Southern California Particle Center

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- University of California, Los Angeles
- University of Southern California
- University of California, Irvine
- Michigan State University
- University of Wisconsin-Madison
- University of Tsukuba, Japan

Characteristics of the Los Angeles Basin Airshed

- Most polluted airshed in the nation, with complex, persistent, unique PM
- 12,000 square miles, population projected at 19 million in 2020, vehicle numbers continue to increase
- Unique, well studied climatology and weather patterns
- Southern California's economy focused in the future on "Goods Movement-Transportation Sector" to replace manufacturing and to be responsive to globalization needs
- Key Sources:
  - Motor vehicles and associated road dusts
  - 300,000 diesel trucks
  - Nation's largest marine port complex – discussion of expansion
  - Airports

Effects of regulation and fleet turnover: Ultrafine particles on the increase

	Kirchstetter Study (1997)	PM Center (2004)
Bore 1 (HDV + LDV)		
PM10 (ug/m3)	130.0	37.2
PM2.5 (ug/m3)	115.7	36.7
PN (particles/cm3)	340,000	550,000

Bore 2 (LDV only)		
PM10 (ug/m3)	40.0	19.4
PM2.5 (ug/m3)	40.9	15.3
PN (particles/cm3)	185,000	450,000

Selected accomplishments (1999-2005)

- Developed mobile size-selective PM concentrators capable of collecting large amounts of ambient samples for in vitro, in vivo and human clinical studies
- Characterized the formation and dynamics of PM near freeways: high levels of ultrafines at freeways has implications for personal exposure and health
- Developed chemical assays for redox and electrophilic activity and demonstrated significant redox activity of PM, particularly ultrafine particles.
- Demonstrated that pro-oxidative chemicals induce hierarchical oxidative stress effects, and developed murine in vivo models for airway inflammation.
- Described in vivo allergic airway responses, neurological and cardiovascular effects in mice resulting from exposure in close proximity to a freeway.
- Human clinical studies using coarse, fine and ultrafine concentrators
- Epidemiological studies on the role of traffic density and asthma and developmental effects

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Overall Research Theme:

Characterizing PM Sources and their Linkages to Toxic Mechanisms and Resulting Health Effects

