Southern California Particle Center

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SOUTHERN CALIFORIA PARTICLE CENTER (SCPC)
Year-3 Progress Report

Date of Report: July 28, 2008
EPA Agreement Number: RD-83241301
Center Name & Internal Number: Southern California Particle Center (SCPC); UCLA Award Number: 018802-001
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Research Category: EPA/STAR Particulate Matter Research Centers (STAR-66509)
EPA Project Officer: Stacey Katz
Period Covered by the Report: August 1, 2007 – July 31, 2008

Southern California Particle Center Overview

The overall objective of the Southern California Particle Center (SCPC) is to bring together outstanding scientists to conduct high priority research to elucidate the underlying basis for health effects associated with exposure to ambient particulate matter (PM). The strengths of the investigators in this center and our demonstrated record of progress, the powerful assortment of equipment available and the unique characteristics of the Los Angeles basin airshed are key factors in why Southern California is an important setting for PM research studies. The overall goal of the SCPC is to establish the linkage between PM source emissions, underlying mechanisms and resulting health effects. Fine and ultrafine particles derived from traffic continue to serve as foci for SCPC interdisciplinary studies that include exposure, toxicology, chemical characterization and epidemiology. Health effects research has centered around identifying mechanisms of PM-induced cardiovascular effects and allergic airways disease and the exposure conditions and components that are associated with observed mechanisms.

The LAB has been described as the most polluted airshed in the nation, with complex, persistent and unique PM. Covering approximately 12,000 square miles, the population is projected to reach 19 million by the year 2020, a 1.5 fold increase from 1990 levels. Vehicle miles traveled are increasing at an even greater rate and the LAB continues to exhibit the most severe ozone and PM air quality problems in the U.S. PM is emitted from numerous sources in the basin, including millions of motor vehicles, nearly 300,000 diesel trucks, the nation's largest marine ports, expanding airports, and tens of thousands of factories.

Major research findings from the initial years of the SCPC have included the following. More recent findings follow:

- The most extensive chemical characterization of PM and intensive monitoring studies in the LAB ever conducted.
- Development of continuous monitors for size-fractionated particle mass and chemical composition, and mobile, size-selective PM concentrators capable of collecting large amounts of ambient samples for toxicological and human studies.
- Characterization of the formation and dynamics of PM near freeways.
- Demonstration of mitochondrial uptake of ultrafine particles and related toxicity.
- Demonstration that pro-oxidative chemicals induce hierarchical oxidative stress effects and development of murine in vivo models for induction of acute airway inflammation.
- Demonstration of in vivo allergic airway responses, neurological and cardiovascular effects in close proximity to a freeway.
- Exposure of healthy and impaired human volunteers to concentrated PM; first CAP exposure studies of human volunteers to coarse and ultrafine particles.
- Demonstration of a linkage between traffic density and human developmental toxicity.
- Demonstration of the relationship between traffic density (at schools and homes) and primary respiratory outcomes.

The research of the Center is divided into five project areas, all of which build on the findings and methodological advances that resulted from the first six years of SCPC

funding. The primary objective of Project 1 is to examine the relationships between PM sources, exposure, and toxicity within the urban atmosphere. This project is an integral part of Projects 2, 3 and 4, by serving as the field operations to collect PM samples for toxicity testing and for providing elevated levels of ambient PM for animal exposure models described in these projects. The primary objective of project 2 is to elucidate the mechanism(s) of PM-induced asthma and atherosclerosis exacerbation in vitro and in vivo. This is accomplished by animal studies in a mobile trailer as well as in vitro studies in representative tissue culture cells. The principal hypothesis is that a major PM injury mechanism is the induction of oxidative stress that promotes respiratory and cardiovascular inflammation as the major pathology feature underlying asthma and atherosclerosis. We propose that oxidative stress is a hierarchical event in which the induction of antioxidant defense pathways in tier 1 is in dynamic equilibrium and defends against the pro-inflammatory (tier 2) pro-apoptotic (tier 3) effects of higher levels of oxidative stress. In Project 3, the catalytic redox and electrophilic properties of ambient PM samples are characterized using cell-free chemical assays. A primary hypothesis is that PM contains constituents capable of inducing cellular stress by redox or other chemical processes, focusing on two general mechanisms, redox and electrophilic reactions.

The overall goal of Project 4 is to advance knowledge on the importance of particle size and composition to the induction of oxidative stress responses in a high-risk population of elderly people with coronary artery disease. Project 5 consists of three subprojects that either directly or indirectly seek to improve our ability to assess health risk of air pollution by chemical and biological assays used by SCPC. The paragraphs that follow provide an overview of SCPC research progress in selected areas during the first three years of the renewed program.

Project 1: The SCPC is building upon its history of research addressing public exposure to and health effects of mobile source PM, by investigating a variety of mobile source types. Field sampling campaigns during the last three years have been conducted at freeways, tunnels, an airport, a seaport, and in communities affected by combinations of these sources. Roadway studies have been a central feature of the SCPC since 1999. An in-vehicle study conducted during this renewal period concluded that 33-45% of a typical Los Angeles resident's exposure to UFP may occur while in a vehicle, confirming the high importance to public health of vehicular emissions. Size segregated chemical composition of freeway samples found that UFP collected at a freeway were composed predominantly of organic carbon and had high acidity relative to larger size fractions. During heavy traffic periods, high concentrations of polycyclic aromatic hydrocarbons (PAH) and organic quinones were detected in traffic-derived particles. Emission factors for particulate matter components have been developed for road and tunnel environments.

A set of studies of ambient PM near busy seaports included chemical characterization of size segregated ambient samples and a focused analysis of spatial variability in particle number concentration and size distribution within communities affected by emissions from the Long Beach and Los Angeles ports. The study incorporated both summer and winter measurements, and toxicological analysis was performed on selected samples. Markers for marine vessel emissions were determined. Traffic sources and secondary formation of UFP were major contributors to the PM, and

prevailed over ship emissions and oil combustion even in the community near the harbor. Considerable spatial variability in size distribution and particle number was observed over small, neighborhood-scale distances; this has important implications for exposure assessments conducted in the context of epidemiological studies.

A pilot study at the Los Angeles Airport was performed, and will be the basis for a more detailed study that will include toxicological analysis over the next two years. This pilot study, and other data collected by SCPC investigators detected high counts of UFP downwind of the airport. Measurements of black carbon and particulate phase PAH were not substantially different upwind and downwind of airport activities. Toxicological analysis of UFP and accumulation mode particles will be undertaken in a follow-up study to better characterize the potential public health relevance of offsite movement of aircraft particulate matter emissions.

Wildfires and intentional woodburning are important non-mobile sources of particulate matter in some parts of the US especially California. SCPC investigators found that water-soluble organic carbon was increased during a wildfire episode. The redox-generating activity, per unit mass, of the PM collected during this episode was elevated over background Los Angeles PM samples.

Project 2: SCPC's interdisciplinary research program on the mechanisms by which particulate matter contributes to cardiovascular morbidity and mortality involves toxicological studies in animal and in vitro model systems and an epidemiological study of oxidative stress biomarkers that is supported by extensive PM characterization to provide a variety of exposure metrics for analysis. Ambient fine and ultrafine particles at a near-freeway location were shown to promote atherosclerotic changes and increase systemic oxidative stress markers in a mouse model. These effects were more associated with ultrafine particles (UFP) than fine particles. In vitro studies of human microvascular endothelial cells are in progress to identify alterations in gene expression involved in the responses to cellular stress that may underlie the PM-induced atherogenesis that was observed in mice, and chemical reactivity studies seek to understand the biomolecular processes at work. Chemical composition of PM may explain the observed health effects

The SCPC is engaged in research on the mechanisms of allergic airways responses, pursuing the hypothesis that oxidative stress plays an important role. In a mouse model for the study of adjuvant effects of particles in promoting allergic sensitization UFP, given by instillation, promoted proinflammatory effects in the lung, epithelial changes and inflammatory cell influx. Immune system responses were skewed toward allergic response in UFP exposed animals. In addition, nasal mucosa changes were observed, providing a possible explanation for associations between PM and allergic rhinitis. The findings are being pursued by performing mechanistic studies in dendritic cells in vitro that characterize the specific pathological processes associated with exposure that may explain how these cells alter immune system responses associated with PM exposure.

The pathophysiological changes that result in PM-associated adverse health effects, such as oxidative stress and gene expression alterations, are being investigated in our epidemiological and animal toxicology studies. We are using genome wide association screening to elucidate gene clusters that can be used for bioassay development that reflect the impact of ambient fine versus UFP exposure in murine lung

tissue. We are currently using lungs collected during atherosclerosis studies with the apoE knockout mice and are the process of analyzing promising gene clusters that reflect the pro-the inflammatory effect of different particle sizes.

Project 3: To understand at a fundamental biochemical level how exposure to particulate matter can produce these changes, and to identify the components and sources that are responsible, the chemical reactivity properties of PM samples require investigation. To date, we have developed three assay procedures that can be applied to PM samples to assess their chemical reactivity and potential toxicity as predicted by the considerations above. The assays can detect the ability to generate redox active species, including organics and metals, and measure covalent modification of target proteins. The assays have been applied to different samples collected at sites in the Los Angeles basin. These assays have been used to characterize PM samples from a variety of sources and conditions of collection as part of project 1. Additionally, we have examined a set of diesel exhaust particles with these procedures and have found that the redox activity is associated with the particle fraction in an aqueous suspension and the electrophiles were soluble in the aqueous phase. In a second study of ambient air samples from Riverside, CA, we found that the redox activity was associated with particles and the electrophiles were mostly in the vapor phase which raises important issues about the role of vapor phase co-pollutants. These studies were enabled by sample collection as an element of **Project 5.** Reductive acetylation studies suggest that quinones or quinone-like compounds may be responsible for the redox and electrophilic activities that partition into the organic and aqueous extracts. These experiments provide a conceptual link between studies of the chemical composition of PM and studies of biological outcomes by attempting to identify the biologically active components of samples.

Project 4: A panel study of elderly individuals with coronary artery disease aims to identify exposure characteristics including particle size and composition that are associated with circulating biomarkers of oxidative stress. The study has found positive associations between a number of measures of particle composition/source and biomarkers of stress responses. Overall, the pattern of results suggests that exposure to primary emissions of UFP and organic carbon in this population may lead to sustained increased systemic inflammation, platelet activation and decreased antioxidant enzymes in erythrocytes. Interindividual variability in responses in the SCPC study was high; possible factors under investigation include statin use and genetic variability at the glutathione S-transferase M1 locus.

In conclusion, the SCPC has been especially successful in collecting and characterizing PM from a wide range of sources; this has been a central theme of the Center. In addition, we have begun to link airborne PM and vapor phase characteristics with toxicological investigations to further identify the underlying mechanisms of action of PM. This represents a unique approach to PM research and the development of assays to conduct such studies has represented a major contribution. We have demonstrated that both fine and ultrafine PM is capable of generating significant atherosclerotic lesions in susceptible mice. The Panel study being conducted by Dr. Delfino represents an innovative approach requiring small numbers of participants and has enabled development of linkages between toxicologic parameters and health considerations.

Overall, the Center has been dramatically successful in meeting the objectives set out in our two original applications. We have been successful in leveraging the U.S. EPA funds to obtain extensive funding from other sources, and our publications record is extraordinary. These results demonstrate the overall contributions of our Center.

Future activities:

In the remaining two years of funding, the SCPC will make further, detailed comparisons of the vapor phase co-pollutants and particles, in terms of physical, chemical and toxicological properties in relation to source, and will integrate this work with the efforts described above. Work will continue on improving technical methods for PM aerosol characterization and exposure studies. Attention will be given to using assays from Projects 2 and 3 to evaluate dose-response relationships. We will also focus on the temporal characteristics of PM emissions. Assays for chemical reactivity of PM samples are being adapted to high-throughput conditions, to enable application to a wider range of ambient and source-specific samples. Studies that further address the underlying mechanisms will be undertaken with reference to downstream signal transduction pathways for toxicity and the link with health outcomes. The animal studies on allergic airways sensitization will followed up with inhalation exposures to solidify the relevance of the observations to public health outcomes. Epidemiological analyses of oxidative response markers in elderly subjects with cardiovascular disease will continue, linking biomarkers of oxidative stress to aerosol properties. Genetic analysis of study subjects will be integrated into the overall study findings. These studies will have enormous significance for policy considerations. The integrated efforts of the SCPC overall will produce a strong body of literature, at the disposal of policy makers. The SCPC has been highly successful for nine years and has contributed unique findings to the field. We anticipate continuing to expand our interdisciplinary work in the future and provide new insights to the field.

