John R. Froines, Ph.D.

- Southern California Particle Center and Supersite

- Participants: UCLA, USC, UCI, UCR, UC Davis, Rancho Los Amigos, Michigan State University, University of Tsukuba (Japan), Sonoma Technology

- Funding sources: U.S. EPA and California Air Resources Board

Southern California Particle Center and Supersite

SCPCS Research is divided into three focus areas:

Investigation of the **biological mechanisms** of particulate matter (PM) effects in relation to PM physical and chemical characteristics

- The Chemical Toxicology of Particulate Matter
  A Cho, J Froines
- Biological Effects of Diesel Exhaust Particles (DEP) and PM
  A Nel
- Aged Rat Pilot Study
  M Kleinman, C Sioutas, A Cho, J Froines
- Particle Dosimetry
  R Phalen

Studies of **emission sources** and adverse health effects

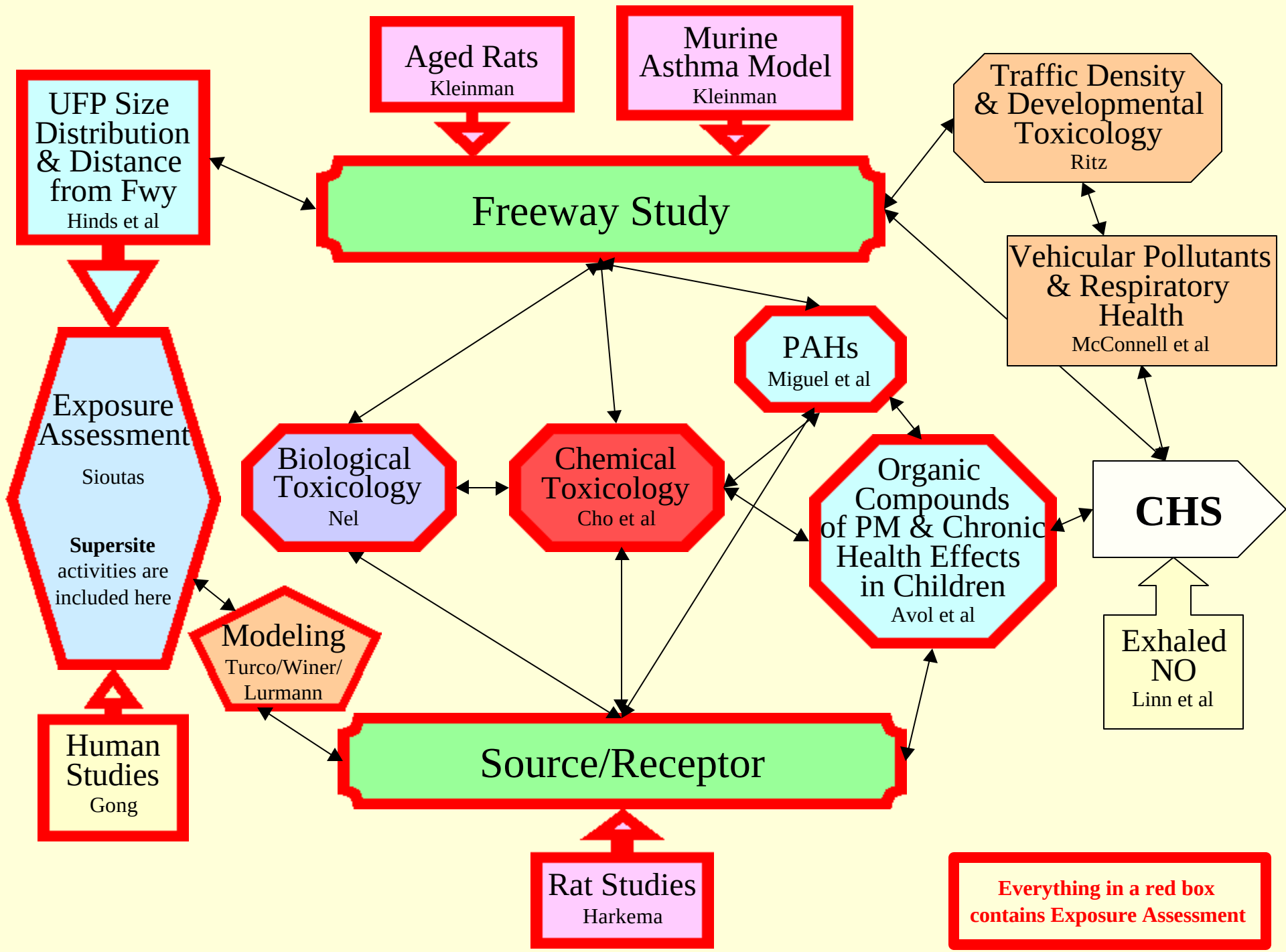
- Relationship Between Ultrafine Particle Size Distribution and Distance From Highways
  W Hinds, Y Zhu, C Sioutas
- Exposure to Vehicular Pollutants and Respiratory Health
  R McConnell, F Lurmann
- Traffic Density and Human Reproductive Health
  B Ritz
- Effects of Exposure to Freeways with Heavy Diesel Traffic/Gasoline Vehicles on Asthma Mouse Model
  M Kleinman, C Sioutas, A Cho, J Froines
- Measurement of the "Effective" Surface Area of Ultrafine and Accumulation Mode PM
  S Friedlander, C Sioutas

Studies of the effects of varying **spatial & temporal patterns** of ambient PM & co-pollutants & resulting health effects with emphasis on the role of atmospheric chemistry

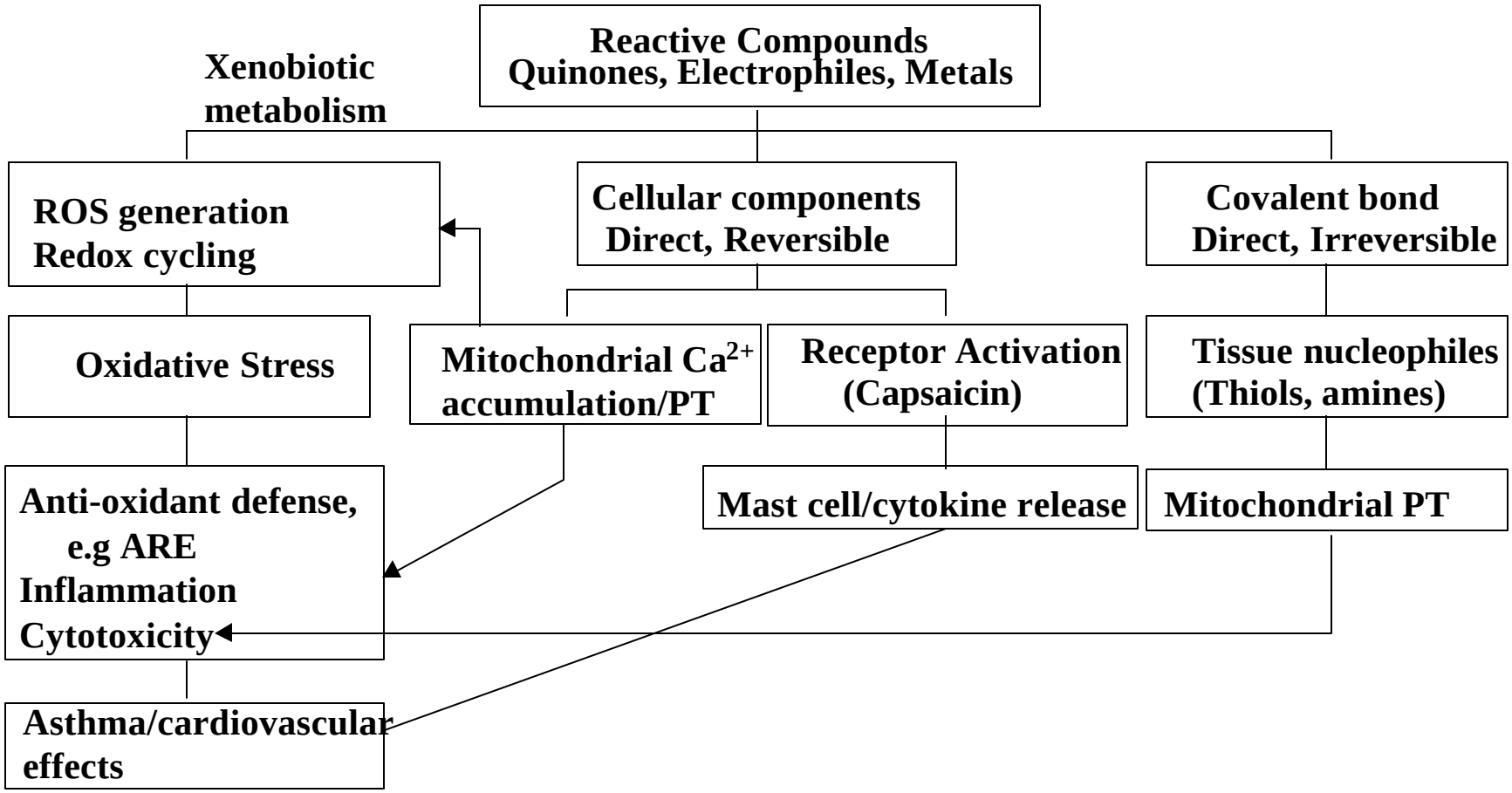
- Role of Quinones, Aldehydes, PAHs, & Other Atmo. Transformation Products on Chronic Health Effects in Children; Phase I–Exposure Assessment
  E Avol, A Miguel, A Cho, J Froines
- Novel Method for Measurement of Acrolein in Aerosols
  J Charles
- Off-Line Sampling of Exhaled Nitric Oxide in Respiratory Health Surveys
  H Gong, Jr., W Linn
- Acute Cardiopulmonary Responses to Concentrated Ambient Particulate Matter in Human Volunteers.
  H Gong, Jr., C Sioutas, W Linn
- Particle Size Distributions of PAHs in the Los Angles Basin
  A Miguel, C Sioutas
- Source/Receptor Study
  C Sioutas, M Kleinman, W Hinds, A Cho, J Froines, J Harkema, A Miguel, A Nel
- Exposure and Airshed Modeling Applications in Support of SCPCS and CHS Projects
  R Turco, A Winer

SCPCS Research Theme:

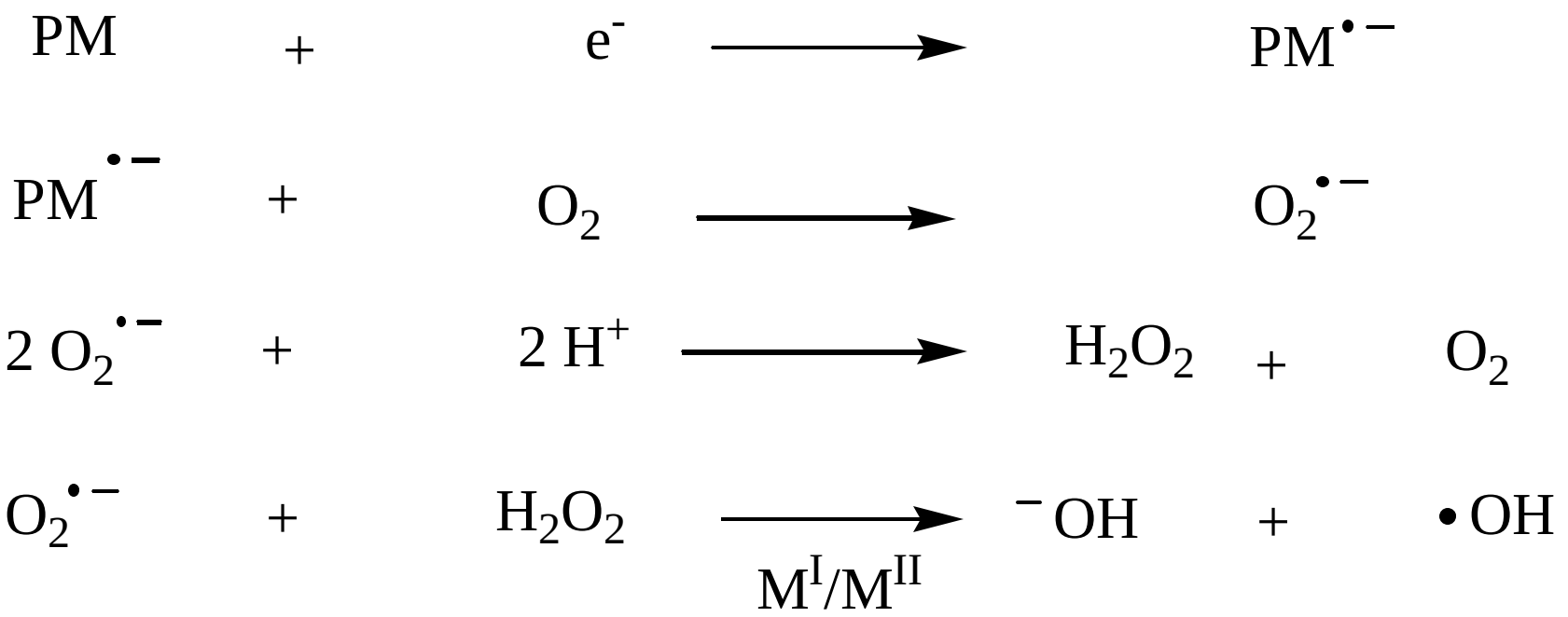
Mobile Source Pollution and the resulting health effects


Aged Rats
Kleinman
Murine Asthma Model
Kleinman
UFP Size Distribution & Distance from Fwy
Hinds et al
Freeway Study
Traffic Density & Developmental Toxicology
Ritz
Vehicular Pollutants & Respiratory Health
McConnell et al
Exposure Assessment
Sioutas
Supersite activities are included here
PAHs
Miguel et al
Biological Toxicology
Nel
Chemical Toxicology
Cho et al
Organic Compounds of PM & Chronic Health Effects in Children
Avol et al
CHS
Modeling
Turco/Winer/ Lurmann
Exhaled NO
Linn et al
Human Studies
Gong
Source/Receptor
Rat Studies
Harkema
Everything in a red box contains Exposure Assessment

Hypotheses


Reactive Compounds
Quinones, Electrophiles, Metals
Xenobiotic metabolism
ROS generation
Redox cycling
Cellular components
Direct, Reversible
Covalent bond
Direct, Irreversible
Oxidative Stress
Mitochondrial Ca2+ accumulation/PT
Receptor Activation (Capsaicin)
Tissue nucleophiles (Thiols, amines)
Anti-oxidant defense, e.g ARE
Inflammation
Cytotoxicity
Mast cell/cytokine release
Mitochondrial PT
Asthma/cardiovascular effects

Generation of ROS



Hypotheses/findings

Toxicity of PM is due to chemically reactive components which include metals and organics

PM and their components exhibit the ability to catalyze electron transfer between reducing agents and oxygen to generate reactive oxygen species

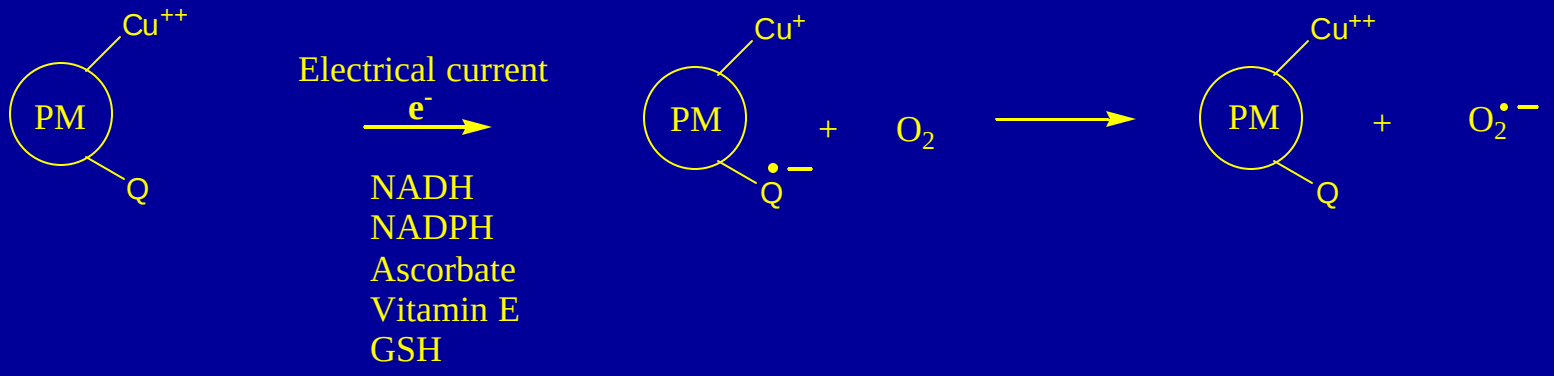
PM is a self-contained, catalytic entity capable of toxic chemistry when in proximity to metabolizing/respiring cells.

