

Ultrafine Particle Health Effects

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Background

- PM2.5 standards based on evidence from Epi. studies
 - consistent associations between outdoor PM concentrations and adverse health effects
- National Research Council, 1997; *Research priorities for airborne particulate matter (PM)*
- Uncertainty due to limited scientific information about
 - specific types of particles and composition causing adverse health effects
 - contributions of particles of outdoor origin to actual human exposures
 - underlying mechanisms (pulmonary, vascular, cardiac) that can explain the epidemiological findings of mortality and morbidity associated with exposure to ambient particulate matter

Summary of Our PM Center Findings

- A wider range of target tissues and health endpoints are associated with PM exposure than was known in 1997
- Mobile sources are highly relevant to the risks posed by ambient PM
- Improved mechanistic understanding of PM toxicity

Ultrafine particles have an important role in toxicity

Health Effects Associated with PM Exposure

- CNS and autonomic nervous system
- Development: Low birth weight/preterm birth
- Increase in asthma and other respiratory disease in children
- Decrease in lung development and function in children
- Cardiovascular disease including atherosclerosis in adults
- Cancer

All Airborne PM is toxic to some degree; potency is based on physical and chemical characteristics

Objectives of this Ultrafine Presentation

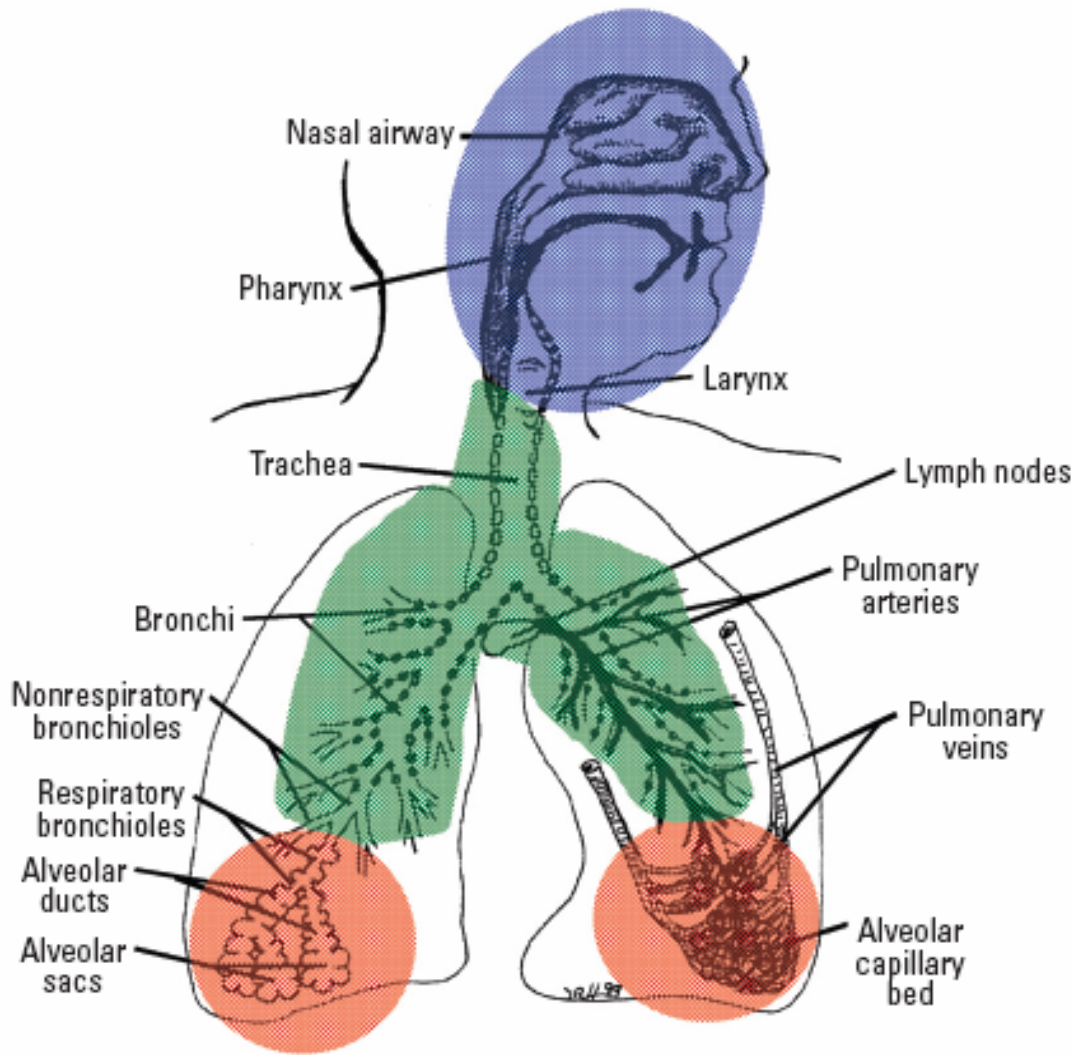
- Characteristics of ultrafine PM
- Some mechanistic features of ultrafine toxicity
- Health effects identified to date

- Central Hypothesis: The high number concentration of UFPs will be of major toxicologic/health significance when these particles interact with cells and subcellular components

Model for the Formation of Airborne Particulate Matter

- Combustion of organic materials produce a variety of compounds.
- Particles generated during combustion of organics will become rapidly coated with gas-phase adsorbates/condensates which will be strongly retained by the surface
- Release dependent on the polarity of the physiological media (membrane vs. aqueous)
- The toxic potential of UFPs is greatly enhanced by their free location and movement within cells which promote interactions with proteins, organelles and DNA

Translocation, cont.



Oberdorster et al., EHP, 2005

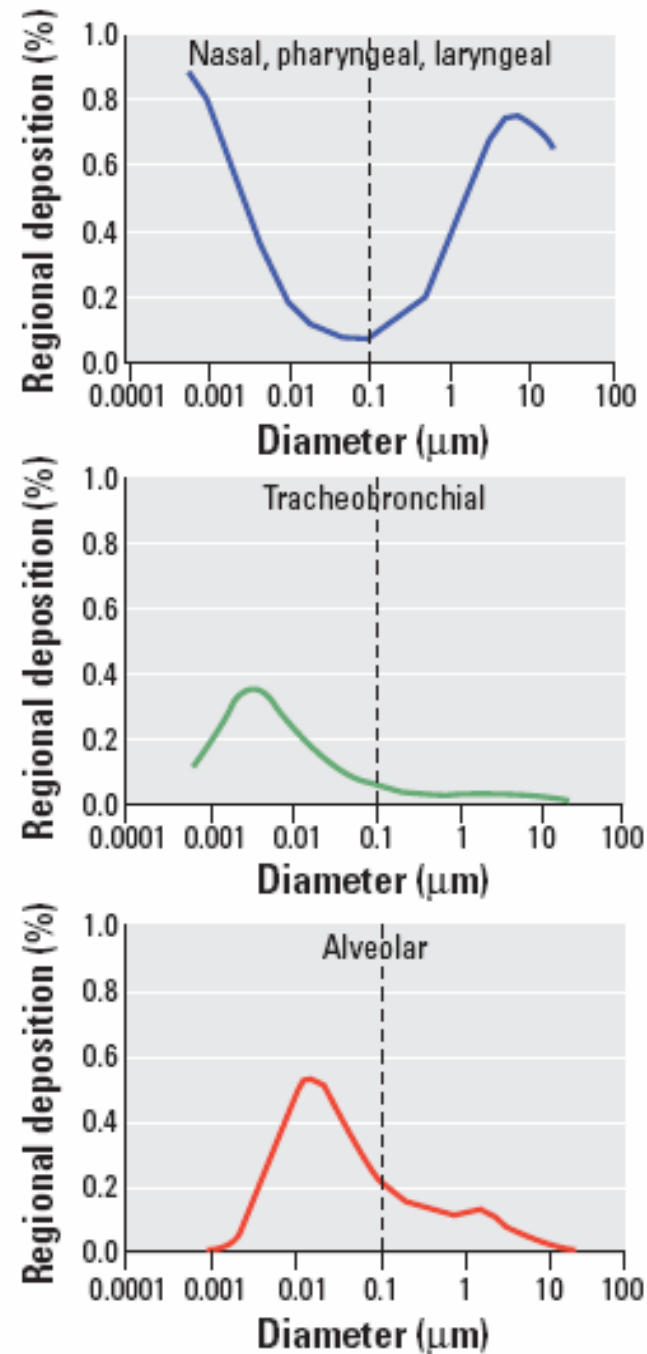
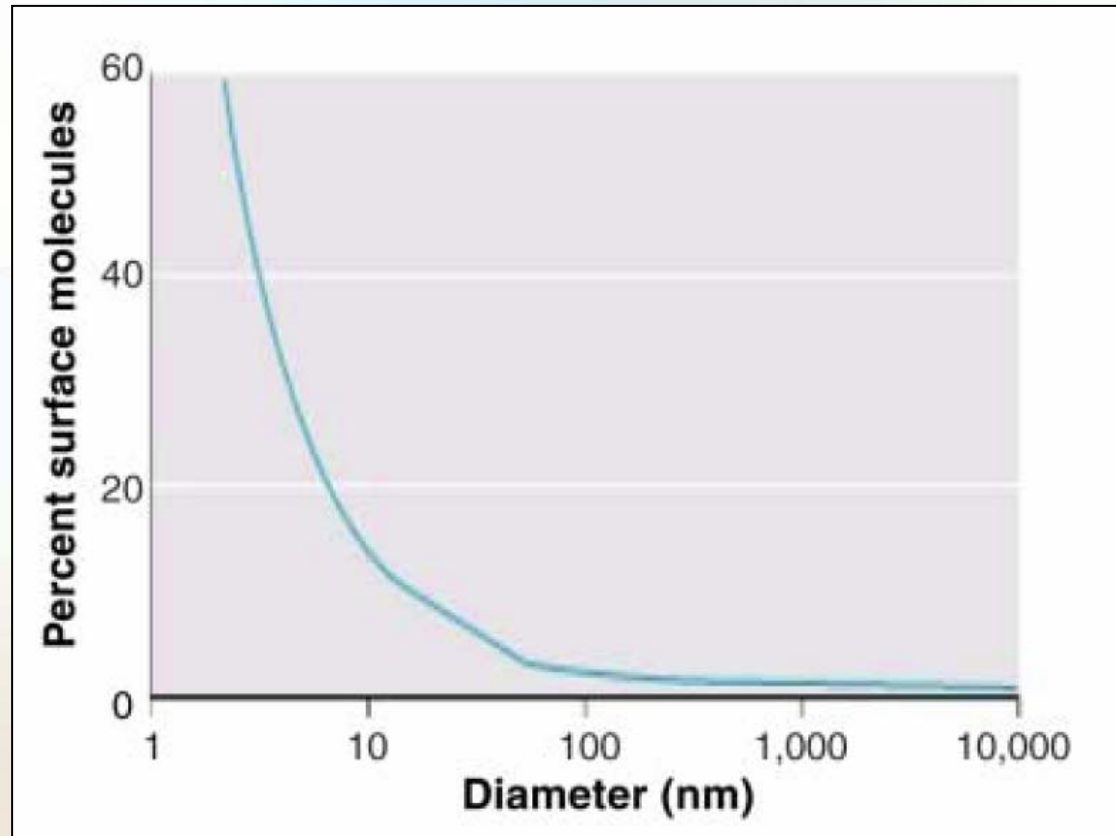


Table 1. Particle number and particle surface area for 10 $\mu\text{g}/\text{m}^3$ airborne particles (5).