Project Title: Project 1: Contribution of Primary and Secondary PM Sources to Exposure and Evaluation of their Relative Toxicity

Investigators: Constantinos Sioutas[1] and James J. Schauer[2]
Institutions: [1]University of Southern California Department of Civil and Environmental Engineering; [2]University of Wisconsin-Madison Department of Civil and Environmental Engineering

Objective of Research: The primary objective of Project 1 is to examine the relationships between PM sources, exposure, and toxicity within the constraints of the urban atmosphere. This project is an integral part of Projects 2, 3 and 4, by serving as the field operations to collect PM samples for toxicity testing and for providing elevated levels of ambient PM for animal exposure models described in these projects. Our major themes are:

1. Physical and chemical properties of PM emitted from different PM sources.
2. Determine the characteristics of the volatile and non-volatile particle components of these sources. Also provide in vitro samples to Projects 2- 4
3. Measure exposure gradients and intra-community variability of PM from complex, unstudied sources such as airports and port activities.
4. To assess the contributions of these outdoor sources to indoor exposure in support of Project 4.

Progress Summary/Accomplishments:

Over the course of the 20 months covered in this report, we carried out field sampling campaigns at the I-710, at the facilities of USC, LAX, and we completed our sampling campaign in Long Beach, CA. These studies were linked with Projects 2-4 and are described below:

Studies at the I-710

The I-710 freeway is a 26 m wide eight-lane highway connecting the ports complex of Long Beach and San Pedro to the shipping yards in East Los Angeles. For this reason, as much as 25% diesel traffic has been reported on this freeway. The I-710 study focused on particle characteristics next to the I-710 freeway and pursued the following concurrent activities:

a. Detail chemical analysis of coarse, fine + ultrafine, and ultrafine PM including major ions, EC, OC, trace metals and organic compounds
b. PAH and tracer concentrations and emission factors from the 710 and comparison to previous measurements in the Caldecott Tunnel
c. In Vitro collection of coarse, fine + ultrafine, and ultrafine PM to be used by Projects 2 and 3 of this center
d. Tandem DMA particle diameter/volatility measurements
e. Particle surface area measurements with TSI active PM surface monitor
f. Ultrafine mass chemical composition measurement

Activities a-f have been successfully completed and 8 manuscripts have been published, accepted or submitted for publication and provide details of these observations and analyses. In vitro PM samples have also been delivered to the investigators of Projects 2 and 3 at UCLA.

Summer Campaign at USC

This study was conducted at the University of Southern California's Particle Instrumentation Unit (PIU) located on the University Park campus near downtown Los Angeles, California. The study was conducted over 4 consecutive 5-day weeks from June 28 through July 27, 2006. The PIU is located within ~ 150 m of a routinely congested freeway (Interstate 110) and near construction and parking facilities. Here we focus on changes occurring at a traditional "source" site. Several different aerosol measurement techniques were used to characterize the physical and chemical properties of the aerosol.

Monthly averages of the data suggest the strong influence of commute traffic emissions on morning observations of ultrafine particle concentrations. By contrast, in the afternoon our measurements provide evidence of secondary photochemical reactions becoming the predominant formation mechanism of ultrafine aerosols. Measurements of the volatility of the ultrafine aerosol are consistent with this interpretation as overall volatility increases in the afternoon and there is less evidence of external mixing. (Moore et al 2007)

During the same campaign, we focused on daily variation of ultrafine (< 180 nm in diameter) particle chemical characteristics. Ultrafine particles (UFP) were collected weekly for two 3-hr periods each day. The relative abundances of alkanes, PAH, and hopanes in the morning denote a strong influence of commute traffic emissions on ultrafine particle concentrations. By contrast, afternoon concentrations of oxygenated organic acids and sulfate rose, while other species were diluted by increased mixing height or lost due to increasing temperature. These are clear indicators that secondary photochemical reactions are a major formation mechanism of ultrafine aerosols in the afternoon. The concentrations of organic species originating from vehicular emissions measured in this study compare favorably to those from freeway-adjacent measurements by using CO2 concentrations to adjust for dilution, demonstrating the effectiveness of this tool for relating sites affected by vehicular emissions. (Ning et al 2007)

Intra-community Variability Studies

One of the major themes of Project 1 is the measurements of exposure gradients and intra-community variability of PM from complex, unstudied sources such as airports and port activities. We conducted a pilot study in the area of Long Beach, CA to investigate the intra-community spatial variation of PM impacted by numerous local and regional sources. This study shows that, although PM mass in different size fractions is spatially homogeneous within a community, the spatial distribution of some elemental components can be heterogeneous. Epidemiological studies using only PM mass concentrations from central sites may not accurately assess exposure to toxicologically-relevant PM components. (Krudysz et al 2008a, 2008b).

Indoor – Outdoor Exposure Characterization Studies (with Project 4)

In collaboration with investigators in Project 4, we measured hourly indoor and outdoor fine particulate matter (PM2.5), organic and elemental carbon (OC and EC, respectively),

and particle number (PN), ozone (O3), carbon monoxide (CO), and nitrogen oxide (NOx) concentrations at two different retirement communities in the Los Angeles, CA, each during winter and summertime periods. This monitoring was part of a larger effort, the Cardiovascular Health and Air Pollution Study (CHAPS), which is also supported by the activities of Project 4. Overall, the magnitude of indoor and outdoor measurements was similar, probably because of the major influence of outdoor sources on indoor particle and gas levels. On average, 36% - 44% of measured indoor OC was composed of outdoor-generated primary OC. contributions of outdoor-generated SOA and primary OC to indoor OC and to demonstrate their importance in indoor environments. The outcomes presented here will be used by CHAPS investigators to determine the relationship between cardiovascular outcomes and hourly retirement community exposures by each resident to PM2.5 of indoor and outdoor origin. (Polidori et al 2007)

Los Angeles – Long Beach Port Studies

During our winter campaign (Arhami et al 2008), PM samples were collected concurrently at six sites for a 7-weeks period between February and May 2007. Four sites were set-up within the communities of Wilmington and Long Beach; one site was located at a background location near the harbors of the Los Angeles port; the sixth site, near downtown Los Angeles, was chosen to represent a typical urban area. Coarse (PM2.5-10), accumulation (PM0.25-2.5), and quasi-ultrafine (PM0.25) mode particles were collected at each site. Vehicular emissions from the nearby traffic were the most prominent anthropogenic sources of both quasi-ultrafine and accumulation mode PM in all port sites (except at the background site located upwind, where ship emissions were the dominant PM source). Overall, traffic sources were major contributors to the PM and organic matter mass, and prevailed over ship emissions and oil combustion even in the studied harbor community. (Arhami et al 2008)

In collaboration with investigators from projects 2-3, we have also examined the ability of PM in that area to induce oxidative stress (Hu et al 2008). The formation of reactive oxygen species (ROS) in cells exposed to particulate matter (PM) results in oxidative stress, which is an important mechanism associated with many adverse health effects caused by PM exposure. The results confirmed our earlier observations that OC (mostly from motor-vehicle emissions) is the most important component influencing the DTT consumption by PM samples. The variability of macrophage ROS was better explained by variations in OC concentrations and water-soluble vanadium (probably from ship emissions – bunker oil combustion). (Hu et al 2008)

In order to better understand and quantify intra-community variability in UFP concentrations in the complex area of the LA- Long Beach port, a dense network of 14 monitoring sites was set-up in Los Angeles in two clusters – San Pedro/Wilmington and West Long Beach – in communities surrounding the Ports of Los Angeles and Long Beach. The intra-urban variability observed in this study is comparable to and exceeds the inter-urban variability observed in a previous study in Los Angeles. UFP concentrations can vary considerably on short spatial scales in source-rich environments, which can strongly influence the accuracy of exposure assessments (Moore et al 2008). In addition to the particle number concentration measurements described above, we

conducted concurrent particle size distribution measurements in an effort to identify UFP sources and types in the LA – Long Beach port area, while providing data to investigate local scale effects of both photochemical and physical processes on UFP. Spatial heterogeneity exists between "background" and "source" sites especially for particles < 40 nm, while spatial homogeneity is observed between geographically close sites. In addition, variations in r-values as a function of particle size are not necessarily consistent with corresponding CODs values, indicating that using results from correlation analysis alone may not accurately assess spatial variability. This study is described in detail by Krudysz et al (2008b).

Los Angeles Airport (LAX) Pilot Study

During this period, we also completed the data analysis and publication of our first pilot studies at LAX (see Westerdahl et al 2008). Air monitoring was performed in the vicinity of the Los Angeles International Airport (LAX) during the spring of 2006. The particle numbers at the upwind site were dominated by particles of approximately 90 nm diameter while downwind sites were dominated by particles peaking at approximately 10-to-15 nm. Additional data obtained from a study of UFP levels conducted subsequently by a co-author indicates that aircraft-generated UFP persist up to 900 meters from an LAX runway (Biswas et al 2007). Considered together, these observations suggest that airport operations are associated with elevated levels of UFP much further downwind in the neighboring community than would have been predicted by prior studies of UFP from roadway-traffic. Based on these results, we intend to conduct at a later time more detailed studies in that area, focusing this time on the collecting of ultrafine and accumulation mode particles for chemical and toxicological analysis in the same locations of the Westerdahl et al (2008) study.

Reconciling Emission Factors of PM Species Emitted by Vehicles in Freeways and Roadway Tunnel Environments

In addition to our tunnel-based emission factors for light and heavy duty vehicles, we estimated emission factors of various particle species from light and heavy duty vehicles (LDVs and HDVs, respectively), including organic and elemental carbon (OC and EC,), sulfate, polycyclic aromatic hydrocarbons (PAHs), hopanes, steranes, trace metals, elements, and particle number (PN), based on roadway measurements (Ning et al 2008). Sampling campaigns were conducted at two different roadways: the CA-110 highway (where only gasoline-powered vehicles are allowed), and the I-710 freeway (where about 20% of the total number of vehicles are diesel-powered trucks). The PM emission factors determined in these roadways were compared to those reconstructed from recent source emission data from the Caldecott tunnel (Phuleria et al 2006), and those from previous tunnel and chassis dynamometer studies. Very good agreement between estimated and reconstructed emission factors was found. This suggests that PM speciated chemical data collected at roadsides can be used to calculate reliable emission factors for several important particulate species at other locations, characterized by a similar mix of on-road motor vehicles. Thus, it may be plausible to estimate the concentration of organic tracers next to the freeway by averaging emission profiles of different vehicles and driving cycles measured on a dynamometer if the dilution factor (typically calculated based on CO2 concentrations) between dynamometer and the freeway is known or can be

determined. (Ning et al 2007). Based on these roadway measurements and average driving time, it appears that 33–45% of total UFP exposure for Los Angeles residents occurs due to time spent traveling in vehicles. (Fruin et al 2008)

Physicochemical and toxicological properties of Particulate Matter from October 2007 Southern California wildfires (collaboration with Project 3 and Dr. Flemming Cassee)
Wood smoke is one of the important sources of PM whose chemistry and toxicity we proposed to investigate. On October 20, 2007, 23 large wildfires ranging from Santa Barbara County to the U.S.– Mexico border, burned more than 500,000 acres of land and destroyed over 1,500 homes with the largest mandatory evacuation (of over 900,000 people) in the state's history. During this period, we conducted measurements of various particle and gaseous pollutants such as CO, nitrogen oxides, O3, PM2.5 (particles with an aerodynamic diameter less that 2.5 μm) mass with its chemical constituents, and size fractionated particle number, measured at a site near the University of Southern California (USC). Detailed chemical and toxicological characteristics have been evaluated from the PM samples collected during the fire event, and compared to those after the October 2007 fire period (when outdoor PM2.5 is mostly impacted by the vehicular traffic emissions). We showed that concentration of water-soluble organic carbon (WSOC) and several organic tracers including levoglucosan were increased by 2-3 fold during the fire period. The per mass PM toxicity expressed in nmoles of DTT consumed per μg of PM and min was also higher for the samples collected during the wildfire episode compared to the PM samples impacted by traffic suggesting that wood smoke on a per PM mass basis is a significant source of PM that are redox active. (Verma et al 2008).

