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Nanomaterial toxicology - Finding the best way forward

Vicki Stone

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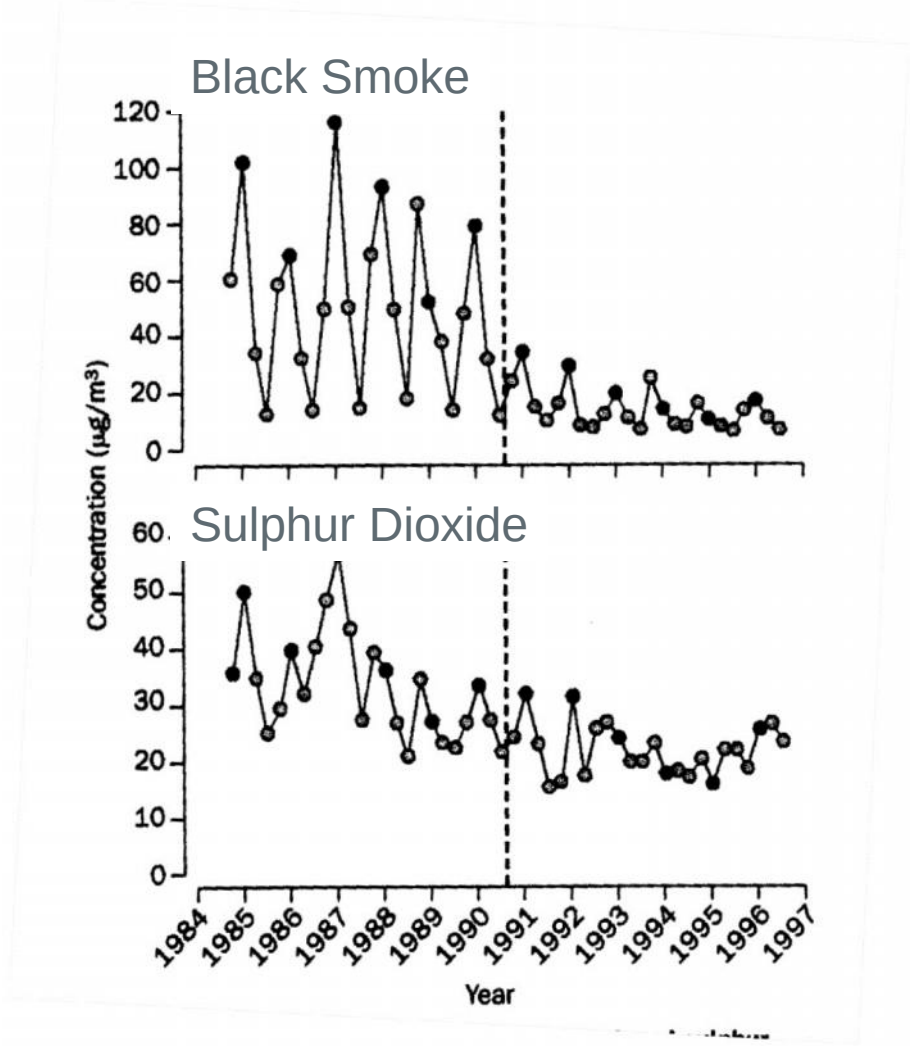
LEADERS IN IDEAS AND SOLUTIONS

Toxicology Award Winner

2015-16

Dublin: Ban on coal sales, Sept 01 1999



On cessation of coal sales, the death rate decreased.

Cause of death	% drop
Non trauma deaths	5.7
Respiratory deaths	15.5
CV deaths dropped	10.3

