Nanotoxicology: an emerging discipline

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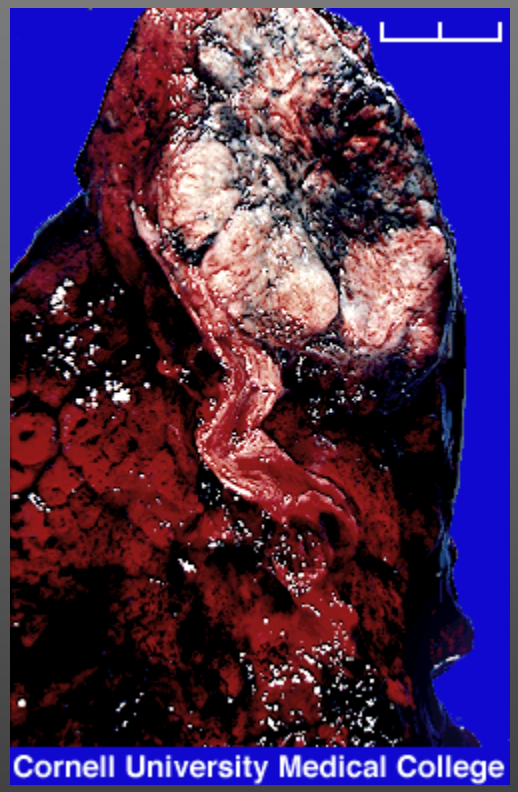
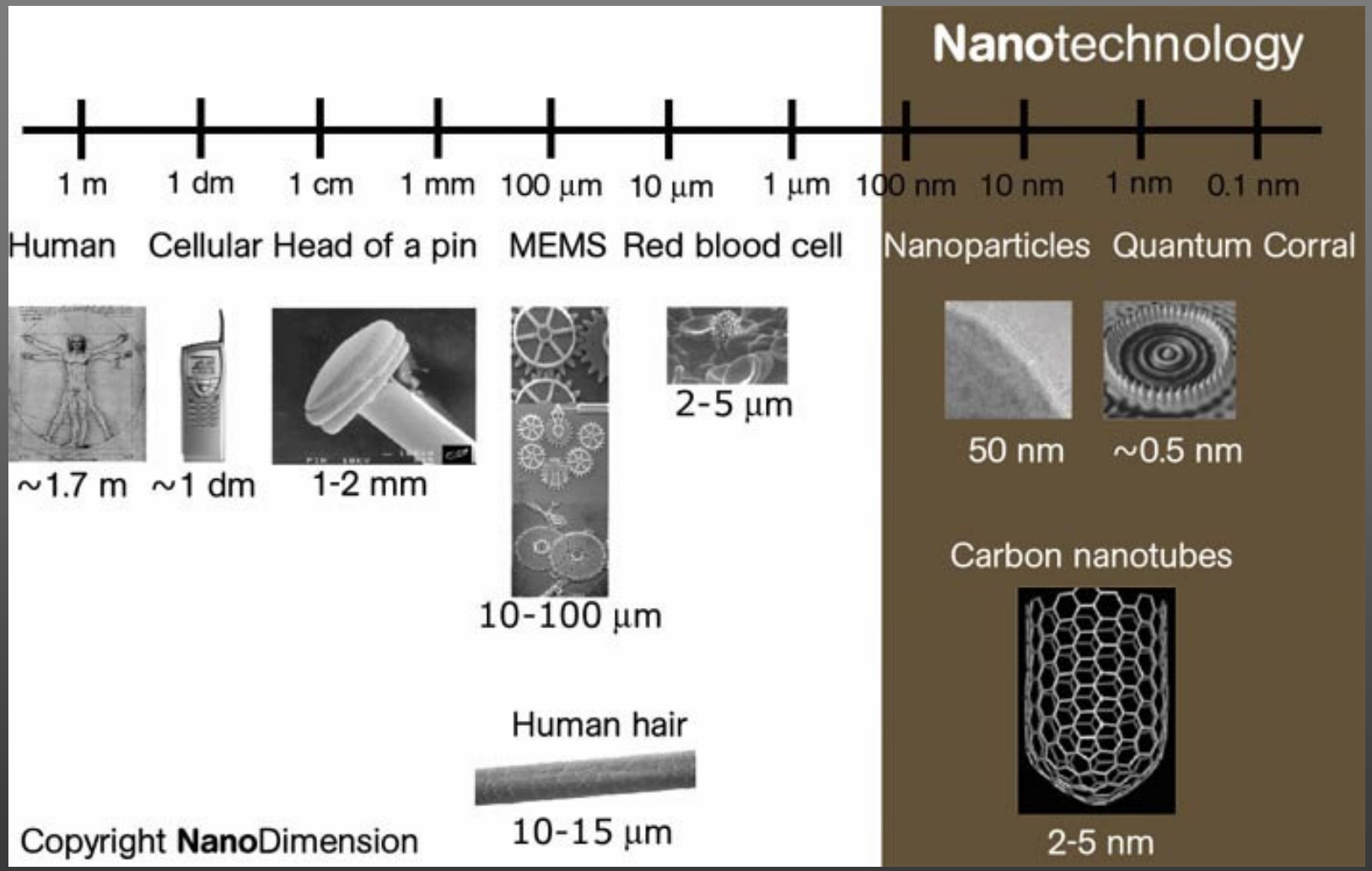
Particles cause lung disease



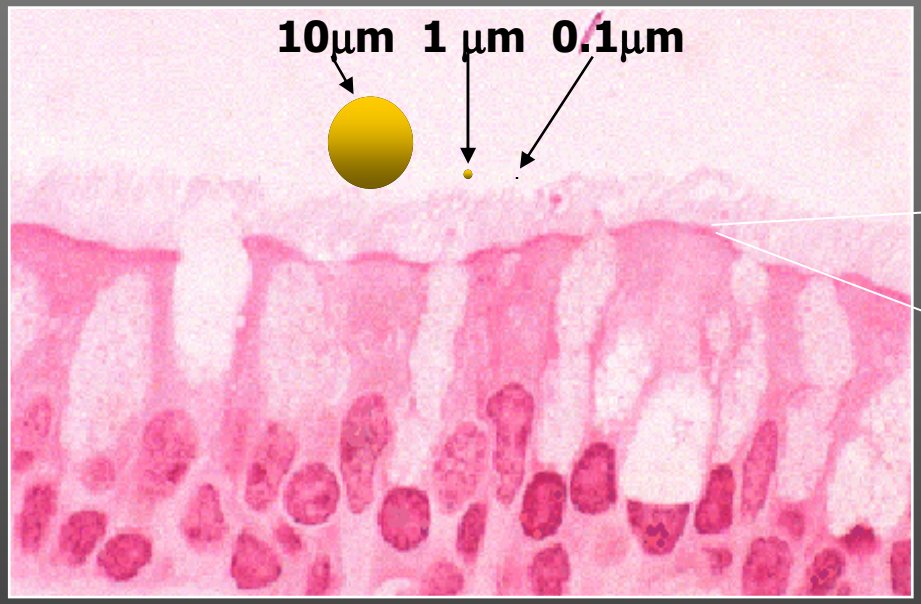
Nanoparticles :

the knowledge gap between 'accidental' versus 'engineered' nanoparticles

Nanoparticles	Source	Exposure	Toxicology
Accidental	from combustion Road vehicles Fossil fuel combustion cooking	Low exposure Everyone	+++++ e.g. diesel, ufCB, welding fume etc
Manufactured	bulk use of nanoparticles in industry e.g. carbon black Nanotechnology	High exposure Workers	++++ e.g uf carbon black, uf TiO2
Manufactured	Nanotechnology	High exposure Workers then Low exposure everyone	+/-


Nanotechnology
1 m
1 dm
1 cm
1 mm
100 µm
10 µm
1 µm
100 nm
10 nm
1 nm
0.1 nm
Human
Cellular
Head of a pin
MEMS
Red blood cell
Nanoparticles
Quantum Corral
~1.7 m
~1 dm
1-2 mm
2-5 µm
50 nm
~0.5 nm
10-100 µm
Carbon nanotubes
Human hair
10-15 µm
2-5 nm
Copyright NanoDimension

Nanoparticles and the lungs



Bronchial epithelium

Cilia 0.25μm diameter

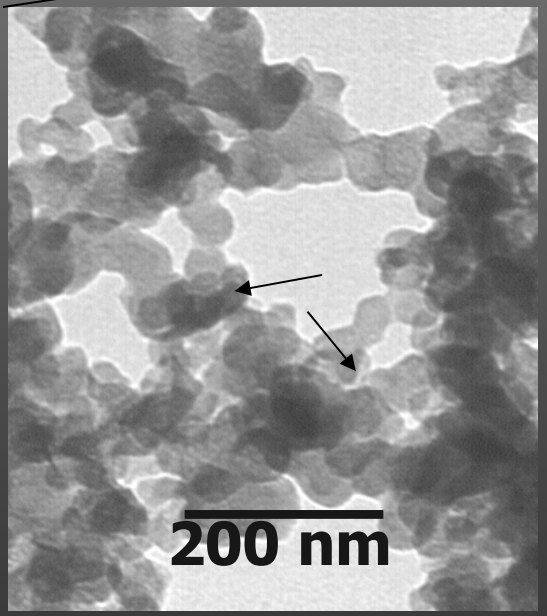
1.0 μm

0.1μm

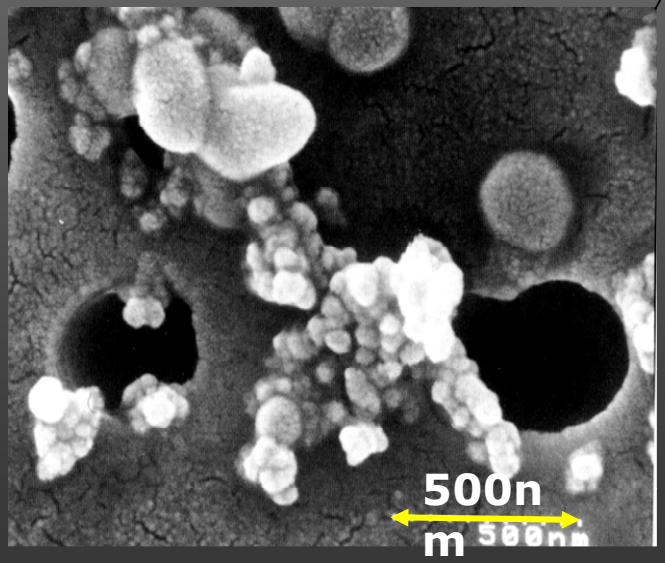
Traffic -derived nanoparticles

defined by particle toxicologists previously as ultrafine particles

singlet particles are <100nm diameter (0.1mm)



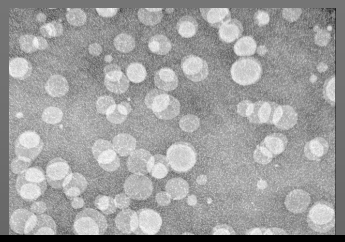
TEM image of Glasgow PM_{10} particles

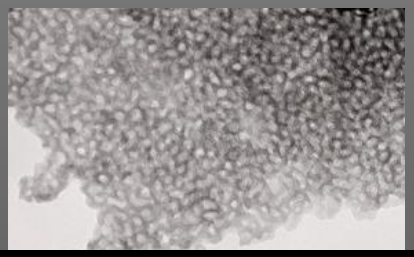


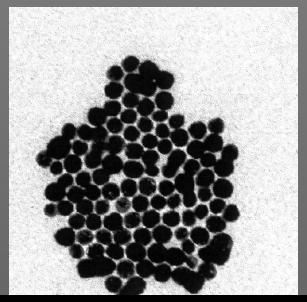
SEM image of Birmingham PM_{10} particle

Engineered nanoparticles

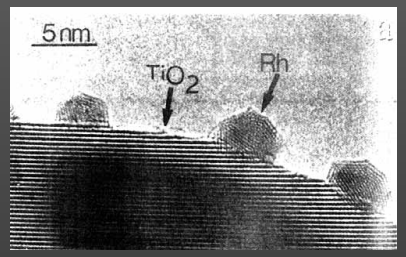
Designed for diverse uses within industry - variable compositions and surfaces

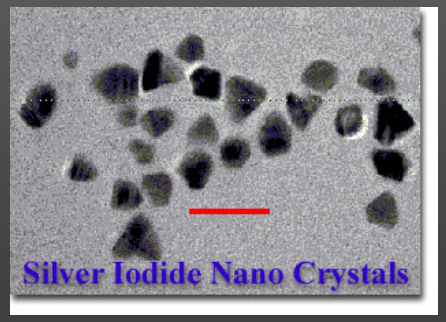


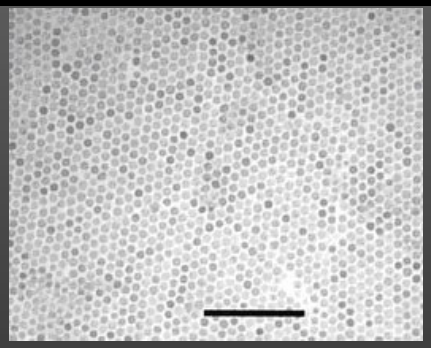




Uses in IT, food, textiles, paints , finishes, plastics, cosmetics, cleaners *etc, etc, etc*. i.e. everything







cobalt

What diseases might nanoparticles cause?

