

Dr. John Froines

Background

- In 1997 EPA developed the standard for airborne particulate matter (PM 2.5) but given scientific uncertainties the National Research Council, 1997 issued a report; *Research priorities for airborne particulate matter (PM)* calling for additional research
- Uncertainty due to limited scientific information about
 - specific types of particles and composition causing adverse health effects
 - contributions of particles to actual human exposures
 - mechanisms to explain the findings of mortality/morbidity associated with PM:
- As a result U.S. EPA established 5 Particle Centers to conduct research on the issues surrounding PM of which one was the Southern California Particle Center



Southern California Particle Center

Director: John R. Froines, Ph.D.

Co-Directors:

Constantinos Sioutas, Ph.D.

Andre Nel, M.D., Ph.D.

Funding
U.S. EPA
ARB
SCAQMD

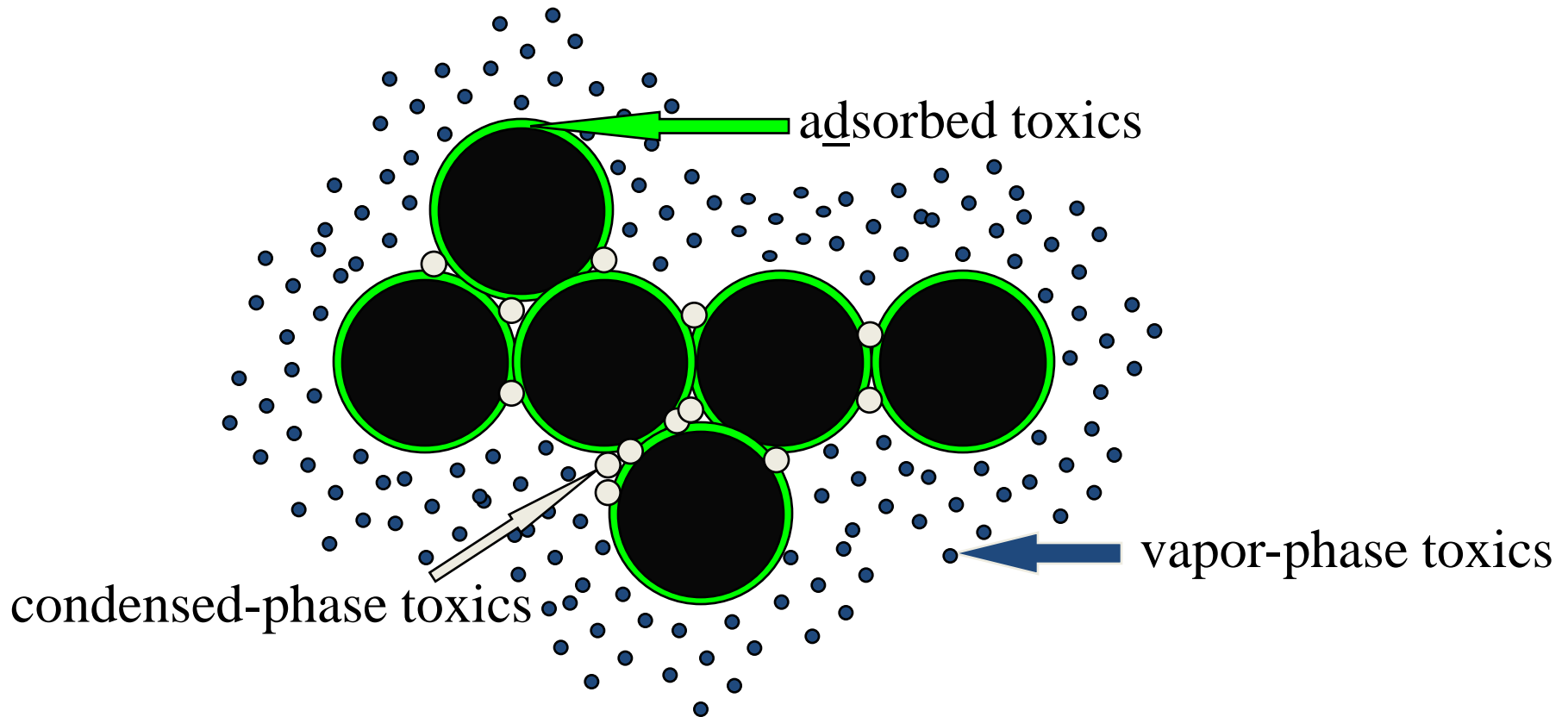
- University of California, Los Angeles
- University of Southern California
- University of California, Irvine
- Michigan State University
- University of Wisconsin-Madison
- University of Tsukuba, Japan