Health Studies – collectively with Project 2 :***The Role of Oxidative Stress in the Susceptibility to PM-Induced Adverse Health Effects***
During this period covered by this report, we have assisted the investigators of project 2 to conduct exposures of genetically susceptible (apoE-deficient) mice to well characterized (physically and chemically) fine and ultrafine CAPs. Following up on the results of the Campbell et al (2008) study, we have further explored the evidence that the brain may also constitute a site adversely effected by the environmental presence of airborne particulate matter by examining the association between exposure to PM and adverse CNS effects in apolipoprotein E knockout (ApoE-/-) mice exposed to two levels of concentrated ultrafine particulate matter in central Los Angeles (USC) using our concentrators. The study provided clear evidence of aberrant immune activation in the brains of exposed animals as judged by a dose-related increase in nuclear translocation of two key transcription factors, NF-kB and AP-1. In order to determine the mechanism by which these events occurred, levels of several MAP kinases involved in activation of these transcription factors were assayed by Western blotting. The results suggest that the signaling pathway by which these transcription factors are activated involves the activation of JNK. (Kleinman et al 2008)

Development of high collection efficiency electrostatic precipitator for in vitro cell exposure to concentrated ambient particulate matter (PM)- projects 2-3

In several discussions with ESAC members (Drs. Flagan, McMurry, Russell and Cassee) over the past year and during our previous meetings, we entertained the idea of utilizing electrostatic precipitation for on-line collections of PM for toxicity studies. To that end, we developed a new sampling system to collect ambient particles for in-vitro studies (Sillanpaa et al 2008). The in-vitro electrostatic collector consists of two units: a Versatile Aerosol Concentration Enrichment System (VACES) and a new design of electrostatic precipitator (ESP). This system allows for shorter sampling durations, which benefits cells that might not be viable after prolonged exposure to the sampling environment. Shorter time interval sampling also enables discovery of possible relationships between health effects and time of day or short-term source emissions. The main advantage of in-vitro electrostatic collector is that it collects >95% of particles - regardless of particle type or diameter - directly to a target area, on which a cell culture can be placed. The unique design implements two metal needles to facilitate a corona discharge, resulting in high particle charging efficiency with little ozone production. These advantages make the in-vitro electrostatic collector a viable in-vitro cell exposure technique.

Novel Zero- Ozone Unipolar Charging of Ultra-fine Aerosol Particles using Carbon Fiber Ionizers

Expanding further our technology and use of ESPs for in vitro PM collections, we replaced the unipolar corona discharge charger of our ESP with a simple and novel unipolar charger using carbon fiber ionizers (Han et al 2008a). This charger was developed to effectively charge ultra-fine aerosol particles without the generation of ozone. The newly developed unipolar charger using carbon fiber ionizers can charge ultra-fine particles at least as effectively as currently available unipolar chargers, but with the major advantage of negligible ozone generation, a highly desirable feature if the charged particles are to be used for chemical or biological analysis. (Han et al 2008b)

Health Studies with Project 3*: *Chemical Reactivity of Particulate Matter Sources, and Modification of Chemical and Cellular Properties of PM-Associated Components by the Particle Matrix

In a collaborative study with investigators from projects 3 and 4, we have collected using our VACES fine and ultrafine PM in the area of Riverside and in one of the retirements homes of the subjects of the study in Project 4, to assist our colleagues in their efforts to develop an analytical procedure to detect electrophiles that can be applied to the study of air pollution samples, using the active site of the thiol enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (EC 1.2.1.12) as the target nucleophile.

Health Studies with Project 4*: *Oxidative Stress Reponses to PM Exposure in Elderly Individuals with Coronary Heart Disease

In collaboration with investigators in Project 4, we explored the association between circulating biomarkers of inflammation, antioxidant activity, and platelet activation with primary combustion and secondary aerosols among the study subjects of that project. Our results suggest traffic emission sources of primary OC and quasi-ultrafine particles lead to increases systemic inflammation and platelet activation, and decreases in antioxidant enzyme activity in elderly people with coronary artery disease. (Delfino et al 2008b)

Publications/Presentations:

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2. Arhami M., Sillanpää M., Hu S., Geller M.D., Schauer J.J. and Sioutas C. "Size-segregated inorganic and organic components of PM in the communities of the Los Angeles Harbor across Southern Los Angeles Basin, California." Submitted for publication to Aerosol Science and Technology, January 2008.

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Future Activities:

In the next year of our activities, we will continue our efforts towards the completion of the following activities:

1. Use the Chemical Mass Balance (CMB) model and our organic tracers' data to do source apportionment in the LA-Long Beach harbor sites for PM2.5 as well as for ultrafine particles. We will also examine the seasonal variability of these chemical species and the differences in the contribution of sources between winter and summer seasons.
2. Our Ning Z. et al, Environmental Science and Technology, 41 (17),6000-6006, 2007 paper identified clearly two distinctly different time periods in the summertime at USC; once impacted by vehicular emissions and the other by secondary formation processes. We will conduct high volume collections (order of 10 mg) of ultrafine and accumulation mode PM during these two periods at USC for in vitro toxicological analysis, thus coupling the chemical work performed by our group previously with toxicological outcomes. These samples will be used by investigators in projects 2 and 3 and will serve as pilot data to a more ambitious study (the trajectory study) that we intend to carry out during the 4^{th} year of our program.
3. We will thus attempt to address the limitations of current state-of-the-art in ultrafine particle charging discussed above by coupling the Versatile Aerosol Concentration Enrichment System (VACES) with the unipolar charger using carbon fiber ionizers that we developed (Han et al 2008a). Our goal is to prove that this is a promising alternative for particle charging, especially in applications involving sampling and collection of atmospheric aerosols, often rich in organic compounds, which could be degraded by reactions with ozone and other radicals produced by corona chargers.
4. Exposure assessment analysis in support of the health investigations of Project 4, which is described in more detail in the summary for Project 4.

Project Title: Project 2: The Role of Oxidative Stress in PM-induced Adverse Health Effects

Investigators: Andre Nel[1], Jack R. Harkema[2], Mike Kleinman[3], Aldonis Lusis[4]
Institutions: [1]University of California, Los Angeles Department of NanoMedicine; Michigan State University Department of Pathology and Diagnostic Investigation; University of California Irvine Department of Community and Environmental Medicine; [4]University of California, Los Angeles Department of Molecular Biology

Objective of Research: The primary objective is to elucidate the mechanism(s) of PM-induced asthma and atherosclerosis exacerbation in vitro and in vivo. This is accomplished by animal studies in the mobile trailer in downtown Los Angeles as well as in vitro studies in representative tissue culture cells. The principal hypothesis is that a major PM injury mechanism is the induction of ROS production and oxidative stress that promotes respiratory and cardiovascular inflammation as the major pathology feature underlying asthma and atherosclerosis (Nel 2005; Xia et al 2006a, 2006b; Nel et al 2006). We propose that oxidative stress is a hierarchical event in which the induction of antioxidant defense pathways in tier 1 is in dynamic equilibrium and defends against the pro-inflammatory (tier 2) pro-apoptotic (tier 3) effects of higher levels of oxidative stress (Nel et al 2006).

Progress Summary/Accomplishments:

ApoE knockout mice exposed to CAPs on the freeway shows increased atherogenic potential of ultrafines (Araujo et al 2008)

Epidemiological studies unveil that exposure to ambient PM increases cardiovascular morbidity and mortality. Both epidemiological and animal-based studies suggest that if the exacerbation of atherosclerosis plays an important role in these outcomes. We hypothesized that PM synergizes with known pro-atherogenic stimuli and mediators in their ability to elicit oxidative stress and promote atherosclerosis, and that most of the pro-inflammatory potential resides in the ultrafine particles (aerodynamic diameter <0.1 μm, UFP) that are highly enriched for redox cycling PM chemicals.

We conducted two experimental protocols (Araujo et al 2008). In the first (chow protocol), 6-week-old male C57BL/6J apoE null mice were placed on a chow diet and exposed to CAPs over a 40-day period, while in the second study (high fat diet protocol), 2-month-old male apoE null mice were fed a HFD over a 56-day period. Food and water were administered *ad libitum*. Mice destined for CAPs exposure were transported to the mobile research laboratory in downtown of Los Angeles, close (~300 m) to the I-110 freeway. The animals were housed in a Hazelton Chamber ventilated with air from which 99.9% of the incident particles were removed using a HEPA filter (chow protocol) or in top-filter cages (HFD protocol). There were three exposure groups (17-18 mice/group), namely filtered air (FA), particles < 2.5 μm (FP) and particles < 0.18 μm (UFP). Whole body exposures were performed simultaneously for five hours per day (exposure session), three days per week, for a combined total of 75 and 125 hours in the chow-fed and HFD-fed protocols, respectively. Animals were euthanized 24-48 hours after completion of the last CAPs exposure, and aortas and various organs harvested.

Chow-fed apoE null mice exposed to concentrated ultrafines developed significantly ($p<0.05$) larger early aortic atherosclerotic lesions (33011 +/- 3741, n=15) than animals exposed to $PM_{2.5}$ (26361 +/- 2275, n= 16), filtered air (21362 +/- 2864, n= 14) or left non-exposed (17261 +/- 1659, n= 17) (Araujo et al 2008). Exposure to ultrafine particles resulted in an inhibition of the anti-inflammatory capacity of plasma high density lipoproteins and increased systemic oxidative stress markers as evidenced by a significant: (i) increase in hepatic malondialdehyde levels, (ii) upregulation of Nrf2-related phase-2 response genes (e.g., catalase, superoxide dismutase) when compared to FA or NE mice (Nel et al 2005). While HFD-fed apoE null mice did not exhibit differences in their aortic atherosclerosis, they still show evidence of increased systemic oxidative stress as evidenced by significant upregulation of Nrf2 and Nrf2-regulated genes.

Genome-wide analysis screening in endothelial cells revealed synergistic cellular stress responses during exposure to organic DEP extracts and oxidized LDL components (Gong et al 2007).

We used human microvascular endothelial cells (HMEC) to test the hypothesis that pollutant particles synergize with known pro-atherogenic stimuli and mediators in their ability to elicit oxidative stress and promote atherosclerosis (Gong et al 2007). We studied the combined effects of a model air pollutant, diesel exhaust particles (DEP), and oxidized 1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phosphorylcholine (ox-PAPC) on genome-wide gene expression. HMEC were treated in triplicate wells with an organic DEP extract (5 μg/ml), ox-PAPC (10, 20 and 40 μg/ml) or combination of both compounds for 4 hours. Gene expression profiles were assessed by Illumina microarray technology. We found that ox-PAPC as well as DEP regulated a large number of genes (up-hand down-regulated) in a dose-dependent fashion (Gong et al 2007). More importantly, a marked degree of co-regulation was present where the combined action of DEP and ox-PAPC resulted in a different effect than DEP and ox-PAPC alone. All together, 1555 genes were significantly upregulated (> 1.5 fold, $p < 0.05$) by the three DEP and ox-PAPC combinatory treatments. Notably, some genes were uniquely regulated by ox-PAPC and not by DEP, and *vice versa*, some genes were regulated by DEP but not by ox-PAPC.

We used weighted gene co-expression network analysis (WGCNA) to identify 12 modules of densely interconnected genes that were given unique color codes (Gong et al 2007). We found three modules (Brown, Green and Yellow) that were most highly enriched in genes that were differentially regulated by the stimuli. Interestingly, all these three modules exhibited patterns of additive/synergistic interaction where the combined action of DEP and ox-PAPC resulted in a greater effect than each compound alone. We developed a novel synergistic index that allows us to differentiate in between additive effects and synergistic effects. Conceptually, we defined synergy as a response to DEP plus ox-PAPC that was greater than the effects induced by either compound alone and greater than the summation of those individual effects. Interestingly, the brown, green and yellow modules concentrated 83% of the synergistically expressed genes identified in the gene network. These three modules were also enriched in synergistically coregulated

genes and pathways relevant to vascular inflammation. We validated our gene expression data by quantitative PCR (qPCR) in the same set of samples analyzed by microarray analysis and in a set of samples from an independent experiment. Representative genes from various pathways were selected including ARE-regulated genes [e.g. HO-1, selenoprotein S (SELS)], inflammatory response genes [e.g. Interleukin 8 (IL-8), chemokine (C-X-C motif) ligand 1 (CXCL1)], immune response genes [e.g. Interleukin 11 (IL-11)], UPR genes [e.g. ATF 4, heat shock 70kDa protein 8 (HSPA8), X-box binding protein 1 (XBP1)], oxygen and reactive oxygen species metabolism genes [e.g. dual specificity phosphatase 1 (DUSP1), PDZ and LIM domain 1 (PDLIM1)]. qPCR could confirm 91 % of the synergistic effects that were revealed by microarray technology (Gong et al 2007). All considered, the synergistic response profile represents a combination of hierarchical oxidative stress as well as protein unfolding response genes.

We validated this synergy on selected genes in vivo by demonstrating that liver gene expression of hypercholesterolemic mice (HFD protocol from section 1) exposed to ambient ultrafine particles exhibited significant upregulation of the module genes (Gong et al 2007). Indeed, liver tissue was assayed for mRNA expression of HO-1, as well as two key UPR transcription factors, XBP1 and ATF4. UFP-exposed animals exhibited a significant up-regulation ($p < 0.05$) of all three genes in comparison with FP, FA and NE mice. These results indicate that the synergistic effects predicted by our in vitro studies have important in vivo outcomes, in which pro-oxidative PM chemicals may gain access to the systemic circulation from the lung and may then be able to synergize with circulating ox-LDL.

