

**SOUTHERN CALIFORNIA PARTICLE CENTER AND SUPERSITE (SCPCS)
Progress Report**

Period Covered by the Report: June 1, 2003 – May 31, 2004

Date of Report: June 30, 2004

EPA Agreement Number: R82735201

Title: Southern California Particle Center and Supersite (SCPCS)

Investigators: *John R. Froines* (jfroines@ucla.edu); Arthur Cho, Jon Fukuto, William Hinds, Antonio Miguel, Andre Nel, Beate Ritz, Richard Turco, Arthur Winer, RC Yu; Henry Gong, William Linn; Jack Harkema; Fred Lurmann; Michael Kleinman, Robert Phalen; Janet Arey, Roger Atkinson; Edward Avol, Kiros Berhane, William Gauderman, Nino Kunzli, Rob McConnell, John M. Peters, Constantinos Sioutas, Sheldon Friedlander, Dan Stram ; Yoshito Kumagai.

Institutions: *University of California, Los Angeles – Lead Institution*; Rancho Los Amigos Medical Center; Michigan State University; Sonoma Technology; University of California, Irvine; University of California, Riverside; University of Southern California; University of Tsukuba, Japan

Research Category and Sorting Code: EPA/STAR Particulate Matter Research Centers (2003-STAR-66500)

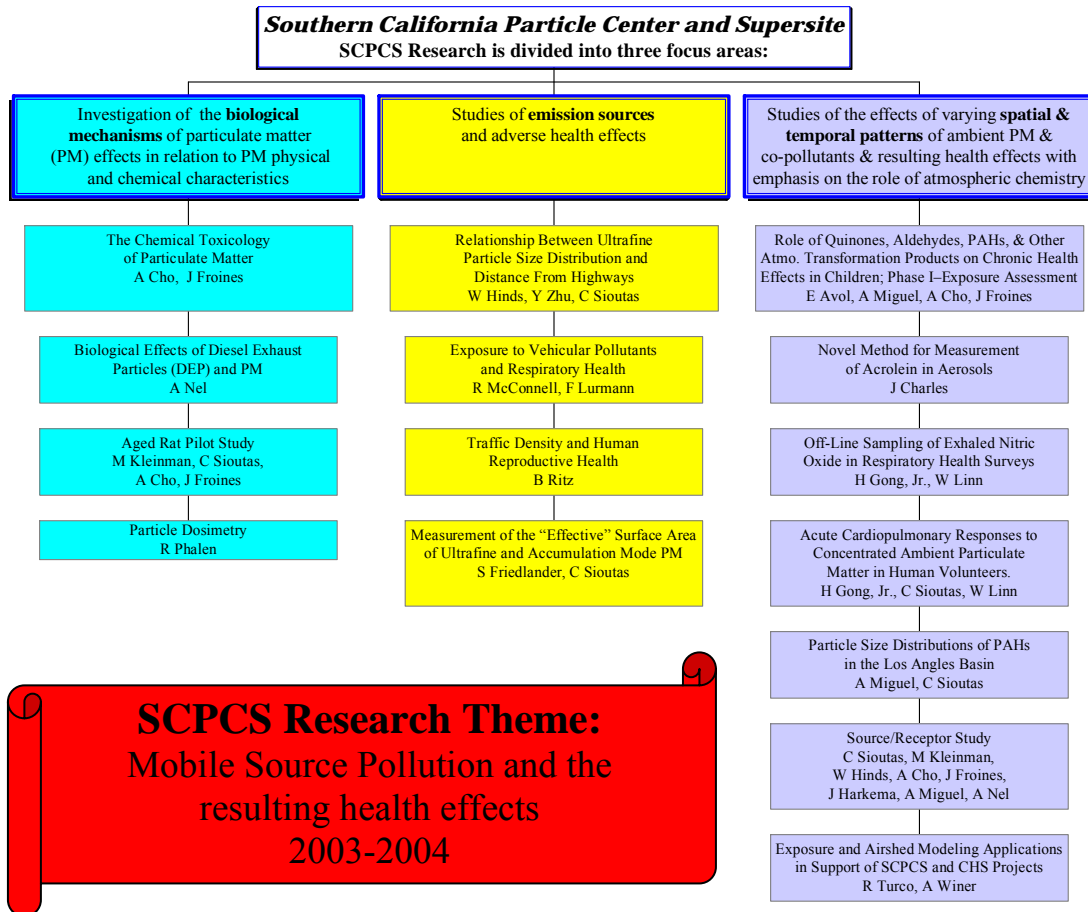
Project Period: 06/01/1999 to 05/31/2005

Overview of the Southern California PM Center:

The overall objective of the Southern California Particle Center and Supersite (SCPCS) is to bring together outstanding scientists from the leading universities in Southern California to identify and conduct high priority research to better understand the effects of particulate matter (PM) and ensure protection of public health. The SCPCS makes use of an integrated approach to address the issues of exposure, dosimetry, toxicology, and epidemiology identified in the EPA's RFA. Integration is accomplished by seeking out and involving in the Center major researchers from all aspects of air quality and environmental health. A team has been assembled for the SCPCS that is committed to the need for strong cross- and interdisciplinary programs of research addressing the challenging issues posed by PM. All studies in the SCPCS are enhanced by the availability of ultrafine, fine, and coarse ambient particle samples collected by mobile concentrators which are linked to in vitro, in vivo, and human clinical studies.

The Los Angeles Basin (LAB) is home to more than 14 million people who breathe the most polluted air in the nation. SCPCS research projects provide the airshed with detailed characterization of exposure by studies to assess PM size, composition, and seasonal and spatial variability. Toxicology investigations are directed toward understanding the mechanisms and the chemical and physical components of PM responsible for health effects and the epidemiologic research enhances our ability to detect human health effects from ambient air pollution.

All studies in the SCPCS are being conducted within the context of the primary theme of "mobile source emissions and resulting health effects." Below is a chart that outlines the projects of the Center broken down into the three major research areas utilized by the Center. All three research areas are complimentary and focus on the role of organic and metal constituents of PM. The research progress for these studies will be summarized in the sections below.



Human Subjects

The SCPCS works in full accordance with UCLA's IRB regulations. The only project requiring human subjects approval is "Acute Cardiopulmonary Responses to Concentrated Ambient Particulate Matter in Human Volunteers." This project has approval at both Rancho Los Amigos and UCLA through May, 2005 and November, 2004 respectively.

QA/QC

The SCPCS maintains a Center Quality Management Plan, which incorporates QA/QC and Data Management, and requires each project to have its own specific plan. Before receiving funding, each investigator must submit a Quality Management Project Plan (QMPP) and a Data Management Plan. These are kept on file in the Center's Administrative Core and are available upon request.

To ensure the quality of our data, we have used an external auditor, Mr. David Bush of Technical & Business Systems, Inc., to review each projects QMPP and audit the laboratories. All audits

have been completed and the reports sent to the laboratories. Where applicable, memos addressing the changes suggested in the audit were written and are on file in the main office.

Biostatistics

Daniel Stram, Ph.D. continues to provide statistical consultation to projects supported by the SCPCS. Dr. Stram has been providing direct assistance to SCPCS principal investigators in two main areas: analyses of human exposure studies conducted at Rancho Los Amigos (Henry Gong, PI) and analysis of the data from Dr. Michael Kleinman's freeway exposure study. Dr. Stram continues to be involved in the analysis and presentation of data from the human exposure studies at Rancho Los Amigos. In the last year, he has focused these efforts on the effect of particle chemistries, especially sulfur, on the modification of the effect of exposure to particles in these studies. He has also continued his work on graphical display of the data from these complicated studies.

Together with a graduate student (Ling Yao) Dr. Stram is completing a manuscript on the effects of the sulfur portion of particle exposure on measured outcomes. For several of these (total lymphocytes, eosinophils), the modification of treatment (exposure to CAPs) effect by the level of sulfur contained in the particles gives statistically significant results. In addition to the ongoing projects, Dr. Stram works with all SCPCS investigators to review proposed analytical approaches upon request.

Theme 1. Investigation of the biological mechanisms of particulate matter (PM) effects in relation to PM physical and chemical characteristics

The Chemical Toxicology of Particulate Matter

Investigators: Arthur K. Cho and John R. Froines

Project Hypothesis: This project is testing the hypothesis that PM contains reactive chemical species and they, either separately or as a mixture, are responsible for the toxicological phenomenon associated with PM. These chemical species can be organic or inorganic and act through several possible chemical reactions with biological substrates. 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. In the electrophilic reactions, a reactive function in PM reacts with nucleophilic functions in biological systems to form covalent bonds. These are irreversible so the biological molecule affected is destroyed. By characterizing and quantitatively determining the reactivity in a given PM sample, we hope to be able to predict its toxicity. The thiol function on biological macromolecules is a likely target of these reactions as they react in both redox and covalent bond forming reactions. Thiols are key functions in biological molecules such as enzymes, transporters and receptors so their modification will result in substantial disruption of cell biochemistry.

Project Objectives: To test our hypotheses, we have developed and continue to develop quantitative chemical assay procedures that can be applied to PM samples to assess their chemical reactivity and toxicity as predicted by the considerations above. Two of the assays, an assay for selected quinones and an assay for redox activity, have been developed and applied to

ambient samples. Additional assays under development include an electrophilic reactivity assay and two biochemical assays.

Progress and Results to Date:

Aim 1: Dithiothreitol (DTT) based redox assay: We have analyzed LAB PM samples from several SCPCS projects to determine the relationships between source and distance, meteorology changes and redox activity in samples collected from various sites. We have made the following observations:

- That redox activity is greatest on a mass basis in UF fractions and correlates with the organic and carbon content of the particles rather than the transition metal content.
- The assay appears to be reproducible in other laboratories as a comparison of results obtained in this laboratory and those obtained by a Mexico City group on the same samples indicates good agreement.
- Initial results of a study comparing our DTT based assay with that based on ascorbate used by Professor Kelly of London suggest that an ascorbate based assay may be measuring metal based redox activity whereas our results with DTT appear to correlate with organic content. We are now investigating the differences in the two reactions by determining the consumption of oxygen as well as the reducing agents to obtain the stoichiometry of the reaction.
- In studies directed at the chemical characterization of PM, we examined the effect of extraction of diesel exhaust particles (DEP) on the residual redox activity. We found that extraction of DEP with dichloromethane had only a slight effect on the redox activity of the particles themselves, but the dichloromethane extract had about 50% of the activity found in the original DEP sample. These results suggest that the chemical properties of particle samples change after extraction and we are now testing this notion with ambient PM samples collected on filters.

Aim 2: Electrophile assay -- To address the second pathway for oxidative stress induction by PM, we have decided to use an enzyme with a key thiol which, when modified by covalent interaction, would lose activity. Because of its catalytic nature enzyme activity can be amplified with a sensitive product assay and longer incubation. In preliminary studies with a common enzyme, glyceraldehydes-3-phosphate dehydrogenase, we have found that electrophilic quinones as well as diesel exhaust extracts irreversibly inhibit this enzyme under anaerobic conditions. DEP extracts will inactivate the enzyme at concentrations of 10 to 100 microgram/mL. Because of the small volumes used in the procedure, the assay can be applied to filter or impinger PM samples collected at sites proximal to freeways.

Aim 3: Yeast based toxicity -- Both redox and electrophilic activity cause oxidative stress to cells but they differ in their dependency on oxygen (Rodriguez et al., 2004). We began studies with yeast to test the hypothesis that the two mechanisms of oxidative stress could be distinguished with this system because these cells grow under both aerobic and anaerobic conditions. In experiments designed to test ambient particle samples, yeast cells were exposed to organic extracts of DEP at concentrations of 10 to 100 micrograms/mL. The cell viability of yeast were inhibited by 50% at about 25 micrograms/mL concentrations. Removal of oxygen reduced the DEP extract toxicity threefold, indicating that most, but not all, of the toxicity was oxygen dependent. Consistent with the notion of oxidative stress, the intracellular concentration

of glutathione, which maintains the redox status of the cell, was decreased by 40% at a concentration of 10 microgram/mL. Although this concentration was not lethal, it clearly subjected the cell to oxidative stress.

Aim 4: Study of intact particles vs. extracts -- Numerous studies have shown that PM can be toxic as intact particles and studies examining their inherent chemical activity have been conducted. In the initial study, we examined DEP using a modification of the DTT assay in which instead of DTT consumption we measured oxygen consumption in an enclosed system. We found that as much as 90% of the activity found in the original particles remained after three extractions with dichloromethane. To test the notion that the residual activity may be due to adsorbed metals, the particles were then extracted with dilute acid (1 N HCl). Again about 70% of the activity remained in the particles (manuscript # 4). These results indicate that particles such as those from diesel exhaust exhibit a reactivity that is not removed by the common extraction procedures for PM studies. Furthermore, as the sum of the extracted activity and the residual particle activity is greater than the original particle activity, dichloromethane extraction appears to alter the redox properties of the residual particle. These studies will continue with filter-collected particles obtained from sites on the USC campus.