Southern California Particle Center

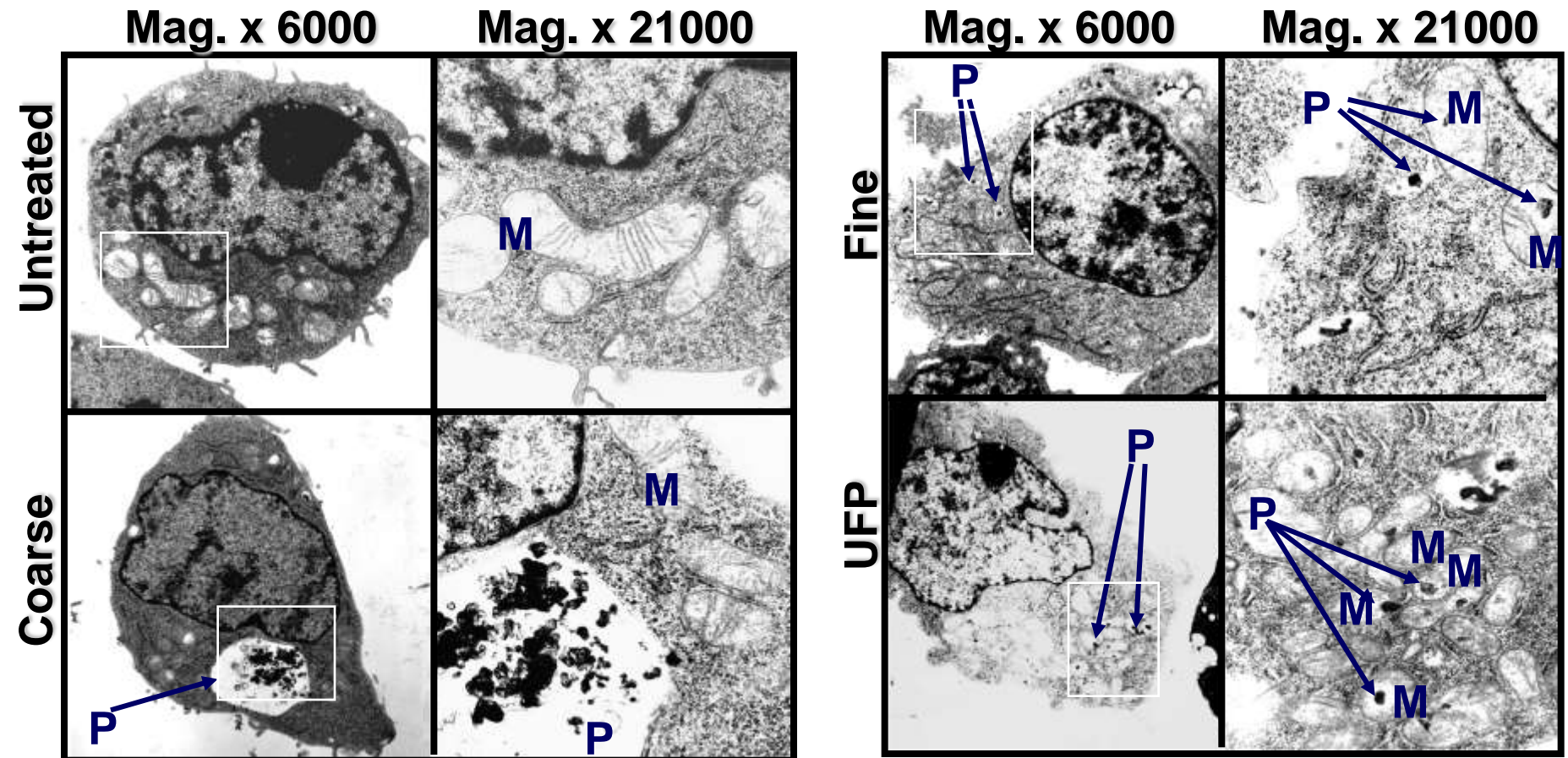
- **The overall objective is to determine the underlying basis for health effects from airborne particulate matter (PM) and vapor co-pollutants.**
- **PM and vapors from mobile sources is a major foci**
- **Determine the physical and chemical properties of PM and vapors emitted.**
- **Identify new adverse health effects and the underlying mechanisms of action.**