= 116 fewer resp deaths/year
+ 243 fewer CV deaths/year

Clancy et al. 2002
The Lancet 360: 1210-14

PM_{10}

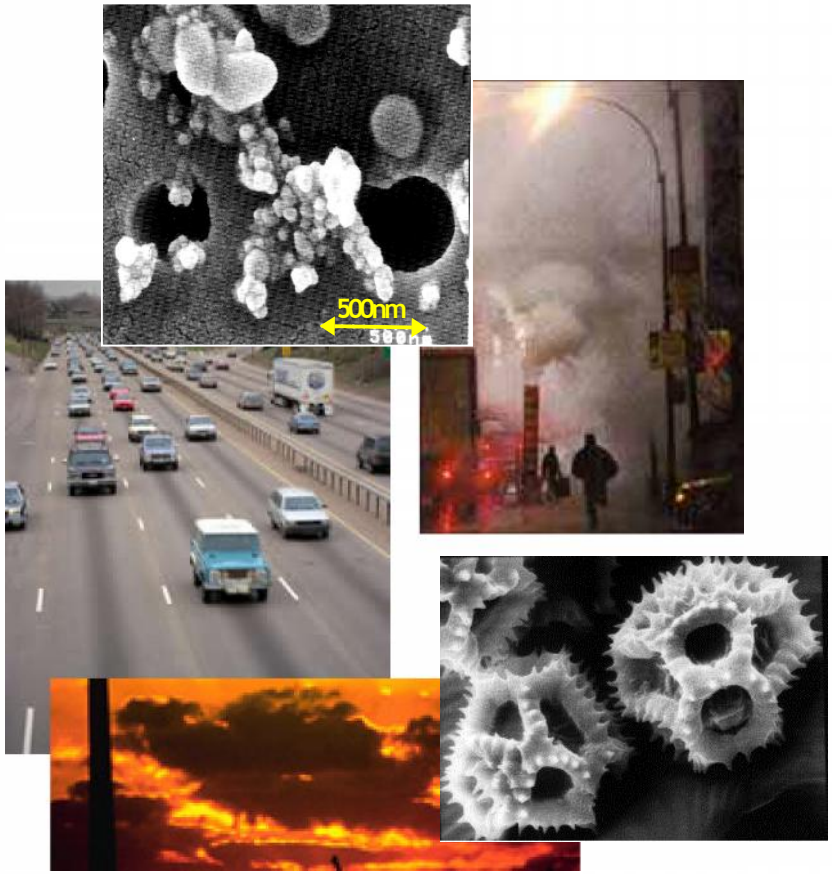
Particulate matter collected through a 10 μm filter with an efficiency of 50%

Carbon based particles
- Traffic and industrial
- Many ultrafine
- Transition metals