The PM10 experience

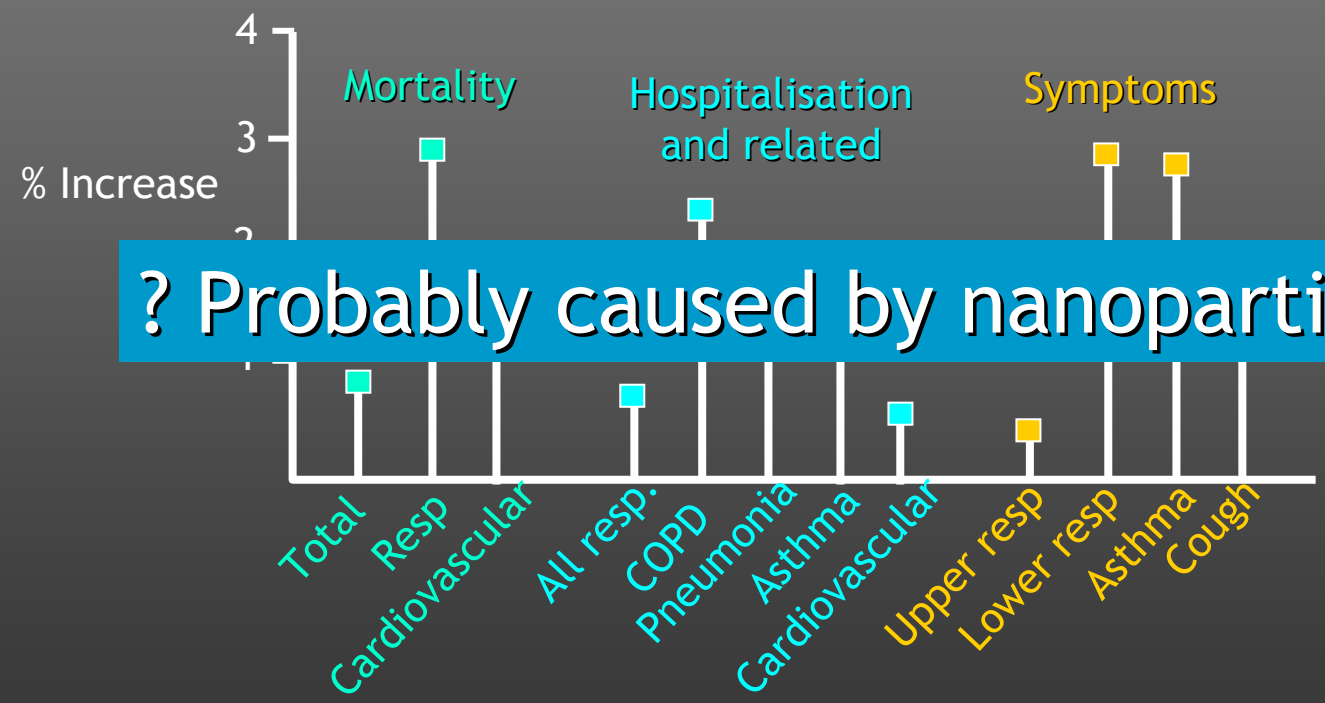
The animal and experimental studies

Bulk nanoparticles in industry : not properly studied for populations exposed to NP

HUMAN DISEASES: PM_{10}

Percentage change in any health end-point for a $10\mu g/m^3$ increase in PM_{10}

(summarised from over 100 studies)



? Probably caused by nanoparticles?

From Pope 2000

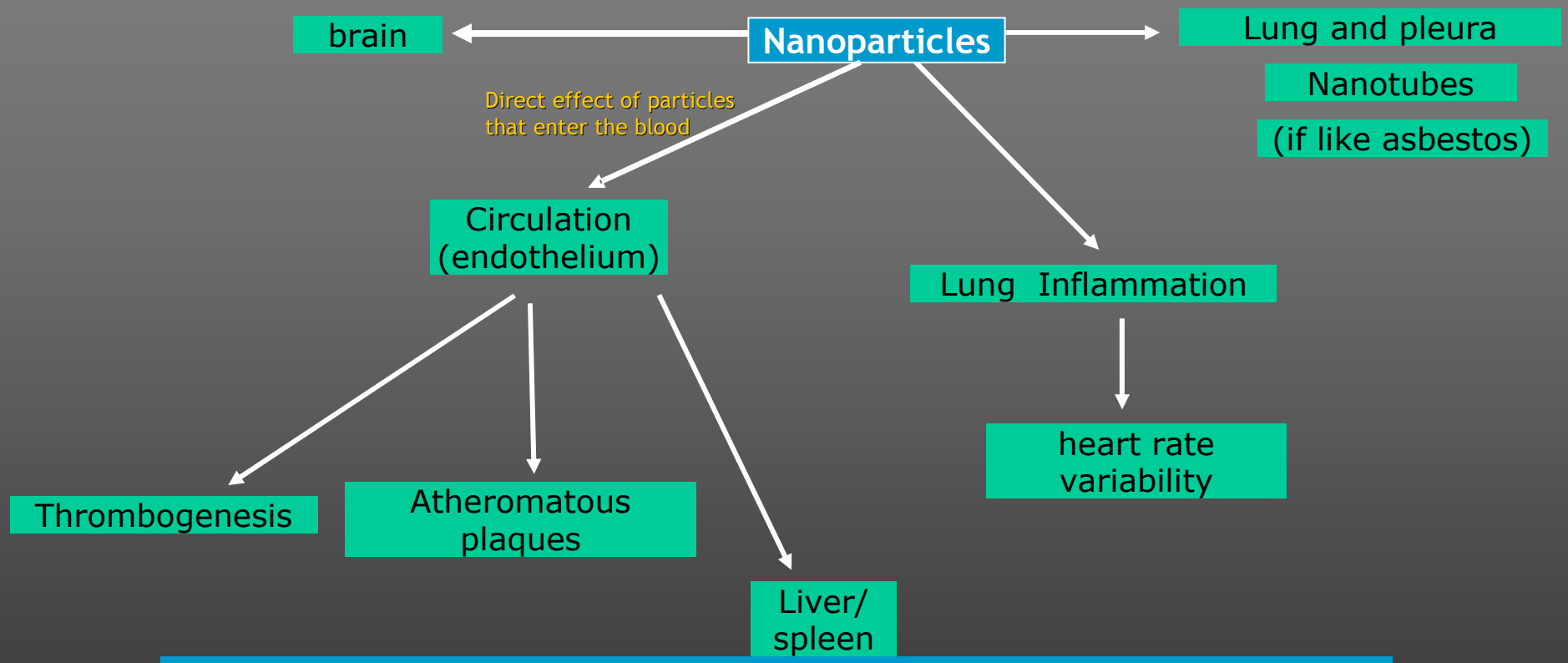
Chronic effects of air pollution Effect of living in a polluted city in the 6 cities study

Cause of death	% increase -most v least polluted	CI
All	1.28	1.1-1.48
Lung cancer	1.43	0.85-2.41
Cardiopulmonary	1.38	1.12-1.69
All others	1.01	0.79-1.3

? Probably caused by nanoparticles?

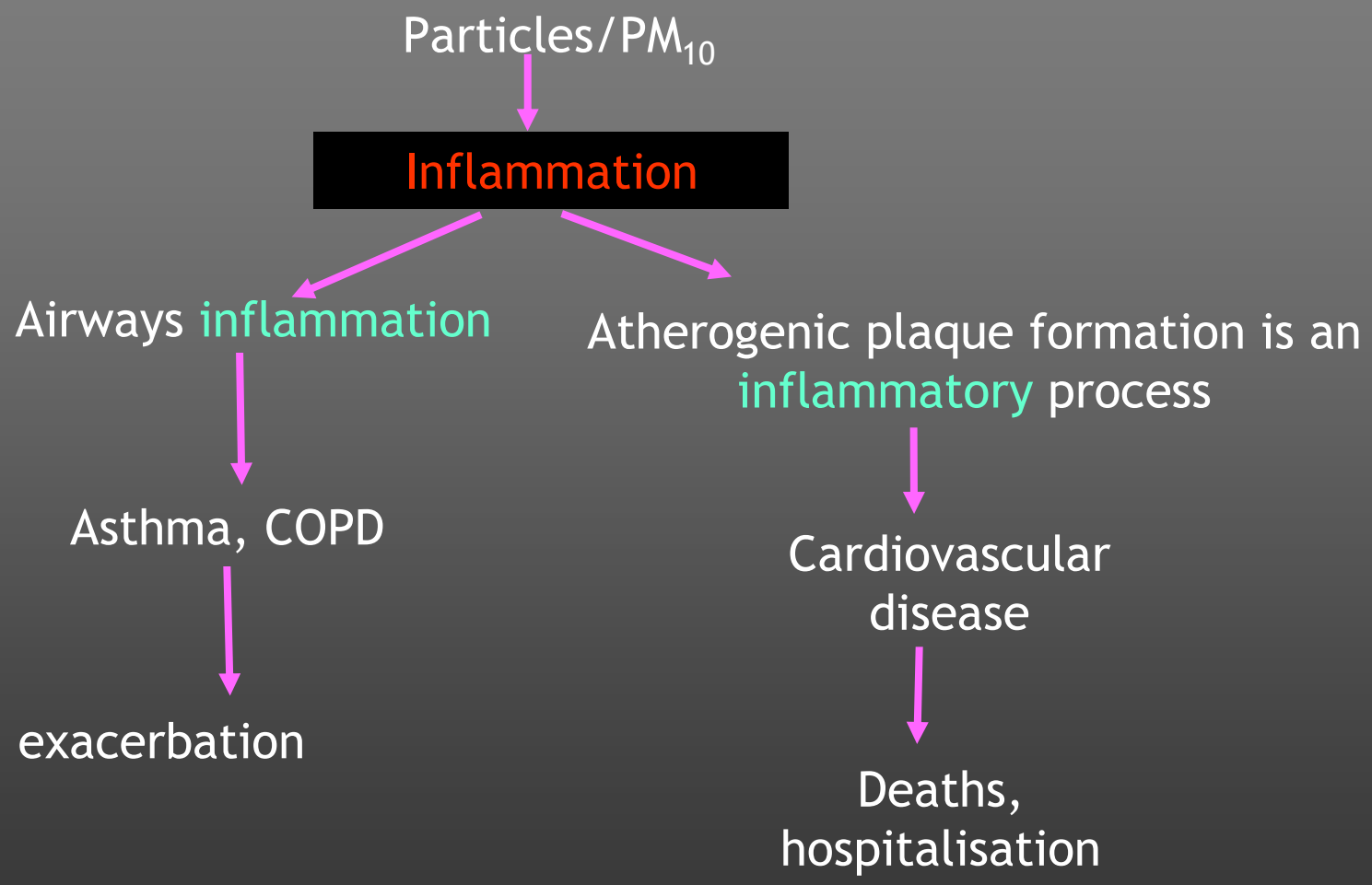
Harvard Six Cities reanalysis report

Animal studies with nanoparticles Evidence of translocation and adverse effects

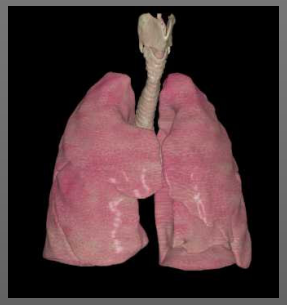


Definitely seen with some types of nanoparticles

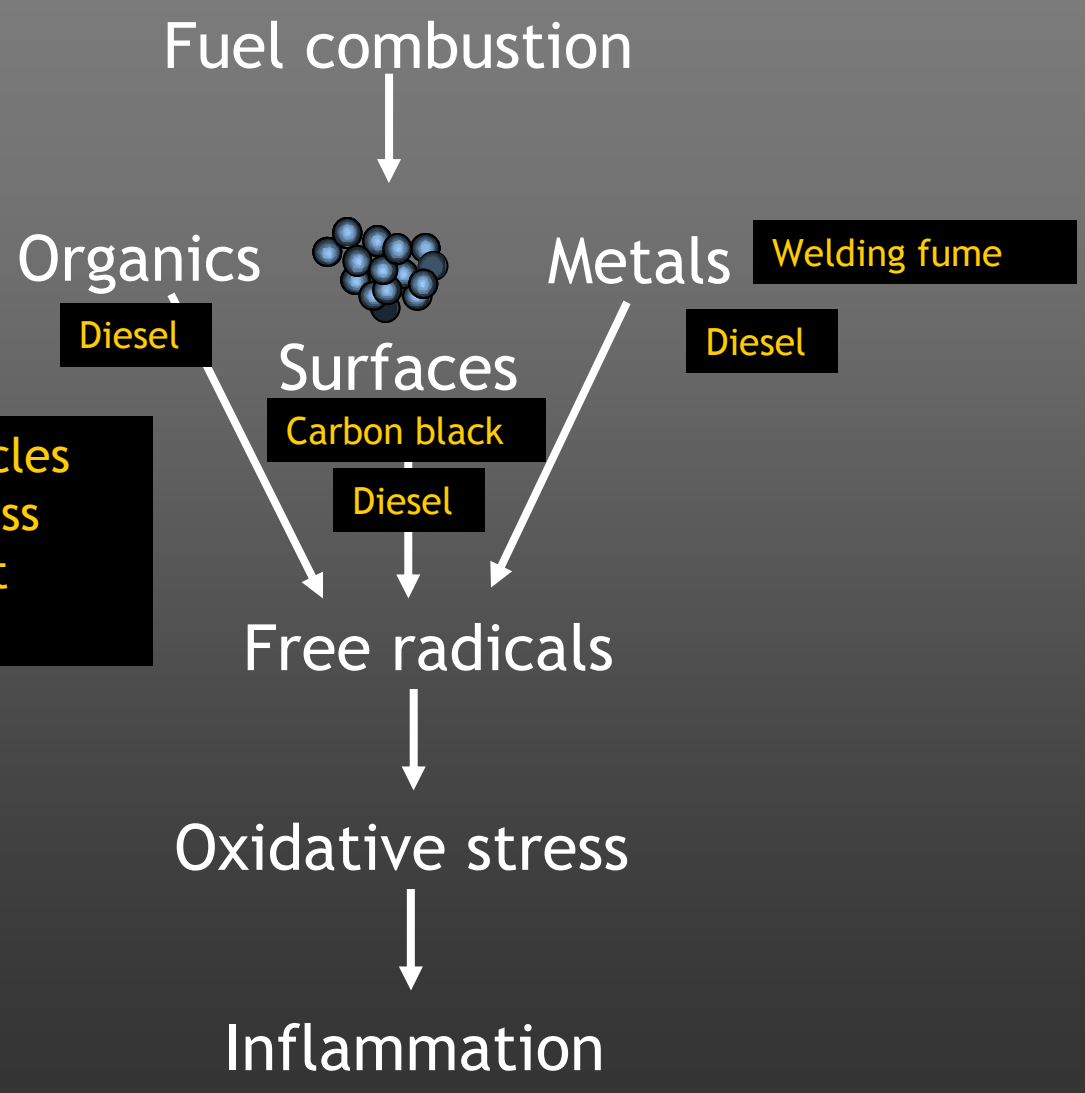
Role of inflammation in causing adverse effects of PM10 and nanoparticles



Nanoparticles in the lungs



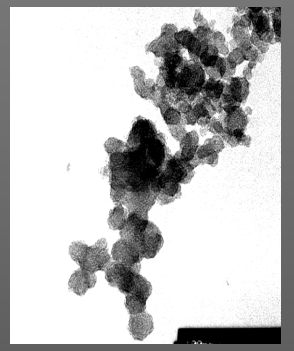
Mechanistic hypothesis linking nanoparticles and inflammation



Different nanoparticles cause oxidative stress because of different properties

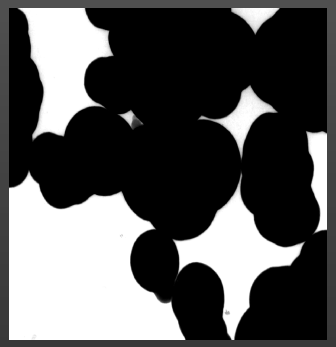
Oxidative activity of fine (Fine CB) and nanoparticle carbon black (NPCB) after 1000 seconds using Dichlorofluorescein