Year 5 Publications and Abstracts:

1. Cho, A. K., DiStefano E, You Y, Rodriguez CE, Schmitz DA, Kumagai Y, Miguel AH, Eiguren-Fernandez A, Kobayashi T, Avol E, Froines JR (2004). "Determination of Four Quinones in Diesel Exhaust Particles, SRM 1649a & Atmospheric PM_{2.5}." *AS&T* **38**(S1): 68-81.
2. Cho, A. K., Sioutas C, Miguel AH, Kumagai Y, Schmitz DA, Singh M, Eiguren-Fernandez A, Froines JR (Submitted, 2004). "Redox Activity of Airborne particulate Matter (PM) at different sites in the Los Angeles Basin." (submitted *Environmental Research*).
3. Rodriguez, CE, Shinyashiki M, Froines J, Yu RC, Fukuto JM, Cho A (2004). "Examination of Quinone Toxicity using Yeast *Saccharomyces cerevisiae* Model System." *Tox. (in press)*.
4. Pan, C. G., Cho A, Froines JR, Fukuto JM (2004). "Characterization of the Redox Properties of Diesel Exhaust Particles and the Toxicological Implications." *Tox. Sciencis (in press)*.
5. Hiyoshi, K., Takano, H., Inoue, K., Ichinose, T., Yanagisawa, R., Tomura, S., Cho, A. K., Froines, J. R., and Kumagai, Y. (2004) "Effects of single intratracheal administration of phenanthraquinone on murine lung". *Journal of Applied Toxicology*, (in press)

Year 6 Research Plan: In Year 6 we plan to continue to develop quantitative assay procedures that can be applied to PM samples to assess their chemical reactivity and toxicity as predicted by the considerations above. In particular, we plan to complete the development of the GAPDH assay and assess the application of assays to Center Projects examining health effects of ambient particles. The purpose of the assay procedures is to characterize the chemical properties of PM with the following objectives: 1) To determine the comparative toxicities of PM at emission sources and at receptor sites in winter and summer, and 2) To determine the comparative toxicities of emissions from gasoline and diesel fuel sources.

Biological Effects of Diesel Exhaust Particles (DEP) and PM **Investigator: Andre Nel**

Project Hypothesis: Our studies are premised on the presence of redox cycling chemicals which may be responsible for the biological and adverse health effects of PM. Our principal hypothesis is that these chemical compounds engage in redox cycling reactions in the lung, where the generation of reactive oxygen species (ROS) and/or covalent modification of critical cellular

molecules induce oxidative stress. We propose that the generation of oxidative stress in epithelial cells and macrophages leads to a hierarchical response, which is protective in nature but may yield to injurious effects if the level of oxidative stress increases. We propose that the pro-inflammatory effects are responsible for the adjuvant effects of DEP on allergic inflammation and asthma.

Project Objectives:

Aim 1: To establish a murine model for ovalbumin (OVA)–induced allergic inflammation and to use that model for studying the adjuvant effects of DEP on allergic inflammation. This model seeks to address the hypotheses that: (i) Particulates induce pro–inflammatory effects in the lung by inducing oxidative stress; (ii) Particle composition determines the level of oxidative stress and the extent of the inflammatory changes.

Aim 2: To establish an in vitro model of toxicity in tissue culture macrophages and epithelial cells, in which the effects of particle composition can be studied in terms of oxidative stress effects, cytokine production and induction of cytotoxicity (apoptosis). This includes the study of CAPS, which are collected in a liquid impinger (BioSampler).

Progress and Results to Date:

Aim 1: We have been experimenting with murine asthma models to elucidate the effects of DEP on allergic airway inflammation and airway hyperreactivity (AHR). In Year 3, we showed that it is possible to use aerosolized DEP in an OVA inhalation-sensitization model to elicit adjuvant effects in terms of OVA-specific IgE and IgG₁ antibody responses. However, this model did not yield an increase in BAL eosinophils or an increase in AHR as determined by whole body plethysmography (WBP). We concluded that combined DEP/allergen inhalation challenge is insufficient for achieving the sensitization threshold that is required to induce widespread allergic inflammation and AHR. Numerous attempts to develop a short-term animal model in which DEP exert adjuvant as well as AHR effects have failed. This includes the use of intraperitoneal (i.p.) OVA sensitization, as well as varying the time point and dose of DEP administration. We did, however, notice that i.p. OVA administration in the absence of alum, can induce a state of mild allergen sensitization in which DEP exert effects on AHR. Since PM exposure can induce an acute asthma exacerbation, we set out to develop protocols that can be used to study DEP effects on AHR independent of the adjuvant effects of these particles.

Outcome: Three protocols were developed, two of which require OVA sensitization while the third was OVA-independent. (i) In the mild sensitization protocol, Balb/c mice received i.p. OVA without alum, and were then challenged with aerosolized OVA +/- DEP. (ii) In the post-challenge model, DEP was delivered after OVA challenge to animals sensitized by i.p. OVA plus alum. (iii) Nebulized DEP was also delivered to IL-5 overexpressing mice that exhibit constitutive airway inflammation. Animals were subjected to whole body plethysmography, and then sacrificed for performance of bronchoalveolar lavage, histology and serology.

We demonstrated that DEP could induce AHR in all 3 protocols. In the mild OVA sensitization protocol, DEP delivery concomitant with OVA challenge induced increased airway obstruction as well as increased inflammatory changes and mucin production in large and intermediary airways (manuscript in preparation). A possible explanation for the impact of nebulized DEP on

large and intermediary airways is the deposition characteristics of the relatively large nebulized droplets. Animals in this protocol did not exhibit an increase in OVA-specific IgE or TH₂ cytokine levels. The second model utilizes constitutive eosinophilic inflammation to elicit an effect on AHR and airway inflammation in IL-5 transgenic mice (manuscript in preparation). The realization that a lesser but above-threshold level of airway inflammation is optimal for elucidating DEP effects, led us to adapt the classical OVA-sensitization model (OVA + alum administration i.p) for DEP-induced AHR. Instead of delivering the DEP at the time of OVA challenge, the nebulized particles were given after the allergen challenge. At this point, there is a decline in OVA-induced allergic inflammation, allowing us to observe the effects of nebulized DEP on AHR and lung morphology. This approach overcomes a previous problems with the classical model in which DEP administration concomitant with OVA challenge, was not potent enough to elicit effects on AHR and airway inflammation. With these exposure protocols in place, we should be able to dissect chemical components by which DEP induce acute asthma exacerbations.

Aim 2: We have extended the concept that the biological effects of DEP are mediated through the generation of oxidative stress. We provided evidence that a range of biological effects indicate a stratified oxidative stress response to DEP chemicals. In the past year we have extended these concepts to study concentrated UF particles collected in the LAB. We have previously shown that these particles induce oxidative stress via a perturbation of mite contra function. For that reason, we began testing these particles for their effects on oxidative stress responses and isolated mitochondrial preparations in vitro.

Outcome: We demonstrated that ambient UF, but not commercial, nanoparticles exert potent functional effects on isolated mitochondria (manuscript in preparation). In addition, we showed that aliphatic, aromatic and polar organic compounds, fractionated from DEP, exert differential toxic effects in RAW 264.7 cells. Cellular analyses showed that the quinone-enriched polar fraction was more potent than the polycyclic aromatic hydrocarbon (PAH)-enriched aromatic fraction in O₂⁻ generation, decrease of $\Delta\Psi_m$, loss of mitochondrial membrane mass, and induction of apoptosis. When studying these chemical fractions in isolated liver mitochondria, a major effect of the polar fraction was to promote CsA-sensitive permeability transition pore (PTP) opening, both directly at low doses as well as by inducing membrane potential ($\Delta\Psi_m$) depolarization at higher doses if mitochondria were first Ca²⁺-loaded. The PTP effect was mimicked by redox cycling DEP quinones. While the aliphatic fraction failed to perturb mitochondrial function, the aromatic fraction at low dose increased the Ca²⁺ retention capacity, while at high dose it induced mitochondrial swelling and decrease in $\Delta\Psi_m$. This swelling effect was mostly cyclosporin A insensitive, and could be reproduced by a mixture of PAHs present in DEP. Above chemical effects on isolated mitochondria could be reproduced by ambient UF particles, which contain an abundance of the same organic chemical compounds. In contrast, commercial polystyrene UF particles failed to exert a mitochondrial effect, suggesting that DEP and UF effects are mediated by their chemical compounds and not by the particles themselves.

The pro-inflammatory effects of particulate pollutants, including DEP, are related to their content of redox cycling chemicals and their ability to generate oxidative stress in the respiratory tract. To prevent this effect, and to protect against asthma, there is an antioxidant defense pathway in place, which mediates the expression of phase II enzymes. This induction is dependent on the

expression of a genetic antioxidant response element (ARE) in the promoters of these antioxidant and detoxification enzymes. We recently investigated the mechanism by which redox cycling organic chemicals, prepared from DEP, induce phase II enzyme expression as a protective response. We demonstrate that aromatic and polar DEP fractions, which are enriched in PAHs and quinones, respectively, induce the expression of heme oxygenase-1 (HO-1), glutathione-S-transferase (GST), and other phase II enzymes in macrophages and epithelial cells. We show that HO-1 expression is mediated through the accumulation of the bZIP transcription factor, Nrf2, in the nucleus. Nrf2 accumulation and ARE activation are regulated by the proteasomal degradation of Nrf2; this pathway is sensitive to the effect of pro-oxidative and electrophilic DEP chemicals. This effect could be reproduced by ambient UF particles, and establishes an important antioxidant defense mechanism that may protect against the development of allergic inflammation and asthma in atopic people that are exposed to particulate pollutants.

Year 5 Publications and Abstracts:

1. Hao, M., Comier S, Wang M, Lee JJ, Nel A (2003). "Diesel Exhaust Particles Exert Acute Effects on Airway Inflammation and Function in Murine Allergen Provocation Models." Journal of Allergy and Clinical Immunology **112**(5): 905-914.
2. Li, N., Hao M, Phalen R, Hinds W, Nel A. (2003). "Particulate Air Pollutants and Asthma: A paradigm for the Role of Oxidative Stress in PM-Induced Adverse Health Effects." Clinical Immunology **109**: 250-265.
3. Li, N., Alam J, Eiguren A, Slaughter N, Wang X, Huang A, Wang M, Sioutas C, Nel AE (Submitted, 2004). "Nrf2 is a Key Transcription Factor in Antioxidant Defense in Macrophages and Epithelial Cells: Protecting Against the Injurious Effects of Pro-oxidative Air Pollutants". Journal of Immunology.

Year 6 Project Plan: Explore a range of organic molecules including PAHs, their oxy-derivatives, aldehydes and ketones, and metals adsorbed on PM as they exert pro-inflammatory and tissue damaging effects through covalent binding of organic pollutants to cellular macromolecules, and generation of ROS which results in cardiopulmonary effects. We also aim to explore the notion that PM from vehicles powered by diesel engines are more toxic than gasoline powered sources based on data from in vitro and source tracer studies by comparing the biological activity of samples collected on freeways and a traffic tunnel using HO-1 expression and regulation by Nrf2, as well as cellular glutathione ratios, as markers for oxidative stress in tissue culture cells.

Particle Dosimetry

Investigator: Robert Phalen

Project Hypothesis: Particle Dosimetry is an essential aspect of PM health-related research. Knowledge about the deposition and fate of inhaled particles is central to identifying susceptible subpopulations, extrapolation of laboratory data to humans, and designing/interpreting a wide variety of research projects. The Dosimetry Core performs both (a) original research within the guidelines set forth by the National Research Council's (NRC) Committee on Research Priorities for Airborne Particulate Matter, and (b) service to investigators in the SCPCS.

Project Objectives: In Year 5 we have senior-authored two papers, coauthored three others, and are preparing another. With respect to recent laboratory activities, we have prepared 14 new mouse lung casts, and performed airway morphometry on two. We have also helped prepare five research proposals, and assisted four SCPCS investigators with either general or dosimetric support.

Progress and Results to Date: The difficult problem of establishing appropriate doses for in-vitro mechanistic PM studies has been a priority in Year 5. Specifically, our calculations in support of Dr. Nel's work on mechanisms of particle-induced asthma were incorporated in a recent publication (Li et al., 2003). In this paper we show that the exposures of cells in-vitro that this group used were realistic for approximating in-vivo environmental PM exposures at specific respiratory tract sites (airway bifurcations) in potentially susceptible subpopulations. We are preparing another paper that will provide dosimetry guidance to other researchers performing in-vitro PM research, and we have been invited to address this topic at the 2005 Society of Toxicology annual conference.

In support of the freeway studies of Dr. Kleinman, we have characterized the exposures of mice to CAPS in their cages. Our paper (Oldham, 2004) demonstrates that the mice do receive the desired doses of PM. This contribution is important for understanding the responses observed (with respect to implications to human populations). In addition, the Core has helped substantially with several other aspects of the freeway studies, including conducting the exposures, acquiring tissues, and publishing the findings (Kleinman, 2004).

Our work on preparing replica in-situ lung casts has continued. A replica cast of the nasal and tracheobronchial tree of the Brown Norway rat was supplied to Dr. Harkema; the cast has been sent to the Battelle Institute for CT scanning to acquire data for particle dosimetry modeling. This important rodent model will be made more useful as a result of the CT analysis of this cast. Another rodent model, the lung tumor susceptible A/J mouse in use for PM studies at the Lovelace Respiratory Research Institute and CIIT, has been used to make 14 in-situ tracheobronchial casts. Two of the casts have been subjected to initial morphometric measurements, indicating that the airways are similar to those of the BALB/C mouse.

With respect to facilitating the development of new investigators, we have been involved in the submission of five research proposals; two with Dr. Arezoo Campbell for investigating the effects of CAPS on the brain (NIH), and for examining the effects of exposure of Parkinson's disease-susceptible mice to ambient air in Riverside (HEI); and three for Dr. Michael Oldham to develop an upper airway model for children (NIH), to perform complete airway modeling of the BALB/C mouse (HEI), and to validate CT scan-derived morphometry methods of rodent models (NIH, with Case Western University). Each of these proposals are responsive to the NRC's recommendations for priority PM research, and each will help new researchers affiliated with the SCPCS research program to develop as independent investigators.

