Mechanistic Aspects of Nanoparticle Toxicology - Particle Deposition and Clearance

What can we learn from the ultrafine particles?

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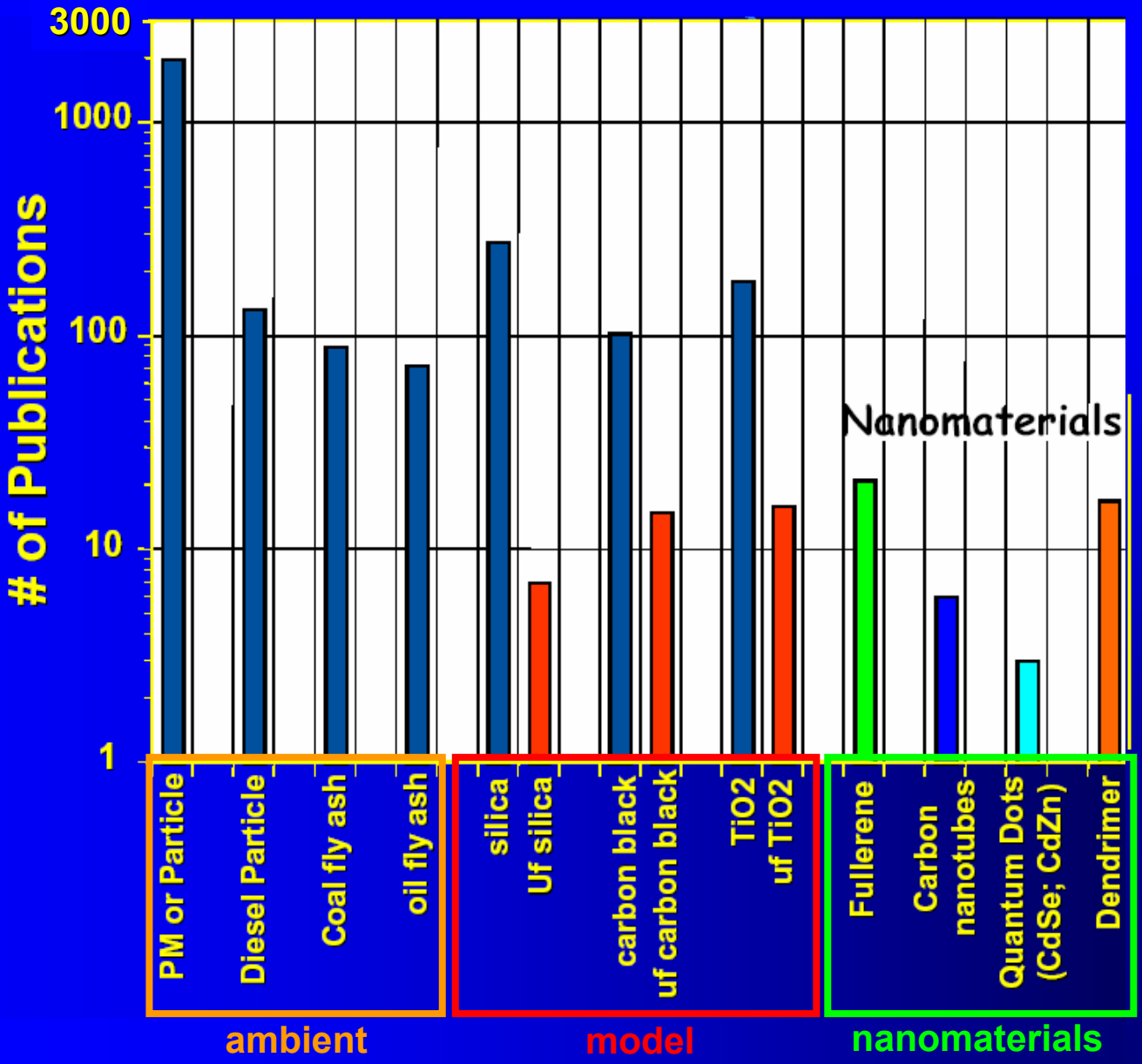
Mechanistic Aspects of Nanoparticle Toxicology - Particle Deposition and Clearance

Object: Draw parallels from NP to PM toxicity

- *Deposition & Clearance Mechanism*
- *Acute Pulmonary Effects*
- *Translocation to Secondary Organs*
- *Extra-pulmonary Effects*

What do we know about Particle-Toxicology?

PubMed

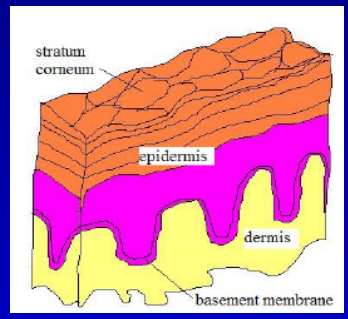


modivied from Kevin Dreher, US EPA: „Nanotechnology for Remediation Technical Nanotechnology for Remediation Technical" Workshop, Okt'05

Routes of Exposure to Ambient Particles:

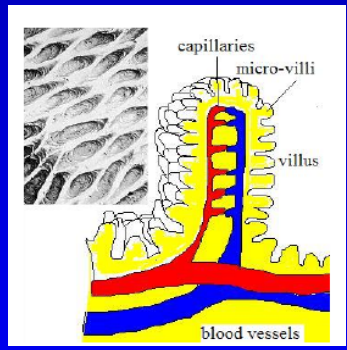
Organ systems exposed:

- **Skin**
  surface area:1.5-2 m^2

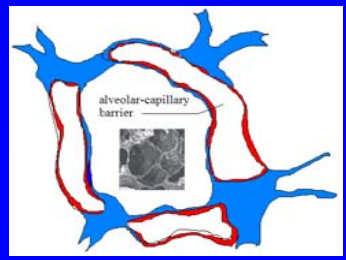


thickness: 0.05 - 1.5 mm

- **Gastrointestinal Tract**
  surface area: 200 m^2
  10^{12}-10^{14} fine particles are ingested per person/day



- **Lung**
  surface area: 150 m^2
  10^{10}-10^{12} fine particles deposit in the lung/day



“Ambient” Nanoparticles, a Fraction of Particulate Matter (PM)

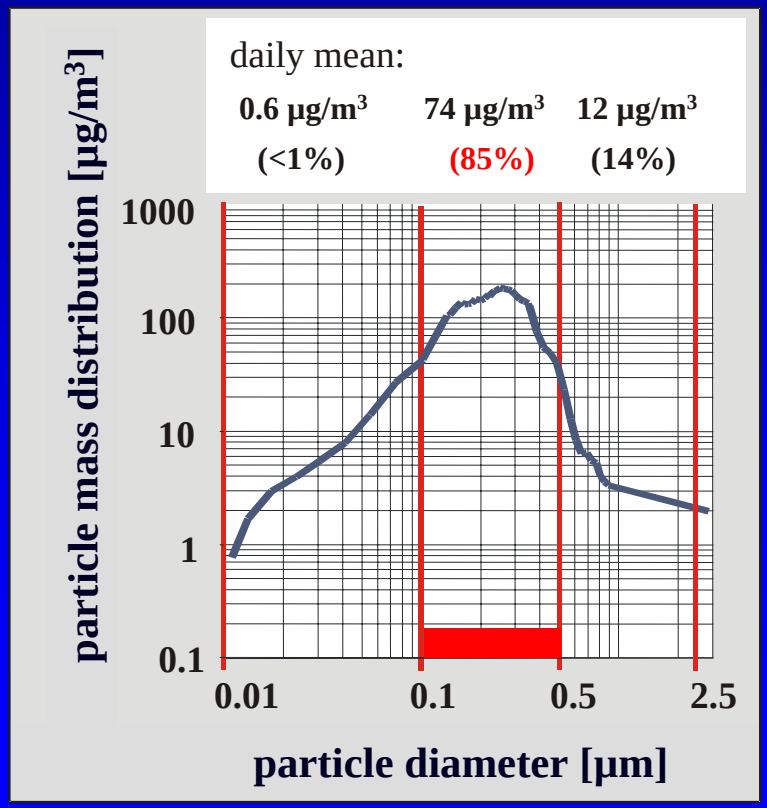
- mainly **carbonaceous** NPs, derived from **combustion** processes
- **low-solubility** in aqueous/physiological solutions
- **low-toxicity,** no cytotoxic effects below 100 µg/ml
  (“the dose makes the poison”)

Size distribution of fine ambient particles ($PM_{2.5}$)

10 nm – 2.5 µm

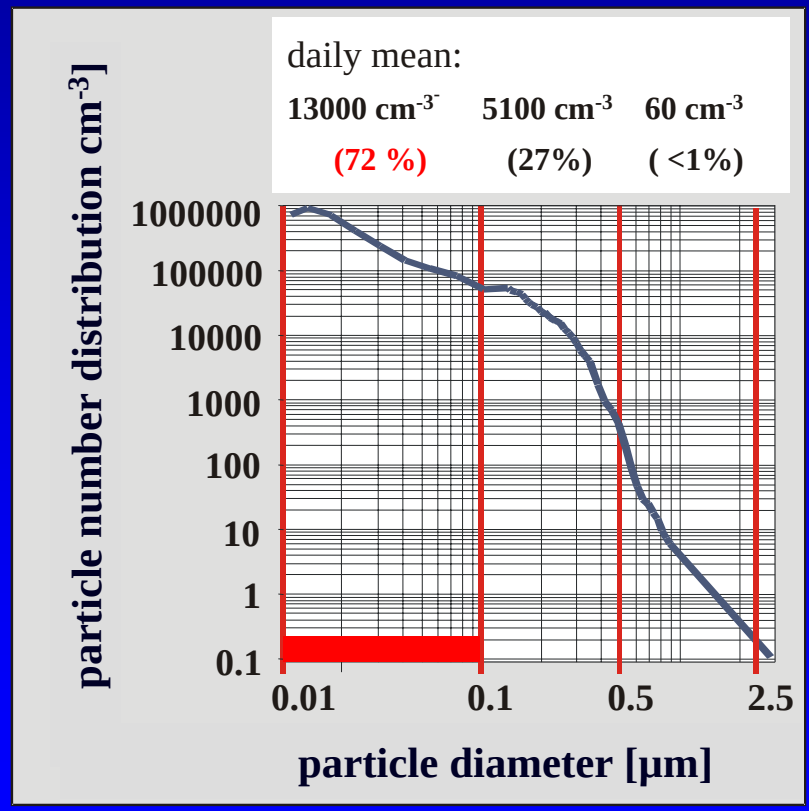
mass burden:

$PM_{2.5}$: 0.1 µm – 2.5 µm



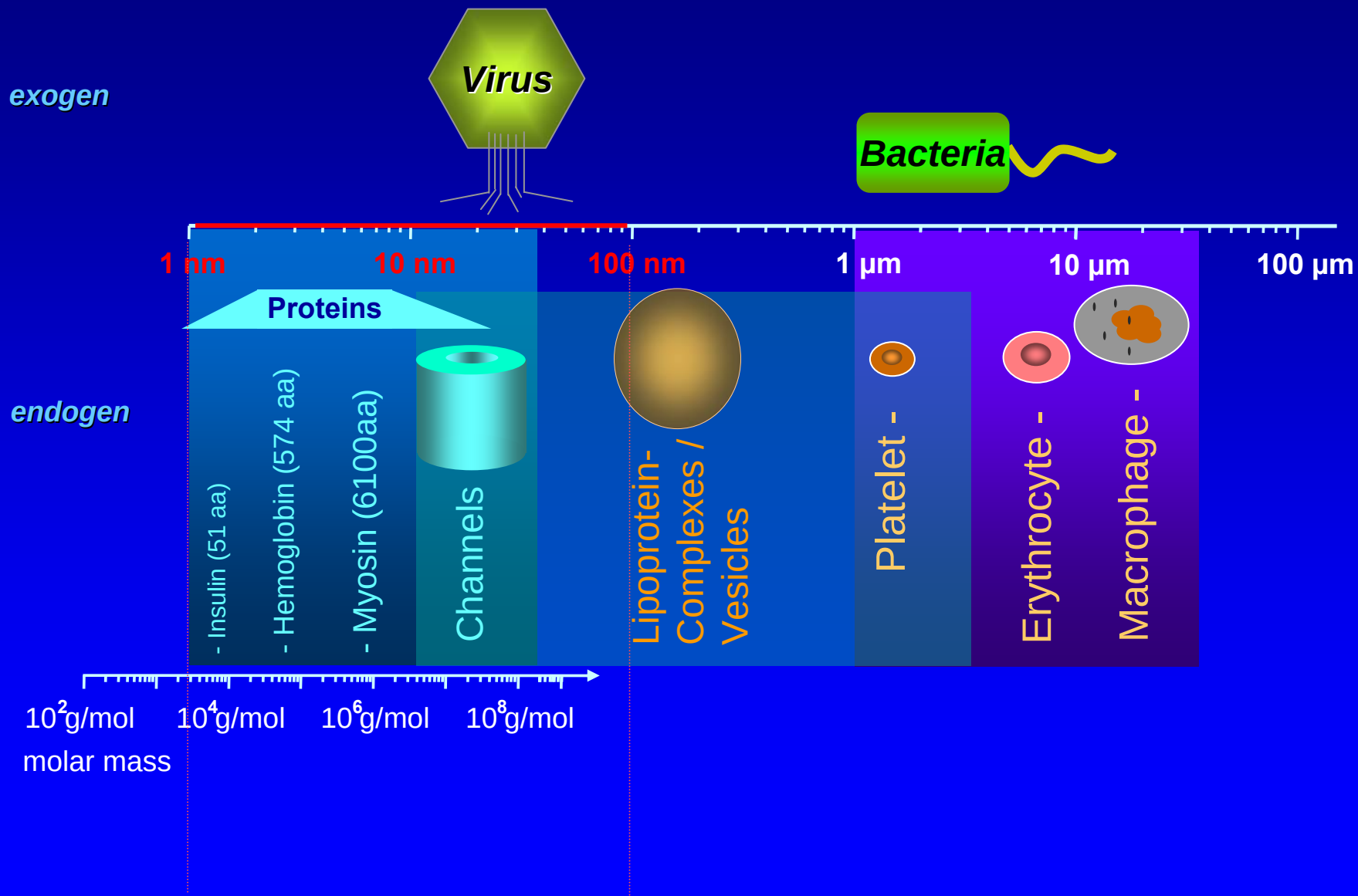
number burden:

ultrafine particles < 0.1 µm



Tuch et al. 1997

Size Relation at Cellular Scale



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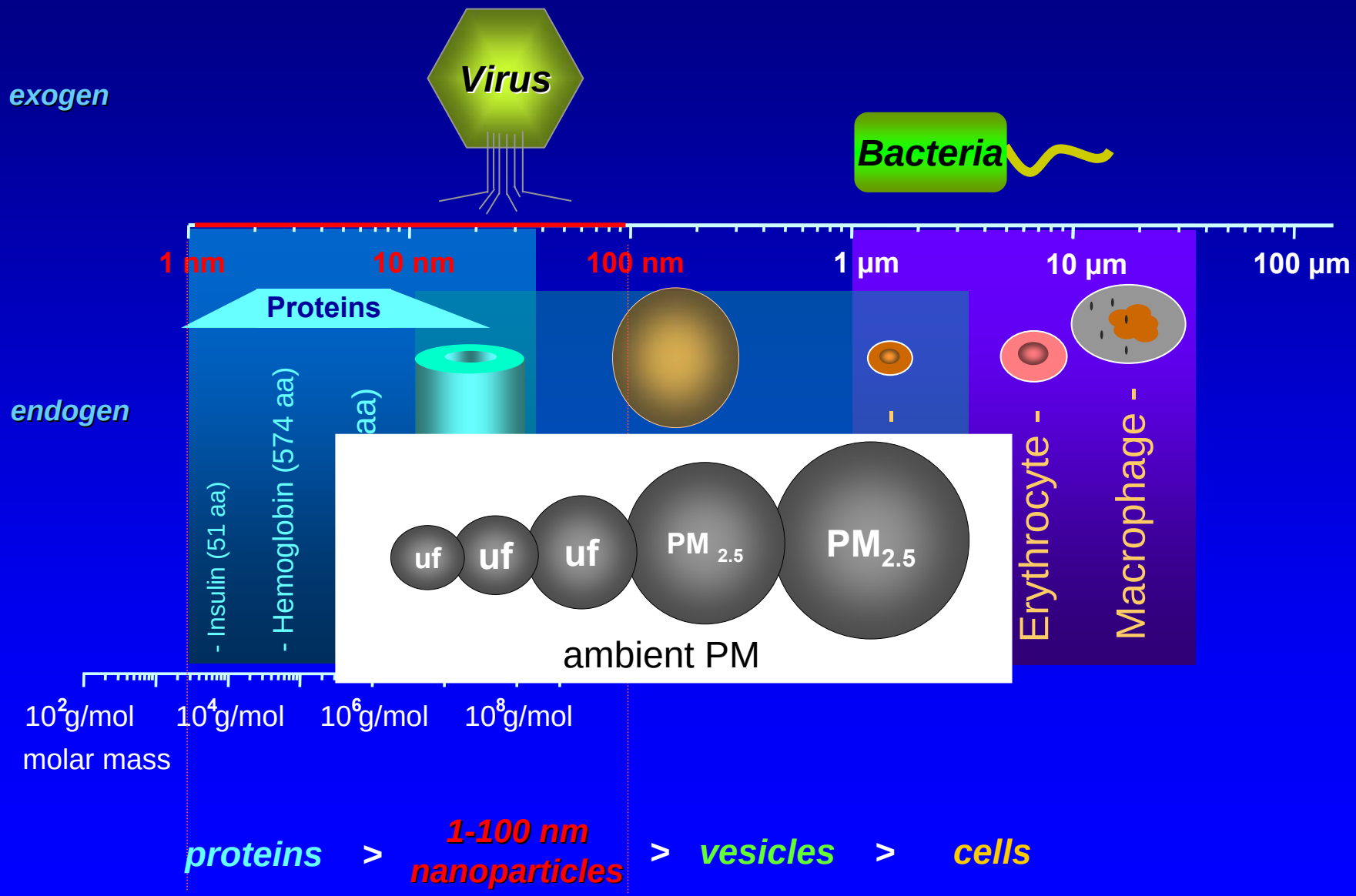
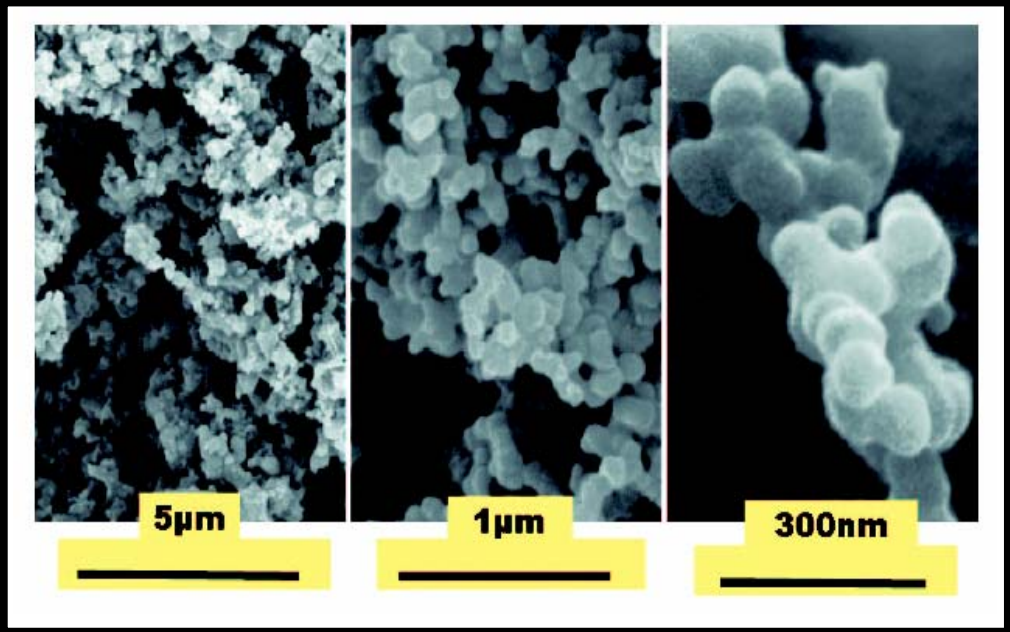
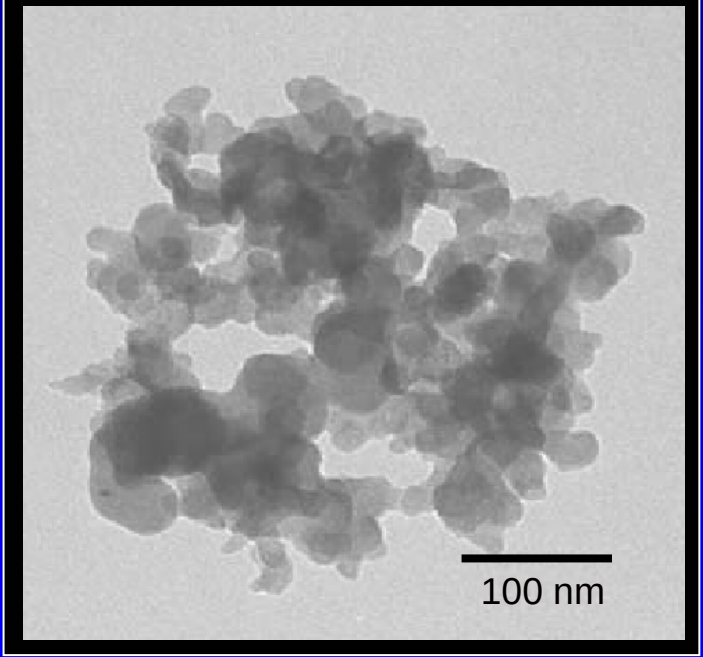


Shape of soot particles

Soot Particles



Diesel Soot (SRM1650a)



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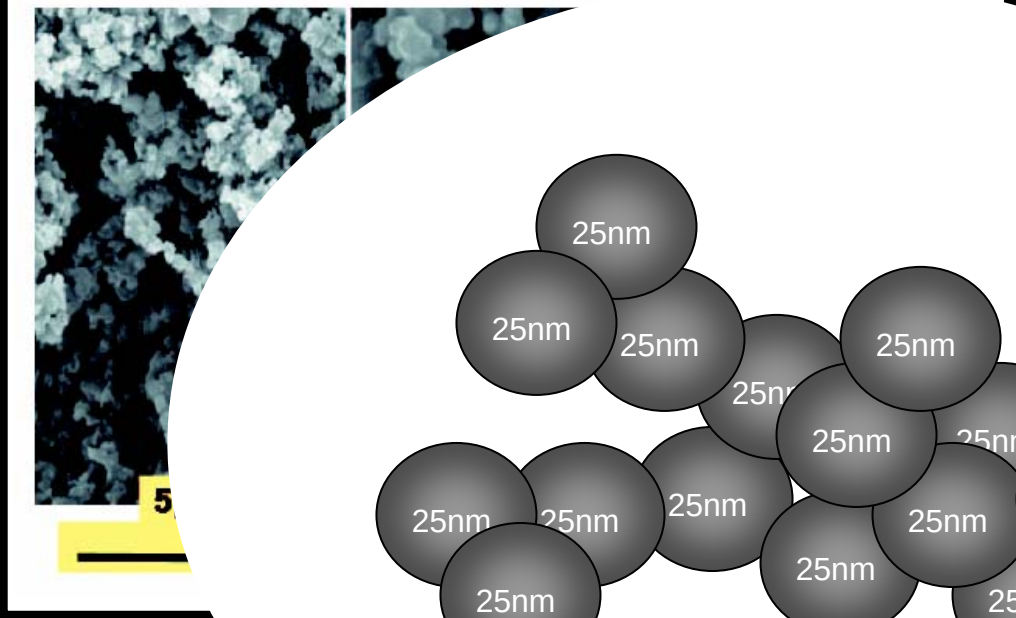

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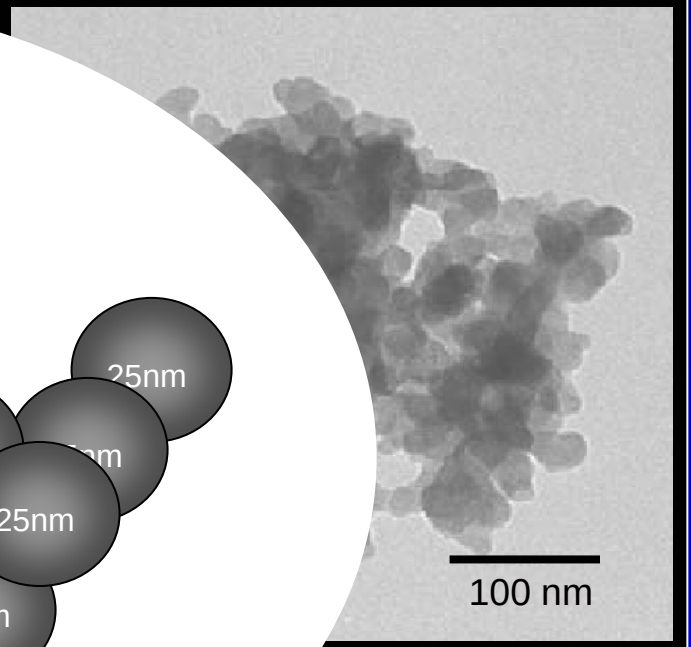
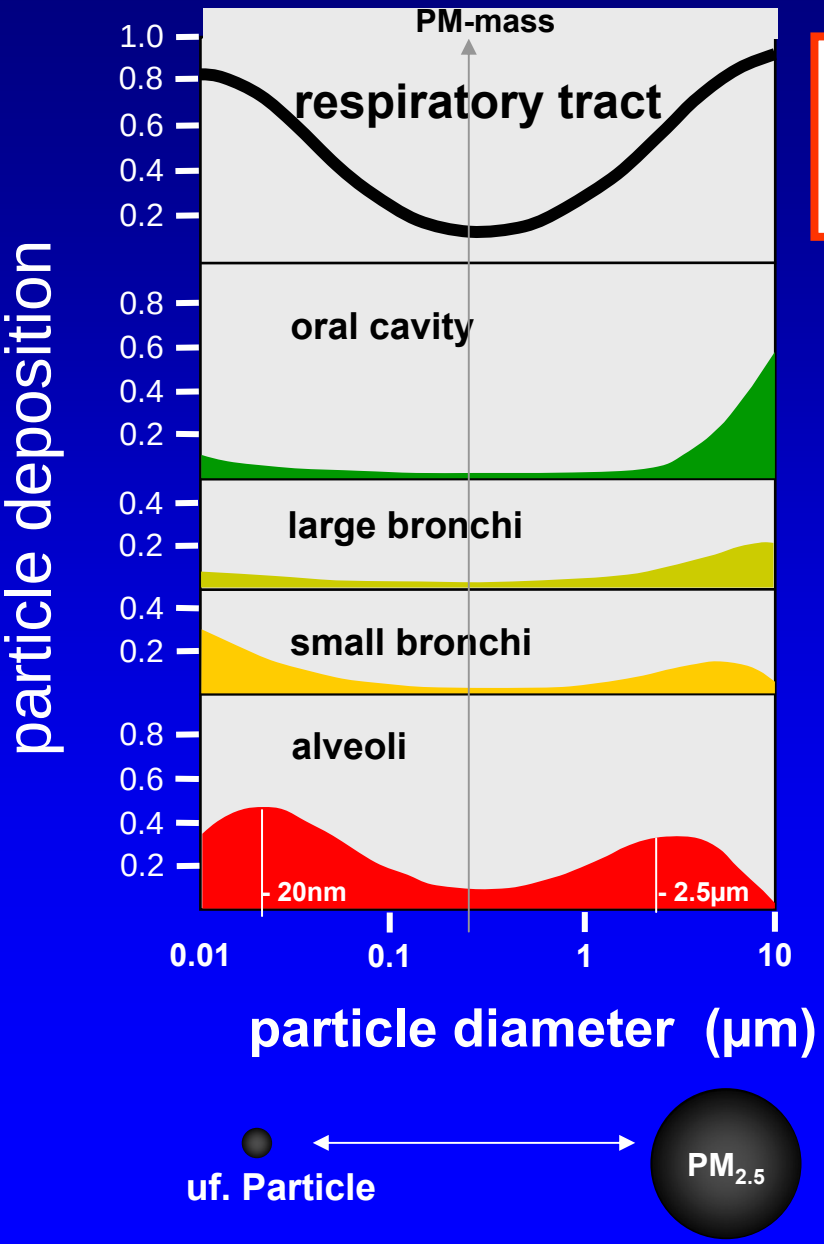


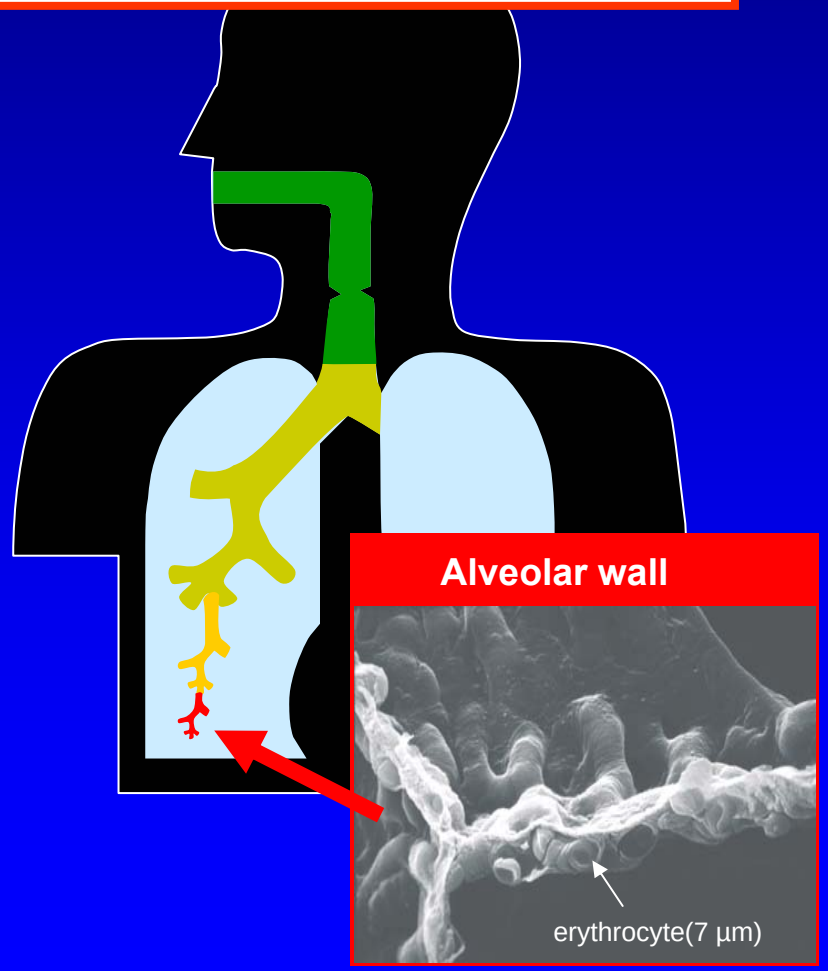
Soot:
aggregates from carbonaceous primary particles