Aerosol Toxics



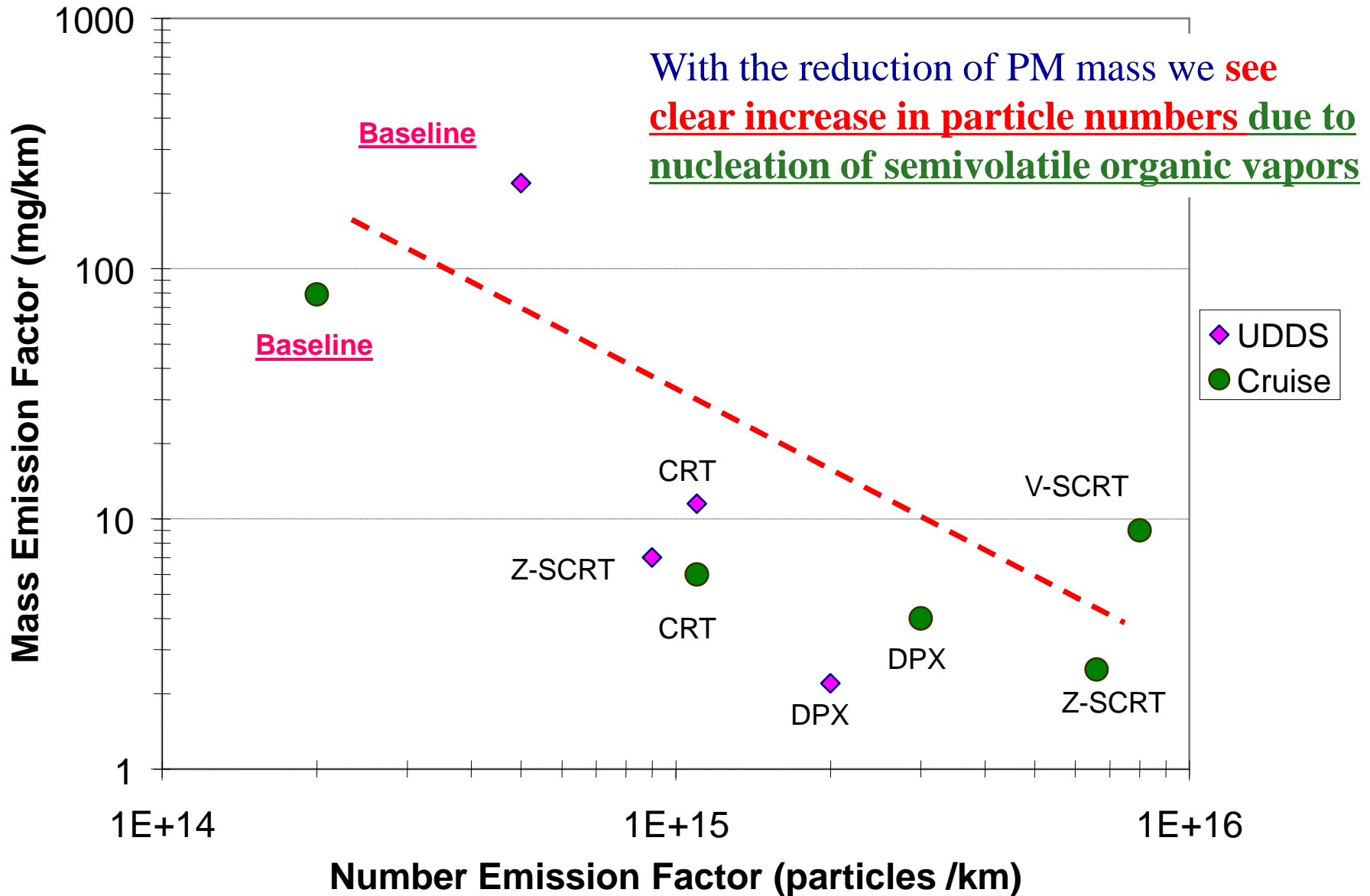
Ultrastructural analysis of lung tissue found inhaled ultrafine particles were located within the epithelial barriers, cytoplasm, mitochondria and the nucleus of cells.

Mitochondria: An Important Subcellular Target of PM and a Source of ROS Generation



RAW 264.7

Particle Number vs Mass Emission Factors (EF) from Older and Newer Diesel Trucks



Biswas et al. Atmos. Environ, 2008

Introductory comments

- The operating hypothesis of our work has been that there are two major chemical reactions that initiate a process that results in adverse health effects.
 - not chemical specific;
 - result of chemical properties of many chemicals
- This mix of reactive chemicals is the “soup” to which we are exposed as part of the ambient air.
- Instead of measuring a specific chemical entity, we measure the total toxicity of each air sample, using a group of assays that are important.

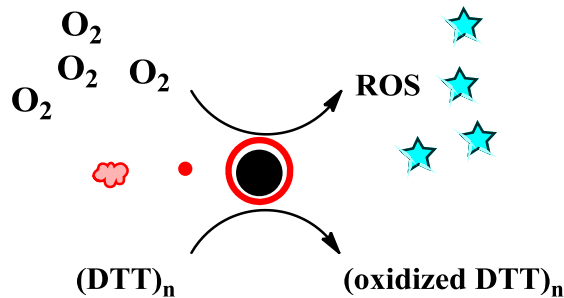
Introductory comments

- The two reactions are:
 - Prooxidant activity: This activity reflects the ability of the sample to generate reactive oxygen species (ROS), which then induce a state of oxidative stress in cells, leading to the inflammatory response.
 - Electrophilic activity: This activity reflects the ability of reactive compounds in the sample to form, irreversible bonds with proteins and DNA. The resulting proteins are irreversibly modified and lose their normal functions.
 - Inflammation in the heart, lung, brain are key to disease or illness development and derive from the two reactions above

Events leading to toxicity

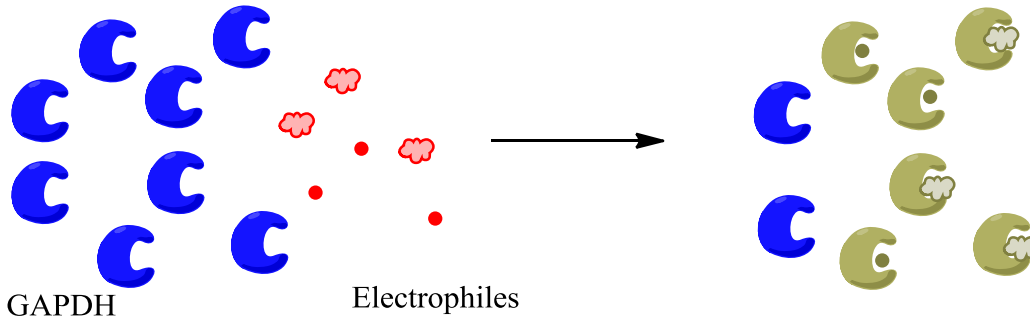
Chemical events		
	Prooxidant	Electrophile
Chemical reaction	Oxidation of biological macromolecule	Covalent modification of biological macromolecule
Cellular events		
	Consequences of protein modification	
Pathways	Proinflammatory	Anti-inflammatory, protective
Regulatory proteins	Protein tyrosine phosphatase, IκB	Keap1
Relevant transcription factors	NFκB, AP-1	Nrf-2
Early expression products	IL6, TNFα	HO-1, GSH synthases
Tissue effects		
Response	Inflammation	Removal of reactive chemicals
Consequences	Exacerbation of pulmonary and cardiovascular disease	Protection against chemical insults by inactivation reactions