Particle diameter (μm)	Particles/ml of air	Particle surface area ($\mu\text{m}^2/\text{ml}$ of air)
2	2	30
0.5	153	120
0.02	2,390,000	3000

Particle number concentrations have been found as high 1.5 million/cc with an average of 300,000-400,000 on a freeway near Long Beach

Inverse Relationship between Particle Size and Number of Particles on the surface



Sixty years ago-
viruses translocate
along axons and
across epithelia

Size nm

30

10

3

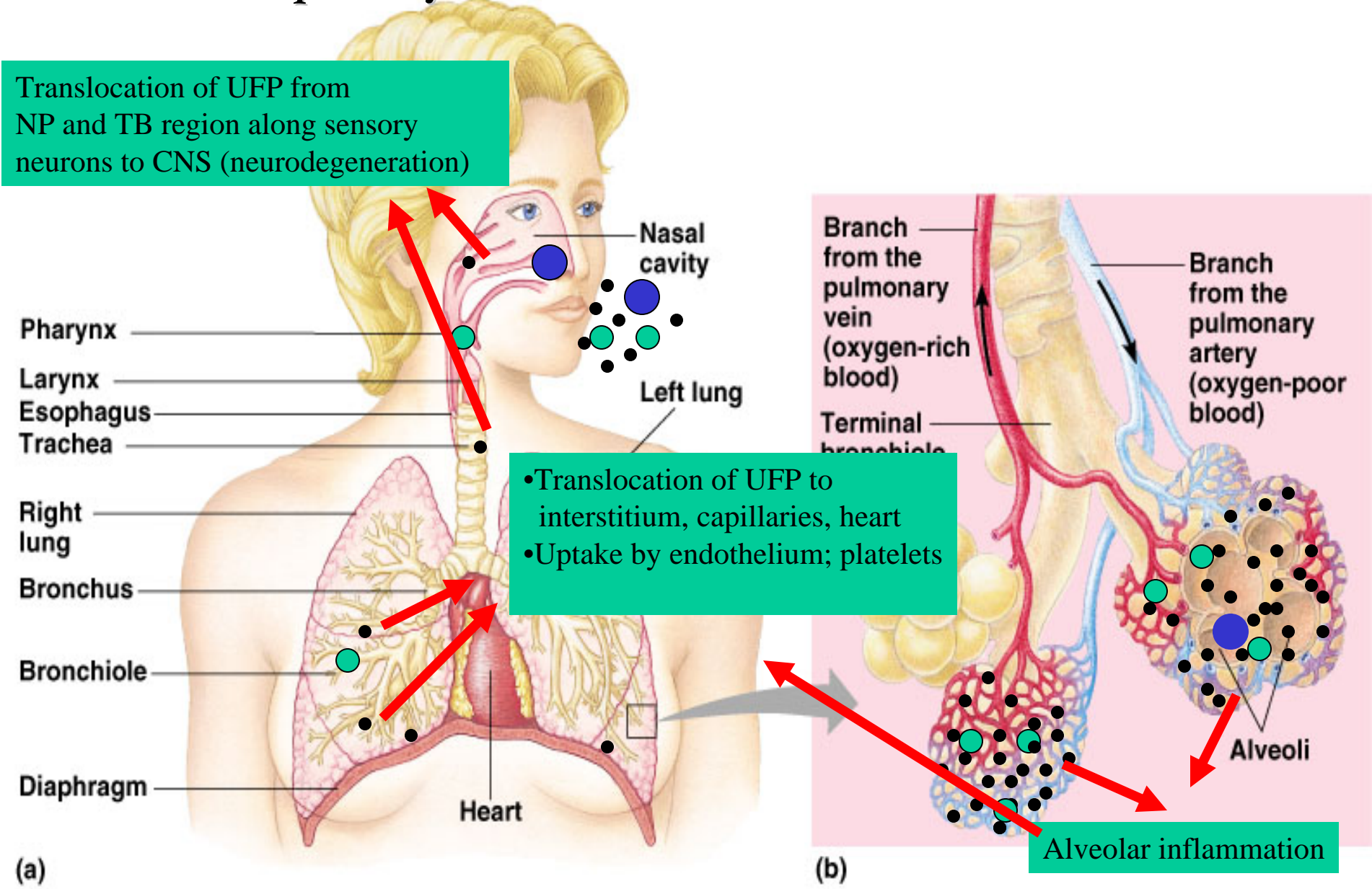
% on surface

10%

20%

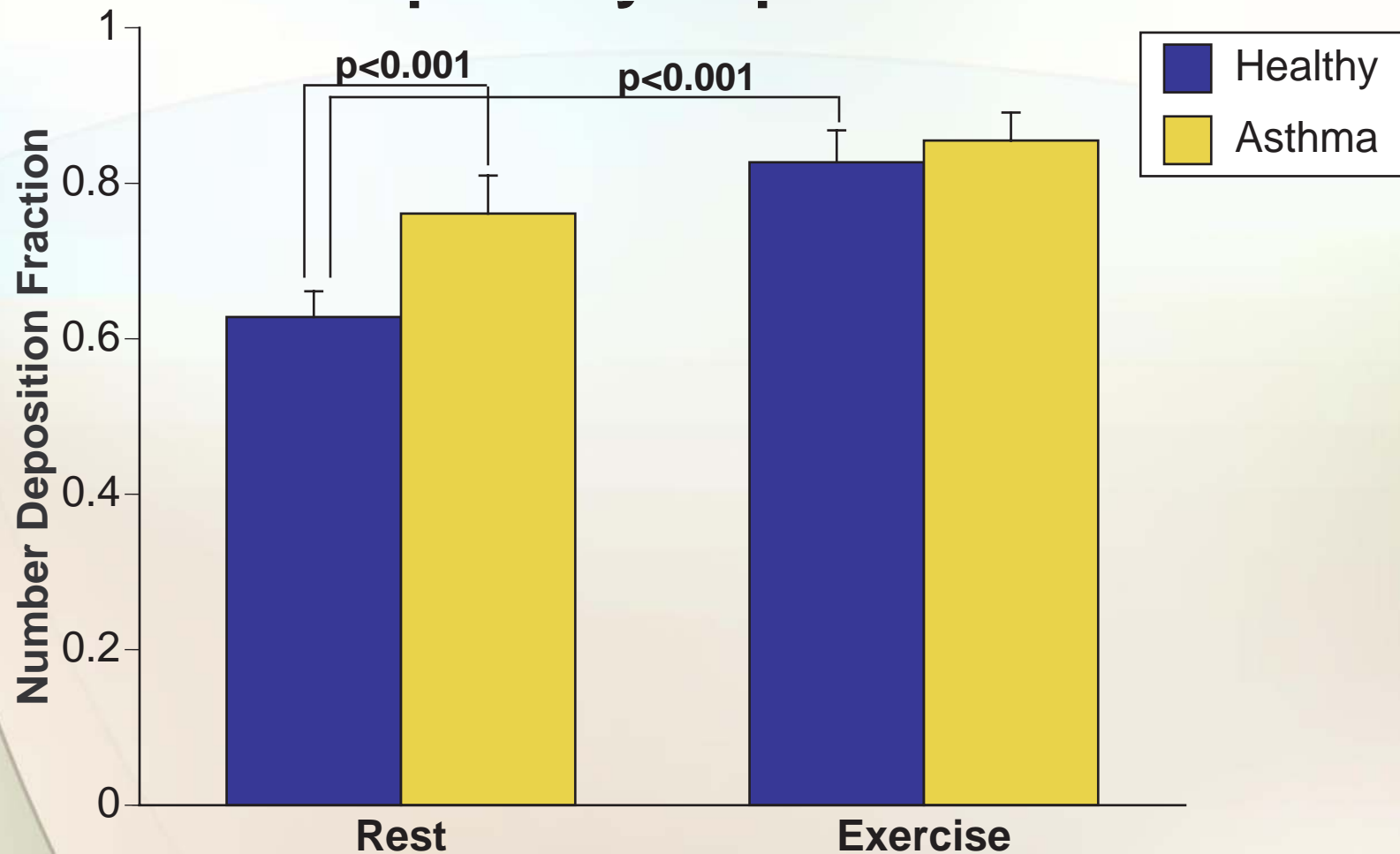
50%

Deposition and Pathways of Particle Translocation Within and Outside Respiratory Tract--Main Mechanism for UFP is Diffusion

