# San Joaquin Valley Health Effects Research Center (SAHERC)

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# Basic Problem

- Epidemiological studies show and association between particulate matter and increased morbidity and mortality
- What is it about particles that cause health effects?
- What health effects do they cause?

# Projects and Cores

- Projects
  - 1: Pulmonary Metabolic Response
  - 2: Cardiovascular Metabolic Response
  - 3: SJV Aerosol Inhalation Exposure
  - 4: Transport and Fate of Particles
  - 5: Architecture Development
- Cores
  - Animal Exposure
  - Particle Generation, Modification, and Characterization
  - San Joaquin Valley
  - Imaging
  - Bioanalytical

  - Quality Management
  - Administrative

# Pulmonary Metabolic Response

**Michelle Fanucchi, Charles Plopper, Alan Buckpitt**

Epidemiological studies have associated infant exposure to environmental particulate air pollution with increased morbidity and mortality.

We have demonstrated that postnatal animals are more susceptible to pulmonary injury by polycyclic aromatic hydrocarbons (PAHs).

The objective of this project is to determine whether increased neonatal vulnerability to bioactivated toxicants is exacerbated when PAHs are adsorbed to particulate matter.

# Aims and Approaches

- ***Overarching hypothesis****: The differentiating epithelium of the neonatal lung is more susceptible to particulate matter-induced pulmonary injury than the differentiated epithelium of the adult lung.*

- **Specific Aim 1**: Compare the pulmonary cytotoxic response of seasonal urban and environmental particulate matter in postnatal and adult rats.

- **Approach:** Environmental particulate matter will be collected. Postnatal and adult rats will be given a single intratracheal insufflation of known particle fraction. At 1 and 24 hr post-insufflation, lungs will be evaluated for: 1) particle clearance; 2) site-specific epithelial injury (permeability and tight junctions); 3) site-specific proliferation; 4)gene expression profiles; and 5)site-specific oxidative stress.

- **Specific Aim 2:** Compare the pulmonary cytotoxic response of a PAH alone and adhered to a particle in postnatal and adult rats.

- **Approach:** Synthetic particles of carbon and a PAH (*currently 1-nitronaphthalene*) will be generated. Postnatal and adult rats will be given a single intratracheal insufflation of the synthetic particle or an instillation of 1NN. At 1 and 24 hr post-insufflation, lungs will be evaluated for: 1) particle clearance; 2) site-specific epithelial injury (permeability and tight junctions); 3) site-specific proliferation; 4)gene expression profiles; and 5)site-specific oxidative stress.

- **Specific Aim 3:** Compare the pulmonary cytotoxic response of a PAH adhered to a carbon particle with a PAH adhered to a carbon particle containing a transitional metal in postnatal and adult rats.

- **Approach:** Synthetic particles of carbon, a PAH (currently 1-nitronaphthalene), and a transitional metal (*currently iron*) will be generated. Postnatal and adult rats will be given a single intratracheal insufflation of the synthetic PAH-carbon particle or th synthetic PAH-carbon-metal particle. At 1 and 24 hr post-insufflation, lungs will be evaluated for: 1) particle clearance; 2) site-specific epithelial injury (permeability and tight junctions); 3) site-specific proliferation; 4)gene expression profiles; and 5)site-specific oxidative stress.

- **Specific Aim 4:** Compare the pulmonary cytotoxic response of a PAH adhered to a carbon particle containing a transitional metal in postnatal and adult rats with and without oxidant stress exposure.

- **Approach:** Synthetic particles generated in Sp Aim 3 will be used. Postnatal and adult rats will be exposed to ozone or filtered air for 90 days. Immediately following the last ozone exposure, the rats will be given a single intratracheal insufflation of the synthetic PAH-carbon particle or the synthetic PAH-carbon-metal particle. At 1 and 24 hr post-insufflation, lungs will be evaluated for: 1) particle clearance; 2) site-specific epithelial injury (permeability and tight junctions); 3) site-specific proliferation; 4)gene expression profiles; and 5)site-specific oxidative stress.

# ENDOTHELIAL CELL RESPONSES TO PM *IN VITRO* AND *IN VIVO*

Dennis Wilson and Jack Rutledge

Associations between episodes of particulate matter (PM) air pollution and hospital admissions for cardiopulmonary disease have been documented worldwide.