Nanoparticle

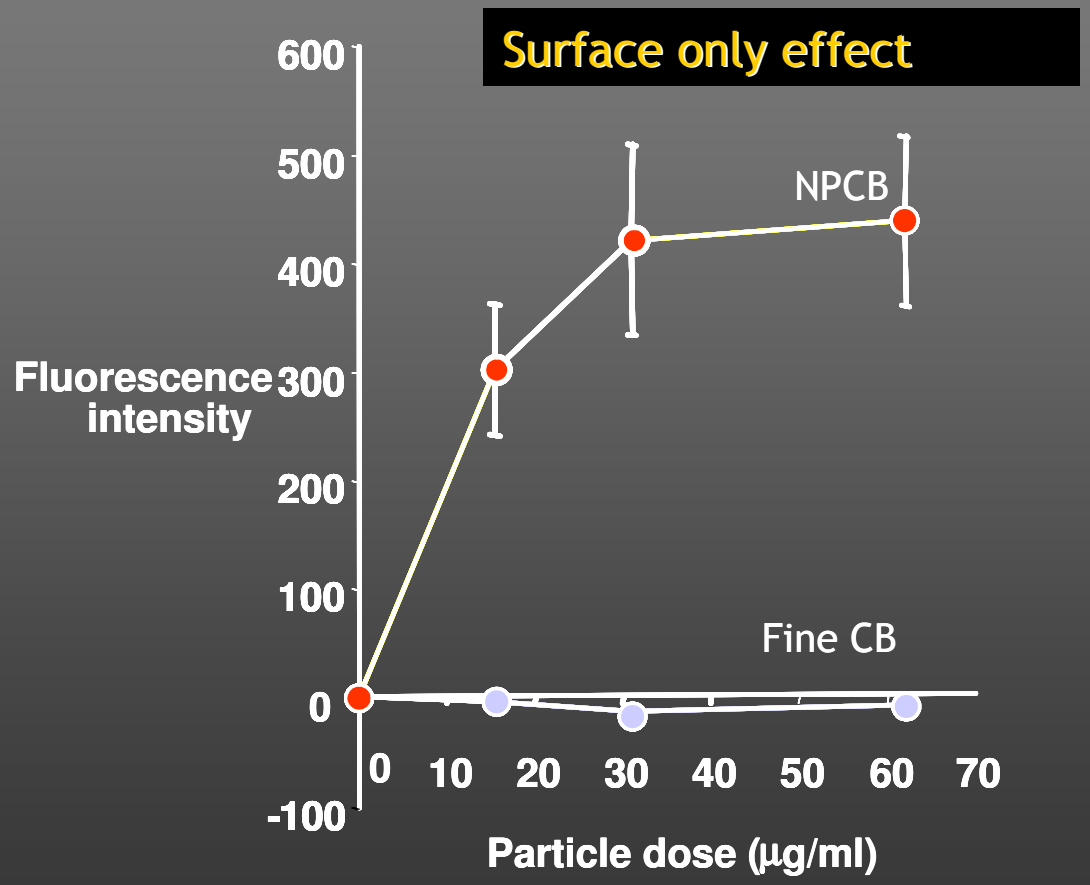


100nm

Fine

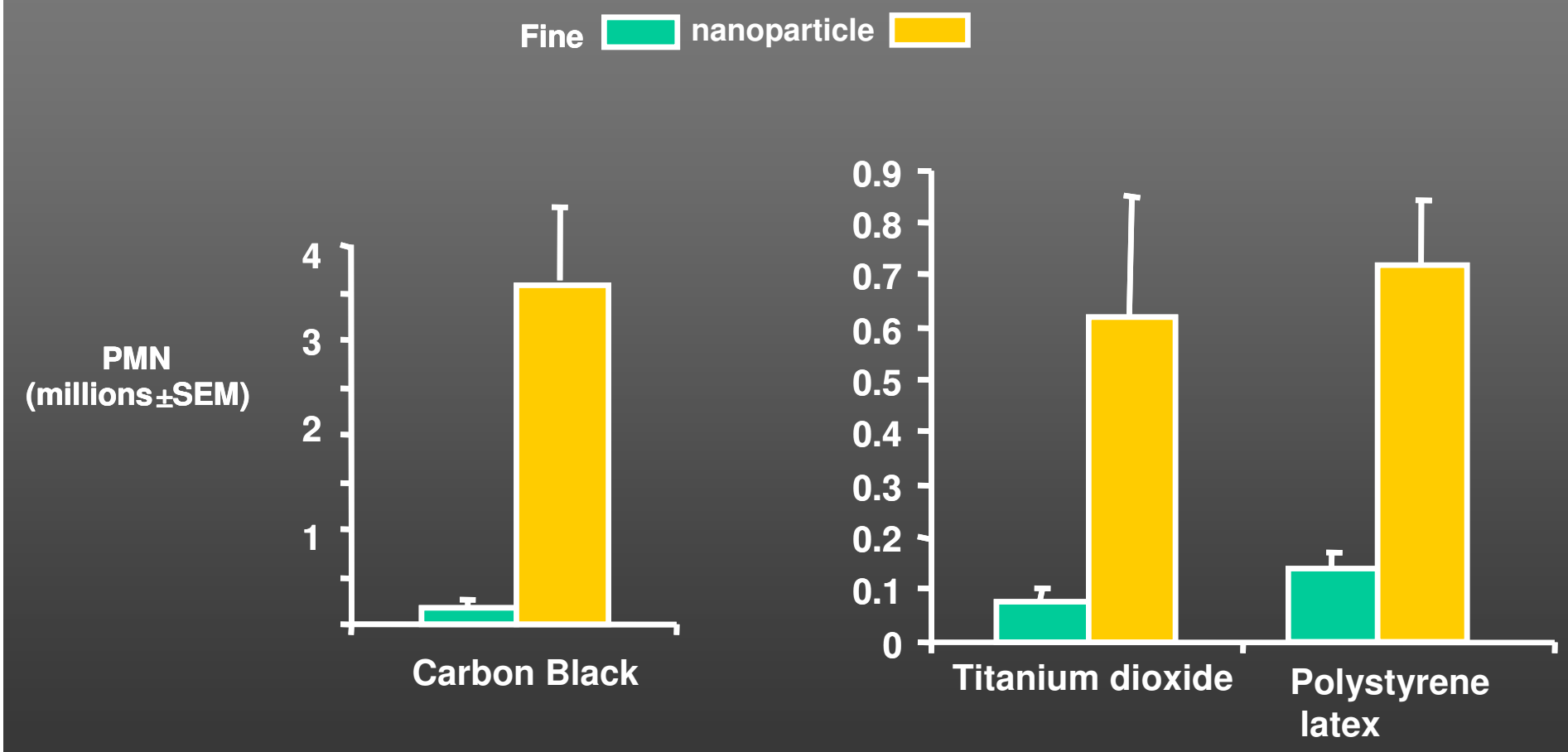


100nm



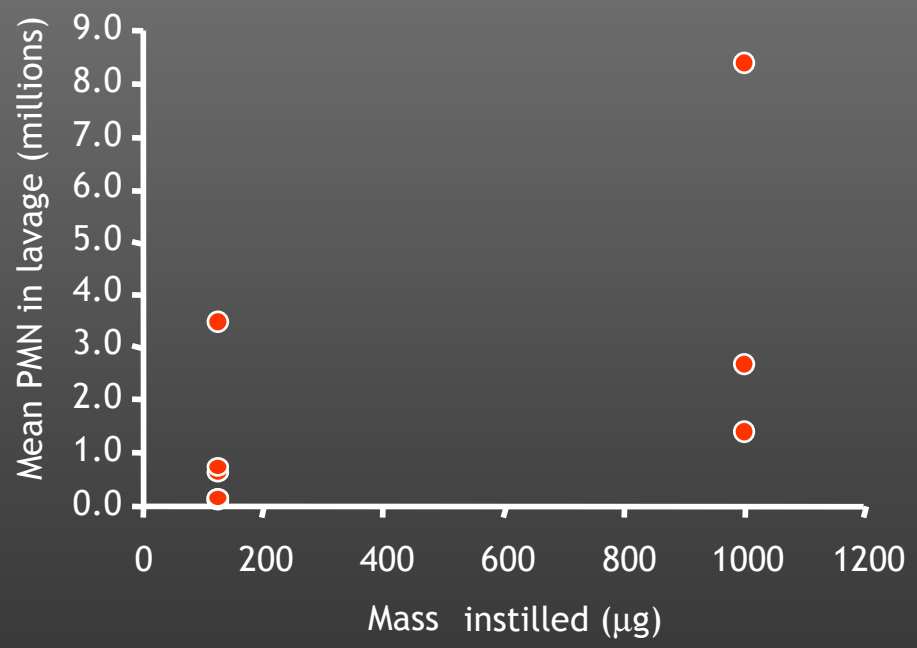
Wilson, M. R., J. H. Lightbody, K. Donaldson, J. Sales, and V. Stone. 2002. Interactions between ultrafine particles and transition metals in vivo and in vitro. *Toxicol.Appl.Pharmacol*. 184:172-179.

Nanoparticles cause more inflammation in BAL than the same mass of fine respirable particles composed of the same material; instillation of 125µg into rats - lavaged 18-24 hours later

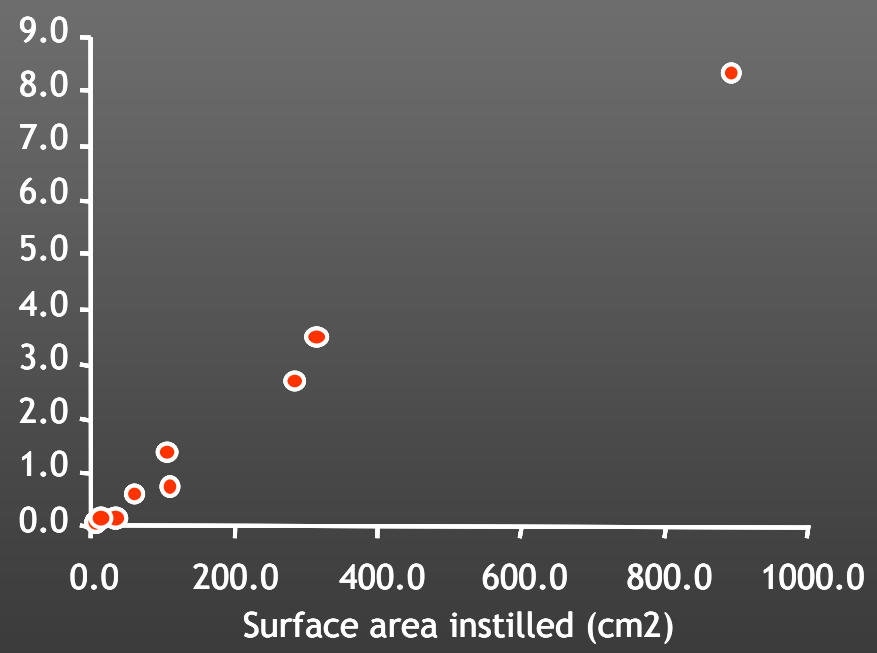


A range of fine and nanoparticle-sized low toxicity, low solubility particles cause inflammation in relation to surface area dose, not mass dose