Deposition & Clearance

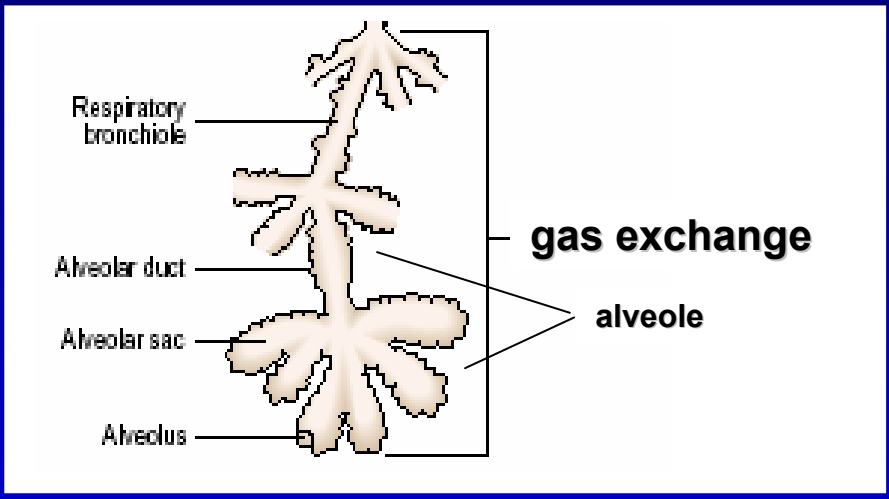
Particle Deposition in the Respiratory System

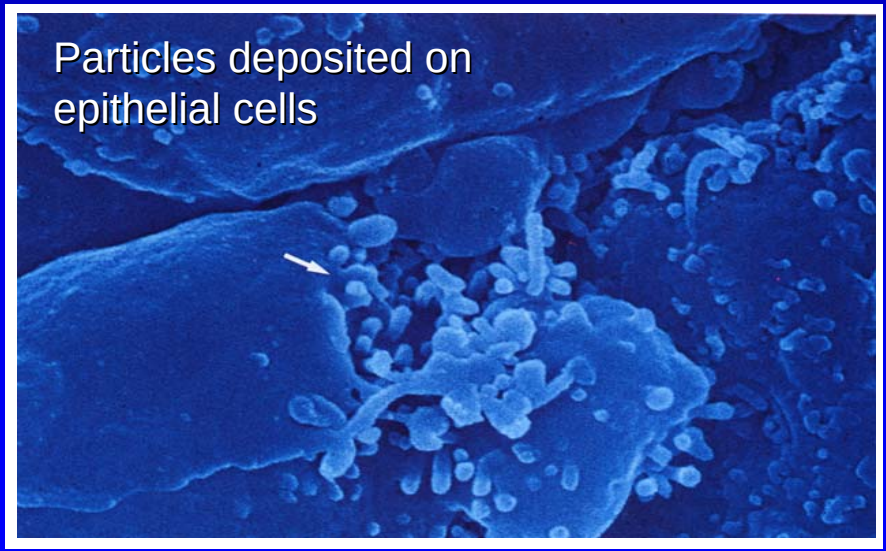


ultrafine particles cause the highest burden to the small bronchial and alveolar region!

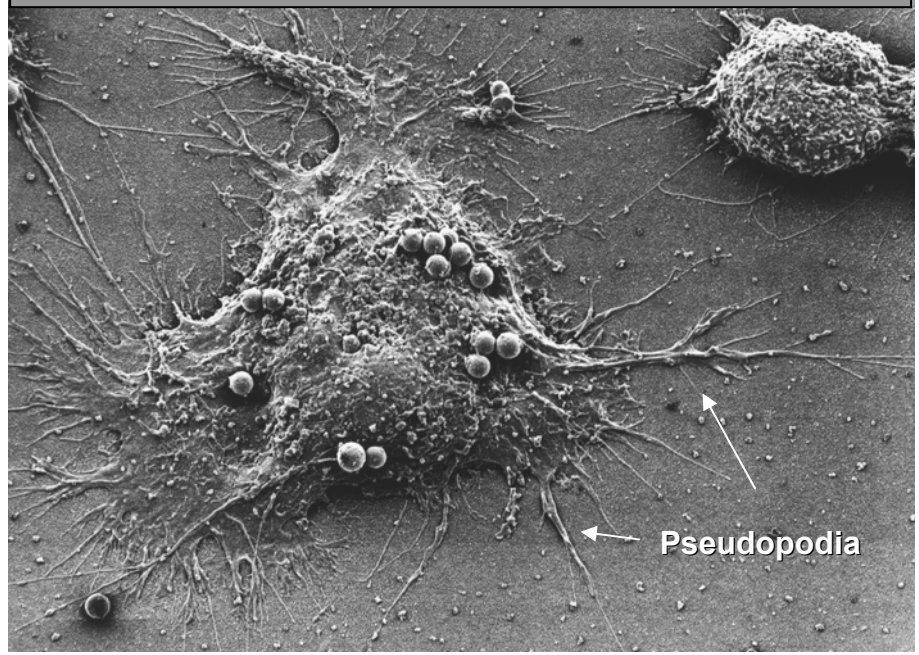


Mechanisms of Defense – Alveolar Region





Phagocytosis of particles by macrophages



Morgenroth & Takenaka

Pathways of Particle Clearance

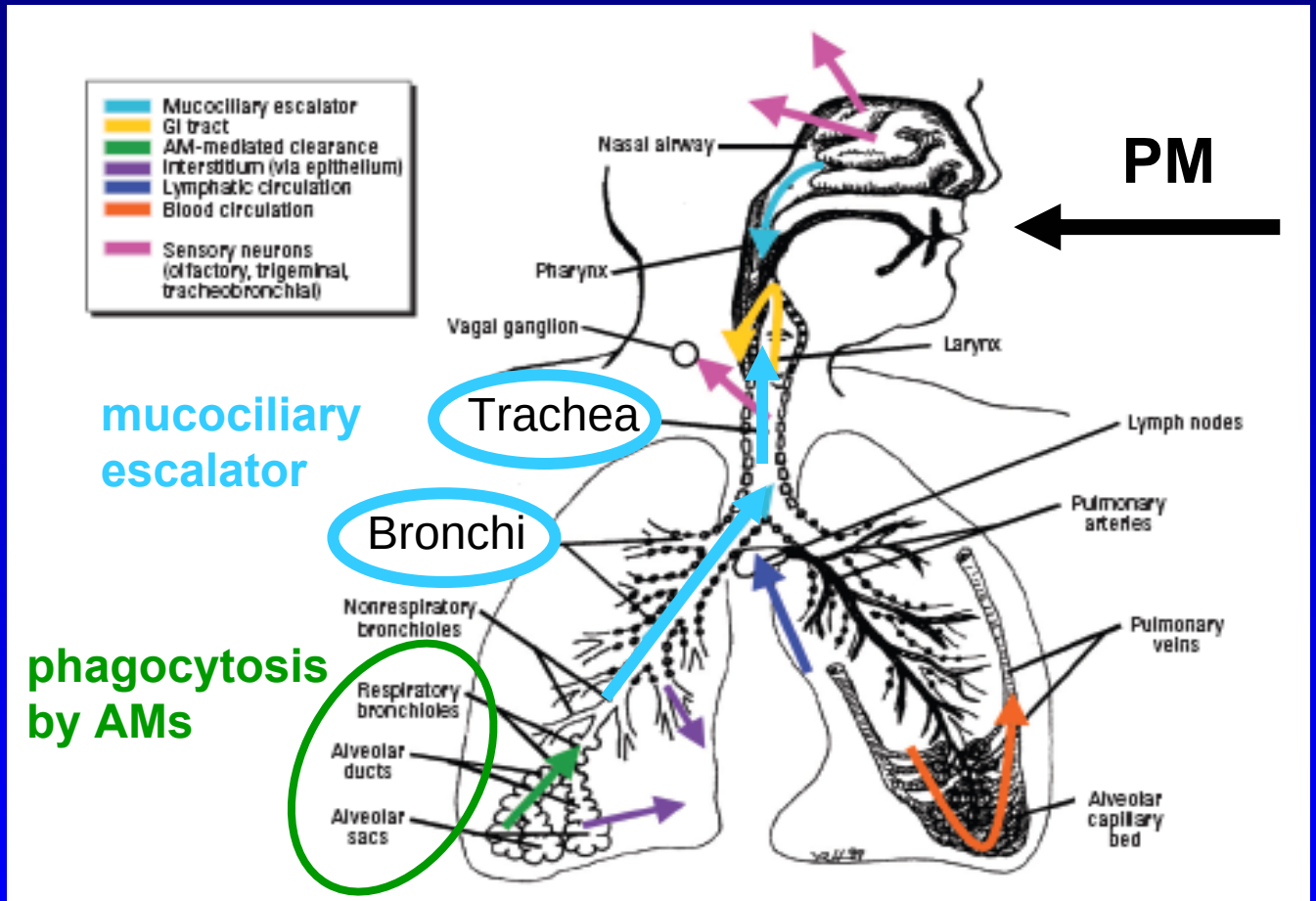
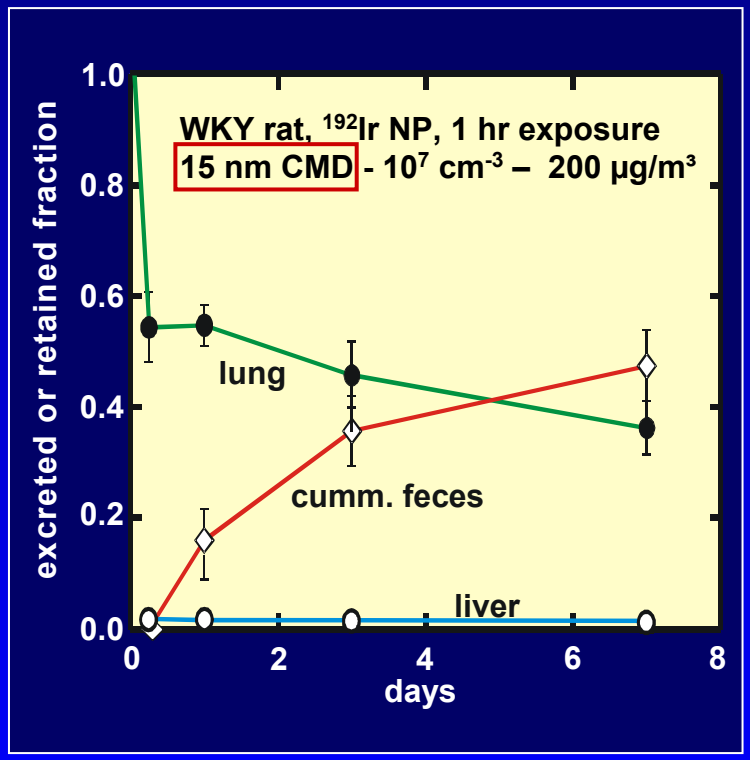


Figure 9. Pathways of particle clearance (disposition) in and out of the respiratory tract. There are significant differences between NSPs and larger particles for some of these pathways (see "Disposition of NSPs in the respiratory tract"). Drawing courtesy of J. Harkema.

Oberdörster 2005

Biphasic Clearance Kinetics



Kreyling 2004

Impact of Particle Size

Particles lavaged or retained 24h post exposure

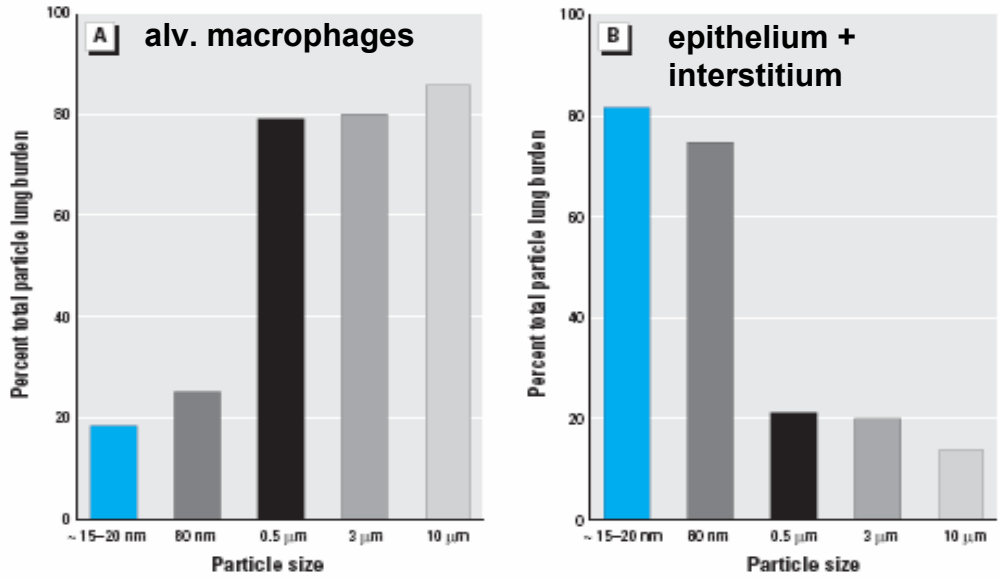
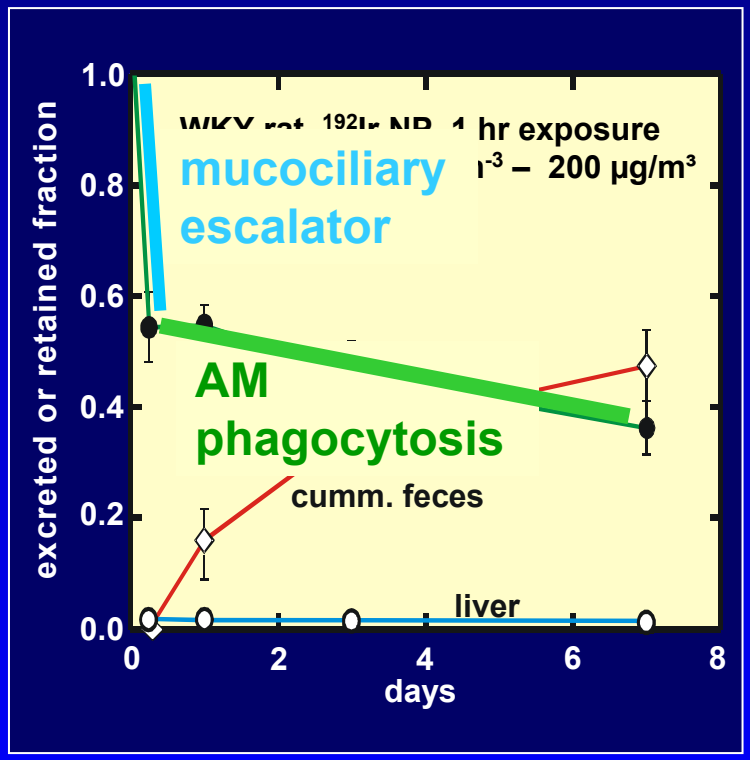


Figure 10. *In vivo* retention of inhaled nanosized and larger particles in alveolar macrophages (*A*) and in exhaustively lavaged lungs (epithelial and interstitial retention; *B*) 24 hr postexposure. The alveolar macrophage is the most important defense mechanism in the alveolar region for fine and coarse particles, yet inhaled singlet NSPs are not efficiently phagocytized by alveolar macrophages.