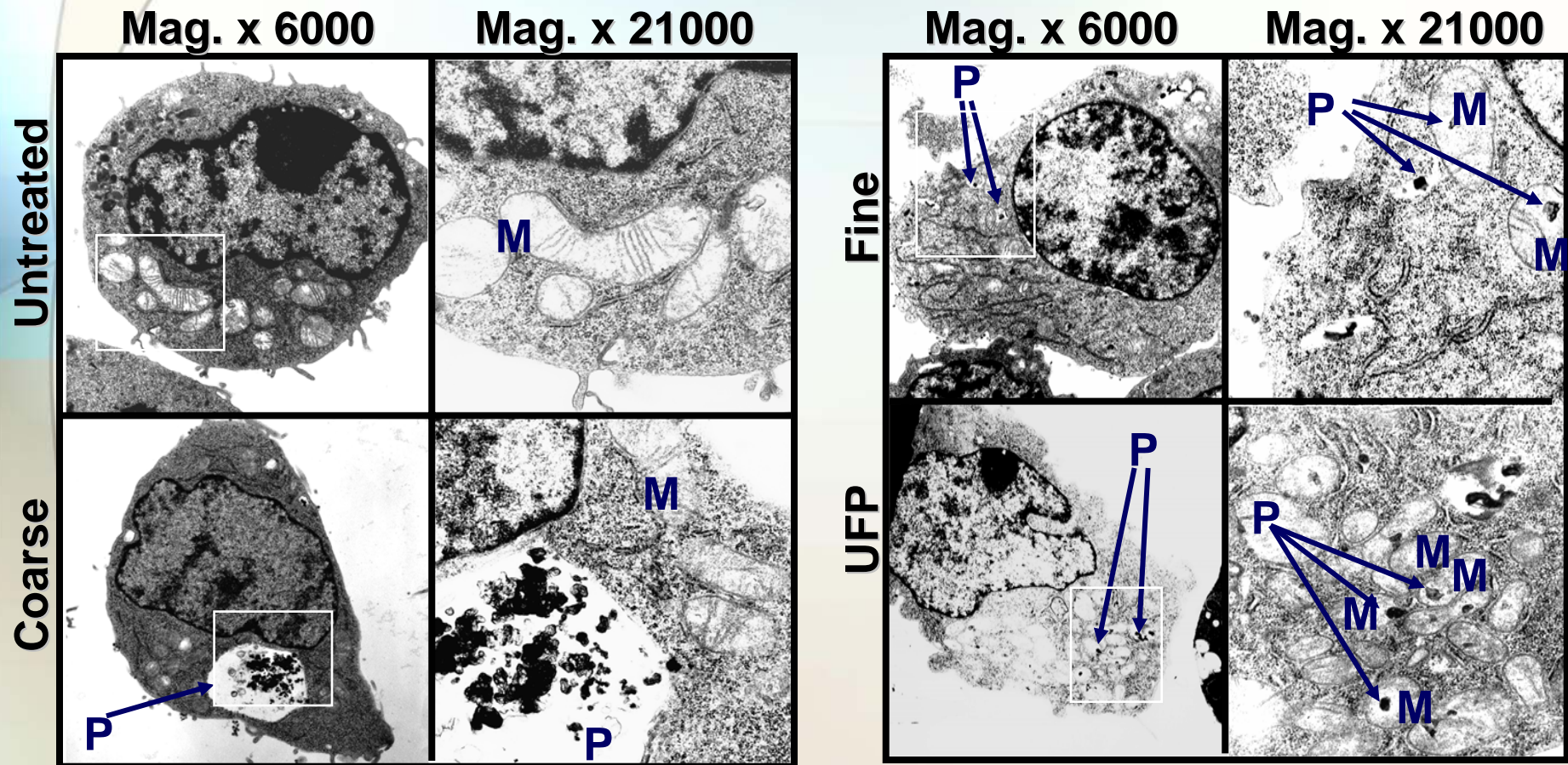


PM and Health

People with asthma have higher total respiratory dose of UFPs



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



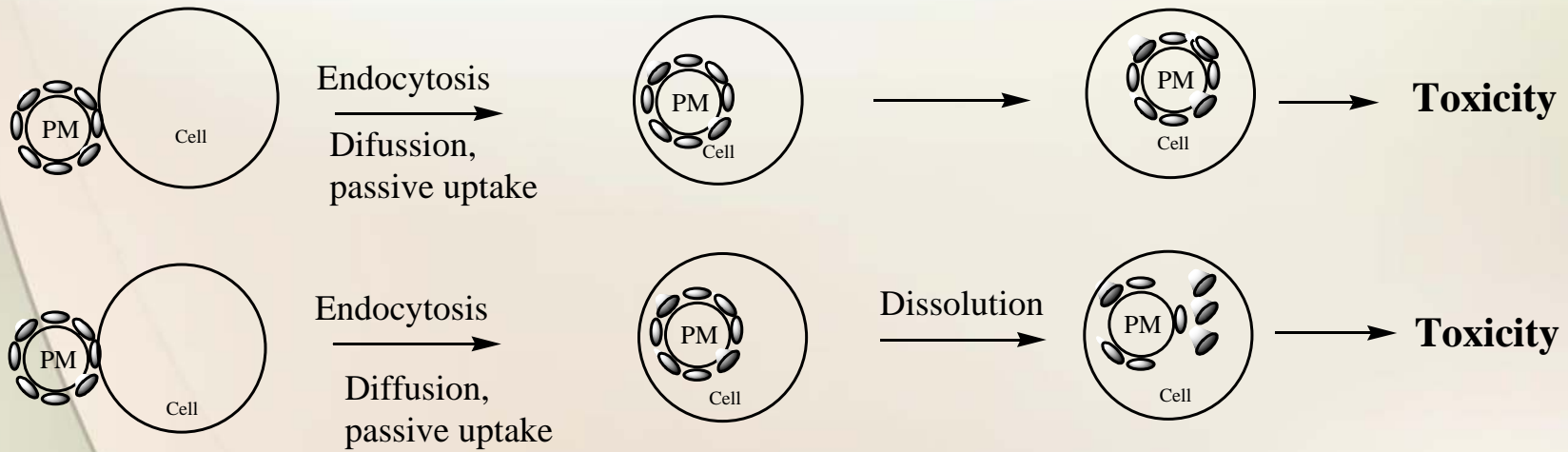
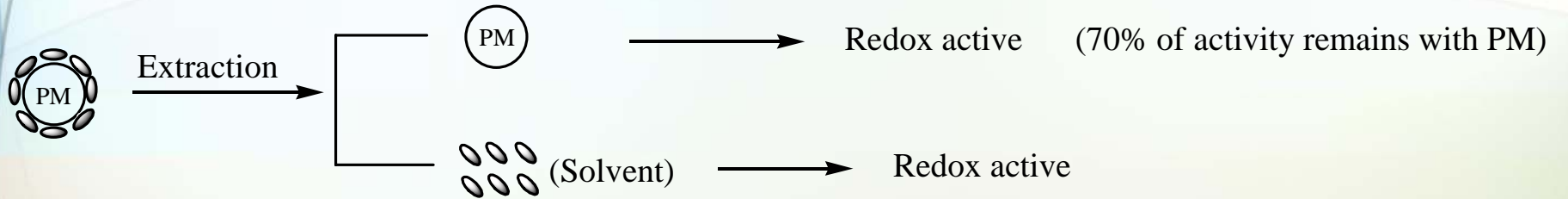
Mitochondria are redox active organelles

RAW 264.7

Ultrafine Particles Cross Cellular Membranes in Lungs and Cultured Cells

- Ultrastructural analysis of lung tissue found inhaled ultrafine particles were located within and beyond the epithelial barrier, in the main lung tissue compartments, cytoplasm and the nucleus of cells
- Particle uptake appears to occur via diffusion or passive uptake
- Particles within cells are not membrane bound and have direct access to intracellular proteins, organelles, and DNA which may greatly enhance their toxic potential

PM Structure and Toxicity–Redox Activity



Exposure to PM (BaP)

- Exposure involves two phases, a rapidly decaying one and one that persists for a long time
- Metabolic processes are also present that convert protoxins to toxins

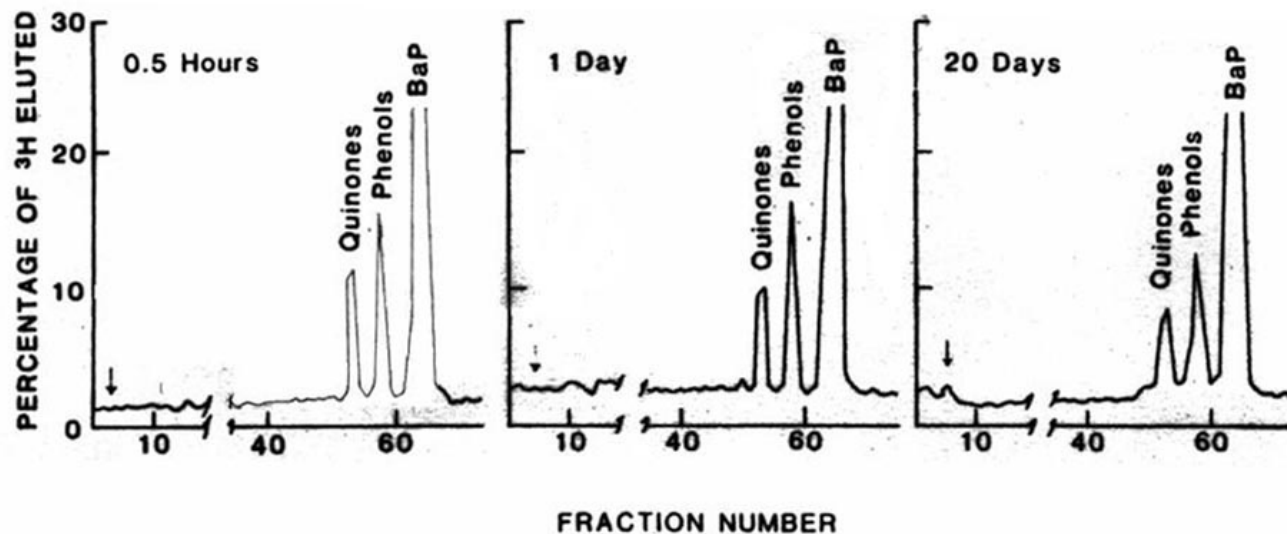


FIG. 6. High-performance liquid chromatographs of ^3H -BaP and ^3H -BaP metabolites organically extracted from lungs taken at various times after nose-only inhalation exposure of rats to diesel soot-associated ^3H -BaP. The arrow indicates the void volume. ^3H -BaP and its metabolites were identified by the comigration of ^3H radioactivity with authentic BaP standards detected by uv absorbance (254 nm).

PM characteristics

- Elemental carbon and PM in the lung increases with age. The load in secondary targets as well (clearance half life can be as great as 700 days in humans)
- PM participates in odd electron or radical chemistry
- PM capable of accepting electrons from reducing agents and pass them on to O_2 to generate toxic, reactive oxygen species
- PM is then capable of oxidative chemistry that is responsible downstream for health effects.

Characteristics of PM continued

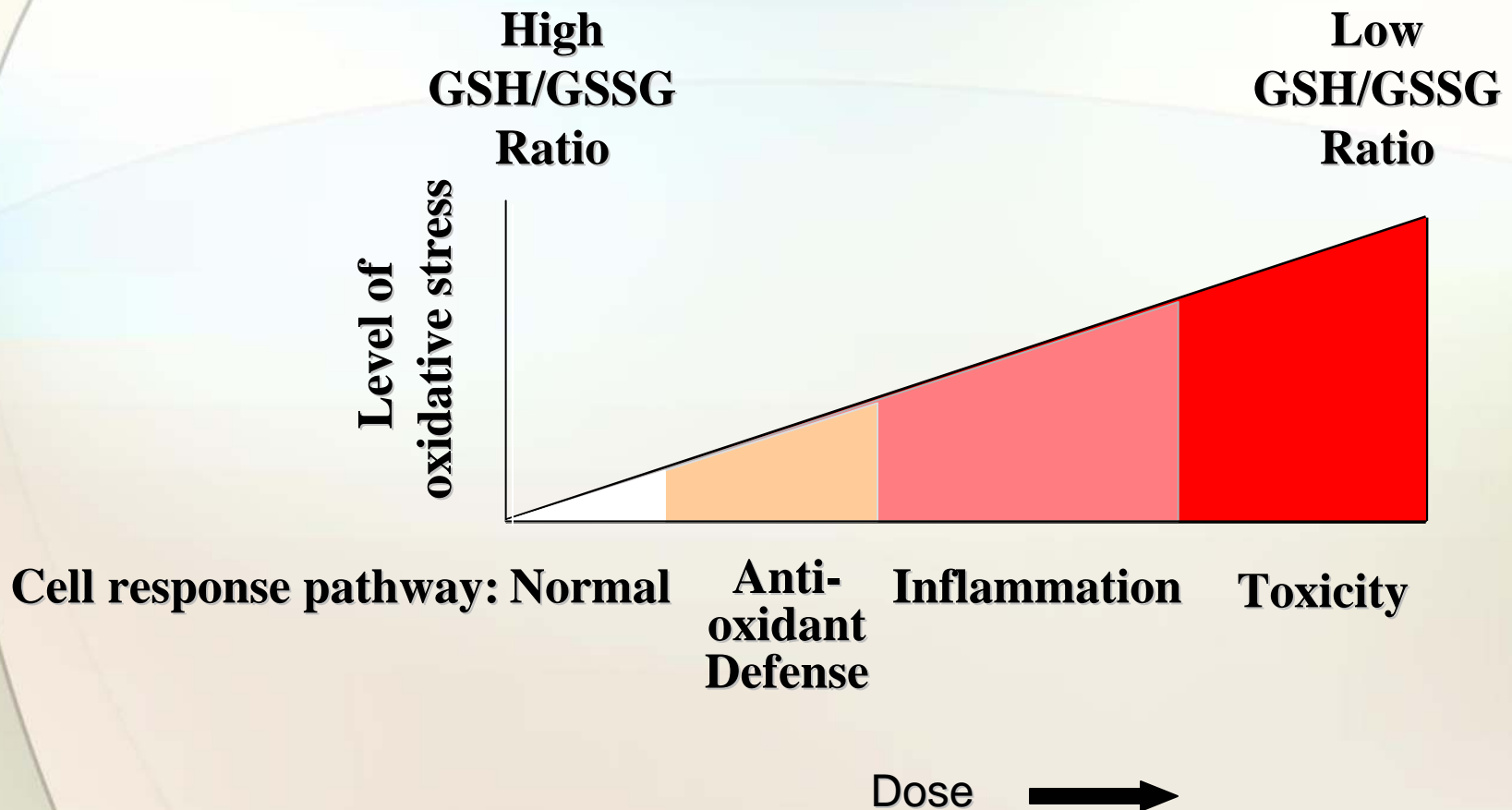
- The reactive chemical species in PM can be organic or inorganic and act via two chemical mechanisms, redox (formation of reactive oxygen species (ROS) and electrophilic reactions
- The most common functional group associated with PM toxicity in the cell are thiol groups on proteins
- Chemical assays have been used to demonstrate that PM can generate reactive oxygen species and thiol chemistry
- Ability of PM to participate in chemical/biochemical reactions can be used to group/rank PM sources in health outcomes

HYPOTHESIS: Organic chemical components and transition metals associated with PM contribute to adverse cardiorespiratory effects based on their ability to induce oxidative stress.