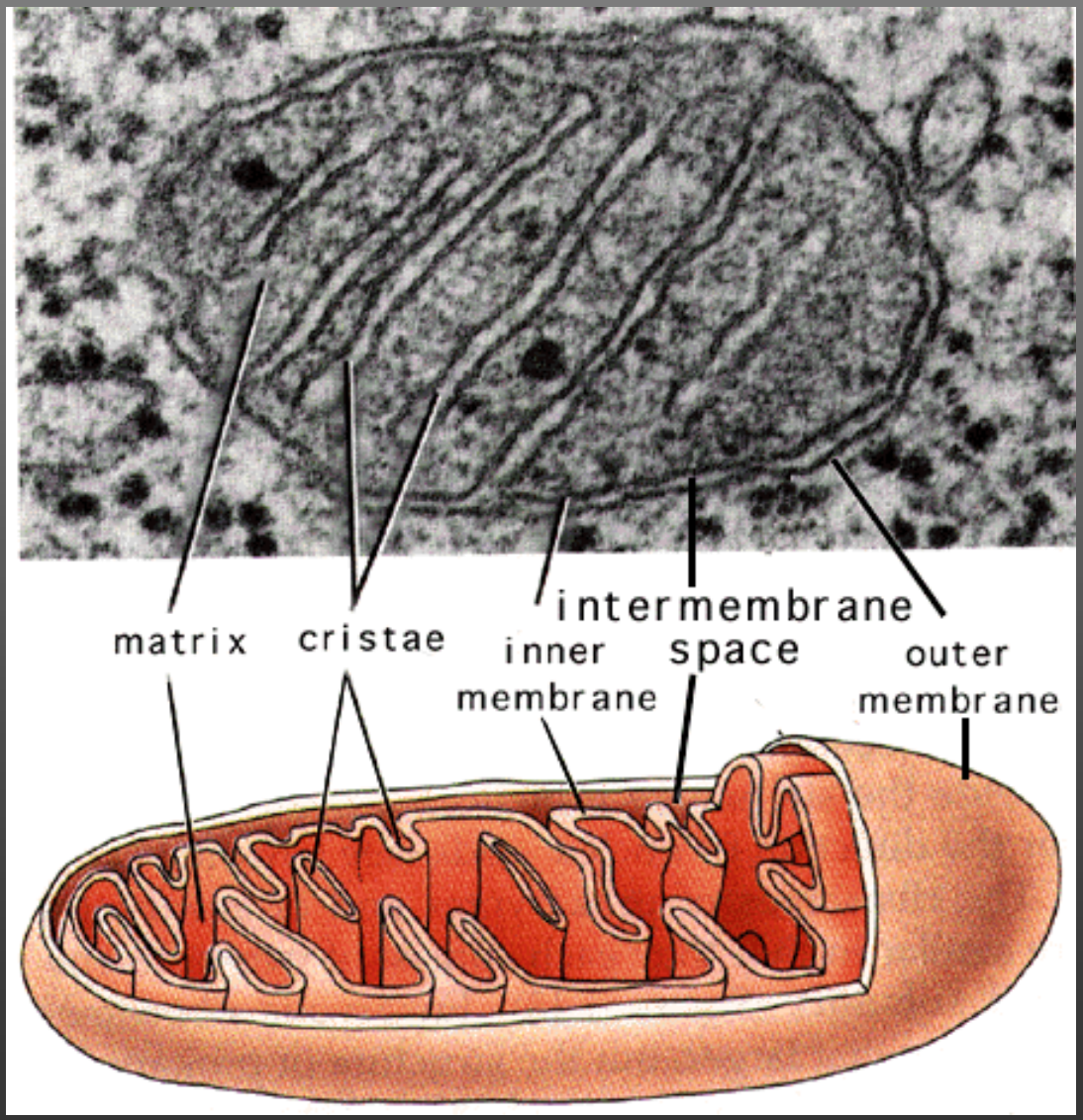
Dose expressed as mass instilled

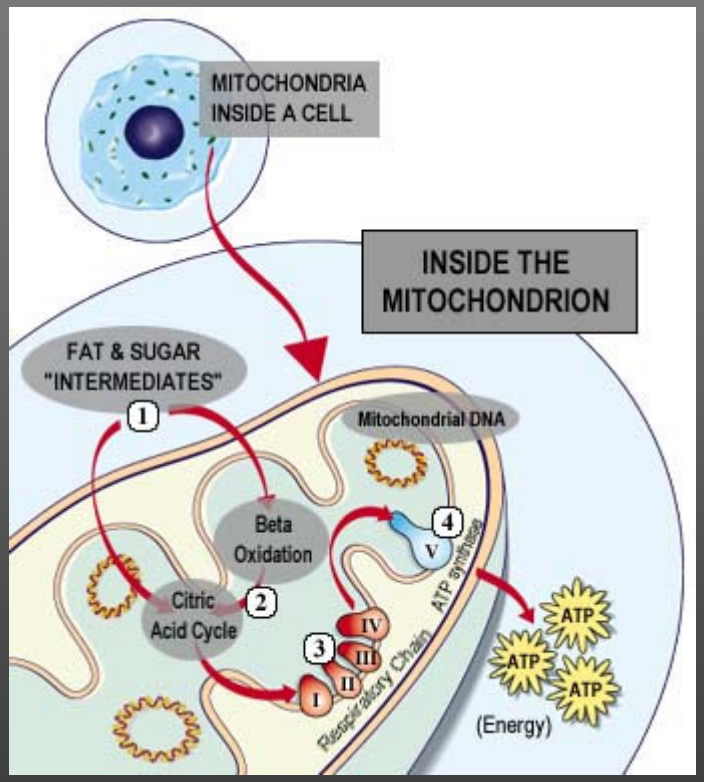


Dose expressed as surface area instilled

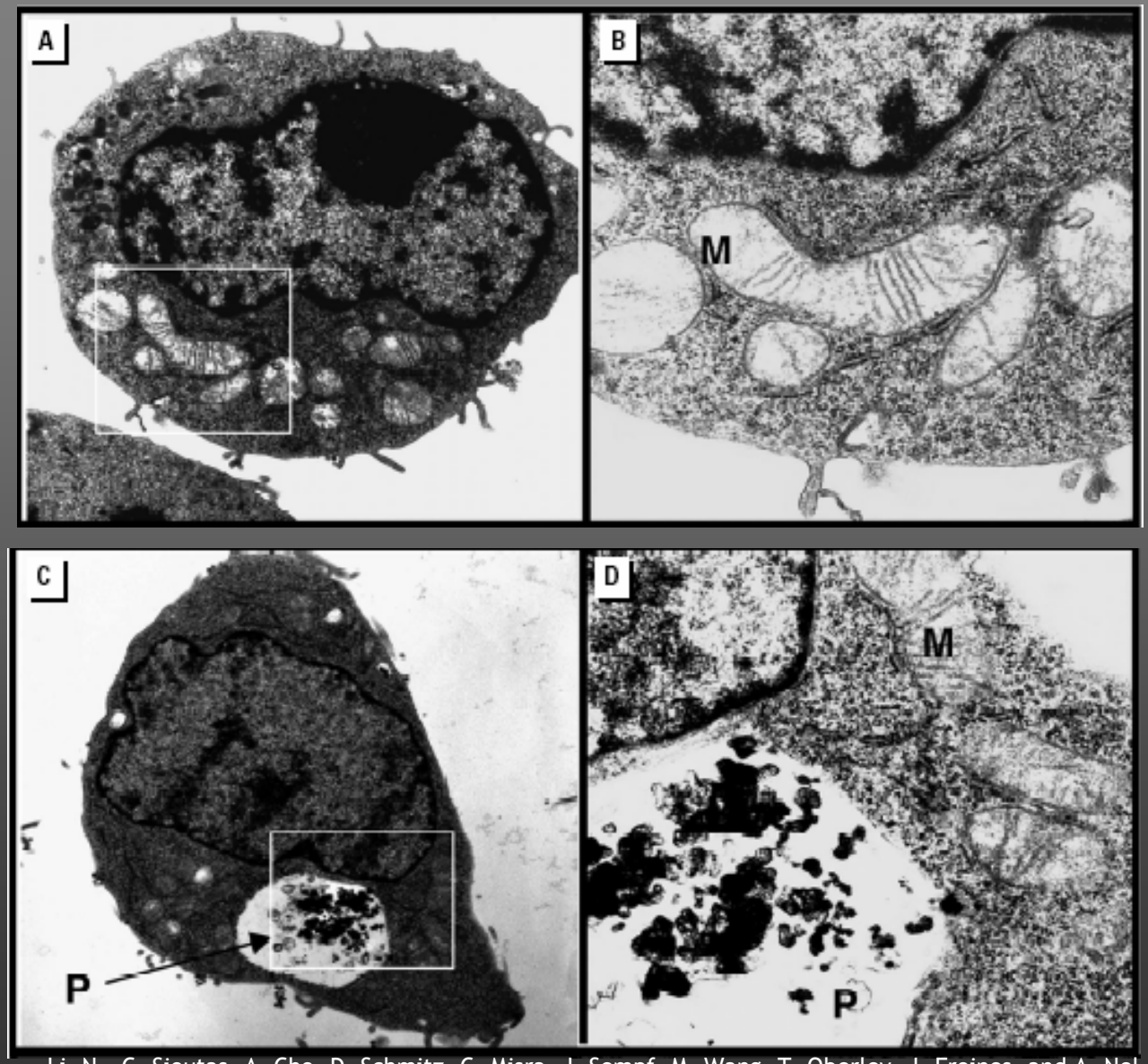


Mitochondria as a target for NPs





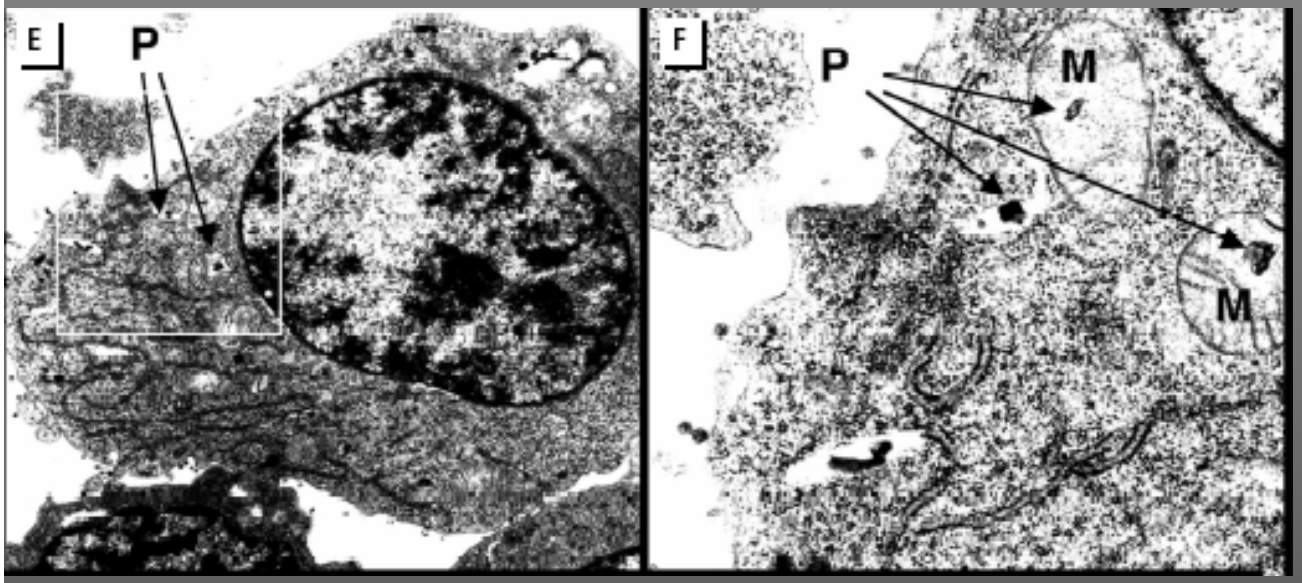
The ultrafine/NP fraction of PM is tropic for the mitochondria and appears to be toxic to them



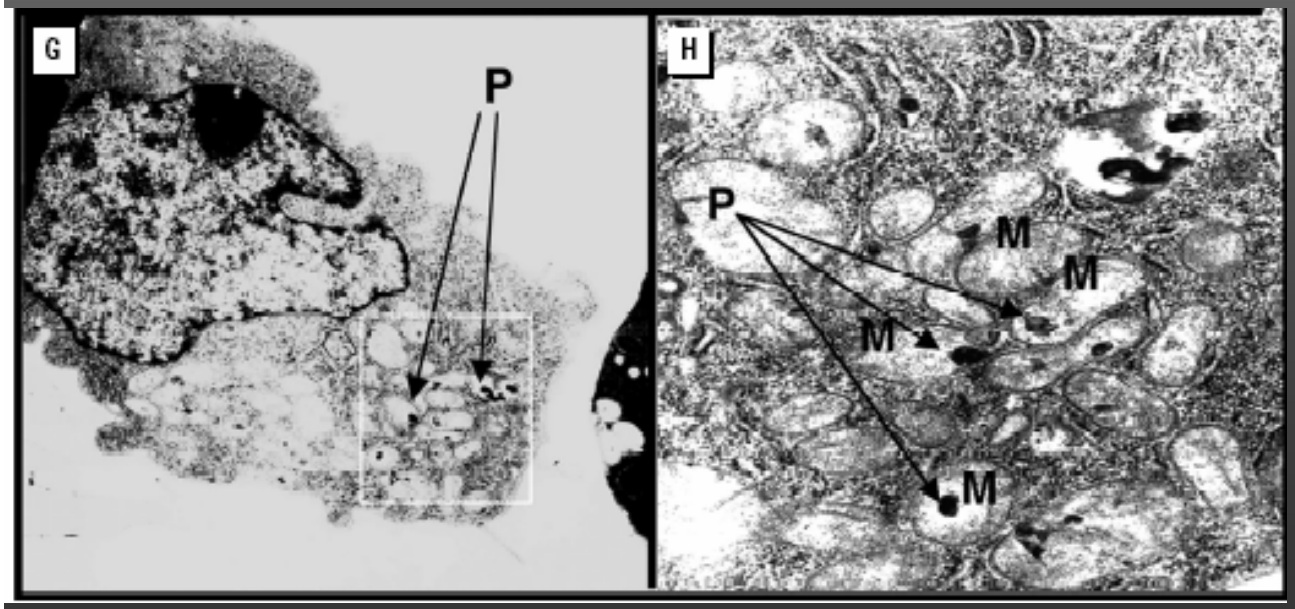
RAW246.7

RAW246.7
+
coarse PM

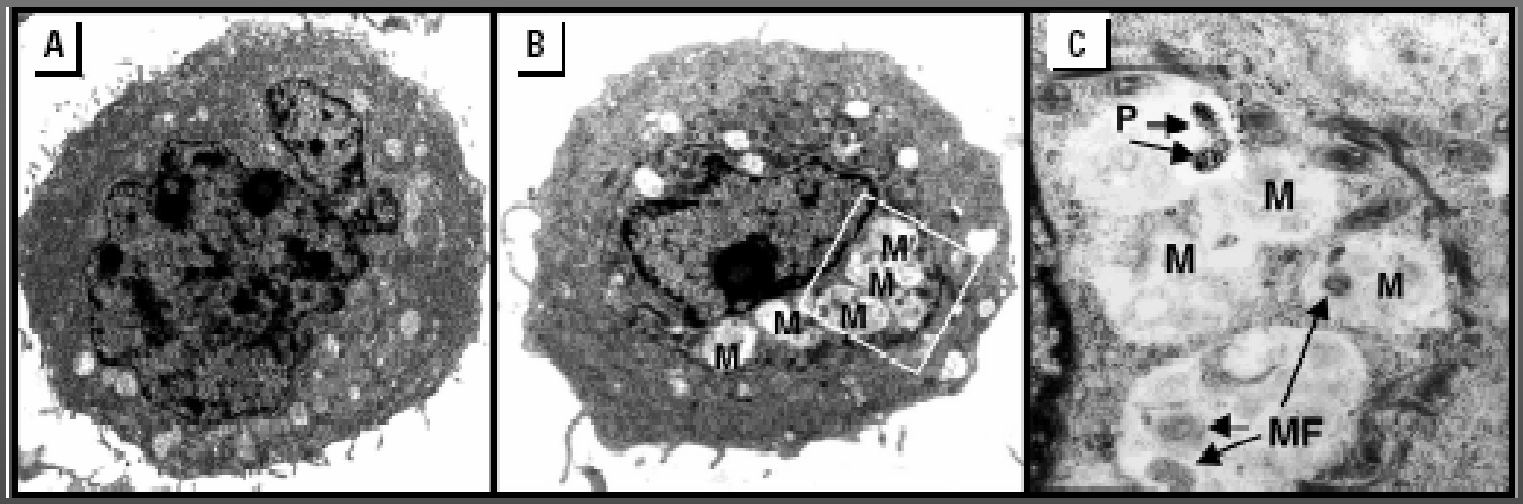
Li, N., C. Sioutas, A. Cho, D. Schmitz, C. Misra, J. Sempf, M. Wang, T. Oberley, J. Froines, and A. Nel. 2003. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. *Environ.Health Perspect*. 111:455-460.