Progress on studies looking at the effect of the pro-oxidative potential of ambient particles on their adjuvant effect on allergic inflammation in a murine asthma model.
Although studies have suggested that ambient PM can act as an adjuvant to promote sensitization to common environmental allergens, there is a paucity of direct evidence showing this effect on allergic sensitization in vivo. We have developed a mouse model, which allows us to demonstrate the adjuvant effect of ambient ultrafine particles (UFP) on ovalbumin (OVA)-induced allergic sensitization in vivo. Intranasal sensitization of Balb/C mice with a low dose of endotoxin-free OVA (10 μg) in the presence of ambient PM followed by OVA (1%) aerosole challenge resulted in a significant increase of allergic inflammation compared to the saline control or OVA alone. At a dose of 0.5 μg/mouse, UFP significantly enhanced OVA-induced eosinophil infiltration, airway inflammation, and serum OVA-specific IgE and IgG1 production. UFP also increased the production of a number of pro-inflammatory cytokines in the lung. Cytometric bead array anaysis of BAL showed that while the animals in OVA/UFP group had significantly enhanced production of TNFα, IL-5, IL-6, IL-13, KC, MCP-1 and MIP-1α, those in other groups (OVA alone, LPS plus OVA, or F/UF plus OVA) were unaffected. The increase of IL-5 and IL-13 by OVA/UFP is important indicator suggesting that ambient UFP be capable of skewing immune response towards Th2 immunity.

Using different controls, we were able to show that neither endotoxin nor ultrafine carbon black particles had any enhancing OVA sensitization. Moreover, side-by-side comparison

of UFP and FP indicate that this adjuvant effect is specific to the UFP since FP failed to enhance the effect of OVA. This approach could allows us to compare the contribution of particle size and accompanying differences in the pro-oxidative potential of PM2.5 and UFP in an in vivo model, similar to what we have previously demonstrated in tissue culture cells (Li et al, Environ. Health Perspect. *111*:455-460 ; 2003). To provide further evidence that the adjuvant effect of UFP correlates to its redox –active organic chemical contents, we characterized the ambient PM for their chemical composition, redox potential, and the ability to induce intracellular oxidative stress. Ambient UFP consistently had higher organic carbon content compared to the fine PM. DTT assay, which measures the redox activity of ambient PM based on the interaction between quinones and DTT, showed that ambient UFP had much stronger oxidant potential than the fine particles. Moreover, ambient UFP also had a greater ability to induce antioxidant enzyme HO-1, a sensitive marker of oxidative stress. Taken together, these data suggest that the degree of adjuvancy is related to the OC content and redox potential of PM.

Morphometric analysis of the lung showed that the major changes in the lungs of OVA/UFP-treated mice consisted of marked mucous cell metaplasia in the surface epithelium lining the conducting airways (large- and small-diameter bronchioles) and an associated mixed inflammatory cell influx consisting mainly of eosinophils, lymphocytes and plasma cells in the interstitial tissues surrounding these airways. Airway lesions were most severe in the main axial airways, but were also present to a slightly lesser degree in the small-diameter, terminal bronchioles of the mice exposed to both OVA and UFP. Along the axial airways, the volume densities of intraepithelial mucosubstances in the proximal and distal generations (5 and 11) were approximately 22 and 24 times greater, respectively, than those measured at the same airway generations in saline-instilled control mice. Mice exposed only to OVA had epithelial and inflammatory alterations that were similar to those in the OVA/UFP mice, but the severity of these changes in the large-diameter, pre-terminal and small-diameter, terminal bronchioles were must less severe. As a comparison, the morphometrically determined volume densities of mucosubstances in the airway epithelium lining the proximal axial airways (generation 5) of OVA/UFP mice were approximately twice as much as those in mice exposed only to OVA. In the distal axial airway (generation 11), OVA/UFP mice had almost five times more intraepithelial mucosubstances compared to those in OVA-alone mice.

In addition to the lower airway, we have investigated changes in the nasal mucosa. Nasal histopathology and morphometry showed that mice exposed to both OVA and UFP had airway epithelial and inflammatory changes consistent with an acute allergic rhinitis. The principal morphologic alterations were mucous cell metaplasia/hyperplasia of airway epithelium accompanied by inflammatory cell influx including eosinophils and mononuclear cells (lymphocytes and plasma cells) in the underlying lamina propria of the nasal mucosa. These changes were restricted to intranasal regions lined by transitional or respiratory epithelium. No alterations were present in regions lined by olfactory epithelium. There was a markedly greater amount of mucosubstances in the nasal transitional epithelium lining the maxilloturbinates compared to the mice in the control or OVA-alone groups. These data suggest that UFP exert its adjuvant effect immediately

upon contact with the allergen in the nasal turbinate and this may explain the association between air pollution and allergic rhinitis.

Taken together, these results suggest that intranasal OVA delivery caused minimal allergic airway, epithelial and inflammatory responses in the lungs and nose of mice without concomitant intranasal UFP instillation. Thus, intranasal exposure to UFP exerts an adjuvant effect that could not be obtained with intranasal fine PM. The results are currently being written up for publication and will serve as the launching pad for carrying out in vivo inhalation exposure studies that will attempt to show that CAPs, UFP in particular, can lead to similar sensitization.

Exposure to pro-oxidative DEP chemicals perturb the antigen-presenting function of dendritic cells, which may explain the adjuvant effect of PM in asthma (Chan et al 2006; Gilmour et al 2006; Phalen et al 2006).

Dendritic cells (DCs) play a key role in antigen presentation in the immune system. There is growing evidence that the redox equilibrium of DCs influence their ability to induce T-cell activation and to regulate the polarity of immune response. Ambient PM acts as an adjuvant that promotes sensitization to common environmental allergens. Systematic dissection of the molecular pathways of PM-induced adjuvancy is of great general interest and it is also a key priority in the research of asthma and allergy (Gilmour et al 2006). We are studying the hypothesis that altered cellular redox equilibrium by PM and adsorbed redox cycling organic chemicals leads to the perturbation of DC function and favors Th2 skewing of the immune response. In year 2 we investigated how DEP chemical-induced oxidative stress interferes with DC function including maturation, antigen uptake, antigen presentation, expression of costimulatory molecule, cytokine/chemokine production, and T-cell activation (Chan et al 2006). DCs were prepared from mouse bone marrow cells. Exposure of DCs to organic DEP extract (DEP_{ext}) resulted in a dose-dependent glutathione depletion and the induction of antioxidant enzyme, heme oxygenase-1 (HO-1) (Chan et al 2006).

Although DEP chemicals per se did not change the expression of DC surface molecules ($I\text{-}A^d$, CD54, and CD86), DEP_{ext} was able to suppress LPS-induced expression of these molecules in a dose-dependent fashion. Similarly, while DEP_{ext} alone failed to exert an effect on IL-12p40 and IL-12p70 production by DCs, it suppressed the LPS-induced production of this cytokine (Chan et al 2006). The inhibitory effects of DEP_{ext} on LPS-induced CD86 expression and IL-12 production could be neutralized by thiol antioxidant, N-acetyl cysteine, indicating the involvement of oxidative stress.

Using $CD4^+$ T cells from a T-cell receptor transgenic mouse strain (DO11.10) that recognizes $OVA_{323\text{-}339}$ in the context of the BALB/c MHC class II ($I\text{-}A^d$), we demonstrate that simultaneous exposure of DCs to DEP_{ext} and LPS induced a significant decrease in IFN-γ production compared with that in the cells treated with LPS only. Furthermore, exposure of DCs to DEP_{ext} alone, before antigen pulsing, significantly increased IL-10, a Th2 cytokine, production in the co-cultured T cells. In addition to inhibiting LPS effect (TLR4), organic DEP chemicals also suppressed CD86 expression induced by TLR2, TLR3, and TLR9 agonists suggesting that TLRs may be targets of DEP chemicals (Chan

et al 2006). Using BMDCs from Nrf2-deficient mice, we show that Nrf2 is required for the suppression of LPS effects by DEP chemicals. We demonstrate that DEP_{ext} inhibits LPS effects on DC by interfering with NFκB signaling pathway. The findings from the our second-year studies indicate that organic DEP chemicals indeed alter the redox equilibrium in DCs and that oxidative stress does interfere with several DC functions leading to the suppression of Th1 response (Chan et al 2006). Our studies also indicate that Nrf2-mediated phase II response and NF-κB signaling pathway play keys roles in modulating DC function under conditions of PM-induced oxidative stress.

The adjuvant effect at the level of DC could play out in select sites of mucosal immunity in the lung. Utilizing tracheobronchial particle dose considerations, we were able to demonstrate that it is theoretically possible to achieve the in vitro levels of oxidative stress that are described in the aforementioned paragraph at so-called hotspots of deposition in the lung (Phalen et al 2006). These deposition sites that are located at point of airway bifurcation could be the mucosal areas where the allergen as well as the inhaled particles come together to drive adjuvant effects in the immune system.

Proteome analysis of the oxidative stress responses in a bronchial epithelial cell line BAL Fluid (Jung et al 2007).

We have previously demonstrated that organic redox cycling diesel exhaust particle (DEP) chemicals induce a tiered oxidative stress response in a macrophage cell line as determined by proteome analysis (Xiao at al; J Biol Chem, 278:50781-50790; 2003). In collaboration with Dr. Joseph Loo from the Keck Proteomics facility at UCLA, a 2D-difference gel electrophoresis (DIGE) technology was used to demonstrate that organic DEP extracts induce a pro-inflammatory response in a BEAS-2B human bronchial epithelial cell line (Jung et al 2007). The DIGE method uncovered induction of an unfolding protein response (UPR) that is characterized by increased IL-6 and IL-8 production in parallel with increased expression of Hsp70, HSF-1, and ATF4 (Jung et al 2007). The UPR pathway is activated under stress conditions in order to govern the protein processing and/or folding in the ER. These results corroborate the demonstration of a protein unfolding response by the gene clustering analysis that was performed in endothelial cells as well as the gene response profile in the livers of apoE mice exposed to concentrated air pollution particles as reported above.

The identification of global protein expression changes in BAL fluid (BALF) and lung tissue from ovalbumin (OVA) sensitized mice could provide new insights into the complex molecular mechanisms involved in asthma. We are using two dimensional polyacrylamide gel electrophoresis (2D-PAGE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify significantly increased protein expression in a murine asthma model and potential protein targets of N-acetylcysteine (NAC) in allergic airway inflammation. Six proteins were found to be significantly increased in BALF from OVA-challenged Balb/c mice compared to a control group: chitinase 3-like protein 3 (Ym1), chitinase 3-like protein 4 (Ym2), acidic mammalian chitinase (AMCase), pulmonary surfactant-associated protein D (SP-D), resistin-like alpha (FIZZ1), and haptoglobin α-subunit. A total of 11 protein spots on 2D-gels were significantly increased in lung tissue from the murine asthma model, including Ym1, Ym2, FIZZ1,

and other lung remodeling related proteins. Western blotting confirmed increased Ym1/Ym2, SP-D, and FIZZ1 expression measured from BAL fluid and lung tissue from OVA-challenged mice. Administration of NAC intraperitoneally before OVA inhalation inhibited Ym1/Ym2, SP-D, and FIZZ1 expression from BALF and lung tissue. Proteins Ym1/Ym2, FIZZ1 and SP-D identified in this study could be associated with the pathogenesis of asthma and suggest a link between oxidative stress-induced inflammation and asthma. A manuscript reporting these findings upon completion of the immunohistochemical analysis is being prepared.

Conclusions: All considered, our data indicate that oxidative stress indeed plays a major role in driving inflammatory responses in the respiratory and cardiovascular systems. We have developed study methods and tools that are capable of following these oxidative stress effects under abiotic and biotic conditions, with the capability of linking that to specific PM chemical components such as the OC fraction. We are capable of using predictive in vitro models to link the oxidant injury to pathological processes in vivo, in particular atherosclerosis and asthma. These data are being integrated in the center with studies source and aerosols studies as well as ongoing research in humans in order to develop a comprehensive understanding of the role of oxidant injury in PM-induced adverse health effects.

Publications/Presentations:

1. Araujo, J. A., Barajas, B., Kleinman, M., Wang, X., Bennett, B.J., Gong, k.W., Navab, M., Harkema, J., Sioutas, C., Lusis, A.J., and Nel, A.E. Ambient particulate pollutants in the ultrafine range promote early atherosclerosis and systemic oxidative stress. Circulation Research. 102:589-96, 2008.

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9. Xia, T., Kovochich, M., and Nel, A. The role of reactive oxygen species and oxidative stress in mediating particulate matter injury. Clin. Occup. Environ. Med. Exposure to airborne particles: health effects and mechanisms. 5:817-826, 2006b.

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Invited Presentations:

1. Invited Symposium Speaker at the American Thoracic Society International International Convention, May 2007, San Francisco, CA. Symposium: "Mechanisms of Particulate Matter Induced Mortality". Title: "Proteomic approaches to particulate matter induced toxicity."