Sulphates/Nitrates
- Traffic/photochemical
- Mainly ultrafine

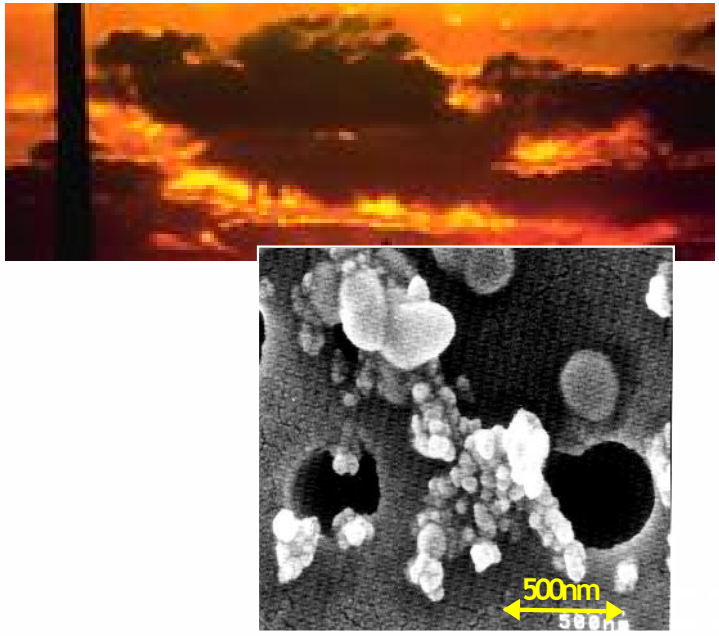
Wind blown dust
- Mainly coarse

Biological components
- Spores
- Pollen
- Mainly fine and course



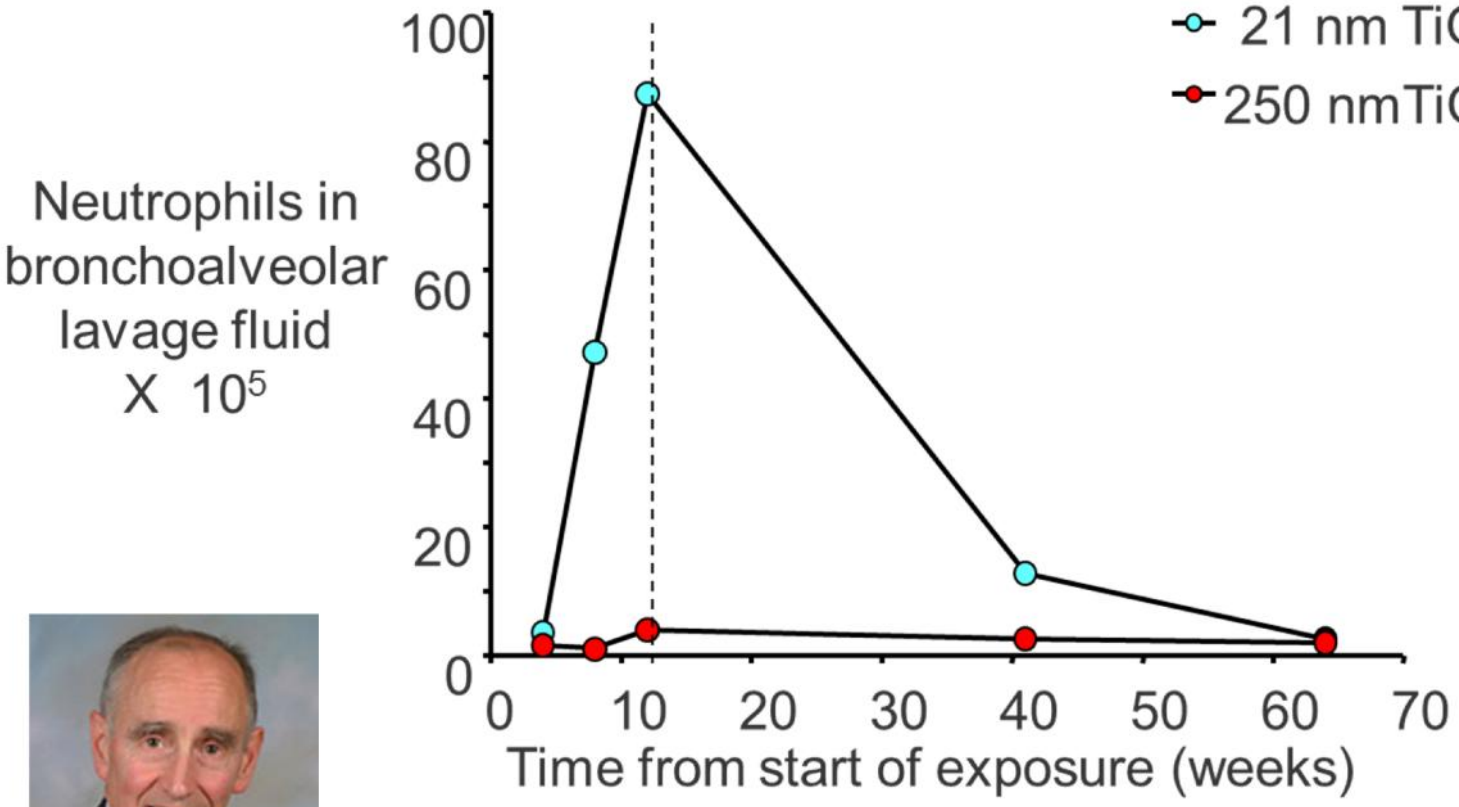
ie Cocktail. Which component is responsible for the health effects?

The Ultrafine Hypothesis



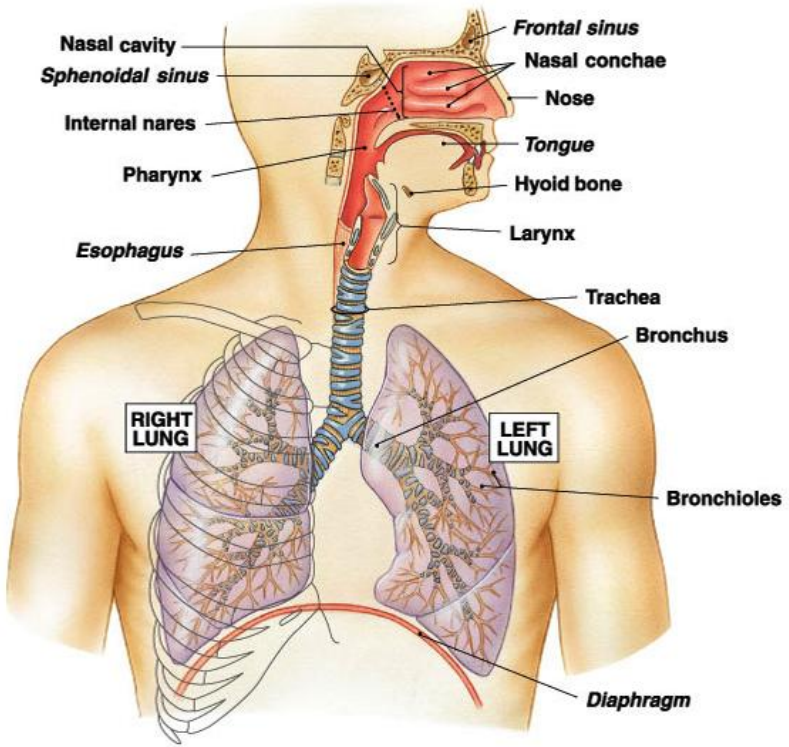
Seaton A., MacNee W., Donaldson K., Godden D. Particulate air pollution and acute health effects. Lancet 1995. 345; 176-178.