RAW246.7
+
fine PM



RAW246.7
+
NP PM



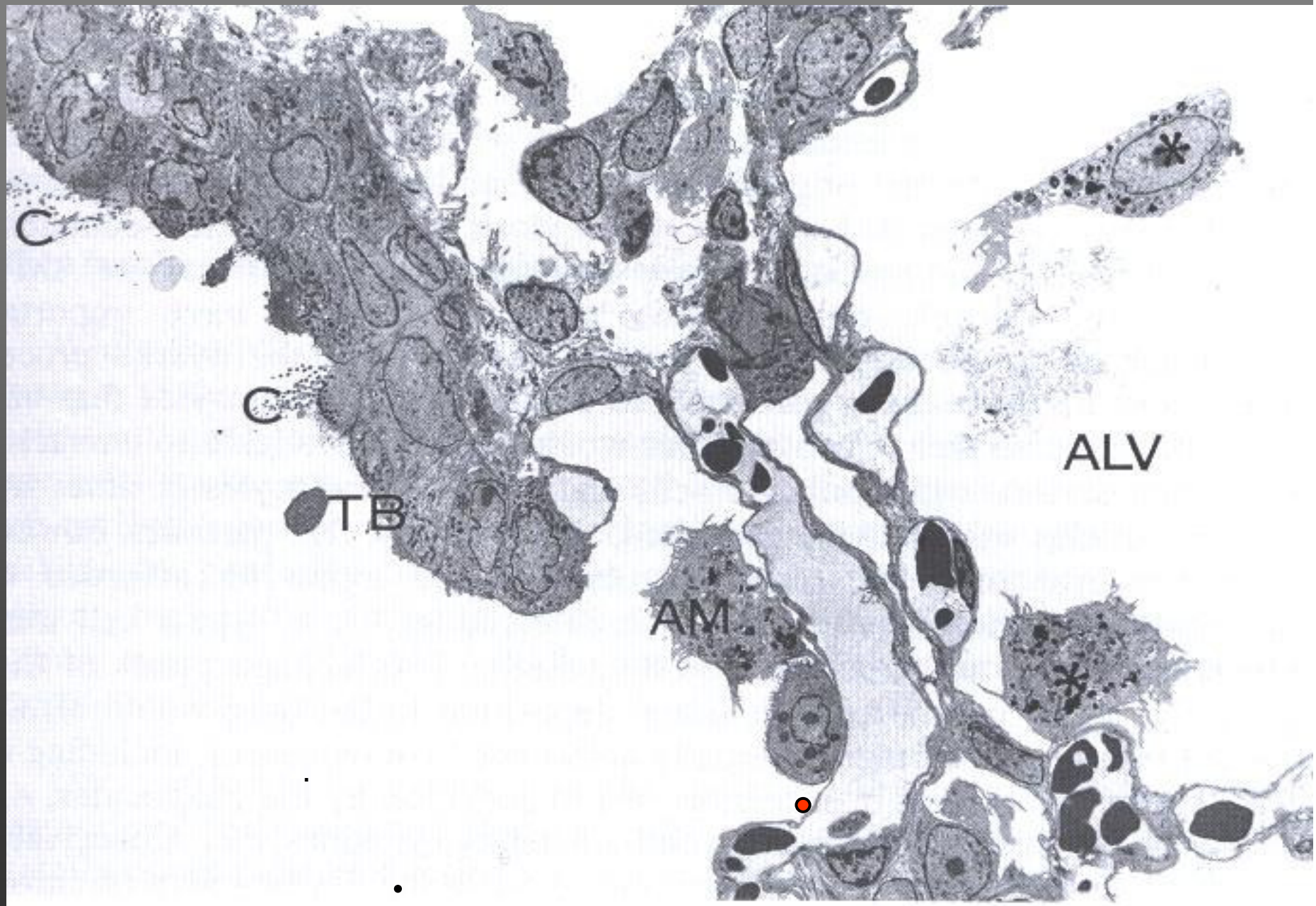
BEAS-3

+

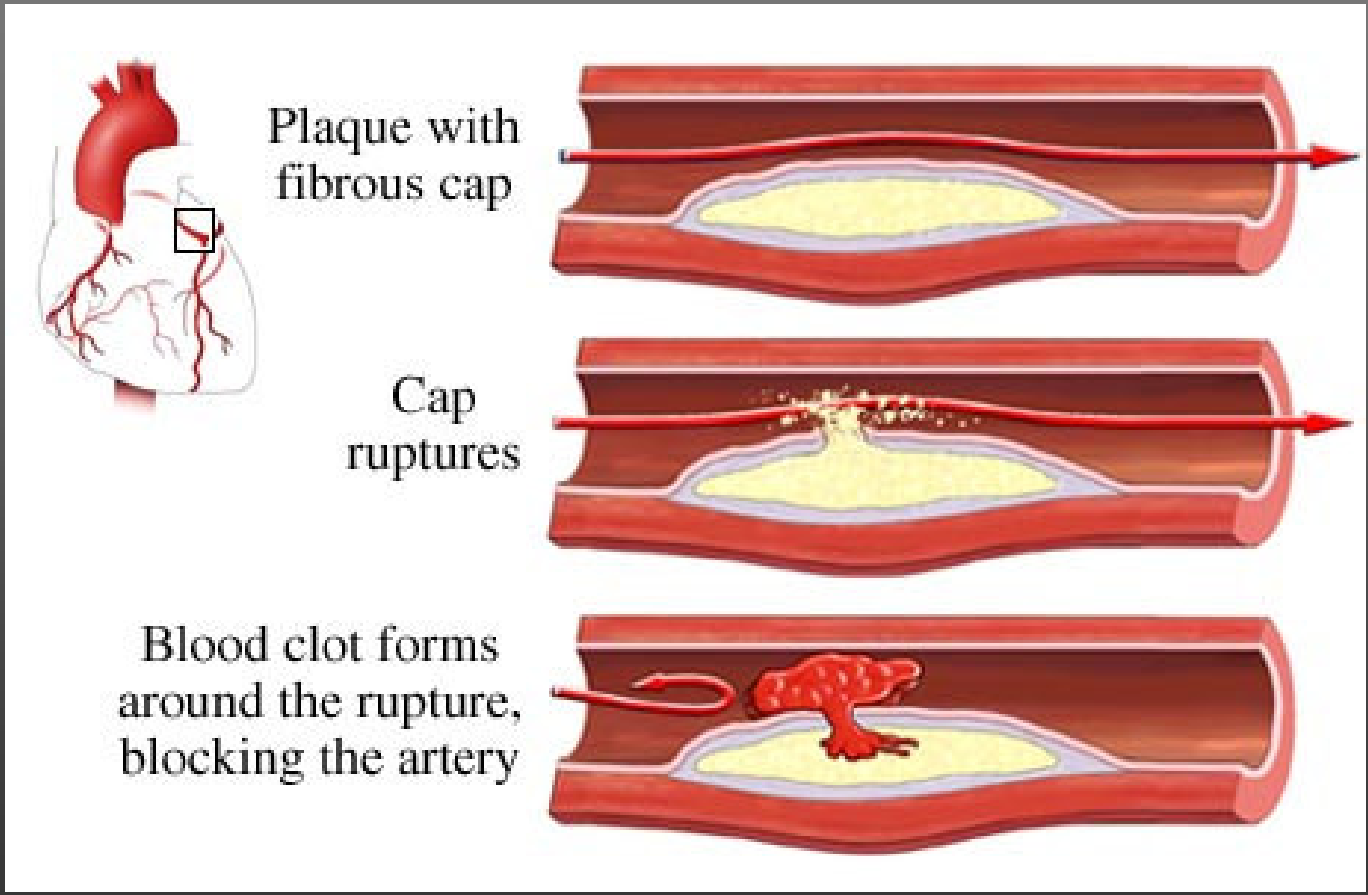
NP PM

Inhaled nanoparticles and the blood

Particles in the centriacinar region and the blood



Atherosclerotic plaques- the main cause of cardiovascular disease and death



Effects of Concentrated ambient particles (CAPS) on atherosclerosis

- ApoE$^{-/-}$ mice (lack ApoE)
- DK – (lack ApoE +lack low density lipoprotein receptor)
- Exposed to CAPS for 5 months 110-130μg/m^3
- Assessment of the atherosclerosis in the root of the aorta

Chen LC, Nadziejko C. Effects of subchronic exposure to concentrated ambient particle (CAPS) in mice: V CAPS exacerbate aortic plaque development in hyperlipidemic mice Inh Toxicol. 17 217-224 , 2005

CAPS causes more atherosclerosis and greater cellularity in the lesions

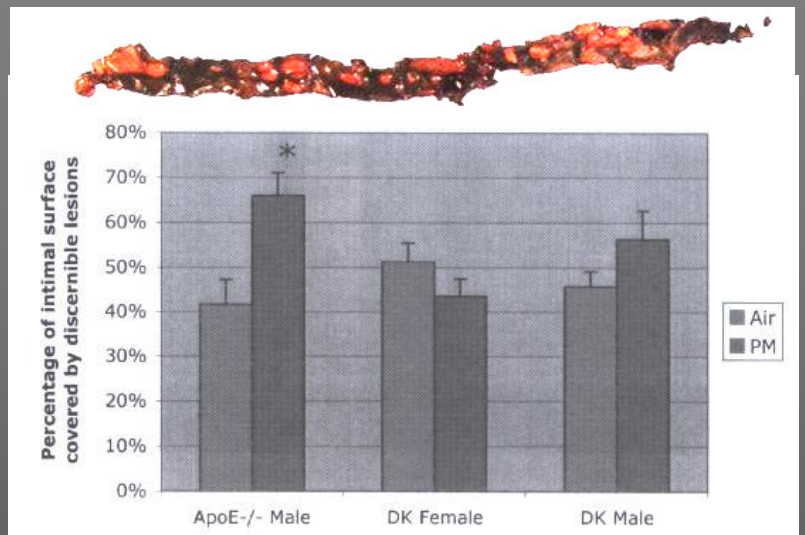


FIG. 8. Percentage of aortic intimal surface covered by grossly discernible atherosclerotic lesion in mice exposed to CAPs.

Percentage area of aorta covered by atherosclerotic lesion

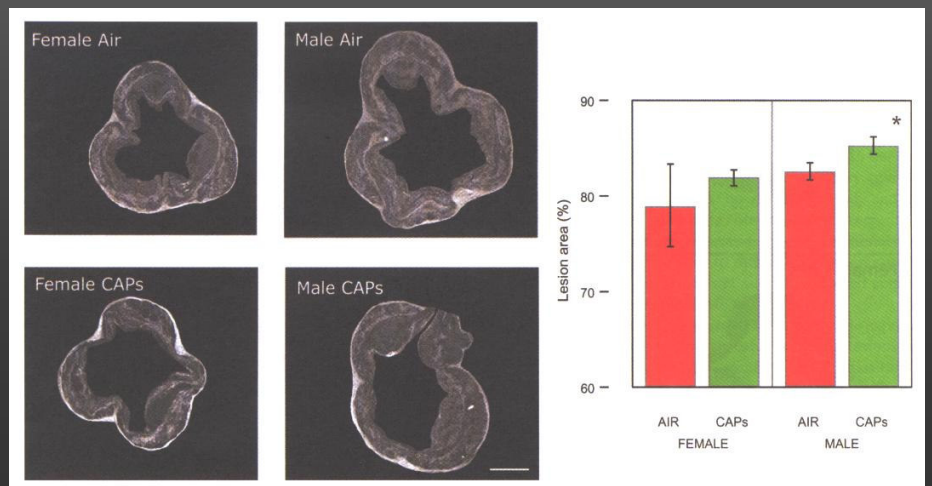
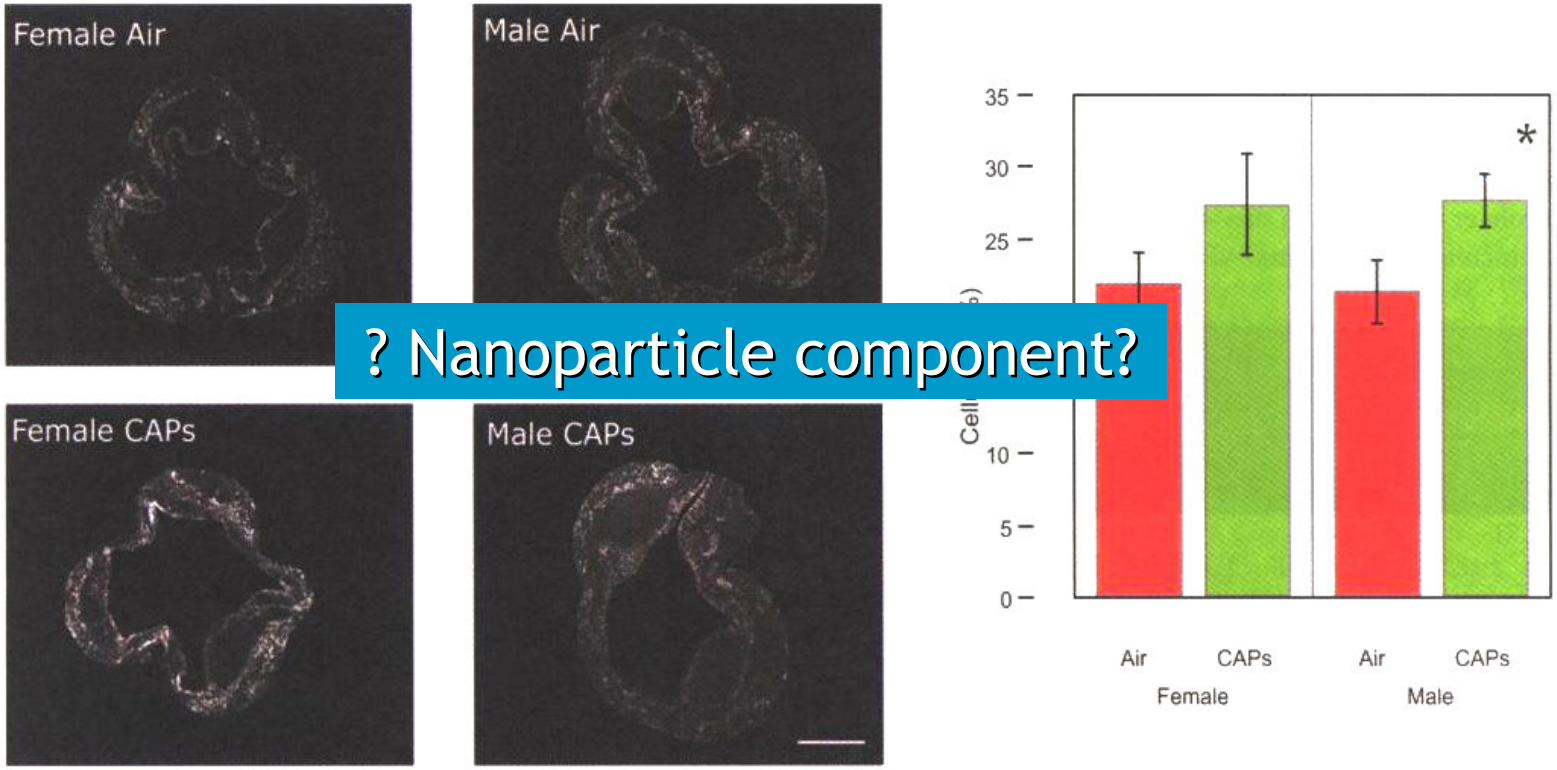


FIG. 2. Lesion areas (mean ± SE) of aorta root in DK mice exposed to CAPs. Asterisk indicates significant at $p = .06$. Bar at the bottom equals 500 μm.

Lesion area by microscopy

CAPS causes more atherosclerosis and greater cellularity in the lesions



? Nanoparticle component?

FIG. 3. Cellularity (mean ± SE) of aorta root in DK mice exposed to CAPs. Asterisk indicates significant at $p = .04$. When data of male and female were combined, difference significant at $p = .01$ between air- and CAPs-exposed animals. Bar at the bottom equals 500 μm.