Oberdörster 2005

Small particles escape form AM clearance!

Biphasic Clearance Kinetics



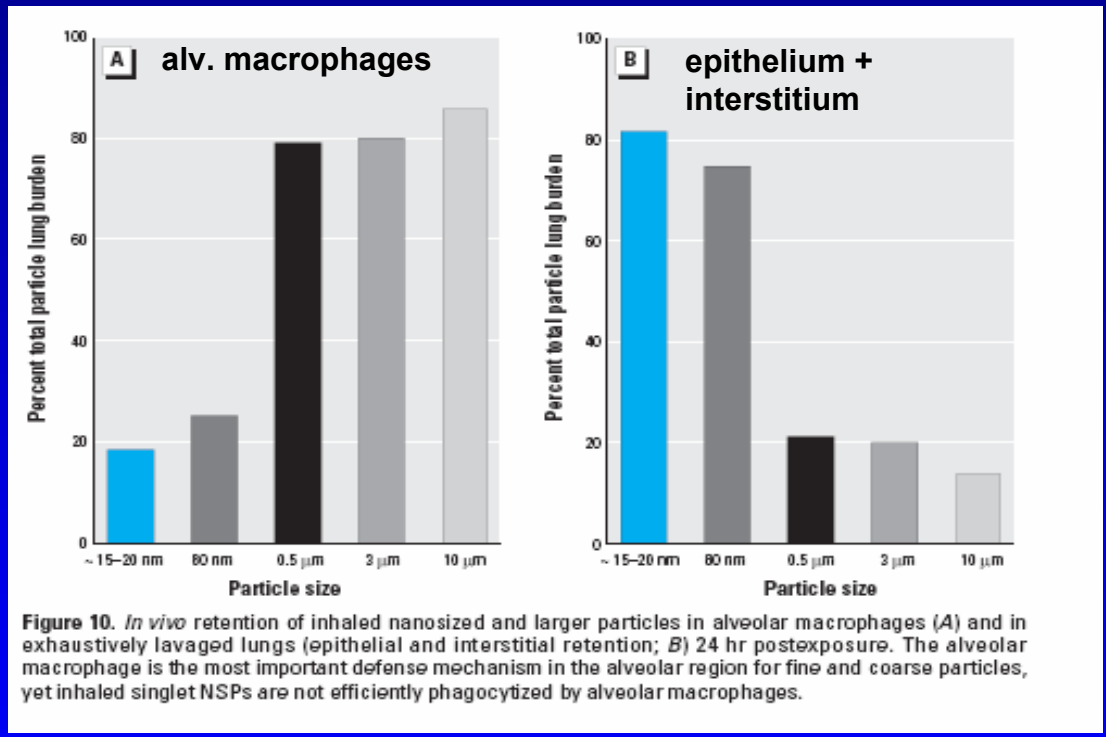
Kreyling 2004

upper airways: fast clearance

alveolar region: slow clearance

Impact of Particle Size

Particles lavaged or retained 24h post exposure



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Oberdörster 2005

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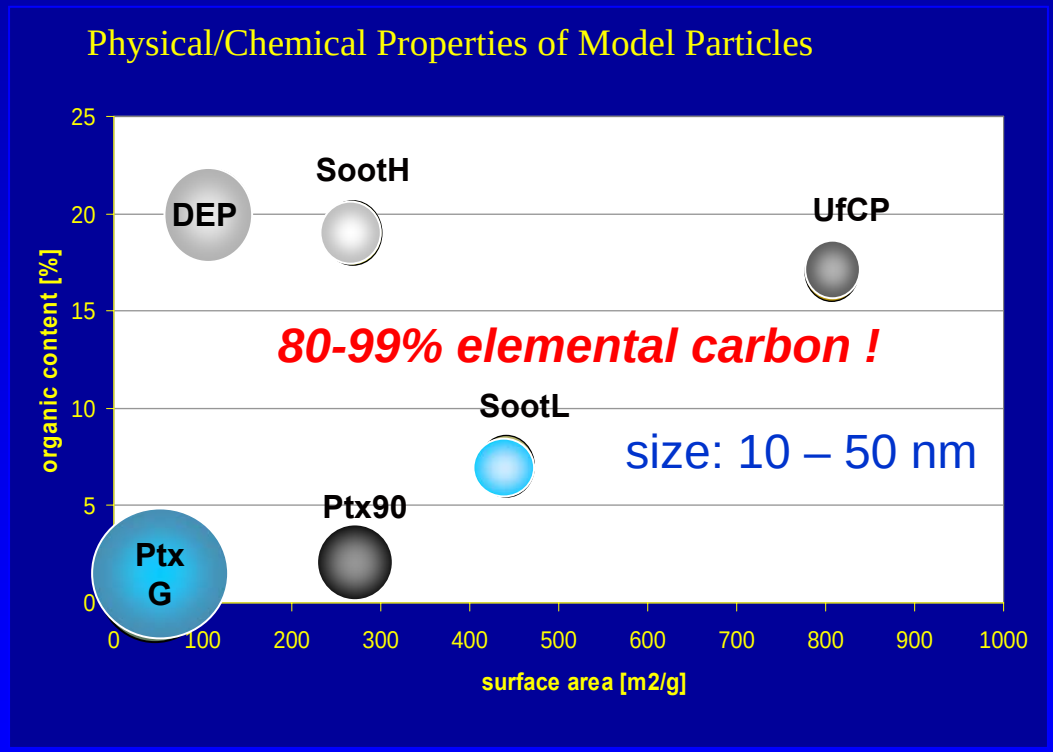
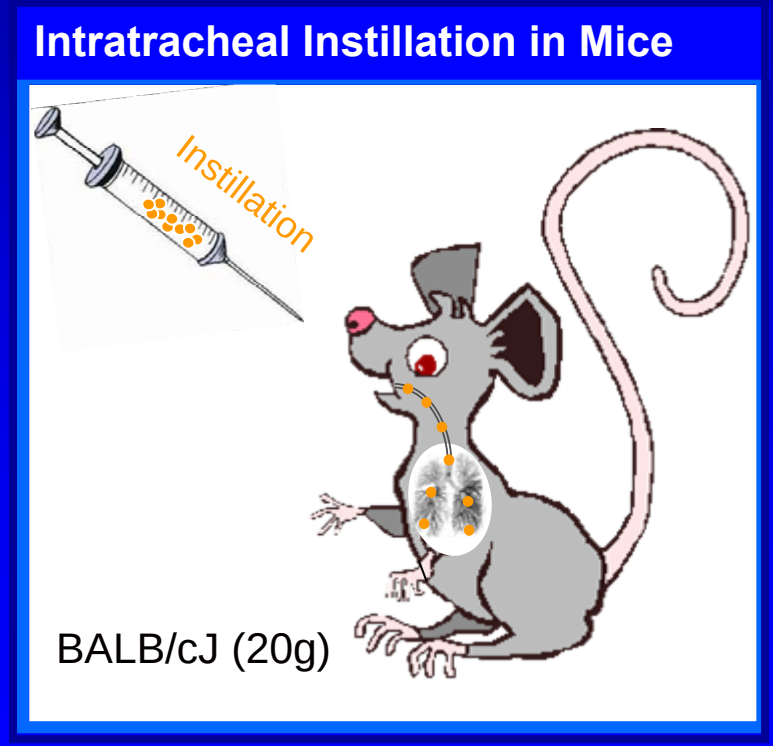
Acute Pulmonary Effects

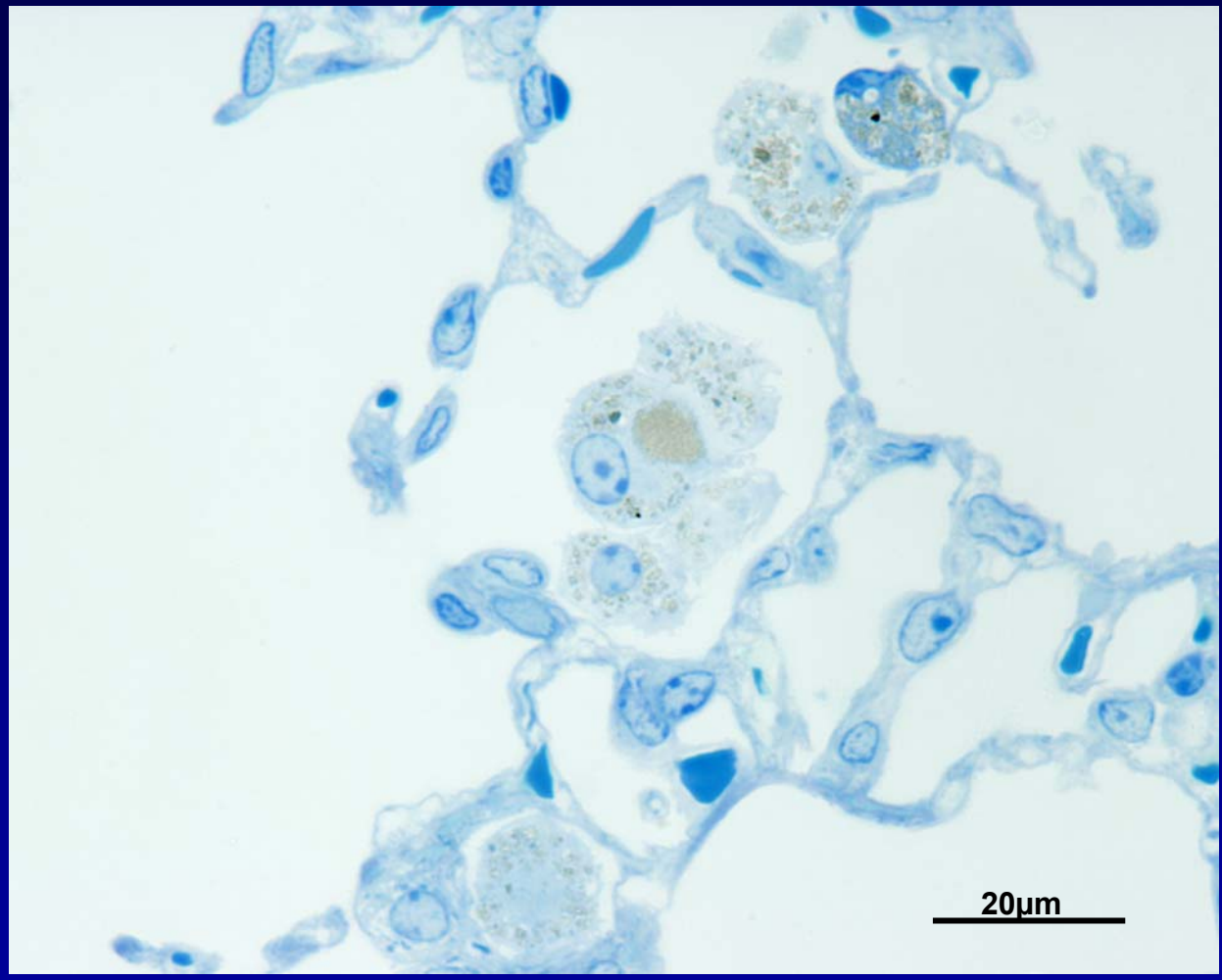
Acute Effects of Pulmonary Deposited Particles?

Particle Exposition:



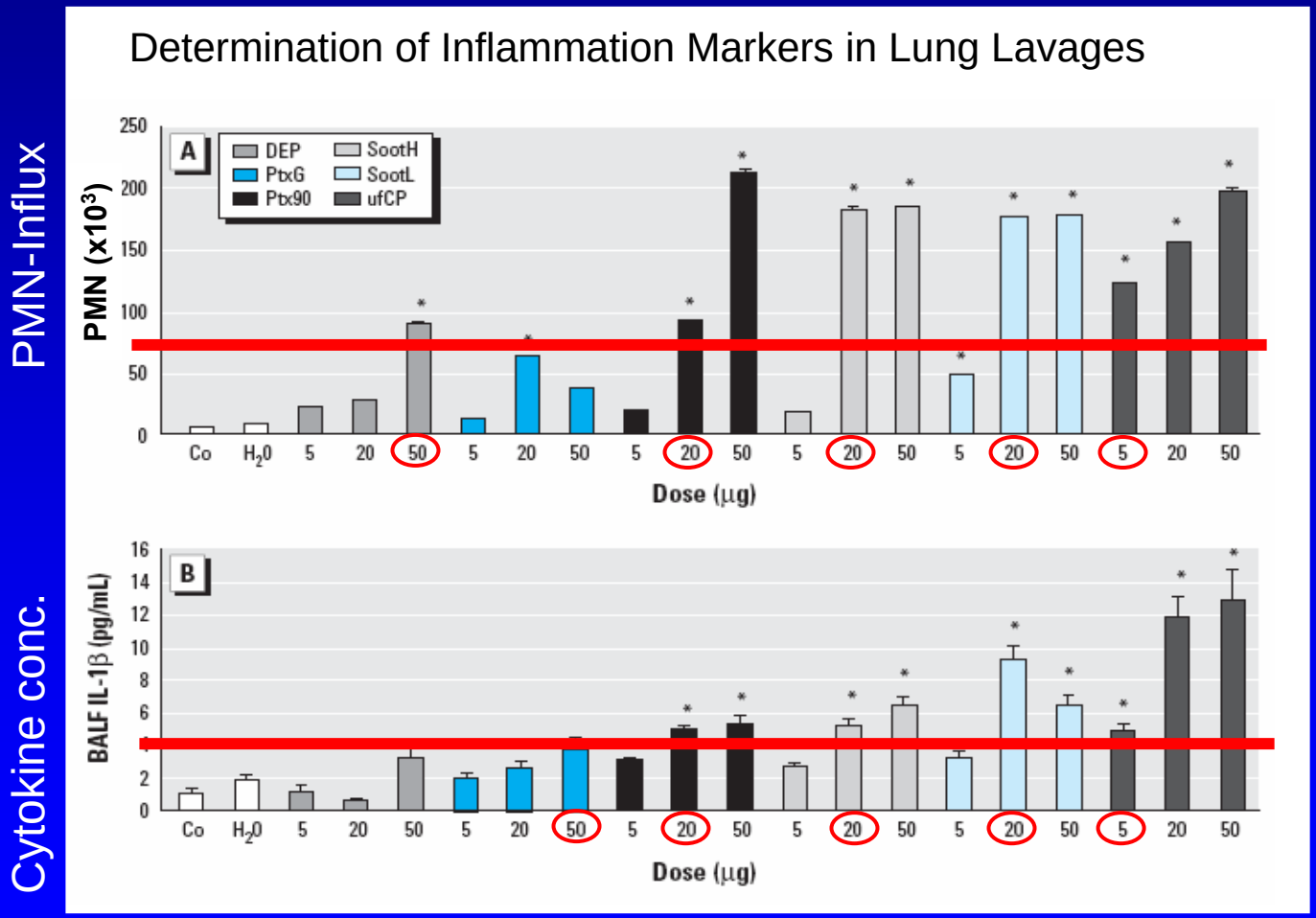


Lung of a mouse 24h after intratracheal instillation of 20 µg ufCP



Semithin section, Staining: toluidine blue, x 63,

Carbonaceous nanoparticles induce acute pulmonary inflammation 24h after instillation