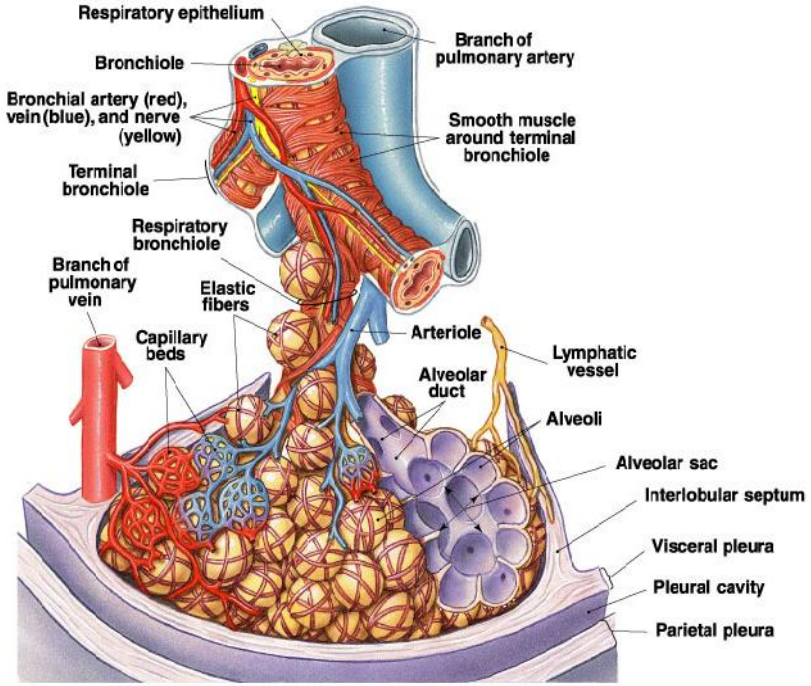
Evidence for Ultrafine Particle effects



Ferin et al. 1992 Am.J.Respir.Cell Mol. Biol. 6: 535-542.

Inhalation as a route of exposure





Martini. Fundamentals of Anatomy and Physiology, 7th edition, 2006.