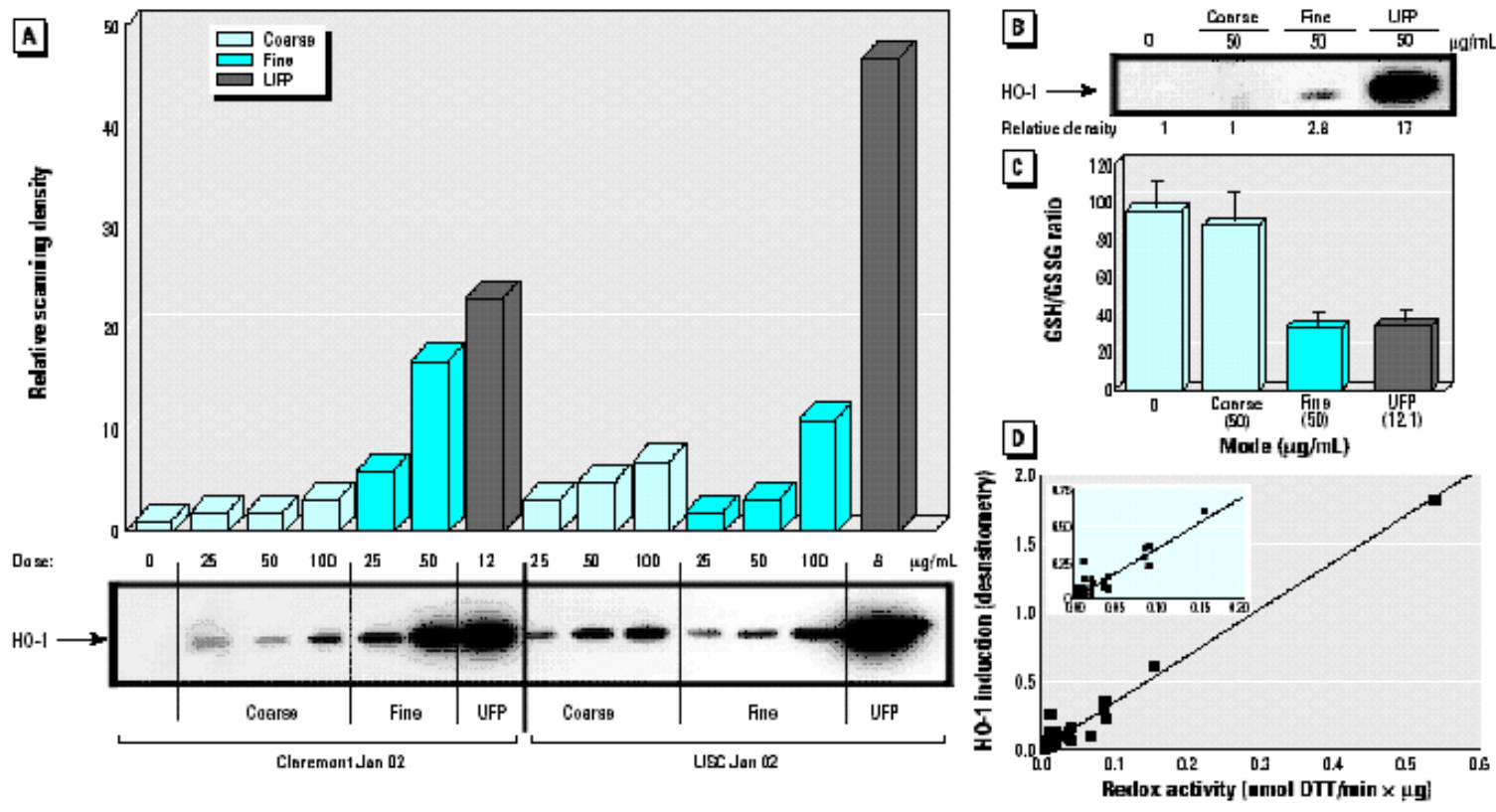
Oxidative stress is responsible for the development of inflammation in the lung and cardiovascular system,

UFP have large surface area and therefore adsorbed chemicals are bioavailable for redox or electrophilic chemistry.

Pathways of Oxidative Stress



Ambient PM Causes Oxidative Stress

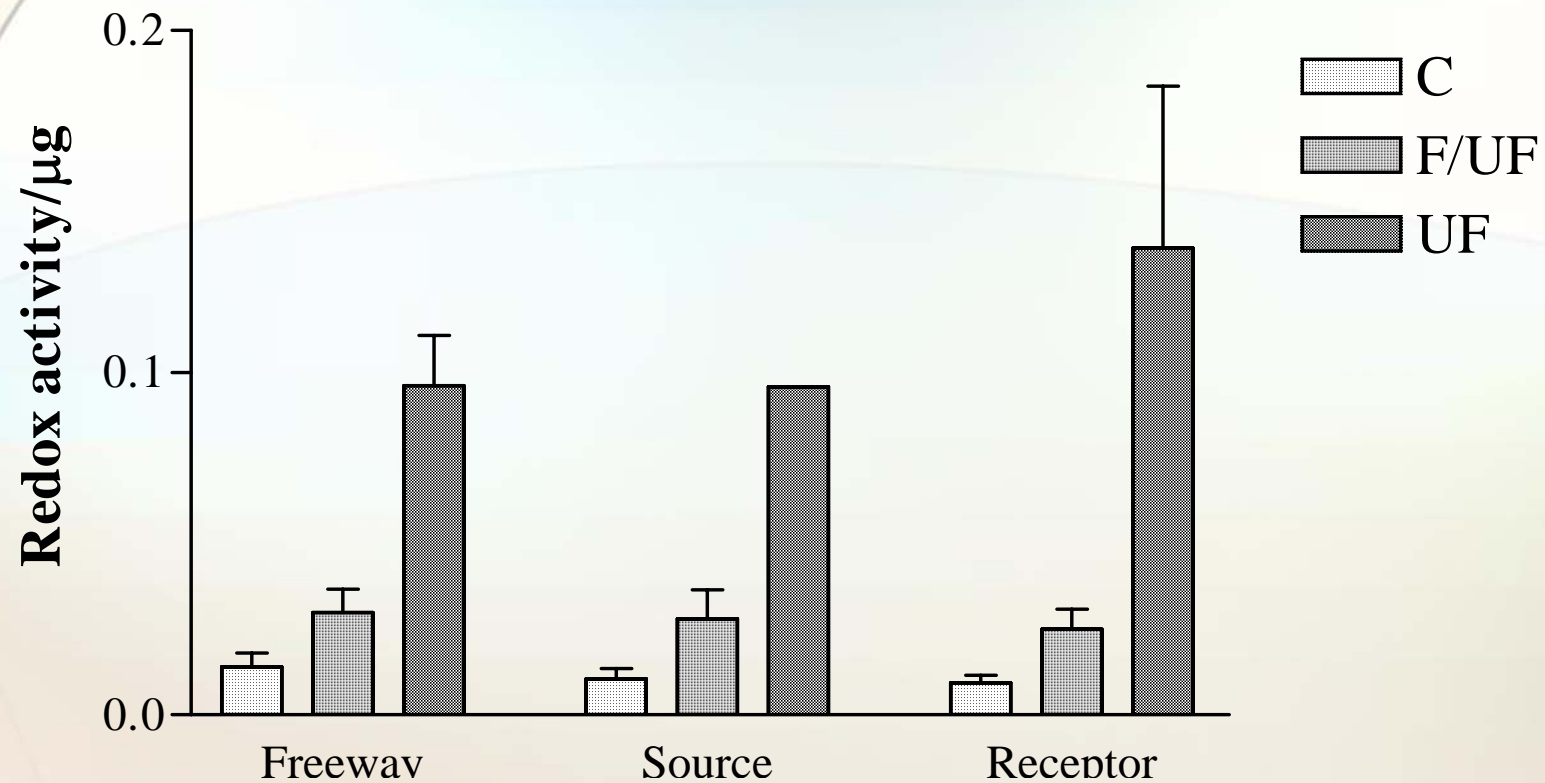


(A) **Dose dependent** induction of the antioxidant enzyme, hemeoxygenase-1 by ambient PM. **Ultrafine PM most active.**

(C) Change in GSH/GSSG ratios with different size fractions. **Ultrafine most potent.**

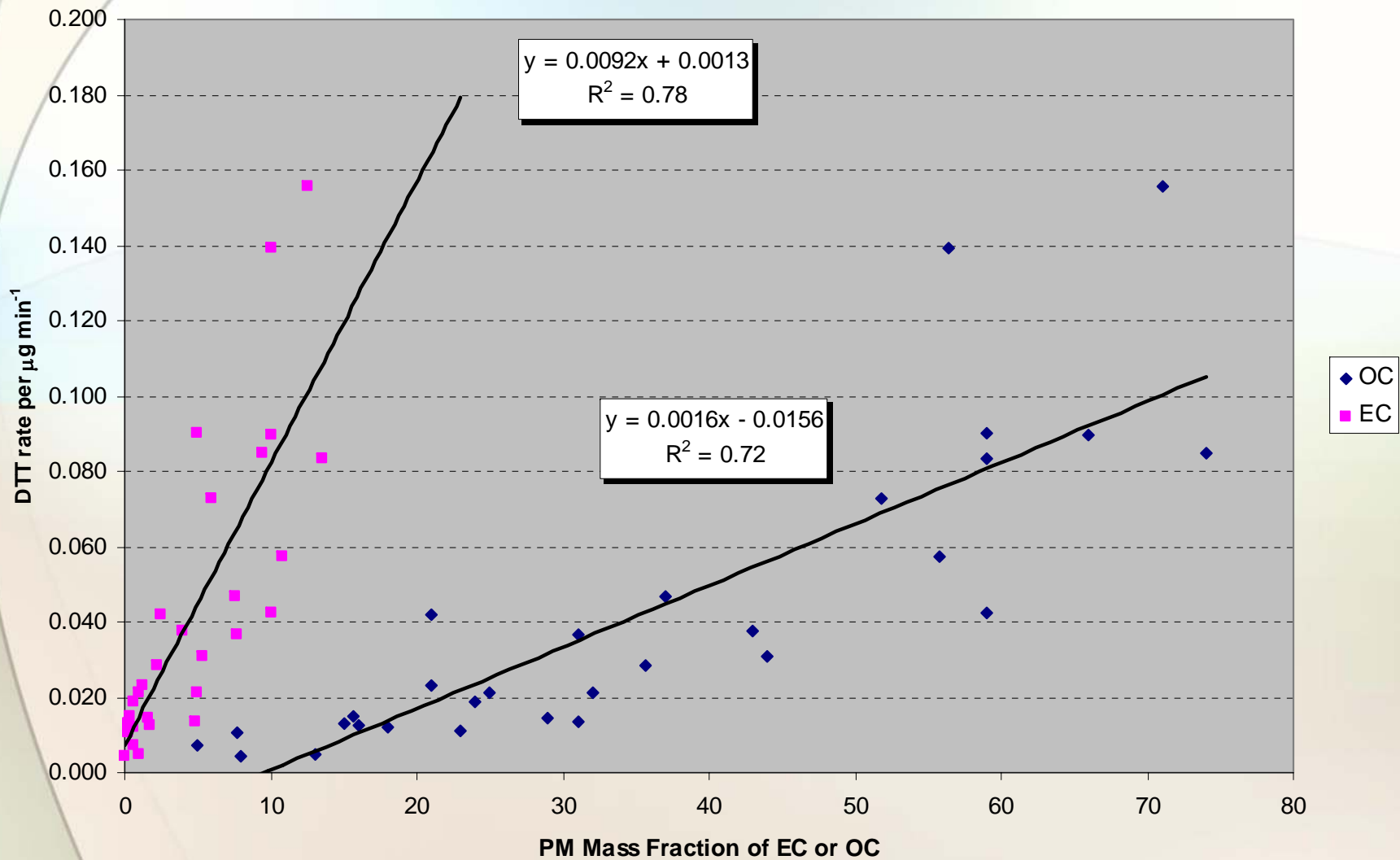
(D) Correlation of HO-1 induction with PM redox activity, assayed by consumption of the dithiol, DTT, a measure of redox activity.