Stoeger 2006, Environ Health Perspect 114:328

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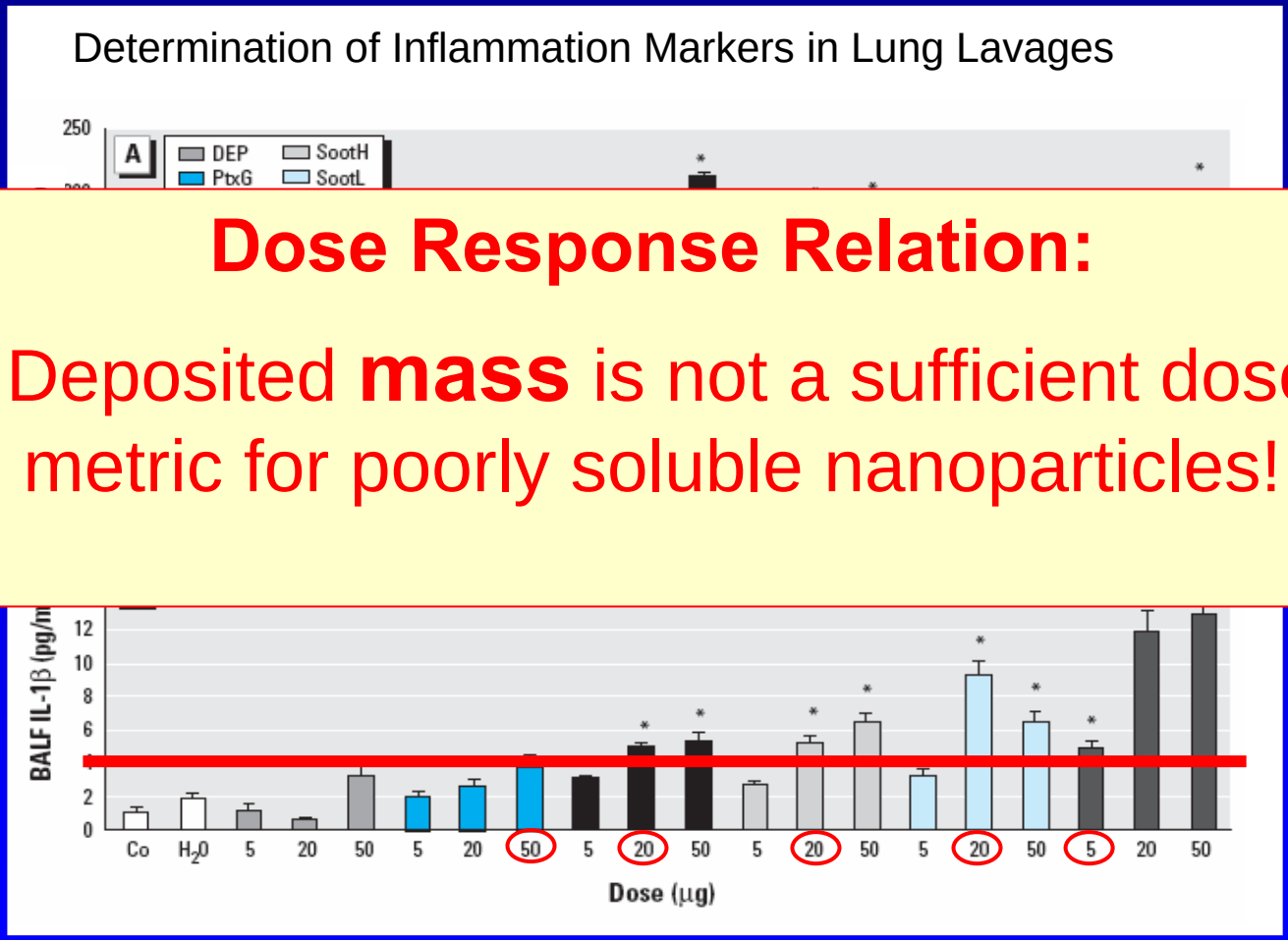
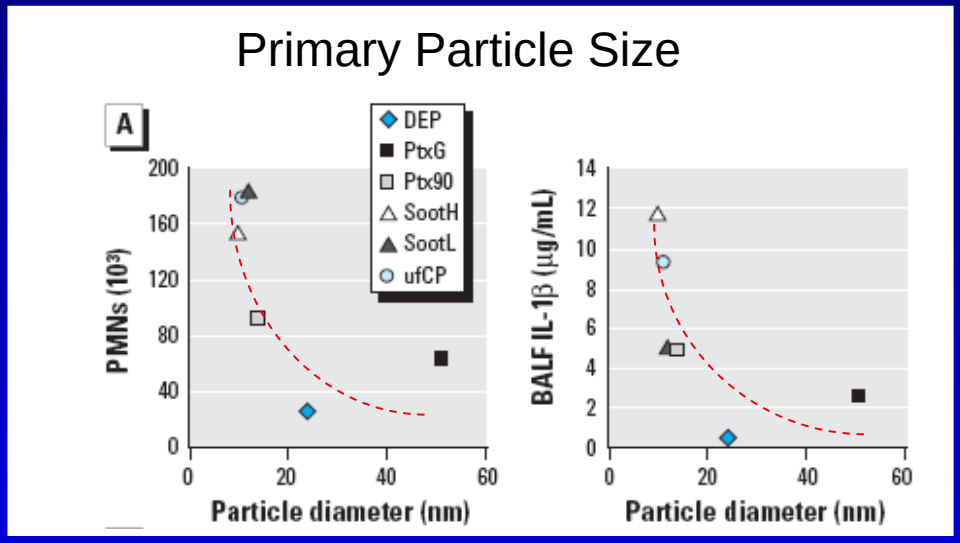


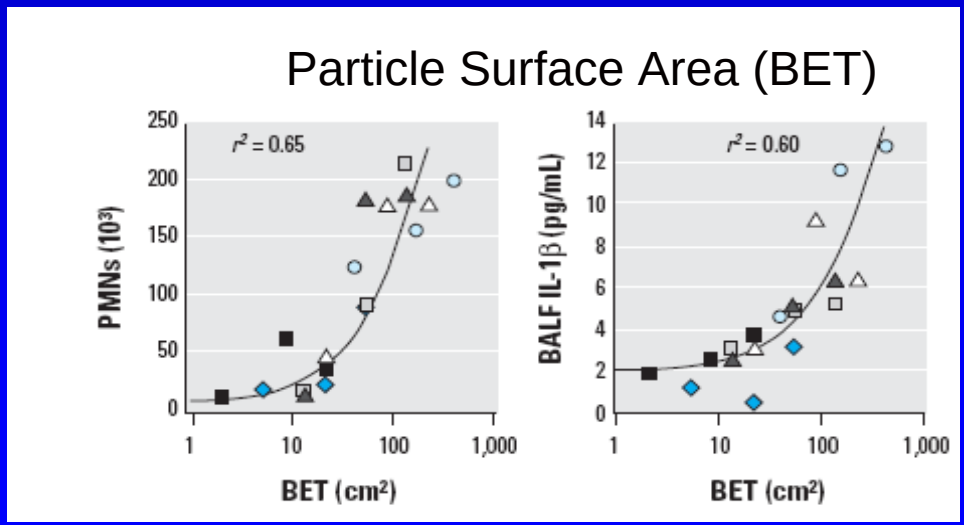
Dose Response Relation:

Deposited **mass** is not a sufficient dose metric for poorly soluble nanoparticles!

Stoeger 2006, Environ Health Perspect 114:328

In search of the most relevant parameter for quantifying pulmonary inflammation ?





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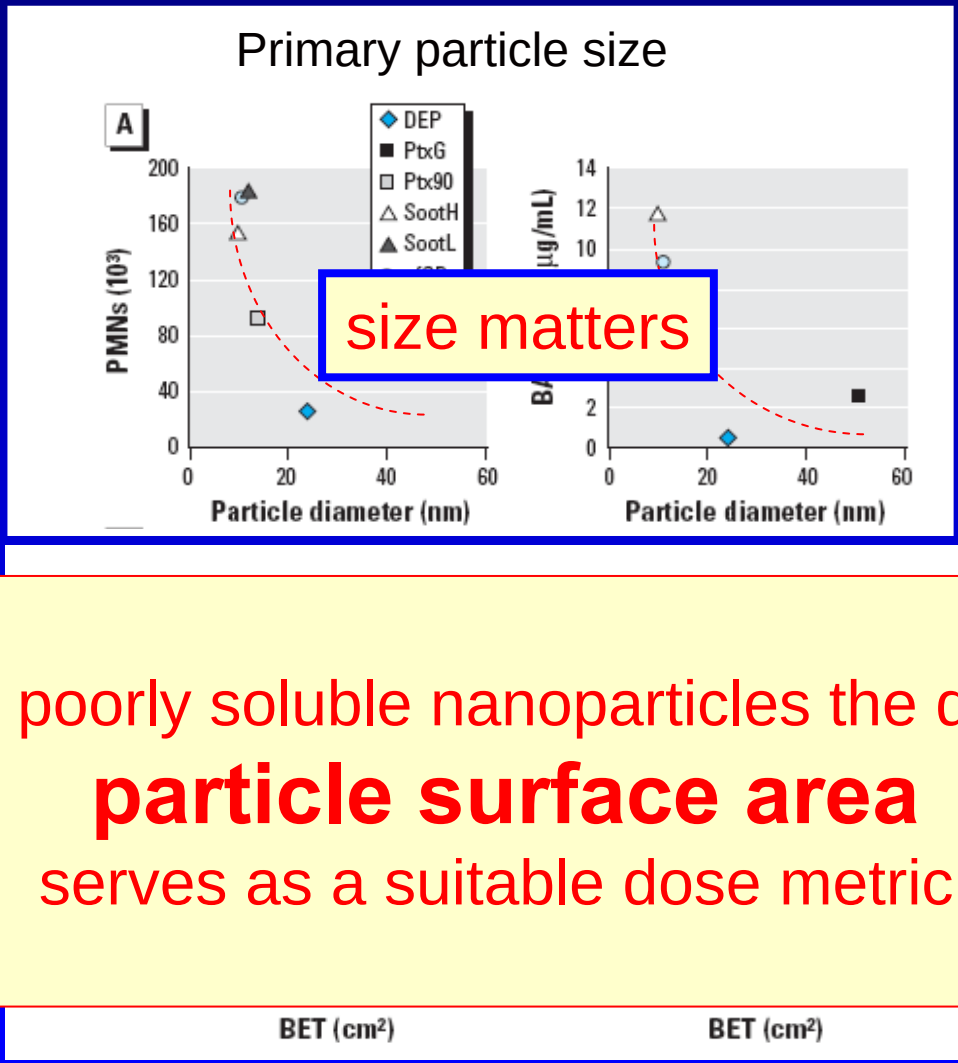


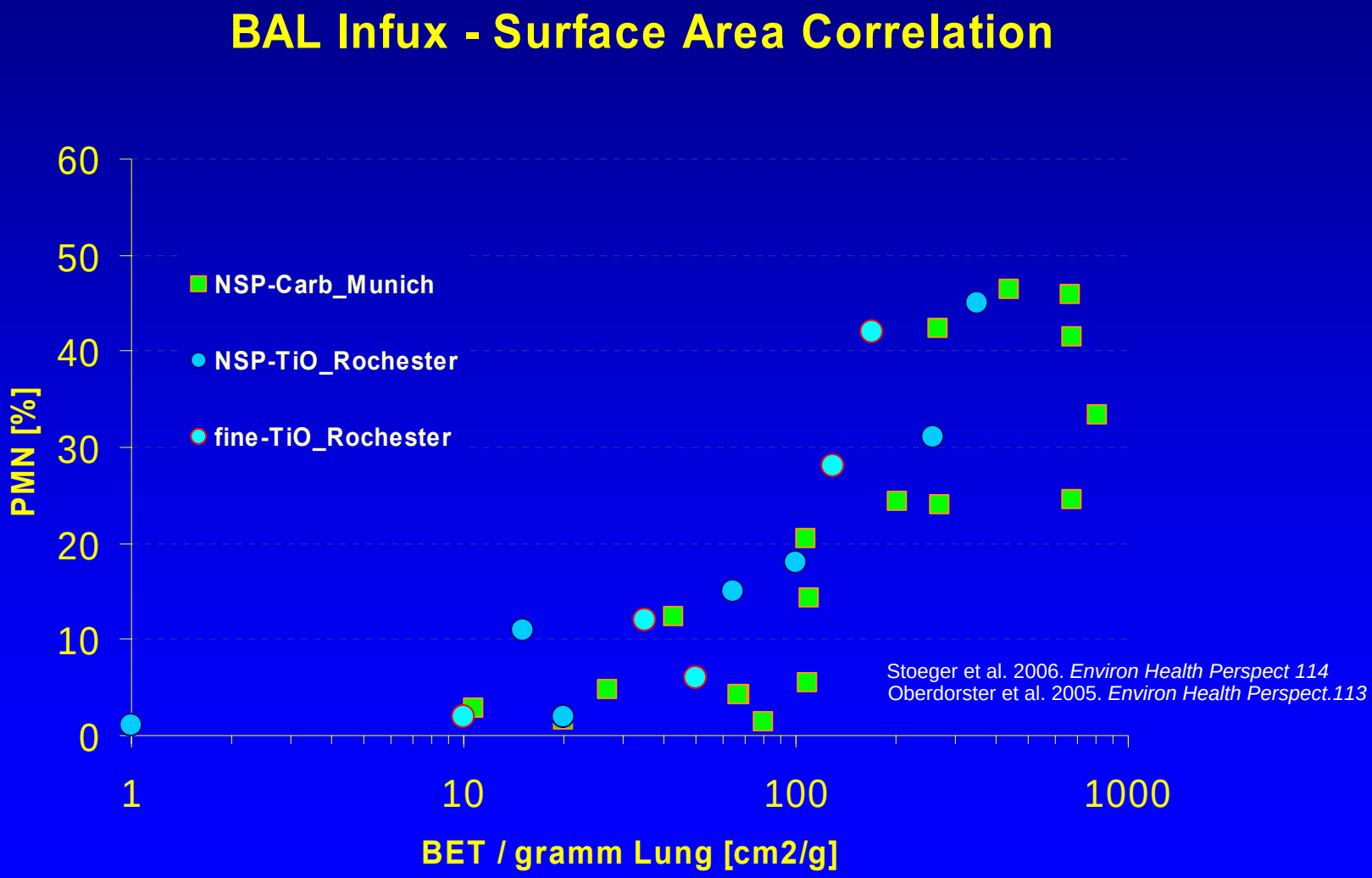
For the poorly soluble nanoparticles the deposited

particle surface area

serves as a suitable dose metric!