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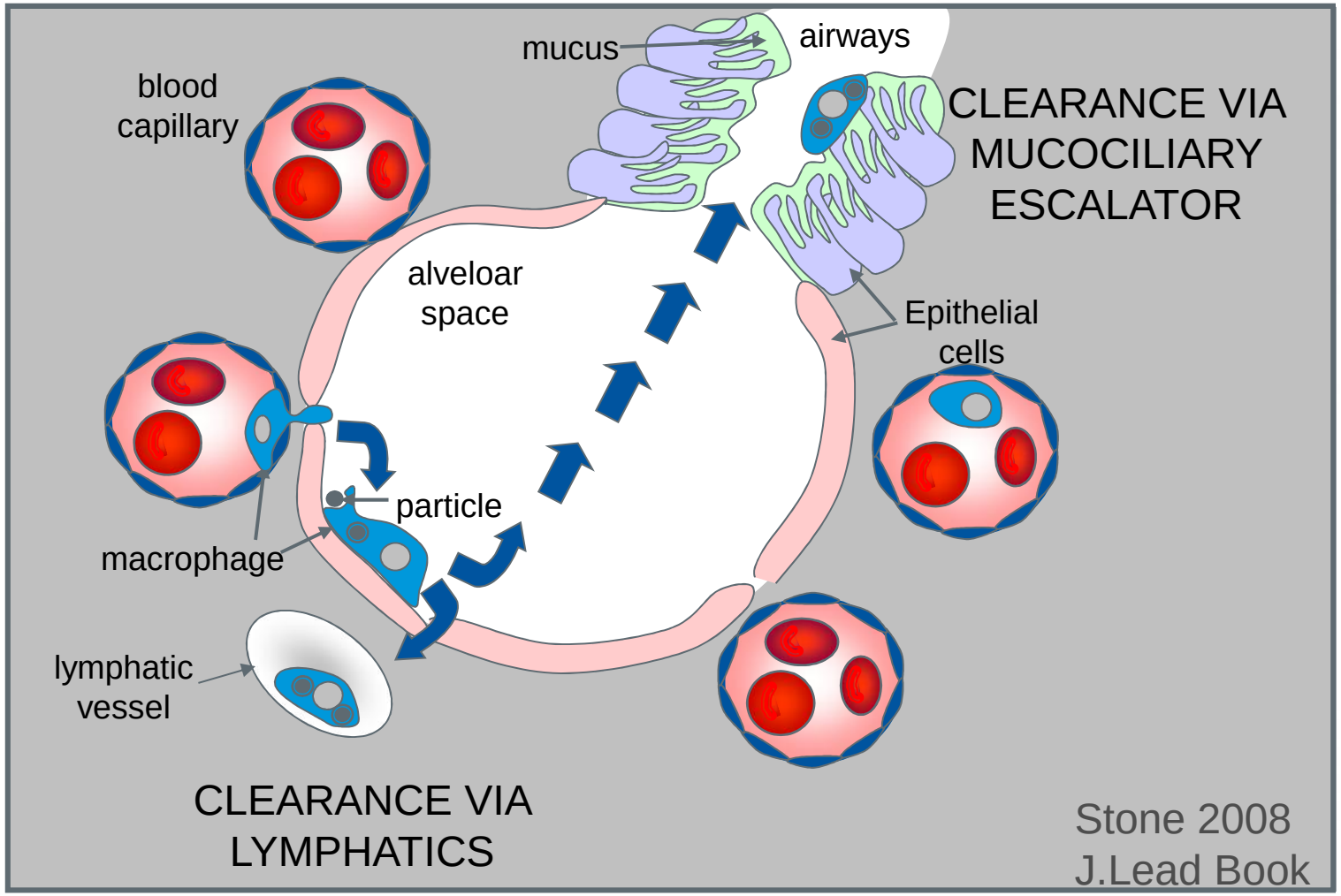