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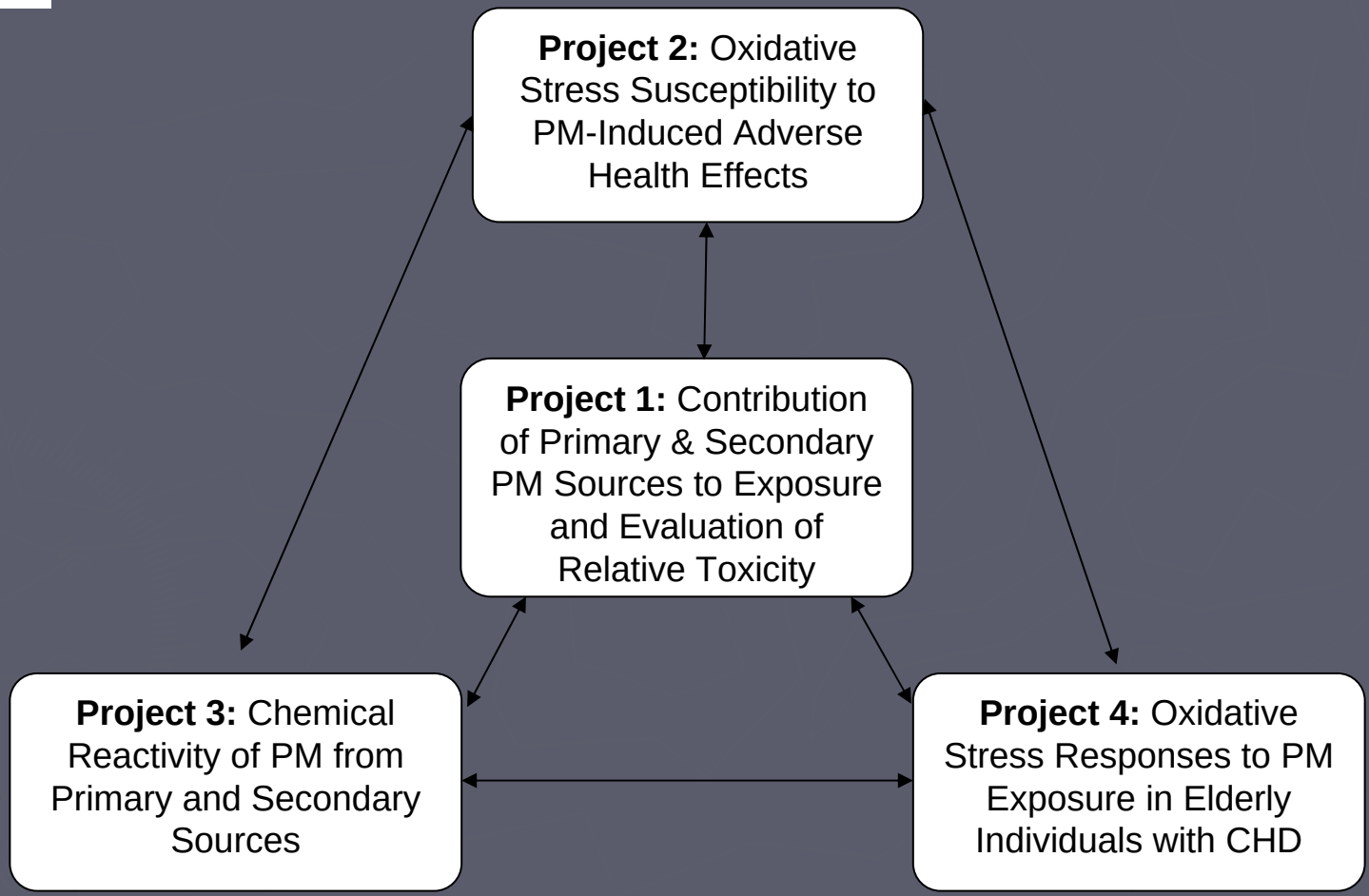

SCPC Hypotheses for PM Toxicity and Health Effects

- **Organic chemicals / metals on the PM matrix are responsible for toxicity and health effects**
- **The chemical species in PM act via two chemical mechanisms, redox and electrophilic reactions.**
  - **PM and constituents generate reactive oxygen species (ROS)**
  - **Thiols are a common target functional group associated with PM toxicity**
- **ROS or electrophilic chemistry → Oxidative stress (alterations in pro-oxidant / antioxidant ratio)**
- **Oxidative stress → pro-inflammatory effects**
- **Inflammation →enhancement of asthma, cardiovascular disease and other health outcomes; failure of antioxidant system enhances susceptibility**

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

Lead Investigator: Constantinos Sioutas
Co- Principal Investigator: Jamie Schauer

This project is an integral part of Projects 2, 3 and 4 , by serving as the field operations to collect PM samples for toxicity testing and for providing elevated levels of ambient PM for animal exposure models described in these projects.

Specific Aims

1. To determine the physical and chemical properties of PM emitted from different PM sources, including secondary formation, to evaluate how exposure to PM and the toxicity of PM from these sources vary with respect to location, season, and particle size.
2. To assess the contributions of these outdoor sources to indoor exposure and toxicity.
3. To determine the physical, chemical and toxicological characteristics of the volatile and non-volatile particle components that originate from mobile sources.
4. To measure the exposure gradients and intra-community variability of PM from such complex, unstudied sources such as airports and port activities on a local scale.