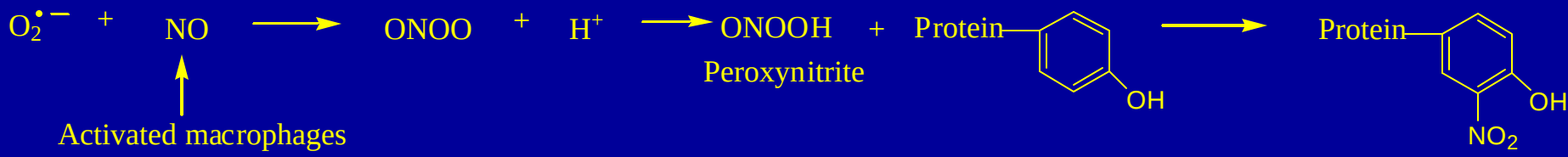
This catalytic ability amplifies exposure effects

PM may be more active than its components because of the structure.

Research in our laboratory indicates that PM is electrochemically active

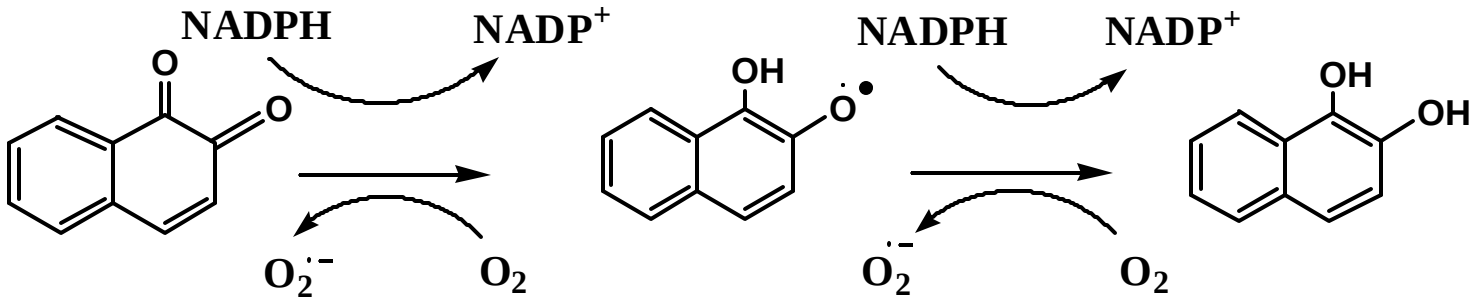
Unique particle characteristics





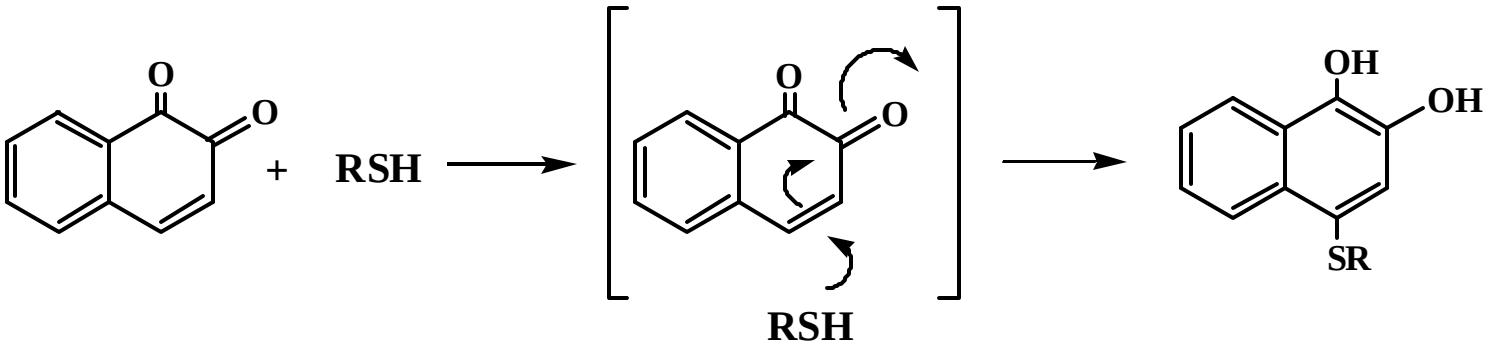
Quinone reactions

Redox reaction

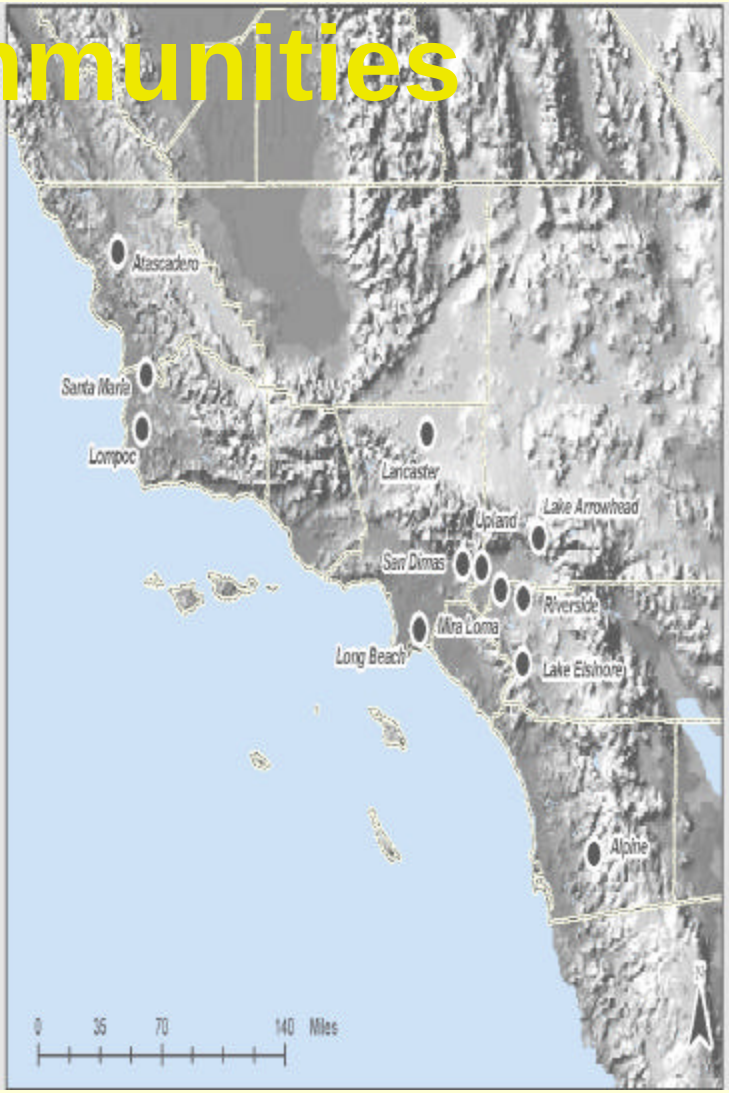


$$2\,O_2^{-\cdot} + 2H^+ \longrightarrow O_2 + H_2O_2$$

Covalent bond formation



12 CHS Communities



Naphthalene across Los Angeles (ng/m^3)

- Lompoc 58.5
- Atascadero 266
- Upland 207
- San Dimas 502
- Mira Loma 518
- Riverside 575
- Highest values: Riverside 6,336; San Dimas 5,618; Mira Loma 4,290
- 1,4-napthoquinone Long Beach 4.3

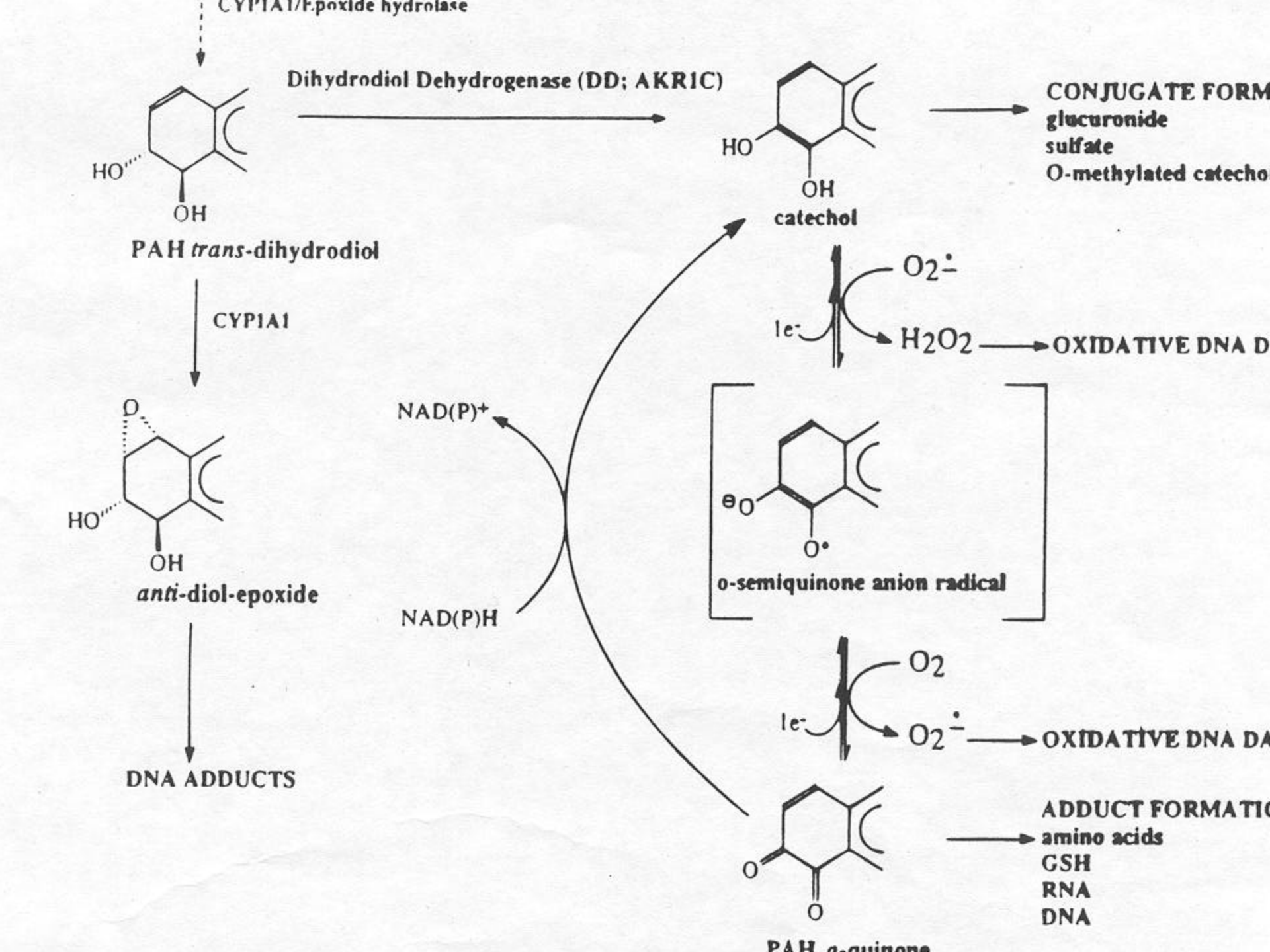
Naphthalene-Levels of evidence

- Male rats-clear evidence
- Nasal cavity adenoma 0/49,6/49, 8/48, 15/48
- Neuroblastoma 0/49, 0/49, 4/48, 3/48

- Female rats-clear evidence
- Nasal cavity adenoma 0/49, 0/49, 4/49, 2/49
- Neuroblastoma 0/49, 2/49, 3/49, 12/49

