Effects of Inhaled Particles in A Sensitive Animal Model

9/19/02

Presentation for the Chairman's Seminar Series

California Air Resources Board

Michael T. Kleinman

University of California, Irvine

Outline

- Brief History
  - Air Pollution Health Effects Lab
  - Kleinman
  - Development of Research Program
- Key ARB Studies
  - Chemical Components
  - Acid Aerosols
  - Diesel ± O3 ± Acids
  - PM10 and PM2.5 Components

Outline (cont.)

- Current Studies
  - Effects of Ultrafine (UF), Fine (F) and Coarse (C) Particles on Cardiopulmonary Responses
  - Effects of Concentrated F and UF Real-World Particles on Development and Exacerbation of Respiratory System Allergies.

Background

- The Air Pollution Health Effects Laboratory built at UCI with predominant support from ARB (~1976).
- Grew rapidly into a strong, multidisciplinary research facility.
  - Crocker – MD and Pulmonary Biologist
  - Phalen – Aerosol Physics
  - McClure – Histopathologist
  - Walters – Engineer
  - Reischel and Mautz – Respiratory Physiologists
  - Bhalla – Cell Biologist
  - Kleinman – Inhalation Toxicologist

Background - Kleinman

- USAEC Health and Safety Laboratory
  - Atmospheric Transport of Weapons Debris
  - Radioactivity and Heavy Metal Contamination of Food (National) and Soil (Global).
- NYU Institute of Environmental Medicine
  - Source Apportionment Method for NYC

Background – Kleinman (cont.)

- Rancho Los Amigos Hospital
  - Particle Exposure and Characterization
  - Human Exposure Studies – Complex Mixtures and Acid Aerosols.
- UCI
  - Human Exposure Studies (Carbon Monoxide)
  - Rodent Studies (Complex Mixtures, PM)

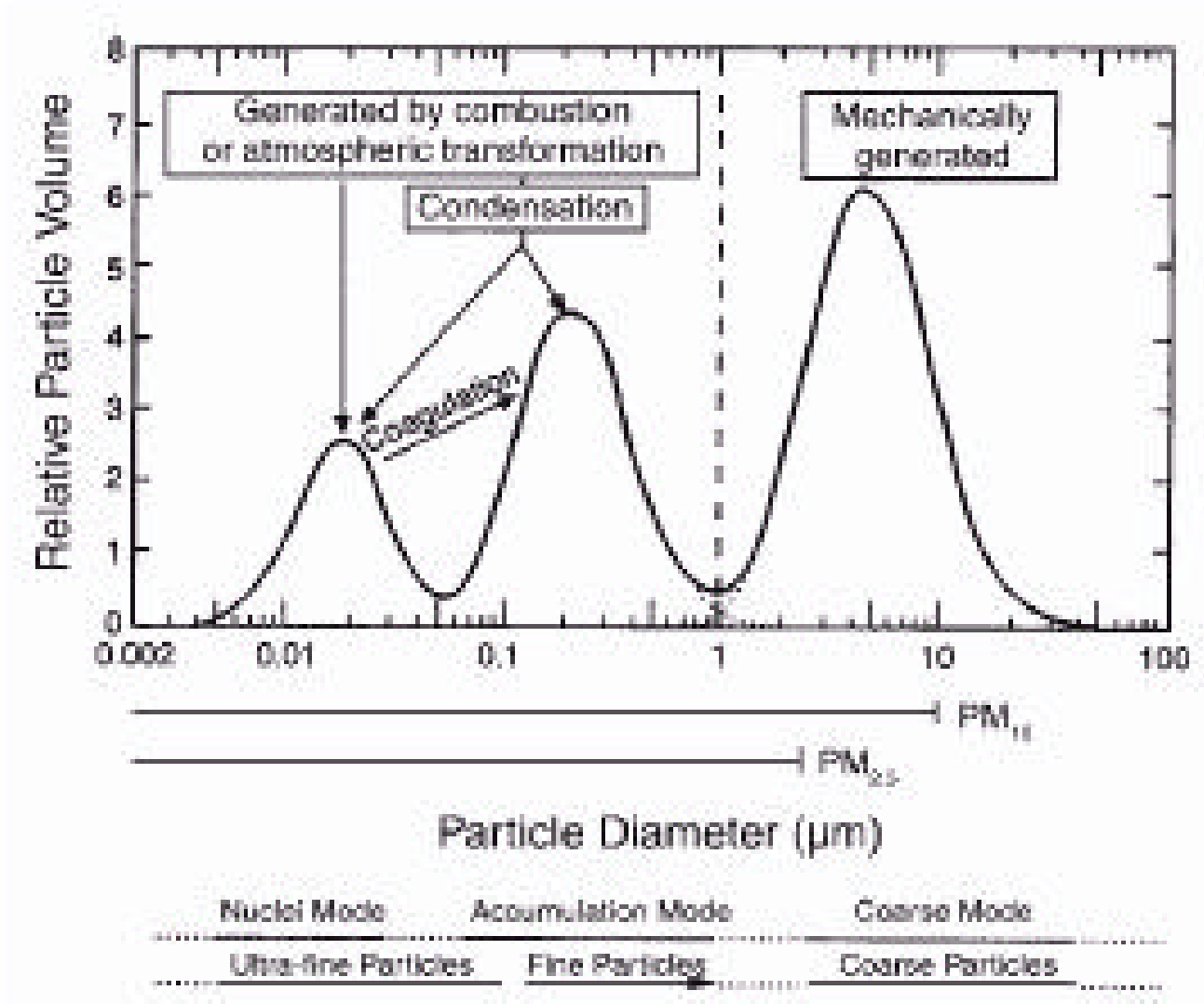
Effects of Ultrafine (UF), Fine (F) and Coarse (C) Particles on Cardiopulmonary Responses

. Recent epidemiological and toxicological studies have associated particle inhalation with human morbidity and mortality, injury to the lung and changes in cardiac functions.

. Ambient particles are a complex mixture, both with respect to composition and size.

. Particle size may play a role in how effects on health are mediated.

Three Size Modes of Particles in Ambient Air Originate from Different Processes.



Concentrations Used Were Greater Than Current Ambient Air Standards for PM

PM Standards

	Time Period	PM_{10} ($\mu g/m^3$)	$PM_{2.5}$ ($\mu g/m^3$)
United States EPA[a]	Daily	150	65
United States EPA[a]	Annual	50	15
California	Daily	50	25 (Proposed 2002)
California	Annual	20	12 (Proposed in 2002)
European Union[b]	Daily	50	Not set
European Union[b]	Annual	20	Not set

[a] Under revision.

[b] To be met in 2010 (to be reviewed in 2003).

Acute Particle-Related Health Effects

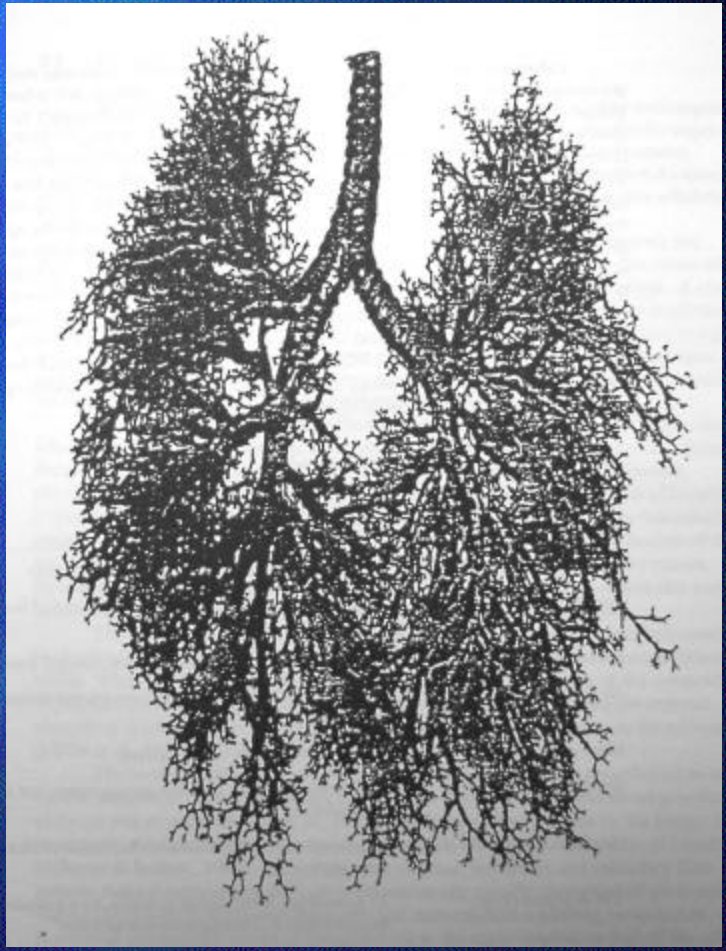
Table 4.6 Estimated acute respiratory health effects of PM_{10}

	Effects [%] of 10 μg/m³ PM_{10} increase (daily mean)
Increase in daily mortality	
Total mortality	1.0
Respiratory mortality	3.4
Cardiovascular mortality	1.4
Increase in hospital usage (respiratory)	
Admissions	0.8
Emergency visits	1.0
Asthma exacerbations	
Asthma attacks	3.0
Bronchodilator use	2.9
Emergency department visits	3.4
Hospital admissions	1.9
Increase in respiratory symptoms	
Lower respiratory symptoms	3.0
Cough	1.2
Upper respiratory symptoms	0.7

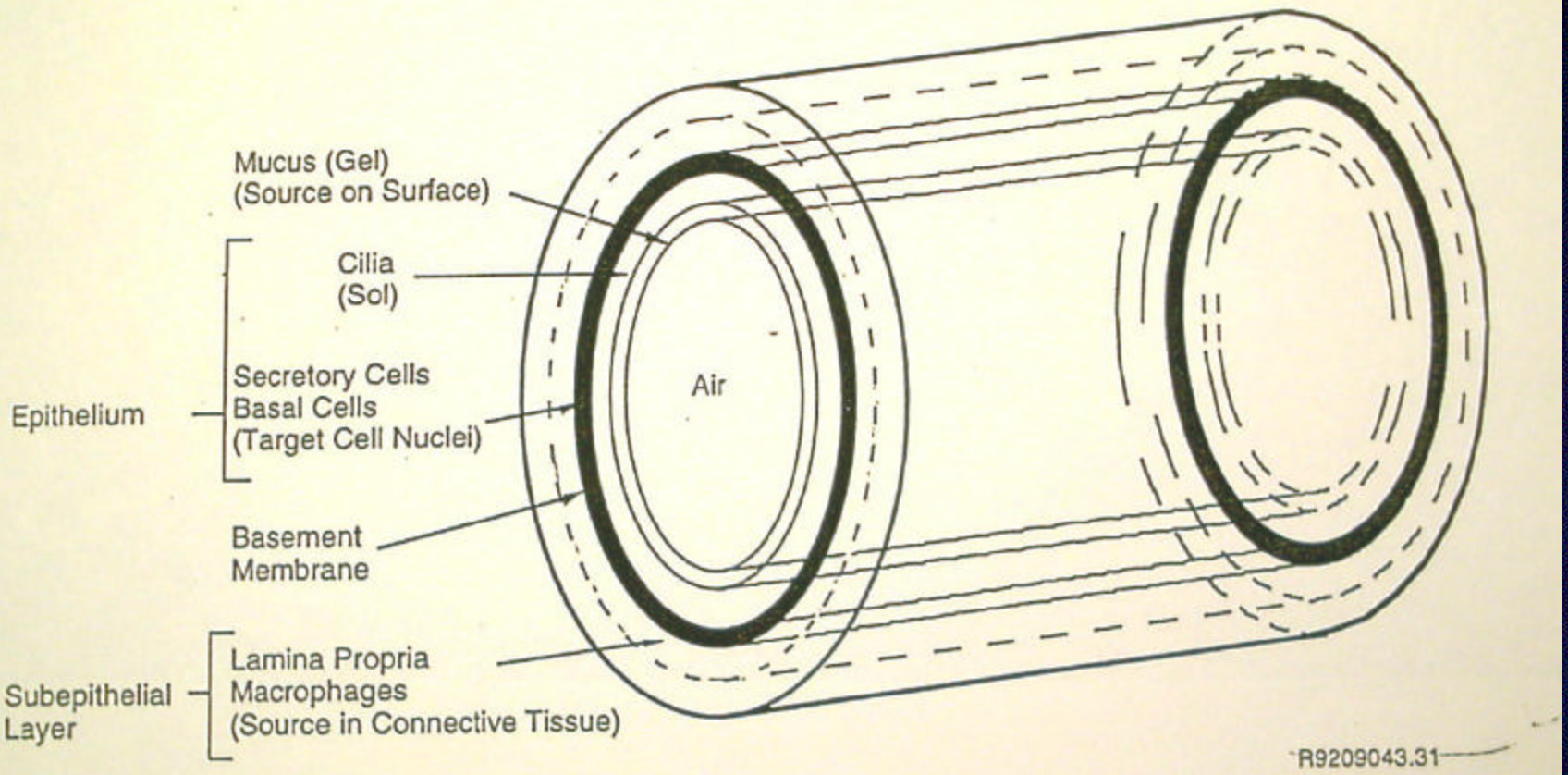
- Acute effects range from mortality to exacerbation of symptoms.
- The most sensitive are those with chronic pre-existing disease.
- Elderly people and children are also sensitive.

Understanding Particle-Induced Health Effects Requires an Understanding of the Lung

- The human lung is a complex, branching structure.
- The structure is also complex at the cellular level .
- This complexity results in differential sensitivity to particles.