Estimates suggest 70% of the increase in cardiac deaths are due to myocardial infarction. Recent studies emphasize the potential importance of systemic circulation of ultrafine particulate air pollution.

The objective of this project is to determine the effects of ultrafine particulates on endothelial and vascular inflammatory responses and evaluate the potential association between atherosclerotic vascular disease and circulating PM.

# Aims and Approaches

- **Overarching hypothesis**: Systemic effects on the cardiovascular system are associated with endothelial cell responses leading to activation of inflammatory or clotting cascades. Circulating PM selectively accumulates in regions of the vasculature with endothelial compromise and stimulates the progression of pre-existing vascular disease.

- **Specific aim 1**: To characterize human endothelial cell culture responses to direct CAPs exposure.

  **Approaches**

  - Microarray analysis of PM exposed human endothelium
  - RT-PCR quantitation of Target genes associated with Inflammation
  - $Ca^{++}$ release responses to PM
  - Examine nuclear translocation of second messengers

- **Specific aim 2**: To determine the effects of direct PM exposure on permeability and inflammatory cell adhesion in vessels

  **Approaches**

  - Endothelial monolayer permeability responses to PM
  - Monocyte adhesion responses to PM

# Aims and Approaches

**Specific aim 3**: To compare the nature and location of endothelial cell responses in vessels of CAPs exposed mice.

**Approaches**

- Immunohistochemistry for pro-inflammatory protein expression in CAPs exposed mice
- Laser capture microdissection of arteries with RT-PCR of response genes identified in aim 1

**Specific Aim 4)** To determine the effects of CAPs exposure on the progression of preexisting vascular disease in apolipoprotein E (ApoE -/-) deficient mice.

**Approaches**

- Monocyte adhesion in isolated carotid arteries from CAPs exposed normal and ApoE -/- mice
- Laser capture microdissection of atheromatous lesions from CAPs exposed ApoE -/- mice

# Inhalation Response of SJV Aerosol

**Kent Pinkerton, Mike Kleeman, Ann Bonham**

Preliminary epidemiological evidence suggests that cardiac mortality in the SJV is strongly correlated with PM10.

We have characterized the spatial and temporal variability of the size and composition of airborne particles in the SJV.

The objective of this project is to determine how variation in particle concentration, size and/or composition affects health outcomes.

# Aims and Approaches

- ***Overarching hypothesis****: the size and composition distribution of airborne particles affects their health outcomes through different:*
  - *(1) mechanisms of oxidative stress, and*
  - *(2) impacts on heart rate variability.*
- **Specific Aim 1**: To test whether the differences in particle size and composition that occur naturally in the SJV as a function of location and season have an effect on health outcomes.
- **Approach:** Mice will be exposed to concentrated airborne particles at an urban and rural location in the SJV during the summer and winter. Heart rate variability and markers for oxidative stress will be monitored. Samples of airborne particles will be collected at the same time so that particle size and composition can be correlated with health outcomes.

- **Specific Aim 2:** To determine the source of particles used in exposure experiments in specific aim 1.
- **Approach:** Chemical "fingerprints" for particles released from different sources will be used to determine the sources of ambient particles collected in Specific Aim 1 that are correlated with severe health outcomes.

- **Specific Aim 3:** To test whether exposure to freshly emitted particles from individual sources causes the same health outcomes as exposure to a mixture of aged particles.
- **Approach:** Mice will be exposed to freshly emitted particles from the sources that dominated their exposure during specific aim 1. Heart rate variability and markers for oxidative stress will be monitored. Samples of airborne particles will be collected at the same time so that particle size and composition can be correlated with health outcomes.
- **Specific Aim 4:** To identify the specific particle size and composition that causes negative health outcomes.
- **Approach:** Plausible mechanisms relating negative health outcomes to particle size and composition will be tested using laboratory particles that mimic features of the particles released directly from sources.

# Transport Mechanisms and Systemic Fate of Inspired Ultrafine Particles

Dennis Wilson, Angelique Louie, Ian Kennedy, Michelle Fanucchi, Alan Buckpitt

Recent evidence demonstrates that ultrafine particles diffuse rapidly from the lungs into the systemic circulation.