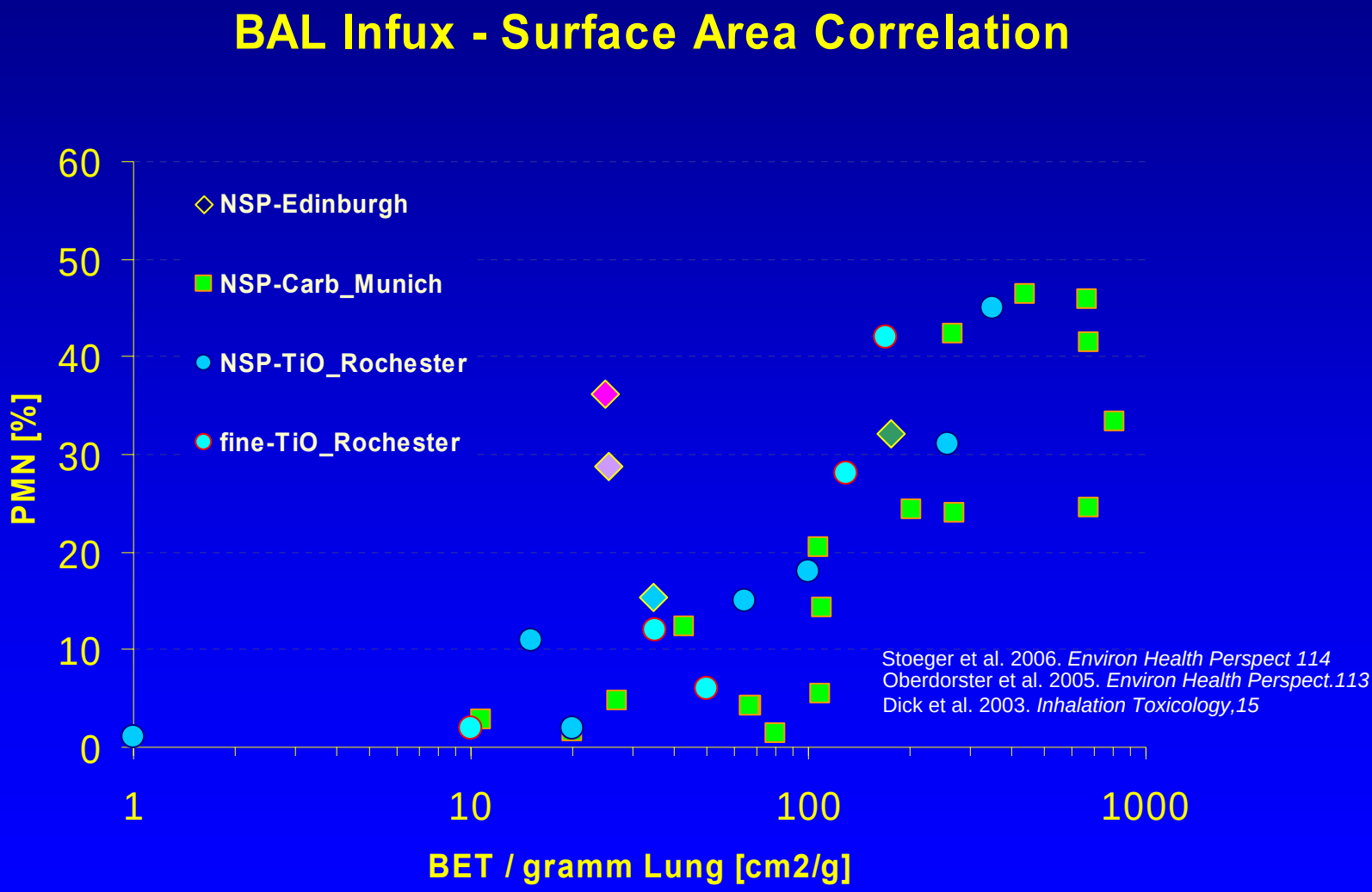
Stoeger 2006, Environ Health Perspect 114:328

Influence of Surface Reactivity



Stoeger et al. 2006. *Environ Health Perspect 114*
Oberdorster et al. 2005. *Environ Health Perspect.113*

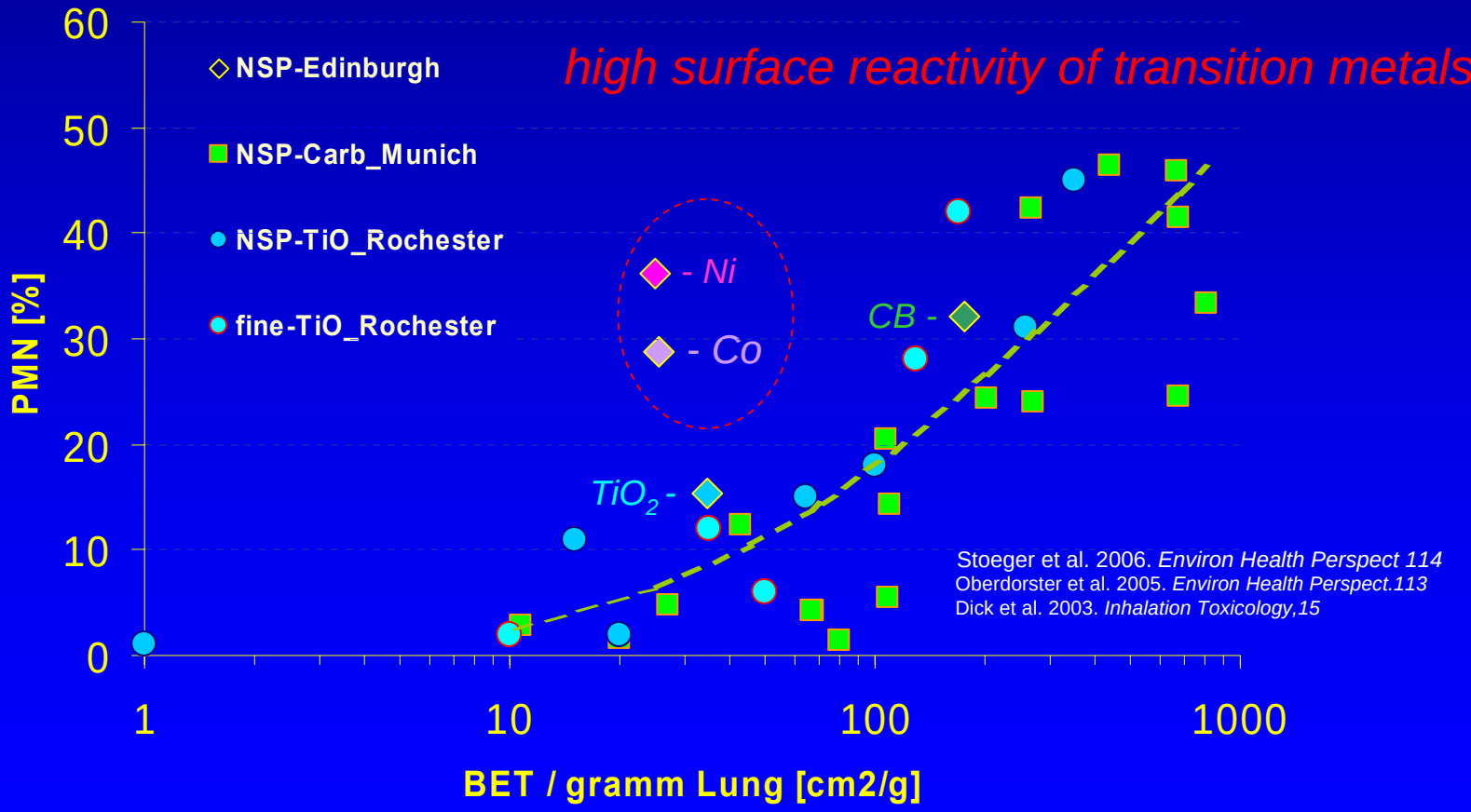
Influence of Surface Reactivity



Stoeger et al. 2006. *Environ Health Perspect 114*
Oberdorster et al. 2005. *Environ Health Perspect.113*
Dick et al. 2003. *Inhalation Toxicology,15*

Influence of Surface Reactivity

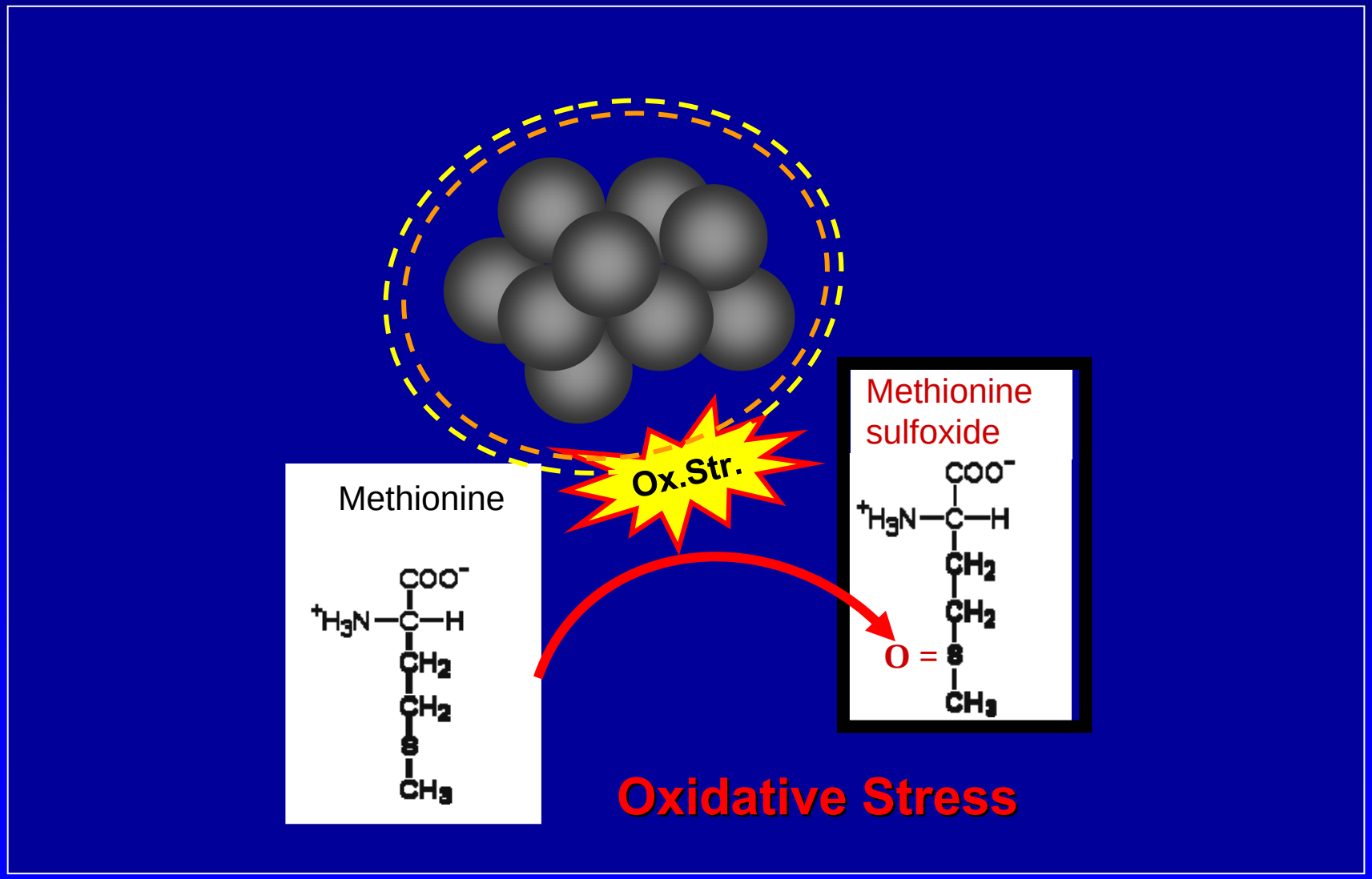
BAL Influx - Surface Area Correlation



high surface reactivity of transition metals

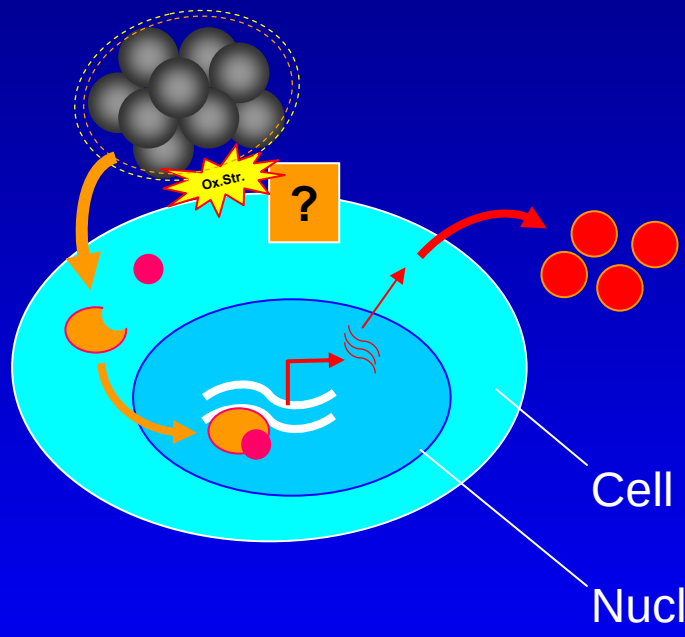
Stoeger et al. 2006. *Environ Health Perspect 114*
Oberdorster et al. 2005. *Environ Health Perspect.113*
Dick et al. 2003. *Inhalation Toxicology,15*

Oxidative Surface Reactivity of Carbon-Nanoparticles



Hypothesis of Particle Induced Inflammation

(Particle – Cell Interaction)



Proinflammatory mediators:
Chemokines/Cytokines,
Prostaglandins …

particle deposition on cell surface → induction of oxidative stress → signalling Cascade (NFkB/AP1) → proinflammatory gene expression → release of proinflammatory mediators

Gene Expression Analysis at "threshold level":
Inhalation of Carbon Nanoparticles Induces Proinflammatory Response

TABLE 1
Changes in gene expression after inhalation of ultrafine particles

Identifier	Name/Description	Gene symbol	4h inhalation	24h inhalation
			Fold Induction	
M12571	Heat shock protein, 1A	Hsp1a	**4**	1,2
L40406	Heat shock protein, 105 kDa	Hsp105	**2,6**	1,1
AF101164	CEA-related cell adhesion molecule 2	Ceacam2	**2,2**	1,3
AW124318	suppression of tumorigenicity 13	St13	**2,2**	1
U27830	stress-induced phosphoprotein 1	Stip1	**2**	1,2
U16162	prolyl 4-hydroxylase alpha(I)-subunit	P4ha1	**2**	1,1
U2392	osmotic stress protein 94	Osp94	**2**	0,9
X03505	Serum amyloid A 3	Saa3	1,1	**5,4**
X69620	Inhibin beta-B	Inhbb	2,5[a]	**3,8**
X13986	osteopontin, secreted phosphoprotein	Spp1	1,1	**3,4**
M34141	Prostaglandin-endoperoxide synthase 1	Ptgs1	1	**3**
AW230891	leucine-rich alpha-2-glycoprotein 1	Lrg1	1,4	**2,8**
V00755	TIMP-1	Timp1	1,3	**2,4**
M64086	spi2 proteinase inhibitor (spi2/eb4)	Serpina3n	1,1	**2,4**
M62470	Thrombospondin 1	Thbs1	3,0[b]	**2,4**
X81627	24p3, lipocalin2	Lcn2	1	**2,4**
L41352	Amphiregulin	Areg	1,2	**2,4**
AV300608	SH2 domain binding protein 1	Sh2bp1	0,8	**2,2**
M26071	Coagulation factor III	F3	1,1	**2,2**
AF023919	PK-120 precursor	itih-4	0,9	**2,2**
M15131	Interleukin 1 beta	Il1b	0,9	**2**
M36120	Keratin complex 1, acidic, gene 19	Krt1-19	1	**2**
M35970	expressed in non-metastatic cells1	Nme1	0,8	**2**
X16834	Galectin-3, Mac-2	Lgals3	1	**2**
			Fold Repression	
K02588	Cytochrome P450,1a1	Cyp1a1	1,2	**2,6**
X58289	Protein tyrosine phosphatase, receptor typeB	Ptprb	1,6	**2,4**
Y07693	Nuclear factor I/C	Nfic	1,2	**2,4**
D38216	RyR1 skeletal muscle ryanodine receptor	Ryr1	1	**2,2**
X84037	E-selectin ligand-1, golgi apparatus protein 1	Glg1	1,4	**2**
AI152867	eukaryotic translation initiation factor 2C	Eif2c2	1,6	**2**