A Typical Airway



Characteristics of Asthmatic Airways

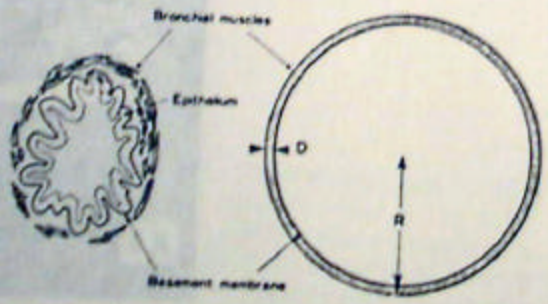
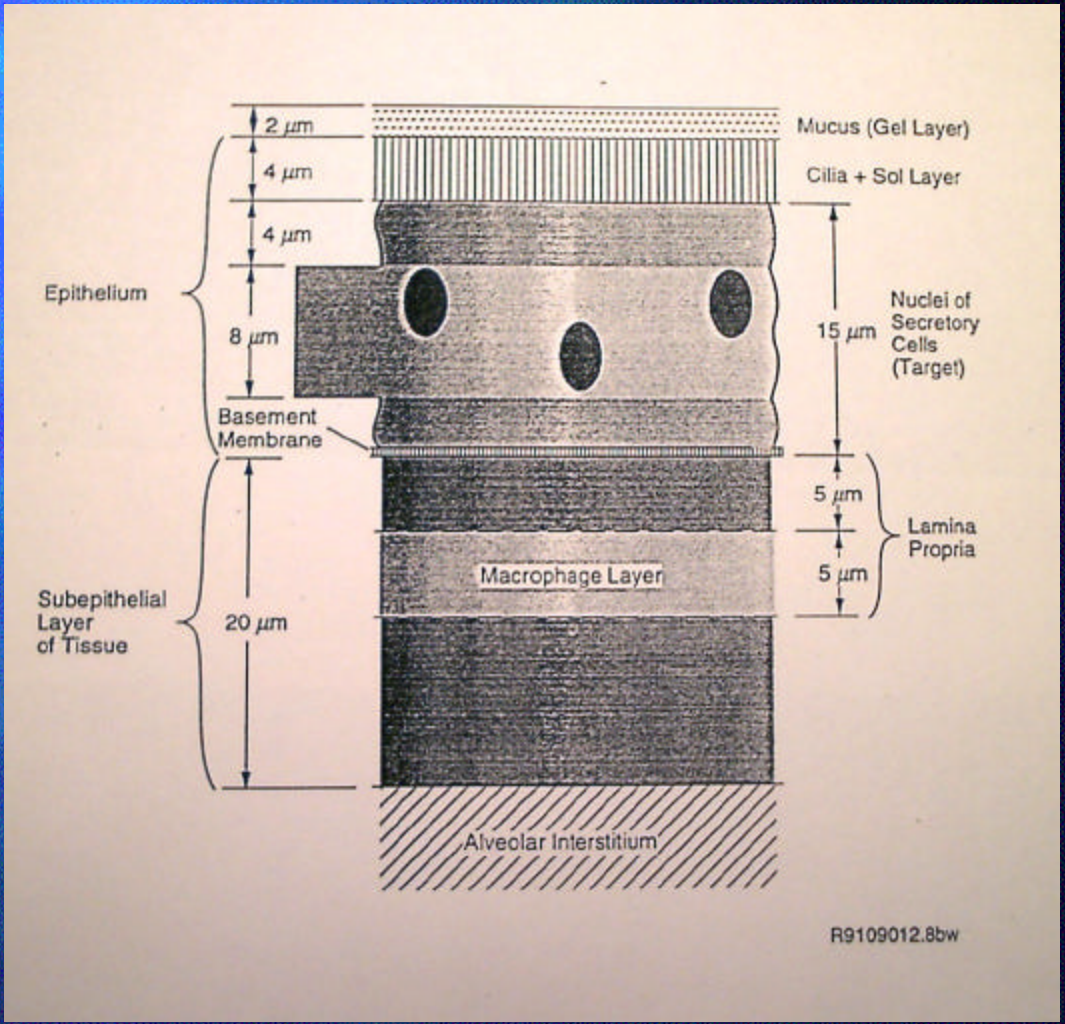


Fig. 1. (A) Constricted airway often found in autopsy of asthmatic lungs, giving an impression of overstressed muscular hypertrophy. H&E stain. ×33. (B) A schema illustrating the morphometric treatment of bronchial muscle. A segment with the basement membrane of perimeter length L and muscles of total areas (*left*) is standardized into a circular state (*right*) with the same L and S, where the anatomic radius R and the muscular thickness D are determined.

The "Target Zone" in Terminal Bronchioles

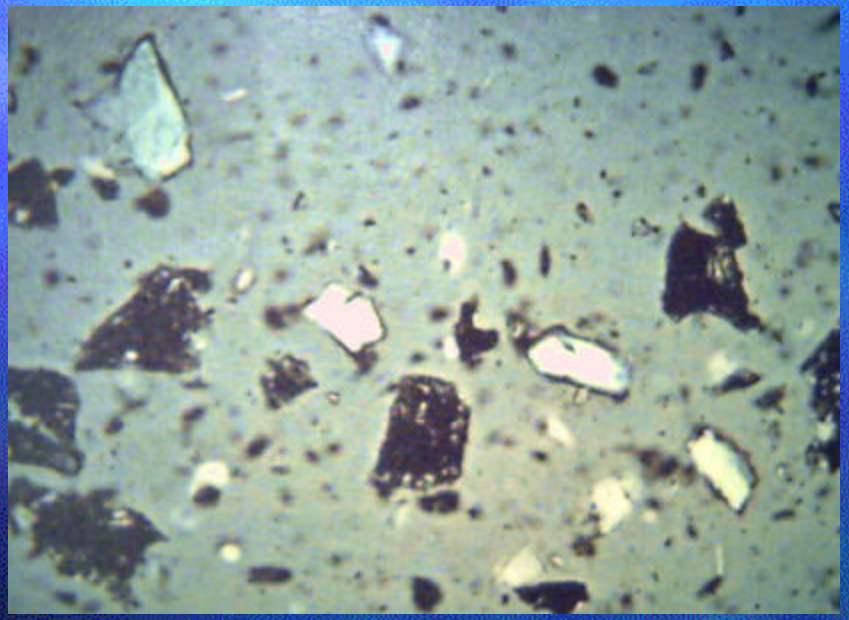
- Effects occur when particles interact with the target cells.
- The steps could include deposition, particle clearance, dissolution and metabolism.



Acute Respiratory Tract Injury

- Conducting airways and gas exchange region of the lung can be injured by inhaled particles.
  - Increased by exercise.
  - At rest, 7-10 LPM ventilation.
  - Lung capillary bed perfused 1 to 5 times by ENTIRE circulating blood volume.
- Injury patterns are different for oxidant gases (e.g. O_3) and particles.
  - O_3 - centriacinar focal lesions.
  - Particles – Diffuse lesions and interstitial inflammation.

How Do Particles Cause Effects?



Chemical Components of PM and Their Biologic Effects

Component	Major Subcomponents	Described Biologic Effects
Metals	Iron, vanadium, nickel, copper, platinum, and others	Can trigger inflammation, cause DNA damage, and alter cell permeability by inducing production of reactive oxygen species (particularly hydroxyl free radicals) in tissues
Organic compounds	Many are adsorbed onto particles; some volatile or semivolatile organic species form particles themselves	Some may cause mutations, some may cause cancer; others can act as irritants and can induce allergic reactions
Biologic origin	Viruses, bacteria and their endotoxins (lipopolysaccharides), animal and plant debris (such as pollen fragments), and fungal spores	Plant pollens can trigger allergic responses in the airways of sensitive individuals; viruses and bacteria can provoke immune defense responses in the airways
Ions	Sulfate[a] (usually as ammonium sulfate), nitrate[b] (usually ammonium or sodium nitrate), and acidity (H^+)	Sulfuric acid at relatively high concentrations can impair mucociliary clearance and increase airway resistance in people with asthma; acidity may change the solubility (and availability) of metals and other compounds adsorbed onto particles
Reactive gases	Ozone, peroxides, aldehydes	May adsorb onto particles and be transported into lower airways, causing injury
Particle core	Carbonaceous material	Carbon induces lung irritation, epithelial cell proliferation, and fibrosis after long-term exposure

[a] Formed from the neutralization of sulfuric acid vapor, which is generated from the oxidation of sulfur dioxide emitted from combustion of fuel containing sulfur, such as that used in motor vehicles and oil- and coal-burning powerplants.

[b] Formed from nitric acid vapor, which is generated in the atmosphere from the reactions of nitrogen oxides.

Measures of Lung Injury

- Cell Proliferation
  - Mechanism for replacement of injured epithelial cells (injury repair)*.
  - Interstitial proliferation may indicate both repair and ‘recruitment’ of inflammatory cells*.
  - Sustained cell proliferation in a tissue may enhance tumor development (review of available evidence is equivocal).

Measures of Lung Injury (Cont.)

- Edema
  - Influx of plasma and cells to interstitial and airway-alveolar airspaces.
  - May be associated with alterations of microvascular and epithelial function (e.g. opening of tight junctions).
  - Runs gamut from minor or quickly reversible ultrastructural changes to disruption of entire cell layer (rapidly fatal).
  - Often accompanied by oxidative stress.
  - Consequences can be gas-exchange compromise and resolution can involve fibrogenesis leading to permanent or progressive damage.

Measures of Lung Injury (Cont.)

- Oxidative Stress
  - Free radicals in lung*
  - NO*
  - Can be produced by inhaled particles (transition metals) or by phagocytic cells secondary to ingesting particles (respiratory burst).
  - Decreased concentrations of anti-oxidants.
  - Inactivation of anti-proteases.
- Inflammatory Mediators
  - Arachidonic Acid Metabolites
    - Prostaglandins
    - Leukotrienes
  - Complement system

Measures of Lung Injury (Cont.)

- Cytokines
  - Small glycosylated proteins (8 to 30 kDa)
  - Regulate cell differentiation, proliferation and activation
  - Interact with specific cell membrane receptors on immune and non-immune system cells.
  - Elaborated by cells to regulate their own physiology (autocrine) or that of other cells (paracrine).
  - Influence physiological processes (e.g. body temperature, apetite, sleep patterns).
  - Role(s) in tissue injury, infection and neoplasia are complex.
  - Can be “initiators” or “recruiters.”

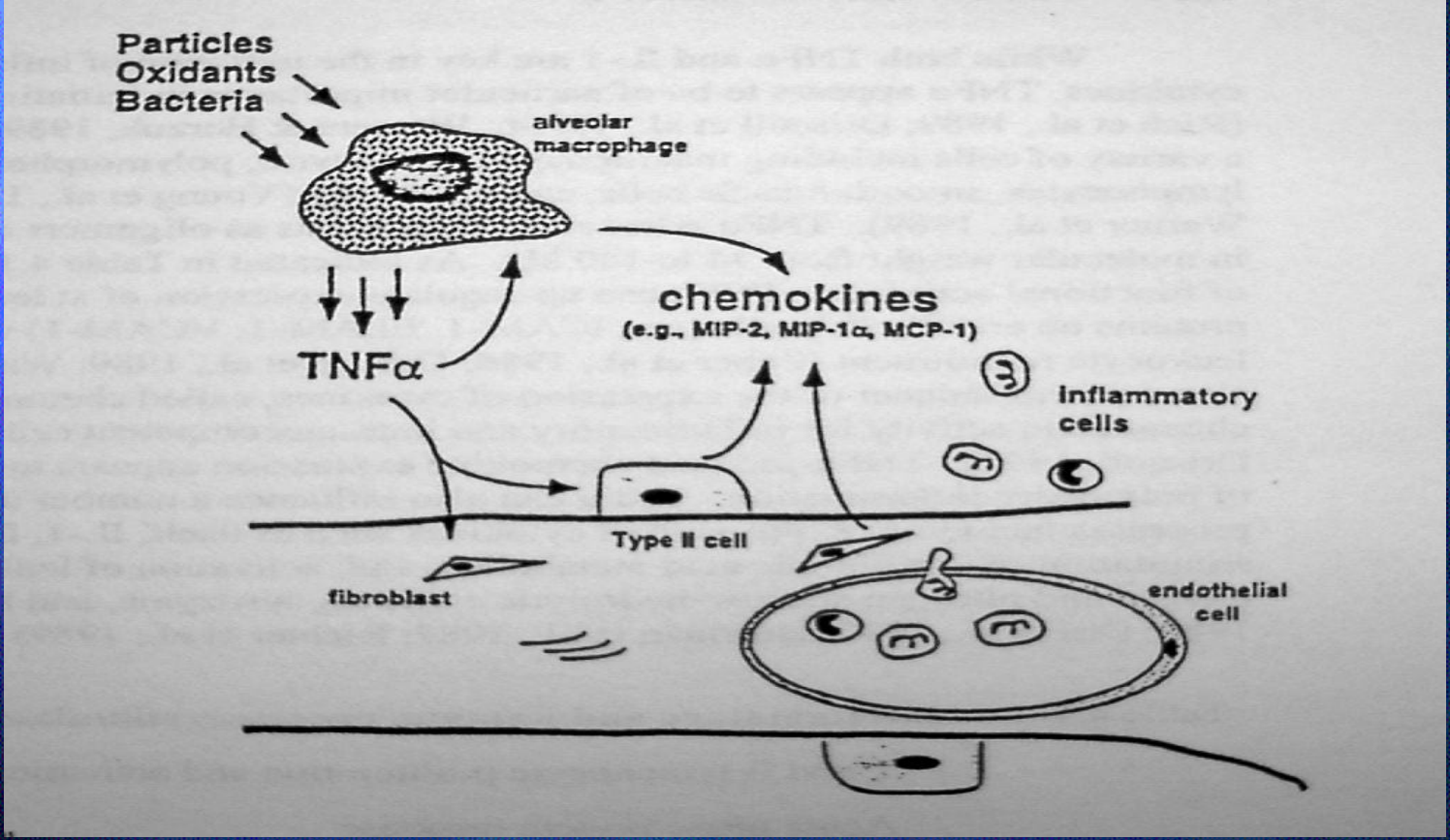
Measures of Lung Injury (Cont.)

- Initiating Cytokines (IL-1, TNFα, TGFβ)
  - Early mediators of response.
  - Expressed rapidly after exposure to toxic agent or recognition by host defenses of infection or neoplasia.
  - Can set ‘cascades’ in motion and can induce cytokine expression and responses in non-immune cells (fibroblast, smooth muscle, epithelial and endothelial cells).
  - Persistent expression of initiating cytokines can be associated with chronic inflammatory or fibrotic lung diseases (e.g. pulmonary granulomas and tissue fibroses).