The mechanisms of ultrafine transport to the systemic circulation and tissues remain largely uncharacterized.

The objective of this project is to determine the effects of size and charge on the time course, distribution and mechanisms of accumulation of PM in circulation and tissues of animals with normal and altered lung structure.

# Aims and Approaches

- **Overarching hypothesis**: Trans-epithelial movement of inhaled PM results in accumulation in target organs based on endothelial cell facilitated transport.

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

  **Approaches**

  - Kinetics of Indium labeled particulates after insufflation
  - Distribution within components of blood
  - Effect of size and charge on absorption and distribution

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

  **Approaches**

  - PET of dextran coated iron oxide particles conjugated to $Cu^{64}$ and labeled with fluorochrome
  - Quantitative distribution of fluorescent particles after inhalation exposure using plastic embedded tissues from target organs
  - Comparison with rats given lifetime $O_3$ exposures

# Aims and Approaches

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

**Approaches**

- Confocal and deconvolution microscopy of HAEC and BEAS2b cells exposed to fluorescent particles of varying size and charge
- Determine effects of inhibitors of specific pathways of endothelial facilitated transport.

**Specific Aim 4)** To characterize the dynamics of interaction between particulates and airways and arterial walls.

**Approaches**

- Fluorescent PM uptake in isolated tracheas and carotid arteries from normal mice
- Tissue localization in plastic embedded sections by confocal microscopy

# Developmental Response and Particle Deposition


## Tony Wexler and Charlie Plopper


Epidemiological evidence suggests that children exposed to air pollution develop impaired lungs.

We have observed alterations in lung architecture in monkeys exposed to ozone during development.

The objective of this project is to quantify the amount and time course of pollutants that lead to these architectural abnormalities and their functional implications.

# Aims and Approaches

- ***Overarching hypothesis****: Exposure of young children to air pollutants during critical windows of postnatal airway development*
  - *(1) compromises airway growth and alters airway architecture, which:*
  - *(2) diminishes lung function due to the development of non-optimal airway architecture and*
  - *(3) shifts intrapulmonary particle deposition patterns due to altered flow rates and airway geometries.*
- **Specific Aim 1**: To test whether the normal pattern of dysanaptic growth of airways in neonates alters airway architecture and patterns of airflow from that in adults.
- **Approach:** Neonates will breathe clean, filtered air during development. Lung function tests will be performed. Their lungs will be fixed, excised, and casted. The casts will be imaged to reveal the lung architecture, which will be compared at sequential stages of development.

- **Specific Aim 2:** To test whether dysanaptic postnatal growth alters the deposition of inhaled particles within the respiratory tract of infants and young children as they grow.
- **Approach:** Neonates and adults will be exposed to different-sized particles, whose deposition pattern will be imaged. Mathematical models of particle deposition will be developed to predict these patterns and identify the cause for the deposition patterns.

- **Specific Aim 3:** To test whether exposure to oxidant air pollutants during critical phases of airway growth compromises postnatal airway growth.
- **Approach:** Neonates will be exposed to ozone for various time courses related to periods of rapid growth. The approaches in Aim 1 will be used to identify the architecture, which will be compared to normals characterized in Aim 1 to quantify their variation and deviation from the norm.

- **Specific Aim 4:** To test whether exposure to particles and ozone during critical phases of airway growth compromises growth to a greater degree than exposure to particles or ozone alone.
- **Approach:** Neonates will be exposed to a range of particle sizes, compositions, and morphologies, with and without ozone, as a function of stage of development. The approaches and normals from Aim 1 will be used to identify the architecture and alteration due to exposure. Comparing these results to those obtained in Aim 3 will elucidate the hypothesis.

- **Specific Aim 5:** To test whether compromised airways produce altered patterns of intrapulmonary particle distribution and deposition.
- **Approach:** Normal adults and those exposed to pollutants during development will be exposed to different-sized particles, whose deposition pattern will be imaged. Mathematical models of particle deposition will be developed to predict these patterns and identify the cause for the deposition patterns, similar to Aim 2.

# Project Timetable