2. Invited Speaker/Visiting Professor, Feinberg School of Medicine, Northwestern University, Chicago, IL, June 2007. Title: "Air Pollution Health Effects: An Understanding of the Role of Oxidative in Heart and Lung Disease"

3. Invited Speaker/Visiting Professor, Keck School of Medicine, University of Southern California, Los Angeles, CA, June 2007. Title: "Air Pollution Health Effects: An Understanding of the Role of Oxidative in Heart and Lung Disease"

4. Invited Plenary Speaker, AAAAI International Convention, February 2007, San Diego, CA, Plenary Session, "Immunopathogenesis of Asthma," Title: "Role of Oxidative Stress by Particulate Air Pollution in Asthma Exacerbations."

5. Invited Speaker, AAAAI International Convention, March 2008, Philadelphia, PA, Symposium, " How do Air Pollutants Promote Airways Inflammation and Contribute to Asthma Morbidity?" Title: "How Does Composition of Air Particulates Cause Airways Injury and Promote Allergic Sensitization?." "Gene, Environment, and Disease Panels", for the US-Japan 6. Cooperative Medical Science Program that is administered by the NIH, San Francisco, CA, March 27-28, 2008. Also functioned as a speaker in the session, Oxidative Stress and Human Disease. Title: "The implications of the hierachical oxidative stress hypothesis for cardiorespiratory disease".

6. Invited speaker and Session Chair, 11th International Inhalation Symposium (INIS), 2008, Fraunhofer ITEM, Hannover, Germany. Title: "Nanoparticle-bio-interactions: controlled design and cellular response".

Future Activities:

1. We will continue the collaboration with Dr. Jake Lusis and Jesus Araujo using the genome wide association screening (GWAS) to elucidate gene clusters that can be used for bioassay development that reflect the impact of ambient fine versus UFP exposure in murine lung tissue. We are currently using lungs that were collected during the performance of atherosclerosis studies with the apoE knockout mice and are the process of analyzing promising gene clusters that reflect the pro-the inflammatory effect of different particle sizes. These studies will be supplemented by further animal exposures in the mobile laboratory as well as using the lung tissue for immunohistochemistry and proteome analysis. We are looking to establish *in vivo* biomarkers for oxidative stress that can be used in air pollution research. We will also use BAL fluid from our OVA adjuvancy model to screen for potential biomarkers of oxidative stress through the use of proteome profiling.

2. We will continue to work on the OVA adjuvancy model including performing studies that will investigate the role of oxidative stress at the level of antigen presenting cells as outlined in our studies using dendritic cells and diesel exhaust particle extracts in year two. We will determine whether *in vivo* inhalation exposure the same outcome and whether bronchial epithelial cells contribute to modification of dendritic cell behavior in vitro and in vivo. These studies will be conducted in collaboration with Project 1, Dr Harkema and Dr Kleinman.

3. In collaboration with Project 3, Project 1 and Dr William Hinds, we will attempt to develop online instrumentation that can be used to monitor the oxidant potential of ambient UFP as a metric for their biological potency. This study is premised on the DTT assay that Dr. Art Cho and Dr. John Froines have previously developed to measure PM oxidant potential based on thiol chemistry. These studies that will be conducted by a new postdoctoral fellow, Dr. Allen Haddrell, who proposes to design and build instrumentation with the ability to monitor the oxidative stress potential of a given sample of UFP in near real time. The proposed operation of the device is as follows: Particles are pulled from the air via a vacuum, once in the system the particles are charged via a corona discharge. The charged particles are then directed into an AC levitation trap where they can be held indefinitely. The levitated particles are subsequently introduced to a primary thiol in the vapor phase. The particles oxidize the thiol vapor, generating thiol radicals that are ionized with a deep UV laser. The thiol ions are ejected from the levitation chamber into an ion trap where they are counted. The number of thiol ions produced is used to predict the oxidative potential of the particles held within the trap. Through the use of standardized materials, the data will be converted to report the oxidative stress potential of the suspended particles in a given fraction of air rather than simply reporting the absolute number.

4. In collaboration with Drs Delfino, Araujo, and Lusis, we will begin the work on Aim 3 that proposes to use serum samples collected from indoor exposed elderly human subjects with ischemic heart disease in Project 4 to determine how oxidation of HDL

affects the anti-inflammatory and anti-oxidative properties of this lipoprotein fraction. The utility of this assesment has been demonstrated in the murine atherosclerosis studies that were discussed above (1). Dr. Jake Lusis and Dr Jesus Araujo will determine whether oxidation of HDL leads to a decline in the anti-inflammatory protective activity of human HDL. Dr. Delfino has collected 156 plasma samples that were preserved in a sucrose cryo-preservative solution. These samples correspond to 5-12 repeated measures in 17 individuals belonging to one out of four retirement communities in Dr Delfino's CHAPS study. These samples can be used to assess HDL antioxidant capacity by employing a fluorescent cell-free assay that allows us to evaluate the ability of HDL to inhibit LDL oxidation. Briefly, HDL fractions will be isolated by magnetic bead separation. Once separated, 0.625 μg of HDL samples will be preincubated with 0.25 μg standard LDL in triplicates on 96-well microplates at 37 ºC for 30 minutes. 5 μg DCF will be then incubated at 37 ºC for 1 hour. Reactive oxygen species will be determined by the degree of DCF-fluorescence read in a fluorescence plate reader at an excitation of 485 nm and emission of 530 nm. HDL anti-inflammatory capacity will be expressed in HDL inflammatory index (HII) units, calculated as the ratio of LDL-induced DCF fluorescence in the presence vs. absence of HDL. HII > 1 indicates a pro-inflammatory potential.

Project Title: Project 3: The Chemical Properties of PM and their Toxicological Implications

Investigators: Arthur Cho[1], John R. Froines[2], Yoshito Kumagai[3]
Institution: [1]University of California, Los Angeles Department of Pharmacology; [2]University of California, Los Angeles Department of Environmental Health Studies; [3]University of Tsukuba, Japan Department of Medical Sciences

Objectives of Research: The objective of the project is to characterize the catalytic redox and electrophilic properties of ambient PM samples using cell-free chemical assays. The hypothesis being tested is that PM contain constituents that are capable of inducing cellular stress by redox or other chemical processes and that such processes can be quantitatively assessed by specific analytical chemical procedures.

We are focusing on two general mechanisms, redox and electrophilic reactions. Redox reactions involve the catalytic reduction of oxygen to reactive oxygen species by components of PM with electrons from biological sources. These reactions are thought to induce a state of oxidative stress in cells. In the electrophilic reactions, a reactive function in PM reacts with nucleophilic functions in biological systems to from covalent bonds. These bonds are irreversible so that the affected biological molecule is destroyed. The thiol group is a likely target of the two reactions as this group participates in both redox and covalent bond forming reactions. Thiols serve key functions in proteins such as enzymes, transporters and receptors so their modification can result in substantial disruption of cell biochemistry. By characterizing and quantitatively determining the reactivity in a given PM sample with respect to these chemical properties, we hope to be able to predict its potential toxicity.

To date, we have developed three assay procedures that can be applied to PM samples to assess their chemical reactivity and potential toxicity as predicted by the considerations above. The assays have been applied to different samples collected at sites in the Los Angeles Basin.

Progress Summary/Accomplishments: Part of the role of the project 3 investigators is to provide data to other projects for their studies in which the assays developed are applied to samples. In addition, project 3 continues to develop or refine the assays used to enable us to provide more detailed analyses. In that context, we are collecting samples specifically for use as research objects. In year 2 and 3, we obtained two sets of such samples. One set was obtained from the EPA and they were used to characterize the physical and chemical properties of the reactive substances found in the media. A second set of samples was collected as part of Project 5 in Riverside, California to assess the relative contributions of particle and vapor phases to the chemical properties of toxicological interest.

Chemical assays:

The chemical reactivity assays provide quantitative data on:

a. Electron transfer capacity of organic and inorganic constituents, using the consumption of dithiothreitol (DTT) as the measure of activity.

b. Electron transfer capacity of redox active metal constituents, using the conversion of salicylate to dihyroxybenzoic acids (DHBAs) by hydroxyl, a reaction dependent on transition metals. A description of this assay and its application to ambient samples has been submitted for publication.

c. The content of electrophilic species, capable of forming covalent bonds with a thiolate enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), in PM samples. The ability of the sample to inactivate the enzyme under anaerobic conditions is determined.

d. Specific chemical species relevant to the operating hypothesis are also measured, specifically:

i. Polynuclear aromatic hydrocarbons
ii. Selected quinones
iii. Where necessary, metal ion species have been measured by inductively coupled plasma mass spectrometry through the analytical services of the Department of Chemistry at UCLA

e. In a cellular assay, changes in oxidative status during exposure is determined. The induction of the stress protein hemeoxygenase-1 and the changes in the redox status of glutathione are measured.

The accomplishments summarized in this report reflect the ongoing development and utilization of the assays to further characterize ambient air.

Accomplishments 2005-06

The GC/MS based quinone assay and the DTT based redox activity assay were used in studies with DEP obtained from Japan and ambient air samples collected on the 110 freeway in Los Angeles and in the Caldecott Tunnel in the Berkeley Area for analysis.

Analyses of the four quinones in the Tunnel samples showed high concentrations of the naphthoquinones in the vapor fraction with relatively high levels of 9,10-anthroquinone in the particle phase. The high levels of the naphthoquinones in the Tunnel were interpreted to indicate quinone formation during combustion rather than from photochemical processes. Redox analyses of freeway and tunnel samples revealed that, on a per mass basis, the activities were similar, although there was considerable day-to-day variation. The difference between the Tunnel and freeway was in particle count; the count was much higher in the Tunnel so exposure was greater on a m^3 basis. Application of the GAPDH assay to DEPs showed, for the first time, that electrophiles are present in the organic extracts of these particles and similar findings were made in the ambient samples tested. The assay is being refined in order to provide a robust and reproducible procedure that will provide quantitative data to allow comparison between samples.

Accomplishments 2006-07

In addition to analyses provided for samples collected in Project 1, work during this project period has continued to develop assay procedures for the ability of PM samples to carry out chemical reactions that reflect mechanisms of their toxicity. We focused on two procedures, one an assay for metal based redox activity and the second, an assay for electrophile content.

Summary: Evaluation of the DHBA procedure for redox active metal species.
An assay for the electron transfer capacity of redox active metal constituents of air pollutants is being developed in which the conversion of salicylate to dihyroxybenzoic acids (DHBAs) by hydroxyl, is monitored. In study, assay results from a VACES-biosampler study of ultrafine particle suspensions, collected indoors and outdoors in the same site, were compared with copper and iron ion concentrations, obtained by IPCMS procedures. The results indicated that copper ion was 60 times more active than iron in the Fenton reaction. Therefore, the iron ion concentration would have to be at least 10 fold higher than that for copper in order to contribute significantly to the observed DHBA formation rate. Thus, with this caveat, the assay can be used to assess metal based redox activity in a sample under conditions resembling those in lung lining fluid where high levels of ascorbate are present. A manuscript has been submitted for publication.

The presence of electrophiles in PM samples
The thiol protein, GAPDH is used as a target nucleophile to determine the concentration of electrophilic species in a given test sample. The reaction of the electrophile with the nucleophilic center in the enzyme is conducted under anaerobic conditions to prevent oxygen based inactivation. The assay was used to measure electrophiles in extracts of diesel exhaust particles and in aqueous suspensions of ambient air samples collected in Riverside and Claremont, sites on the eastern end of the Los Angeles Basin. The electrophile content in DEP extracts were found to correlate with PAH and with quinone content, indicating that the electrophiles measured had similar physical chemical properties to these classes of organic compounds. Because of the irreversible nature of electrophile action on thiols, effects of chronic exposure to low levels of electrophiles could be cumulative, increasing the levels of protein thiol inactivation with time. This action differs from the oxidation of thiols by ROS, which is reversible because cells can reduce oxidized thiols by processes involving disulfide isomerases and disulfide reductases[2,4] .

Accomplishments 2007-08
In addition to the analysis of samples from project 1, additional studies utilized the assays developed to address the following questions:

1. In the course of normal exposure, individuals are exposed to particle and vapor phase components of ambient air. How are the reactive components of air, as defined by the assays, distributed between water and particles in suspension?
2. Does the vapor phase contain reactive compounds relevant to toxicity? If so, what types of compounds and what kind of exposure is involved?

These questions were addressed with large scale samples, including DEP samples obtained from the EPA and samples from several large scale collections made in Riverside, CA.

Study 1. Characterization of 7 diesel exhaust particle (DEP) samples collected at the EPA

Initially, the physical properties of the reactive species were determined by filtering aqueous suspensions of the samples into particle (filter bound) and soluble (filtrate) fractions. The activities of the two fractions were determined by assaying the total suspension and the filtrate, with the particle phase determined by difference. Next, the activities of dichloromethane (DCM) extracts of the samples were compared to assess the polarity of the reactive species. Finally, the DCM extracts were subjected to reductive acetylation to determine the role of quinone like species in the reactions. Reductive acetylation is used in stabilizing quinones for a GC/MS assay[1] and will block the redox and electrophilic reactions of quinones.