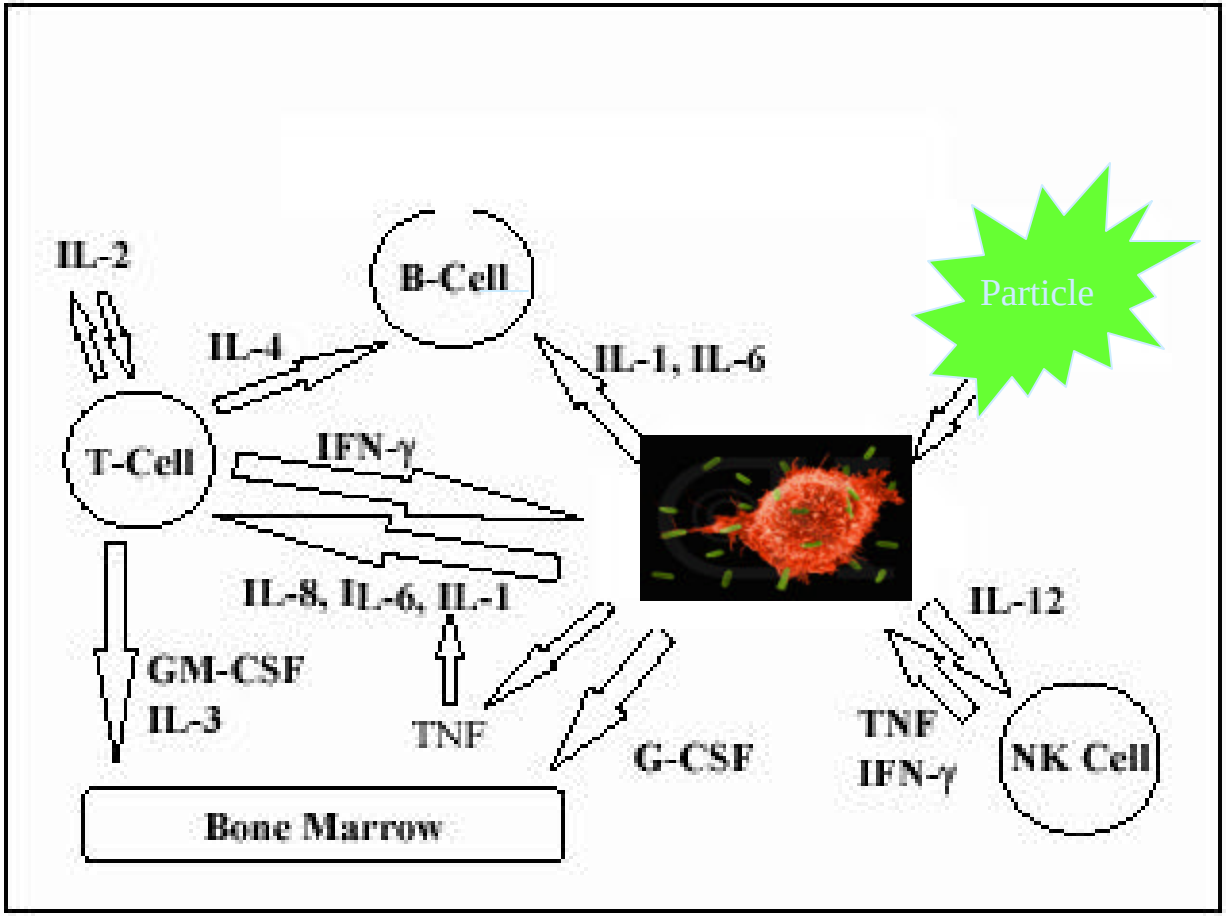
Measures of Lung Injury (Cont.)

- Cytokines recruit inflammatory cells.
- Cytokines elicit changes that allow recruited cells to “acquire” targets.
  - Elicit adhesion molecules.
  - Stimulate proliferation of epithelial cells.

Particles Induce Release of Cytokines and Chemokines



Cytokine Cascade



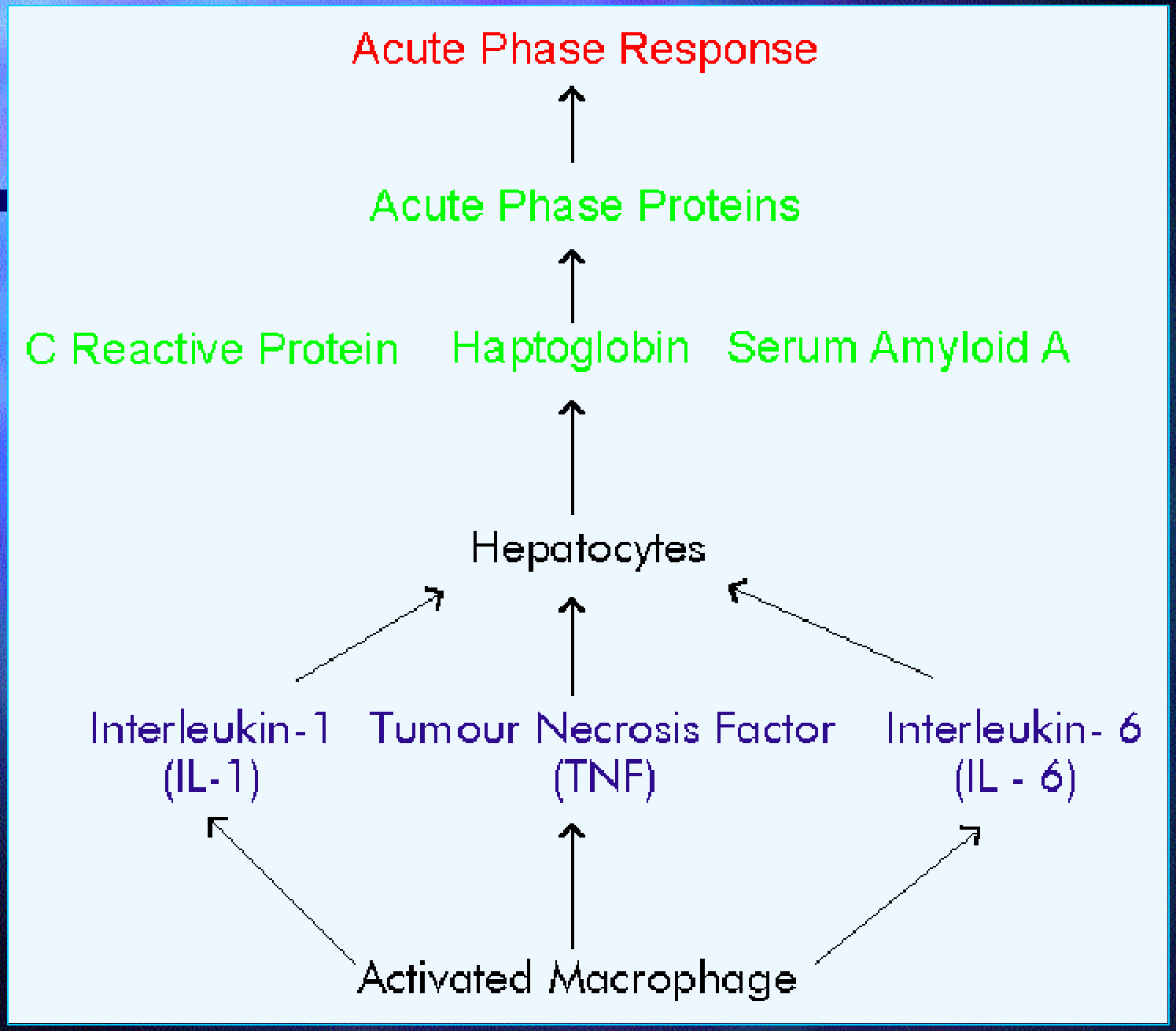
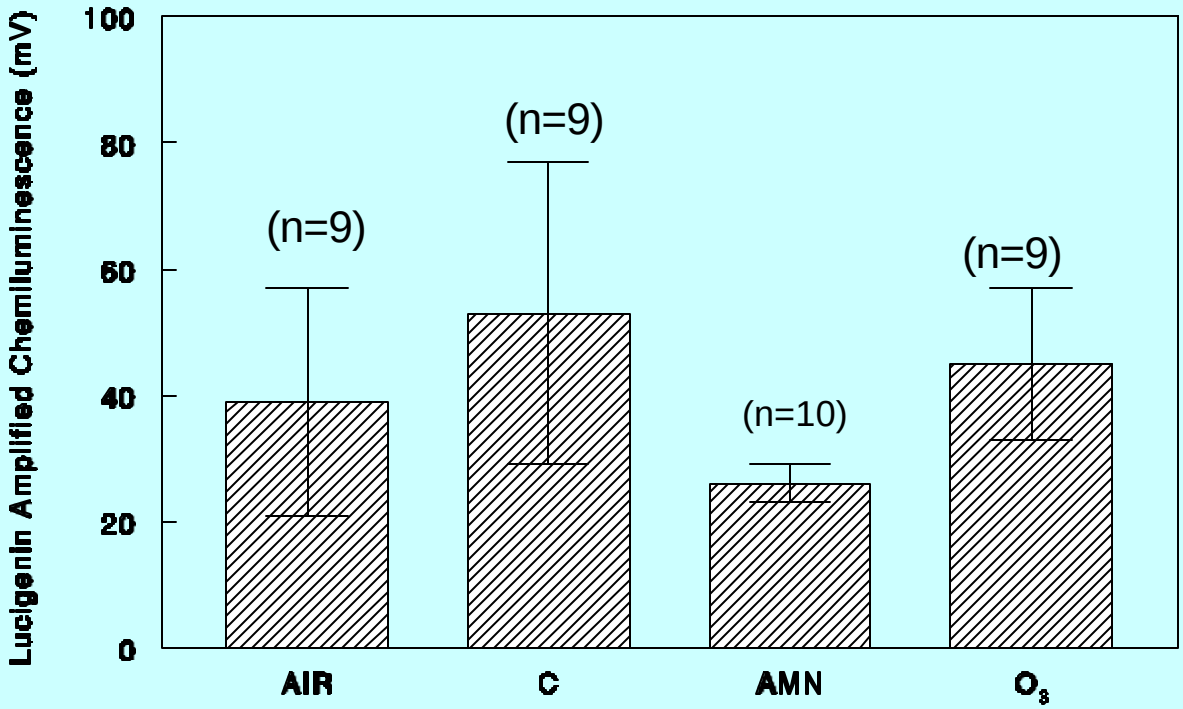

Acute Phase Response
Acute Phase Proteins
C Reactive Protein
Haptoglobin
Serum Amyloid A
Hepatocytes
Interleukin-1
(IL-1)
Tumour Necrosis Factor
(TNF)
Interleukin- 6
(IL - 6)
Activated Macrophage

Table 1 Origin and Composition of Ambient Particles

Size Range (µm)	Particle Class	Predominant Generation Source	Environmental Composition Characteristics
$D_p \leq 0.1$	Ultrafine	gas to particle conversion, condensation	organic C, SO_4, NO_3, elemental C, metals
$0.1 < d_p \leq 2.5$	Fine	combustion, condensation, particle coagulation	organic C, SO_4, NO_3, elemental C, metals
$2.5 < d_p \leq 10.0$	Coarse	Mechanical processes	soil, resuspended matter from paved and unpaved roads, industrial materials, brake linings, tire residues, SO_4, NO_3, elemental C, metals

Figure 1. Initial Studies – No Effects of Particles Alone



Methods (Exposure)

- Rats were exposed nose-only for 6 hr.
- Atmospheres contained ammonium nitrate (AMN), elemental carbon (C) and ozone (O_3).
- Rats were exposed to large (>1.0 μm), fine (0.4 to 0.7 μm) and ultrafine (< 0.1 μm) particles.

Methods (Endpoints)

- Blood pressure measured before exposure, after exposure, and after 18 hr.
- Rats were euthanized 18-hr post-exposure.
- Left lung was lavaged; right lung was inflated and fixed for histology.
- Macrophages analyzed for free radical production.

Results

- Exposure Data
- Macrophage Free Radical Production
- Macrophage FcR Binding
- Evidence of Oxidative Stress
- Changes in Hemodynamic Functions
- Nitric Oxide in Breath

Exposure Conditions

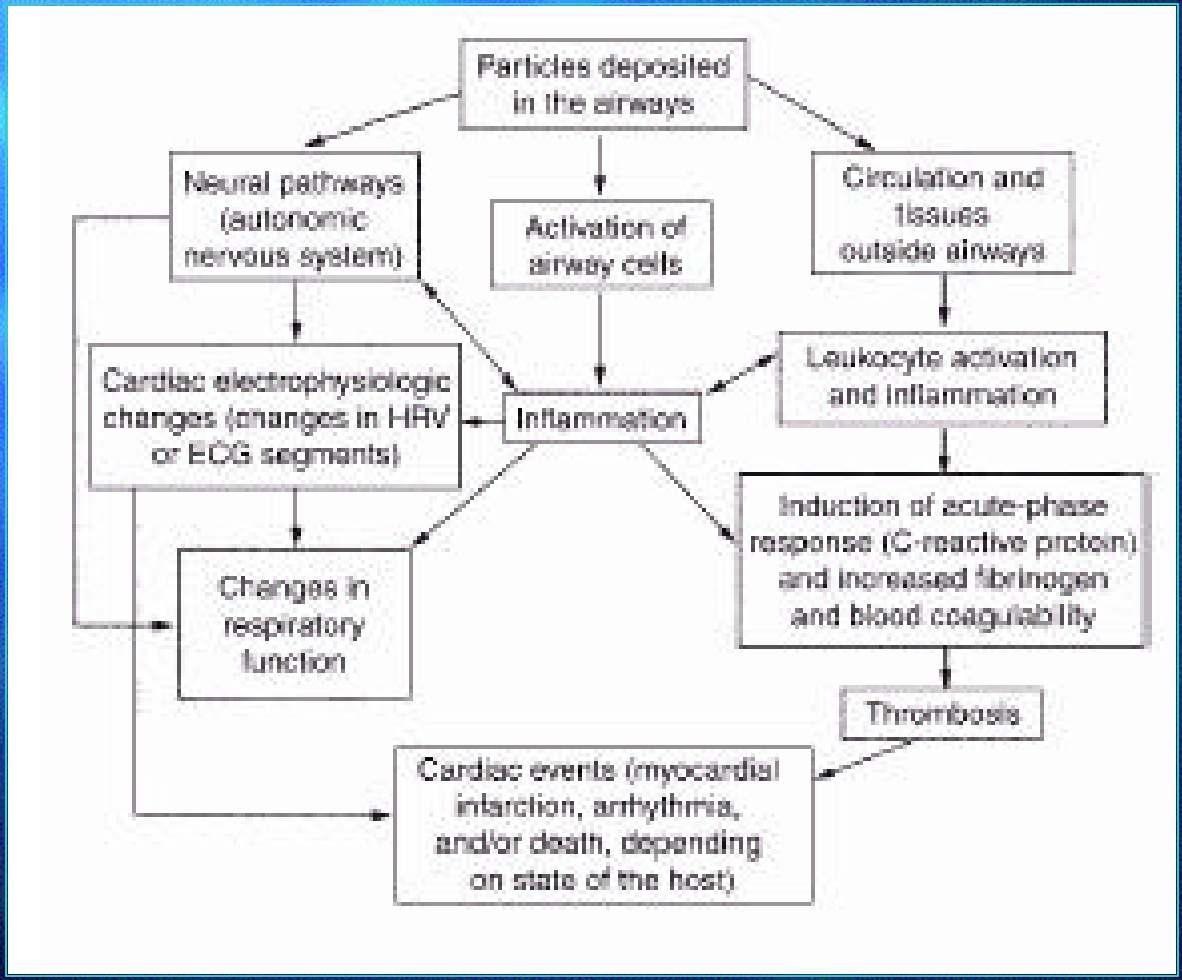
Young Rats

Gp #	Atmos.	Particle Size MMAD (µm); GSD	Conc.	RH
1	Purified Air	NA[a]	NA	59.88 ± 0.84
2	"ultrafine" Carbon + AMN + O_3	0.21 µm; 3.0	253 ± 7 276 ± 25 0.21 ± 0.03	
3	"Fine" Carbon + AMN + O_3	0.68 µm; 2.8	172 ± 14 288 ± 49 0.19 ± 0.03	
4	"Coarse" Carbon + AMN + O_3	1.7 µm; 3.0	189 ± 26 263 ± 30 0.22 ± 0.02	