Assays



Loss of DTT/min = formation of ROS/min

Rate is proportional to prooxidant content



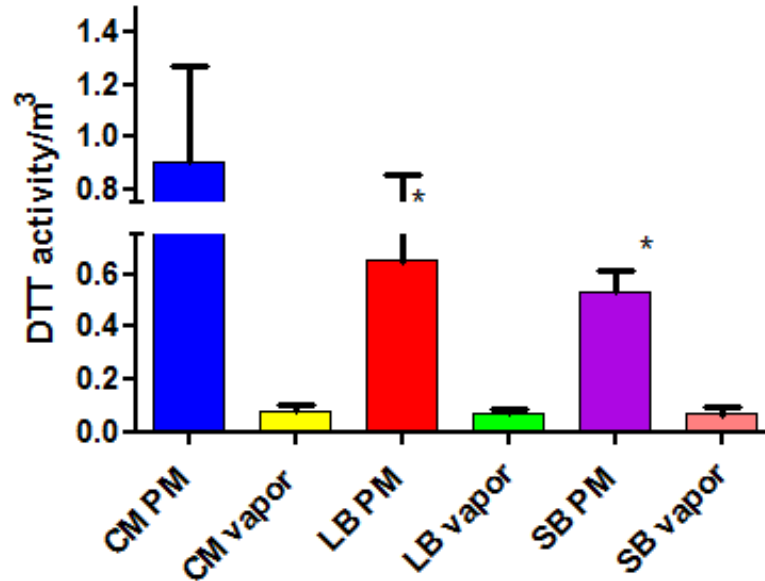
Loss of active protein is proportional to electrophile content

1. The DTT assay is dependant on a chemical reaction analogous to that generating ROS by pollutants.
2. Prooxidants can be metals or organic compounds; the contribution of metals is blocked by DTPA.
3. The GAPDH assay uses a particularly reactive protein as a target for electrophiles which inactivate the enzyme.
4. These assays are performed in cell free conditions, so there are no limitations of cellular access such as those shown earlier.

AQMD Railyard Study

- **Use quantitative chemical and cellular tests to describe the toxicological properties of air samples collected in residential neighborhoods close to four most polluted rail yards.**
- To assess the ability of railyard emissions to activate inflammatory pathways
- Determine the cellular effects of the air samples
- Engage community residents living near 4 rail yard facilities to determine sampling locations and share research results to develop community awareness

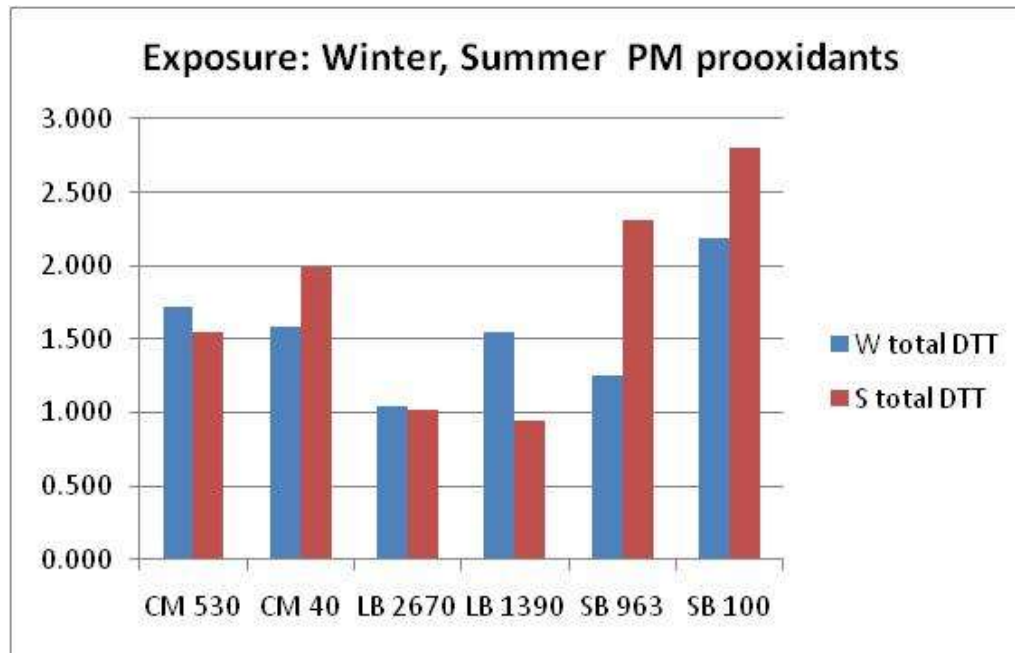
Prooxidant content in particles and vapors



* Significantly different from CM particle activity

1. When the data from each site is pooled, the prooxidant content can be statistically analyzed.
2. Air samples from Commerce have significantly higher prooxidant content than do Long Beach and San Bernadino. Long Beach content may be higher than San Bernadino.
3. Most (>90%) of the prooxidants are found in the particle fraction.