Transport to the blood means that other organs can be exposed

Localisation of bloodborne carbon particles to the liver and spleen

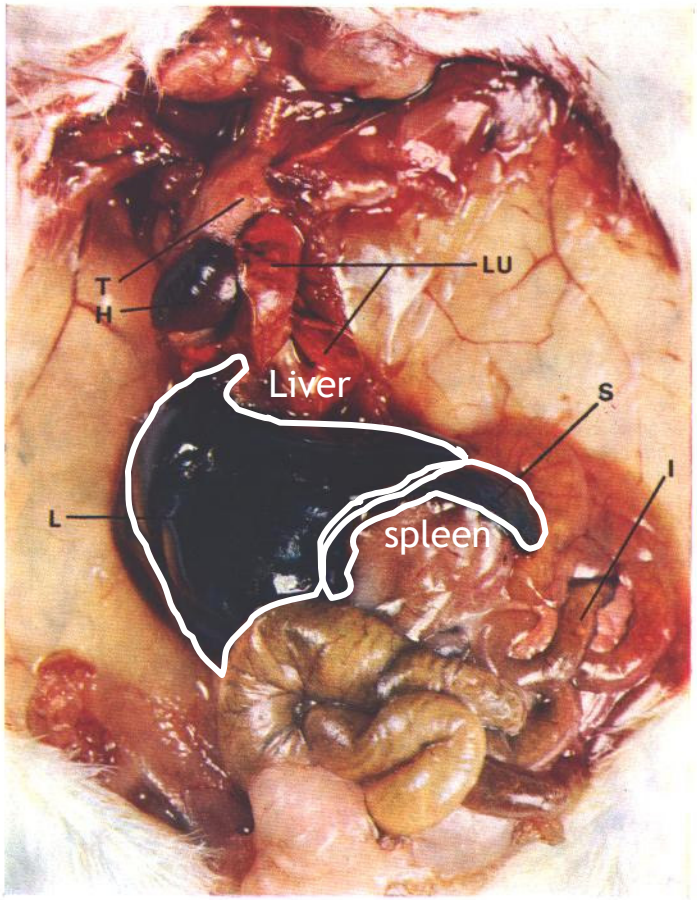
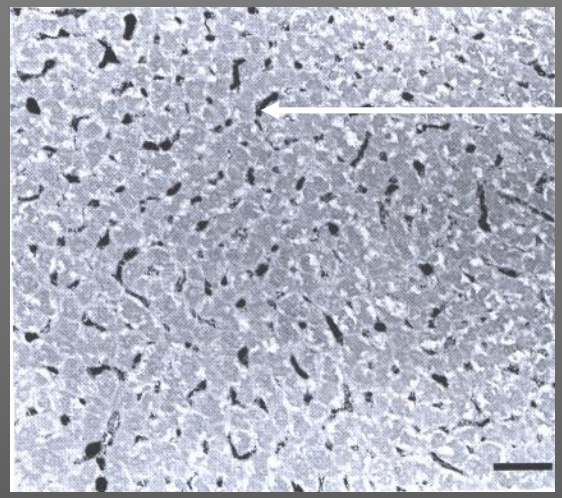
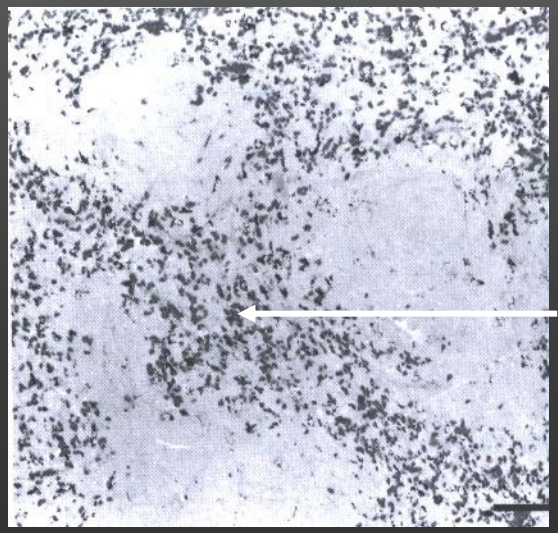


FIG. 33

Dissection of mouse after a single intravenous injection of colloidal carbon. Note that the liver (L) and spleen (S) have turned black: the heart (H), thymus (T), lungs (LU) and intestine (I) are unchanged. ×2.

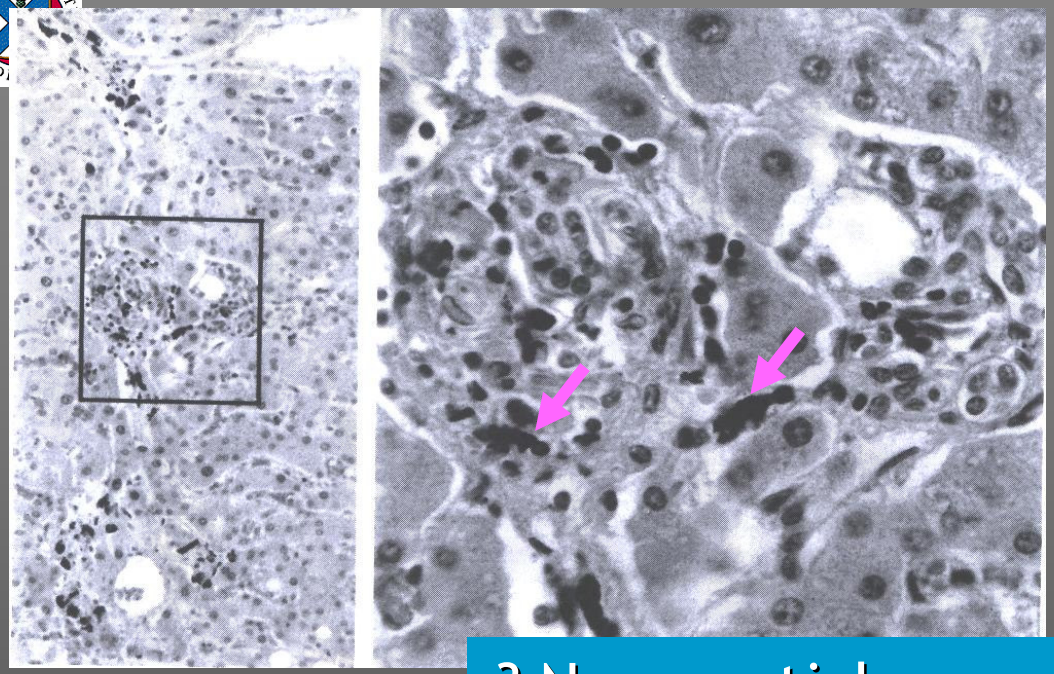


Carbon-loaded Kupfer cells



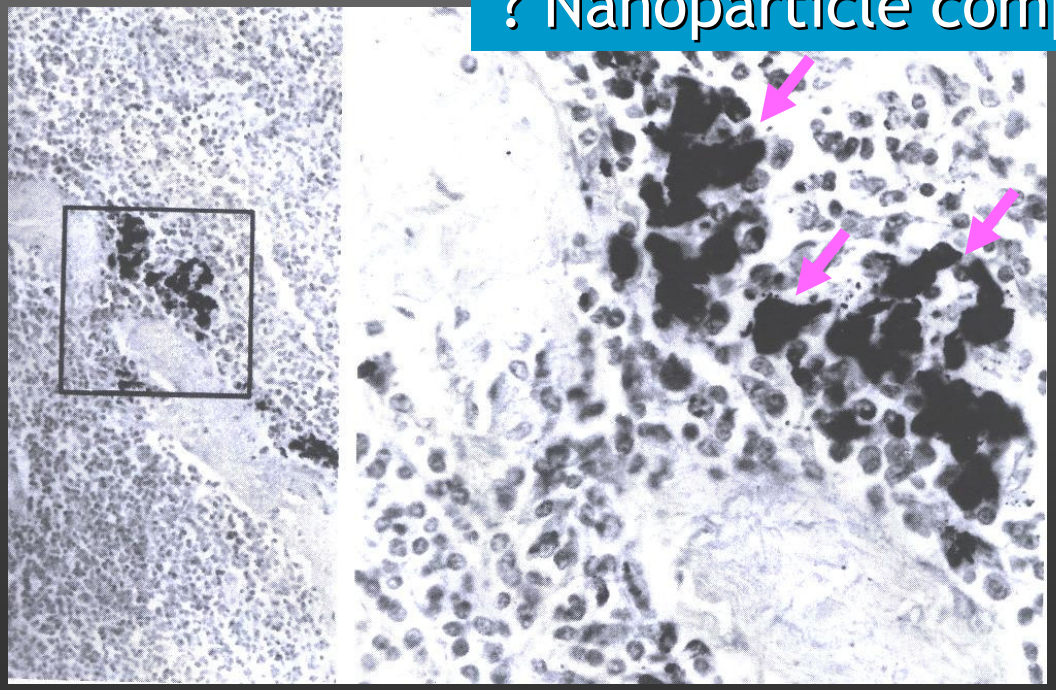
Carbon-loaded sinusoidal macrphages

Accumulation of dust particles in the liver and spleen of coalworkers



liver

? Nanoparticle component?



spleen

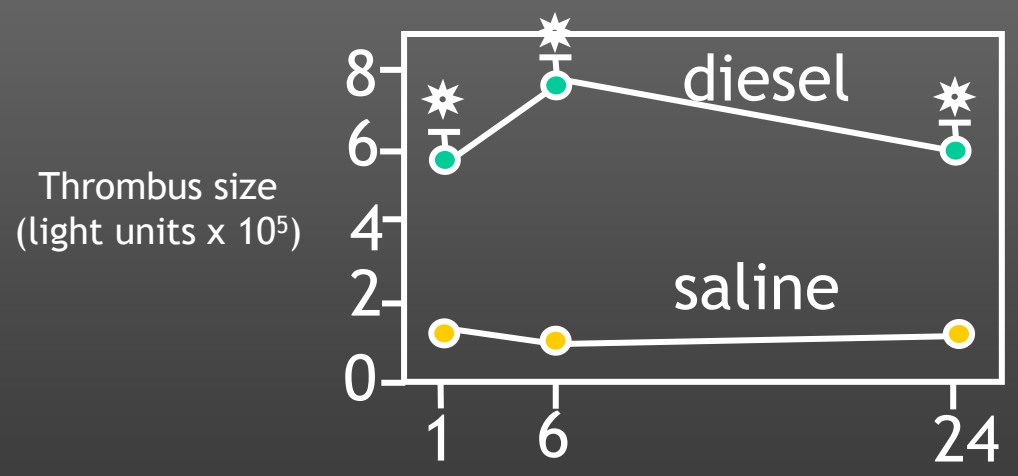
LeFevre, M. E., F. H. Green, D. D. Joel, and W. Laqueur. 1982. Frequency of black pigment in livers and spleens of coal workers: correlation with pulmonary pathology and occupational information. Hum.Pathol. 13:1121-1126.

Pulmonary deposition of particles enhances experimental thrombosis

Pulmonary deposition of diesel nanoparticles enhances experimental thrombosis in hamsters

The right femoral vein exposed and mounted on a transilluminator.
Intravenous administration of Rose Bengal
Femoral vein irradiated with green light
Endothelial injury and thrombus formation
The thrombus monitored under a microscope at 40 magnification.
The change over time in light transmission through the blood vessel is related to extent of thrombus

saline control hamsters and in hamsters intratracheally instilled with diesel exhaust particles (DEP, 50 µg/animal), 1 h, 6 h and 24 h earlier.



Nemmar, A., P. H. Hoet, D. Dinsdale, J. Vermylen, M. F. Hoylaerts, and B. Nemery. 2003. Diesel exhaust particles in lung acutely enhance experimental peripheral thrombosis. *Circulation* 107:1202-1208.

Transport of nanoparticles to the brain

TRANSLOCATION OF INHALED ULTRAFINE PARTICLES TO THE BRAIN

G. Oberdörster, Z.Sharp, V. Atudorei, A. Elder, R. Gelein, W. Kreyling, C. Cox

University of Rochester, Rochester, NY; University of New Mexico, Albuquerque, NM; and GSF, Neuherberg/Munich, Germany; 4NIH, Bethesda, MD

Inhalation Toxicology 16, 437-445. 2004.

- Ultrafine elemental ^{13}C particles (CMD = 36 nm; GSD = 1.66) from ^{13}C graphite rods by electric spark discharge in an argon atmosphere

- Attained AMC 160 μg/m^3.

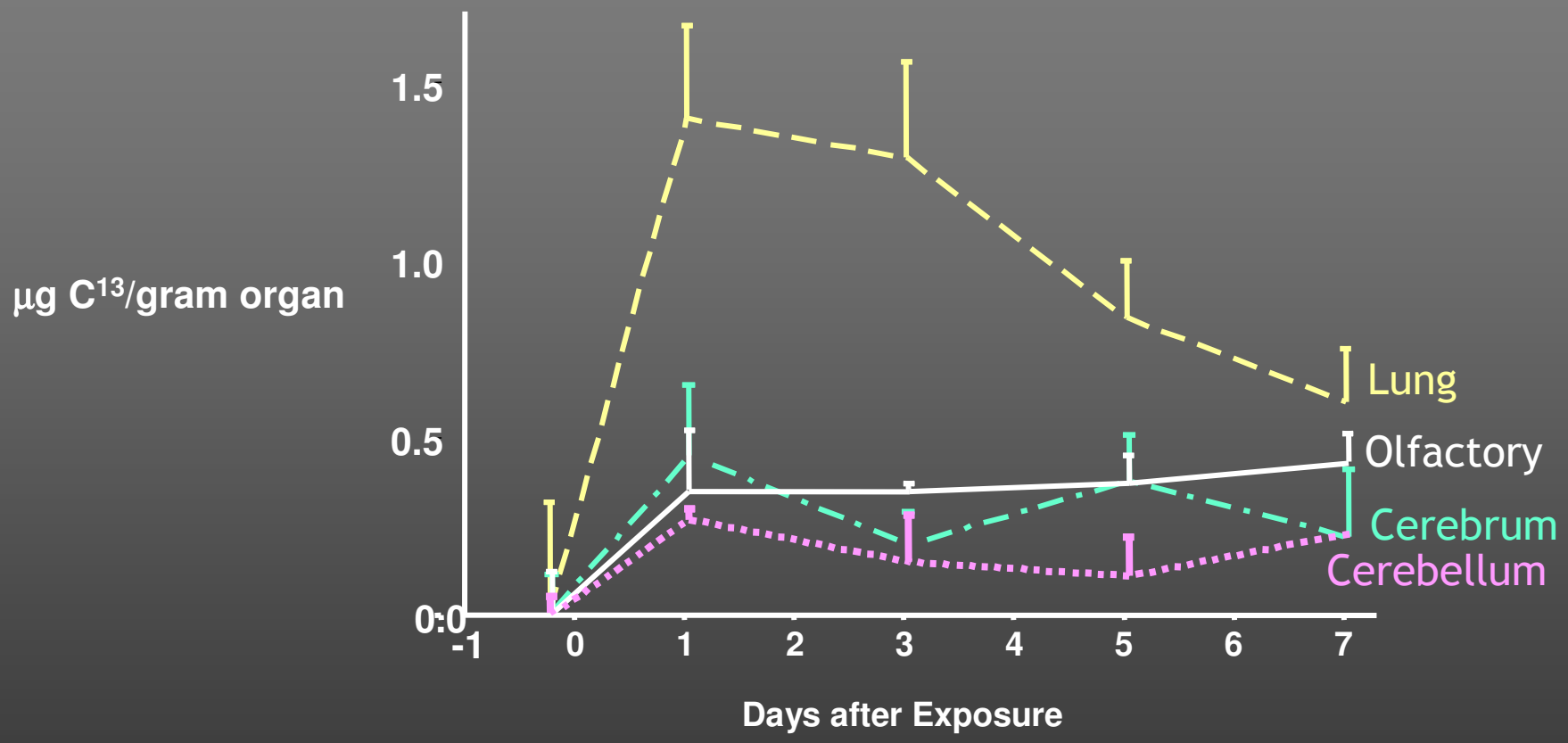
- Exposed for 6 hrs.