Results: Experiments with aqueous suspensions of DEP showed that the chemical species associated with redox activity are bound to particles, whereas electrophiles are mostly water soluble. However, this extract does not exhibit metal based redox activity and both the redox and most of the electrophilic activities are lost with reductive acetylation. One possible explanation for the fractionation results is the association of humic like substances with the particles. Humic like substances have been reported in diesel exhaust particles[3] which are thought to be polymeric, highly polar organic materials[5]. Included in the functional groups are quinones, catechols and multiple carboxyl and hydroxyl functions. The latter two functions could act as chelating functions for metals and the first two functions could account for the electrophilic and redox activities found in the particles. Reductive acetylation of the DCM extracted provided support for the involvement of quinone-like organic species in both redox and electrophilic activities of this fraction.

Study 2: Large scale sample collection at Riverside. Tisch sampler with filter and XAD resin.

In collaboration with Professors Hinds and Kleinman, large scale collections of particles and volatile components of ambient air were collected in Riverside, California using a Tisch Sampler with a 10 cm Teflon coated glass filter and a bed of XAD resin to collect PM2.5 and the corresponding vapor fraction, respectively. The objective of the study was to conduct comprehensive analyses, from physical characterization to *in vivo* animal responses on these air samples. By collecting both particle and volatile fractions, the chemical analyses would provide the chemical properties of the particle and vapor phase of an air sample. The collections represented a total of about 300 m^3 of air, a sufficiently large volume to perform multiple assay procedures. Preliminary studies of the two fractions showed that aqueous suspensions of the particulate fraction were more redox active than the volatile fraction and that electrophilic activity was higher in the vapor phase. The inhibitory effects of the vapor fraction on protein tyrosine phosphatase1B (PTP1B) were also determined. PTP1B, like GAPDH, is also a thiolate enzyme and can be inactivated by electrophiles in a similar manner.

Results: This study of ambient air is the first, to our knowledge, in which chemical properties of the particle and vapor phase of the same air mass are being investigated. The redox properties of the air mass in the three samples were predominately in the particle phase whereas the electrophiles were predominately in the vapor phase. The observations made earlier with the DEPs would suggest that the redox activity in particle

phase was particle bound. The presence of electrophiles in the vapor phase indicate that they are of lower molecular weight and as quinone data collected in Riverside earlier showed that the naphthoquinones were present in high levels, they may be major consituents. Since the TOF/MS results from the protein bound species indicate a molecular weight of 422 for the bound species, it is likely that multiple equivalents bind to PTP.

Cellular studies showed that the electrophiles in air volumes of 1-2 m^3 can activate the MAPkinase cascade which results in cell proliferation and cytokine expression. In other cells, protective events can occur through the activation of the Nrf2-keap1 system. Nrf2 is a transcription factor that when activated will initiate the synthesis of multiple protective proteins such as glutathione transferases, multidrug resistant proteins, quinone reductases and glutathione synthases. Thus, components of the vapor phase can elicit both adverse and protective effects, depending on their content and concentrations.

The assays developed in this project provide quantitative estimates of both redox and electrophilic activity in a given sample. In this period, their quantitative nature has been used to characterize the physical and chemical properties of the components responsible for the activities. While the results do not provide information on the specific chemical species responsible for the effect, they can provide information on the chemical class involved.

Results from studies with multiple DEP samples indicate that the redox activity, both metal- and organic-based, is particle bound in aqueous media. In contrast, electrophilic species are water soluble and are present in significant levels in the vapor phase. Support for the presence of quinone like compounds in the organic components was provided by reductive acetylation which converts quinones to their inactive hydroquinone esters. This reaction, which inactivates quinones, caused a loss of both redox and electrophilic activity of the organic fraction of DEPs. The distribution of activities between particles and water are relevant to particle exposure, for they suggest that redox based reactions associated with particles will remain with the particles upon their deposition into lung tissue, whereas electrophiles will be more soluble. Particle access to cells is variable, as multiple pathways are possible, but if the particle deposits on the cell surface, the redox reactions observed can take place on the cell surface, using electrons available from compounds such as ascorbate and glutathione, which are present in lung lining fluid. In contrast, electrophiles appear to dissociate from the particles and can diffuse into cells. Thus, the bioavailability of the redox active species in DEPs will be very different from that of electrophiles. Electrophiles also have distinctive exposure properties because they form covalent bonds with tissue nucleophiles such as protein thiols groups to inactivate them. Depending on the turnover of the protein involved, the actions of these electrophiles can be cumulative and become significant with chronic exposure.

The initial studies of vapor phase samples from Riverside show that this phase contains substantial levels of electrophiles and emphasize the importance of this component of air pollution. Experiments examining the cellular effects of the extracts showed that both inflammatory and protective responses occur, mediated by different cellular signaling

pathways. In summary, in the course of the first three years of project 3, we have developed and applied three assays for toxicologically relevant chemical reactivity in ambient air samples. Their quantitative nature has allowed a comparison of activities of samples, reflecting differences in sites of collection, multiple collections at the same site, and fractionation of DEPs.

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2. Eiguren-Ferndandez A, Avol E, Thurairatnam S, Hakami M, Froines J, Miguel AH. Seasonal influence on vapor- and particle-phase polycyclic aromatic hydrocarbons concentrations in school communities located in Southern California, Aerosol Science and Technology, 41(4):438-446, 2007.

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Presentations:

1. Testimony at the Santa Monica Airport Panel meeting, April 2006, Los Angeles, CA.

2. Presentation at the AQMD Ultrafine Particles conference, *Ultrafine Particle Health Effects*, April 2006, Los Angeles, CA

3. Interview with Randy Paige, CBS, *Ultrafine Particles*, May 2006, Los Angeles, CA

4. *Southern California Particle Center Studies on Ultrafine Particles*. Presentation to the Southern California Association of Governments' Goods Movement Task Force, May 2006.

5. Testimony at the Goods Movement Task Force meeting, *Health Effects of Ultrafine Particles*, May 2006, Los Angeles, CA.

6. Interview with NPR: Living on Earth (Ingrid Lobet), *Health Effects of Perchloroethylene*, May 2006, Los Angeles, CA.

7. *Ultrafine Particles: Exposure, Toxicity and Health Studies*. Presentation to the Board of Harbor Commissioners (at their invitation), July 2006, Port of Los Angeles, CA.

8. Presentation at ARB, *Research Progress of the Southern California Particle Center: Health and Mechanisms Studies*, February 2007, Sacramento, CA.

9. Presentation at SCEHSC retreat, *Approaches to Exposure and Physical Activity Assessment in Environmental Health Studies*, March 2007, Laguna Niguel, CA.

10. *Research Progress of the Southern California Particle Center: Health and Mechanisms Studies*. Presentation to the Columbia NIEHS External Advisory Committee. June 2007, New York, NY.

11. Interview with *Fresno Bee, Health Effects of Ultrafine Particles*, July 2007.

12. Interview with NPR: *Health Effects of Ultrafine Particles,* October 2007.

13. Presentation at CNS – UCSB Nanotech conference, *Nanotechnology – how to define risks and control them*, November 2007, Santa Barbara, CA.

14. Presentation, "Moving Forward" Conference, *Health Effects of Ultrafine Particles*, November 2007, Carson, CA.

15. Interview with NPR: *Health Effects of Ultrafine Particles*, January 2008.

16. Presentation at ARB Symposium on Particulate Matter, *Health Effects of Particulate Matter*, March 2008, Sacramento, CA.

17. Presentation at The Future of Nanotechnology: A Legislative Summit, *Environmental and Health Implications of Nanotechnology: Narrowing our Knowledge Gap*, April 2008, Los Angeles CA.

18. Presentation at Fogarty Meeting on NIH Campus, *Research Status of the UCLA Fogarty Program on Occupational and Environmental Health*, May 2008, Bethesda, MD.

19. Testimony at the LAUSD Air Quality Working Group meeting, June 2008, Los Angeles, CA.

20. Presentation at the Center for Occupational and Environmental Health Advances in Biological Exposure Assessment Workshop, July 2008, Los Angeles, CA.

Future Activities:

1. The DEP studies showed that redox active metals are tightly bound to particles in an aqueous suspension. One explanation was the notion that organic functions on the particles such as carboxyl groups may chelate metals. To investigate this possibility, approaches to particle modification such as esterification will be examined. In addition, DEPs doped with specific metals may be available from the EPA. These particles would be particularly useful in planned studies of the availability of bound metals for participation in chemical reactions.

2. The studies of particle and vapor phase components of ambient air will continue, initially with completion of studies of the particle phase of the current samples. Then, collections will be made at different sites and at different seasons to determine the roles of these parameters in the reactivities of the two phases.

Project Title: Project 4: Oxidative Stress Responses to PM Exposure in Elderly Individuals with Coronary Heart Disease

Investigators: Ralph Delfino[1], Norbert Staimer[1]; Susan Neuhausen[2]; Nosratola D. Vaziri[3]; Dan Gillen[4]

Institutions: [1]University of California, Irvine Department of Epidemiology; [2] University of California, Irvine Department of Genetic Epidemiology; [3] University of California, Irvine Department of Physiology and Oxidative Stress; [4] University of California, Irvine Department of Toxicology

Objectives of Research: The overall goal of this study is to advance knowledge on the importance of particle size and composition to the induction of oxidative stress responses in a high-risk population of elderly people with coronary artery disease. We hypothesize that biomarkers of oxidative stress responses will be associated with indoor and outdoor home PM mass and total particle number concentration. Given the interplay between oxidative stress and inflammation, we anticipate this would support the view that PM leads to systemic inflammatory responses. We further hypothesize that biomarkers will be more strongly associated with predicted indoor exposure to PM of outdoor origin (from source tracer analyses). We will also evaluate effects of exposure to specific metals, elemental and organic carbon, and specific organic components used as source tracers. We further hypothesize that biomarker associations with ultrafine and fine PM will be better explained by chemical assays that measure reactive oxygen species and electrophilic activity. Individual susceptibility will also be assessed, including medication use and polymorphisms in genes coding for proteins involved in oxidative stress responses.

Progress Summary/Accomplishments: Over the period of 9 months of the third year of study, tasks related to data management and analysis has been ongoing. Results from the first one of two years of study panel follow-up were published this year (Delfino et al. 2008). Below we summarized preliminary results from the analysis of both years of data involving repeated measures in 60 subjects. This work represents cumulative progress over three years of funding. Additional laboratory work for biomarkers of oxidative stress is underway. Only methods are briefly described below. Genotyping work for GST M1 and T1 is complete, and analyses of gene-environment interactions are underway.

Analysis of the relationship between biomarkers of antioxidant activity and air pollutants:

As proposed, the Cardiovascular Health and Air Pollution Study (CHAPS) is a panel study with daily repeated measurements of health outcomes and exposures in elderly individuals with a history coronary artery disease. Study subjects ages 65 years or older lived in retirement communities prohibiting indoor tobacco smoke at shared locations and in buildings with common ventilation systems. We have completed follow-up in all four communities in the LA Basin. Retirement communities being studied are located in inland urban areas of the LA Basin considered down-wind smog receptor sites with aged PM, but also affected by local traffic with freshly emitted PM. Evidence from the SCPC show that PM concentrations and components by size-fraction in the LA Basin are

expected to vary across sites because of traffic density and transport, and between our two seasonal study periods described below.[1-3]

Over a seven month period, each subject is followed in two 6-week blocks with blood draws for circulating biomarkers of inflammation, thrombosis, oxidative stress and antioxidant activity (described below), and measurements of exhaled nitric oxide (NO) at the end of each of 12 weeks. During the 12 weeks of panel follow-up, subjects complete daily personal digital assistant (PDA) or paper diaries for time-place activity, psychosocial stress, medications, and antioxidant vitamin and mineral supplements. The effect of medications such as statins on exposure-response relationships is currently being investigated.