Redox Activity

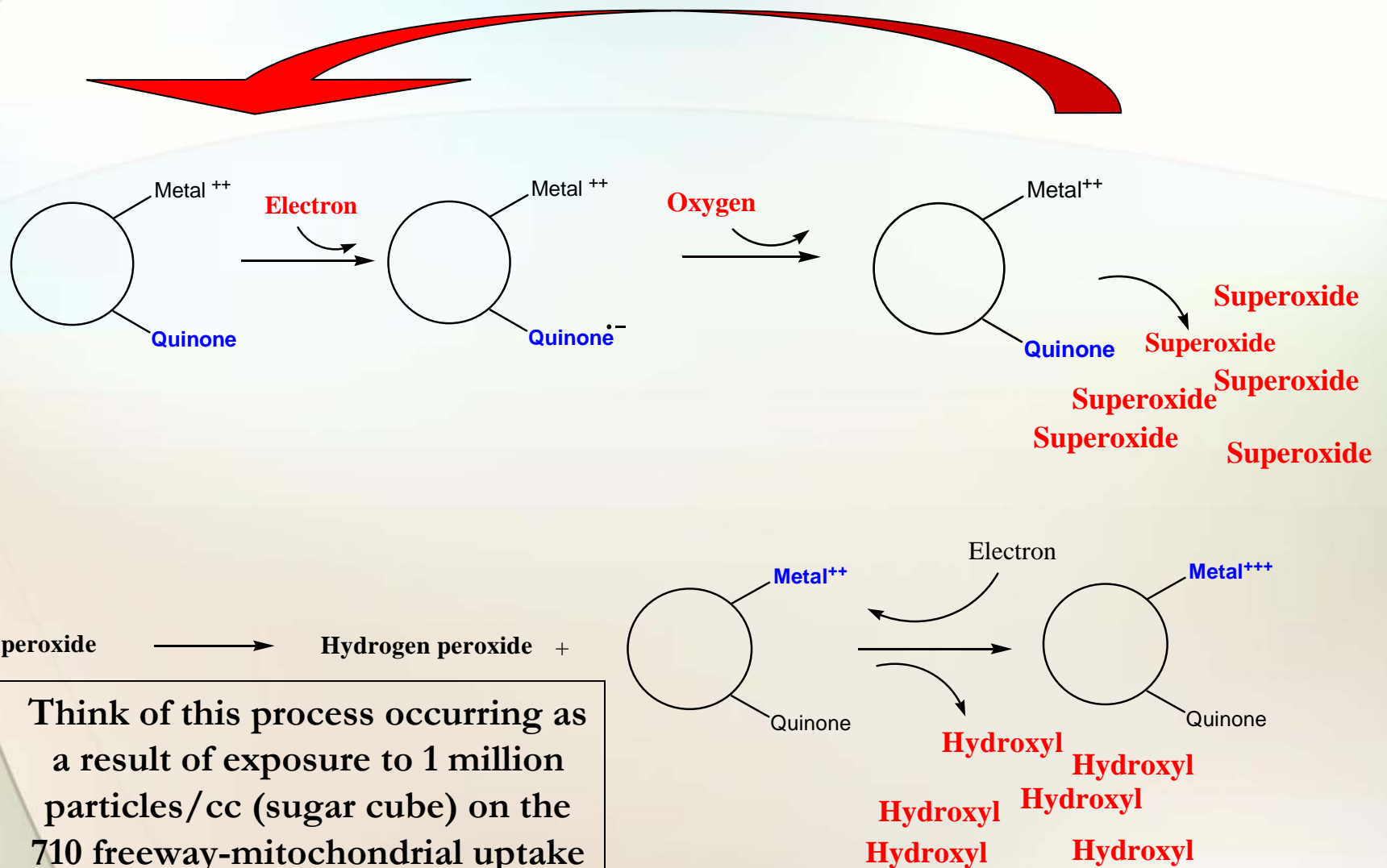


When activity is expressed per mass, the results reflect the potency of the sample

DTT Activity and Carbon



Compounds Capable of Catalytic Redox Activity and Oxidative Stress Production



Think of this process occurring as a result of exposure to 1 million particles/cc (sugar cube) on the 710 freeway-mitochondrial uptake

Particle Size and Composition Relation to Toxicity

Table 5
Contrasting features of coarse, fine, and ultrafine particles^a

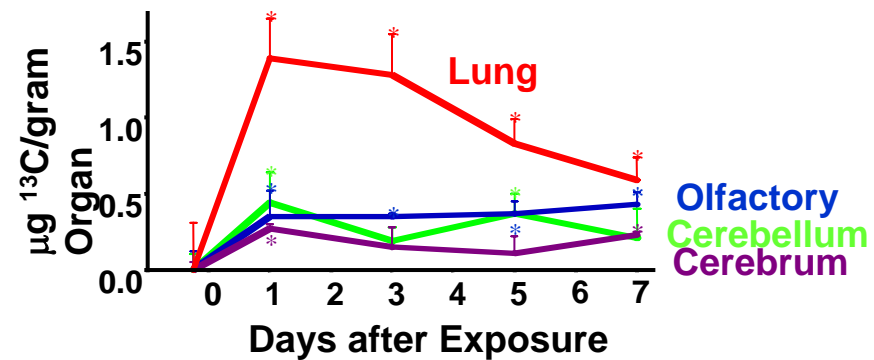
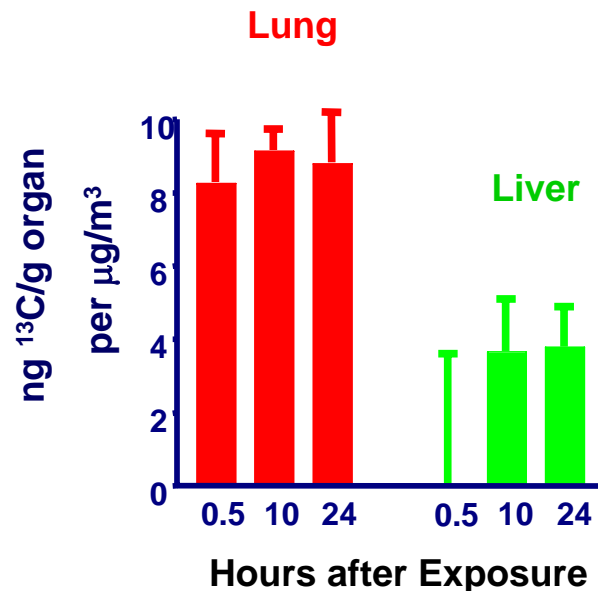
Parameters	Particle mode		
	Coarse (PM ₁₀)	Fine (PM _{2.5})	Ultrafine
Size	2.5–10 μm	2.5–0.15 μm	<0.15 μm
Organic carbon content	+	++	+++
Elemental carbon content	+	++	+++
Metals as % of total elements	+++	++	+
PAH content	+	+	+++
Redox activity (DTT assay)	+	++	+++
HO-1 induction	+	++	+++
GSH depletion	+	+++	+++
Mitochondrial damage	None	Some	Extensive

^a [85].

(sampled at source site)

Does PM Affect other Organ Systems?

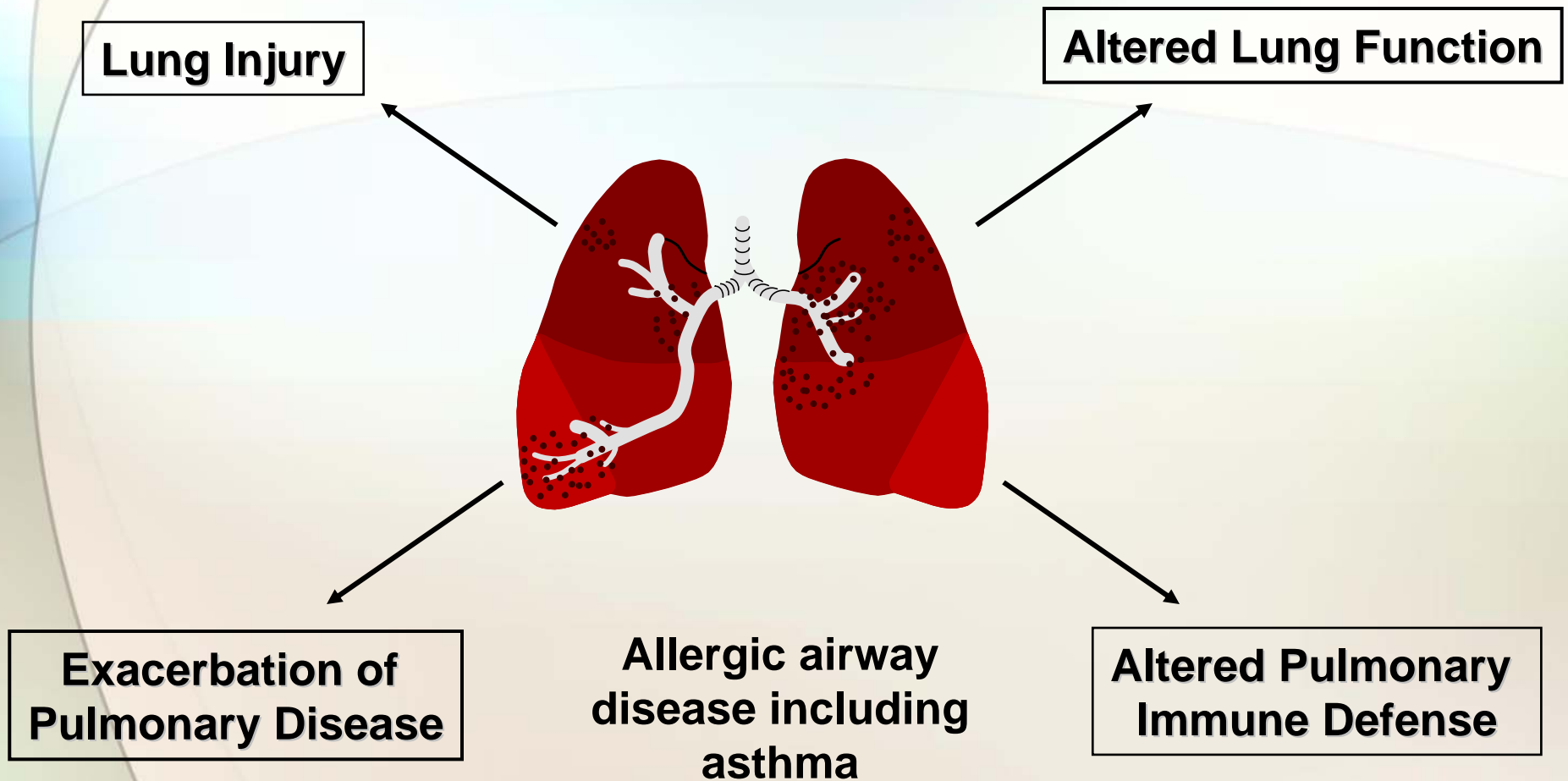
Labeled ultrafine particles are translocated to the lung and to specific regions of the brain, where they stimulate brain cells to produce pro-inflammatory markers: the particles appear to be preferentially located in the mitochondria: redox active



Brain Inflammation Markers
Tissue from Mice Exposed at BH2 2002

	Control	UF	F+UF
TNF α (ng/mL)	2.0 \pm 0.1	2.2 \pm 0.1	2.5 \pm 0.2
IL-1 α (ng/mL)	1.6 \pm 0.2	2.7 \pm 0.3*	2.0 \pm 0.4*
NF κ B (units x 10 ⁻³)	8.5 \pm 4.4	11.0 \pm 1.6**	10.7 \pm 3.0**