- Lungs, cerebrum, cerebellum and olfactory bulbs removed 1,3,5 and 7 days after exposure.

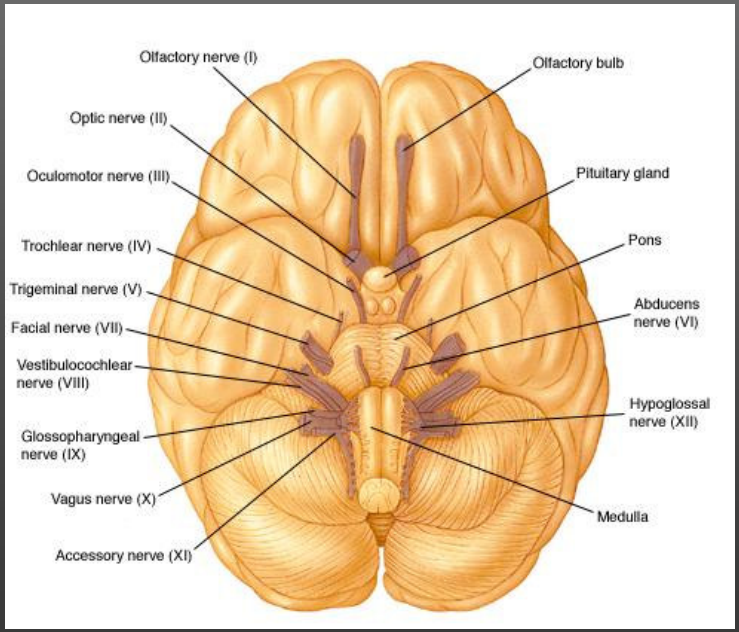
- ^{13}C concentrations determined by isotope ratio mass spectroscopy and compared to background ^{13}C levels of sham-exposed controls (day 0).

- The background-corrected pulmonary ^{13}C added as ultrafine ^{13}C particles on day 1 post-exposure was 1.34 μg/lung.

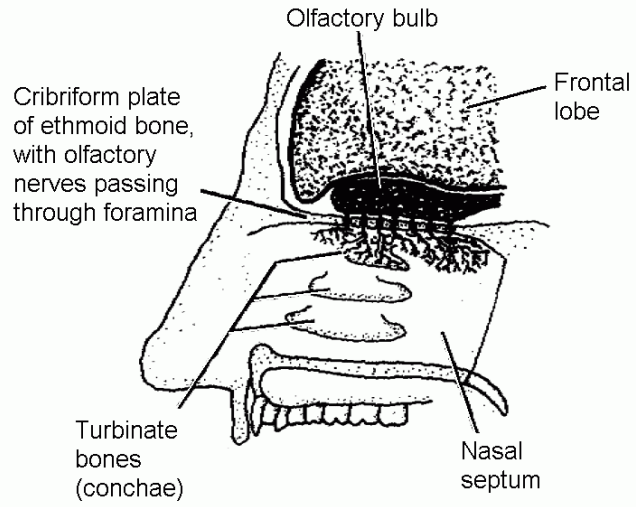
Translocation of inhaled ultrafine particles to the brain



G. Oberdörster, Z.Sharp, V. Atudorei, A. Elder, R. Gelein, W. Kreyling, C. Cox Translocation of inhaled ultrafine particles to the brain Inhalation Toxicology in Press



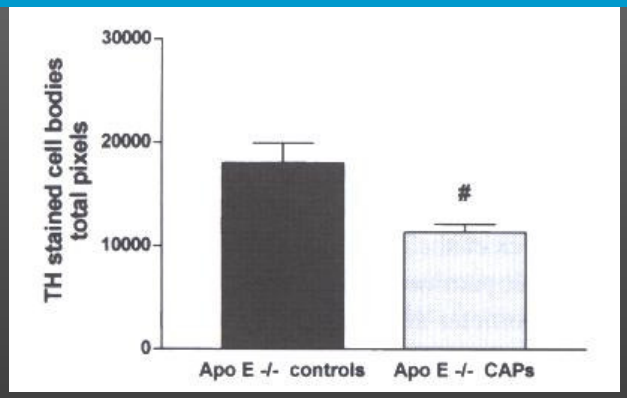
The olfactory epithelium is located in the upper part of the nasal cavity, on the nasal septum and superior concha.



Loss of dopaminergic neurons in the brain of mice exposed to CAPS for 4 months

Coronal sections of brain stained with tyrosine hydroxylase, a marker of dopaminergic neurones

? Nanoparticle component?



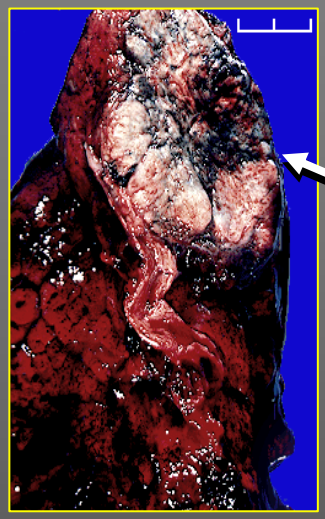
Similar degeneration of dopaminergic neurones is seen in clinical and experimental models of Parkinson's disease

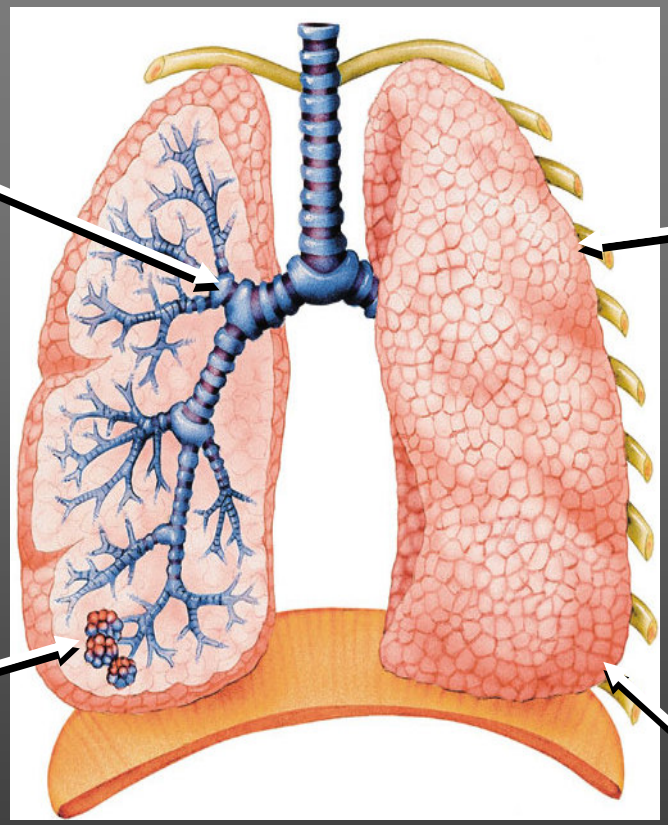
Effects of subchronic exposure to concentrated ambient particles: VII Degeneration of dopaminergic neurons in ApoE mice. Veroniesi B, Makwana O, Pooler M, Chen LC. Inhal Toxicol 17, 235-241 2005

Carbon nanotubes

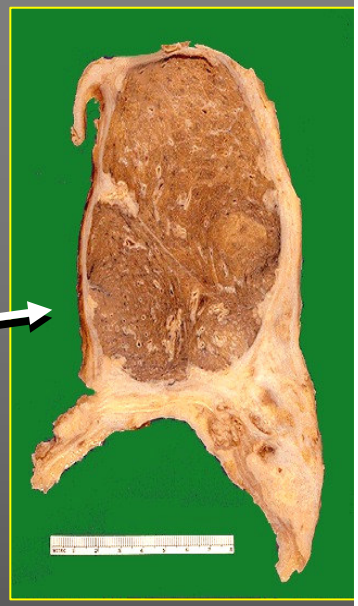
Asbestos -related lung diseases

Bronchogenic carcinoma





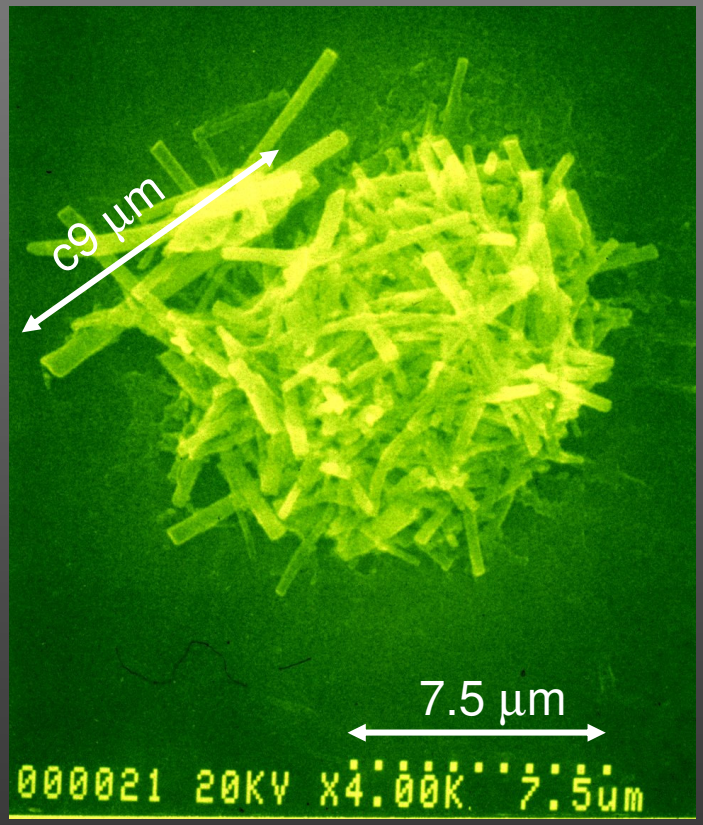
Pleural mesothelioma



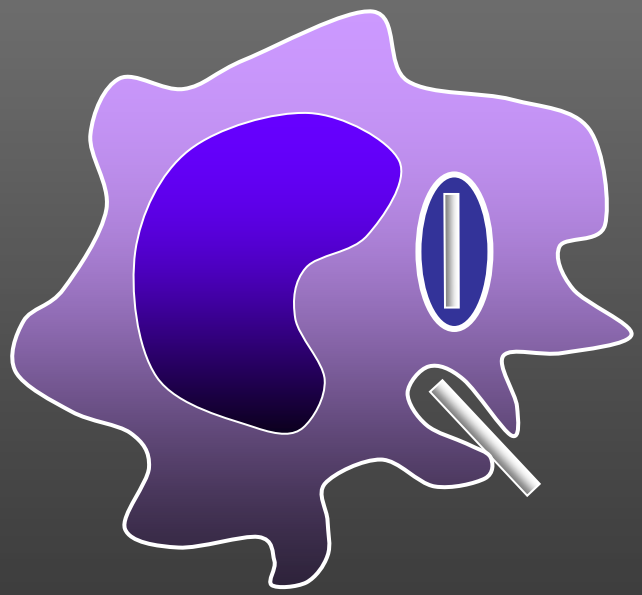
Asbestosis
Honeycomb lung

Pleural plaque

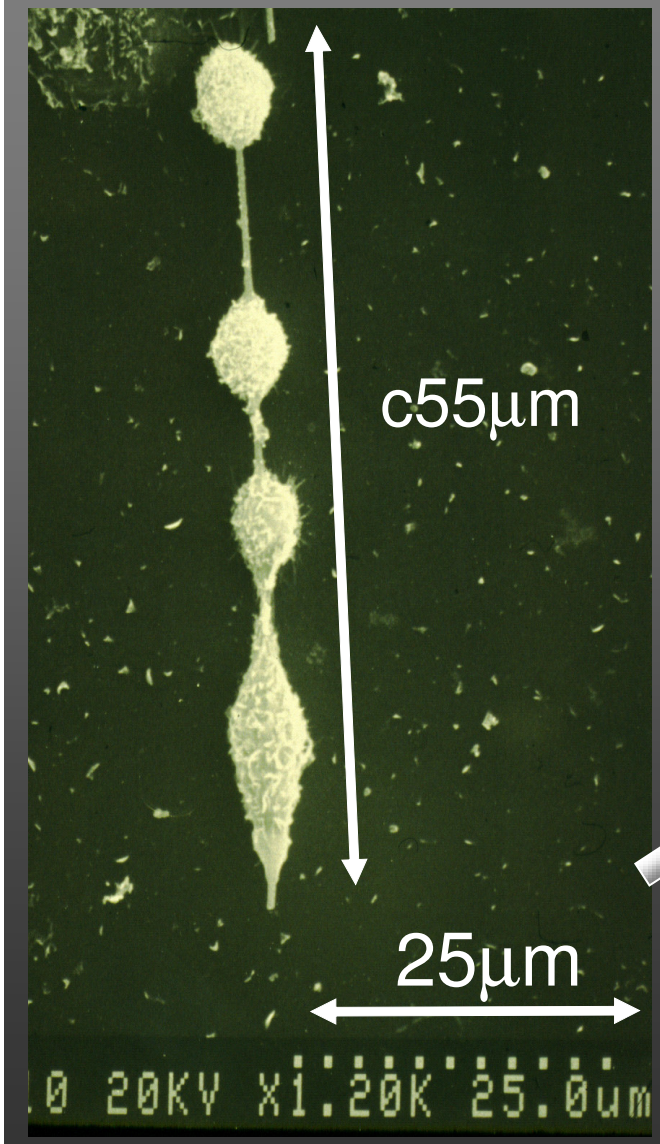
Macrophages can phagocytose many short fibres without problem and move them out of the lungs