Old Rats

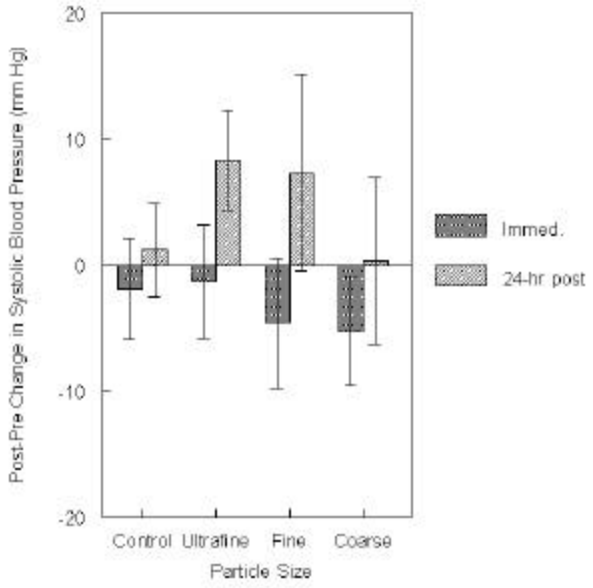
Gp #	Atmos.	Particle Size MMAD (µm); GSD	Conc.	RH
1	Purified Air	NA[a]	NA	59.88 ± 0.84
2	"ultrafine" Carbon + AMN + O_3	0.21 µm; 2.8	87.8 ± 19 166 ± 28 0.198 ± 0.004	
3	"Fine" Carbon + AMN + O_3	0.68 µm; 2.5	91.35 ± 12.15 176.25 ± 38.36 0.194 ± 0.004	
4	"Coarse" Carbon + AMN + O_3	1.8 µm; 2.3	92.35 ± 18.51 136.29 ± 27.61 0.197 ± 0.003	

Pathways by which particle deposition in airways can induce adverse effects in airways and the cardiovascular system.

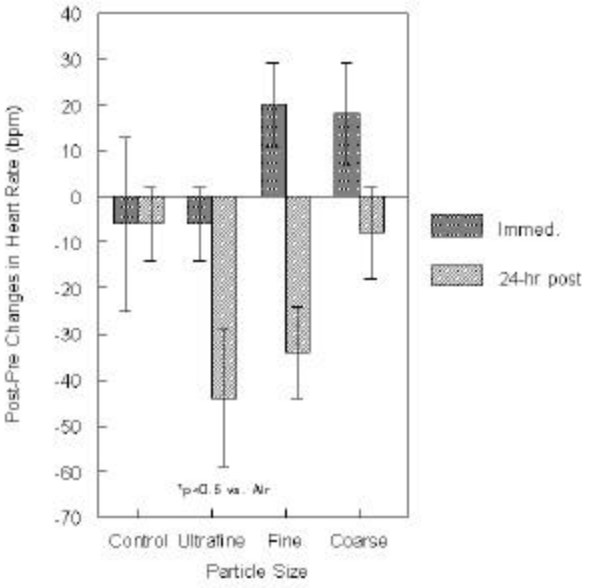


Hemodynamic Changes After Particle Exposures in Young Rats

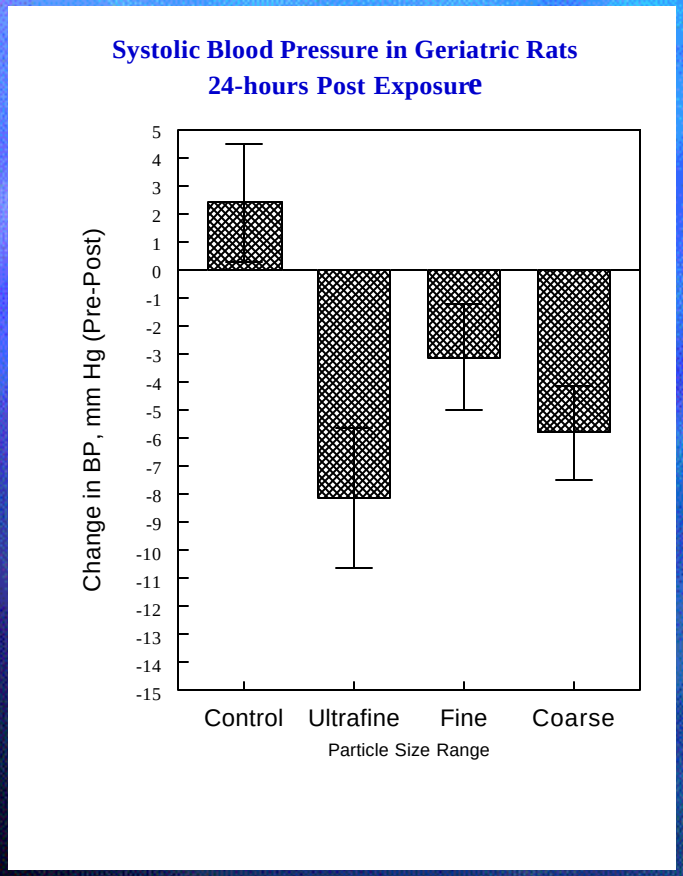
Systolic Blood Pressure in Young Adult Rats Immediately and 24-hours Post Exposure

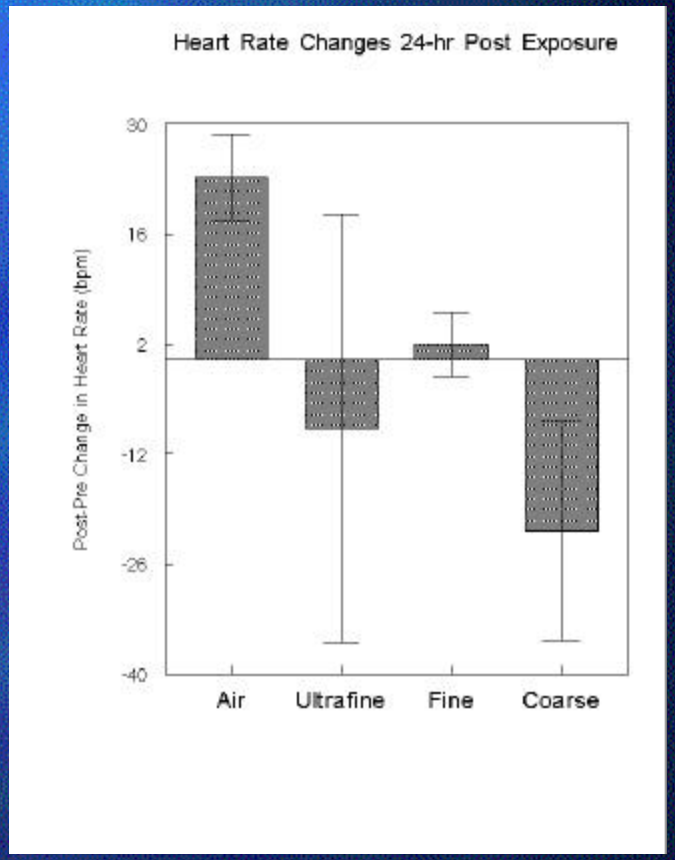


Heart Rate in Young Adult Rats Immediately and 24-hours Post Exposure



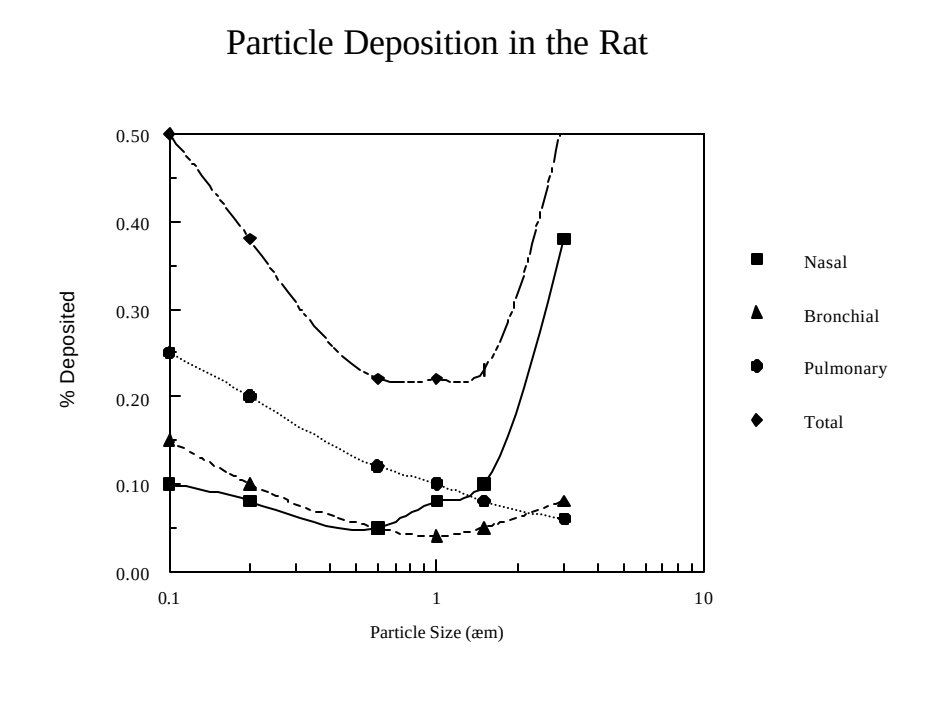
Hemodynamic Changes After Particle Exposures in Geriatric Rats





Particle Size Determines Deposition Fraction and Dose

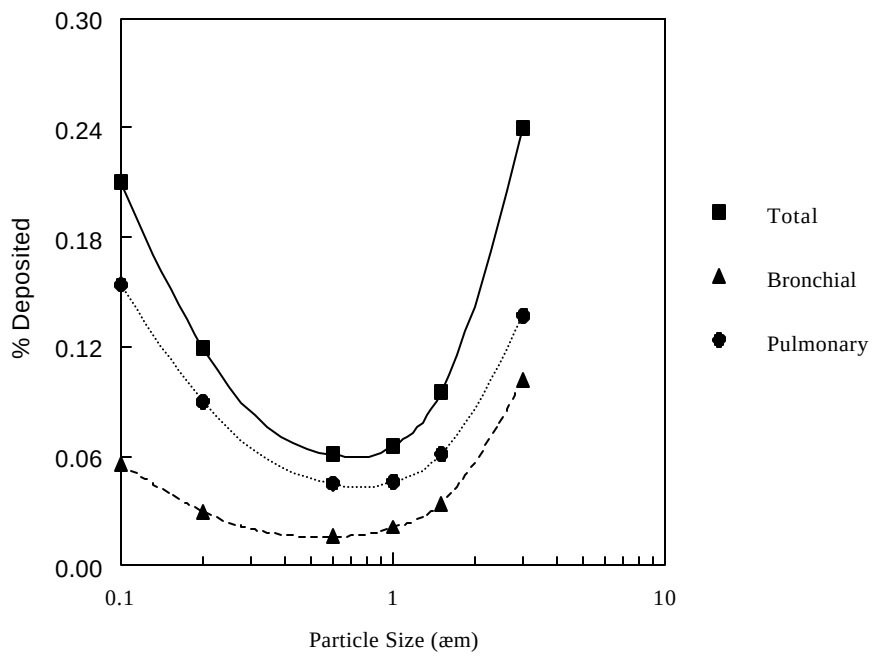
- Nasal deposition is markedly greater for the Coarse Particles.
- Deposition in the gas exchange region of the lung increases with decreasing particle size.