Potential Effects of PM on the Pulmonary System



Respiratory Health and Traffic

European studies with better estimates of traffic exposure

- Adverse respiratory health associated with exposure to nearby traffic
- Truck traffic (diesel) may be more important
- Effects close to heavy motorways
- Effect may be more prominent in girls

Traffic Related Studies

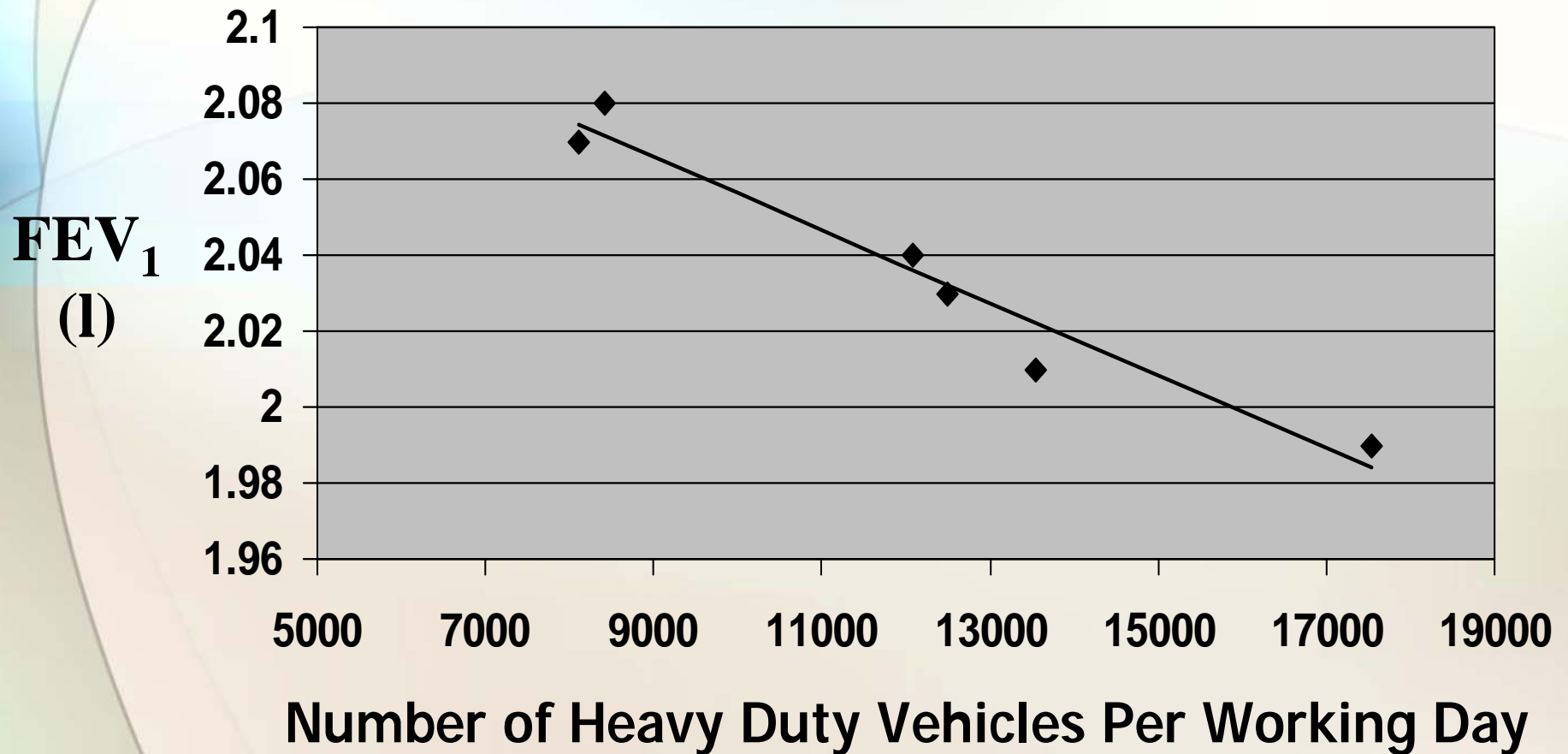
- Cardiopulmonary mortality (Netherlands) and road proximity: 1.95 (1.09-3.52); black smoke: 1.71 (1.10-2.67)
- Wheezing in school children (England): 1.08 (1.00-1.16) (Venn et al, Am J Crit Care Med, 2001) living within 150m of a main road
- Lung function changes in children less than 300 m to freeway (Netherlands): FVC -3.6; FEV1 -4.1 (truck traffic density; FVC -2.7; FEV1 -3.7 (black smoke)

Netherlands: Brunekreef and co-investigators

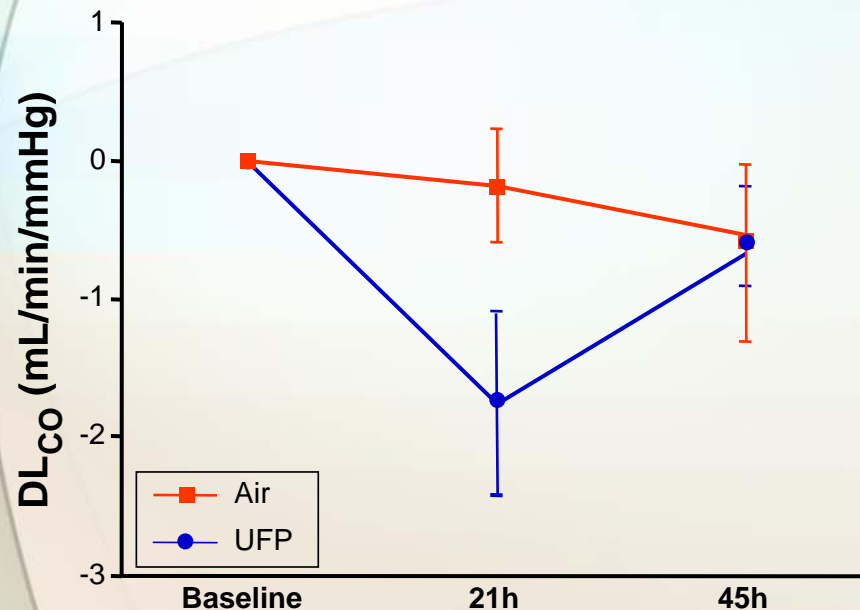
Respiratory Effects and Ultrafine Particles

- **Peters:** Study in asthmatics: Decreases in peak expiratory flow, feeling ill during the day and cough were associated with the number concentration and the mass concentration of fine and ultrafine PM. Peters et al., Am J Crit Care Med, 1997
- Models suggest the decrease in peak expiratory flow were most closely associated with 5 day means of ultrafines as well as health endpoints.
- **Pettinen:** The present results (PEF) show that particle number concentrations (especially UF) are negatively associated with the respiratory health of adult asthmatics.
- Findings suggest that particle number concentrations in addition to mass measurements should be used in air quality monitoring. Pettinen et al, Eur Resp J. 2001
- **Brunekreef:** Growing body of evidence incriminating traffic fumes and respiratory disease. Brunekreef and Sunyer, Eur Resp. J, 2003

Living within 300m of Major Roadways Affects Lung Function



PM Causes Changes in Lung Function



Humans exposed to ultrafine particles have decreased diffusing capacity, a measure of oxygen transfer from the lung to the blood (vasoconstrictive effect)

Traffic, Susceptibility and Childhood Asthma

(McConnell et al., EHP, May, 2006)

- Asthma and wheeze were strongly associated with residential proximity to a major road (75m from a major road)
- These associations were strongest among children with no parental history of asthma who had lived at the same address since early in life
- In this group, the highest risk occurred adjacent to a major road and risk decreased to background rates at 150-200 m from the road
- Larger risks of asthma associated with long-term residence within 75m of a major road were observed among girls than among boys

Respiratory Toxicity and Traffic

Study of exhaled nitric oxide (Delfino)

Hypothesis

- Airway inflammation in asthmatic children as measured by exhaled nitric oxide (eNO) will increase with exposure to fine PM

Conclusion

- eNO increased with higher concentrations of both personal and regional outdoor elemental carbon levels and outdoor organic carbon. Stronger association with EC suggests diesel exhaust exposure may lead to airway inflammation in children with asthma.

Clinical exposures to PM (Gong)

Hypothesis

- Short-term exposures to concentrated ambient ultrafine particles in Los Angeles cause acute cardiopulmonary responses

Conclusion

- Reduction of O₂ in arterial blood noted: clinical significance in susceptible individuals with compromised cardiorespiratory status

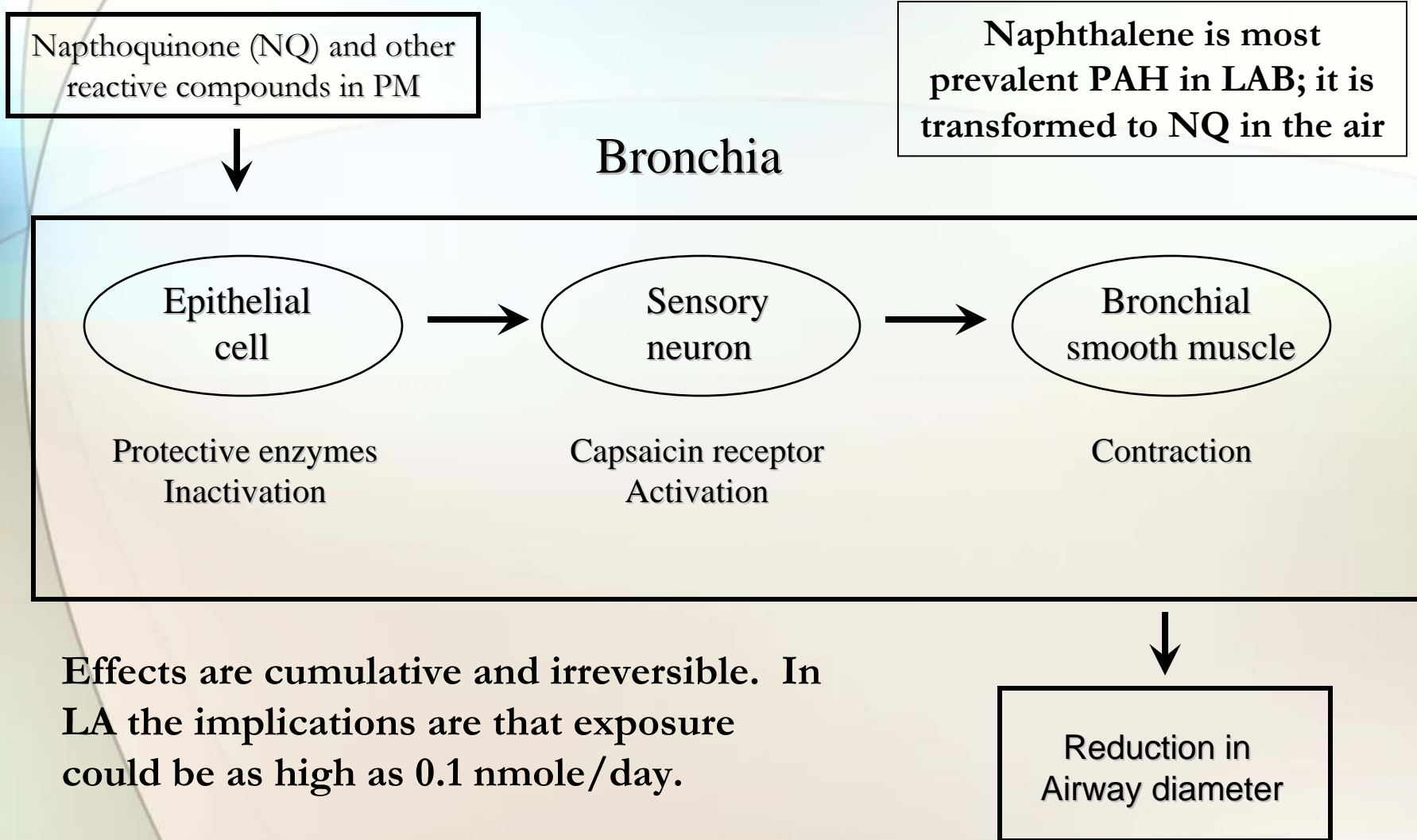
Source Related Investigations – Freeway Studies/Traffic Density