Aim 1. The Relationship Between PM Sources and Particle Characteristics, Including Toxicity

Investigate how contributions of the following PM sources:

- **Diesel traffic**
- **Gasoline traffic, including cold-start conditions**
- **Secondary Formation**
- **Road and Brake Dust**
- **Aircraft and Airport Activities**
- **Ships and Port Activities**
- **Residential Wood Combustion**

vary with respect to:

- **Upwind Source and Downwind Receptor Sites**
- **Season (Winter vs. Summer)**
- **Ultrafine, Accumulation, and Coarse Mode PM concentrations and chemistry**
- **Chemical reactivity, *in vivo* and *in vitro* PM toxicity (with *Projects 2 and 3*)**

Aim 2. Exposure to Outdoor vs. Indoor Sources

Determine how the relative contributions to indoor exposure of the following ambient and non-ambient sources:

- Diesel and gasoline vehicles
- Secondary Formation
- Road and Brake Dust
- Ambient and indoor residential wood combustion
- Indoor cooking
- Indoor house dust

vary with respect to:

- Particle size (coarse, accumulation, and ultrafine PM modes)
- Season, meteorology, home characteristics, and air exchange rates

Determine the implications of source contributions in terms of toxicity and health impacts (integration with Projects 2, 3, 4).

Aim 3. Toxicity of Semi-Volatile and Non-Volatile Components of PM from Motor Vehicles

A combination of ultrafine aerosol concentrators (VACES) and thermodenuder technologies can be applied to study the semi-volatile and non-volatile fractions of PM separately.

Conduct in vivo and in vitro toxicity studies using the VACES-thermodenuder tandem in:

- Dynamometer facilities—can test heavy duty, light duty, +/- PM filter traps, catalysts, etc.
- Roadway tunnels
- Near freeways– gasoline or heavy duty traffic

Aim 4. The Intra-Community Variability of Exposure to Different PM Sources

Determine intra-community variability of exposure to PM of different sizes and from different sources in a complex air pollution setting (Long Beach, CA), an urban study area of the USC Children's Health Study.

- Analyze archived filters from the Long Beach study for speciated organics by GC/MS and trace elements by ICP-MS.

- The additional data will allow a complete source apportionment by chemical mass balance (CMB) methods.

Chemical and Physical Characterization

Physical Characterization

Particle Size Distributions

Particle Number Concentrations

Particle Number Distributions

Chemical Characterization

Secondary ions - IC

Organic and elemental carbon (ECOC) – NIOSH 5040

Water soluble organic carbon - TOC

Trace metals - ICPMS

Water soluble trace metals - ICPMS

Iron oxidation state (Fe(II) and Fe(III)) – Spectrometric Methods

Molecular markers - GCMS

PAH and substituted PAH – GCMS and GC-NCI-MS

Macromolecules and HULIS - LCMSMS

Project 2: The Role of Oxidative Stress in the Susceptibility to PM-Induced Adverse Health Effects

- Lead Investigator: Andre Nel
- Costas Sioutas
- Ralph Delfino
- Mike Kleinman
- Jake Lusis
- Jack Harkema

Aim 1: The role of oxidative stress in PM-induced exacerbation of asthma and atherosclerosis in normal and genetically susceptible mice.

Asthma:

Premise: Fine and ultrafine particles exacerbate allergic airway inflammation, oxidative stress, IgE production, mucus hypersecretion, and airway hyperreactivity (AHR). The effects of PM may be augmented by weakened antioxidant defenses.

Approach:

- Low grade, alum-free OVA sensitization protocol in BALB/c mice, +/- exposure to concentrated particles.

- Use a similar protocol in Nrf2 knockout mice with weakened antioxidant defenses to determine whether ➔ augmentation of OVA-induced and PM-enhanced effects?

Aim 1, cont.

Atherosclerosis:

Premise:

PM synergize with oxidized LDL components to increase pro-inflammatory and apoptotic events that contribute to atherogenesis.