Particle Size Determines Deposition Fractions and Sites

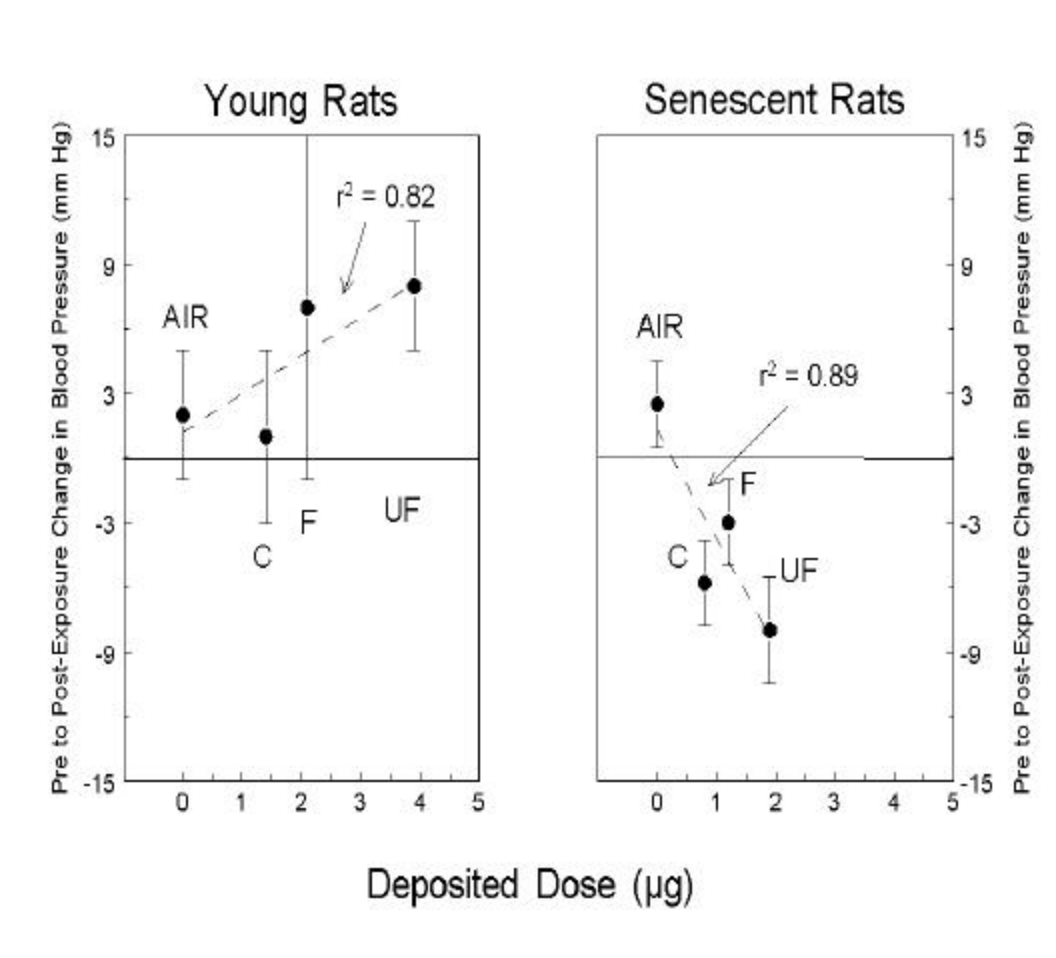
- Thoracic deposition can be estimated using appropriate models after correcting for nasal deposition.
- Of the aerosol that penetrates through the URT, most deposit in the gas exchange region.

Figure 2. Estimated Regional Particle Deposition in the Rat



Particle Dose Can Explain The Observed Responses

- We can estimate deposition fractions using models.
- We can estimate ventilation from observations or scaling models.
- We can calculate a deposited dose.



Is the mouse model relevant?

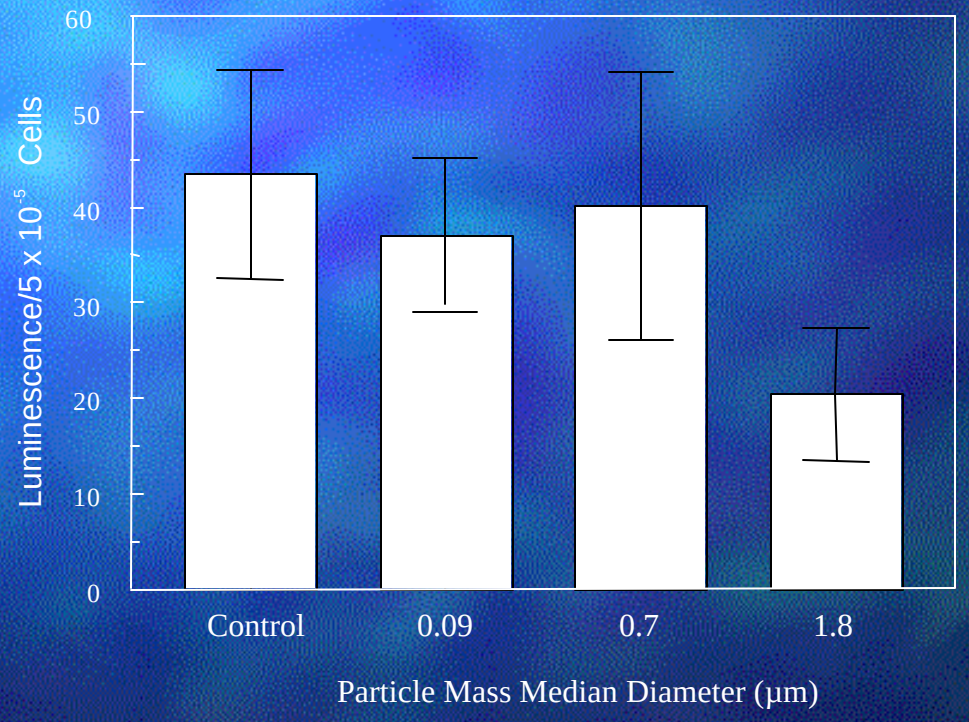
- Based on above we can scale results to a human equivalent –
  - Healthy Adult – Significant Effect at 6 $\mu g/m^3$

$6\ \mu g/0.25\ kg \times 70\ kg/21.6\ m^3 = 78\ \mu g/m^3$

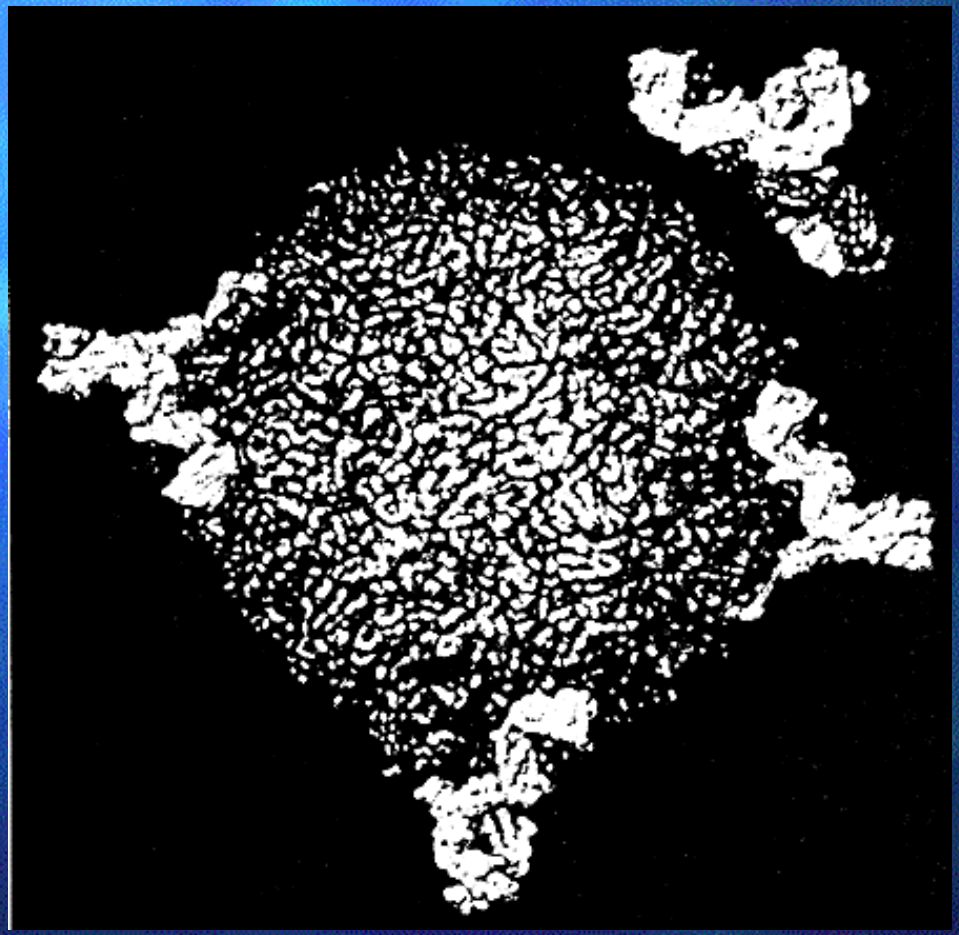
  - Old Rat – Significant Effect at 1 $\mu g/m^3$