Naphthalene-relevance of animal evidence

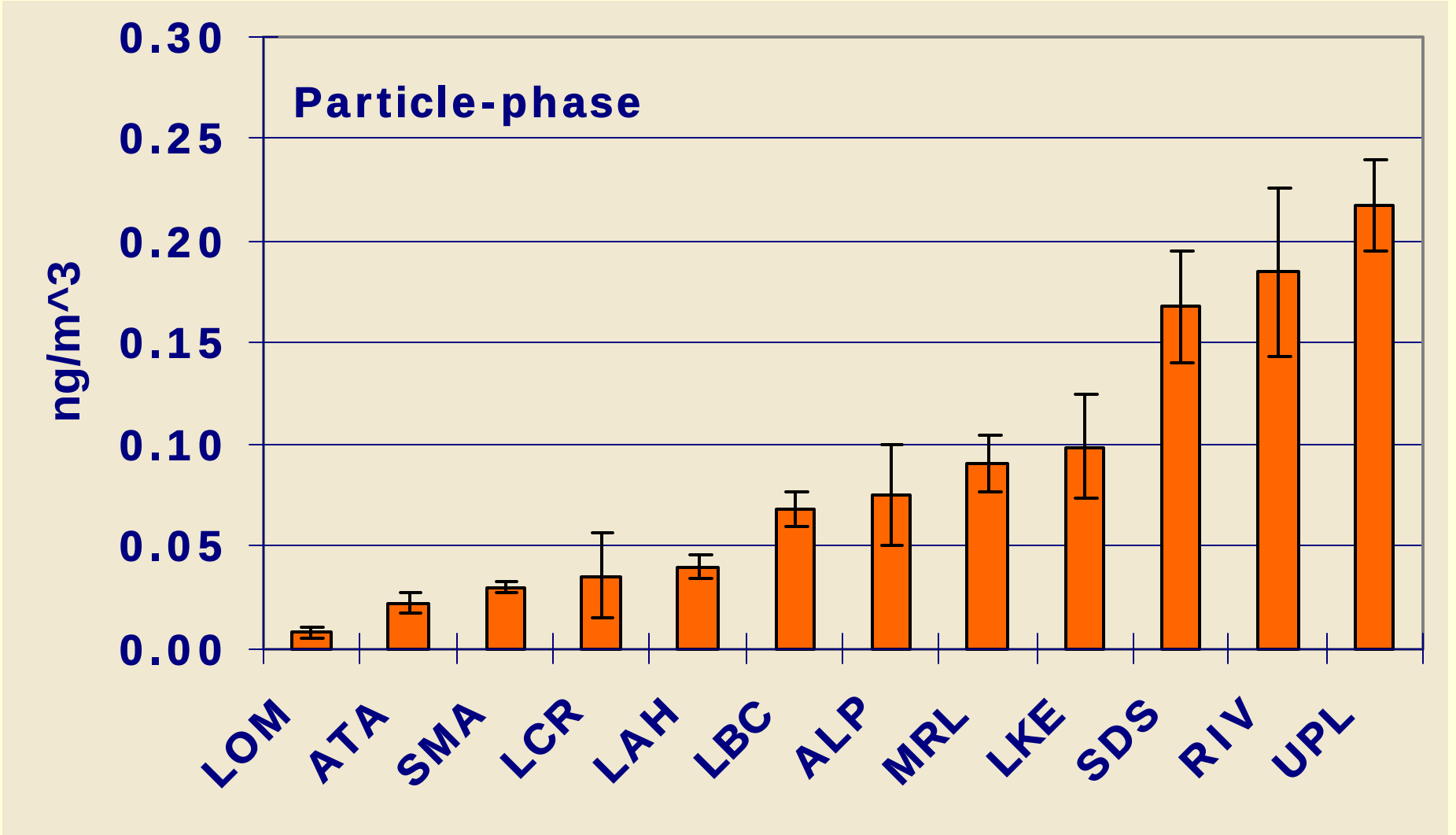
- Neuroblastomas and nasal adenomas have not been observed in the chamber controls
- Neuroblastomas and nasal adenomas have not been observed in historical controls
- Therefore, incidences of these cancers were considered to be related to naphthalene exposure
- NTP Board of Scientific Counselors Com. unanimously voted to list


CYP1A1/Epoxide hydrolase
Dihydrodiol Dehydrogenase (DD; AKR1C)
CONJUGATE FORM
glucuronide
sulfate
O-methylated catecho
HO
OH
PAH trans-dihydrodiol
catechol
CYP1A1
O2·-
1e-
H2O2
OXIDATIVE DNA D
NAD(P)+
NAD(P)H
o-semiquinone anion radical
O2
1e-
O2·-
OXIDATIVE DNA DA
anti-diol-epoxide
DNA ADDUCTS
ADDUCT FORMATI
amino acids
GSH
RNA
DNA
PAH o-quinone

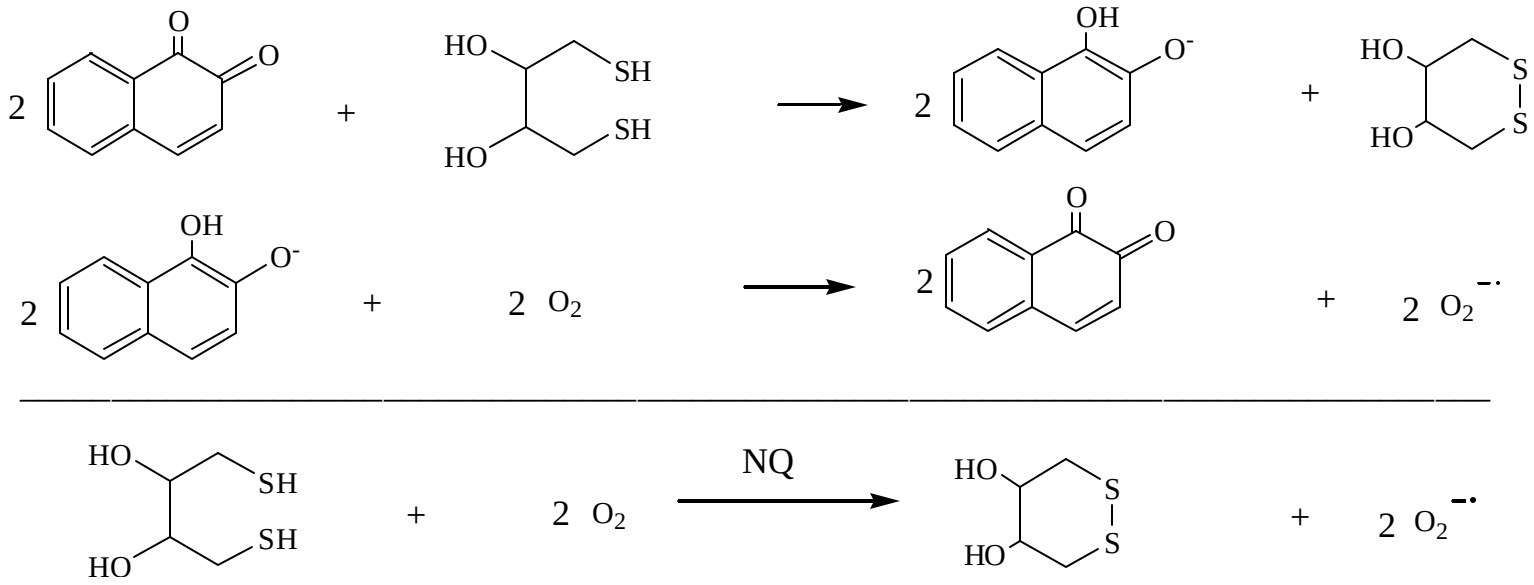
Quinone genotoxicity

- NPQ
- TA97a, TA98, TA100, TA104
- PQ (1,2)
- TA97a, TA98, TA104
- PQ (9,10)
- TA97a, TA100
- Yu et al, 2002: NP-1,2-dione mutates p53 under redox cycling conditions. Explains mutational spectra in p53 in lung cancer
- Population risk for cancer at measured levels is 40 cases/million (potentially underestimated)

Spatial Distribution of PQ



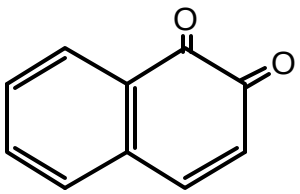
Redox activity: Quinone catalysis of oxygen reduction by DTT



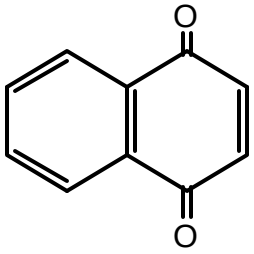
- Q catalyzes reduction of oxygen by DTT
- Significant concentrations of quinones in LAB
- Similar reaction observed for PM

Redox activity of quinones: Naphthoquinones

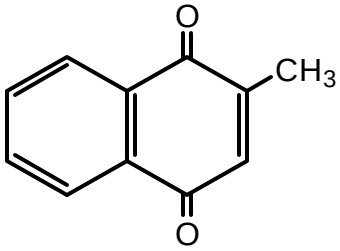
Naphthoquinones	DTT activity (nmol/min*µg)
1,2	8.9
1,4	9.1
2-Me-1,4	15.8
5-OH-1,4	5.8
9,10-PQ	55.5



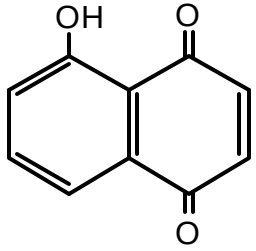
1,2 NQ



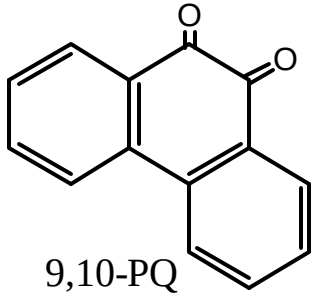
1,4 NQ



2-Me-1,4-NQ

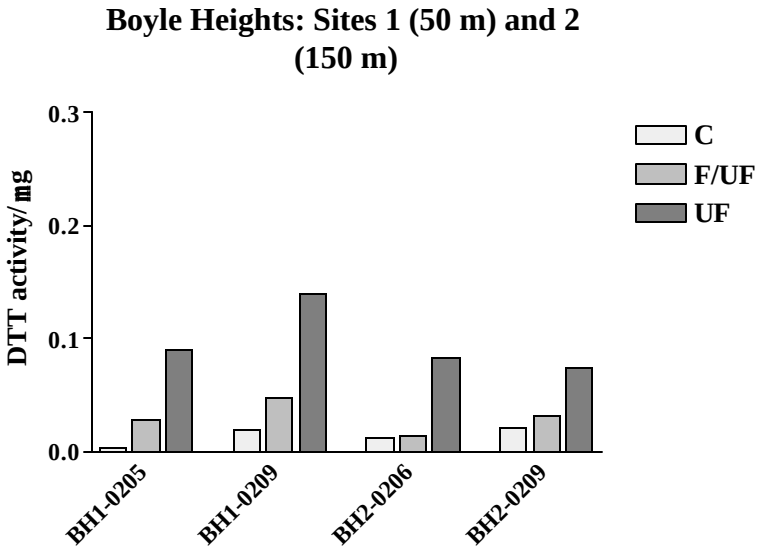


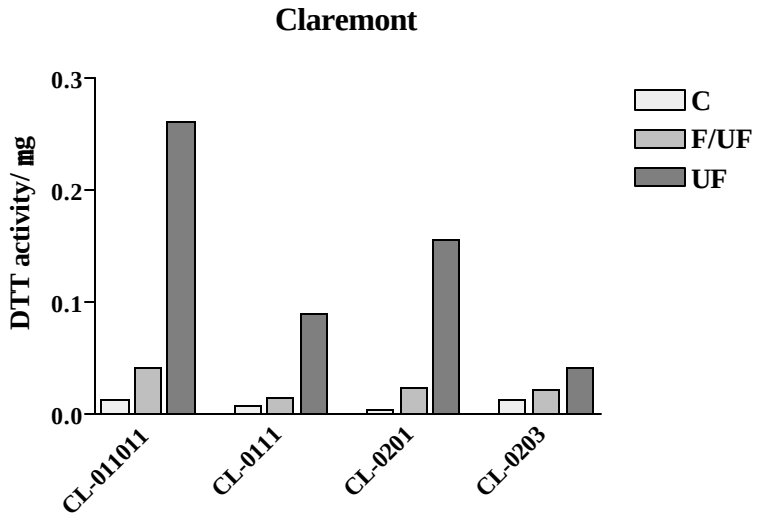
5-OH-1,4-NQ

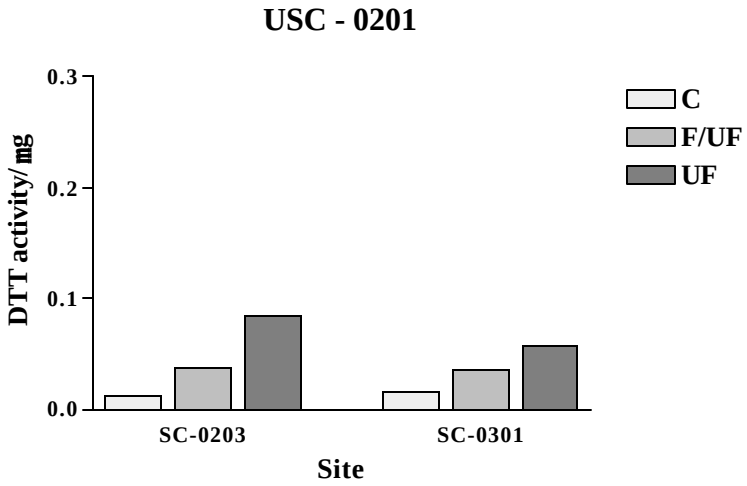


9,10-PQ

DTT activity across the LAB/ug







The DTT assay gives the relative potency for the three sites

Claremont(receptor) 137

Boyle Heights (freeway) 96 (BH1-115, BH2-78)