On the national/international scene, Dosimetry Core staff have edited two dedicated peer-reviewed issues of *Inhalation Toxicology* that contain papers from the AAAR PM conference held in Pittsburgh in March-April 2003. Also, the Core director has prepared a review paper on the current health effects research on PM (Phalen, 2004, in press), presented a workshop, "The Particulate Air Pollution Issue: Basics and an Update", for the American Industrial Hygiene Association, and accepted an invitation by the Society of Toxicology to present a dosimetry talk as part of a session on "In vitro toxicity testing of air pollutants: pros and cons."

Year 5 Publications and Abstracts:

1. Li, N., Hao M, Phalen R, Hinds W, Nel A. (2003). "Particulate Air Pollutants and Asthma: A paradigm for the Role of Oxidative Stress in PM-Induced Adverse Health Effects." *Clinical Immunology* **109**: 250-265.

2. Oldham, M. J., Phalen RF, Robinson RJ, Kleinman MT (2004, in press). "Performance of a Portable Whole Body Mouse Exposure System." Inhalation Toxicology.
3. Phalen, R. F. (2004, in press). "The particulate air pollution controversy." Nonlinearity in Biology, Toxicology, and Medicine.
4. Kleinman, M., Sioutas C, Stram D, Froines J, Cho A, Chakrabarti B, Hamade A, Meacher D, Oldham M (Submitted, 2004). "Inhalation of concentrated ambient particulate matter near a heavily trafficked road stimulates antigen-induced airway responses in mice." JAWMA.
5. Campbell, A., Becaria A, Bondy SC, Meacher D, Oldham M, Sioutas C, Misra C, Kleinman M. (2004, in revision). "Exposure to Particulate Matter in Air Pollution Increases Inflammatory Parameters in Mouse Brain." Neurotoxicology.

Year 6 Research Plan: The Dosimetry Core will focus on three major projects in Year 6:

Project 1: "Dose Calculations for Geriatric Rats." The SCPCS-related freeway study of Drs. Kleinman and Sioutas will use aged rats as a potential model for elderly humans. The use of such compromised models, and research on their PM doses, is a priority in the NRC recommended research portfolio. We hypothesize that age, per se, is associated with dilation of bronchial airways (due to loss of muscle tone) which leads to uneven PM deposition (due to airflow distribution inhomogeneity). In-situ casting of airways using physiological saline replacement followed by detailed morphometry will allow us to test our hypothesis.

Project 2: "Transfer Coefficient Study for Rats in Mobile Exposure Unit." This project involves using fluorescent monodisperse polystyrene-latex particles to quantify the exposure of rats used in the freeway study. The hypothesis is that the freeway exposure cage will deliver different PM doses than does a nose-only system. A publication is planned.

Project 3: "Workshop on the Potential Effects of Particle Electrical Charge on Toxicity." Although much is known regarding the distribution of electrical charge on laboratory particles and the effect of such charge on the deposition of inhaled particles, there is a clear need to explore this topic further. Specifically, the charge states of aerosols in the LAB and the implications to their toxicity are largely unknown. As a start, we propose to organize a 1-day workshop at UCLA to define the state of current knowledge, assess the implications to SCPCS research projects, and define future research needs.

Theme 2. Studies of emission sources and adverse health effects

Relationships between Ultrafine Particle Size Distribution and Distance from Freeways Investigators: William Hinds, Yifang Zhu, Constantinos Sioutas

Project Hypothesis: The overall objective of this project is to improve our knowledge of the indoor levels of UF particles from outdoor origin, especially those from motor vehicles in the vicinity of freeways.

Project Objectives: Previously we have reported high concentrations of ultrafine particles near major freeways. Many urban residences are located in close proximity of high-density roadways. Given that people spend over 80% of their time indoors, understanding transport of UF particles from outdoor to indoor environments is important for assessing impacts of outdoor PM on human health. The extent of particle penetration into indoor environments is governed by indoor and outdoor sources, exchange rates, and particle physico-chemical characteristics. Indoor particle concentrations, therefore, depend on the dynamics of the transport and fate of outdoor particles in the indoor environments. Previous research in this area has focused on PM_{2.5} and

PM10 properties and behavior (Jones et al., 2000; Thatcher and Layton, 1995). These studies found outdoor particles to be present at significant concentration in indoor spaces. Considering health implications of UF particle exposure, it is important to assess particles' penetration characteristics into indoor environments and the relationship between their physical and chemical properties and infiltration.

Progress and Results to Date: Four apartments near the 405 Freeway in Los Angeles were recruited for this study. Three of the apartments (Apt 1, 2 and 3) are on the eastern side of the 405 Freeway. They are on the third floor with windows 3 m above a sound barrier wall. The horizontal distances between apartments 1-3 and the wall range from 15 m to 40 m. The fourth apartment (Apt 4) is on the opposite, western, side of the 405 Fwy, 15 m from the sound barrier wall. Apt 4 is on the second floor with windows 0.5 m above the wall. All the apartments are about 8 years old with central mechanical ventilation systems that can be turned off. Figure 1 illustrates the location of the sampling site, with relative distances and positions of the four study apartments and the 405 freeway.

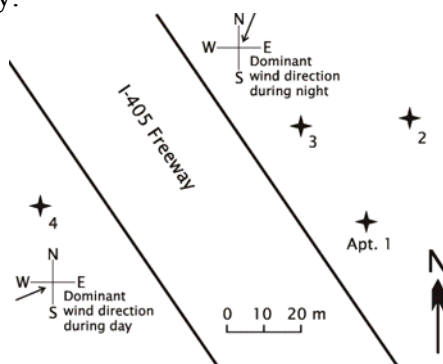


Figure 1. Schematic diagram of sampling site and dominant wind directions.

Indoor and outdoor UF particle size distributions (6 nm to 220 nm) were measured concurrently under different ventilation conditions without indoor sources or aerosol generation activities. Figures 2 shows averaged particle size distributions and indoor/outdoor ratios for apartment 1. Figure 2a shows day time (10 am – 5 pm) and figure 2b shows night time indoor and outdoor particle size distributions, with the x- and y-axes indicating particle diameter (nm) and particle number concentration as $dN/d\text{Log}D_p$ (cm^{-3}), respectively. Figure 2c shows size dependent indoor/outdoor ratios during day and night times.

Figure 2(a) shows a daytime outdoor particle size mode near 20 nm, consistent with previous reports (Zhu et al., 2002b). No such mode exists for indoor observations, and indoor particle number concentration (PNC) is much more stable than outdoors. Nighttime PNC, shown in figure 2(b), are comparable to their daytime values. Although traffic densities are lower during the night, vehicle speeds on the freeway are much faster. It has been shown previously that faster vehicles generate more particles (Zhu et al., 2002b). Lower nighttime temperatures, may also result in higher emission factors for particle number, as described by Kittelson (1998). Another reason for higher PNCs during the night may be lower wind speeds and a lower atmospheric mixing height, thus weaker atmospheric dilution effects.

As figure 2(c) shows, I/O ratios during day and night times exhibit similar trends and shapes. Day and night I/O profiles for particles above 20 nm are consistent with theoretical curve shapes.

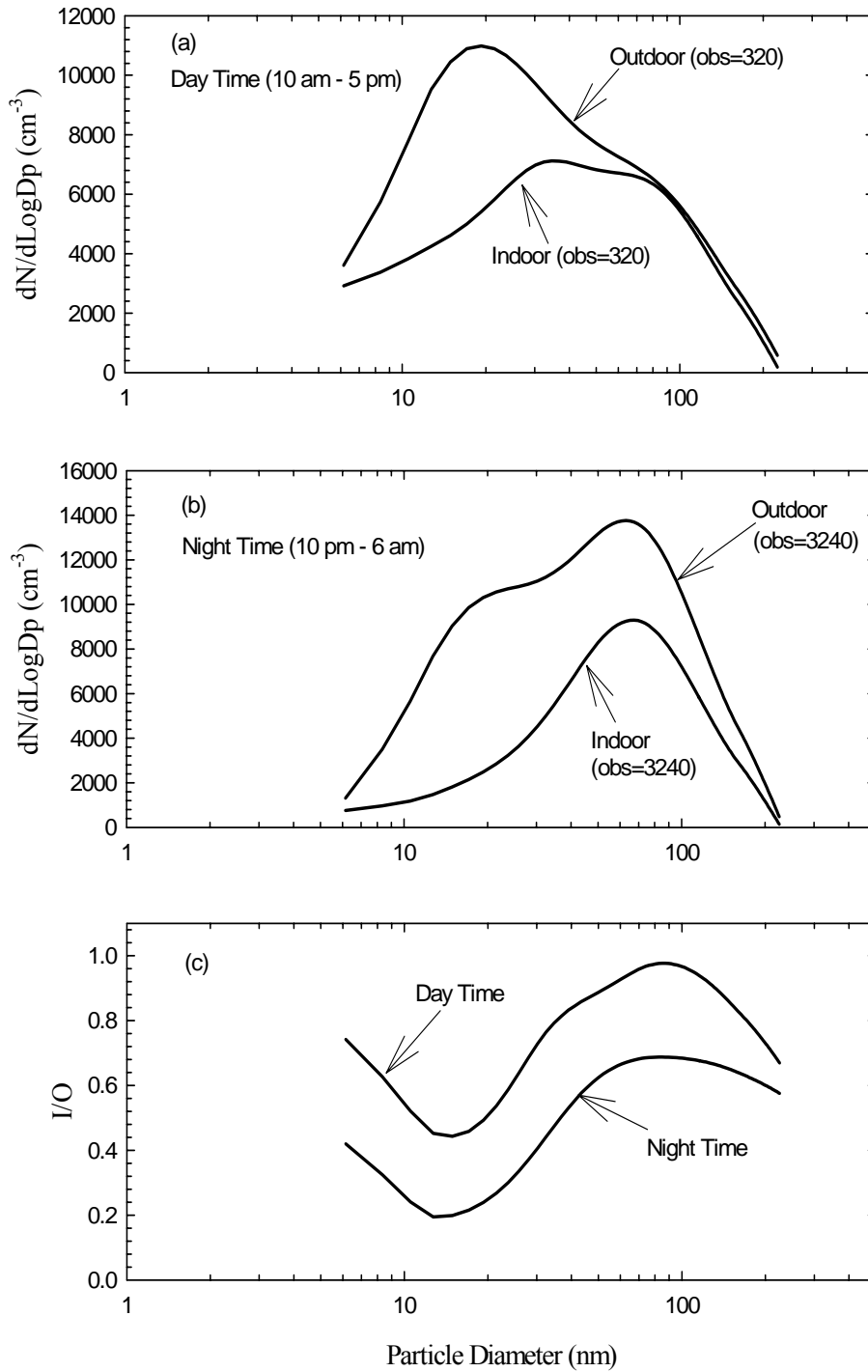


Figure 2. Averaged (a) day time, (b) night time outdoor and indoor particle size distributions and (c) size dependent I/O ratios in Apt 1.

Curves for particles below 20 nm do not correspond to the accepted theory, as no downward trend is observed for both day and night time observations. One possible reason is the low instrument detection limit in that size range, and thus large variability and less statistical confidence in data below 20 nm. Another possible reason may be the unique, semi-volatile, nature of freeway UF particles. Theories always assume particles are 100% non-volatile. Although this may be true for most previous studies carried out under urban background conditions, freshly emitted freeway UF particles are known to have a considerable fraction of volatile components, especially particles below 50 nm (Kittelson, 1998). For example, some of the particles in the 20-40 nm size range may lose their volatile components and become particles of 20 nm or less. Such loss of volatile components has been observed previously (Lunden et al., 2003). The difference between day and night I/O may be due to higher air exchange rates during daytime.

Year 5 Publications and Abstracts:

1. Zhang, K. M., Wexler A, Zhu Y, Hinds W, Sioutas C. (Submitted, 2003). "Evolution of Particle Number Distribution near Roadways, Part II: The 'Road-to-Ambient' Process." Atmospheric Environment.

Year 6 Research Plan: (William Hinds, Antonio Miguel, and Constantinos Sioutas)

As exposure assessment for UF PM will require detailed site-specific data, microenvironment data, or the use of concentration models, the objective of Year 6 is to improve our knowledge of the spatial and temporal distribution of UF PM. The basic hypothesis is: locations with high concentrations of UF PM can dominate an individual's daily dose of UF PM in urban areas. We propose the following projects for Year 6: a) Spatial profiles of UF PM near freeways at night, b) UF PM concentrations inside and outside vehicles driving on freeways, c) UF PM exposure on or near busy streets, d) Measurement of UF PM near freeways over a wider range of ambient temperatures than previous measurements.

Exposure to Vehicular Pollutants and Respiratory Health

Investigators: Rob McConnell, Fred Lurmann

Project Hypotheses: We have examined the relationship between estimates of exposure to traffic related pollutants, derived from CALINE4, and the primary respiratory outcomes in the CHS. During the past year, we have focused on: 1) prevalence of lifetime asthma at entry into the study; 2) incidence of asthma during follow-up; 3) lung function at study entry and its growth during follow-up; 4) school absence and traffic (a new outcome of interest for traffic); 5) chronic asthma exacerbation associated with yearly variation in particulate metrics; 6) NO in fresh traffic exhaust may reduce exposures to ozone; therefore, the effect of traffic density within a community may vary depending on ambient ozone levels.