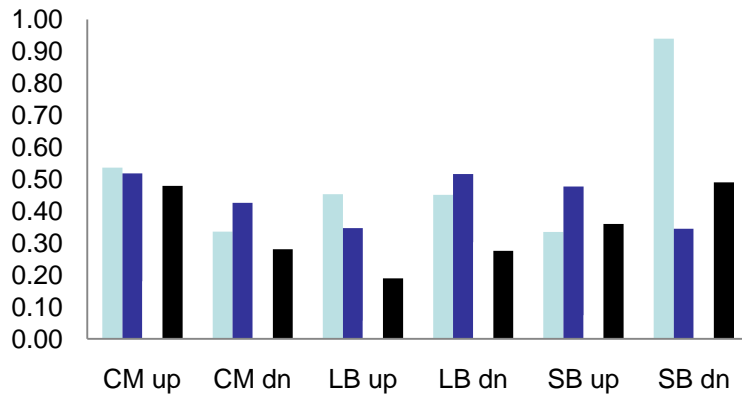
Total exposure to PM prooxidants.



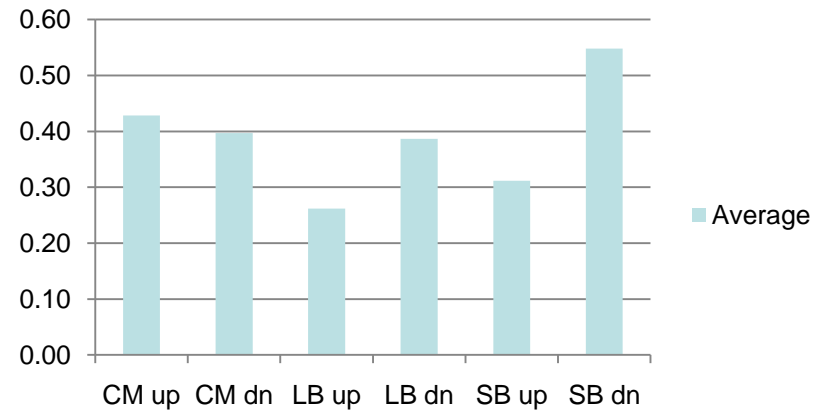
1. There is clear evidence for prooxidant activity which will lead to health effects
2. Atmospheric chemistry occurs and the result appears to be increased toxicity of the air pollution