We recruited four retirement communities with an average of 365 residents (range 182-575). From these communities, we recruited 102 subjects who underwent baseline clinical evaluation on site at the University of California Irvine Senior Mobile Van. The clinical work-up included an intake history and physical by study cardiologists, 12-lead ECG, complete pulmonary function tests, blood tests including CBC, fasting lipid profile and fasting glucose. Confirmation of CAD diagnosis was made with a medical records review (e.g. positive stress test, MI history). Twenty-one subjects were not eligible and 17 dropped out prior to or after the start of the panel study, or had too few biomarker observations (< 5 of 12 weeks) leaving 64 subjects, 32 from the first year of study (2005-06), and 32 currently being followed in year 2. In year 1, two of the 32 subjects had insufficient or invalid biomarker data, in part due to exclusions for frequent infections. One of 32 subjects in year 2 had frequent infections and another had biomarker data that was beyond the upper range of standard curve, leaving 60 subjects ages 71-96 years with 5-12 weekly blood draws (N=578) (Table 1***).***

Table 1. Characteristics of subjects in CHAPS (N=60)

Characteristic	Mean ±SD or N (%)
Age (years)	84.1 ± 5.60
BMI (kg/m^2)	26.8 ±3.87
Gender	34 (56.7%) Males, 26 (43.3%) Females
Cardiovascular History	
Confirmation of CAD:[a]	
-Myocardial infarction	27 (45.0%)
-Coronary artery bypass graft or angioplasty	20 (33.3%)
-Positive angiogram or stress test	10 (16.7%)
-Clinical diagnosis[b]	3 (5.0%)
Current angina pectoris	18 (30.0%)
Pacemaker or defibrillator	13 (21.7%)
Cardiac arrhythmia	16 (26.7%)
Congestive heart failure	13 (21.7%)
Hypertension	42 (70.0%)
Hypercholesterolemia	43 (71.7%)
Other Medical History	
Type II Diabetes	8 (13.3%)

COPD or Asthma	9 (15.0%)
Stroke or transient ischemic attack	8 (13.3%)
Medications	
ACE inhibitors and Angiotensin II receptor antagonists	24 (40.0%)
HMG-CoA reductase inhibitors (statins)	31 (51.7%)
Platelet Aggregation Inhibitors or Coumadin	21 (35.0%)
Calcium Channel Blockers	21 (35.0%)
Smoking history	
Never smoker	34 (57.6%)
Ex-smoker (no smoking last 12 months)	25 (42.4%)

[a] Each category is hierarchical - excludes being in above diagnostic category
[b] coronary microvascular disease

In 2005-2006, subjects in two retirement communities were followed in four alternating six-week phases (groups 1-2). Again in 2006-2007, subjects in another two retirement communities were followed in four alternating six-week periods (groups 3-4). We collected six weeks of data in each community during a warmer period of higher photochemical activity (July-early Oct, phase 1), and six weeks of data in each community during a cooler period of higher air stagnation (late Oct-Feb, phase 2).

We recently published results of an analysis of biomarker data from the first of two years of the panel study (Delfino et al. 2008). The analysis included 30 elderly residents in the first two retirement communities. We are also completing a similar analysis of the 60 subjects that includes data from the second year. We focused analyses of data for both years on biomarkers that were informative in the year 1 analysis. We assayed weekly concentrations of plasma CRP, TNF-α and its soluble receptor (sTNF RII), IL-6 and its receptor IL-6 sR, and soluble P-selectin by ELISA. Erythrocyte GPx and SOD activities were assayed spectrophotometrically.
Air pollutant exposure measurements included hourly indoor (i) and outdoor (o) home pollutant gases, total particle number (PN), $PM_{2.5}$ elemental carbon (EC), $PM_{2.5}$ organic carbon (the most volatile fraction, OC_1, and the least volatile fractions, OC_{2-4}), and aethelometer black carbon (BC). We also measured size fractionated PM mass, condensation mode (quasi-ultrafine) particles, 0-0.25 μm in diameter ($PM_{0.25}$), accumulation mode particles, 0.25-2.5 μm in diameter ($PM_{0.25-2.5}$), and coarse mode particles, 2.5-10 μm in diameter ($PM_{2.5-10}$) collected with impactor samplers

We also estimated indoor and outdoor secondary organic carbon (SOC) from total OC and primary OC (OC_{pri}) from total OC as described in our recent publication.[4] Briefly, the contributions of primary and secondary OC to measured outdoor OC were estimated from collected OC and EC concentrations using EC as a tracer of primary combustion-generated OC (i.e. "EC tracer method").[5] The study average outdoor SOC accounted for 40% of outdoor particulate OC (40-45% in the summer and 32-40% in the winter). Air exchange rates and infiltration factors (F_{inf}) at each site were also determined. Estimated F_{inf} and measured particle concentrations were then used in a single compartment mass balance model to assess the contributions of indoor and outdoor sources to measured

indoor OC, EC, $PM_{2.5}$ and PN. We assume that indoor exposures to PM of outdoor origin are relevant to personal exposures given that people spend most of their time indoors.

Exposure-response data were analyzed with mixed linear models controlling for temperature and excluding weeks subjects had infections. The model also takes into account between-group exposure effect; within-group, between-phase exposure effect; and the within-subject, within-phase exposure effect that is the parameter of interest.

In the analysis of the two years of data, we confirmed our published findings involving the first year of data, and many associations were more significant as anticipated. Many positive associations were found for EC, OC (mostly OC_{pri}), BC, PN, CO and NO_2 with IL-6, sTNF RII, and sP-selectin. Associations of these air pollutants with CRP and TNF-α were also found, but limited to the upper distribution of thee biomarkers. Generally, the strongest and most significant associations for particle mass were for quasi-ultrafine $PM_{0.25}$, except for sP-selectin, which was more strongly associated with $PM_{0.25-2.5}$. Most associations were strongest for longer-term averages out to the last 9 days. In addition, associations were stronger for estimated indoor PM of outdoor origin than for raw indoor exposures. This suggests that outdoor particles were important. P-selectin is derived predominantly from activated platelets. It is critical for initial leukocyte adhesion to platelets and endothelial cells, activates leukocytes and endothelial cells,[6] and may play a crucial role in the development of neointimal formation after arterial injury.[7] Associations of air pollutants with sP-selectin were stronger in subjects not taking platelet aggregation inhibitors while association with sTNF RII and CRP were stronger among subjects not taking statins.

Using a leave-one-out approach and individual autoregression models we found that five influential subjects showed significant positive associations between pollutants and SOD, and three influential subjects showed significant positive associations between pollutants and GPx. In the remaining subjects, significant inverse associations were found for erythrocyte SOD and GPx with the same exposures above. Inverse associations were found for erythrocyte SOD and quasi-ultrafine $PM_{0.25}$ plus $PM_{0.25-2.5}$ and $PM_{2.5-10}$, although associations were stronger overall for SOD with quasi-ultrafine $PM_{0.25}$. Inverse associations were similarly stronger for GPx with both $PM_{0.25}$ and $PM_{2.5-10}$ as compared with $PM_{0.25-2.5}$. These findings suggest enzyme inactivation within erythrocytes by pollutant components or ultrafine particles in most subjects. We do not know why a small number of other subjects show positive responses. Again, for SOD and GPx we found that indoor PM of outdoor origin was often more informative. As with the similar finding for biomarkers of inflammation like IL-6, this suggests that outdoor particles were important even though subjects spend a majority of their time indoors.

It is conceivable that antioxidant enzyme inactivation is in part responsible for pollutant-related increase in biomarkers of inflammation and thrombosis such as IL-6 and sP-selectin. This is supported by a finding of within-subject inverse associations of IL-6 with GPx, and sP-selectin with SOD. In our publication of the year 1 results (Delfino et al. 2008) we present a discussion of the possible role of these erythrocyte antioxidant

enzymes in air pollution health effects.

In addition, associations of some biomarkers (IL-6, sP-selectin, and SOD) with markers of primary combustion (EC, BC, OC_{pri}), particle number, and $PM_{0.25}$ were stronger in the cooler 6-week period than the warmer 6- week period of follow-up. Primary OC and particle number were higher in cooler months characterized by air stagnation and lower secondary particle growth. Interestingly, concentrations of other pollutants that were more strongly associated with IL-6 and SOD in the cooler phase (EC, BC, and $PM_{0.25}$) were not higher then, suggesting differences in particle composition were important, perhaps as better reflected by OC_{pri}.

These results suggest that emission sources of primary $PM_{2.5}$ OC, quasi-ultrafine particles and related particle number concentrations lead to sustained increased systemic inflammation, platelet activation, and decreased circulating erythrocyte antioxidant enzymes in elderly people with coronary artery disease. Such effects may be partly behind reported morbidity and mortality associations.

Pilot Study of Exhaled Hydrocarbons:

We tested the utility of using exhaled breath gases as a marker for oxidative stress in one of four retirement communities (Riverside). A series of different trace hydrocarbons are being evaluated as possible exposure or response biomarkers. We first tested whether exhaled ethane and *n*-pentane predicted exhaled NO and to indoor/outdoor gas pollutants in order to evaluate ethane and *n*-pentane as potential markers of oxidative stress, which is potentially related to airway inflammation. Exhaled ethane and pentane are possible lipid peroxidation products. In this study, 16 elderly subjects were sampled and the exhaled breath was analyzed using canister sampling and GC/GC-MS analysis (Sherwood Rowland-Blake Laboratory, Department of Chemistry, University of California Irvine). Exhaled ethane and pentane concentrations were subtracted from indoor ethane and pentane concentrations measured in the same manner. Offline exhaled NO was determined using procedures recommended by the American Thoracic Society and European Respiratory Society. We tested criteria pollutant gases as the only predictor variables of exhaled ethane and pentane. We only used pollutant gases since they were measured with fewer missing data on the 12 days of sampling (Friday afternoons) and we were also interested in testing whether ethane and pentane were simply exposure markers of volatile air pollutants.

There was a strong correlation between the breath and room hydrocarbon concentrations (R^2 = 0.99 and 0.37, p = 0.0005 and 0.02, for ethane and *n*-pentane, respectively). Assuming a linear relationship between breath and room hydrocarbon concentrations, least-squares analysis of the data give slopes of 1.06 ± 0.05 for ethane and 1.06 ± 0.10 for *n*-pentane. Slopes larger than one indicate that the rate of clearance from the lung is larger than the uptake within the lung. Thus, both hydrocarbons are slightly elevated in the breath samples in comparison to the room samples, although within the uncertainties, the breath/room ratio for *n*-pentane is indistinguishable from one.

No associations were found between exhaled hydrocarbon species and exhaled NO.

Instead, exhaled hydrocarbons adjusted for indoor hydrocarbon concentrations were associated with indoor and outdoor air pollutants (NO, NO_2 and CO), suggesting air pollutant exposures are driving exhaled hydrocarbon concentrations. This may be secondary to residual ethane and *n*-pentane in the airways not adjusted for by subtracting room ethane and *n*-pentane from exhaled breath concentrations. However, because exhaled NO is not necessarily reflected by levels of lipid peroxidation, additional work will include an analysis of the relationship of biomarkers of oxidative stress (especially carbonylated proteins) to ethane and *n*-pentane.

Genotyping work:

The Glutathione S-transferase M1 (GSTM1) gene is located at chromosome 1p13.3. The homologous recombination between two almost identical 4.2 Kb regions flanking the GSTM1 gene resulted in a 16Kb deletion containing the entire gene. It is so-called null allele of the GSTM1 gene. Similarly, null allele was reported in the GSTT1 gene, another member of the glutathione S-transferase family. A triplex PCR were used to amplify the wild-type allele of the GSTM1 gene, the wild-type allele of the GSTT1 gene, and the interleukin 5 receptor-alpha (IL5RA) gene that served as the internal control to monitor the DNA quality of each sample. PCR primers are listed in the Table 2.

Table 2 primer sequences for the triplex PCR

Primers*	primer sequence	expected size of PCR product
GSTM1-1F:	CTGCCCTACTTGATTGATGGG	273 bp
GSTM1-1R:	CTGGATTGTAGCAGATCATGC	
GSTT1-1F	TTCCTTACTGGTCCTCACATCTC	480 bp
GSTT1-1R	TCACCGGATCATGGCCAGCA	
IL5RA-3602F	TTCACCGCACATTGCACATTAGGG	334 bp
IL5RA-3936R	AGTGGAAATGGTGTTGCCACCTTG	

*Primer sequences for the amplification of GSTT1 gene and GSTM1 gene are from Bailey et al [8]

The absence of 273-bp PCR product in a DNA sample indicates of the presence of the homozygous null allele of GSTM1 gene, and, on the other hand, the absence of the 480-bp PCR product indicates the presence of the homozygous null allele of GSTT1 gene, if good DNA quality in that sample can be confirmed by the success amplification of 334 bp PCR product in the IL5RA gene. PCR amplification in 4 subjects failed for all of the three genes, which indicate that DNA quality for those samples may be poor. After being purified by Zymo-clean column, those samples worked well in the following PCR except one sample. For that sample, DNA was re-extracted from 0.5 ml of another batch of whole blood and PCR reactions were done using this DNA. PCR bands were successfully observed for both the GSTM1 and the GSTT1 gene. Out of 56 subjects genotyped so far, we found 27 subjects (48%) were homozygous GSTM1 null, and 6 subjects (11%) were homozygous GSTT1 null.