Project Objectives: Our approach has been to geo-code addresses of residences and schools and then to assign traffic exposures to these addresses, based on traffic estimates available from the CALTRANS. Using modifications of statistical modeling strategies we have developed for the CHS, the effect of traffic exposure on lung function and asthma prevalence and incidence has been examined. In previous years' reports, we presented data suggesting that the effect of traffic may be an early life effect of residential proximity to freeways. We have examined the association of residential and school proximity to heavy traffic corridors with lung function, lung function growth, asthma prevalence and incidence and school absence. We have relied heavily

on the results of Fred Lurmann's efforts to better model PM and other exposures resulting from residential and school proximity to traffic. This year, we have concentrated on consolidating this work.

Aim 1: *Effect of traffic on asthma prevalence.* We have completed our analyses of lifetime asthma at study entry and traffic related pollutant exposure. We observed large risks associated with measured home NO₂ and with residential distance to a freeway. (Manuscript currently under review). There were no associations with other CALINE4 particulate metrics. In a preliminary examination of risks associated with early life asthma in younger children (5-6 years old) in Long Beach, we are also seeing large risks associated with traffic related pollution, in spite of relatively small size. Among children in the upper decile of traffic density within 150 meters and among children with more than 20,000 average annual daily traffic counts within 50 meters of the home, we see greater than 5-fold increased risks of lifetime asthma. Analyses are ongoing.

Aim 2: *Effect of traffic on asthma incidence and Estimating competing risks for ozone and PM.* We have completed analyses to evaluate systematically how ozone varies within communities based on traffic patterns, using a sample of homes for which ozone was measured simultaneously at the home and the central site monitor, as part of a previous study (Avol et al. 1998). Using the proposed prediction model from this manuscript, we have observed large risks of new onset asthma associated with within-community variability in ozone exposure. We also examined residential distance to a freeway, because this metric indicative of particulate exposure was associated with lifetime asthma at study entry. There was no significant association with freeway distance by itself, but if we adjusted for the effect of ozone, there was a significant association with freeway distance. We believe that because distance to a freeway and ozone are inversely correlated that an effect of the particulate exposure from freeways was only observed after accounting for the competing risk of asthma associated with ozone. The effect of ozone is unlikely to be explained by confounding, because the effect was unchanged in models controlling for demographic characteristics, income, education, indicators of access to care, and indoor exposures. In addition, the effect was modified by family history of asthma (bigger risk of asthma among those children with no family history), and the effect of within community variability in ozone is larger in communities with higher average background concentrations of ozone. These results are compatible with previous associations of incident asthma with ozone we have observed in the CHS, effects which were also larger in children without an atopic history (McConnell et al. 2002). They are also compatible with recent toxicologic evidence that air pollutant exposure causes asthma (Schelegle et al. 2003, Miller et al. 2003, Larson et al, 2003). A manuscript is in preparation.

Aim 3: *Effect of traffic on lung function and lung function growth.* We have examined the association of lung function deficits at study entry with a novel Bayesian estimator of NO₂ exposure at homes. Local variation in NO₂ within communities may be considered surrogate of particulate exposure associated with fresh traffic emissions. In addition, lung function growth curves for eight years of follow-up of the CHS have recently become available, and preliminary analyses suggest that these are associated with traffic related particulate pollutants derived from CALINE4, as well as with background pollution measured at the central site monitor in each community.

Aim 4: *School absence and traffic.* School absences are an outcome not proposed for study in this year's work plan, but we have observed strong associations with CALINE4 derived traffic metrics and distance to a freeway. The effects were observed exclusively among children with asthma. A manuscript is in preparation.

Aim 5: *Chronic asthma exacerbation is associated with yearly variation in central site particulate metrics.* Studies of the toxicology and chemistry of PM by other SCPCS co-investigators suggest that organic carbon (OC) may be more biologically active than other PM components. Pollutants modeled from traffic estimates *spatial* variation in pollution within communities. An alternative approach to evaluating within-community variability in pollutant exposure is to examine the effect of the *temporal* variation from year-to-year in the average ambient pollutants measured at the central monitoring sites within each community. The association with organic carbon is strong and robust to confounding by other particulate pollutants.

Progress and Results to Date:

- Proximity to high traffic corridors in early life is associated with asthma before school entry.
- In later childhood, it is possible that there are competing exposures to ozone and traffic related pollutants that are involved in asthma pathogenesis.
- School absence is associated with traffic related air pollution at home and school among children with asthma.
- Yearly variation in OC is strongly associated with bronchitis among children with asthma.
- Lung function and lung function growth are associated with traffic related air pollution estimated both at the home and at the central site monitor.

We have focused on traffic as a source of particulate emissions likely to cause these health effects. However, because particulate exposure is expensive to measure at multiple sites within communities, we have relied on distance to major roadways and samples of NO₂ at homes as surrogates for particulate exposure. This is a limitation we will address in Year 6.

Year 6 Research Plan: The case-control study of early life asthma, using time resolved nephelometry, PM₁₀, PM_{2.5}, and PM_{0.25} measured at homes in Long Beach has been reviewed and approved by EPA. This study will address a limitation to our studies to date that have relied on NO₂ and other surrogates for particulate exposure.

Traffic Density and Human Reproductive Health

Investigator: Beate Ritz

Project Hypothesis: The overall research goal of this project is to determine whether exposure to motor vehicle related air pollution affects the risk of low birth weight (LBW) and preterm birth in infants born to women living in Los Angeles County, California. Residential proximity to heavy traffic roadways is used as a surrogate measure of exposure to motor vehicle exhaust.

Project Objectives: In a previous study we evaluated the relationship between distance-weighted traffic density (DWTG) – a surrogate measure of residential exposure to elevated air

pollution levels in the immediate vicinity of roadways – and low weight and preterm birth for infants born during 1994-1996 to women living in LA County. We observed an approximately 10-20% increase in risk of term LBW and preterm birth in infants born to women living close to heavy traffic roadways. In Year 5, we expanded our analysis to the time period 1994-2000 and incorporated available information on the number of trucks frequenting freeways in our study area.

Progress and Results to Date: We mapped subject home locations at birth and estimated DWTD and the number of trucks on freeways within 750 feet of each residence. The Caltrans truck data list the annual average number and percents of 2-, 3-, 4- and 5 or more axle trucks passing a given freeway count location within a 24-hour period. We used these data to determine the total number of trucks on freeways within 750 feet of subject residences. We also estimated the number of heavy-duty diesel trucks assuming 43% of the 2-axle (6 tire), 90% of the 3-axle and 100% of the 4-axle and 5 or more axle trucks were heavy-duty diesel. Odds ratios (ORs) for term LBW and preterm and LBW (preterm-LBW) birth, and risk ratios (RRs) for preterm birth were estimated based on quintiles of the DWTD distribution and the 90th and 95th percentiles of the freeway truck distributions using logistic regression. We also evaluated the associations between background air pollution concentrations (as measured at monitoring stations) and the risk of these three outcomes.

Although our results for births during 1994-1996 were similar to those reported previously, we did not observe associations between DWTD and term LBW and preterm-LBW birth for 1997-2000. And associations between DWTD and preterm birth for this time period were observed only for certain subgroups: women whose third trimesters fell primarily during fall/winter months (November-April) (RR=1.07, 95% confidence interval (CI) = 0.99-1.16, comparing the highest to lowest DWTD quintile) and women living in census block groups with a fraction of children in poverty at or above the median value (RR=1.08; 95% CI=1.00-1.18). We observed an 11% increase in the risk of term LBW, a 10% increase in the risk of preterm-LBW birth, and a 4% increase in the risk of preterm birth per one ppm increase in annual average background CO (OR=1.11, 95% CI=0.97-1.28; OR=1.10, 95% CI=0.95-1.27; and RR=1.04, 95% CI=0.97-1.12, respectively) for births during 1997-2000. We also observed a 23% greater risk of preterm-LBW birth for women with $\geq 13,290$ freeway trucks passing within 750 feet of their residence per day (95th percentile) (OR=1.23, 95% CI=1.06-1.43) for births during the later time period.

The limitations associated with using DWTD as a measure of motor vehicle exhaust exposure in pregnant women were discussed previously. We noted that the DWTD model assumes motor vehicle exhaust dispersion follows a Gaussian curve centered on a given roadway with 96% decay occurring at 500 feet (152.4 meters) and that such a curve may not adequately represent dispersion conditions since meteorologic factors such as wind direction, wind speed and inversion layer height may be important. We did try and address this issue in this study by incorporating some simple adjustments for wind direction and wind speed into our DWTD measure. In general, we did not observe overall patterns of greater positive association estimates and clearer exposure-response patterns between DWTD and the outcomes after incorporating simple wind direction and wind speed adjustments.

In summary, although residential proximity to traffic did not appear to be associated with higher risks of term LBW or preterm-LBW birth in the later time period included in this analysis (1997-2000), residential proximity to trucks on freeways did appear to be associated with greater risks of these outcomes, especially preterm-LBW, during 1997-2000. This suggests more heavily polluting vehicles within the overall cleaner motor vehicle fleet, such as trucks, may now be more important for these outcomes. Our finding of positive associations between background CO concentrations and term LBW, preterm-LBW birth, and preterm birth in 1997-2000 suggests, overall, air pollution may still be harmful. We feel a more refined exposure assessment approach is needed at this point to derive further conclusions on the associations we have seen in our studies.

Year 5 Publications and Abstracts:

1. Wilhelm, M., Ritz B (2004). Motor-Vehicle Related Air Pollution and Adverse Birth Outcomes in Los Angeles County, California, 1994-2000. Sixteenth Conference of the International Society for Environmental Epidemiology, New York City. August 1-4, 2004.

Year 6 Research Plan: The goal of our Year 6 project is to evaluate whether maternal in-vehicle air pollutant exposures during commutes (either in passenger cases, buses or other means of public transportation) affected the risk of low birth weight (LBW) and preterm birth in infants born to women living in Los Angeles County, California between 2003-2004. We will use data from a nested case-control study in which we surveyed a sample of 2,500 women in LA County (1,250 women who gave birth to a low weight or preterm infant and 1,250 women whose infants were born at term and weighing over 2,500 grams). This study will provide an important source of information on time-activity patterns of pregnant women in a demographically diverse urban population and important information on the potential health risks of extended commuting and high in-vehicle air pollutant exposures to pregnant women.

Measurement of the “Effective” Surface Area of Ultrafine and Accumulation Mode PM Investigator: Sheldon K. Friedlander, Constantinos Sioutas

Project Hypothesis: A major contribution to the atmospheric aerosol surface area comes from the UF particle size range; in certain cases, much of the surface area is associated with fractal-like aggregates. The large surface area allows this aerosol size range to carry high concentrations of adsorbed compounds which may be biochemically active and likely to cause adverse health effects. Surface areas of fractal structures cannot be estimated accurately using electrical mobility or aerodynamic diameters alone. Better estimates are possible using electron microscopy but current techniques are tedious and faster methods are being sought.

Project Objects: This study provides new data on the surface area, primary particle size, and effective density of ambient UF PM. The methodology permits direct comparison of the electrical mobility diameter measured with the differential mobility analyzer (DMA) and the aerodynamic diameter measured with an impactor. New measurements of aggregate morphology and surface area were made in conjunction with a comparative study of indoor and outdoor aerosols by Profs. Hinds and Sioutas.

A workshop on “Emerging Issues in Nanoparticle Aerosol Science and Technology” was held at UCLA chaired by the PI. More than 30 leading researchers in the nanoparticle aerosol field

worked together to write a 120 page report with recommendations. SCPCS investigators participating include Professors Sioutas, Hinds and Turco. The Workshop Report is available on www.nano.gov under the resources bar, or on the SCPCS website.

Progress and Results to Date:

Aim 1: *Comparison of Aerodynamic and Electrical Mobility Diameters for Atmospheric Aerosols.* In this study, a comparison was made of the aerodynamic and electrical mobility diameters. The goal was to relate data from impactors and mobility analyzers which provide complementary information on aerosol characteristics.

Atmospheric aerosol was sampled using a DMA followed by an 8-stage low pressure impactor. All samples were collected on October 17, 2003, between 1 and 4 pm, during three, 30 min sampling periods. Each sampling period was followed by 15 min of measurement of the particle size distribution with the DMA and condensation particle counter and an additional 15 min of experimental setup. An important finding was that atmospheric UF particles with a narrow range of electrical mobility diameters can have a correspondingly wide range of aerodynamic diameters: articles in each narrow electrical mobility diameter range had aerodynamic diameters in the range of 50 to 500 nm. For particles with an electrical mobility diameter of 60, 90 or 180 nm the count mode of particles measured was in the aerodynamic range of 75 – 120 nm, 240 – 500 nm or 50 – 75 nm, respectively. Therefore the aerodynamic diameter of the count mode of particles was larger than the electrical mobility diameter for particles of 60 and 90 nm. However the aerodynamic diameter was smaller than the electrical mobility diameter for particles of 180 nm. These results agree with the findings of Maricq et al., (Aerosol Sci. Technol., 2000) in which aerodynamic size distributions were obtained for mobility size selected diesel exhaust particles.