USC (source) 71

Total DTT activity

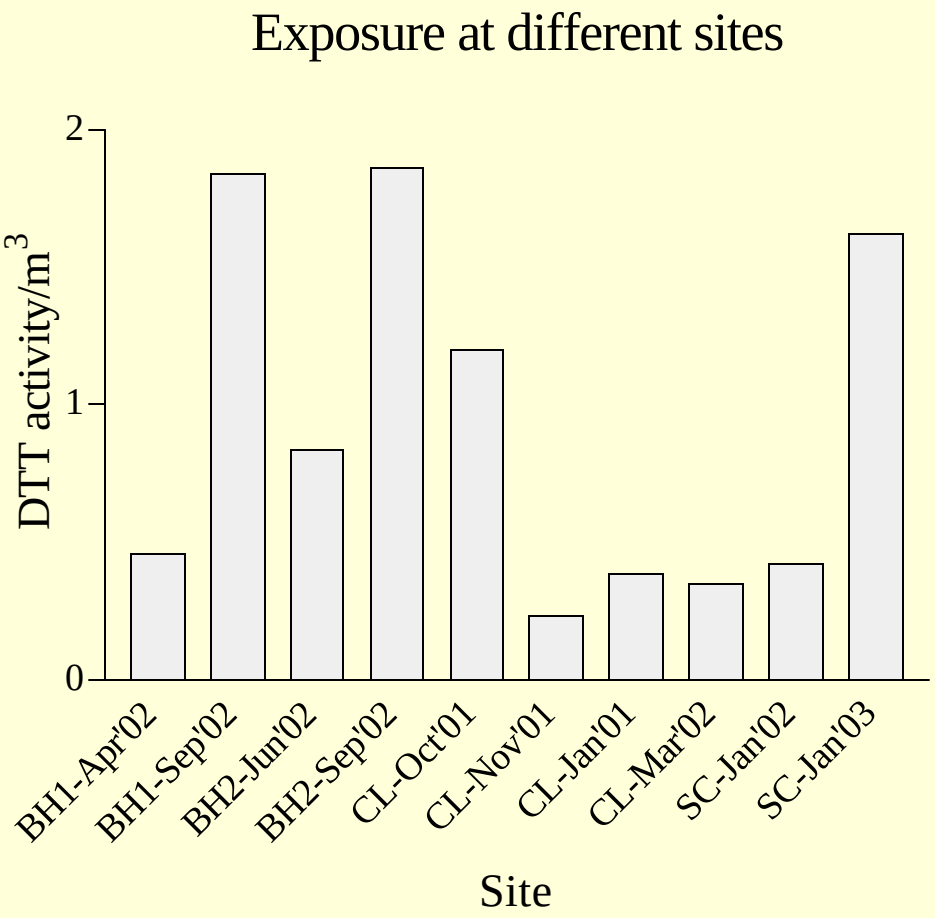


Figure 5

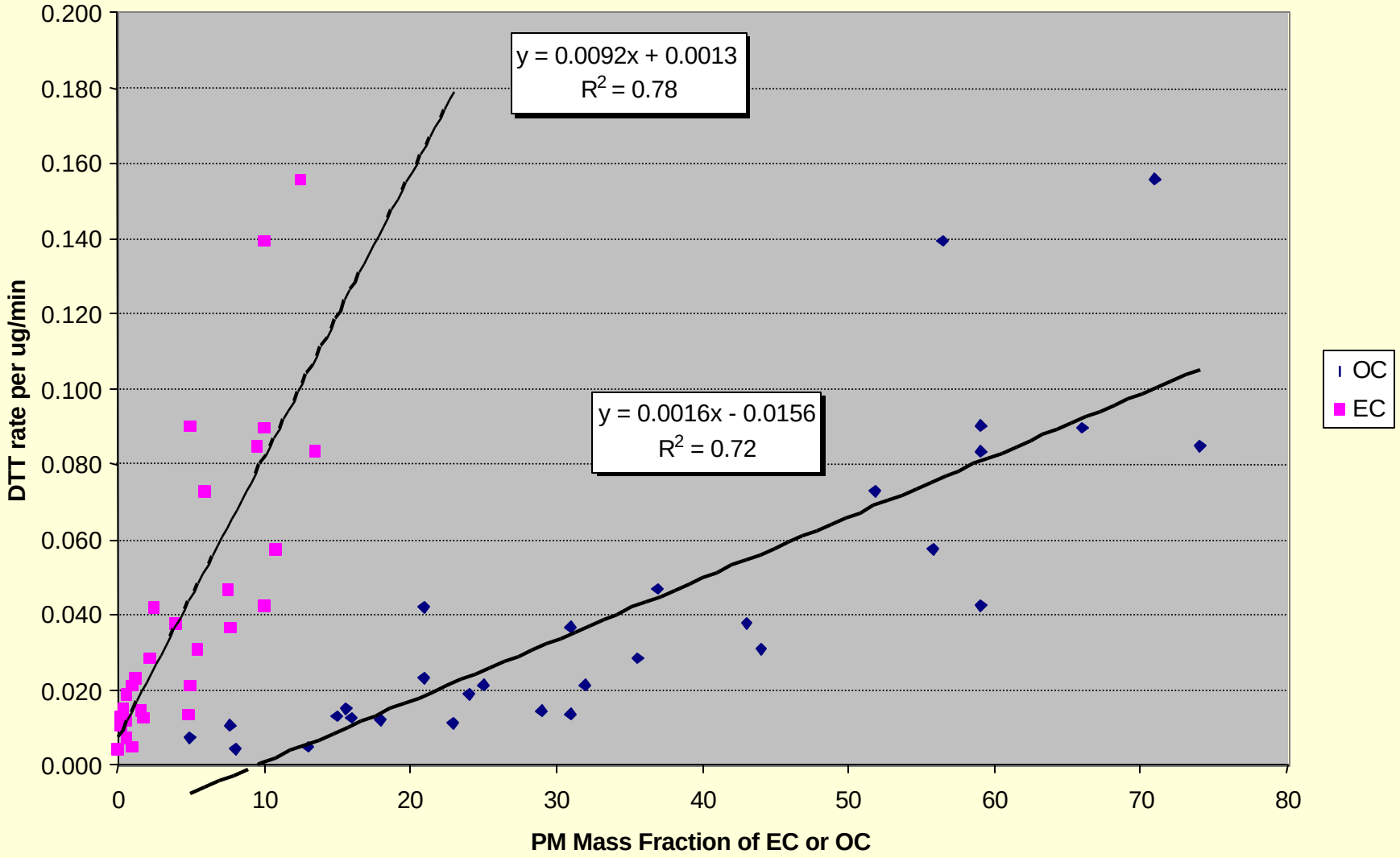
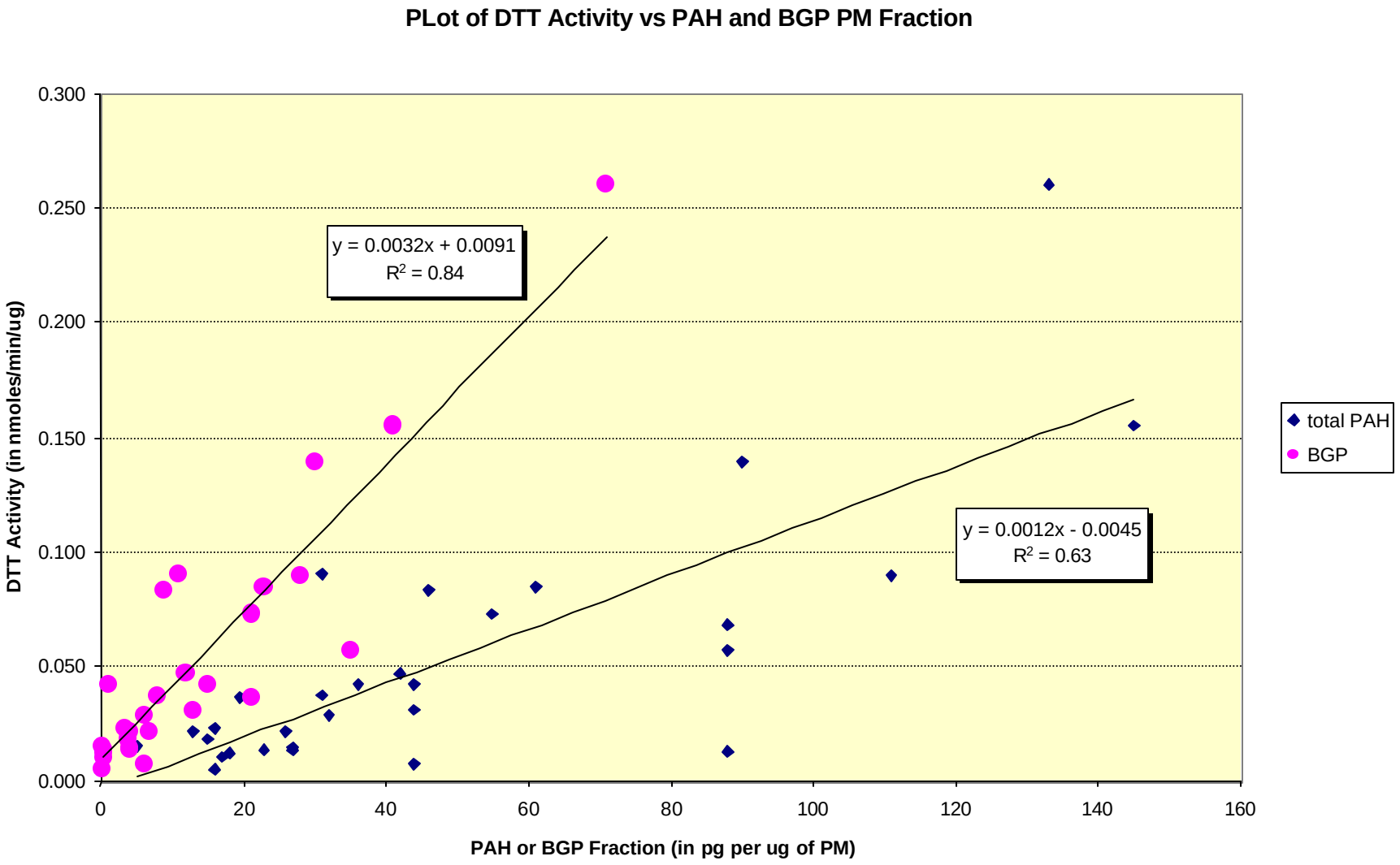

y = 0.0092x + 0.0013
R2 = 0.78
y = 0.0016x - 0.0156
R2 = 0.72
OC
EC
DTT rate per ug/min
PM Mass Fraction of EC or OC
0.200
0.180
0.160
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
0
10
20
30
40
50
60
70
80

Figure 6


PLot of DTT Activity vs PAH and BGP PM Fraction
DTT Activity (in nmoles/min/ug)
0.300
0.250
0.200
0.150
0.100
0.050
0.000
0
20
40
60
80
100
120
140
160
PAH or BGP Fraction (in pg per ug of PM)
y = 0.0032x + 0.0091
R2 = 0.84
y = 0.0012x - 0.0045
R2 = 0.63
total PAH
BGP

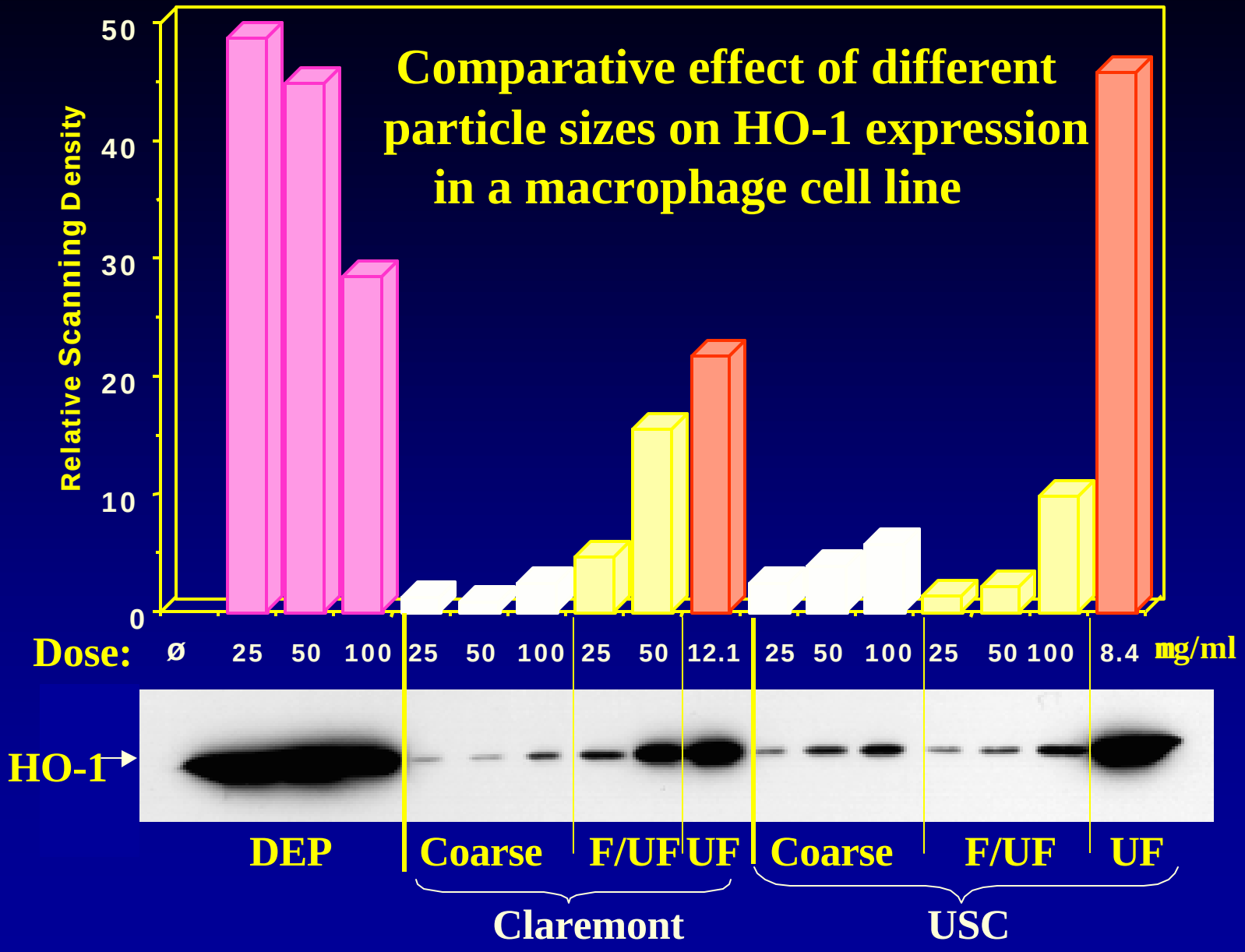
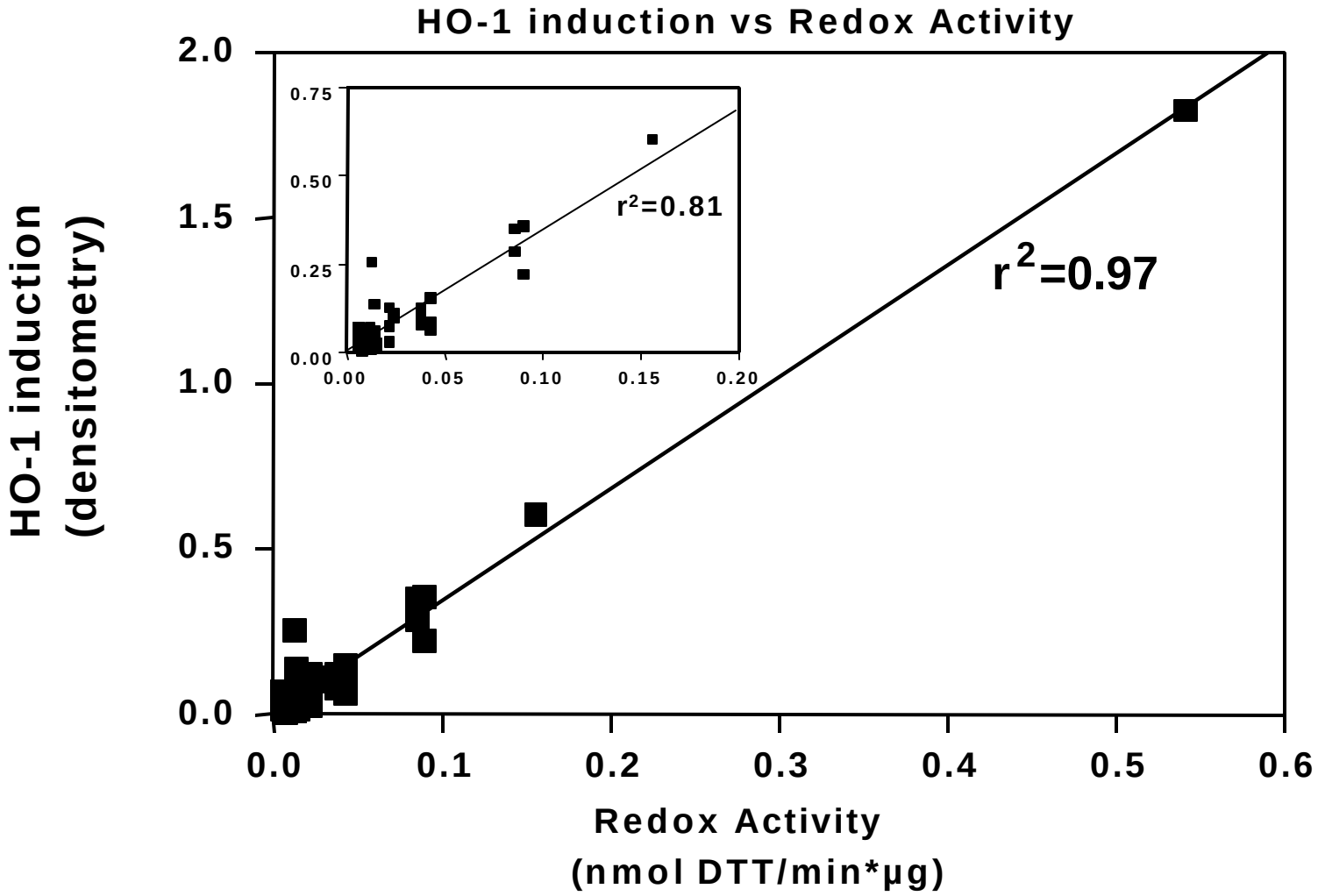