$1\ \mu g/0.45\ kg \times 70\ kg/21.6\ m^3 = 7\ \mu g/m^3$

Respiratory Burst Activity In Macrophages from Aged Rats

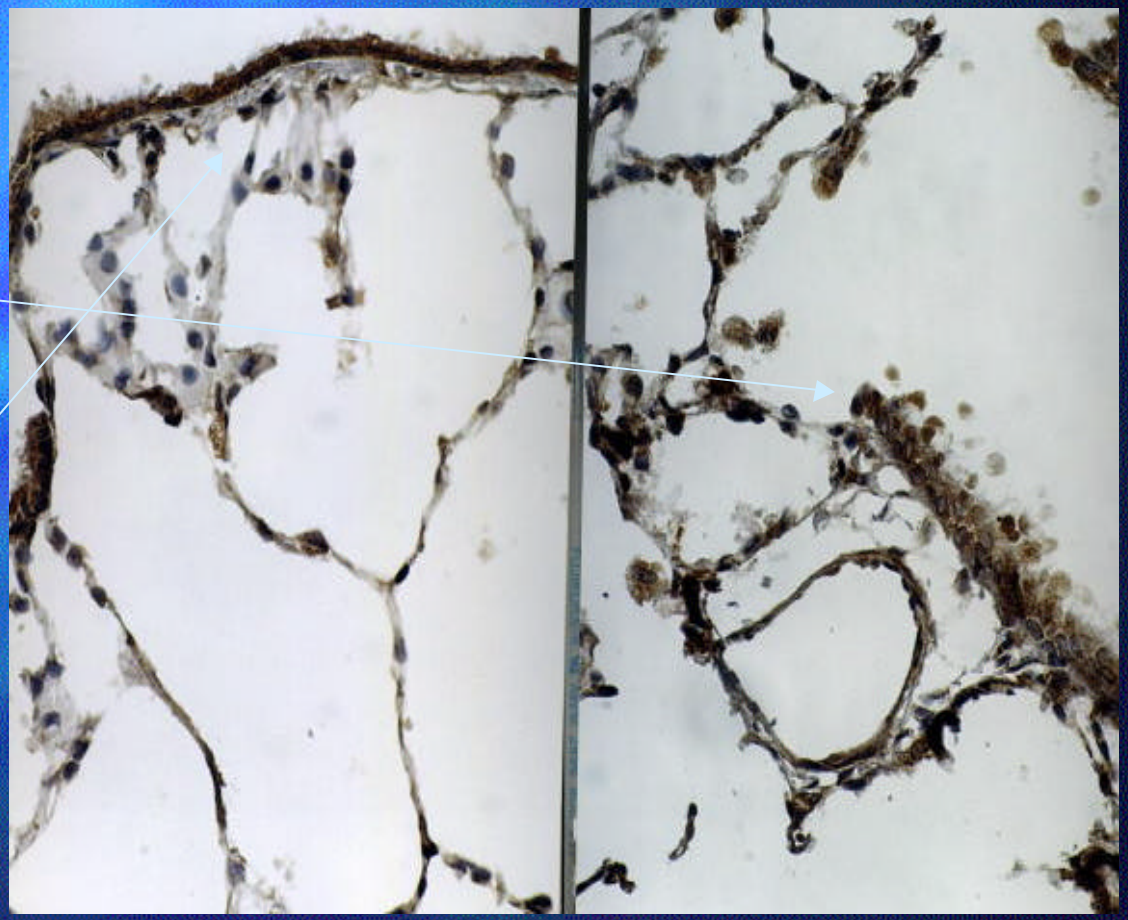


Fc Receptor Binding

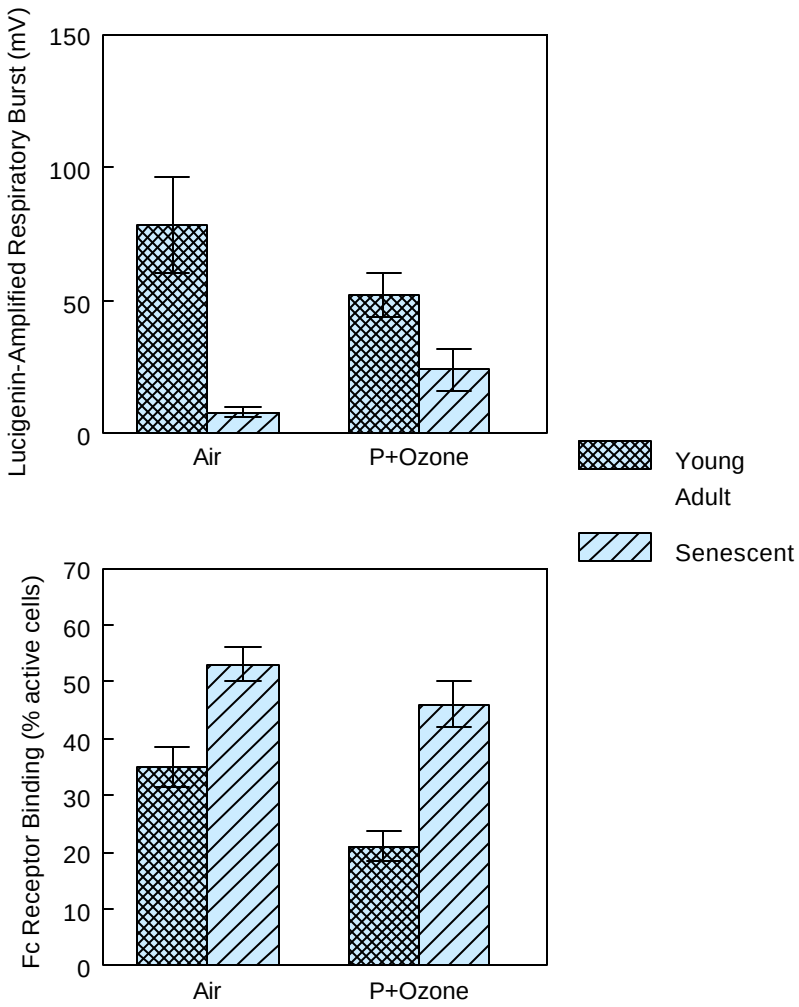


Evidence of Oxidative Stress in Lungs of Rats

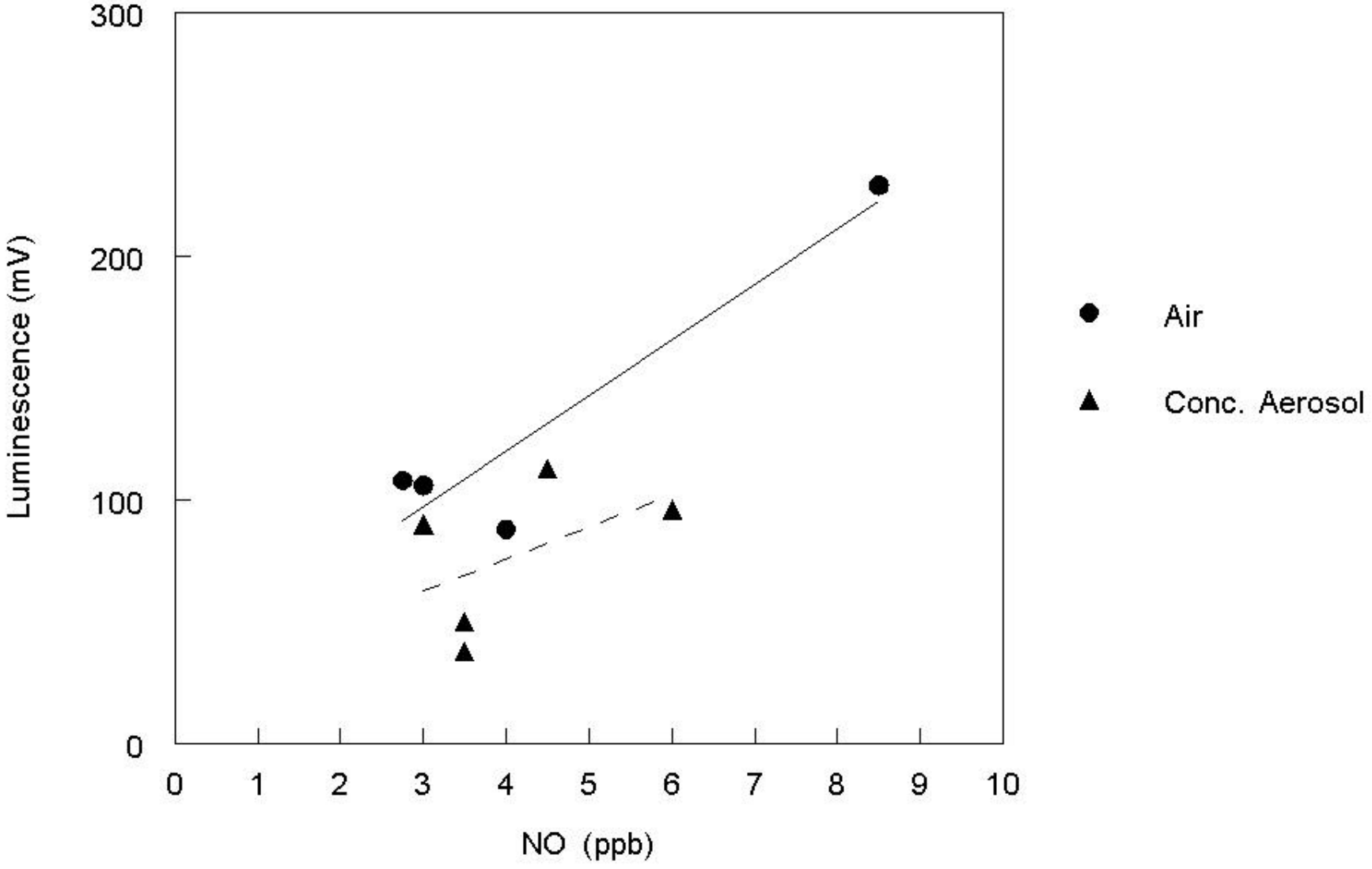
- Terminal bronchioles stained for nitrotyrosine
- Note intense staining associated with inflammatory cells in Panel B.
- Note unstained alveolar epithelium in control (Panel A).



Macrophage Response Differences Between Young and Old Rats



Relationship Between Exhaled NO and Macrophage Respiratory Burst Activity



Discussion

- Coarse particles tend to suppress macrophage free radical production more than fine or ultrafine particles.
- Ultrafine and Coarse Particles Significantly Depressed Systolic Blood Pressure at 18 hr Post-exposure, but not Immediately Post-Exposure.
- Heart Rate and Rate x Pressure Product Were Depressed Indicative of Depressed Ejection Fraction.
- Hypotension is Associated with Pulmonary Oxidative Stress.
- NO data are consistent with above.

Conclusions

- “The dose makes the poison” - Paracelsus
- Particle size is an important parameter for understanding health effects.
- This experiment demonstrates that particle size clearly influences dose.
- Depressed lung ejection fraction at 18 hr, but not immediately post exposure, is consistent with the elaboration of systemic mediators, such as cytokines.
- Holding composition constant, 0.2 μ particles were not more toxic than larger particles.
- Studies with real-world and real UF particles are needed.

SCPCS Freeway Study

Investigators:

M. Kleinman, C. Sioutas, A. Nel, A. Cho, J. Froines, A. Miguel

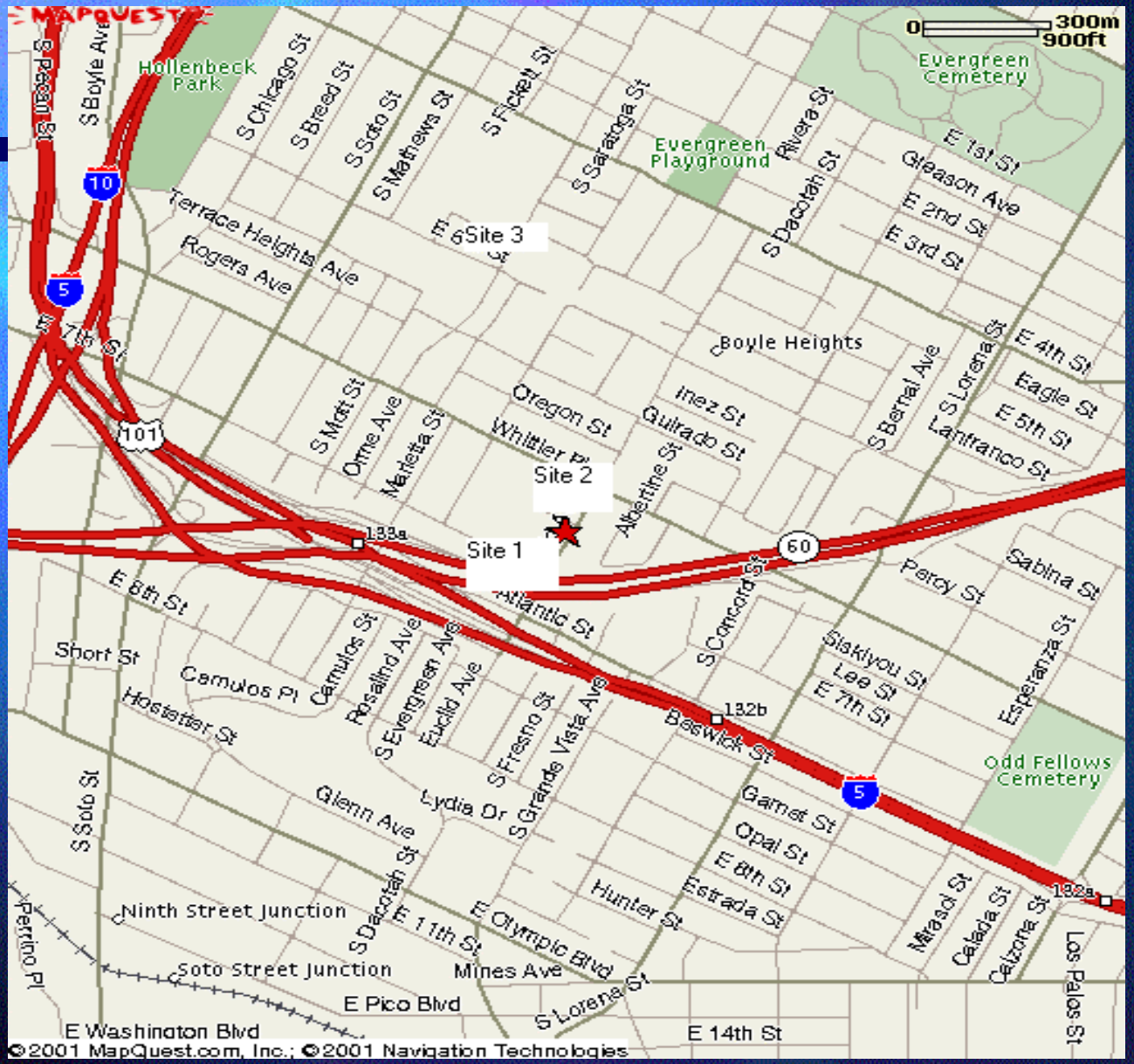
Hypotheses

- Mobile emissions will exacerbate airway inflammation and allergic airway disease.
- The magnitude of allergic airway disease responses will be greater at sites with higher concentrations of UF particles.
- Organic and inorganic PM constituents that can generate ROS will be associated with responses.

Approach

- Ovalbumin-sensitized mice will be exposed to freeway-derived, concentrated fine and ultrafine particles at sites that are at progressively increasing distances downwind from a freeway.
- The effect of background aerosol will be assessed by including an upwind site that is not influenced by the freeway.
- Particle mass concentrations will be held constant at each exposure site.

Freeway Study Sites



Hypotheses

- Mobile emissions will exacerbate airway inflammation and allergic airway disease.
- The magnitude of allergic airway disease responses will be greater at sites with higher concentrations of UF particles.
- Organic and inorganic PM constituents that can generate ROS will be associated with responses

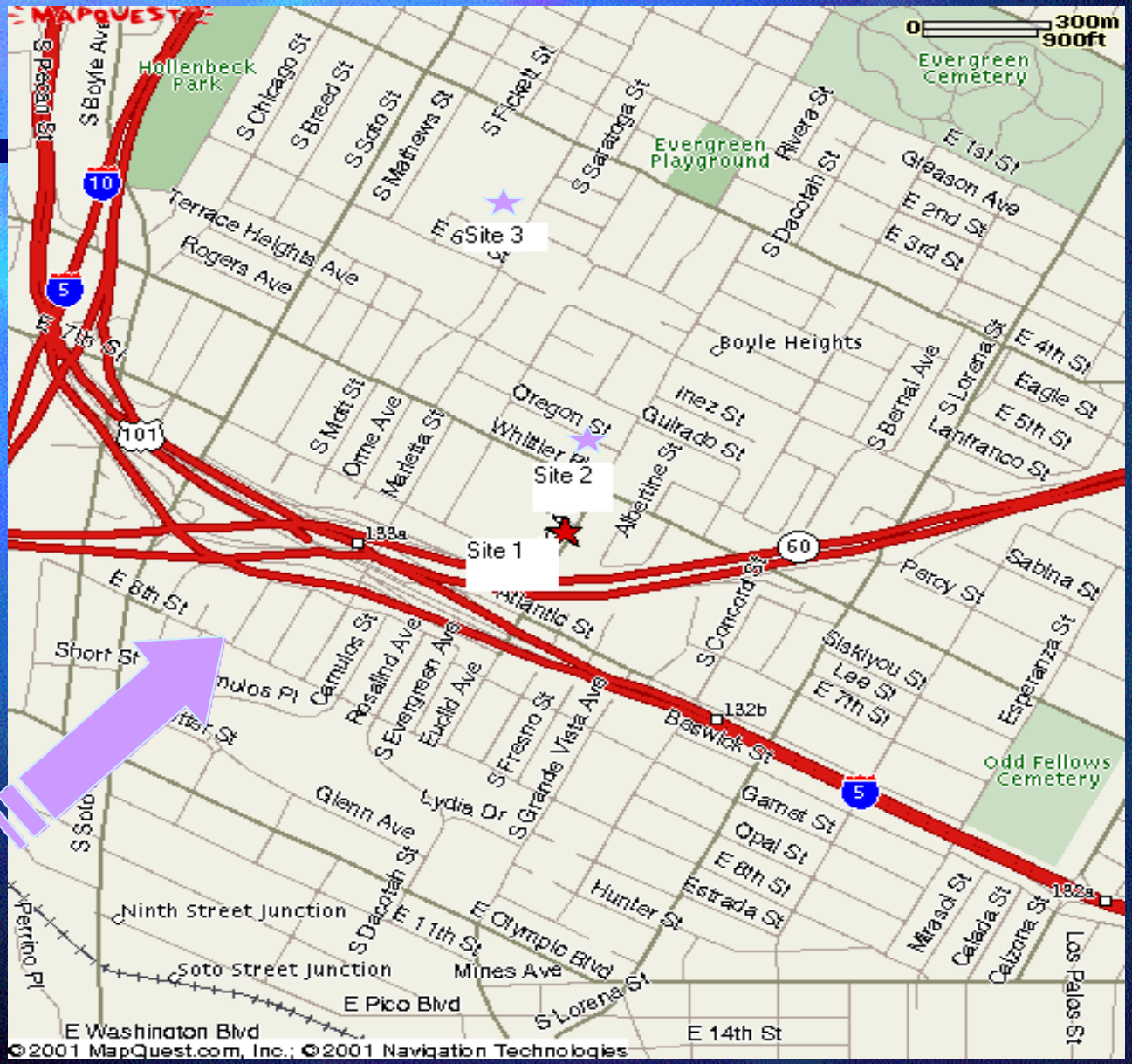
Approach

- Ovalbumin-sensitized mice will be exposed to freeway-derived, concentrated fine and ultrafine particles at sites that are at progressively increasing distances downwind from a freeway.
- The effect of background aerosol will be assessed by including an upwind site that is not influenced by the freeway.
- Particle mass concentrations will be held constant at each exposure site.