Aim 2: *Comparison of Morphology and Surface Area of Indoor/Outdoor Ultrafine Aerosols.* As part of the collaborative study with Professors Sioutas and Hinds, indoor and outdoor aerosols were sampled with the LPI in parallel with a Scanning Mobility Particle Sizer (SMPS) at a graduate housing complex located a few miles south of UCLA close to the I-10 and I-405 junction. Sampling was conducted during the morning of January 14, 2004 in one of the unit's bedrooms, which had its windows shut and doors closed off to the rest of the living area. The sampling port led to the LPI, an eight-stage low-pressure impactor, with 50% aerodynamic diameter cutoff points of 4.0, 2.0, 1.0, 0.5, 0.26, 0.11, 0.075, and 0.05 μm for stages 1 through 8. The impactor samples were analyzed by transmission electron microscopy (TEM) in the morphology studies. Twenty aggregates were analyzed on each stage for a total of 80. Each aggregate was analyzed for fractal dimension (Df), number of primary particles (Np), primary particle size distribution, and total and projected surface area. Particle counts for the LPI samples were made from the TEM images for the LPI samples and compared to SMPS measurements. The fraction of the total particles sampled that consisted of aggregates was determined for the LPI samples. The average primary particle size was about the same for the indoor and outdoor samples. For a given aerodynamic diameter, indoor aggregates had a higher fractal dimension and were composed of more primary particles than outdoor aggregates. Indoor and outdoor particles deposited on stage 8 were composed of the same percentage of aggregates (65 to 70%), while indoor particles deposited on stage 7 consisted of a higher percentage of aggregates (63%) than outdoor particles (46%). These were the first morphological studies of their kind to our knowledge.

Year 5 Publications and Abstracts:

1. T. Barone: "The Nature of the Atmospheric Aerosol: Comparison of Aerodynamic and Electrical Mobility Diameter and Spatial Variation" American Institute of Chemical Engineers Annual Meeting, Nov. 18, 2003, San Francisco, CA

Theme 3: Studies of the effects of varying spatial and temporal patterns of ambient PM and co-pollutants and resulting health effects with emphasis on the role of atmospheric chemistry

The Role of Quinones, Aldehydes, PAHs, and Other Atmospheric Transformation Products on Chronic Health Effects in Children; Phase I – Exposure Assessment Investigators: Ed Avol, Antonio Miguel, Arthur Cho, John R. Froines

Project Hypothesis: This report summarizes project efforts to assess the inter-community variability of ambient PAHs, quinones, and carbonyls. Field operations were performed at sampling locations in twelve Southern California communities participating in the California Air Resources Board (CARB)-sponsored CHS. Field sampling was completed in October 2003. Post sampling parallel sampler calibration conducted in October 2003 provided the necessary statistical data for the continuing analyses with observed health outcomes in the CHS. Chemical analyses were released in May 2004, and statistical analyses are underway.

Project Objectives: Two primary objectives guided the sampling study: 1) to develop an informed estimate of seasonal variability and annual levels of specific ambient air organic species in the twelve CHS communities (Atascadero, Santa Maria, Lompoc, Lancaster, Long Beach, San Dimas, Upland, Mira Loma, Riverside, Lake Arrowhead, Lake Elsinore, and Alpine), and; 2) to assess the possible association of those annual estimates with observed health outcomes, as measured by lung function and health status.

Three sets of vapor- and particle-phase samplers were available for field operations. Samplers were deployed, for alternating two-month periods beginning in mid-2001, in three CHS communities at a time. Samples were collected over a 24-hour continuous period once every eight days during each sampling period, beginning at midnight. Upon completion of seven sampling days, samplers were relocated to a second set of three study communities. There, sampling was performed in analogous fashion (e.g., the one-in-eight-day schedule), after which the samplers are re-calibrated and returned to the former three sampling sites for a repetition of the one-in-eight-day, two-month sampling protocol. In this manner, the three sets of samplers were used to collect speciated chemical data across six sampling sites each year; Data was collected at the twelve sampling locations.

In each sampling location, two types of samplers were deployed: A Tisch Environmental Model 1202 Semi-Volatile Organic Compound (SVOC) sampling system was used to collect PAHs and quinones, oxidative compounds of potential research interest (see Table 1 for a listing of analytes of study interest). In addition to the Tisch sampler, an in-house carbonyls sampler was

fabricated to collect ambient carbonyls. (Target carbonyls of study interest are listed in Table 1). Sampling was performed over a continuous 24hr period (midnight to midnight) and was initiated by an electronic timer. Sampling matrices, transported to and from the site in insulated coolers, were deployed the day prior to sampling, and collected the morning following sampling. Additional sampling details have been reported in earlier documents and in the standard operating protocols developed for the project.

Table 1. Target analytes of the Organics Sampling Study.

<i>PAHs</i>	<i>Quinones</i>	<i>Carbonyls</i>
Naphthalene (NAP)	1,2-Naphthoquinone (1,2NQ)	Formaldehyde (FOR)
Acenaphthene (ACE)	1,4-Naphthoquinone (1,4NQ)	Acetaldehyde (ACD)
Fluorene (FLU)	9,10-Phenanthroquinone (PQ)	Acetone (ACE)
Phenanthrene (PHE)	9,10-Anthroquinone (AQ)	Acrolein (ACR)
Anthracene (ANT)		Propionaldehyde (PRO)
Fluoranthene (FLT)		Crotonaldehyde (CRO)
Pyrene (PYR)		Butanone (BUT)
Benz[<i>a</i>]anthracene (BAA)		Butyraldehyde (MET)
Chrysene (CRY)		Benzandehyde (BEN)
Benzo[<i>b</i>]fluoranthene (BBF)		Isovaleraldehyde
Benzo[<i>k</i>]fluoranthene (BKF)		Valeraldehyde (VAL)
Benzo[<i>a</i>]pyrene (BAP)		o-tolualdehyde (OTO)
Indeno[<i>1,2,3-c,d</i>]pyrene (IND)		m-tolualdehyde (MTO)
Dibenz[<i>a,h</i>]anthracene (DBA)		p-tolualdehyde (PTO)
Benzo[<i>g,h,i</i>]perylene (BGP)		Hexaldehyde (HEX)

Progress and Results to Date: Data analysis is currently underway, but several observations are worth noting at this time. First, the field collection and stabilization of ambient quinones has proven to be a challenging undertaking. Improvements in the sampling matrix over the course of the field collection effort resulted in improved collection of the vapor-phase quinones component, and may have resulted in an artificial step-change in the observed ambient values. Handling of this subtlety in the review and interpretation of the data is a focus of ongoing discussion. With regard to aspects of the PAH data, recent analyses of the data set have underscored the importance of surface inversions (and warmer ambient temperatures) in observed particle-phase PAH levels, which were 15- and 20-fold higher during colder periods of the first and the second cycle, respectively. In contrast, naphthalene, acenaphthene, anthracene, and phenanthrene levels varied with season by a factor of ~2. At all sites, measured concentrations of benzo[*ghi*]perylene --a tracer of gasoline exhaust emissions-- suggest an important contribution of gasoline engine exhaust. An abstract describing these results has been prepared and submitted for presentation at the Annual meeting of the AAAR in Atlanta GA in October 2004.

Field operations were completed in late October 2003. The final cycle of sampling and extraction of samplers from several of the field sites were hampered by concurrent fires that engulfed portions of Southern California in October 2003. Laboratory analyses of field samples were performed in Winter and Spring 2004. Quinones data were circulated among investigators in May 2004, and the PAH/carbonyls data set is expected in May 2004, as well. Following a review of the data set, investigators will meet to discuss analytical assignments and approaches to best exploit the collected data. Currently, it is anticipated that data analyses will be performed in June 2004, and several manuscripts will be prepared in mid-summer 2004.

Year 5 Publications and Abstracts:

1. Eiguren-Fernandez, A., Miguel AH, Froines JR, Thurairatnam S, Avol E (Submitted, 2004). "Seasonal and Spatial Variations of Polycyclic Aromatic Hydrocarbons in Vapor-Phase and PM_{2.5} in Southern California Urban and Rural Communities." Aerosol Science & Technology.
2. Cho, A. K., DiStefano E, You Y, Rodriquez CE, Schmitz DA, Kumagai Y, Miguel AH, Eiguren-Fernandez A, Kobayashi T, Avol E, Froines JR (2004). "Determination of Four Quinones in Diesel Exhaust Particles, SRM 1649a and Atmospheric PM_{2.5}." Aerosol Science & Technology **38**(S1): 68-81.
3. Eiguren-Fernandez, A., Thurairatnam S, Avol E, Miguel AH. (Submitted, 2004). Seasonal and Spatial Variation of Polycyclic Aromatic Hydrocarbons (PAHs) in Vapor-Phase and PM_{2.5} in the California Children's Health Study. American Association for Aerosol Research, Atlanta, GA.

Novel Method for Measurement of Acrolein in Aerosols

Investigator: Judith Charles

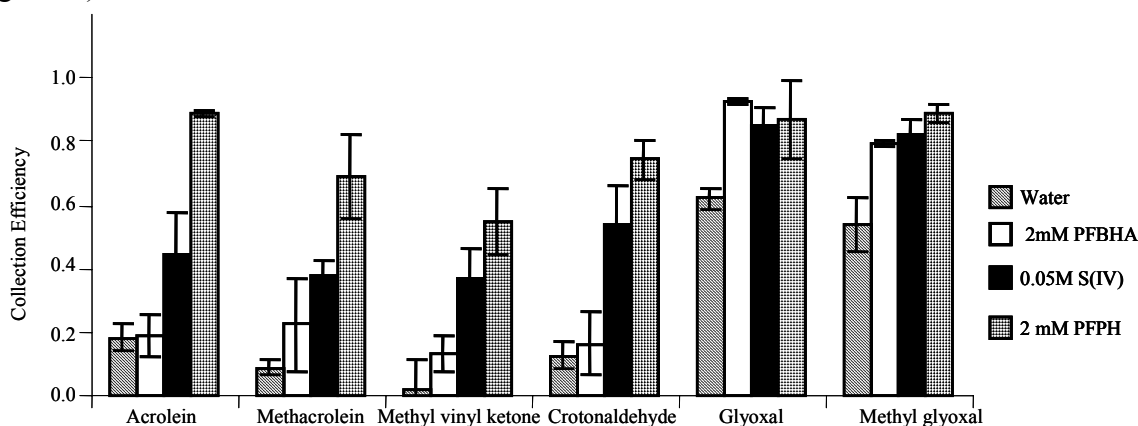
Project Hypothesis: The overall objective of the project is to develop a method to measure acrolein, and other carbonyls that either are directly emitted from motor vehicles or are photooxidation products of hydrocarbons in motor vehicle exhaust (*e.g.*, crotonaldehyde, hydroxyl acetone, glycolaldehyde, methyl glyoxal, glyoxal) that is accurate and precise, and that affords a short sampling time.

Project Objectives: The objective of this project is to develop a new method to measure acrolein and other toxic carbonyls in air that affords part-per-trillion detection limits and short sampling times (10 minutes). The proposed method will rely on using a mist chamber to sample carbonyls, followed by detection of the compounds by using derivatization along with gas chromatography/mass spectrometry (GC/MS).

Progress and Results to Date: Initial work was conducted to explore whether carbonyls could be sampled into a mist chamber by using an aqueous bisulfite solution, and whether the carbonyls could be analyzed by releasing the carbonyl-bisulfite adduct and then derivatizing the "free" carbonyl with *o*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA) prior to detection by using gas chromatography/ mass spectrometry. Specifically, experiments were conducted to: 1) to establish the formation of carbonyl-bisulfite adducts by measuring the formation constants (k_f) for formaldehyde, methyl glyoxal, acrolein, glyoxal, methacrolein, crotonaldehyde, hydroxy acetone and glycolaldehyde, 2) to investigate the effect of pH on PFBHA derivatization of the carbonyls in the presences and absence of bisulfite, and 3) the effect of bisulfite concentration on PFBHA derivatization. Once optimum conditions were determined for formation and derivatization of the carbonyls, we compared the collection efficiency in the mist chamber using an aqueous solution, a 0.001M bisulfite solution, a 2mM PFBHA solution and a 2mM pentafluorophenylhydrazine (PFPH) solution. The collection efficiency is a measure of the efficiency of the mist chamber to capture the carbonyls is the first mist chamber, using 2 mist chambers in series.

The k_f of the carbonyls were determined at pH=5.0 using *pseudo*-first order kinetics. The k_f for formaldehyde was $15.15 \pm 1.36 \text{ M}^{-1}\text{s}^{-1}$ which is in close agreement with the literature value of $12.83 \text{ M}^{-1}\text{s}^{-1}$, thereby validating the analytical approach. The formation constant for acrolein was $0.73 \pm 0.10 \text{ M}^{-1}\text{s}^{-1}$ with a 96% yield in 10 minutes. These k_f and the k_f obtained for the other

compounds establish the formation of carbonyl-bisulfite adducts at pH=5.0 with >80% yields in 10 minutes. Since samples will be collected in the field, and then prepared for analysis in the laboratory, we also explored the stability of the bisulfite adducts over a 6 day period. The effect of pH on PFBHA derivatization was explored at pH=1, 5 and 12 in the absence of bisulfite. The results were consistent with previous data that demonstrate that PFBHA derivatization occurs at neutral and acidic, but not basic pH. The experiment was repeated at pH=5.0 in the presence of 0.05 M bisulfite. The concentration of the PFBHA derivatives was lower in the presence of bisulfite than in the absence of bisulfite, indicating that the bisulfite hinders PFBHA derivatization of the carbonyls. Further experiments established that derivatization was also compromised at 0.005M and 0.001 M bisulfite concentrations, with higher concentrations of derivatives being formed at the lowest bisulfite concentration. The finding that bisulfite hinders or interferes with PFBHA derivatization was significant and may be a deterrent to using an aqueous solution bisulfite as the collecting medium in the mist chamber. Comparison of the collection efficiency for acrolein, methacrolein, methyl vinyl ketone, crotonaldehyde, glyoxal and methyl glyoxal using water, a 0.001M bisulfite solution, a 2mM PFBHA solution and a 2mM PFPH solution demonstrated this occurrence. Overall the collection efficiency followed the order of water < PFBHA solution < 0.001M bisulfite solution < PFPH solution. For example for acrolein, the collection efficiency in water, a 0.001M bisulfite solution, a 2mM PFBHA solution and a 2mM PFPH solution was 0.18 ± 0.04 , 0.448 ± 0.13 , 0.19 ± 0.07 , and 0.89 ± 0.07 , respectively. The reason that the collection efficiency for PFPH is higher than PFBHA is not clear, but may be due to greater water solubility and lower volatility of the hydrazone derivatives, and faster and more expedient derivatization of the carbonyls using PFBHA compared to PFPH. (See Figure 1.)