Comparative effect of different particle sizes on HO-1 expression in a macrophage cell line
Relative Scanning Density
50
40
30
20
10
0
Dose:
Ø
25
50
100
25
50
100
25
50
12.1
25
50
100
25
50
100
8.4
mg/ml
HO-1
DEP
Coarse
F/UF
UF
Coarse
F/UF
UF
Claremont
USC

Figure 2D


HO-1 induction vs Redox Activity
HO-1 induction
(densitometry)
2.0
1.5
1.0
0.5
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Redox Activity
(nmol DTT/min*µg)
r2=0.97
0.75
0.50
0.25
0.00
0.00
0.05
0.10
0.15
0.20
r2=0.81

Conclusions

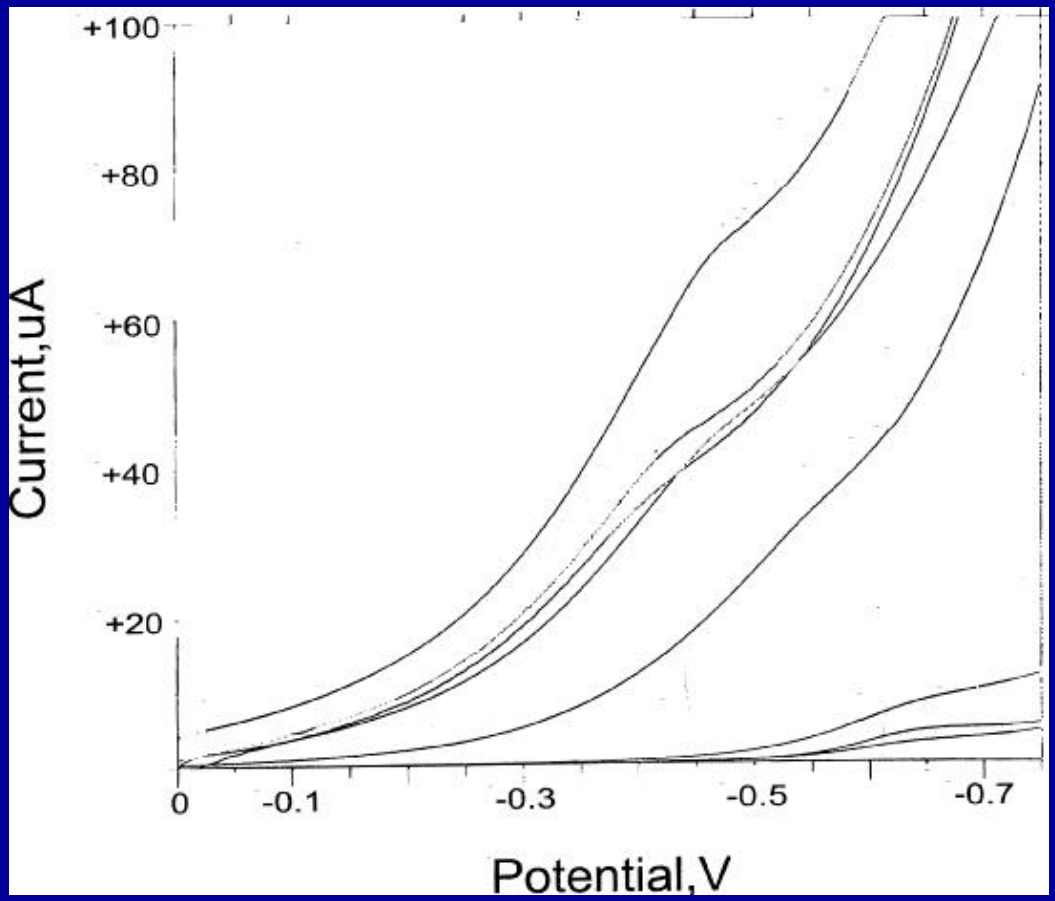
Overall, there is a strong correlation between particle size, chemical composition, ROS generating capacity, and cellular oxidative stress

Ultrafine PM is most potent on a per ug basis

PM may be more active than its components because of the structure.

Redox activity of DEP: Electrochemistry

- DEP mixed with carbon paste and mixture evaluated for oxygen reduction conditions.
- Oxygen reduction appears to occur at a lower voltage suggesting electron transfer by DEP



Redox activity of DEP: Oxygen consumption

Reducing agent	O2 consumption	% of DTT
NADH	0.600	8.7
Trolox	0.100	1.4
Ascorbate	5.900	85.5
GSH	0.270	3.9
DTT	6.900	100.0

- DEP (0.4 mg/mL) were incubated with the indicated reducing agents (500 mM) and oxygen consumption determined.
- O_2 consumption is expressed as micromoles consumed/min*mg DEP

Objective

- To apply a mechanism based approach to quantitative toxicological analysis.
- Assess toxicity by IC50 values in yeast using mutants, aerobic and anaerobic growing conditions. ROS generation requires oxygen.
- To gain mechanistic insights and procedures for quantitative analysis of PM samples

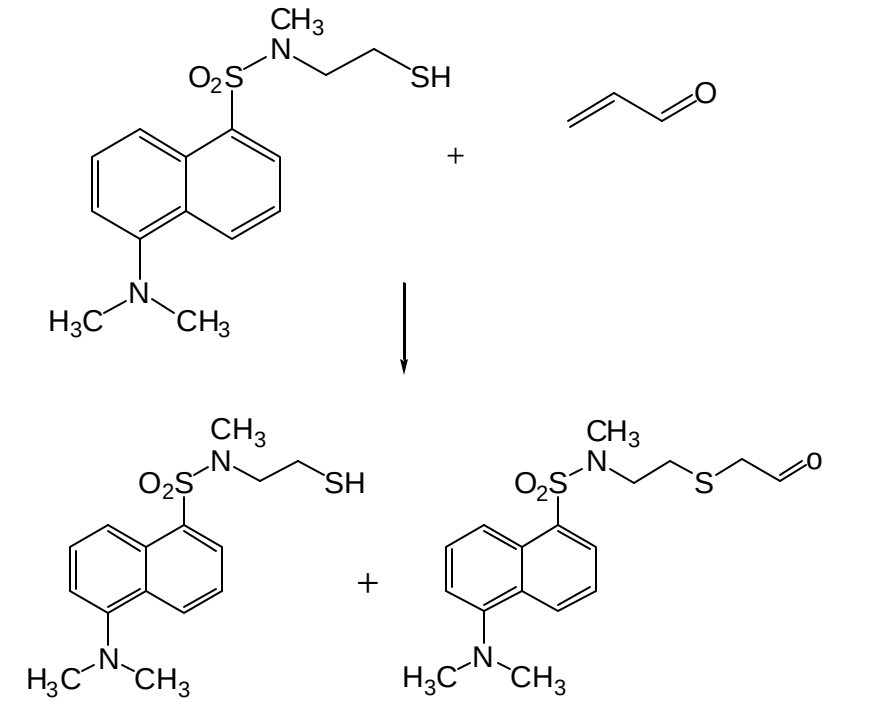
Summary of Growth Inhibition Data

Quinone	Aerobic IC_{50} (mM)	Anaerobic IC_{50} (mM)	SOD1-Delete IC_{50} (mM)	SOD1,2-Delete IC_{50} (mM)
1,2-Naphthoquinone	56.9	142.3	13.0	3.9
1,4-Naphthoquinone	19.4	90.1	8.0	5.8
9,10-Phenanthrenequinone	14.3	36.4	2.7	1.9
9,10-Anthraquinone	>100.0	>100.0	ND	ND
1,4-Benzoquinone	45.9	36.8	19.1	35.3
DEP	327.4	365.2	231.8	ND

* Work in progress
** Not determined

Covalent Probe: A Dansyl Nucleophile

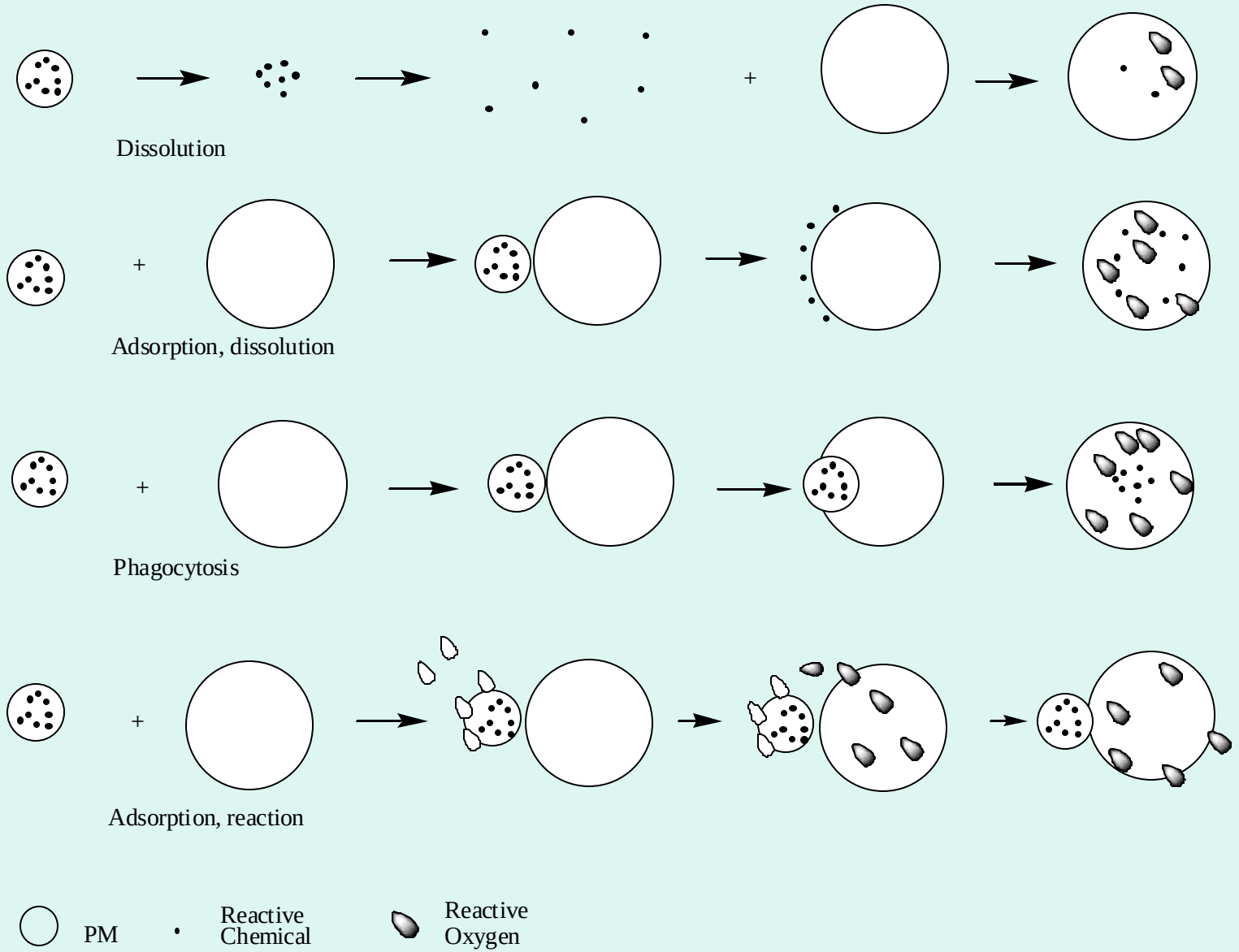
- A fluorescent nucleophile, capable of detecting electrophilic functions in PM.
- Reaction product can be separated from reagent by extraction with base.



Biological aspects of chemical toxicology

- Quinones as prototypical reactive species found in PM
- Smooth muscle preparations. Rat aorta and guinea pig trachea as targets.
- **Results: Ca^{++} and 1,2-NQ on Tracheal Rings**
- 9,10-PQ and 1,2-NQ cause a contraction.
- 9,10-PQ appears to act through ROS since its actions are blocked by SOD
- 1,2-NQ effects appear to be direct, and are blocked by the capsaicin receptor antagonist (Receptors present on bronchial cells that respond by contraction.