Numbers in bold indicate genes induced or repressed twofold or more after 4h or 24h ufCB particle inhalation
[a] excluded from analysis because absent in test sample (see methods)
[b] excluded from analysis because fold change between clean air controls (4h and 24h) twofold or more

4h

24 h

Biphasic reaction:

1. Stress response:
Heat shock proteins (*hsp70*)

2. Proinflammatory response:
- *Interleukin 1 beta*
- *Cyclooxygenase 1*
- *Serum amyloid A3*
- *Osteopontin*
- *TIMP 1*
- *Lipocalin 2*
- *Galectin 3*
- *Amphiregulin*
- *Coagulation factor III*
- *Thrombospondin 1*
- *…*

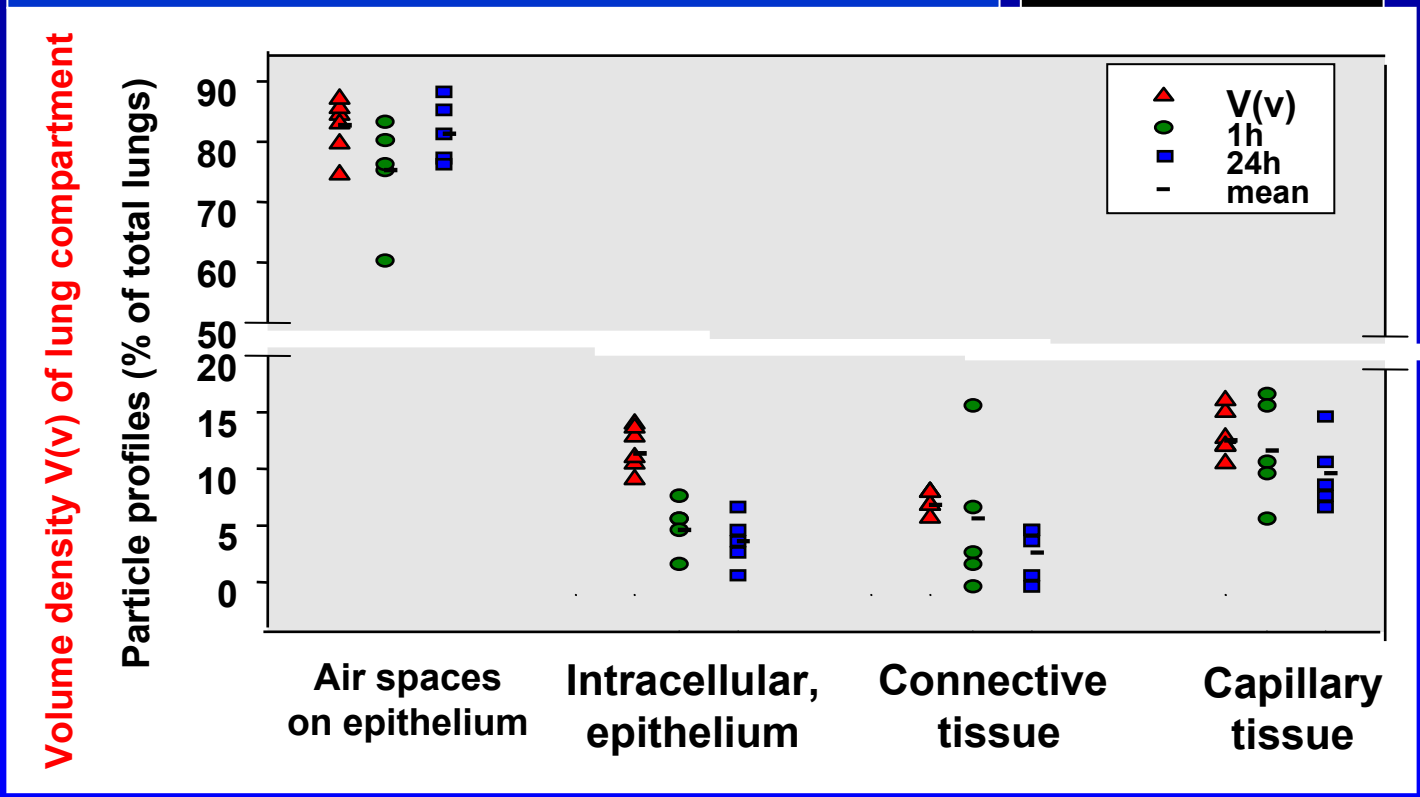
Andre and Stoeger 2006, *Eur Respir J.*

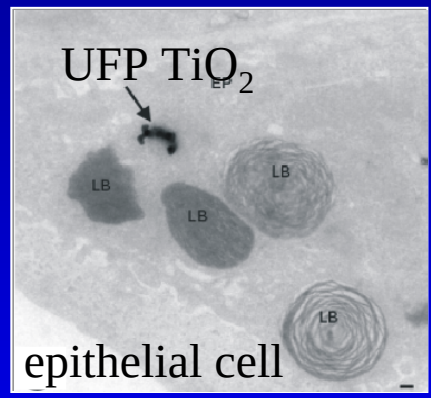
Translocation to Secondary Organs

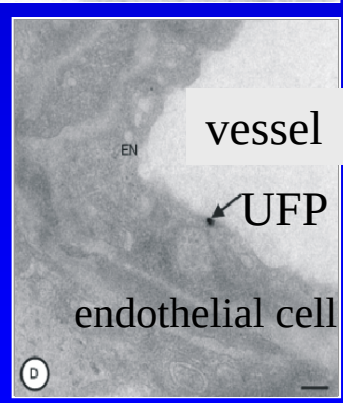
Do inhaled nanoparticles penetrate the tissue of the lung?

Experimental design

- Intubated ventilated WKY rat,
- TiO_2 NP, 1 hr exposure
- 22 nm CMD, 10^7 cm^{-3}, 0.1 mg/m^3
- Lung morphometry 1 & 24 h after inhalation







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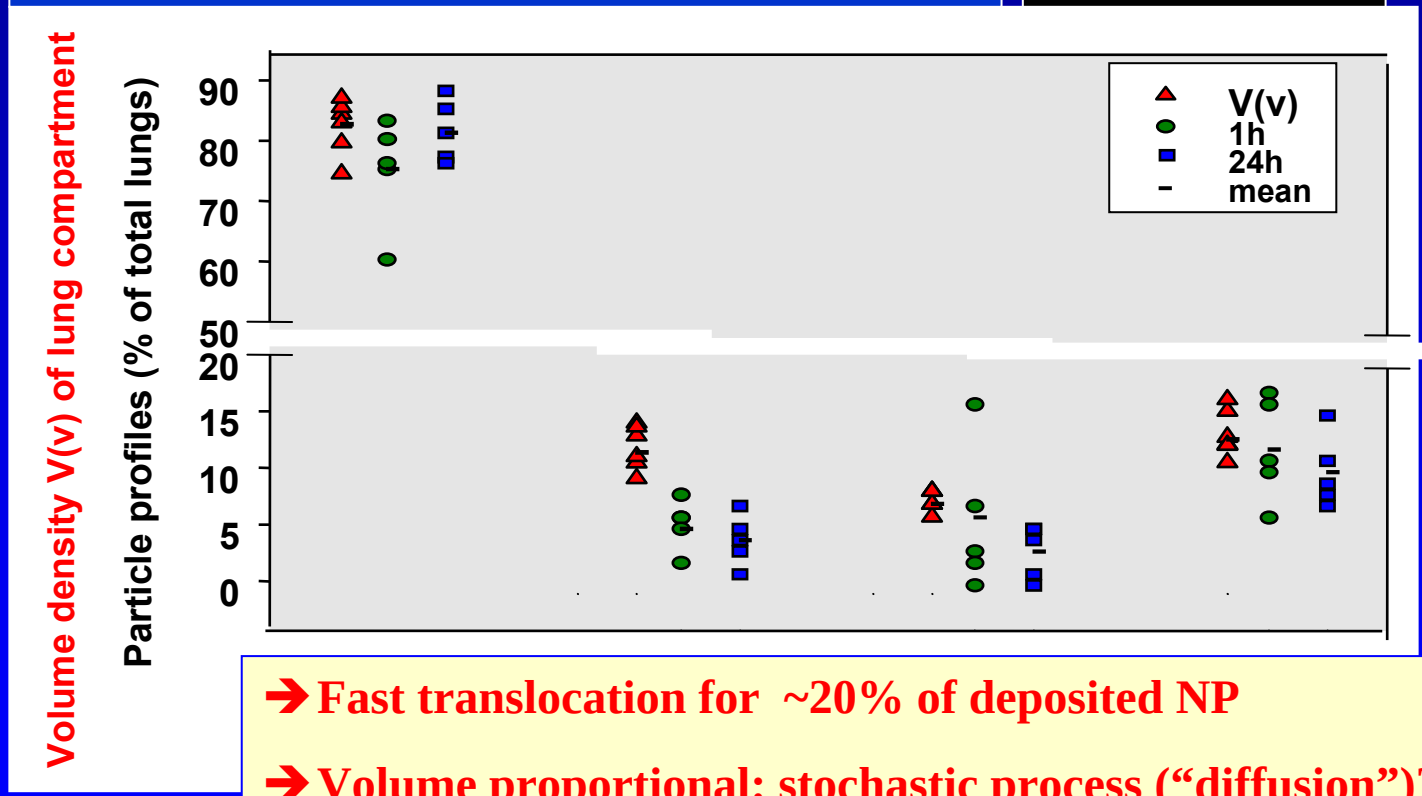
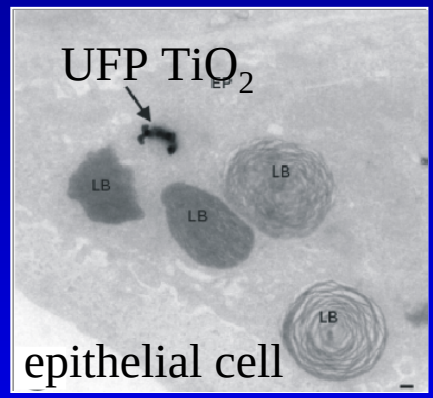
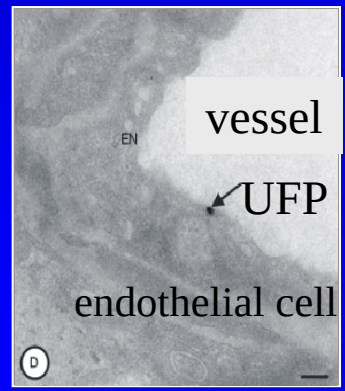






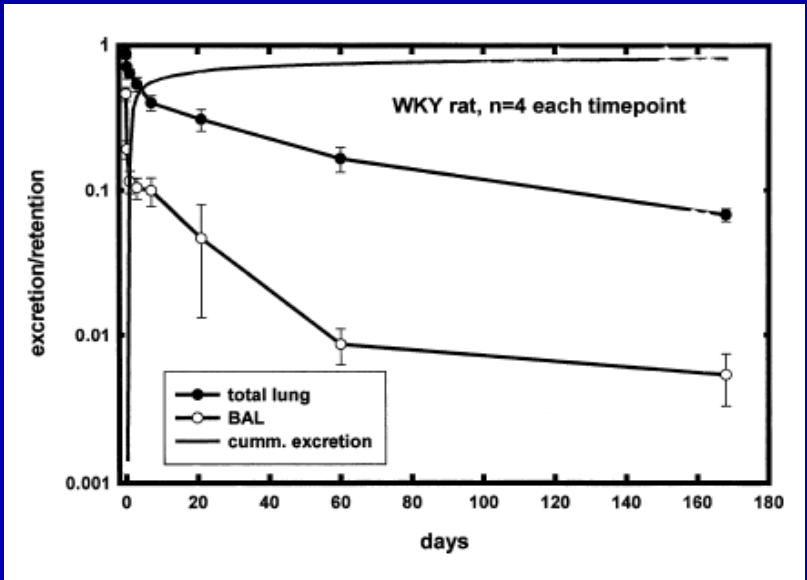
➔ **Fast translocation for ~20% of deposited NP**

➔ **Volume proportional: stochastic process ("diffusion")?**

Translocation into secondary target organs

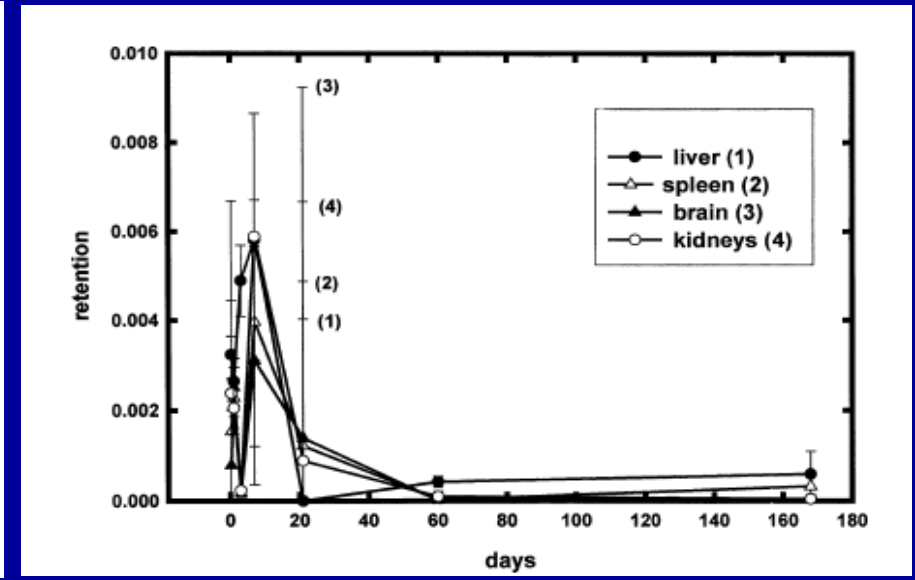
WKY Rats exposed via endotracheal intubation, single 1- to 1.5-h inhalation of 192Ir UFP, mass conc.: 0.2 mg m^{-3}, CMD: 15 nm

Lung



Pulmonary retention and excretion during 6 mo after inhalation of ^{192}IrUFP.

Extra Pulmonary Organs



Translocated fractions into secondary target organs during 6 mo after inhalation of ^{192}IrUFP.