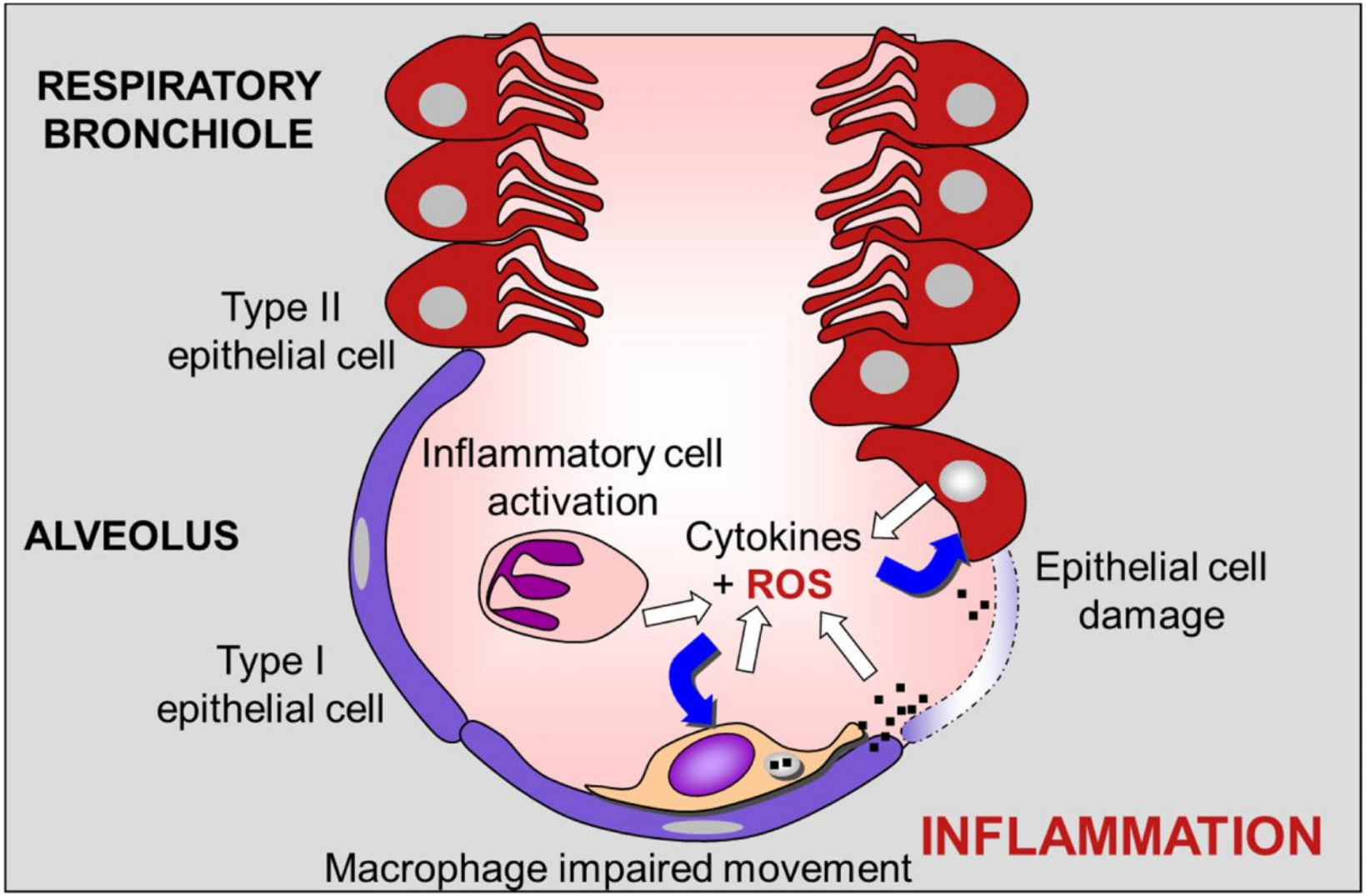
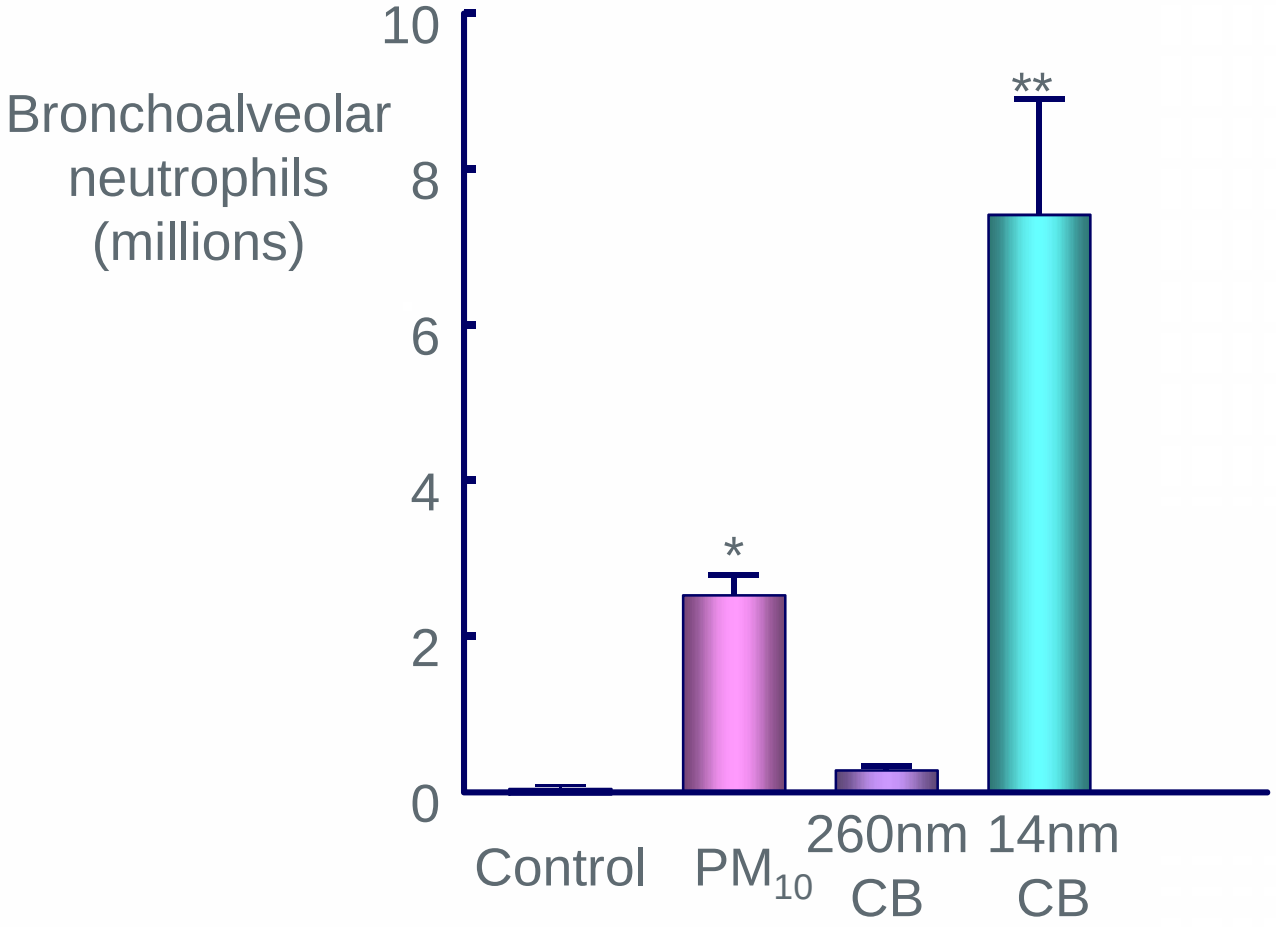
Clearing particles from the lung