Filter DHBA data

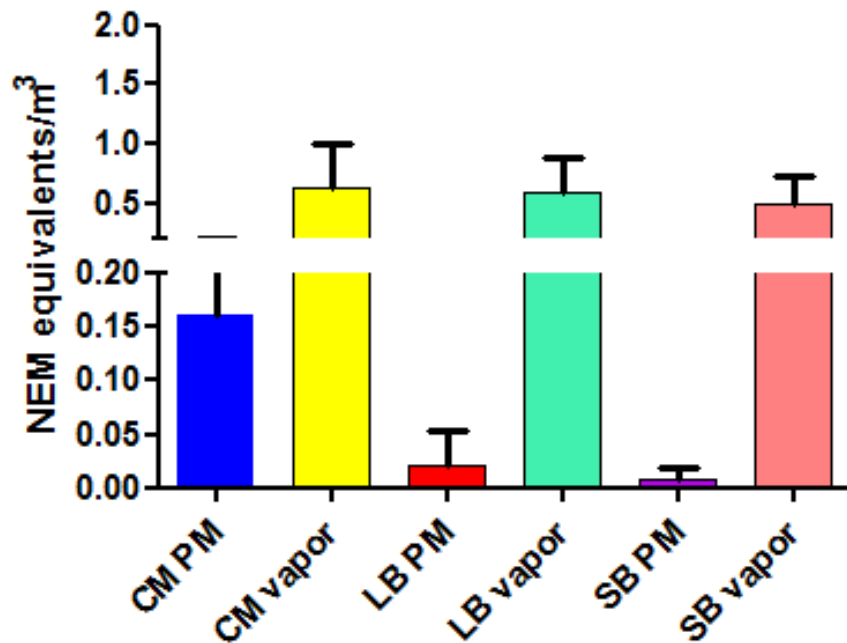
DHBA activity



DHBA Averages

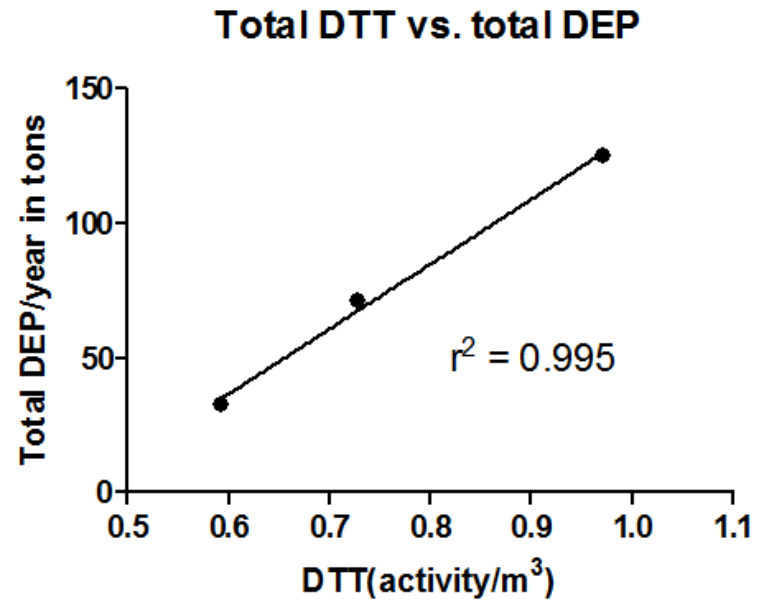
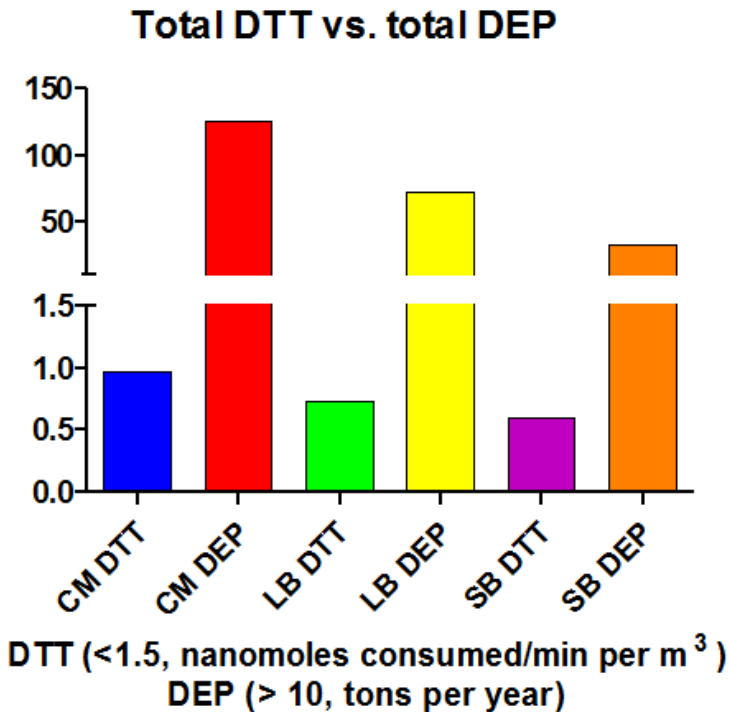


Electrophile content of particles and vapors



Most of the electrophiles are found in the vapors. CM particles appear to have more electrophiles than LB or SB, but the vapor content of all air samples is about the same.

Comparison of total prooxidant content of winter air samples and total diesel exhaust particles emitted per year



Total particle (Fine/ultrafine) prooxidant content is proportional to total diesel exhaust emissions, from trucks and locomotives near the railyards.

Health research findings of the research center

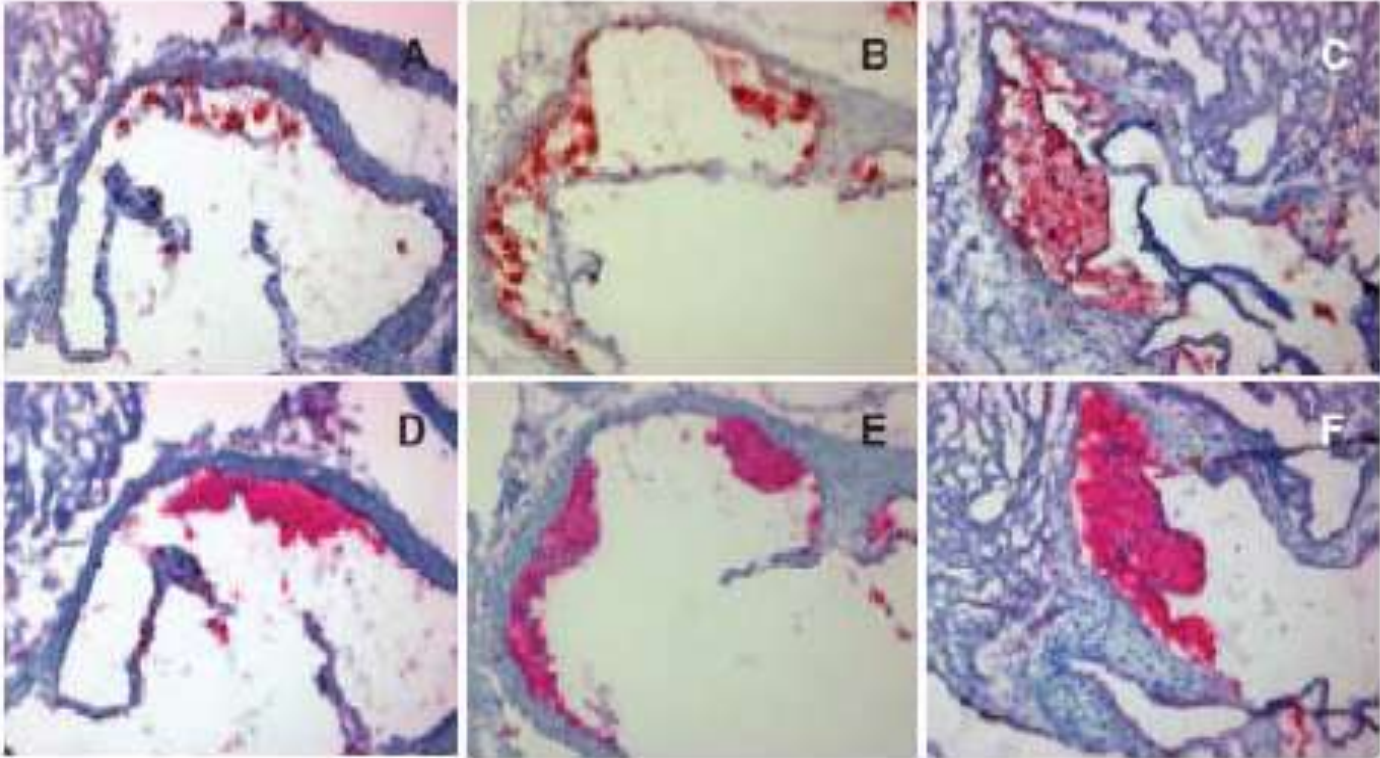
- Cardiovascular disease-Ultrafine particles (UF)
- Exacerbation of allergic airway disease-UF PM
- Asthma-distance from roadway
- Neurological effects
- Low birthweight/preterm birth and possible birth defects
- Theory of disease process