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Publications/Presentations:

1. Delfino RJ, Staimer N, Tjoa T, Polidori A, Arhami M, Gillen D, Kleinman MT, Vaziri N, Zaldivar F, Longhurst J, Sioutas C. Circulating biomarkers of inflammation, antioxidant activity, and platelet activation are associated with urban air pollution in elderly subjects with a history of coronary artery disease. Environmental Health Perspectives. In press, 2008. (NOW PUBLISHED)

Presentations:

1. Delfino RJ, Staimer N, Tjoa T, Polidori A, Arhami M, Kleinman MT, Vaziri N, Sioutas C. Circulating biomarkers of oxidative stress, inflammation, and thrombosis are associated with urban air pollution in elderly subjects with a history of coronary artery disease. Presented to: American Thoracic Society, International Conference, May 18-23, 2007, San Francisco, CA. Proceedings of the American Thoracic Society, 2007; 175:A959.

2. Delfino RJ, Staimer N, Tjoa T, Polidori A, Arhami M, Gillen D, Sioutas C. Exhaled NO in elderly subjects with a history of coronary artery disease and urban air pollution. Presented to: American Thoracic Society, International Conference, May 18-23, 2007, San Francisco, CA. Proceedings of the American Thoracic Society, 2007; 175:A960.

Future Activities:

Analysis of stable peroxidation end products to assess oxidative stress

1. *Lipid peroxidation products:* Cooperation has been established between UCI's mass spectrometry facility (Department of Chemistry) and our laboratory. As a result we have started to develop an immunoaffinity chromatography linked LC/MS method for 8-isoprostane measurements in plasma that combines the simple sample preparation of immunoassays with the sensitivity and selectivity of mass spectroscopic technologies. Preliminary analytical recovery studies succeeded in the detection of deuterated 8-isoprostane standards by LC/MS in the lower pg range. We are in the process of optimizing the method for analyzing actual plasma by adding an immunoaffinity purification step.

2. *Oxidized proteins:* The alteration of proteins as a result of oxidative stress can be assessed by measuring the carbonyl groups of the oxidized proteins. We are in the process of evaluating Cell Biolabs' Protein Carbonyl ELISA kit for rapid detection and quantitation of protein carbonyls in plasma samples. In this assay, the wells of a 96-well plate are first coated with protein samples and then react with dinitrophenylhydrazine (DNP) to mark the carbonyl residues. The derivatized protein carbonyls will be probed with an anti-DNP antibody, followed by an HRP conjugated secondary antibody. The protein carbonyl concentration in unknown plasma samples will be quantified by comparing with known concentrations of reduced and oxidized BSA standards.

Analysis of the above biomarkers of oxidative stress in relation to air pollutant measurements will be conducted in the next year. We have analyzed data for exhaled hydrocarbons to estimate *in vivo* lipid peroxidation and will compare this data with carbonylated proteins in plasma to assess the validity of the hydrocarbons as noninvasive biomarker.

Other genotyping work proposed will begin in year 4. We will complete some analyses of gene-environment interactions starting with GSTM1 and GSTT1.

Project Title: Project 5: Ultrafine Particles on and Near Freeways
Investigators: William Hinds[1], Art Cho[2], John Froines[1], Michael Kleinman[3]
Institutions: [1]University of California, Los Angeles Department of Environmental Health Sciences; [21]University of California, Los Angeles Department of Pharmacology; [3]University of California, Irvine Department of Community and Environmental Medicine

Objectives of Research: To determine the relative contributions of gaseous and particle components of ambient air samples to oxidative stress related health effects. Also, to obtain samples to assess the effect of age and freeze-thaw cycles on redox activity of the samples as measured by the DTT assay.

Project Summary/Accomplishments: Project 5 consists of three subprojects that either directly or indirectly seek to improve our ability to assess health risk of air pollution by chemical and biological assays used by SCPC. Subproject 1 will obtain a large simultaneous sample of both particulate and gas phase contaminants from the same volume of air. Both phases will be used for the full slate of bioassays and detailed chemical analysis. Samples will be taken at different locations having a different mix of fresh , aged, and photochemically produced contaminants: an urban freeway site and a receptor site. We have completed the first phase sampling at UC Riverside and have started the second phase at an urban freeway site. Subproject 2 will provide concentrator capability for the Southern California Particle Center investigators and their projects. Work is continuing on testing and modifying the concentrator to make it suitable for our needs. Subproject 3 will use the concentrator to obtain particle samples at UCLA that will be analyzed by our DTT redox activity assay immediately after collection and after a series of aging and freeze-thaw cycles. The objective is to determine the effect of age and freeze-thaw cycles on redox activity of the samples as measured by the DTT assay.

Subproject 1:Simultaneous sampling of particles and vapors for assays
We setup the sampling system in the animal exposure trailer at the UC Riverside agricultural facility in Riverside, CA. Riverside is receptor site with significant photochemical component. At this site we have completed two major multi-day sampling campaigns, one in April-May and one in October-November. We collected particles on Teflon coated glass fiber filters and gas phase contaminants onto XAD resin at 226 Lpm through a PM-2.5 inlet for six days at approximately five hours per day for a total sample volume of approximately 410 m^3. This was repeated two more time, Filters and XAD resin were stored on-site in a refrigerator and periodically transferred to UC Irvine for freezer storage. Gas phase and particle phase samples have been analyzed or assayed. Results are reported in Project 3. Composite sample volumes were 399, 423, and 415 m^3 for the three replicate samples for the first campaign and 232, 245, and 339 m^3 for the second campaign. A portion of the XAD resin was sent to Professor Kumagai in Japan for analysis by his assay. The next set of samples will also be done in triplicate, but samples will be taken at an urban freeway site

An outgrowth of the activity on this subproject led to a collaborative effort between Mike Kleinman, Bill Hinds, and Art Cho for a more comprehensive sampling scheme. We prepared and submitted a proposal to the AQMD Asthma Consortium for funding to conduct simultaneous sampling for (1) animal exposure (direct with a concentrator); (2)

the full battery of chemical and biological assays (samples taken with the concentrator plus impinger); (3) detailed physical characterization including number concentration, size distribution, PM-2.5 mass concentration, elemental carbon, particle bound PAHs; and (4) filter and XAD resin samples for detailed chemical analysis of the gas phase and particle phase. This will provide a direct comparison of in-vivo response, chemical and bioassay response for gas and particle phases and detailed physical and chemical analysis from simultaneous, collocated samplers. The project has recently been approved and funded and we are planning the sampling and exposure campaign.

Subproject 2: Concentrator testing, modification, and deployment

Our three channel Sioutas aerosol concentrator has been modified to make it more portable and easier to use. The refrigeration unit has been mounted on a separate platform truck and the other components on a second platform truck. A drain with a valve has been installed at the bottom of the humidifier tank. The large rotary vane pump is housed in a noise control box on wheels. The second platform truck has a detachable vertical frame section to support the condenser columns, virtual impactors (concentrators), a control panel, and associated tubing. This arrangement allows easy transportation in a van or small truck.

The inlet to the saturator now floats so that it maintains a precisely controlled gap between the airflow entering the saturator and the water surface. The gap can be adjusted without stopping the air flow or shutting down the system. The control panel houses two pressure gauges, two flow control rotameters, and a differential temperature controller. The latter maintains a constant (within +/- 0.2 ºC) differential temperature between the incoming air and the humidified air exiting the saturator. The control panel also has mounts for an impinger, filter holder, and personal sampling pumps.

We have added a thermometer to continuously measure water temperature in the saturator. This facilitates determining when the whole system has reached thermal equilibrium and helps in determining if we have incomplete saturation. We have insulated the saturator tank to improve the saturation process. For more than a month we were able to borrow a second long DMA, which enabled simultaneous upstream and downstream SMPS measurements of number size distribution. This has greatly facilitated measurement of concentration enrichment factors as a function of particle size and checking on various aspect of concentrator performance.

We have added flow control valves downstream of the virtual impactors to control the major flow in each channel,. This permits running one, two, or three channels or any combination thereof. Experiments were conducted to evaluate the concentration enrichment factors as a function of particle size (7 – 300 nm) for different combinations of channels. For these experiments two SMPSs (model 3936 TSI Inc.) were used simultaneously, one upstream and one downstream of the concentrator. Twenty simultaneous upstream and downstream were run for each condition. These measurements were made while sampling outdoor air. Concentration enrichment factors were calculated as the ratio of downstream to upstream concentration as a function of particle size.

An average concentration enrichment factor was calculated for each condition and the results compared. Single channels operated individually were similar although channel three had a lower concentration enrichment factor for particles below 150 nm. All showed a peak in concentration enrichment factor around 20-25 nm but the magnitude of the peaks were different. When the concentrator was operated using two or three channels at a time, average concentration enrichment factors were lower and somewhat different for each channel combination. The peak in concentration enrichment factor at 20-25 nm was still observable.

Subproject 3: Redox Decay Study

This subproject seeks to systematically determine the extent to which redox activity of a sample of CAPs diminishes as a result of storage and/or freeze/thaw cycling. Our observations suggest this may be happening and other investigators have observed such changes. When the UCLA concentrator is fully validated, six eight-hour concentrator/impinger samples will be collected above the Center for Health Sciences loading dock, through a window in the analytical laboratory. At the conclusion of each sampling period a portion will be immediately assayed by the DTT assay and the remainder split with a portion stored in a refrigerator and the rest stored in a freezer (-4 °C). Over the next six weeks DTT assays will be performed on samples with various storage and freeze/thaw histories. All tests will be conducted in triplicate. Once a consistent decay can be demonstrated, we will define and test conditions or treatments that minimize loss of DTT activity.

CARB study: Cardiovascular Health Effects of Fine and Ultrafine Particles during Freeway Travel

In a related project, funded by California Air Resources Board (CARB), we have developed an
instrumented van for human exposure to freeway air while traveling on a freeway. EPA and SCPC contributed to this project through partial salary support for Dr. Yifang Zhu. The study seeks to evaluate short term measures of exposure and response by measuring heart rate variability, and 26 cytokines and other blood factors before, after, and 20 hours after a two-hour exposure to freeway or filtered air. The van includes a HEPA air filtration system, a two-person exposure chamber, a vibration isolation table, nine near real-time instruments, and a battery power supply. Instruments include a CPC, SMPS, aethelometer, particle-bound PAH, PM-10, PM-2.5, NOx, CO2, CO, temperature, relative humidity, and GPS. The van has and will benefit the SCPC for the projects described in this report. We have complete exposure runs for all 19 subjects.

Prior to our measurements with the van we conducted preliminary studies to obtain data for Inside/Outside (I/O) of particulates for vehicle while driving on a freeway. Particle number concentrations and size distributions were measured under different operating conditions of vehicle ventilation system (windows open, AC on/off, recirculation on/off). In-vehicle air change rates were 60 – 120/hr. Maximum in-cabin protection (~85%) was obtained with ventilation conditions of “recirculation on” and high fan speeds. In-cabin and outdoor particle size distributions in the 7 – 300 nm range were observed to be

mostly bimodal, with the smaller-sized peak occurring at 10-30 nm and the larger-sized peak occurring at 60-100 nm. The factory-installed particulate filter in the vehicle ventilation system offered an in-cabin protection of about 50% for particles in the 7-40 nm size range, and 20-30% for particles in the 40-~200 nm size range. The modified van described above contributed to the development of methodology for this project.

We were able to use the instrumented van to continue our study of changes in ultrafine particles near freeways by taking TEM samples for morphological analysis of 50 nm mobility diameter particles. Samples were taken on-freeway and at 30, 60, and 90 m downwind of the freeway. For samples collected on and near I-405, most (>90%) opaque particles were surrounded by a transparent material. This suggests that the aerosol was internally mixed. The number of particles with multiple inclusions increased with distance from the freeway, suggesting that dilution does not prevent particles from colliding and merging.

Publications/Presentations:

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2. Zhu, Y., Fung, D., Eiguren-Fernandez, A., Hinds, W.C. "An on-freeway exposure and measurement system for freeway aerosol health effects study." Presented at the 2007 AAAR Conference Reno, NV. September 25, 2007.

3. Zhu, Y., Eiguren-Fernandez, A., Hinds, W.C., and Miguel, A.H., "In-vehicle exposure to ultrafine particles on Los Angeles freeways," Environmental Science and Technology. 41, 2138-2145, 2007.

4. Zhu, Y., Fung, D.C., Kennedy, N., Hinds, W.C. and Eiguren-Fernandez, A, "Measurements of ultrafine particles and other vehicular pollutants inside a mobile exposure system on Los Angeles freeways." Journal of Air and Waste Management Association. 58:424-434, 2008.

Presentations:

W. Hinds gave a seminar in the UCLA School of Public Health on April 26, 2007 titled, "Ultrafine Particles and Freeway Traffic."

Future Activities

As outlined above we will continue to take the particle and gas phase samples for subproject 1 at the urban/freeway site. All current assays will be conducted on the samples and the chemical and physical analyses will be used in interpreting the results. We will continue working with the concentrator until we are confident we can get reliable and reproducible results with it. We expect this phase to be completed by summer 2008. Once we reach that stage we will use our concentrator to take samples for the redox decay study and other studies.

An accident with the van has delayed progress on the CARB freeway project, but we expect finish all exposures by August 2008. Then we will focus on the analysis of the data.