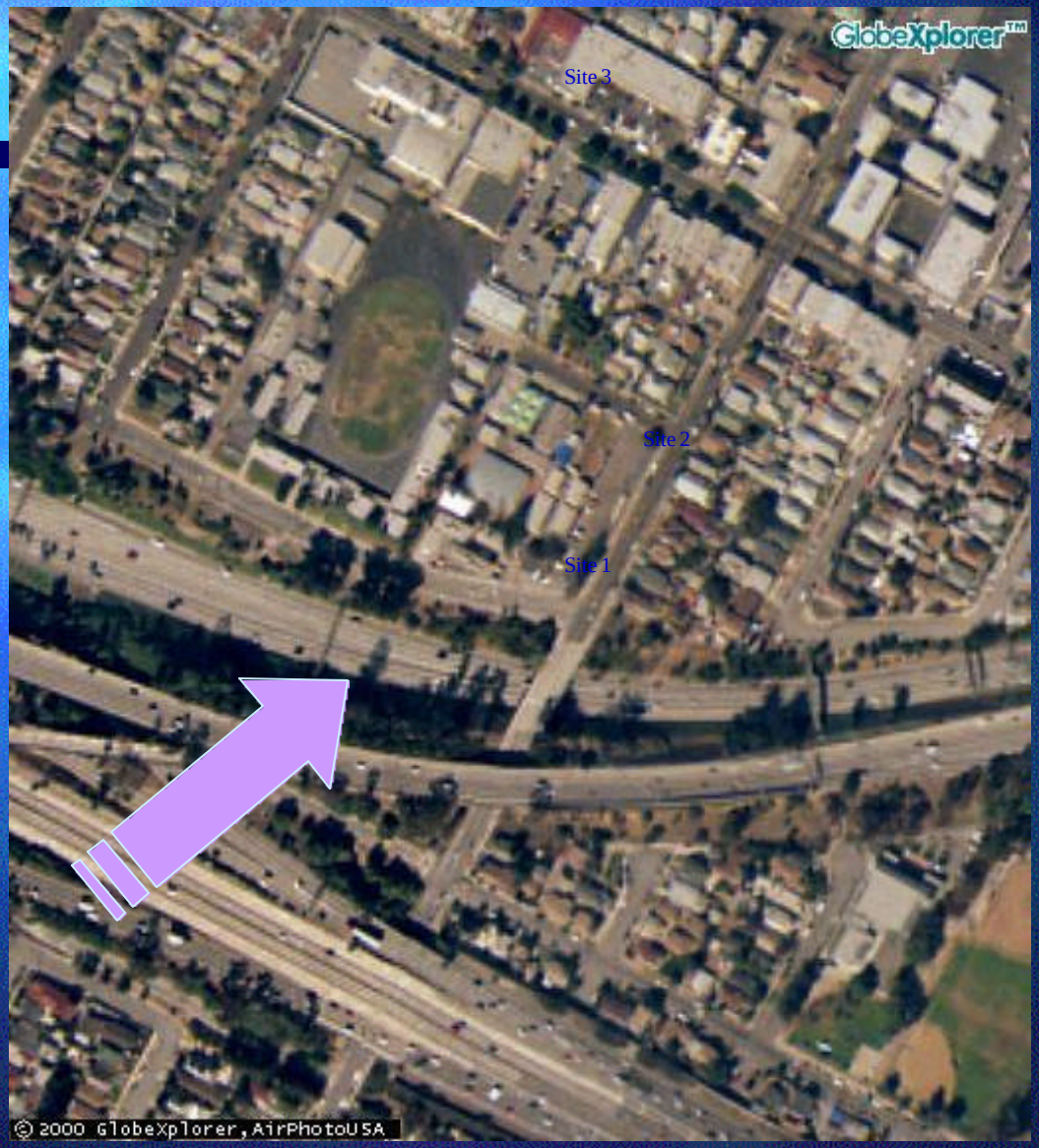
Sites

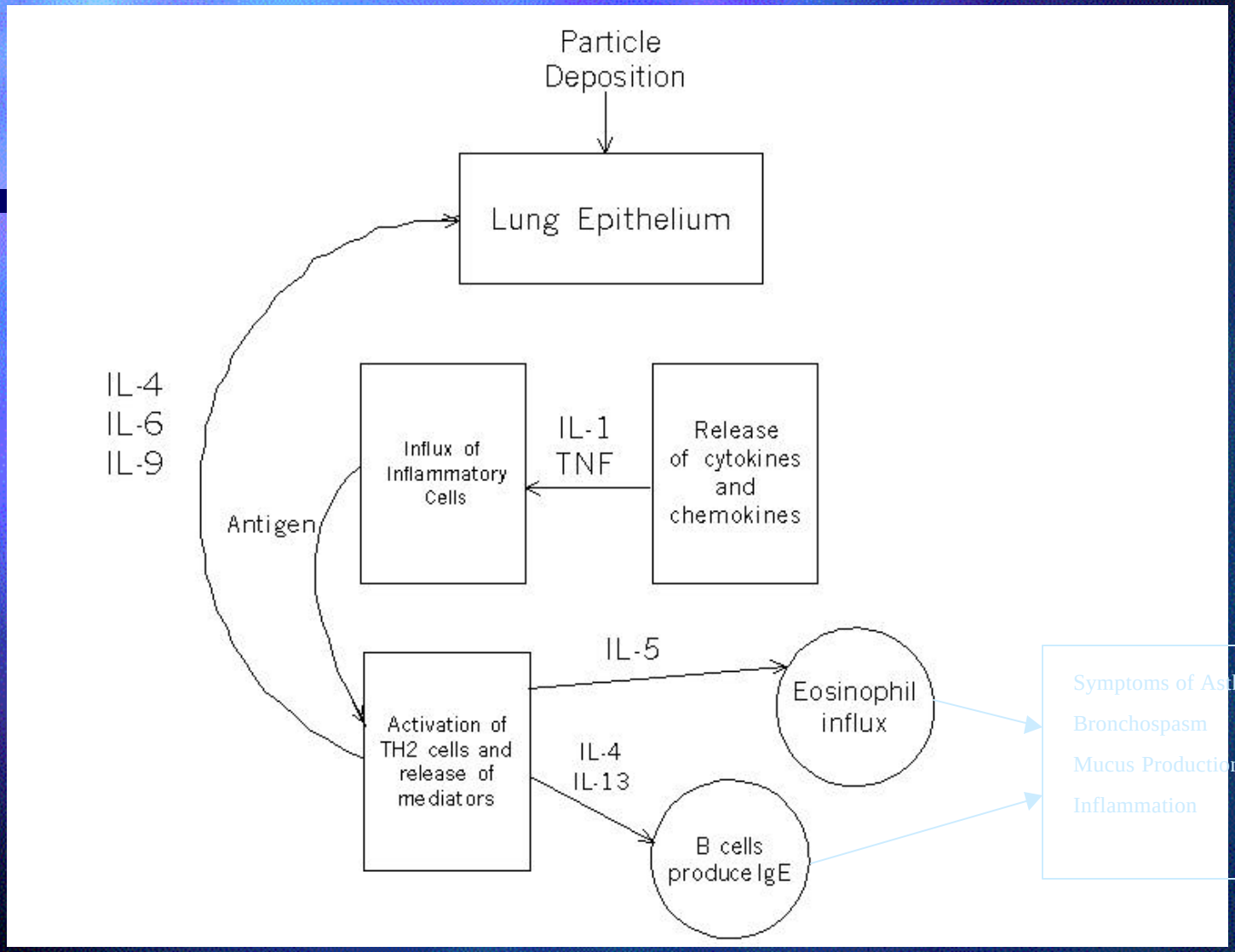
- Three downwind sites have been selected in Boyle Heights.
  - LA School for Mathematics Site A ~50 m downwind of the Freeway.
  - LA School for Mathematics Site B ~150 m downwind of the Freeway.
  - Hollenbeck Middle School (AQMD monitoring station) ~ 500 m downwind
- Prevailing wind direction South to Southwest.

Freeway Study Sites



Freeway Site Area





Symptoms of Asthma

Bronchospasm

Mucus Production

Inflammation

Van Features

- Self-contained exposure system.
- Generator for power deployed downwind of exposure system.
- Purified air transport for animals.
- Rapid assembly and disassembly for exposure system.
- Can be deployed to any accessible site.
- Use of facility approved by ARC

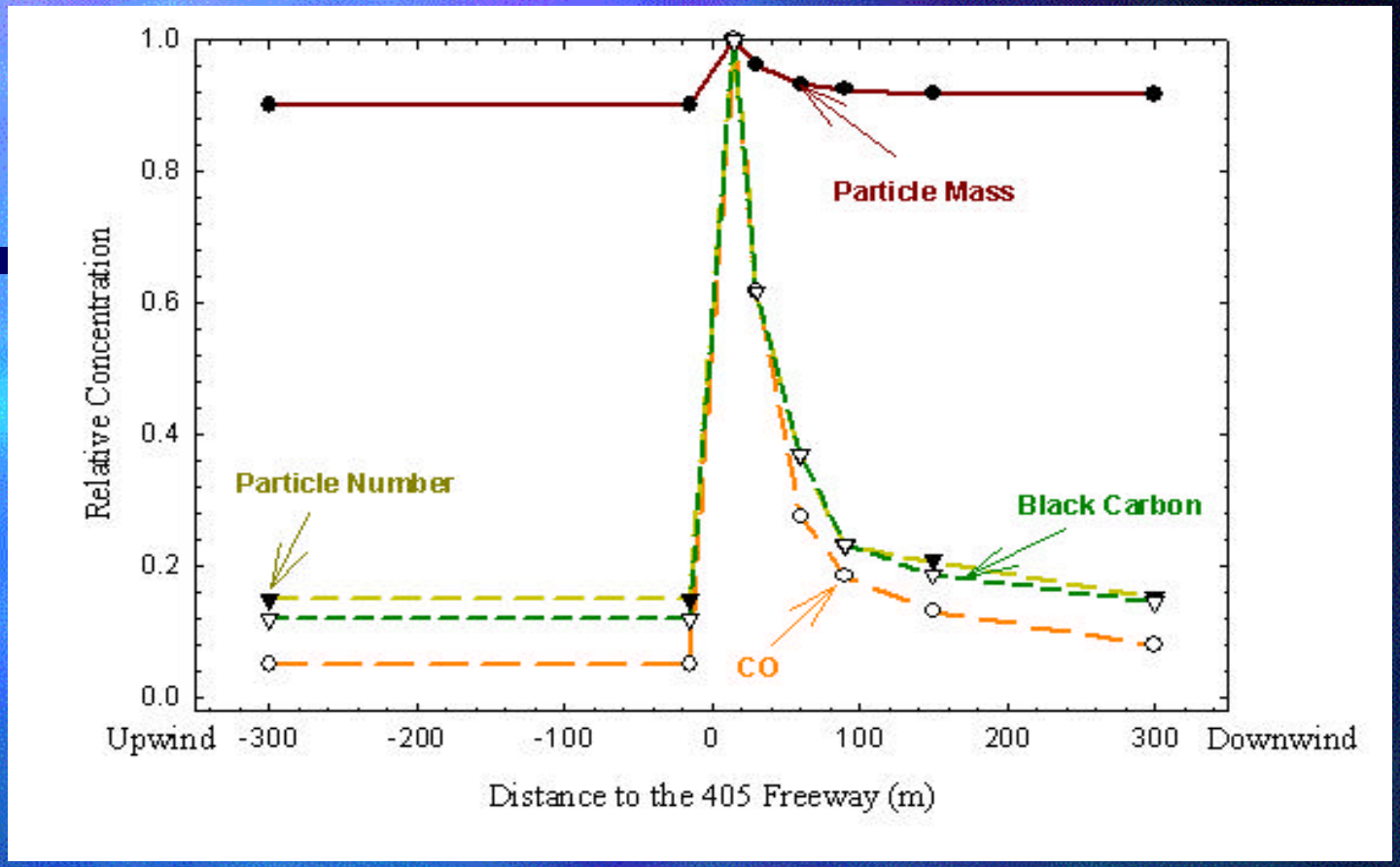
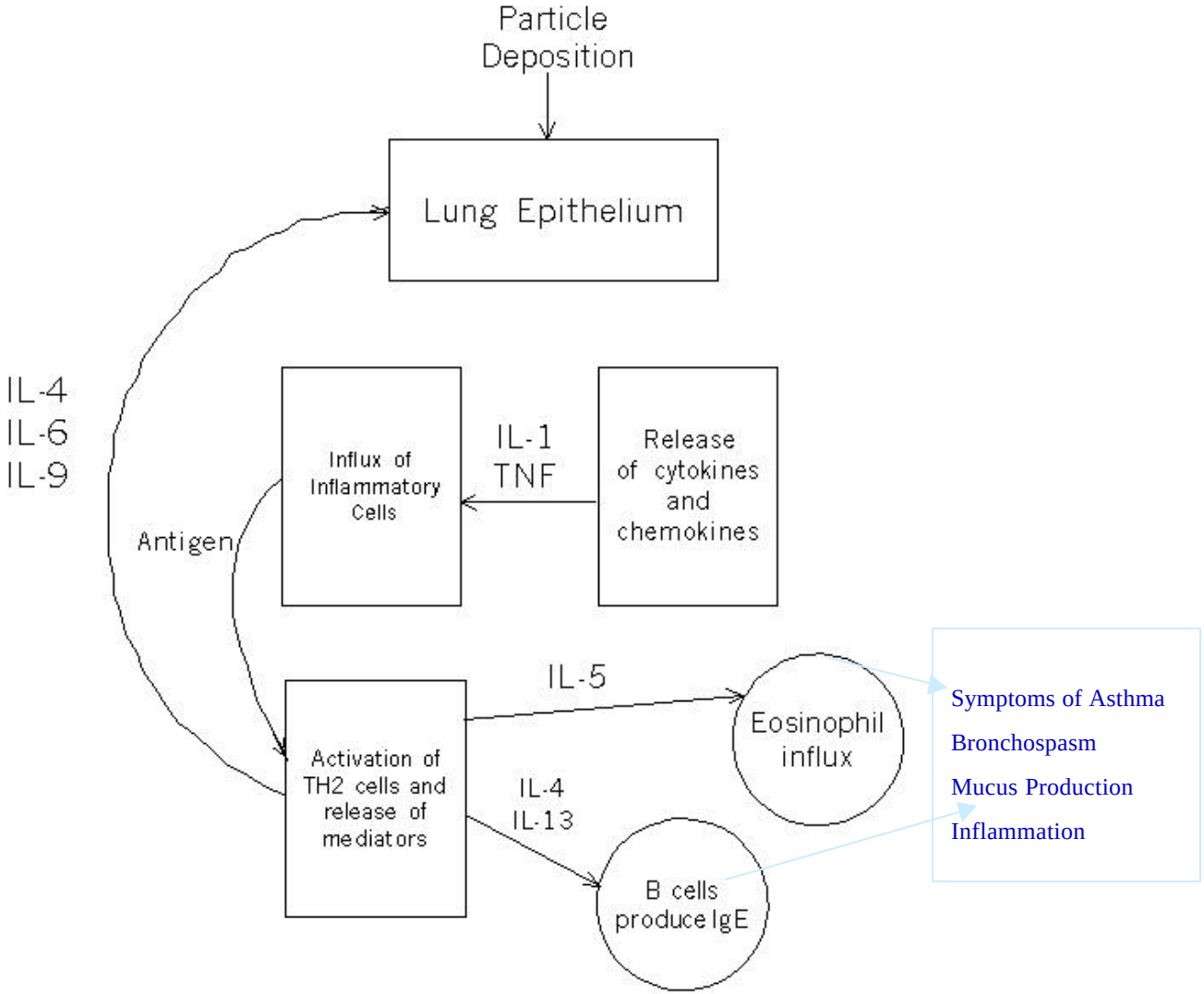
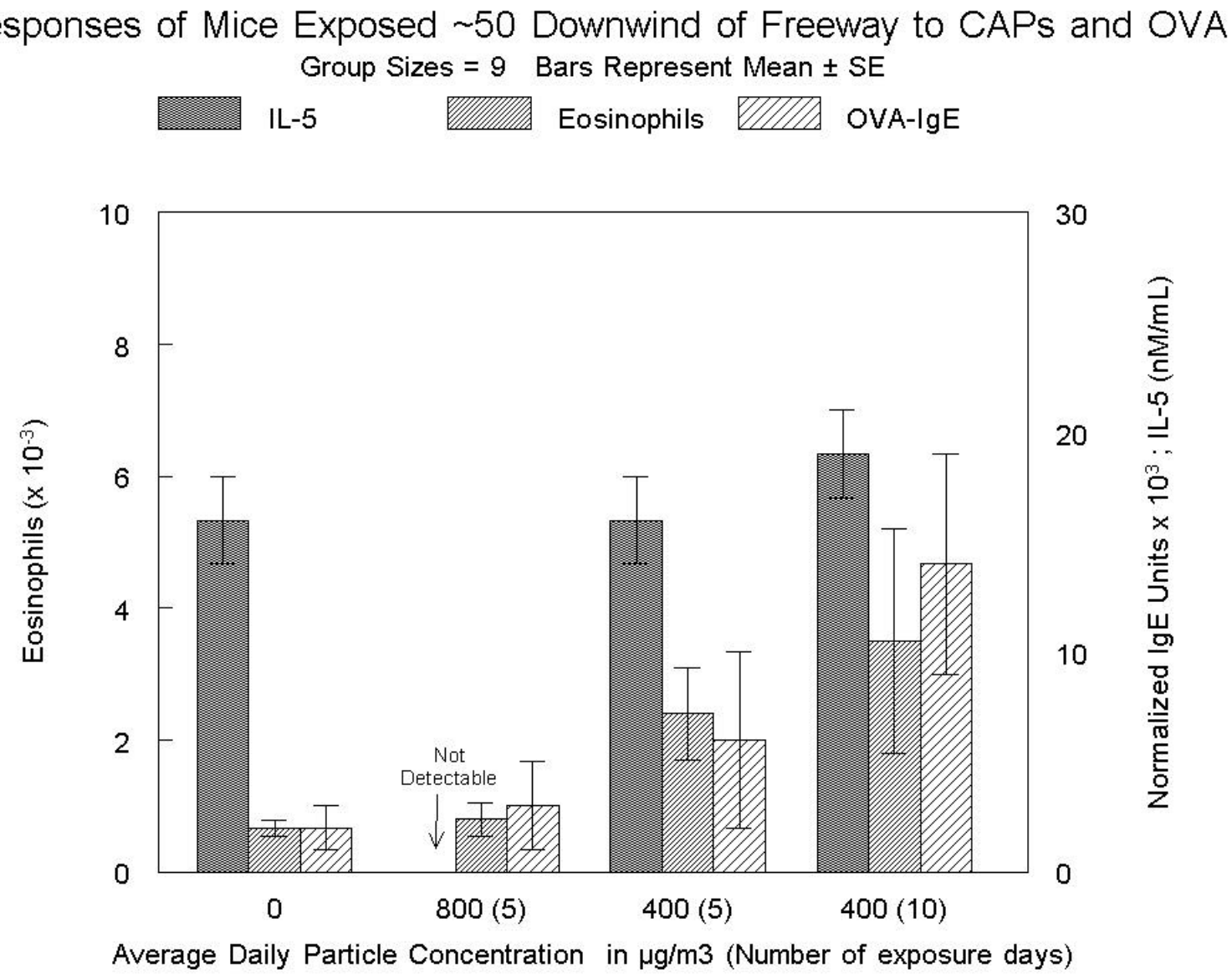