Aortic lesions-UFP result in most significant impact with

FA

FP

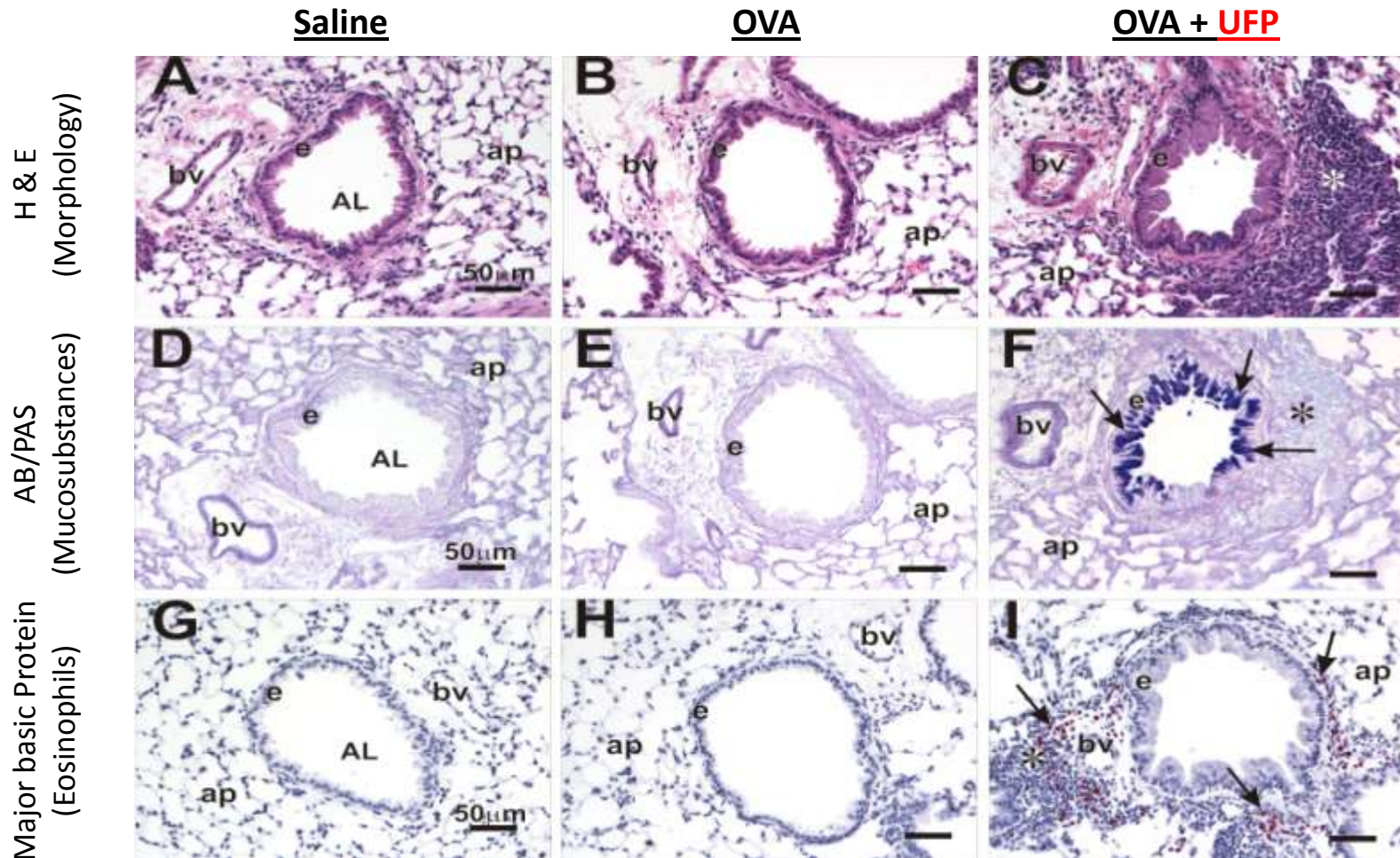
UFP



Conclusions for the blood pressure study

- **Air pollutant particles are associated with increased BP in elderly subjects with coronary heart disease.**
- **Strongest associations were for multi-day exposure suggesting longer-term impacts on vascular function.**
- **The stronger OC associations appear to be attributable to the primary products of fossil fuel combustion.**