The ultrafine particle hypothesis



Ultrafine carbon particles induce inflammation



Time: 6 h
Dose: 125 μg

* p<0.05
** p<0.005

Li *et al*. Thorax1996, 51; 1216-1222.

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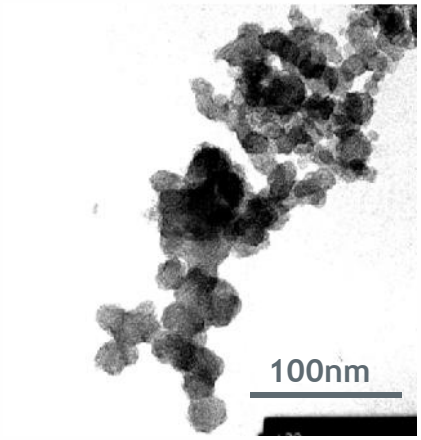
Funding Ultrafine particle Toxicology

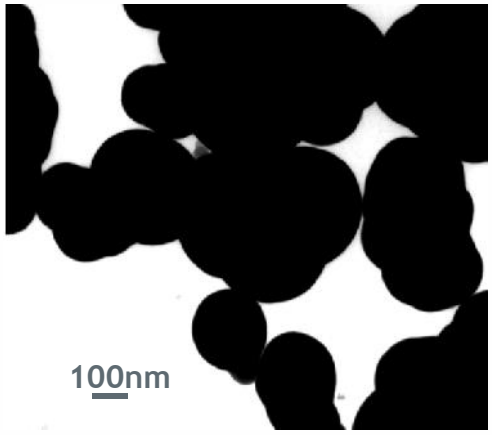
THE COLT FOUNDATION

OCCUPATIONAL AND ENVIRONMENTAL MEDICINE