Approach:

Assess the dose-dependent exacerbation of atherosclerotic lesions in the aorta by CAPS in atherosclerosis-prone apoE knockout mice.

Endpoints: examine hearts for myocardial degeneration, inflammation, interstitial fibrosis and calcium deposition.

Aim 2: In vitro toxicology

1. Determine the effects of varying ambient PM composition and characteristics on induction of oxidative stress and inflammation in tissue culture macrophages, airway epithelial and endothelial cells.
    - Phase II enzyme induction
    - Induction of cytokine and chemokine expression
    - Perturbation of mitochondrial function

2. Compare the observed biological responses with:
    - Chemical composition of the particles
    - Markers of specific outdoor and indoor sources
    - Activity in chemical assays (Project 3)
    - Ability to promote asthma and atherosclerosis in animal models.

Aim 3: Use serum samples collected from indoor exposed elderly human subjects with ischemic heart disease (Project 4) to determine how oxidation of HDL affects the anti-inflammatory and anti-oxidative properties of this lipoprotein fraction.

Premise: PM-induced oxidative stress could enhance the pro-inflammatory and pro-atherogenic effects of oxidized LDL.

Approach:

- Assay the LDL fraction of project 4 serum samples for oxidized phospholipids and for their pro-inflammatory effects during coculture with monocytes.
- Determine whether PM-enhanced oxidation of HDL-associated paraoxonase leads to a decline in HDL anti-inflammatory effects, which in turn facilitates the pro-inflammatory effects of LDL.

Project 3: Chemical Reactivity of PM from Primary and Secondary Sources

Lead investigator: Arthur Cho
Co-P.I. John Froines
Yoshito Kumagai
Elinor Fanning

Specific aims

Objectives: estimate potential of PM to induce redox chemistry and oxidative stress, to study particle matrix and size effects on cellular uptake, intracellular disposition, and biotransformation of components, and to characterize chemical interactions

- Determine the redox and electrophilic properties ambient PM samples obtained in project 1.
- Fractionate PM to determine the roles of adsorbed species vs. the PM matrix in these chemical properties.
- Determine changes in properties of selected chemical species upon adsorption onto particle matrices.

Aim 1: Redox assays

- **Dithiothreitol (DTT) based redox activity.**
  - Consumption of DTT by electron transfer to oxygen through a catalytic process involving redox active organic compounds.
- **Ascorbate (Asc) based redox activity**
  - Consumption of Asc by electron transfer to oxygen through a catalytic process involving redox active metals and organic compounds.
- **Dihydroxybenzoic acids (DHBA):**
  - DHBA are formed from salicylate (SA) by reaction with hydroxyl.
  - Hydroxyl radical is generated by the Fenton reaction from hydrogen peroxide and a transition metal.
  - DHBA may provide a measure of transition metal based redox activity, whereas Asc alone may reflect all redox active species.

Aim 1: GAPDH as a nucleophile

- Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has a highly reactive thiol function essential to its catalytic action.
- Electrophiles such as acrolein and benzoquinone react with the thiol to irreversibly inactivate the enzyme in a time and concentration dependent manner.
- The catalytic activity of the enzyme is used to amplify the inactivation process.

Exploratory Aim: Biological markers for PM exposure assessment

- Key biological targets of PM reactive species include:
  - thiol groups on proteins
  - nucleic acids
  - mitochondria
- Goal: Develop quantitative exposure assessment tools that are based on PM chemical interactions with molecular targets, e.g. macromolecule binding.
- In addition to measurements of chemical concentrations, using biologically relevant dose measures provides a logical linkage between dosimetry and mechanism, biological downstream events and health outcomes.