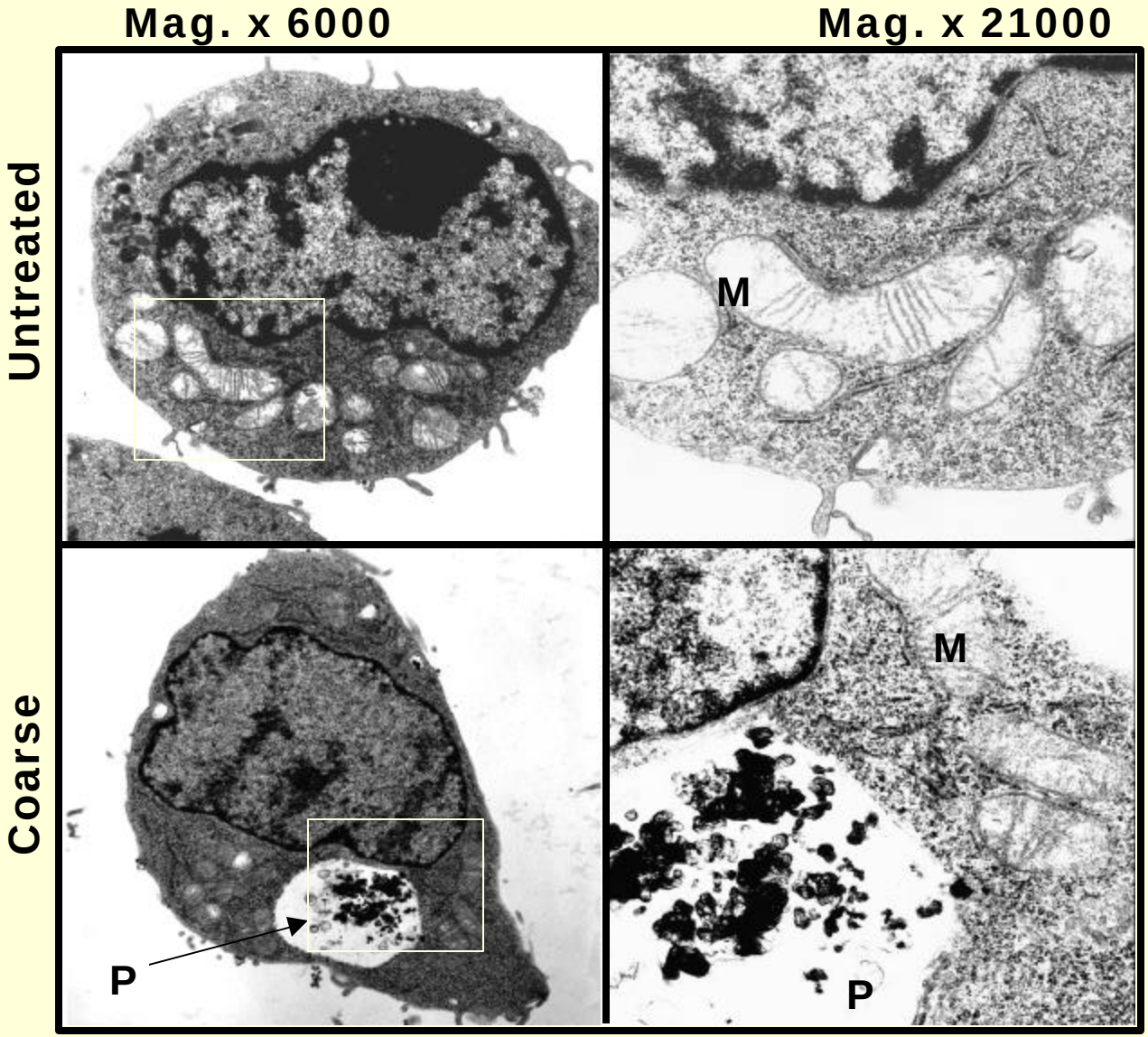
Exposure models



Brauer et al. – Environmental Health Perspectives, 109(10), 2001

- “Lungs (Mexico City) contained numerous chain-aggregated masses of ultrafine carbonaceous spheres

- “Long term exposure to ambient particles, especially to aggregated ambient ultrafine combustion products, results in higher retention of these particles (fine and ultrafine) in lung tissue.”

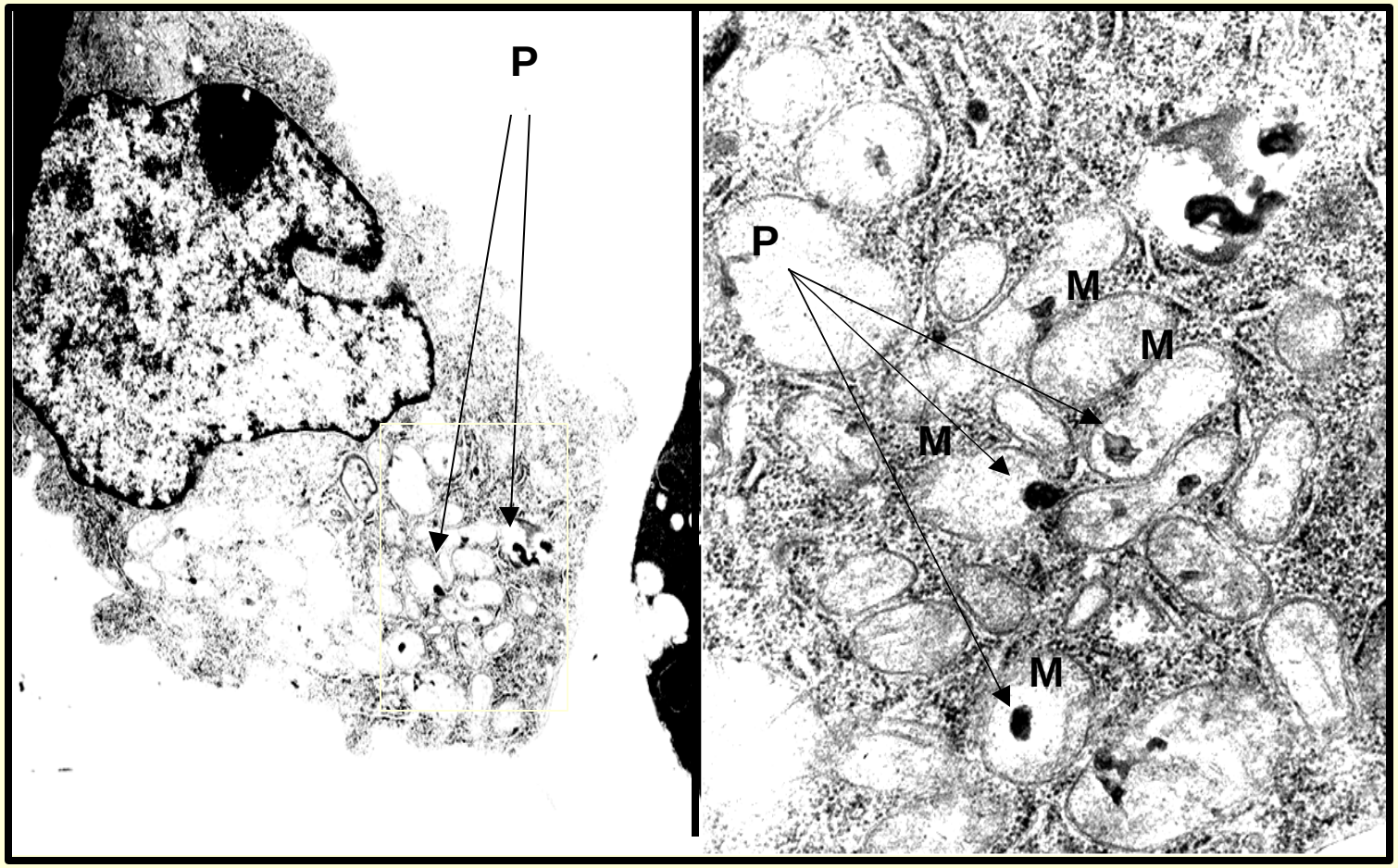

Mag. x 6000
Mag. x 21000
Untreated
Coarse
M
M
P
P

Ultrafines lodge in and destroy mitochondria

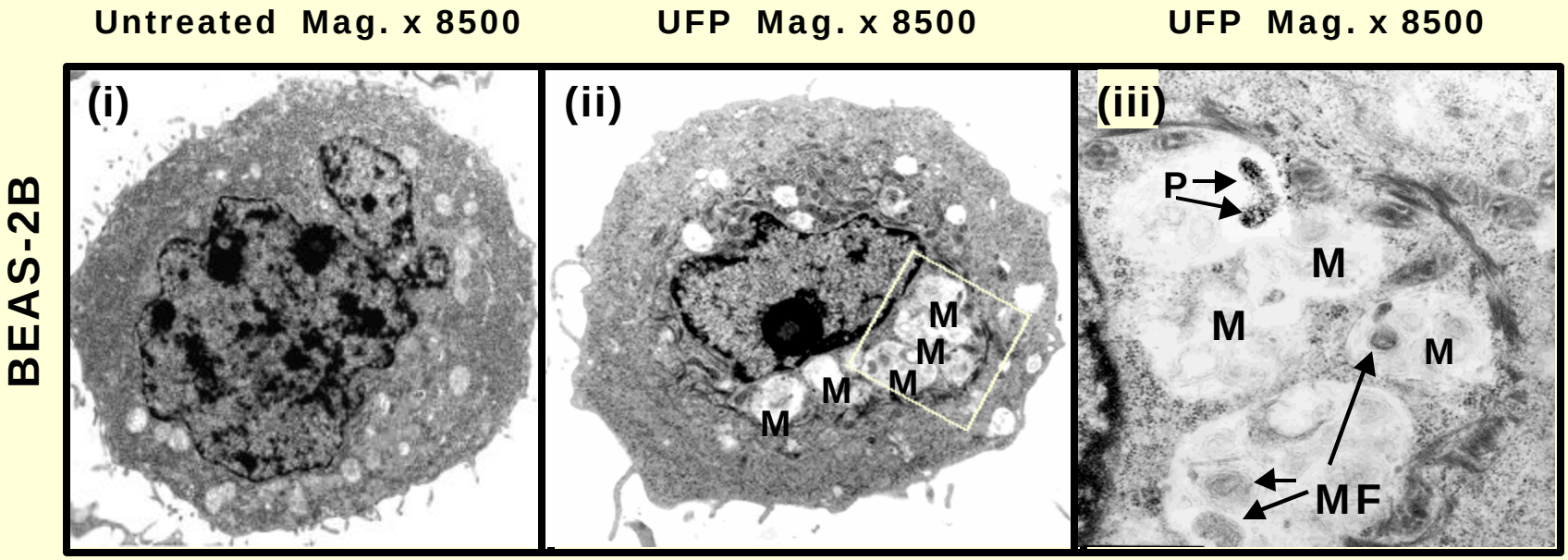
RAW 267.4

Mag. x 6000

Mag. x 21000

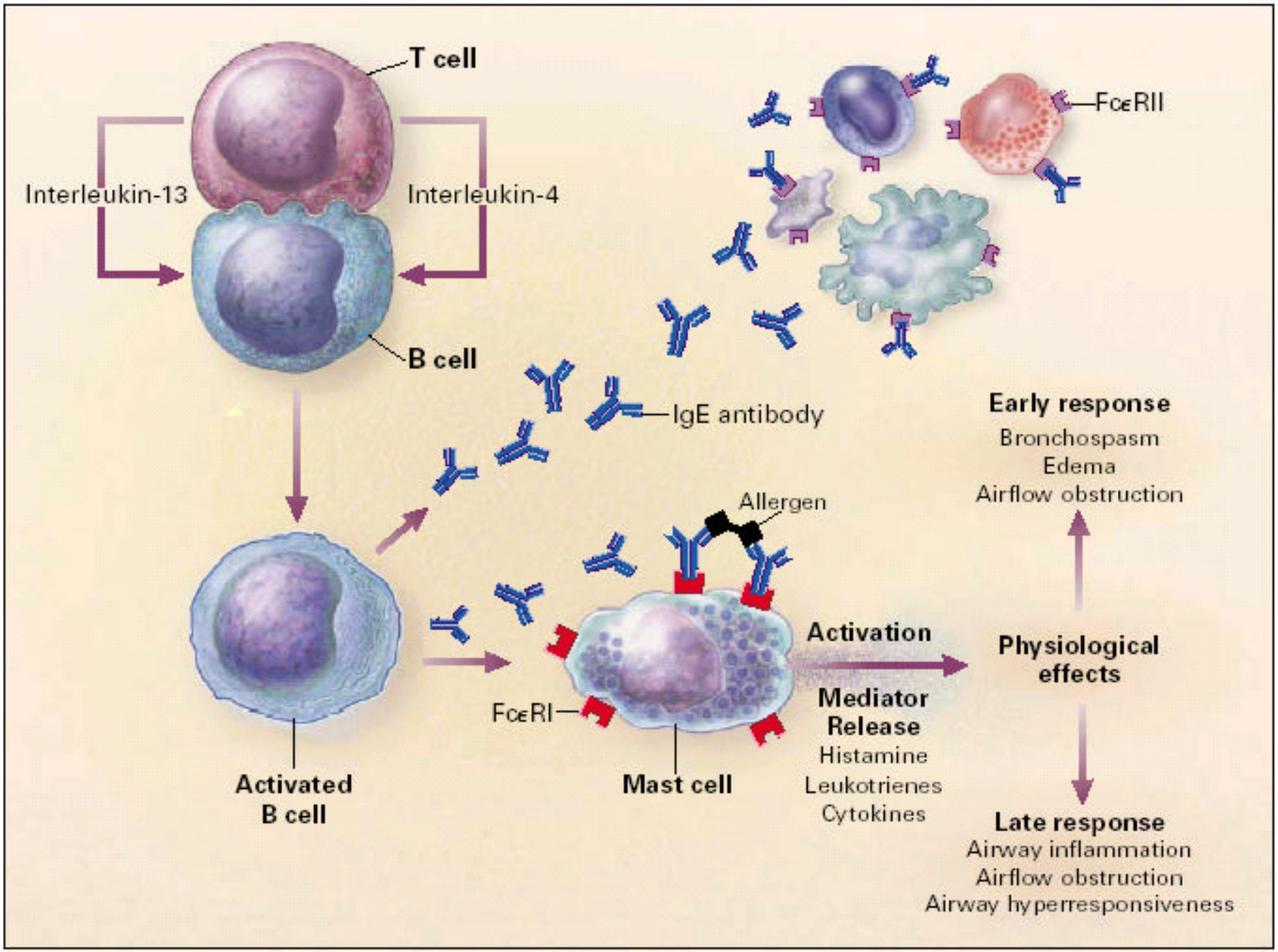


Ultrafines lodge in and destroy mitochondria

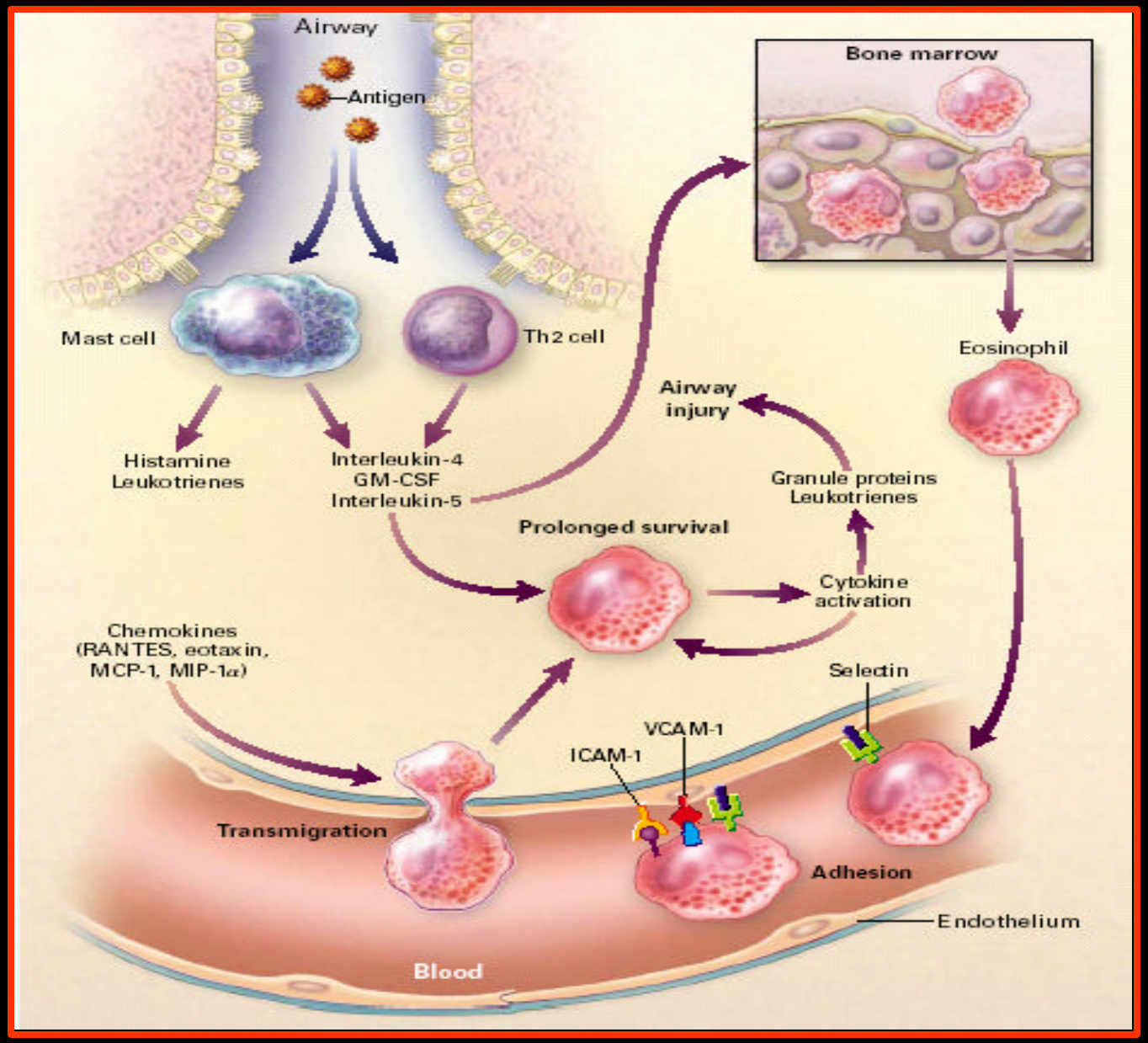


Asthma is a complex disease

- Allergen Sensitization
- Allergic airway inflammation
- Airway hyperreactivity, e.g.methacholine