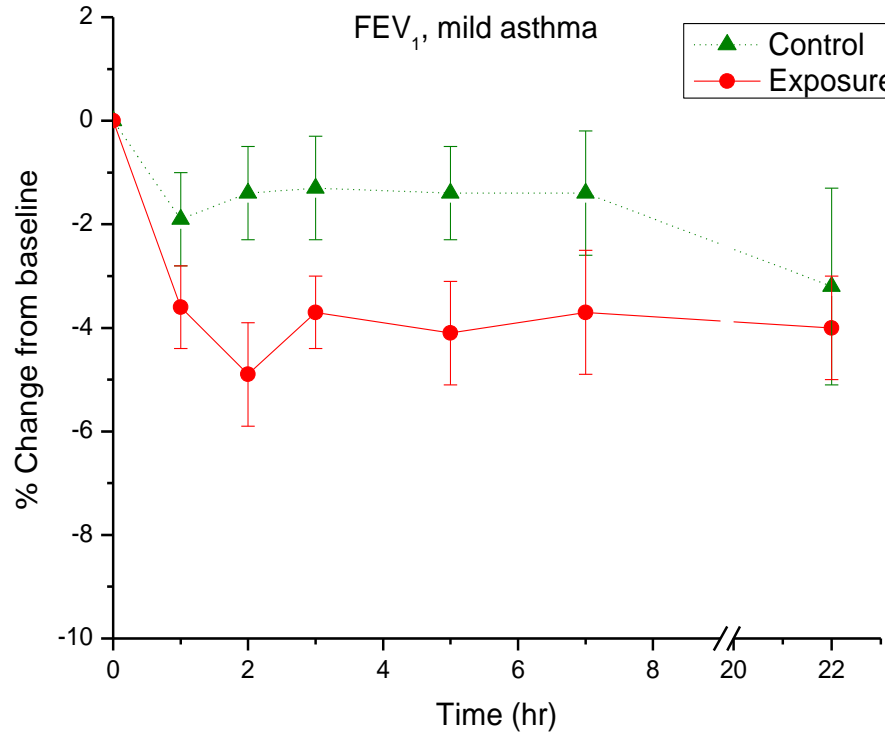
Animal Model to Study PM Effect on Allergic Sensitization



Summary

- ❖ UFP are capable of acting to enhance the primary allergic sensitization response to experimental allergens
- ❖ Inhalation of “real-life” UFP could lead to a profound allergic inflammation deep in the lung in previously sensitized animals

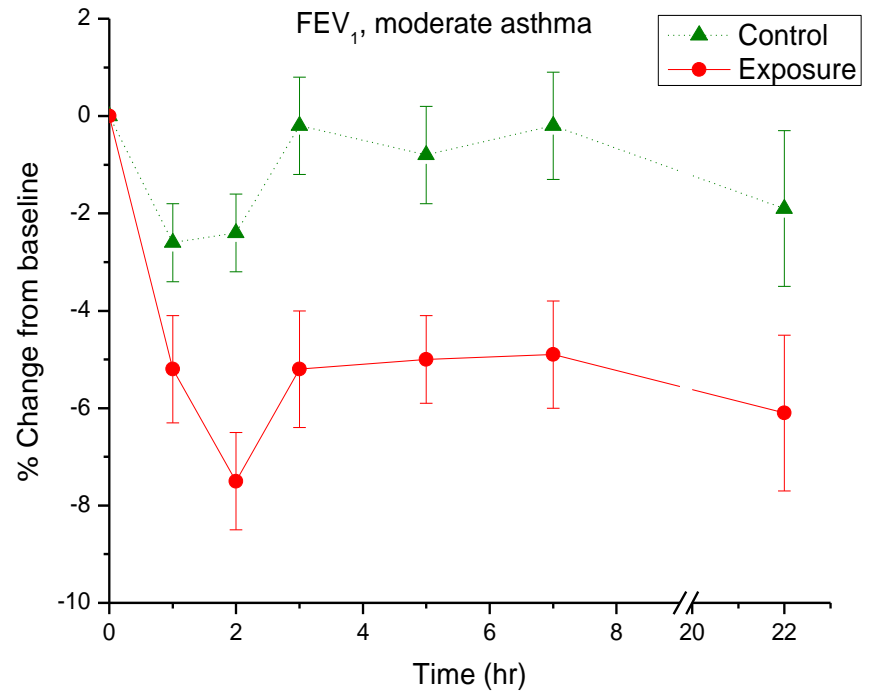
Mean Change from Baseline for FEV₁ (FEV 1 is the amount one can breathout in one second)



d.f=(6, 272)

F=0.86

p=0.525



d.f=(6, 244)

F=1.84

p=0.093

Adverse Effects on Lung Function and Airway Inflammation

The reduction in lung function (FEV_1 and FVC (5 seconds)) was accompanied by increased airway inflammation

We consider inflammation of the lung and heart to be fundamental issues in disease and illness especially lung and cardiovascular

We have shown the pathway (roadmap) from exposure to changes in cells to inflammation and health effects

Findings To Date

1. Prooxidants and quinones are present in ambient air and in diesel exhaust particles, together with their precursors.
2. Electrophiles are also found in ambient air and diesel exhaust. They generate protein adducts which alter the cell's homeostasis or balance.
3. Prooxidants and electrophiles have been found and their levels determined in samples from sites associated with railyards.
4. Reactive organic species such as quinones are generated directly from exhaust and formed from precursors in atmospheric reactions as a given air parcel moves across the Los Angeles Basin.
5. Induction of the protective protein, hemeoxygenase-1 (HO-1), and activation of signaling pathways that lead to the expression of inflammatory cytokines is caused by air pollutants and can be mimicked by quinones, organic air pollution components formed by combustion and atmospheric chemistry.
6. The content of prooxidants in ambient and diesel particles correlates with the induction of HO-1 in macrophages.
7. Electrophiles in air pollution mixtures including both particles and vapors induce HO-1 and activate downstream signaling pathways.