Comparison of collection efficiencies of acrolein and other carbonyls using different solutions in a mist chamber.

In view of the established k_f for the carbonyls, and the water-solubility and stability of the carbonyl-bisulfite adducts, the lower collection efficiency in the presence of the bisulfite solution compared to using PFPH is likely a result of poor derivatization of the carbonyls with PFBHA in the presence of bisulfite. The difference between the collection efficiency when water is used as the collection media vs. an aqueous solution of PFPH indicates that formation of the derivatives is critical to efficiently capturing the carbonyls.

Previous research conducted in our laboratory demonstrate the ability of a 2mM solution in a mist chamber to sample carbonyls with Henry's law constants $< 10^3$. To sample less polar carbonyls, such as acrolein, a 0.001 M bisulfite or a 2 mM PFPH solution can be used. Although

the use of the bisulfite solution to collect the carbonyls and form carbonyl-bisulfite adducts however is an attractive approach due to the water solubility of carbonyl-bisulfite adducts, PFBHA derivatization of the carbonyls is hindered in the presence of S(IV). For this reason, if bisulfite is used in the mist chamber to collect the carbonyls, we suggest that further research be conducted to investigate the direct detection of the carbonyl-bisulfite adducts. The high collection efficiencies for acrolein (CE=8.0), and overall good collection efficiency (CE > ~6.0) for methacrolein, methyl vinyl ketone, crotonaldehyde, glyoxal and methyl glyoxal using a 2mM PFPH solution in the mist chamber indicates that use of PFPH along with detection by using GC/MS will afford sensitive detection of acrolein and other toxic carbonyls.

Acute Cardiopulmonary Responses to Concentrated Ambient PM in Human Volunteers **Investigators: Henry Gong, Constantinos Sioutas, William Linn**

Project Hypothesis: We hypothesize that 1) ambient particles are responsible for short-term cardiopulmonary health effects observed in Los Angeles and other urban areas on "high-pollution" days; 2) specific effects depend on particle size, composition, dosing characteristics, and preexisting health risk factors; and 3) these effects can be demonstrated clearly by controlled exposures of volunteers representing "high-risk" segments of the exposed population.

Project Objectives: We employ ambient particle concentrators interfaced to exposure chambers to investigate these effects in human volunteers. We have documented effects of fine (PM_{2.5}) and coarse (PM_{10-2.5}) particles, and are currently investigating UF particles (PM_{0.1}) in the primary-pollution (coastal) region of Los Angeles. Volunteer subjects have included younger adults, both healthy and with asthma, and older adults, both healthy and with chronic obstructive pulmonary disease (COPD).

Progress and Results to Date: To date, nine subjects (4 healthy, 5 asthmatic) have completed exposure studies with concentrated UF particles, as well as sham-control studies with filtered air. Initial exposures exhibited inconsistent (frequently low) particle counts relative to mass concentration. This was eventually found attributable to a combination of intermittently low output of UF particles from some concentrator modules and leakage of ambient air (containing larger particles) into the exposure chamber. To alleviate these problems, exposures via facemask have been adopted in place of whole-body exposures. The revised system appears to be functioning well, achieving generally higher and more consistent particle concentrations in the subject's breathing zone (typically 125,000 per ml or more by condensation particle counter measurement; 200 ug/m³ or more by gravimetric analysis). No overall net loss of lung function (forced expired volume in 1 second) was found after UF particle exposure. However, asthmatics on average showed a slight decline in lung function (mean 15 ml), significantly different (P < 0.05) from healthy subjects, who showed a slight gain (mean 61 ml). It is currently unclear whether this effect relates to asthmatic status or to generally higher exposure levels in asthmatics.

Year 5 Publications and Abstracts:

1. Gong, H., Linn W, Terrell S, Anderson K, Clark K, Sioutas C, Cascio W, Alexis N, Devlin R. (2004). "Altered Heart Rate Variability in Asthmatic and Healthy Volunteers Exposed to Concentrated Ambient Coarse Particles." *Inhalation Toxicology* **16**: 335-343.

2. Gong, H., Linn WS, Terrell SL, Anderson KR, Clark KW, Sioutas C, Cascio WE, Alexis N, Devlin RB (Submitted, 2004). "Exposures of Elderly Volunteers with and without Chronic Obstructive Pulmonary Disease (COPD) to Concentrated Ambient Fine Particulate Pollution." Inhalation Toxicology.
3. Linn, W. S., Avila M, Gong H. (In press, 2004). "Offline Measurement of Exhaled Nitric Oxide: Sources of Error." Archives of Environmental Health.

Year 6 Project Plan: The current plan is to continue testing the hypothesis that short-term cardiorespiratory effects of ambient UF particles can be demonstrated by proper experimental combinations of ambient pollution and susceptible subjects. This will be done through additional exposures of asthmatic volunteers at the current laboratory site in Downey (typical of the central LA region with heavy primary particle pollution), using the current experimental protocol incorporating a wide range of heart, lung, and blood measurements. Given that only modest health effects have been found in preliminary analyses of UF exposure data, the accumulation of a larger sample size for the existing subject groups is indicated (rather than a shift to elderly/COPD subjects). A larger sample will improve statistical power to detect subtle effects. At the conclusion of Year 6 exposure studies, our pooled exposure and response data set will include 60 or more individuals with 2-6 studies each, 3 different risk factors (advanced age, asthma, COPD), and 3 different particle size ranges (UF, fine, coarse). Analysis of this pooled data set can potentially identify relationships between health endpoints and exposure factors that are not obvious within data from individual experiments. This will be pursued in collaboration with the SCPCS statistician (Daniel Stram, Ph.D).

Particle Size Distribution of PAHs in the Los Angeles Basin **Investigators: Antonio Miguel and Constantinos Sioutas**

Project Hypothesis: Particles with diameters (dp) in the 3-10nm size range --formed by photochemical nucleation-- have been measured in urban air and in remote locations (McMurry et al., 2000; Kulmala et al., 2004). Combustion-generated particles in the 10~40nm range have been observed in tunnel-diluted diesel exhaust, and in motorway measurement studies (Collins and Graskow, 2000). Observations in tunnels and ambient air reported around the world since the mid 60's revealed that the size distribution of elemental carbon (EC) and PAHs measured down to 50nm showed similar shape and form. This association has been hypothesized as being the result of i) adsorption of PAHs on the surface of EC and/or ii) absorption onto the liquid organic carbon (OC) layer associated with the aerosol particles. To date, limited information is available on the composition of chemical species that make up the Aitken size range ($1 < dp < 50\text{nm}$); only major species, including ammonium sulfate, elemental and organic carbon have been observed (McMurry, personal communication, 2004). Until recently, the size resolution of PAH measurements was limited to 50nm. However, recent improvements in sampling instrumentation and analytical protocols permit the examination of PAHs down to 10nm.

Project Objectives: In the work described here, we report measurements of PAHs from 10nm to $2.5\mu\text{m}$ obtained by combining a MOUDI with a nano-MOUDI impactor, and using a fluorescence detector under excitation and emission conditions optimized for groups of PAH separated by HPLC. These measurements include, to the best of our knowledge for the first time, PAH measurements in the Aitken size range.

Progress and Results to Date: In the present report we examine and analyze the results of size distribution measurements of PAHs collected during five 11.5 hrs periods (August 26-30, 2002), from 7:00pm to 6:30 am, in Riverside, located ~70Km downwind from central LA. Samples were composited together to allow sufficient mass on each size bin for accurate PAH quantification by HPLC with selective fluorescence detection (Eiguren-Fernandez and Miguel, 2003). The sampling system consisted of a nano-MOUDI impactor ($10\text{ nm} < dp < 180\text{ nm}$) behind a MOUDI impactor ($180\text{ nm} < dp < 2.5\text{ }\mu\text{m}$).

A significant fraction of the target PAH mass is found in the Aitken size range (10-32nm), regardless of MW or vapor pressure (Figure 1). The distribution of individual PAH mass in the 10-18, 10-32 and 10-56nm size range as a percentage of the total PAH mass measured in the 10nm-2.5 μm size range, and the species log sub-cooled liquid vapor pressures (atm), are shown in Figure 2. It is interesting to note that, for PHE, ANT and FLT, up to ~45% of their mass is found in the narrow 10-56nm size range, an observation consistent with their absorption in the liquid organic carbon (OC) layer, and possibly, adsorption on the surface of entrained EC. For the remaining PAHs, most of the mass in the Aitken range is found in the narrow 10-18nm range. A reasonable hypothesis is that, similarly to the ultrafine mode, these PAHs are adsorbed on the surface of EC. It is noteworthy to mention that, among the PAHs found mostly in the particle-phase (BKF to BGP) a comparatively larger fraction of DBA is found in the 10-32nm size range (Figure 2). This behavior may explain why DBA concentrations observed in PM_{2.5} collected in six CHS sites (Eiguren-Fernandez, 2004) are generally lower than we would expect, based on its sub-cooled liquid vapor pressure. Such large fraction of DBA (a IARC class 2a and EPA class B2 toxic species) would be expected to be lost to a largest extent to cyclones and the tubing walls that lead the aerosol to a filter or impactor stage. If fractal-like agglomerates (Figure 3) produced by spark-ignition and diesel engines contain PAHs it may be argued that these particles could eventually end up in brain cells, mitochondria, and other eukariotes. Finally, collection and chemical analysis of source and ambient nanoparticles with 1-4 hr time resolution, using an electrostatic (non-MOUDI) sampling system and a higher sensitivity analytical method considered by McMurry and Miguel (proposal in preparation) may provide further insights into the mechanism of formation of PAHs in the Aitken size mode.

Our observations suggest that, if fractal-like particles contain PAHs and other air toxics, they may have increased deposition efficiency in the upper region of the respiratory tract. Further more, consideration of aerosol dynamics on the behavior of particles of 10nm or smaller suggests that these particles may be lost to the walls of denuders, commonly used to remove vapor-phase species. When such sampling artifacts occur, interpretation of partitioning data could suffer from severe sampling artifact, as PAHs present in the particle-phase could be erroneously assigned to the vapor-phase.

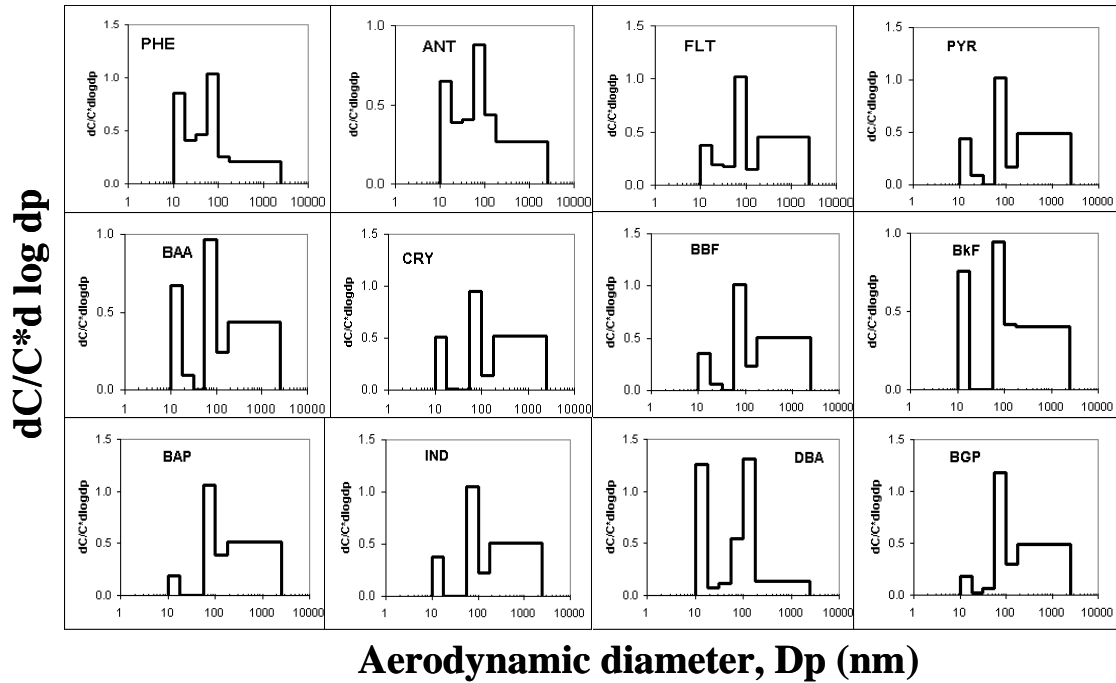


Figure 1. Nighttime PAH size distributions measured in Riverside, California. Samples are five-day 11.5 hr composites taken in Aug 26-30, 2002. Night time temperatures ranged from 13 to 28 °C, a variation of 15 °C. PAH abbreviations: PHE=phenanthrene; ANT= anthracene; FLT=fluoranthene; PYR=pyrene, BAA= benz[a]anthracene; CRY=chrysene; BBF=benzo[b]fluoranthene; BKF=benzo[k]fluoranthene; BAP=benzo[a]pyrene; IND=indeno(1,2,3)pyrene; DBA=dibenz[b]anthracene; BGP=benzo[g,h,i]perylene.