- Hypothesis 1: Mobile source emissions will exacerbate airway inflammation and allergic airway disease and produce cardiopulmonary effects
- Hypothesis 2: The magnitude of allergic airway disease and cardiovascular effects from mobile sources are a function of the size distribution of PM
- Hypothesis 3: Exposure to ultrafine particles at very close proximity to a freeway will result in the most severe effects

Conclusions

- Allergic responses including enhancement of sensitization were seen in mice exposed at the site closest to the freeway
- Hypothesis of ultrafine particles as mediators of health effects is supported

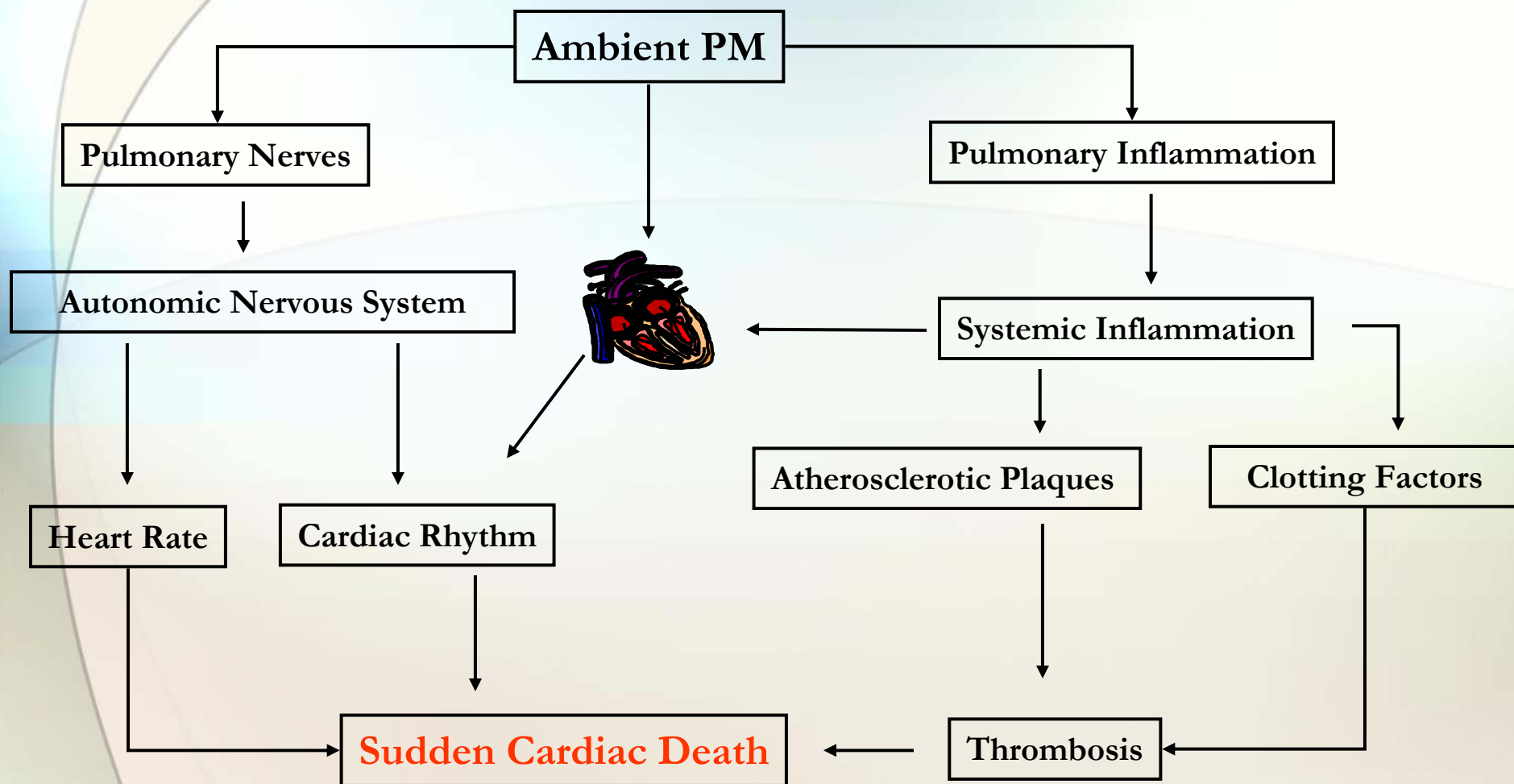
Exacerbation of Asthma by PM Components from Fossil Fuel Combustion



Ultrafines and Surface Area

- Quantitative comparison of acute adverse effects of different UFPs at a dose range causing a moderate inflammatory response in lungs in mice.
- Study confirms the surface area concept is a valuable reference for the assessment of causative health effects for carbonaceous UFPs.
- Particle surface area may be most appropriate parameter to evaluate inflammatory potential and predict adverse effects of UFP

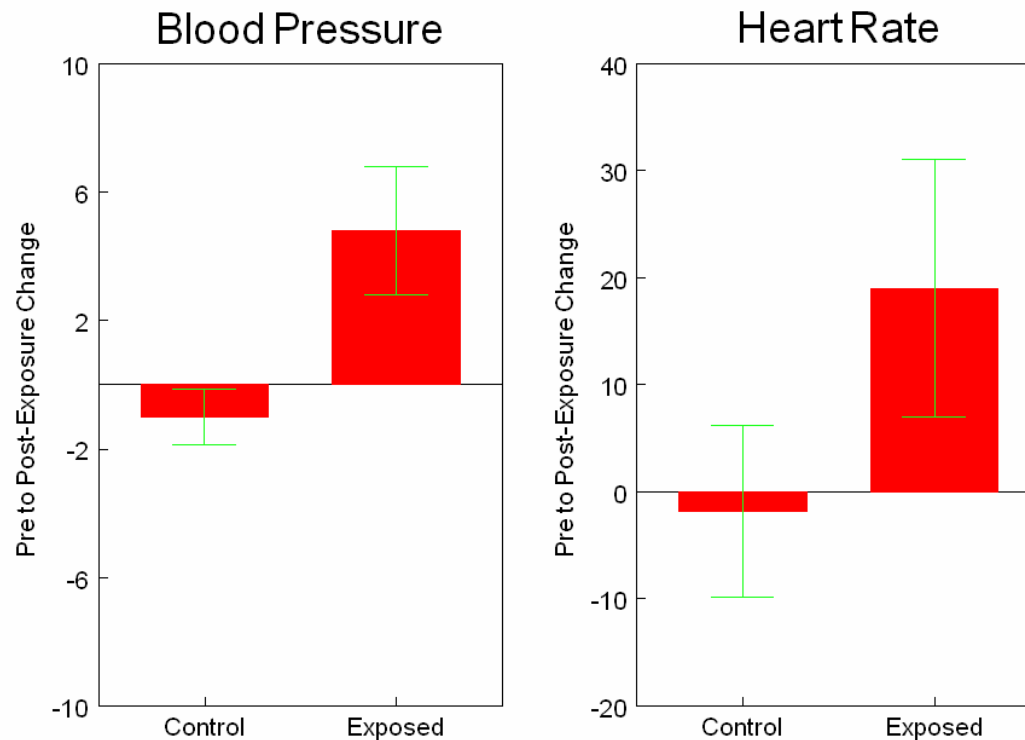
Potential Effects of PM on the Cardiovascular System



Hypothesis: High UFP exposures lead to systemic inflammation through oxidative stress and promote the progression of atherosclerosis?

In Vivo Studies--The Geriatric Rat

Blood pressure and heart rate were increased after CAPs exposures



Panel Studies of Cardiovascular Health

Panel studies with repeated measures show associations between PM and risk of:

- Cardiac ischemia and arrhythmias
- Increased blood pressure
- Decreased heart rate variability
- Increased circulating markers of thrombosis and inflammation

Ultrafine Studies

- Wichmann et al., 2000: Associations between ambient UFPs and mortality
- Pekkanen et al., 2002: Cardiac ischemia in relation to UF particles. Odds ratio – 2.84 and 10,000 ufp/cm³
- Chan et al., 2004: Personal exposure to UFP was associated with decreases in heart rate variability-
autonomic control of cardiac rhythm associated with increased mortality after MI and related to sudden arrhythmic death
- Devlin et al., 2003: Significant decreases in HRV in 10 elderly adults exposed to CAPs from mobile sources

Traffic Studies

- Riediker et al., 2004: Potential physiologic effects of in-vehicle roadside exposures were investigated in North Carolina Highway Patrol troopers
- Findings: decreased lymphocytes, increased red blood cell indices, von Willebrand factor, next morning heart beat cycle length, next morning HRV and ectopic beats

“In vehicle exposure may cause pathophysiological changes that involve inflammation, coagulation and cardiac rhythm.”

Exposure to Traffic and the Onset of Myocardial Infarction

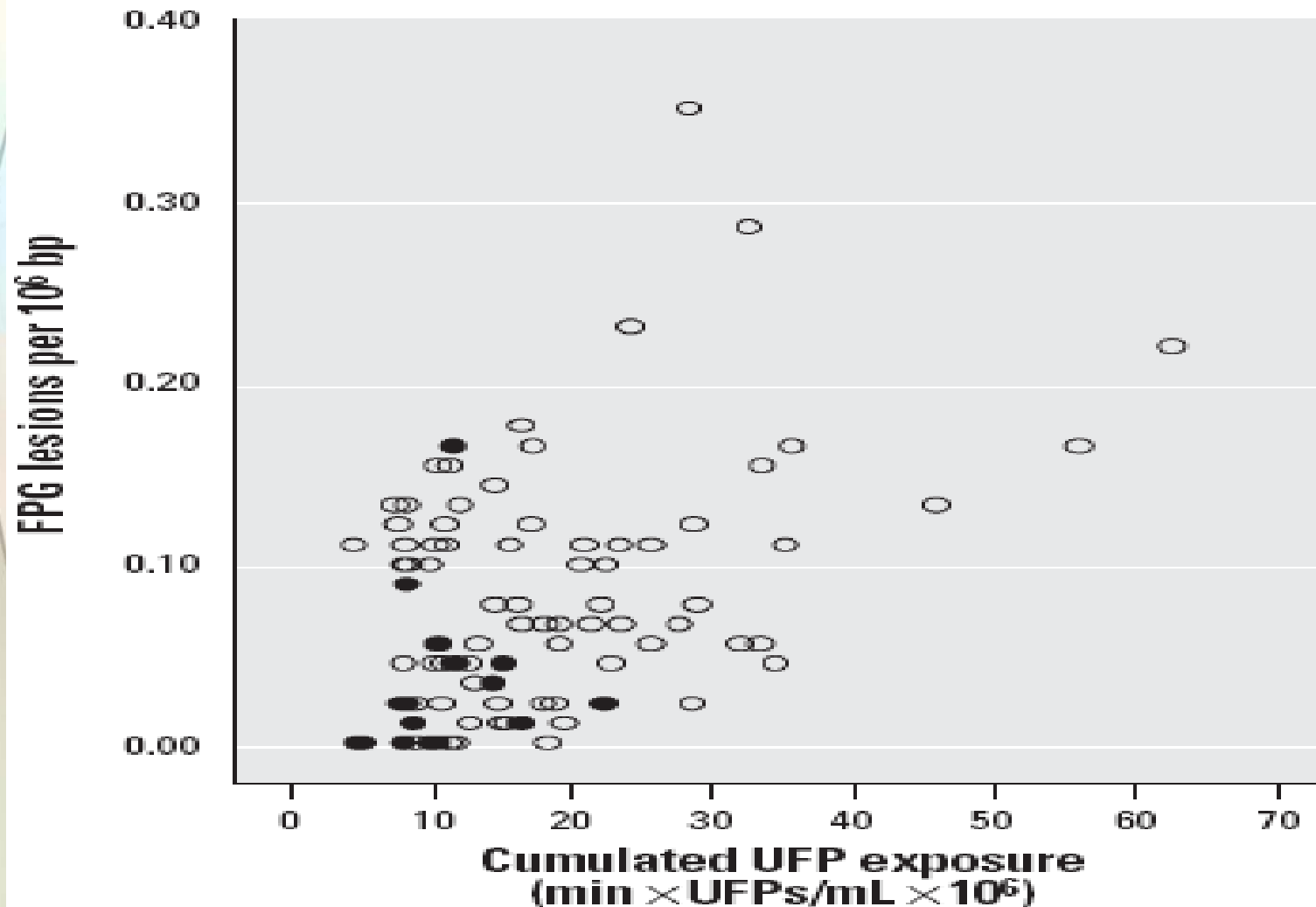
- **Study intent:** Assess whether exposure to traffic can trigger myocardial infarction
- **Results:** An association between exposure to traffic and myocardial infarction onset one hour later was observed (odds ratio: 2.9; 95% confidence interval: 2.2 to 3.8, $p < 0.001$)
- Time spent in cars, public transport and on bicycles was consistently connected with an increased risk for myocardial infarction
- **Conclusions:** Transient exposure to traffic might pose a risk in persons vulnerable to myocardial infarctions