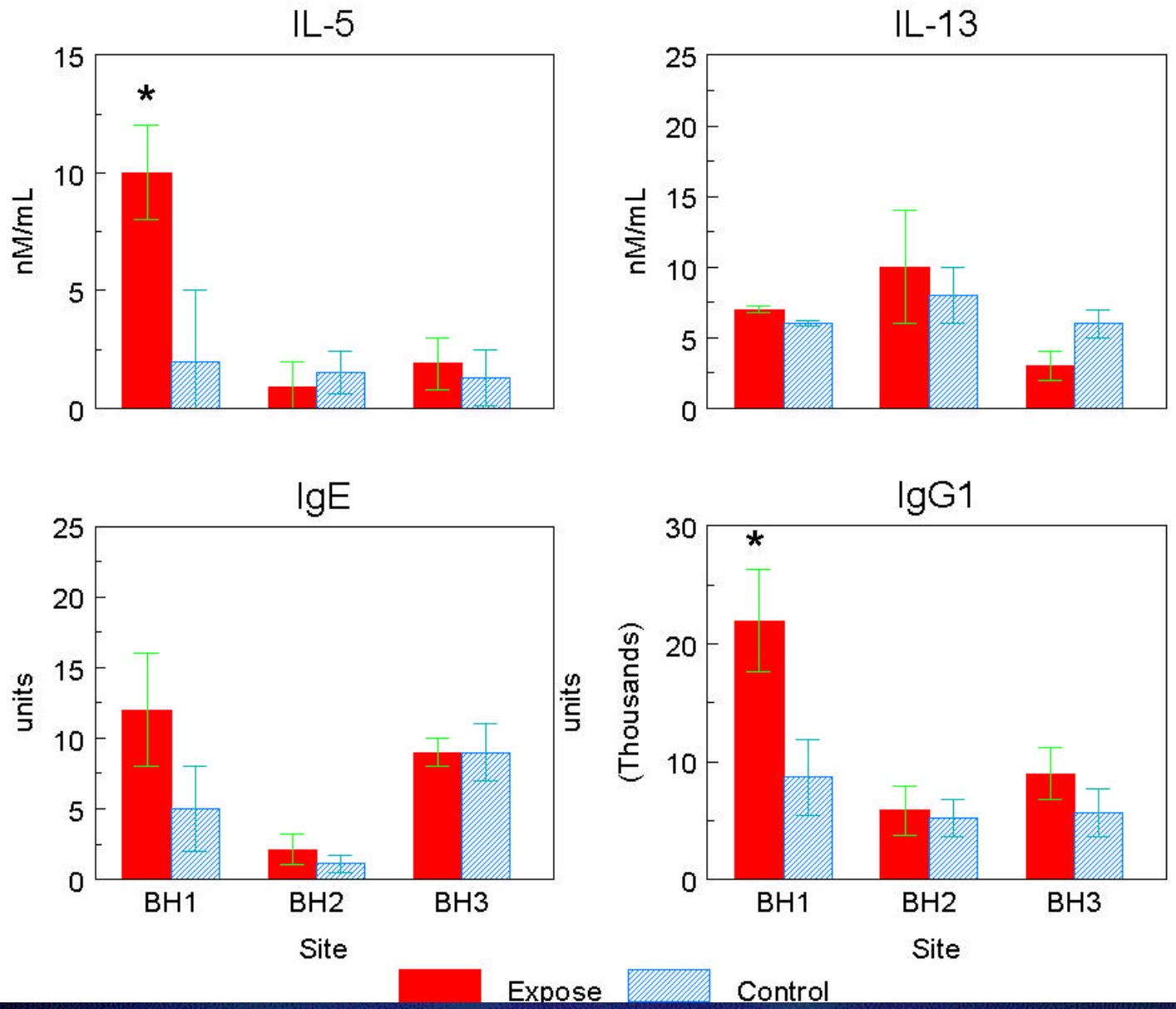
Figure 1. Ambient carbon monoxide (CO) is highly correlated with the number of ultrafine particles and black carbon but not to PM2.5 particle mass. Black carbon is a well accepted tracer for emissions from diesel engines. The concentrations of CO and ultrafine particles are high near the edge of a heavily trafficked freeway (U.S. 405) but the concentrations nearly 5-fold 50 to 100 m downwind.

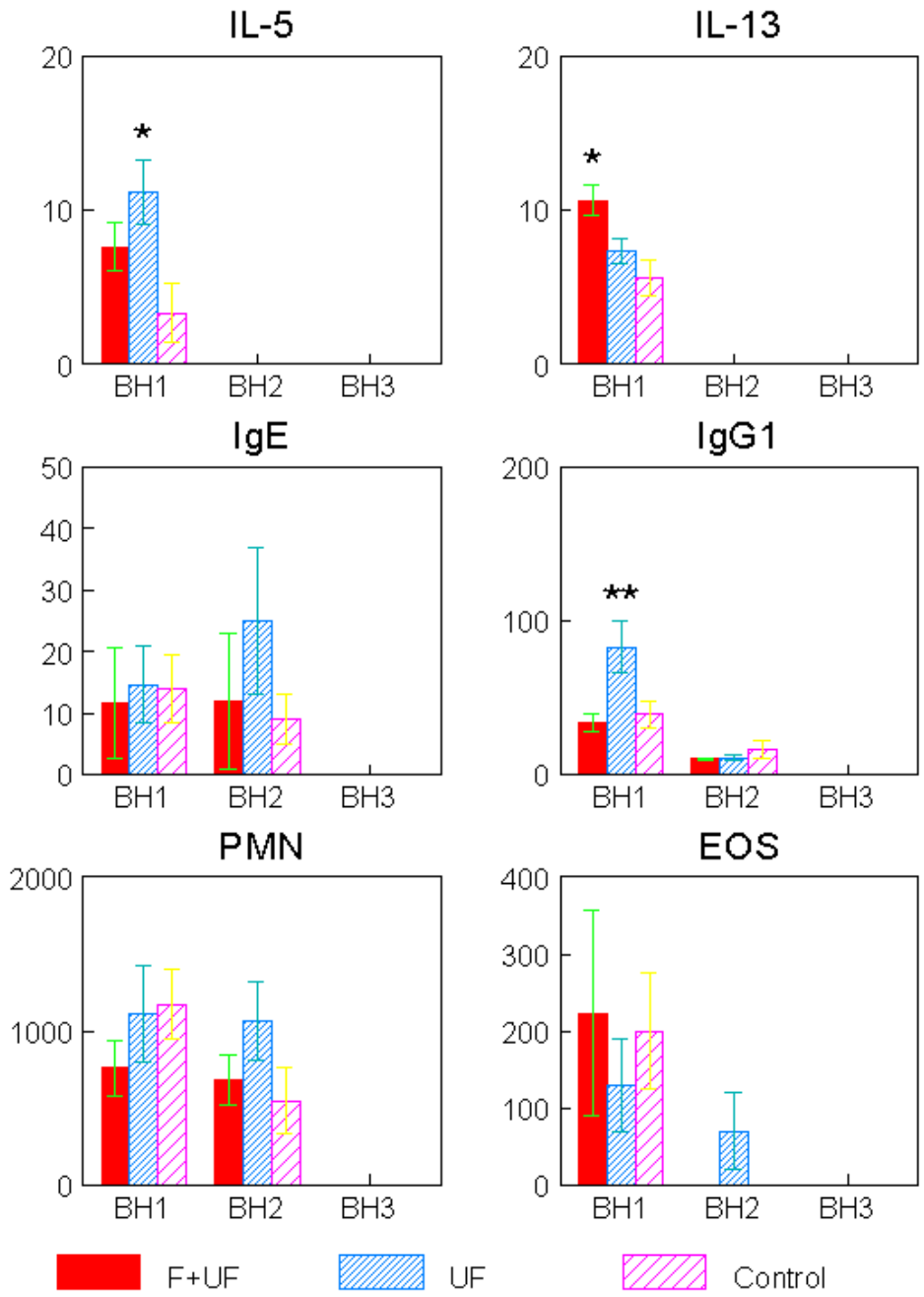

Particle Deposition
Lung Epithelium
IL-4
IL-6
IL-9
Antigen
Influx of Inflammatory Cells
IL-1
TNF
Release of cytokines and chemokines
IL-5
Activation of TH2 cells and release of mediators
Eosinophil influx
IL-4
IL-13
B cells produce IgE
Symptoms of Asthma
Bronchospasm
Mucus Production
Inflammation

Multiphase Model

- Airway allergy responses to CAPs may be dose-dependent.
- Triggering events are particle-induced inflammatory responses leading to oxidative stress.
  - Low Level -- Induce defenses (i.e. GSH and heme oxygenase)
  - Intermediate Levels -- Antigent presentation and T cell activation
  - High Levels -- Cytotoxicity (necrosis and apoptosis)


Responses of Mice Exposed ~50 Downwind of Freeway to CAPs and OVA
Group Sizes = 9 Bars Represent Mean ± SE
IL-5
Eosinophils
OVA-IgE
Eosinophils (x 10-3)
Normalized IgE Units x 10-3 ; IL-5 (nM/mL)
10
8
6
4
2
0
30
20
10
0
Not Detectable
0
800 (5)
400 (5)
400 (10)
Average Daily Particle Concentration in µg/m3 (Number of exposure days)


IL-5
nM/mL
15
10
5
0
*
IL-13
nM/mL
25
20
15
10
5
0
IgE
units
25
20
15
10
5
0
BH1
BH2
BH3
Site
IgG1
(Thousands)
30
20
10
0
*
BH1
BH2
BH3
Site
Expose
Control


IL-5
20
10
0
*
BH1
BH2
BH3
IL-13
20
10
0
*
BH1
BH2
BH3
IgE
50
40
30
20
10
0
BH1
BH2
BH3
IgG1
200
100
0
**
BH1
BH2
BH3
PMN
2000
1000
0
BH1
BH2
BH3
EOS
400
300
200
100
0
BH1
BH2
BH3
F+UF
UF
Control

Conclusion

- 10-day exposure at 400 μg/m^3 yielded significant increases in eosinophils and OVA-specific IgE. IL-5 increased also, but not significantly.
- High dose rates produced less responses than lower doses. This agrees with the multiphasic mechanistic model proposed by Nel.
- Responses decreased with increasing distance from freeway.