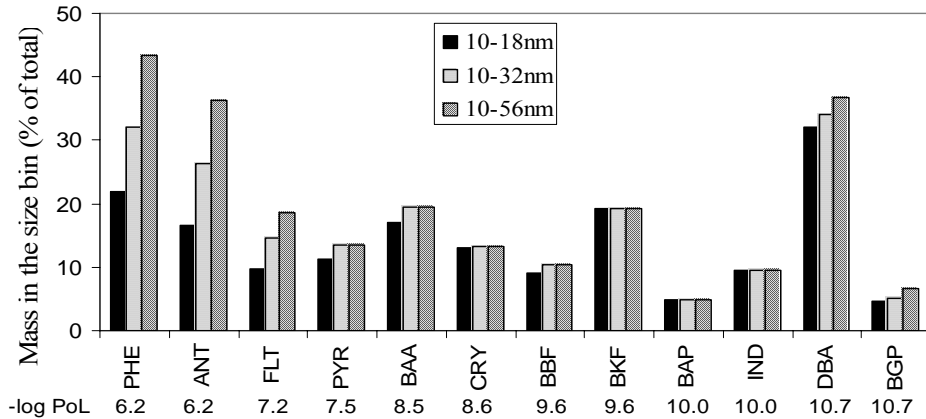


Figure 2. Distribution of individual PAH mass in the 10-56nm dp size range as a % of the total PAH mass measured in the 10nm-2.5µm size range. Also shown are the log sub-cooled liquid vapor pressures (atm) for the target PAHs.

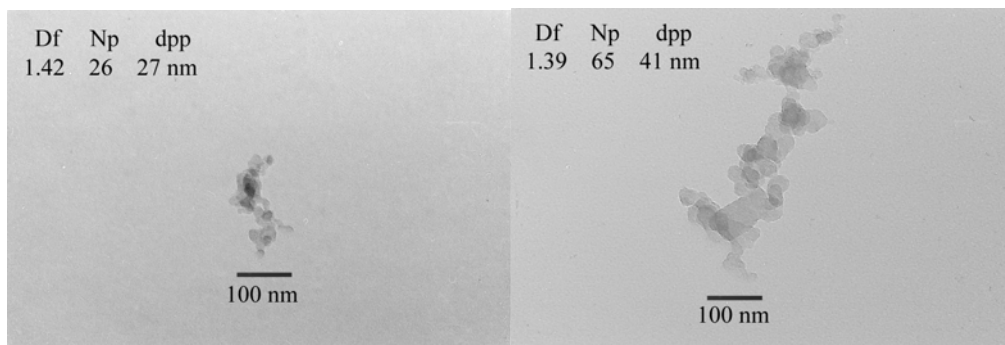


Figure 3. TEMs of fractal-like particles collected at UCLA's SPH loading dock during deliveries by spark-ignition and diesel vehicles. The samples were collected in December 2003 on Formvar® coated grids, using a TSI model 3089 nanometer aerosol sampler operated at 1 LPM, and -10 Kev, after passing through a TSI model 3080 electrostatic classifier. TEM pictures courtesy of Sheldon K. Friendlander and Abert Chong (2004).

Year 5 Publications and Abstracts:

1. Cho, AK., DiStefano, E., You, Y., Rodriguez, CE., Schmitz, DA., Kumagai, Y., Miguel, AH., Eiguren-Fernandez, A., Kobayashi, T., Avol, E., Froines, JR. Determination of Four Quinones in Diesel Exhaust Particles, SRM 1649a and Atmospheric PM_{2.5}. *Aerosol Science & Technology* 38(S1):68-81, 2004.
2. Eiguren-Fernandez, A., Miguel, AH., Froines, JR., Thurairatnam, S., Avol, E. Seasonal and Spatial Variation of Polycyclic Aromatic Hydrocarbons in Vapor-Phase and PM_{2.5} in Southern California Urban and Rural Communities. *Aerosol Science & Tech.* 38:447-45, 2004.
3. Miguel, AH., Eiguren-Fernandez, A., Jaques, PA., Grant, WL., Mayo, PR., Sioutas, C. 2003 - Seasonal Variation of the Particle Size Distribution of Carbonaceous and Ionic Species in Claremont, California. *Atmos. Environ.* 38:3241-3251, 2004.

Exposure Assessment, Source/Receptor

Investigator: Constantinos Sioutas

Project Hypothesis: Mobile sources constitute one of the most important PM sources in large metropolitan areas such as the LAB. Within this basin (and without a doubt in several metropolitan areas of similar morphology), there are three distinct air pollution (hence exposure) regimes that can be identified with respect to mobile sources:

- a) areas that fall within the "zone of influence" of a freeway or a busy thoroughfare;
- b) areas that represent a typical "urban background", but not immediately affected by a freeway, and;
- c) areas downwind from urban sources, in which the main aerosol source is advection of air parcels originally emitted from the polluted upwind urban areas. It is common to refer to the former air pollution regime as "source" and to the latter as "receptor" sites.

Project Objectives: To address this theme, we conduct toxicological studies, which make use of our coarse, fine, and ultrafine concentrators for animal, in vitro, and human clinical research. These studies emphasize the mechanistic based hypothesis testing with particular reference to allergic airway and cardiovascular disease from exposure to concentrated air particles (CAP) and co-pollutants using our mobile particle concentrators. Central to these studies is the ability to characterize the exposure atmosphere of these toxicological studies. Detailed, state-of-the-art characterization of the airborne concentrations, size distribution, and other chemical/physical elements of ambient air particulate within the LAB in relation to health studies were conducted.

Progress and Results to Date: *Seasonal and Spatial Variability of the Size-Resolved Chemical Composition of Particulate Matter (PM₁₀) in the Los Angeles Basin.* For a period of almost three years, sampling of size-fractionated ambient PM₁₀ was performed at urban source sites (Downey and USC) and inland receptor sites (Claremont, and Riverside) in the Los Angeles Basin as part of the Southern California Particle Center and Supersite. Results for size-resolved PM₁₀ mass, inorganic ions (sulfate and nitrate), metals, elemental carbon and organic carbon were obtained. Three collocated MOUDI impactors were deployed to collect 24-h samples roughly once a week. Ultrafine particle concentrations ($dp < 0.1 \mu\text{m}$) were found to be the highest at the source sites resulting from fresh vehicular emissions. Mass concentrations in the accumulation mode ($0.1 < dp < 2.5 \mu\text{m}$) were lower in winter than in summer, especially at the receptor sites. PM concentrations in the coarse mode ($2.5 < dp < 10 \mu\text{m}$) were lower in winter and were composed mostly of nitrate and crustal elements (iron, calcium, potassium, silicon and aluminum). Consistent relative levels of these elements indicate a common source of soil and/or road dust. In the accumulation mode, nitrate and organic carbon were predominant with higher nitrate levels found at the receptor sites. The ultrafine mode PM consisted of mostly organic carbon, with higher wintertime levels at the source sites due to increased organic vapor condensation from vehicles at lower temperatures. Conversely, higher ultrafine organic carbon levels at the receptor areas are due to secondary organic aerosol formation by photochemical reactions as well as increased advection of polluted air masses from upwind. This study is described in greater detail by Sardar et al (2004).

Diurnal Variations of Individual Organic Compound Constituents of Ultrafine and Accumulation Mode PM in the Los Angeles Basin. Individual organic compounds can be used as tracers for primary sources of ambient particulate matter in chemical mass balance receptor models. Previous work has examined PM_{2.5} only and usually over long sampling periods encompassing entire days or longer. In this study, a high-flow rate, low pressure-drop ultrafine particle separator was deployed to collect sufficient mass for organic speciation of ultrafine and accumulation mode aerosol on a diurnal basis. Particles between 0.18 and 2.5 μm in diameter were collected on a quartz-fiber impaction substrate and ultrafine particles below 0.18 μm were collected downstream on a high-volume filter. Four daily time period samples (morning, midday, evening, and overnight) were sampled over five weekdays to form a weekly average composite for each diurnal period. Sampling was conducted at two sites over two seasons; summer (August) and winter (January) samples were collected at both an urban site near downtown Los Angeles (USC) and a downwind, inland site in Riverside, California. Hopanes, used as organic markers for vehicular emissions were found to exist primarily in the ultrafine mode. Levoglucosan, an indicator of wood combustion, was quantified in both size ranges but more was present in the accumulation mode particles. An indicator of photochemical secondary organic aerosol formation, 1,2 benzenedicarboxylic acid, was found primarily in the accumulation mode and varied with site, season and time of day as one would expect for a photochemical product. The atmospheric variations of particulate cholesterol and other organic acids were also considered. By examining the diurnal variation, size-fractionation, and inter-correlations of individual organic compounds, the sources and atmospheric fate of these tracers can be better understood and their utility as molecular markers can be assessed. This study is described in greater detail by Fine et al (2004).

Size-Fractionated Measurements of Ambient Ultrafine Particle Chemical Composition in Los Angeles Using the NanoMOUDI. Ambient ultrafine particles have gained attention with recent evidence showing them to be more toxic than larger ambient particles. Few studies have investigated the distribution of chemical constituents within the ultrafine range. The current study explores the size-fractionated ultrafine (10–180 nm) chemical composition at urban source sites (USC and Long Beach) and inland receptor sites (Riverside and Upland) in the Los Angeles basin over three different seasons. Size-fractionated ultrafine particles were collected by a NanoMOUDI over a period of 2 weeks at each site. Measurements of ultrafine mass concentrations varied from 0.86 to 3.5 $\mu\text{g}/\text{m}^3$ with the highest concentrations observed in the fall. The chemical composition of ultrafine particles ranged from 32–69% for organic carbon (OC), 1–34% for elemental carbon (EC), 0–24% for sulfate and 0–4% for nitrate. A distinct OC mode was observed between 18 and 56 nm in the summer, possibly indicating photochemical secondary organic aerosol formation. The EC levels are higher in winter at the source sites due to lower inversion heights, and are higher in summer at the receptor sites due to increased long-range transport from upwind source areas. Nitrate and sulfate were measurable only in the larger particle size ranges of ultrafine PM. Collocated continuous measurements of particle size distributions and gaseous pollutants helped to differentiate ultrafine particle sources at each site. This study is described in greater detail by Sardar et al (2004).

Year 5 Publications and Abstracts:

1. Miguel, A.H., Eiguren-Fernandez, A., Jaques, P.A., Mayo, P.R. and Sioutas, C. “Seasonal variation of the particle size distribution of polycyclic aromatic hydrocarbons and of major aerosol species in Claremont, California” *Atmospheric Environment*, 38: 3241-3251, 2004.
2. Geller, M.D., Fine, P.M. and Sioutas*, C. “The Relationship Between Real-Time and Time-Integrated Fine and Coarse Particle Concentrations at an Urban Site in Los Angeles, CA”. *Journal of Air and Waste Management Association*, in press, November 2003.
3. Sardar, S.B., Fine P.M., Jaques, P.A and Sioutas*, C. “Seasonal and Spatial Variability of the Size-Resolved Chemical Composition of PM_{2.5} in the Los Angeles Basin” Submitted to *Journal of Geophysical Research*, December 2003
4. Gong, H., Jr., Linn, W.S., Terrell, S.L., Clark, Geller, M.D and Sioutas, C. “Altered Heart Rate Variability In Asthmatic and Healthy Volunteers Exposed to Concentrated Ambient Coarse Particles”. *Inhalation Toxicology*, 16: 335-343, 2004.
5. Jaques, P.A., Ambs, J.L., Hering, S.V., Fine, P.M. and Sioutas*, C. “Field Assessment of the Dynamics of Particulate Nitrate Vaporization Using Differential TEOM® and Automated Nitrate Monitors”. *Atmospheric Environment*, 38(51): 49-59, 2004.
6. Fine, P.M., Chakrabarti, B, Krudysz M., Schauer J.J. and Sioutas*, C. “Seasonal, Spatial, and Diurnal Variations of Individual Organic Compound Constituents of Ultrafine and Accumulation Mode PM in the Los Angeles Basin”. *Environmental Science and Technology*, 1296 – 1304, April 2004.
7. Sardar, S.B., Fine P.M., and Sioutas*, C. “The Relationship Between Particle Number and Co-pollutant Concentrations in the Los Angeles Basin” *Journal of Air and Waste Management Association*, accepted for publication, January 2004
8. Kleinman M.T, Sioutas, C., Stram, D., Froines, J.R., Cho, A.K., Chakrabarti, B., Meacher, D., and Oldham M. “Inhalation of concentrated ambient particulate matter near a heavily trafficked road stimulates antigen-induced airway responses in mice”. Submitted to *Journal of Air and Waste Management Association*, February 2004
9. Zhang, K.M., Wexler, A.S., Zhu, Y., Hinds, W.C. and Sioutas, C. “Evolution of Particle Number Distributions Near Roadways. Part II: The “Road-to-Ambient Process”. Submitted to *Atmospheric Environment*, December 2003.
10. Zhao, Y., Bein, K.J., Wexler, A.S., Misra, C., Fine, P.M. and C. Sioutas*, C. “Using a Particle Concentrator to Increase the Hit Rates of Single Particle Mass Spectrometers”. Submitted to *Journal of Geophysical Research*, January 2004
11. Sardar, S., Fine, M., Mayo, P.R. and Sioutas*, C. “Size Fractionated Chemical Speciation Measurements of Ultrafine Particles in Los Angeles Using the NanoMOUDI”. Submitted to *Environmental Science and*

Technology, January 2004

12. Phuleria, H., Fine, P.M., Zhu, Y. and Sioutas*, C. "Characterization of Particulate Matter and co-Pollutants During the Fall 2003 Southern California Fires". Submitted to *Journal of Geophysical Research*, January 2004
13. Cho A.K., Sioutas C., Schmitz, D.A., Kumagai Y., Singh M., Miguel A.H and Froines, J.R. "Redox activity of airborne particulate matter (PM) at different sites in the Los Angeles Basin" Submitted to *Environmental Science and Technol.*

Exposure and Airshed Modeling Application in Support of SCPCS and CHS Projects **Investigators: Richard Turco, Arthur Winer**

Project Hypothesis and Objectives: Utilizing UCLA's Regional Human Exposure Model (REHEX) and Surface Meteorology and Ozone Generation (SMOG) airshed models, the overall goal of this research is to develop more precise and comprehensive human exposure models that 1) provide an accurate and comprehensive assessment of exposure to PM and other vehicle-related pollutants in Southern California populations; 2) enhance health effects investigations underway in the SCPCS and CHS; and 3) create linkages between emission sources, exposures, and health endpoints.