T cell
Interleukin-13
Interleukin-4
B cell
FcεRII
IgE antibody
Allergen
Activated
B cell
FcεRI
Mast cell
Activation
Mediator
Release
Histamine
Leukotrienes
Cytokines
Physiological
effects
Early response
Bronchospasm
Edema
Airflow obstruction
Late response
Airway inflammation
Airflow obstruction
Airway hyperresponsiveness

Role of IL-5 in Allergic inflammation



Busse & Lemanske. NEJM. 20001; 344: 350- 362.

Adjuvancy and Oxidative Stress: Hypothesis

1. DEP generate ROS $\rightarrow$ Oxidative stress

2. Oxidative stress initiates pro- inflammatory effects in pulmonary target cells (epithelial cells & macrophages)

3. Inflammation is responsible for the adjuvant effects of DEP

Source related investigations – Freeway studies/traffic density

- **Hypothesis 1: Mobile source emissions will exacerbate airway inflammation and allergic airway disease and produce cardiopulmonary effects;**

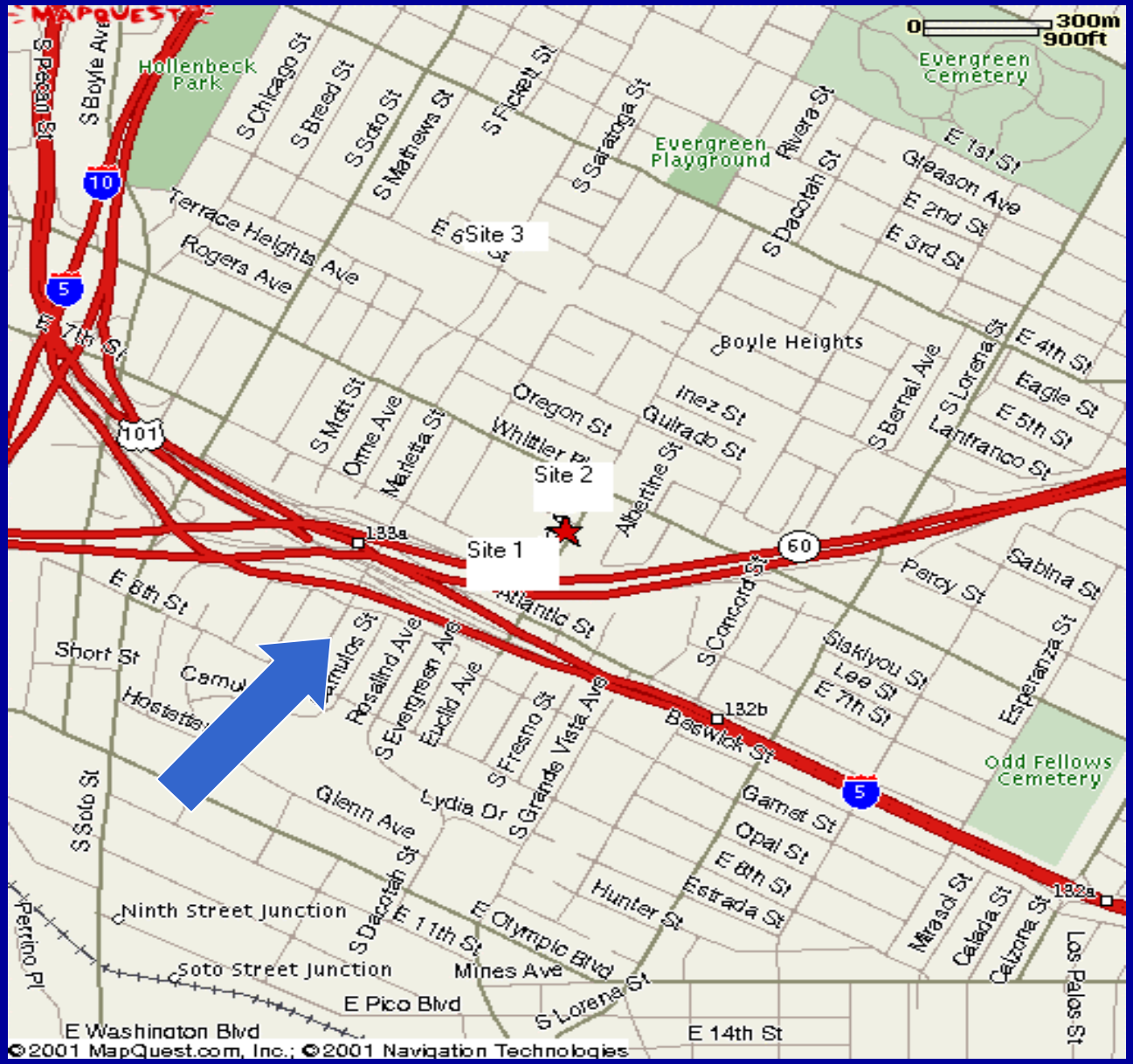
- **Hypothesis 2: The magnitude of allergic airway disease and cardiovascular effects from mobile sources are a function of the size distribution of PM;**

- **Hypothesis 3: Exposure to ultrafine particles at very close proximity to a freeway will result in the most severe effects.**

Description

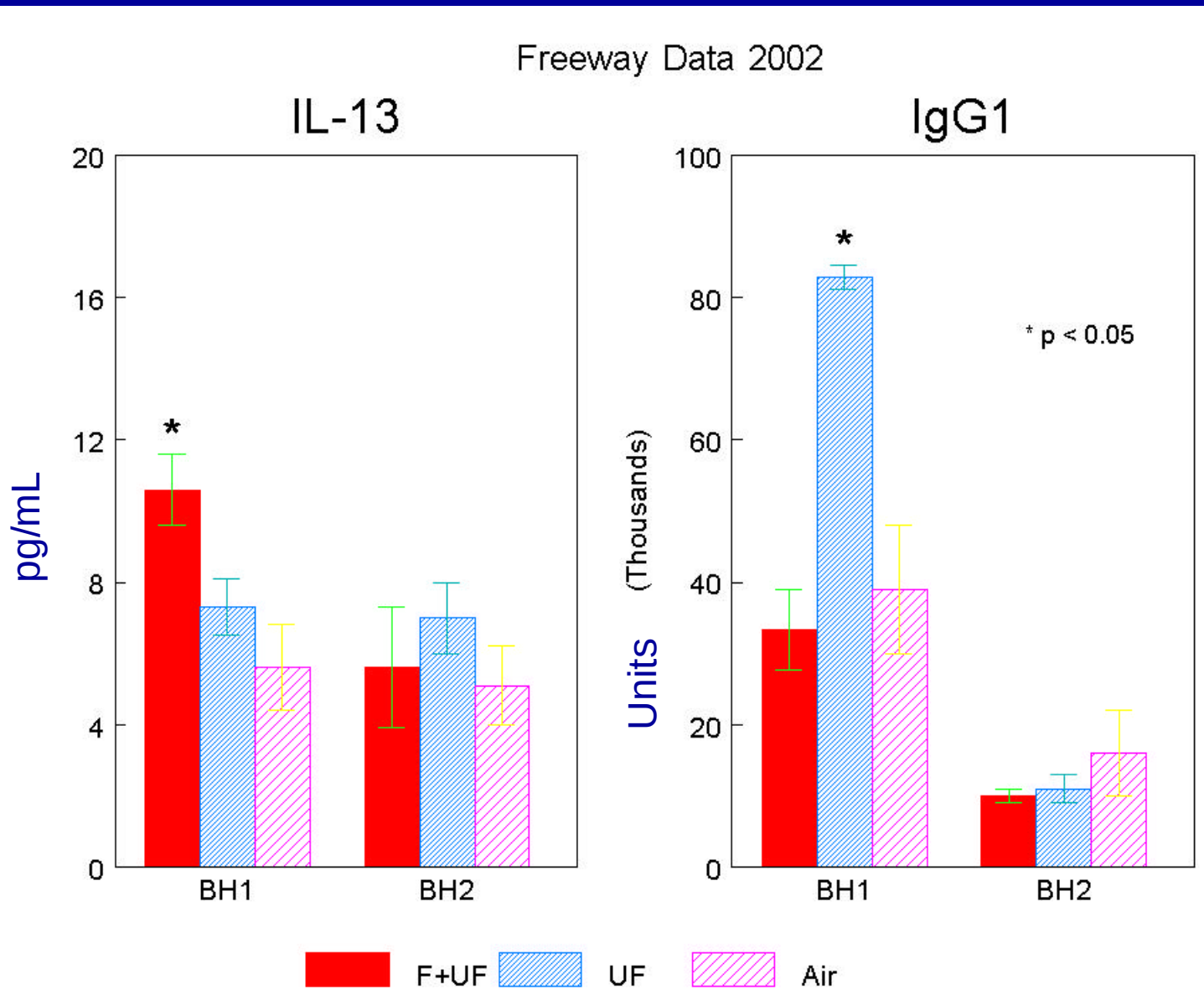
- Ovalbumin-sensitized mice are exposed to freeway-derived, concentrated ultrafine (UF) and fine+ultrafine (F+UF) particles at sites that are at progressively increasing distances downwind from a freeway.
- Particle mass concentrations are held constant (400 $\mu g/m^3$).
- Control animals are exposed to purified air.

Freeway Study Sites



Statistical Analyses

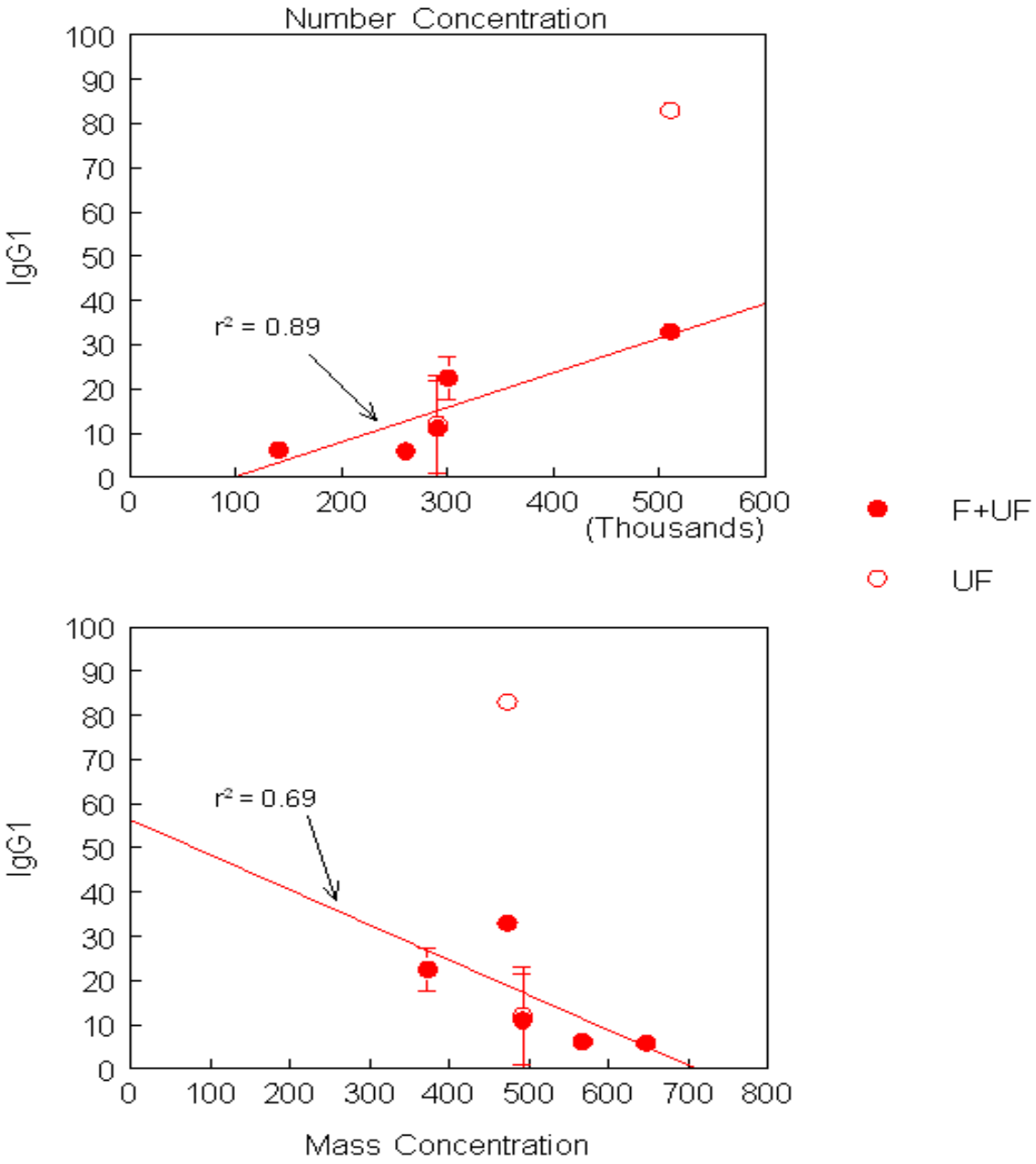
- 2-way ANOVA
  - Site vs. Exposure
- IL-5 Significant increase BH1 CAPs exposed vs. other exposed or controls.
- IgG1 Significant increase BH1 CAPs exposed vs. other exposed or controls.
- EOS Significant increase BH1 CAPs exposed vs. other exposed.
- IL-13 Significant increase BH1 CAPs


Freeway Data 2002
IL-13
IgG1
pg/mL
Units
(Thousands)
20
16
12
8
4
0
100
80
60
40
20
0
*
*
* p < 0.05
BH1
BH2
BH1
BH2
F+UF
UF
Air

Do the data fit our hypothesis?