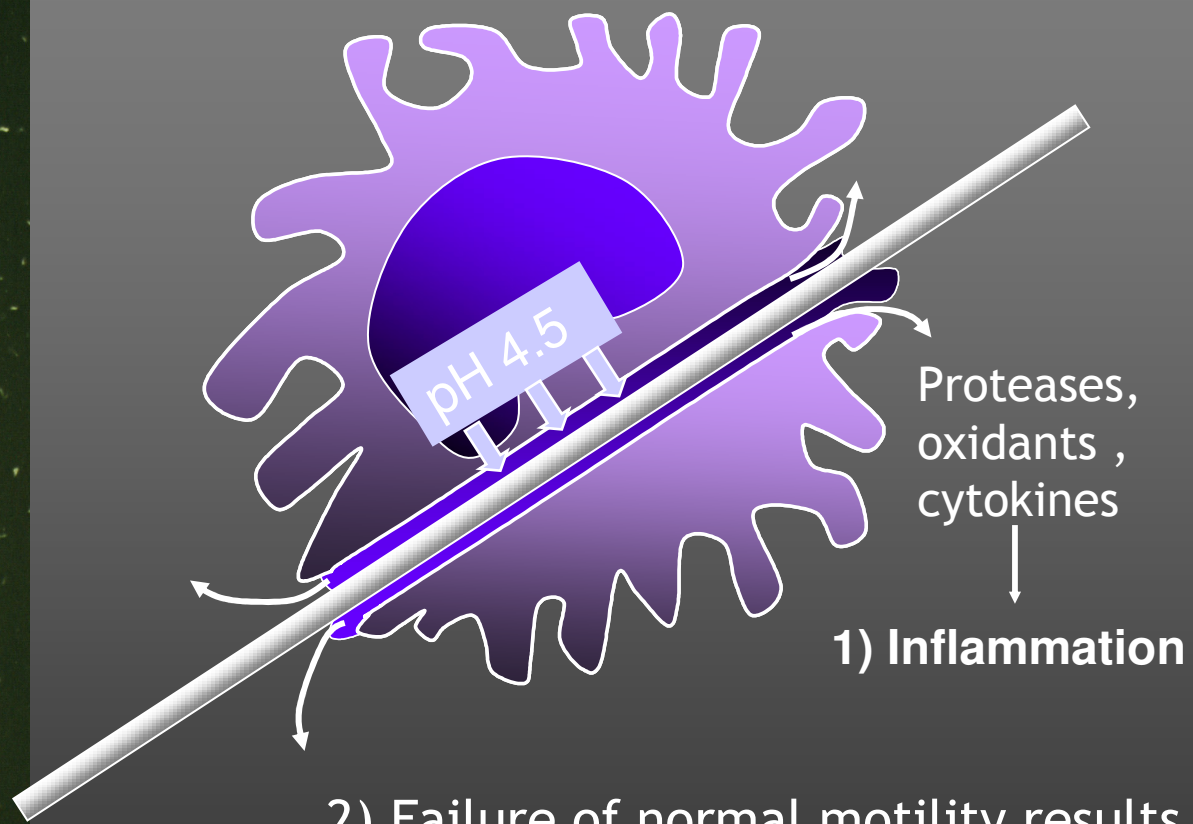


Ashed ghost of a macrophage from the lungs of a rat inhaling Code 100/475 fibre glass



Macrophages cannot completely phagocytose long fibres causing inflammation and impaired clearance





2) Failure of normal motility results in an inability to clear the long fibres out of the lungs

Long thin biopersistent fibres are most pathogenic

•Thinness – contributes to a small aerodynamic diameter so renders fibre respirable

•Length – slowly cleared, stimulate macrophages by incomplete phagocytosis to release oxidants,, proteases, cytokine, mitogens

•Biopersistence – long fibres do not shorten by breakage, or dissolve

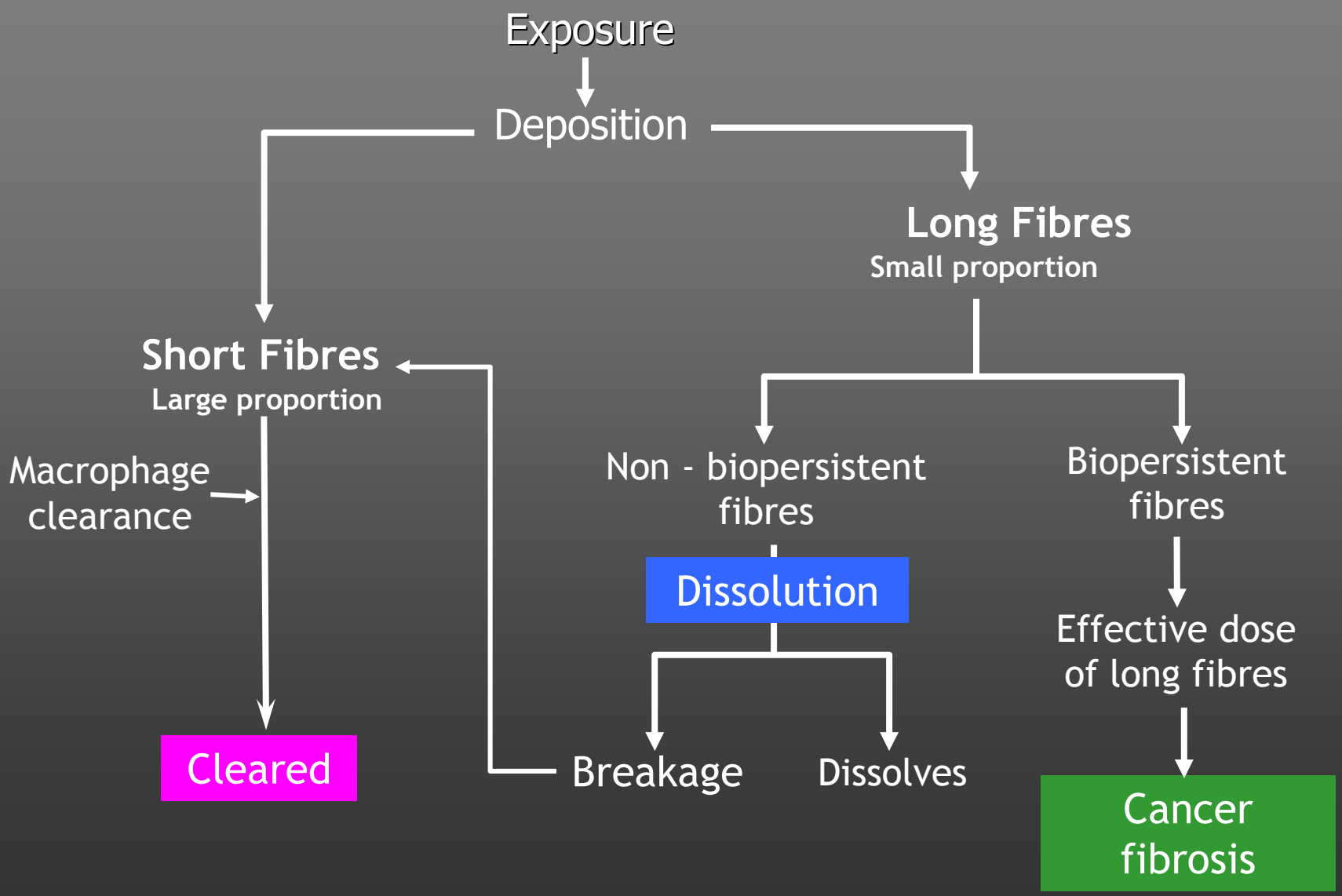
•Thickness – more than about 10 μm means not respirable

•Shortness - easily phagocytosed, doesn't stimulate macrophages

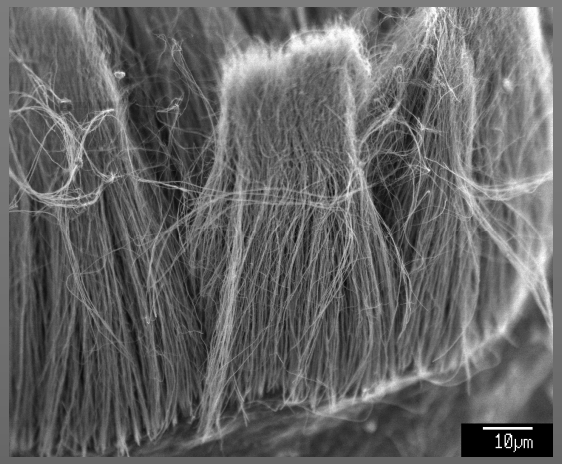
•Blopersistence – it doesn't matter whether short fibres are biopersistent or not

Carbon nanotubes are thin (<10nm) long (mm) and tough

Role of fibre length and biopersistence in determining effective dose



Nanotubes

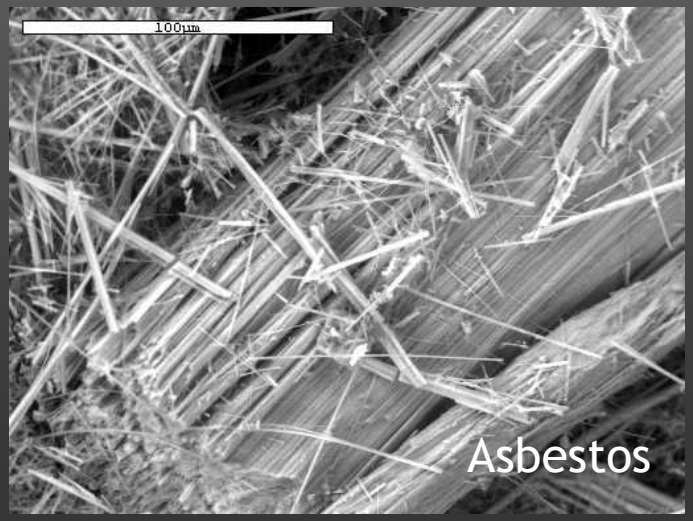


Multi-walled carbon nanotubes 50µm x <100nm

Long – up to mm!

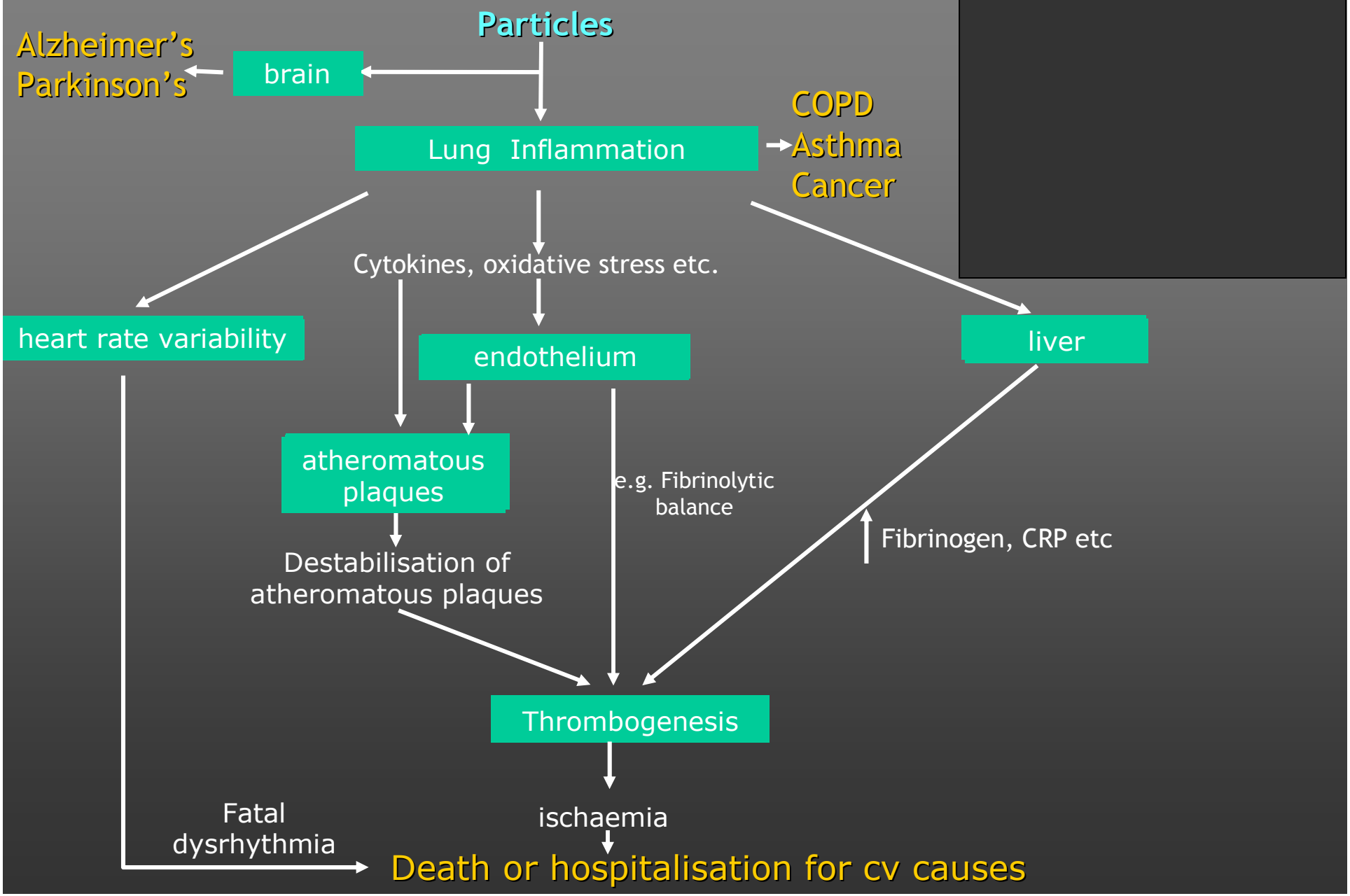
Thin - SW<5nm MW < 200nm

Durable – boiled in acid to clean them



Summing up the potential targets for NPs

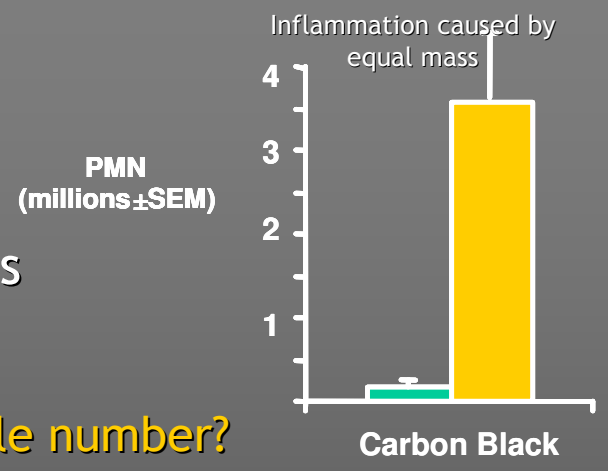
New targets for inhaled particles



Summing up carbon nanotubes

If they can be long, thin and persistent in the lungs then they may behave like asbestos

From nanotoxicology to occupational hygiene



1) Dusts in workplaces is regulated by mass

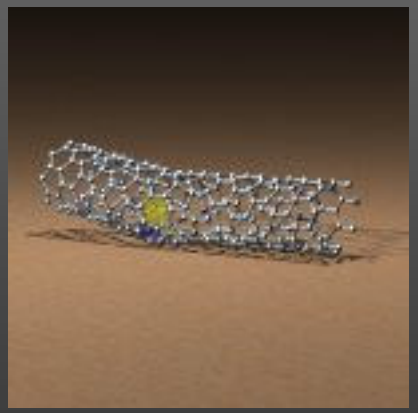
What is the best metric for nanoparticles – particle number? Surface area?

2) Asbestos fibres are regulated by number per ml of air by PCOM

Single walled CNT are 1-5nm:

The resolution of the light microscope is 200nm

How should we regulate nanotubes?



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