Progress on airshed modeling -- Progress made included the assessment of the regional distributions of, and human exposure to, naphthalene and naphthoquinones in the LAB, application of the SMOG model in support of the SCPCS and CHS, and initial development of a detailed model for ultrafine particle size distributions for freeway exposure studies.

Exposure to naphthalene and naphthoquinones may have significant adverse health effects. We investigated the regional distributions of naphthalene and its byproducts over Southern California. We found that gasoline and diesel engine exhaust, with related fuel vaporization, contribute roughly half of the daily total naphthalene burden in Southern California. The spatial distribution patterns of emissions are highly correlated with major roadways in the region owing to the importance of vehicular exhaust as a naphthalene source. We verified the emission inventory for naphthalene against new field measurements undertaken by the SCPCS (Miguel et al.) at the Sepulveda tunnel near LAX and in ambient air samples collected at the California Institute of Technology, Pasadena. The agreement between modeled and measured naphthalene-to-benzene ratios indicates the relative contributions to the total naphthalene emissions from mobile sources are represented reasonably well in our emission inventory.

The SMOG airshed model was used to compute the spatial and temporal distributions of naphthalene and its photooxidation products throughout the region. Our simulations are unique, offering for the first time a comprehensive and unified picture of airborne naphthalene for health impact assessment. The SMOG simulations reveal definite patterns in naphthalene distribution and exposure, with large spatial variations. The results also show large diurnal and seasonal variations, to a much greater degree than can be inferred from existing measurements. Peak naphthalene concentrations are estimated to occur in the early morning hours in the winter.

To improve our predictions of particulate pollutant distributions for exposure assessment in the SoCAB, we updated the primary emission inventories, incorporated a secondary organic aerosol (SOA) module, and integrated a biogenic emission database into the SMOG model. The latest emission inventory developed for SCAQMD 2003 Air Quantity Management Plan was adopted

to estimate CO, NO_x, SO_x, VOC and PM emissions in the SoCAB. The enhancements to the SMOG model have significantly improved our ability to model the distributions and organic composition of particulates and their precursors in the SoCAB. The updated emission inventories also allow more reliable simulations of criteria pollutants of interest in exposure assessments for the individual children in the Children's Health Study (CHS).

We set up a high-resolution two-dimensional version of the SMOG model that includes explicit treatments of aerosol microphysics and chemistry in the configuration of a line emission source. This model, which requires more development in Year 6, will provide a comprehensive basis for investigating local exposure to ultrafine particles.

Progress on Exposure Modeling -- In year 5, significant progress has been made in improving individual and population exposure assessments for vehicle-related pollutants in the South Coast Air Basin. We investigated spatial accuracy issues in roadway data and geocoding processes to provide the most accurate data for exposure and epidemiological studies concerned with vehicle-related air pollution. Large discrepancies, up to hundreds of meters, were found between a widely used U.S. Geological Survey (USGS) roadway network containing traffic activity data and a global positioning system (GPS)-validated road network without traffic information, with the GPS-validated network having higher spatial accuracy. We demonstrated discrepancies in roadway geometry of this magnitude, as well as geocoding errors, can lead to serious exposure misclassifications, on the order of factors of three to ten in assigned pollutant concentrations. The GIS-based algorithm we developed was effective in transferring vehicle activity information from the less accurate USGS roadway network to a GPS-accurate road network, with a match rate exceeding 95%. This method is portable and can be employed to transfer vehicle activity and other data between roadway networks.

By linking the SMOG and REHEX models, we quantified the population exposure to naphthalene for the first time for the California South Coast Air Basin. Average hourly naphthalene exposures in the SoCAB under summer and winter conditions were 270 ng m⁻³ and 430 ng m⁻³, respectively. More than one million and one thousand individuals were estimated to experience naphthalene exposure greater than 1000 and 3000 ng m⁻³, respectively. Substantial spatial and temporal variations exist for naphthalene exposures, with populations living, working, or attending school adjacent to major roadways experiencing the highest exposures. Indoor sources and travel by vehicles accounted for 40% and 4% of the total exposure, respectively, while ETS accounted for less than 5% of total naphthalene exposures. The highest naphthalene exposures estimated from this modeling study exceed the reference concentration for chronic inhalation exposure adapted by the U.S. Environmental Protection Agency.

Environmental inequities in Southern California were also investigated with respect to disproportionate exposure to vehicle-generated pollutants. Minority and high-poverty neighborhoods were found to have a higher population density, lower levels of educational attainment, higher rates of unemployment, lower housing value, older and more multifamily housing, higher density of highways and major roads, and fewer transportation resources. We determined that these neighborhoods bear over two times the level of traffic density compared to the rest of the Southern California region, which may associate them with a higher risk of

exposure to vehicle-related pollutants, both directly and due to penetration of outdoor air into older, more poorly constructed housing with higher than average air exchange rates.

Year 5 Publications and Abstracts:

1. Houston, D., Ong P, Wu J, Winer A (In press - Dec03). "Structural Disparities of Urban Traffic in Southern California: Implications for Vehicle-related Air Pollution Exposure in Minority and High-poverty Neighborhoods." Journal of Urban Affairs.
2. Lu, R., Wu J, Turco RP, Winer AM, Atkinson R, Arey J, Paulson S, Lurmann F, Miguel AH, Eiguren-Fernandez A. (Submitted, 2004). "Naphthalene Distributions and Human Exposure in Southern California." Atmospheric Environment.
3. Wu, J., Lurmann F, Winer A, Lu R, Turco R, Funk TH. (Submitted 2004). "Development of an Individual Exposure Model for Use in Epidemiological Studies: Application to the USC Children's Health Study." Atmospheric Environment.
4. Wu, J., Funk TH, Lurmann F, Winer AM. (Submitted, 2004). "Improving Positional Accuracy of Roadway Networks and Geocoded Addresses in Epidemiological Studies." Transactions in GIS.
5. Lu, R., Wu J, Turco R, Winer A, Atkinson R, Arey J, Paulson S, Lurmann F. Naphthalene and Naphthoquinone: Distributions and Human Exposure in the Los Angeles Basin, (8 - 12 December 2003, San Francisco, CA) *Eos Trans. AGU*, 84(46), Fall Meet. Suppl., Abstract A31D-0079, 2003
6. Wu, J., Lu R, Lurmann F, Winer AM, Turco R: An Exposure Modeling System for Use in Epidemiological Studies: Application to the USC Children's Health Study. Presented at ISEA 2003 Conference, STRESA Piemonte, Italy, Sept. 21-25, 2003
7. Wu, J., and Winer, A.M.: Geocoding and Roadway Geometry Errors: Application to the Exposure Assessment of Vehicle-Related Pollutants in the CHS Study. Presented at the Workshop of "Mapping Community Health: Using Geographic Information Systems (GIS) in Health Research", University of California, Los Angeles, May 7, 2004.

Year 6 Research Plan: The specific objectives of the SCPCS modeling program during Year 6 are to:

- Simulate the dispersion and aging of ultrafine particles downwind of major roadways under field conditions, and to model both individual and regional exposure to ultrafine particles using appropriate exposure models.
- Calculate the regional distributions of organic particulate and elemental carbon with the SMOG model, and the resulting regional population exposures using the REHEX model.
- Correlate the emission sources of key species with specific human receptor exposures.
- Further improve assessments of in-vehicle exposures to ultrafine particles and other vehicle-related pollutants for the SoCAB population and individuals (e.g. CHS children)
- Investigate the relationship between ethnicity and socioeconomic status and spatially-resolved population exposures to vehicle-related pollutants.

REFERENCES

Avol, E. L., W. C. Navidi, and S. D. Colome. 1998. Modeling ozone levels in and around Southern California homes. *Environ Sci Technol* 32:463-468.

Collings, N., Graskow, B.R. (2000). Particles from internal combustion engines - what we need to know. *Philosophical Transactions of the Royal Society of London Series A-Mathematical Physical and Engineering Sciences* 358(1775): 2611-2622.

ICPR (1994). Human Respiratory Tract Model for Radiological Protection, *A Report of a Task Group of the International Commission on Radiological Protection*. ICPR Publication 66 (Smith H. ed). Ann ICRP 24:1-3.

Jones, N.C., Thornton, C.A., Mark, D. and Harrison, R.M., 2000. Indoor/outdoor relationships of particulate matter in domestic homes with roadside, urban and rural locations. *Atmospheric Environment* [ATMOS ENVIRON], 34(16): 2603-2612.

Kittelson, D.B., 1998. Engines and nanoparticles: A review. *Journal of Aerosol Science*, 29(5-6): 575-588.

Kulmala, M., Vehkamäki, H., Petäjä, T., Dal Maso, M., Lauri, A., Kerminen, V.M., Birmili, W., McMurry, P.H. (2004). Formation and growth rates of ultrafine atmospheric particles: a review of observations *Journal of Aerosol Science* 35(2):143-176.

Larson, S. D., E. S. Schelegle, W. F. Walby, L. J. Gershwin, M. V. Fanuccihi, M. J. Evans, J. P. Joad, B. K. Tarkington, D. M. Hyde, and C. G. Plopper. 2004. Postnatal remodeling of the neural components of the epithelial-mesenchymal trophic unit in the proximal airways of infant rhesus monkeys exposed to ozone and allergen. *Toxicol Appl Pharmacol* 194(3):211-20.

Lunden, M.M. et al., 2003. The transformation of outdoor ammonium nitrate aerosols in the indoor environment. *Atmospheric Environment*, 37(39-40): 5633-5644.

McConnell, R., K. Berhane, F. Gilliland, S. J. London, T. Islam, W. J. Gauderman, E. Avol, H. G. Margolis, and J. M. Peters. 2002. Asthma in exercising children exposed to ozone: a cohort study. *Lancet* 359(9304):386-91.

McMurry, P.H., Woo, K.S., Weber, R., Chen, D.R., Pui, D.Y.H. (2000). Size distributions of 3-10 nm atmospheric particles: implications for nucleation mechanisms. *Philosophical Transactions of the Royal Society of London Series A-Mathematical Physical and Engineering Sciences* 358(1775):2625-2642.

Miller, L. A., D. M. Hyde, L. J. Gershwin, E. S. Schelegle, M. V. Fanucchi, M. J. Evans, J. E. Gerriets, L. F. Putney, M. Y. Stovall, N. K. Tyler, J. L. Usachenko, and C. G. Plopper. 2003. The effect of house dust mite aeroallergen and air pollutant exposures during infancy. *Chest* 123(3 Suppl):434S.

Oberdorster, G., Utell, M.J. (2002). Ultrafine particles in the urban air: To the respiratory tract - And beyond? *Environmental Health Perspectives* 110 (8): A440-A441.

Schelegle, E. S., L. A. Miller, L. J. Gershwin, M. V. Fanucchi, L. S. Van Winkle, J. E. Gerriets, W. F. Walby, V. Mitchell, B. K. Tarkington, V. J. Wong, G. L. Baker, L. M. Pantle, J. P. Joad, K. E. Pinkerton, R. Wu, M. J. Evans, D. M. Hyde, and C. G. Plopper. 2003. Repeated episodes of ozone inhalation amplifies the effects of allergen sensitization and inhalation on airway immune and structural development in Rhesus monkeys. *Toxicol Appl Pharmacol* 191(1):74-85.

Thatcher, T.L. and Layton, D.W., 1995. Deposition, Resuspension, and Penetration of Particles within a Residence. *Atmospheric Environment*, 29(13): 1487-1497.

Zhu, Y., Hinds, W.C., Kim, S., Shen, S. and Sioutas, C., 2002a. Study of Ultrafine Particles near a Major Highway with Heavy-duty Diesel Traffic. *Atmospheric Environment*, 36: 4323-4335.

Zhu, Y., Hinds, W.C., Kim, S. and Sioutas, C., 2002b. Concentration and size distribution of ultrafine particles near a major highway. *J Air Waste Manag Assoc*, 52(9): 1032-42.

Zhu, Y., Hinds, W.C., Kim, S. and Sioutas, C., 2002c. Concentration and Size Distribution of Ultrafine Particles near a Major Highway. *Journal of Air and Waste Management Assoc.*, 52: 174-185.

Zhu, Y., Hinds, W.C., Shen, S. and Sioutas, C., 2003. Seasonal trends of concentration and size distribution of ultrafine particles near major highways in Los Angeles. Accepted for publication in *Aerosol Science and Technology*.