- If UF are the key driving force for responses than we should see a significant relationship between response and particle number, EC, OC and perhaps chemical species.
- However, we have seen a negative relationship with particle mass at high concentrations.

Biological Response is Affected by Particle Number and Mass



Inflammatory Mediators in the Brain

- There have been speculations that non-familial neurodegenerative diseases are:
  - related to exposures to environmental toxins
  - at least, in part, due to oxidant-related mechanisms
- Dogs in exposed to air pollution in Mexico City exhibit lesions in brain tissue.
- Brain tissues from mice exposed at BH2 were analyzed for expression of IL-1, TNF and NFkB.

Data From Brain Tissue Analyses

Brain Inflammation Markers Tissue from Mice Exposed at BH2 2002			
	Control	UF	F+UF
TNFα (ng/mL)	2.0±0.1	2.2±0.1	2.5±0.2
IL-1α (ng/mL)	1.6±0.2	2.7±0.3*	2.0±0.4*
NF_kB (units x 10^{-3})	8.5±4.4	11.0±1.6**	10.7±3.0**

Note:
***p< 0.05**
****p< 0.01**

Addition of a new model – the Geriatric Rat

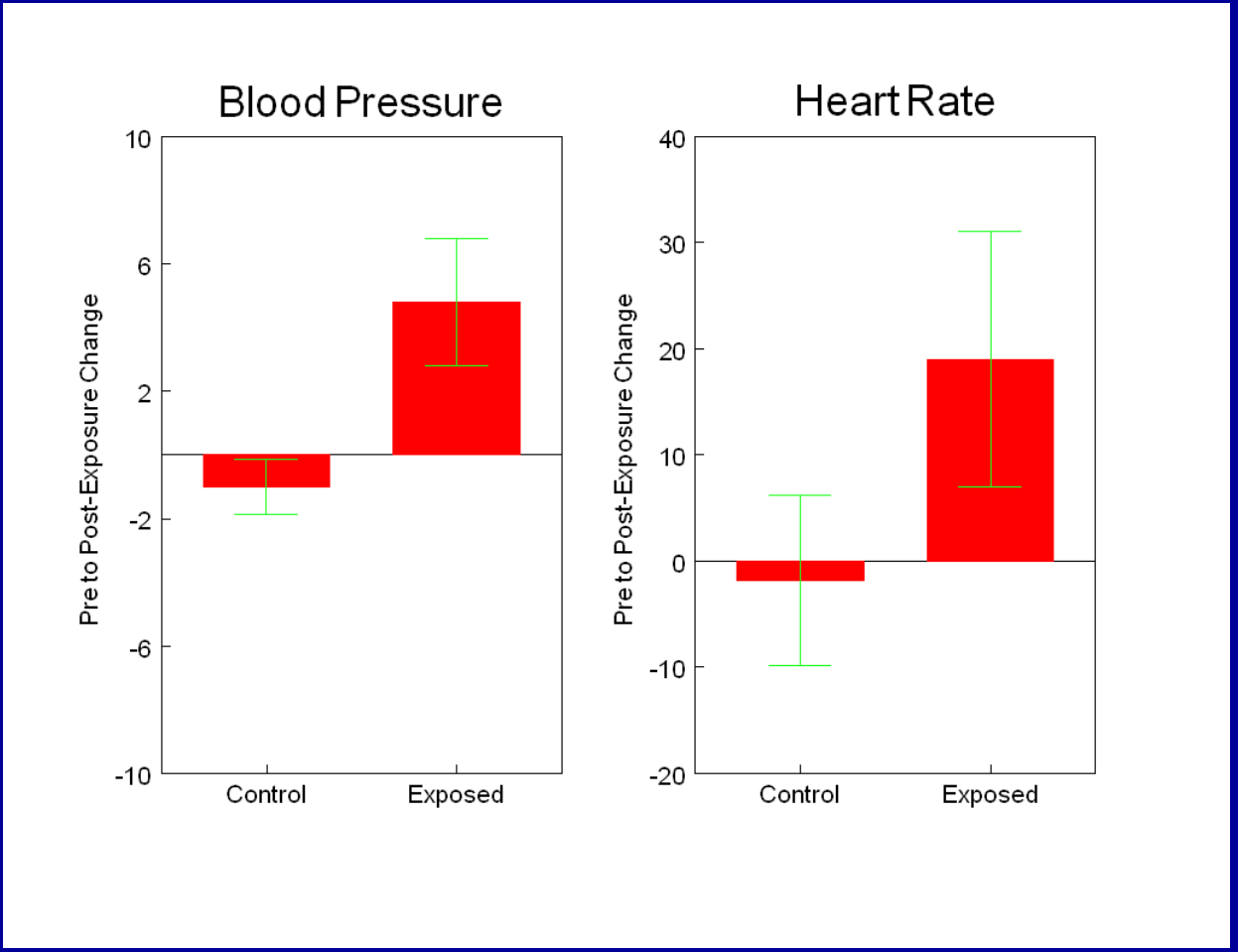
- Previous studies had shown that the ‘geriatric’ rat was sensitive to particle-induced inflammation and hemodynamic effects.

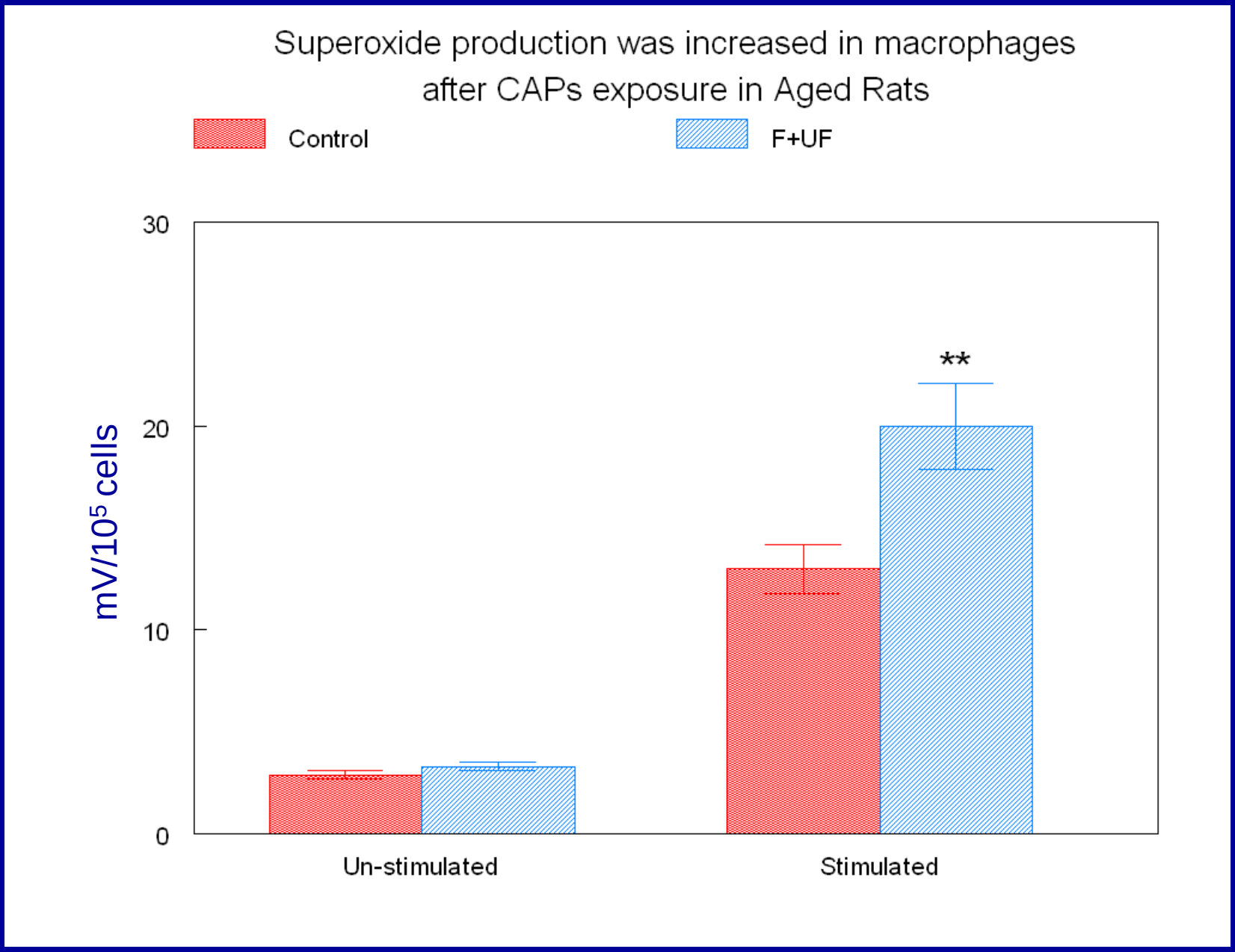
- The geriatric rat is a model for particle effects in a sensitive human population.

Approach

- Rats aged 24-26 months exposed to the highest achievable F+UF concentration.
- Exposures were 4 hours per day for 3 consecutive days. There were 8 controls and 8 exposed rats.
- 4 additional rats were implanted with blood pressure and EKG transponders. They were exposed to F+UF.

Blood Pressure and Heart Rate Were Increased After CAPs Exposures




Superoxide production was increased in macrophages after CAPs exposure in Aged Rats
Control
F+UF
mV/10^5 cells
30
20
10
0
**
Un-stimulated
Stimulated

Conclusions

- Allergic responses were seen in mice exposed at the site closest to the freeway.
- Responses appear to be a function of the number of particles to which the mice were exposed.
- Hypothesis that Ultrafine particles are important mediators of health effects is supported by data.

Conclusions

- Significant inflammatory responses are observed in brain tissue 2 weeks after CAPs exposure; possible relevance to neurological mechanisms.

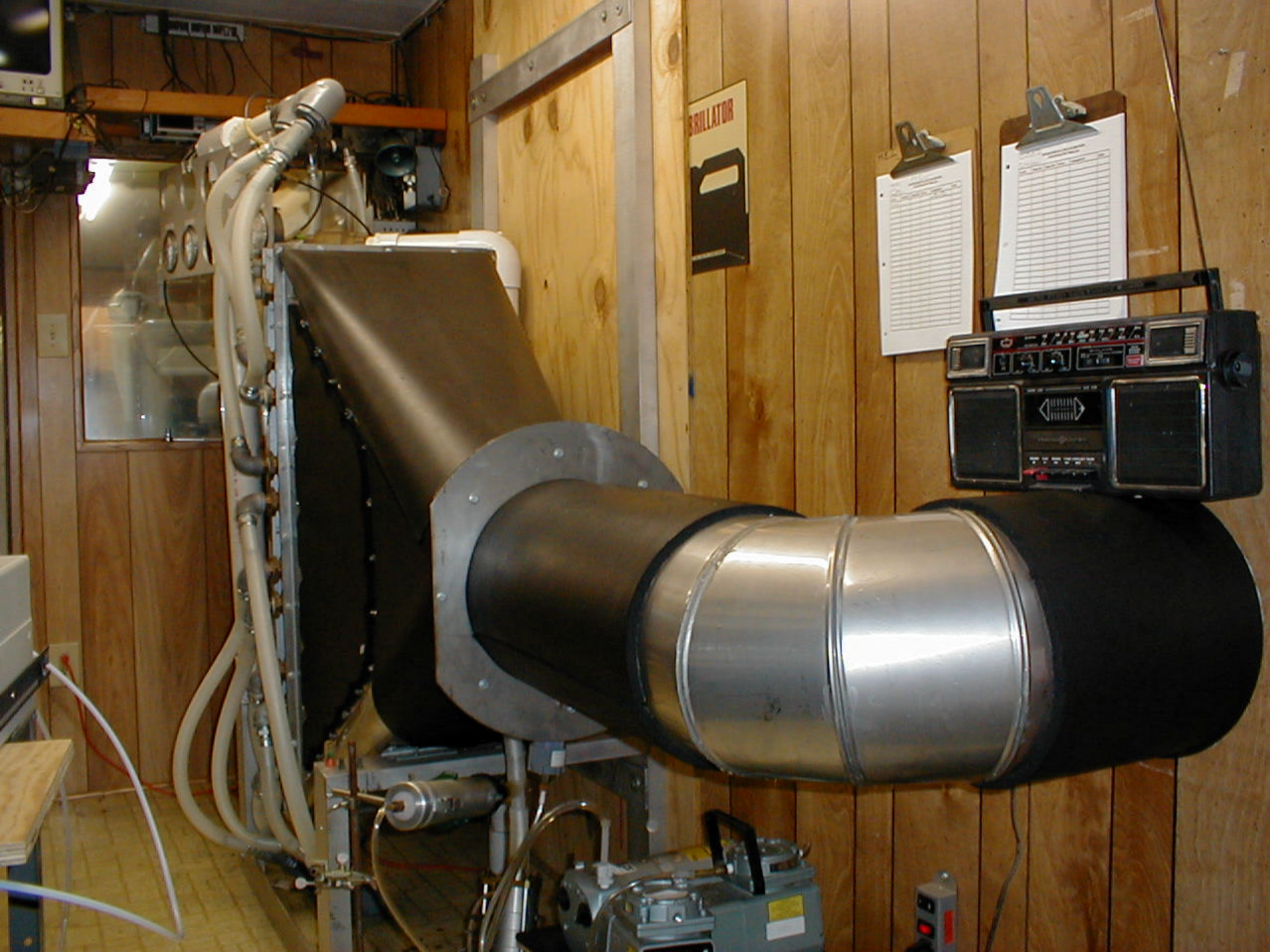
- The geriatric rat model was, so far, successful.
  - Changes in Heart Rate, Blood Pressure and macrophage responses were seen.

Hypothesis: Short-term exposures to CAP cause acute cardiopulmonary responses

PARTICLES	SUBJECTS	ENDPOINTS
Fine $PM_{2.5}$	Healthy Asthmatic Elderly* COPD*	Lung function Symptoms Pulse oximetry Airway inflammation markers Systemic inflammation markers Blood coagulability Heart rate variability Arrhythmia incidence
Coarse $PM_{(10-2.5)}$	Healthy Asthmatic	
Ultrafine $PM_{0.1}$	Healthy (pending) Asthmatic (pending)	

*With & without NO_2

Trailer Housing Fine PM Concentrator at Rancho Los Amigos



CONCLUSIONS
[Healthy & Asthmatic Subjects, Coarse PM]

- First clinical study with coarse PM
- No indication of airway effects from coarse PM exposure
- Increase in cardiovascular stress suggested by increased heart rate/decreased heart rate variability
- Blood & sputum biochemical analyses pending