Translocation into secondary target organs

WKY Rats exposed via endotracheal intubation, single 1- to 1.5-h inhalation of 192Ir UFP, mass conc.: 0.2 mg m^{-3}, CMD: 15 nm

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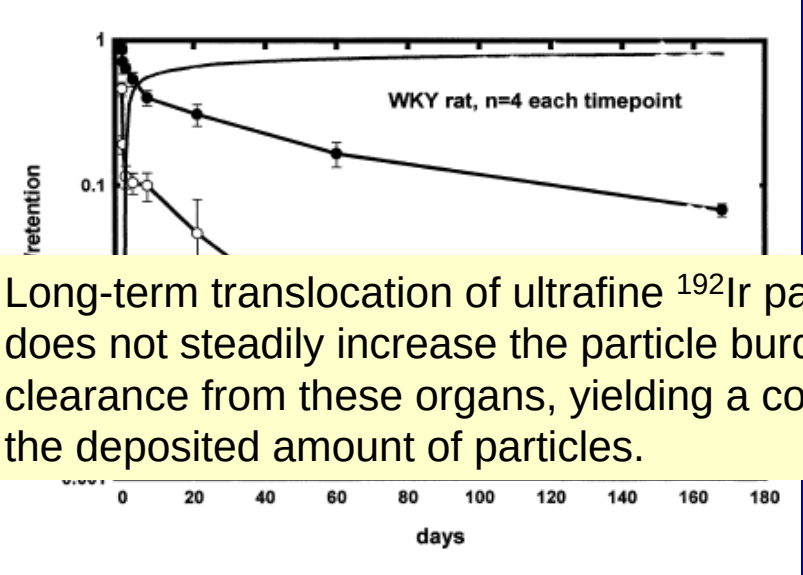

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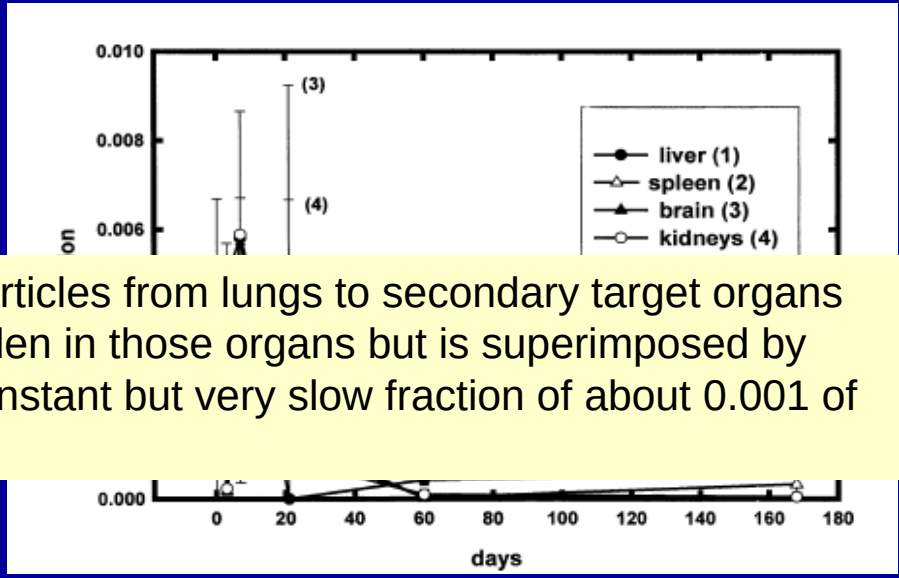

Translocated fractions into secondary target organs during 6 mo after inhalation of ^{192}IrUFP.

Long-term translocation of ultrafine ^{192}Ir particles from lungs to secondary target organs does not steadily increase the particle burden in those organs but is superimposed by clearance from these organs, yielding a constant but very slow fraction of about 0.001 of the deposited amount of particles.

Extra-pulmonary Effects

Which kind of extra-pulmonary PM- effects have been described?

Cardio Vascular System (CVS)

- Disturbance of **vegetative** balance – stress response
- Disturbance of vasomotor function – **vasoconstriction**
- Cardiac **Arrhythmia**
- Systemic/endothelial **inflammation**
- **Procoagulative** state
- Endothelial dysfunktion
- Aggravation of **atherosclerotic** process

=> Infarction – Coronary failure

Central Nervous System (CNS)

- Inflammation / neurodegeneration (activation of microglia cells)

ApoE-Modell + Mexico City Daten

Which kind of extrapulmonary PM- effects have been described?

Cardio Vascular System (CVS)

- Disturbance of **vegetative** balance – stress response

Harder 2005:

Rats exposed to UfCP (180µg/m^3) for 24h

⇒ increased heart rate

⇒ reduced heart rate variability

sympathetic stress response ?

=> Infarction – Coronary failure

Central Nervous System (CNS)

- Inflammation / neurodegeneration (activation of microglia cells)

ApoE-Modell + Mexico City Daten

Which kind of extrapulmonary PM- effects have been described?

Cardio Vascular System (CVS)

- Disturbance of **vegetative** balance – stress response

- Disturbance of vasomotor function – **vasoconstriction**

Harder 2005: *(Telemetric study)*
Hypertensive rats exposed to UfCP (180µg/m^3) for 24h
⇒ increased heart rate
⇒ increased diastolic blood pressure

Elder 2004:
On-Road Exposure of Aged Rats
⇒ Increased plasma levels of Endothelin

Dvonch 2004:
Rats exposed to $PM_{2.5}$ (Detroit, 354µg/m^3, 3 days, 8h/day)
⇒ Increased plasma levels of ADMA
(asymmetric dimethylarginie, eNOS inhibitor)

! vasoconstriction, endothelial dysfunktion !

oglia cells)

ApoE-Modell + Mexico City Daten

Which kind of extrapulmonary PM- effects have been described?

Cardio Vascular System (CVS)

- Disturbance of **vegetative** balance – stress response
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- Cardiac **Arrhythmia**
- Systemic/endothelial **inflammation**
- **Procoagulative** state

Khandoga 2004:
intra-arterial infusion of UfCP in healthy mice (10^7/ mouse)
⇒ induced platelet adhesion
⇒ increased fibrin deposition and vWF expression on the endothelial surface

! **Prothrombotic effect !**

- Inflammation / neurodegeneration (activation of microglia cells)

ApoE-Modell + Mexico City Daten

Which kind of extrapulmonary PM- effects have been described?

Cardio Vascular System (CVS)

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- Disturbance of vasomotor function – **vasoconstriction**
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- Systemic/endothelial **inflammation**
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- Endothelial dysfunktion
- Aggravation of **atherosclerotic** process

Sun 2005:

ApoE$^{-/-}$ mice (+ high fat diet) exposed to 85 µg/m^3 $PM_{2.5}$ for 6 month

⇒ increased atherosclerotic plaque development
⇒ increased vascular inflammation
⇒ increased vasomotor tone

! potentiated atherosclerosis !

Which kind of extrapulmonary PM- effects have been described?

Cardio Vascular System (CVS)

- Disturbance of **vegetative** balance – stress response
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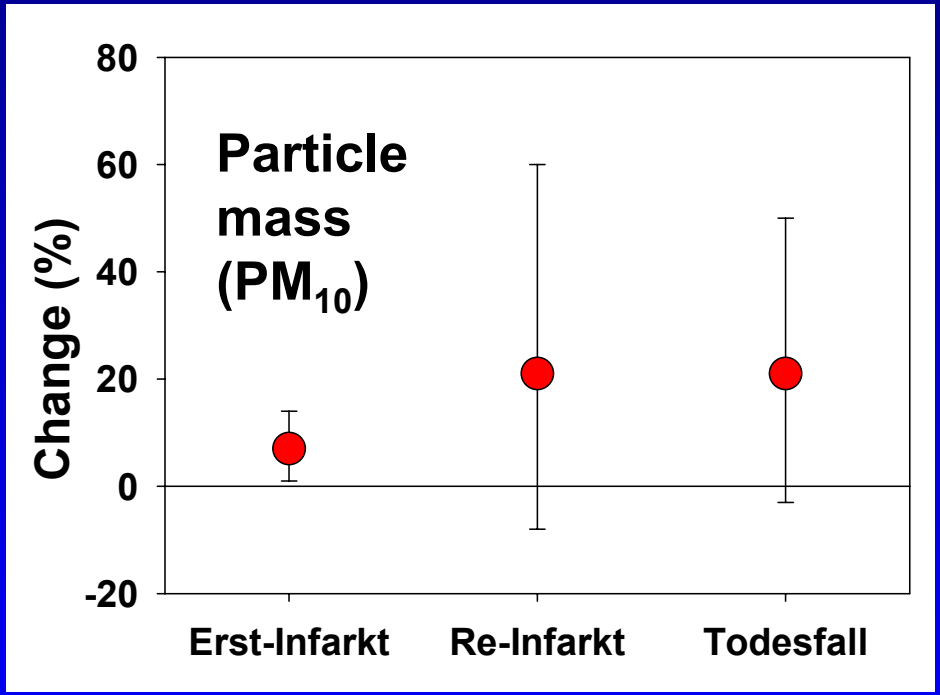
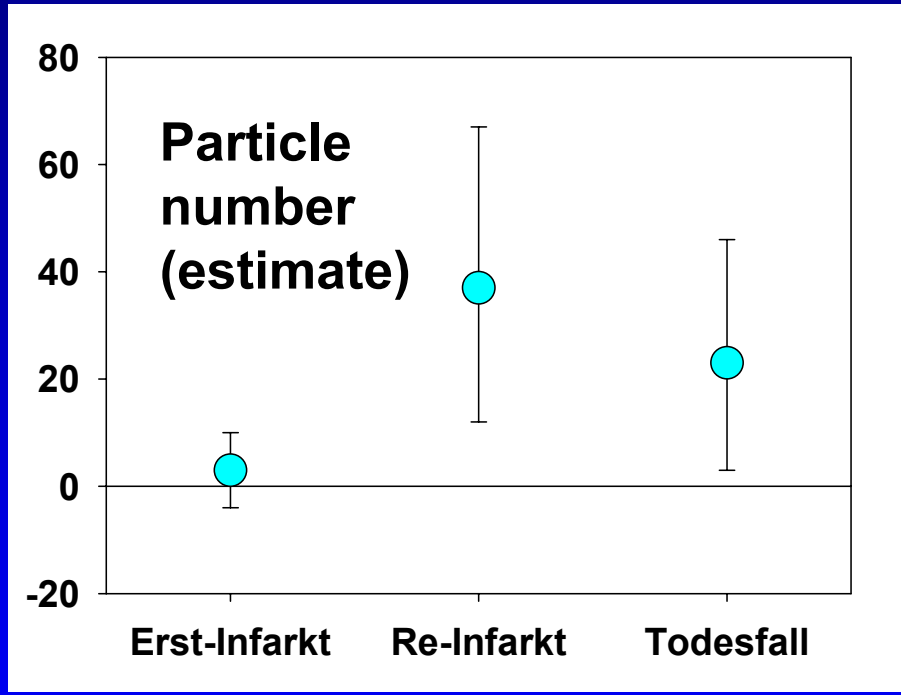
ApoE-Modell + Mexico City Daten

Epidemiological data

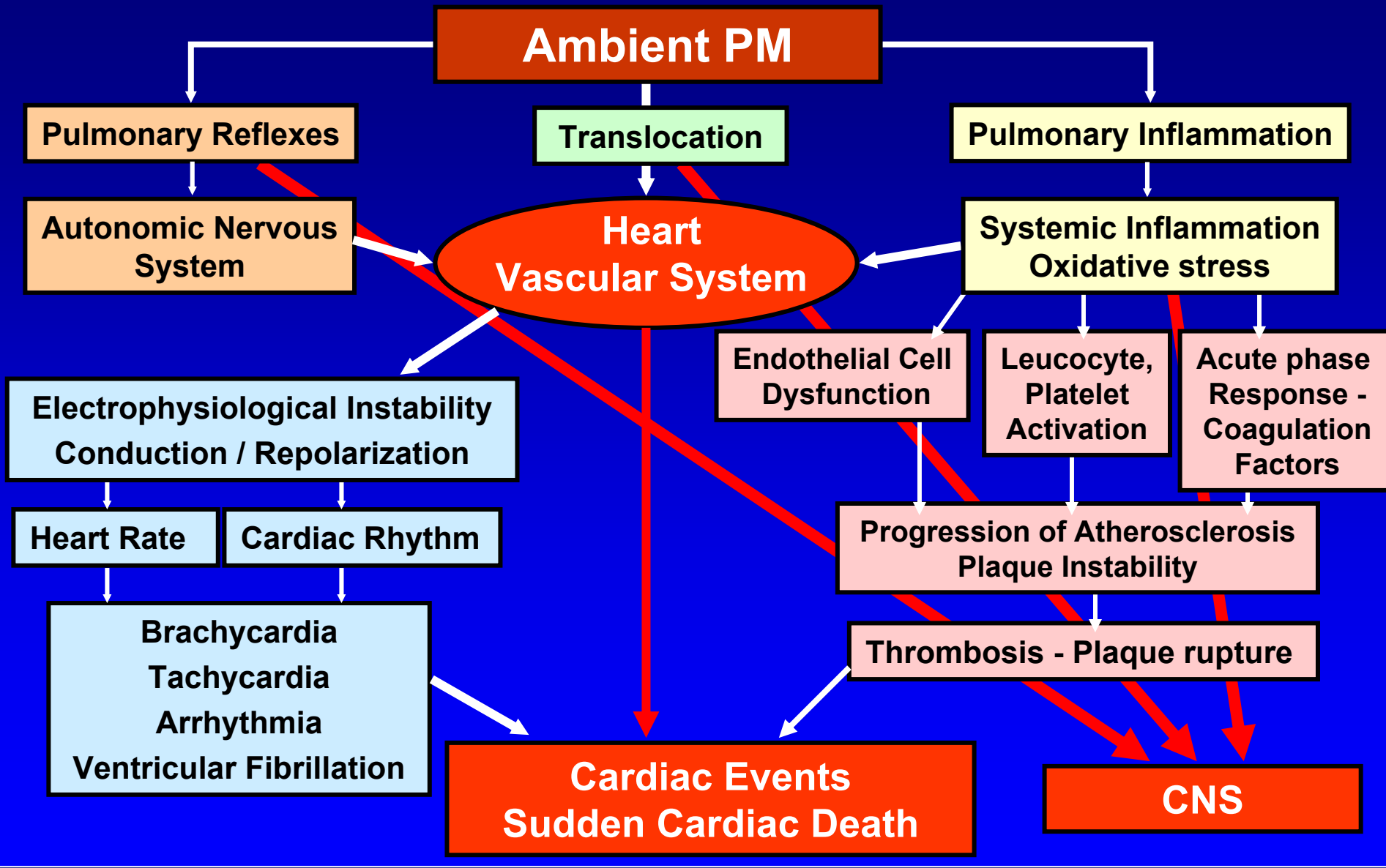
PM and frequency in heart attacks (Augsburg 1995-2000)





Association of particle mass - or number-concentration with infarct rate

Mechanism of cardiovascular effects



Mechanistic Aspects of Nanoparticle Toxicology - Particle Deposition and Clearance

1. *Deposition & Clearance Mechanism*
   - *high alveolar deposition*
   - *ineffective clearance*

2. *Acute Pulmonary Effects*
   - *surface area as dose metric*
   - *NP cause oxidative stress to cellular structures*

3. *Translocation to Secondary Organs*
   - *translocation efficiency ~0.1%*

4. *Extra-pulmonary Effects*
   - *aggravation of inflammatory & cardiovascular diseases*

Mechanistic Aspects of Nanoparticle Toxicology - Particle Deposition and Clearance and Effects

Thank you for your attention!

And special thanks to:

Prof. Holger Schulz

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member of the Helmholtz Association

IHB-Institute for
Inhalation Biology