Conclusions–Role of Ultrafines

- The role of ultrafine particles have not been fully investigated due to lack of data
- Data from epidemiological studies indirectly link implicate traffic and other combustion related pollutants which include ultrafines
- Evidence shows inflammation and oxidative stress are related to both acute and chronic processes in cardiovascular health including atherosclerosis
- Redox active components of ultrafine particles reach target sites in the lungs, vasculature and heart to induce inflammation and oxidative stress*

Ultrafine Particles and DNA Damage

Oxidative DNA Base Damage was Associated with Personal Exposure To UFPs



Four Main Properties of Diesel Particles

- High proportion of Elemental Carbon
- Large surface area (a carbon core)
- Enrichment of PAHs
- Most diesel particles (50-90%) are **SMALL** (~.005 to 0.05um)

Lung Cancer in Railroad Workers Exposed to Diesel Exhaust

Eric Garshick,^{1,2} Francine Laden,^{2,3} Jaime E. Hart,^{2,3} Bernard Rosner,² Thomas J. Smith,³ Douglas W. Dockery,^{2,3} and Frank E. Speizer^{2,3}

¹Pulmonary and Critical Care Medicine Section, Medical Service, Veterans Affairs Boston Healthcare System, Boston, Massachusetts, USA; ²Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA; ³Exposure, Epidemiology and Risk Program, Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, USA

Diesel exhaust has been suspected to be a lung carcinogen. The assessment of this lung cancer risk has been limited by lack of studies of exposed workers followed for many years. In this study, we assessed lung cancer mortality in 54,973 U.S. railroad workers between 1959 and 1996 (38 years). By 1959, the U.S. railroad industry had largely converted from coal-fired to diesel-powered locomotives. We obtained work histories from the U.S. Railroad Retirement Board, and ascertained mortality using Railroad Retirement Board, Social Security, and Health Care Financing Administration records. Cause of death was obtained from the National Death Index and death certificates. There were 43,593 total deaths including 4,351 lung cancer deaths. Adjusting for a healthy worker survivor effect and age, railroad workers in jobs associated with operating trains had a relative risk of lung cancer mortality of 1.40 (95% confidence interval, 1.30–1.51). Lung cancer mortality did not increase with increasing years of work in these jobs. Lung cancer mortality was elevated in jobs associated with work on trains powered by diesel locomotives. Although a contribution from exposure to coal combustion products before 1959 cannot be excluded, these results suggest that exposure to diesel exhaust contributed to lung cancer mortality in this cohort. *Key words:* diesel exhaust, lung cancer, occupational exposure. *Environ Health Perspect* 112:1539–1543 (2004). doi:10.1289/ehp.7195 available via <http://dx.doi.org/> [Online 5 August 2004]

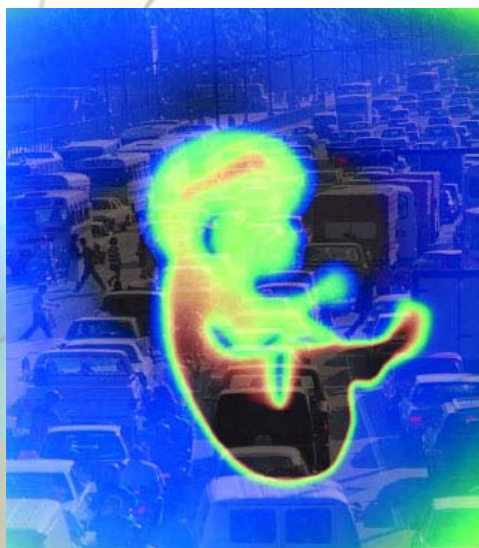
1959. In 1981, men 40–64 years of age with 10–20 years of railroad service in 1959 were selected for data extraction. Based on job in 1959, we identified 39 job codes with exposure to diesel exhaust characterized during an industrial hygiene survey (Woskie et al. 1988a, 1988b). We sampled 56,208 workers in these job codes, including *a*) every third engineer (engineers and firemen), *b*) every third conductor (conductors, brakemen, and hostlers), *c*) all shop workers (shop supervisors, machinists, and electricians), and *d*) a referent group of less-exposed workers (ticket agents, station agents, and signal maintainers, and every fourth clerk). By design, approximately 75% of the workers in the sample were in diesel-exposed jobs and 25% were in low- or no-exposure jobs. The RRB provided a listing of yearly job code, months of railroad service, and mortality

Occupational Diesel Exposure and Lung Cancer

- “Our observation of lung cancer risk [*in railroad workers*] is similar to the risk noted by others in the literature. **In more than 35 studies** of workers with occupational exposure to diesel exhaust, **excess risk of lung cancer** is consistently elevated by 20–50%.”
- “These results indicate that **the association between diesel exhaust exposure and lung cancer is real.**”

Residential Proximity to Freeway Truck Traffic and Preterm & LBW babies

Infants born between 1997-2000 in Los Angeles County



Number of freeway trucks passing within 750 feet of a home per day	Odds Ratio (95% CI)
$\geq 13,290$ trucks	1.23 (1.06-1.43)
$\geq 8,684$ heavy-duty diesel vehicles	1.18 (1.02-1.37)

Model adjusted for all maternal risk factors as covariates, background air pollution concentrations and census block-group level socio-economic status

Key Issues for Future Work

- Key finding to date: UFP: Particle surface area may be the best measure of toxicity over that of mass or particle number*
- Good toxicological evidence that UFP cause inflammation in the lungs
- What are critical characteristics of PM in relation to toxicity?
 - Further evaluation of size fractions needed-Research on UFP should be a major priority given the data to date: focused research is a very high priority
 - Additional studies on particle surface area vis a vis toxicity
 - Relationship between toxic mechanisms and specific toxic components

Research Collaborators

- **Constantinos Sioutas, USC**
- **Arthur Cho, UCLA**
- **Elinor Fanning, UCLA**
- **John Fukuto, UCLA**
- **Andre Nel, UCLA**
- **Yoshito Kumagai, Tsukuba University, Japan**
- **Michael Kleinman, UC Irvine**
- **Henry Gong, Rancho Los Amigos Rehab Ctr**

Table 2. Odds Ratios for the Onset of Myocardial Infarction (MI) after Time Spent in Traffic, According to the Means of Transportation.*

Type of Transportation and Hours before MI	No. of Subjects	Frequency of Exposure in Case Period on Day of MI (%)	Odds Ratio (95% CI)	P Value
Any means of transportation†				
Concurrent	585	8.0	1.50 (1.07–2.09)	0.02
1 hr	625	12.1	2.92 (2.22–3.83)	<0.001
2 hr	634	8.9	2.01 (1.49–2.72)	<0.001
3 hr	635	5.5	1.15 (0.79–1.66)	0.47
Cars				
Concurrent	585	5.6	1.33 (0.90–1.99)	0.15
1 hr	625	8.3	2.60 (1.89–3.57)	<0.001
2 hr	634	6.5	1.94 (1.37–2.76)	<0.001
3 hr	635	4.2	1.16 (0.76–1.78)	0.49
Bicycles				
Concurrent	585	1.8	2.59 (1.27–5.29)	0.009
1 hr	625	2.4	3.94 (2.14–7.24)	<0.001
2 hr	634	1.6	2.70 (1.37–5.33)	0.004
3 hr	635	1.0	1.66 (0.74–3.74)	0.22
Public transportation				
Concurrent	585	0.5	1.08 (0.33–3.55)	0.90
1 hr	625	1.2	3.09 (1.41–6.75)	0.005
2 hr	634	0.9	2.13 (0.91–5.23)	0.08
3 hr	635	0.3	0.69 (0.17–2.88)	0.62

* Analyses have been adjusted for the time of day to control for the potential effects of circadian variation with the use of 23 indicator variables. Vulnerable case periods were 0 to 6 hours before the onset of myocardial infarction, and control periods 24 to 71 hours before onset. The analyses were restricted to time (hours) spent within the study area (defined as the city of Augsburg and two adjacent rural districts) during the case periods and the control periods, to exclude time spent in long-distance travel. Data are from the KORA Myocardial Infarction Registry, February 1999 to December 2001. CI denotes confidence interval.

† "Any means of transportation" combines times spent in cars, in public transportation, and on motorcycles or bicycles.

Traffic, Susceptibility and Childhood Asthma

Table 4. Association of asthma and wheeze with distance to a major road among long term residents, by child's history of allergy [OR (95% CI)].^a

Major road distance (m)	No allergic symptoms (n = 942)	Allergic symptoms (n = 723)
Lifetime asthma^b		
> 300	1.00	1.00
150–300	0.92 (0.43–1.97)	0.87 (0.53–1.41)
75–150	1.04 (0.41–2.62)	0.96 (0.57–1.61)
< 75	2.27 (1.04–4.94)*	1.31 (0.76–2.25)
Prevalent asthma		
> 300	1.00	1.00
150–300	0.98 (0.42–2.26)	0.77 (0.46–1.27)
75–150	0.81 (0.25–2.55)	1.01 (0.60–1.69)
< 75	2.52 (1.07–5.93)*	1.29 (0.76–2.21)
Current wheeze		
> 300	1.00	1.00
150–300	1.50 (0.72–3.12)	0.80 (0.50–1.28)
75–150	0.72 (0.23–2.25)	1.03 (0.63–1.68)
< 75	2.58 (1.14–5.86)*	1.25 (0.75–2.07)

^aAdjusted for age, sex, language of questionnaire, community, and race. ^bParticipants from Lake Arrowhead were excluded from models for stratum without allergy for prevalent and lifetime asthma, because otherwise the models failed to converge. * $p < 0.05$.

Statistical Analyses

- 2-way ANOVA on Site vs. Exposure
- IL-5 Significant increase BH1 CAPs exposed vs. other exposed or controls
- IgG1 Significant increase BH1 CAPs exposed vs. other exposed or controls
- EOS Significant increase BH1 CAPs exposed vs. other exposed