Aim 2: Chemical reactivity of PM fractions

- Determine effects of extracting PM with:
  - Organic solvent (dichloromethane/acetonitrile)
  - Dilute acid (1 M HCl)
  - Artificial lung solution
- On
  - DTT redox activity
  - Asc/DHBA formation
  - GAPDH inactivation

Aim 3:
Matrix and adsorbed chemicals

- Carbon black as the matrix
- Absorption of reactive species:
  - Quinones
  - Transition metal salts
- Absorption of organic precursors:
  - PAHs
- Effect of matrix on the chemical properties measured in aim 1.
- Effect of matrix on cellular disposition

Project 4:

Oxidative Stress responses to PM exposure in elderly persons with coronary heart disease

Lead investigator: Ralph J. Delfino

Specific Aim 1

Examine relationships of PM exposures with circulating biomarkers of oxidative stress & antioxidant capacity.

- Exposure monitoring: $PM_{0.2}$, $PM_{0.2\text{-}2.5}$ & $PM_{2.5\text{-}10}$, and total PN daily at indoor and outdoor retirement home sites over five days prior to each weekly blood draw (4,320 person-days).
- Biomarkers:
  - oxidative stress: GSSG and 8-iso-PGF2α;
  - antioxidant capacity: GSH, activity of EC-SOD, erythrocyte SOD, and GPx-1;
  - balance of capacity and stress: ratio GSH/GSSG;

Specific Aims 2-3

Test hypotheses that associations with particle mass and number in Aim 1 will be better explained by:

2) PM exposures of outdoor origin and by certain markers of PM sources and components ($PM_{2.5}$ EC-OC, transition metals, source tracers for vehicular emissions and photochemical activity) *(linkage to Project 1)*;

3) Quantitative chemical markers for the formation of ROS and electrophilic activity from PM using *in vitro* bioassays of concentrated particle suspensions collected at indoor and outdoor home sites *(linkage to Project 3)*.

Exploratory Aims

- Evaluate relationships of biomarkers of oxidative stress to cardiovascular function (BP and ECG changes) and inflammatory biomarkers measured in the parent NIEHS study;

- To test association of PM and oxidative modification of LDL and HDL, leading to altered pro-inflammatory and anti-inflammatory properties, respectively *(Linkage to Project 2)*;

- To explore genetic susceptibility to oxidative damage by genotyping each study subject for polymorphisms likely involved in oxidative stress responses.

Design

- **Panel study**: repeated measures in 72 subjects:
  - age $\geq$ 65, living in a monitored retirement home, nonsmoking, Dx with CAD;
  - 2 panels of 36 subjects each
- 12 total weekly blood draws in each subject:
  - 6-wk photochemically active period (July-Sep);
  - 6-wk cooler period of air stagnation (late Oct-Jan).

Exposure Assessment

- Organic source tracers in indoor & outdoor PM (sources as in Proj.1)
- ROS/electrophilic activity of concentrated PM samples
- PAH/quinone content of concentrated PM samples
- All timed to coincide with blood samples for biomarkers (centrifuged, aliquoted and frozen within 30 min of draw).

NIEHS, ARB, AQMD & SCPC funded work

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Constantinos Sioutas, ScD, Phil Fine, PhD	Environmental engineering, source apportionment	USC
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Mike Kleinman, PhD	Air pollution toxicology	UCI
Christine E. McLaren , PhD	Biostatistics	UCI
Larry Jamner, PhD	Ambulatory physiology & stress	UCI
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Susan Neuhausen, PhD	Genetic Epidemiology	UCI
Steven George MD, PhD	eNO and inflammation	UCI

Summary of outcomes

1) characterized the physical/chemical characteristics of the wide range of sources and conditions in the LAB;

2) determined the toxicological potential of PM from the respective sources and the importance of varying physical/chemical characteristics, with special attention to fine and ultrafine particles;

3) developed insights into the underlying mechanism of PM toxicity in relation to its chemical components and physical makeup;

4) identified exposure-response relationships for toxicity in relation to a range of parameters;

5) determined the role of susceptibility through in vivo studies using genetically altered murine models and a panel study of elderly persons with CHD.