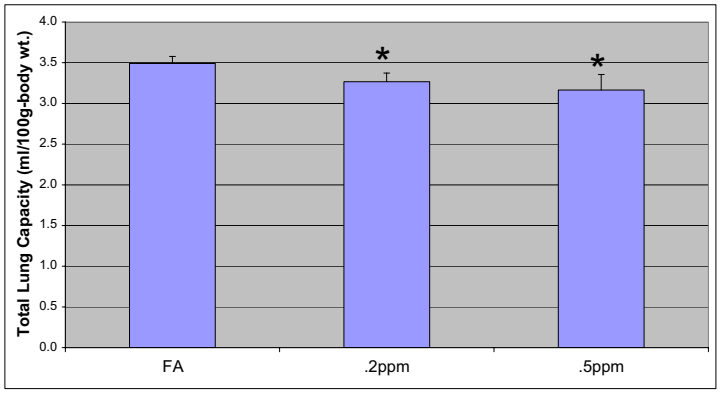


Figure 5. Total Lung Capacity (TLC)

3. *Development of a combustion particle generator for exposing animals to PM during development*

Combustion particles for inhalation studies are generated using an ethylene-fueled burner that can be configured to generate different types of soot. Studies thus far have looked at soot from a diffusion flame where fuel is allowed to mix with a surrounding air flow and excess fuel that escapes from the flame is pyrolized into particulate matter composed almost entirely of elemental carbon. This particulate matter is then ported directly into an exposure chamber where it is mixed with clean air without the need for collecting and re-aerosolizing the particles. Particle concentration can be varied with fuel flow rate, and is held at a mass concentration of 50-80 $\mu g/m^3$ for developmental studies, which translates to a number concentration of approximately 20,000 particles/cm^3.

The same particle generator is capable of generating soot of different sizes and concentrations by operating in a premixed flame mode with an inert gas sheath surrounding the flame. Through careful controlling the fuel-air mixture and overall flow rate, it is possible to select

a particular peak particle diameter within a range of 50-200 nanometers, to vary the concentration of the particles, and to vary the level of PAH produced in the flame. The burner development project has been jointly funded by Projects 1 and 5, and has been used for controlled exposures for both projects.

FUTURE ACTIVITIES

We will analyze rat lungs exposed to particles of different compositions, and compare them to normal lungs. The first particle exposures have been completed and data analyses are currently underway.

PUBLICATIONS AND PRESENTATIONS

Journal Articles

Tebockhorst, S., D.Y. Lee, A.S. Wexler, and M. Oldham, Interaction of epithelium with mesenchyme affects global features of lung architecture: A computer model of development. *J. Appl. Physiol.* 102:294-305, 2007.

Lee DY, Wexler AS, Fanucchi MV, Plopper CG. Expiration rate drives human airway design. *J. Theor. Biol.* 253:381-387, 2008

Lee DY, Park SS, Ban-Weiss G, Fanucchi MV, Plopper CG, Wexler AS. Bifurcation model for characterization of pulmonary architecture. *Anat. Rec.* 291:379-389, 2008

Lee DY, Fanucchi MV., Plopper CG, Fung J, Wexler AS. Pulmonary architecture in conducting regions of six rats. *Anat. Rec.* 291:916-926, 2008

SUPPLEMENTAL KEYWORDS

None.