Model ultrafine particles

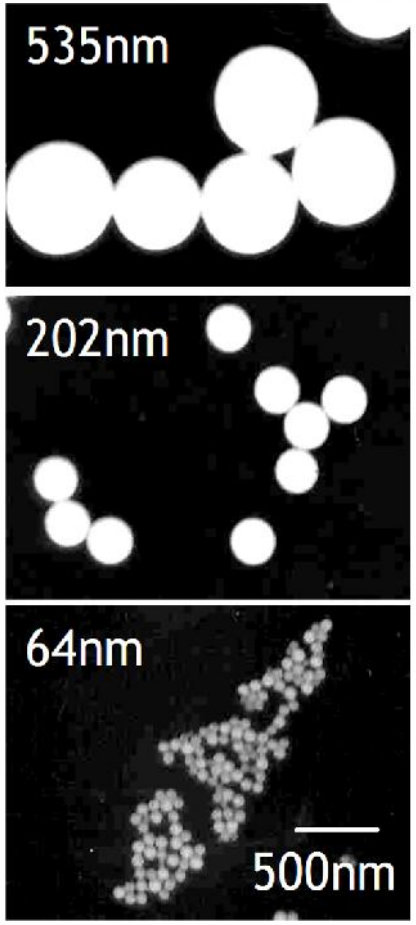


14 nm
254 m^2/g



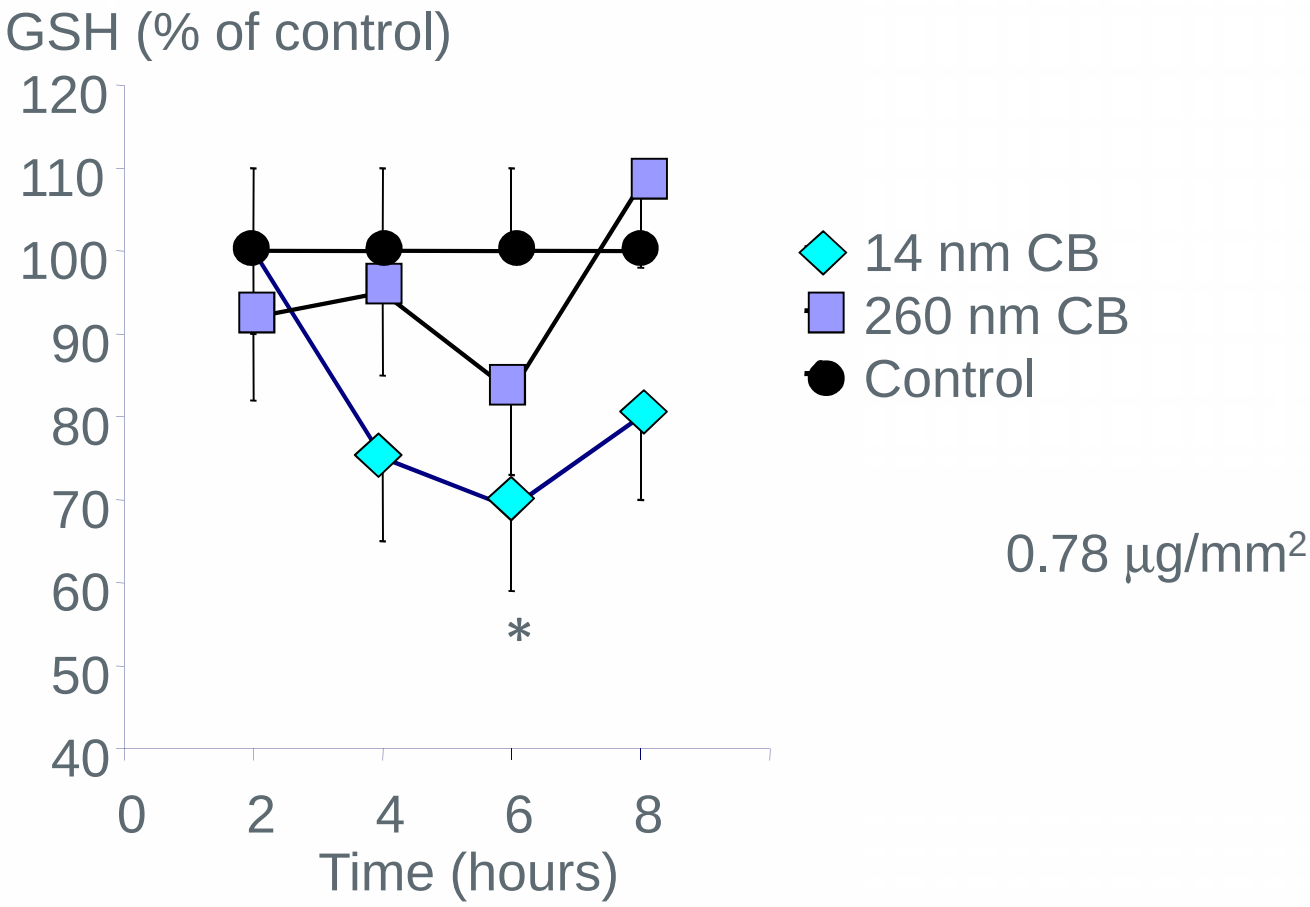
260 nm
8 m^2/g

Carbon black



Polystyrene beads

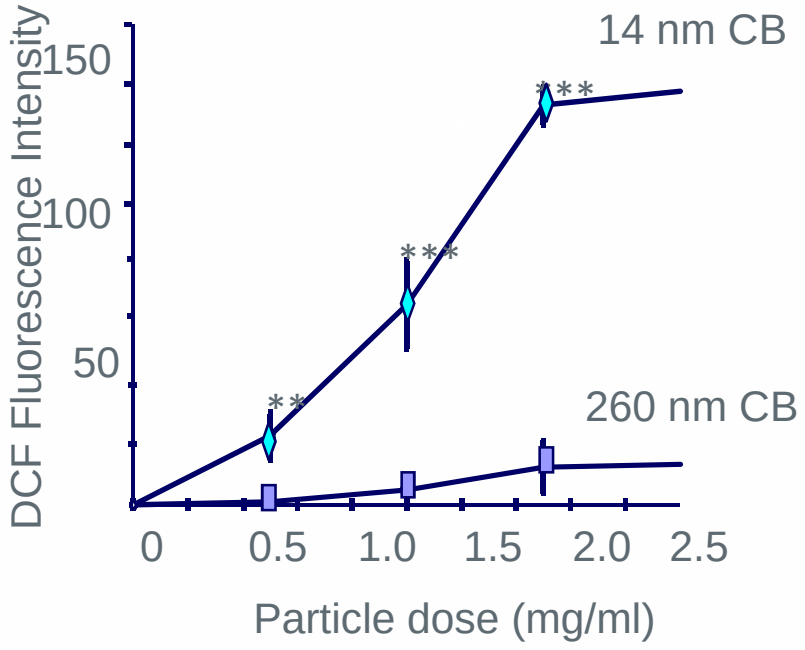
Inducing oxidative stress



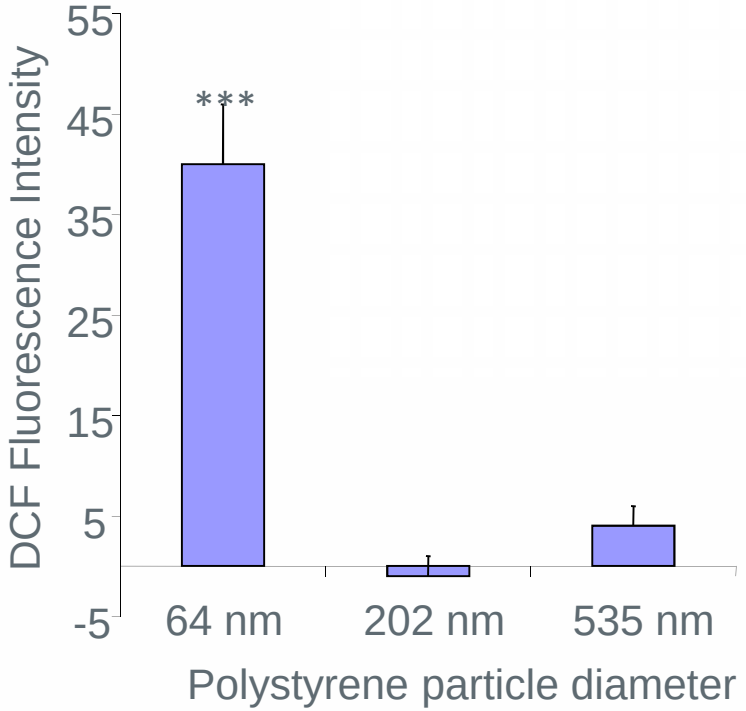
0.78 μg/mm^2

Stone *et al.* 1998 TIV 12: 649-659.

ROS produced by ultrafine particles



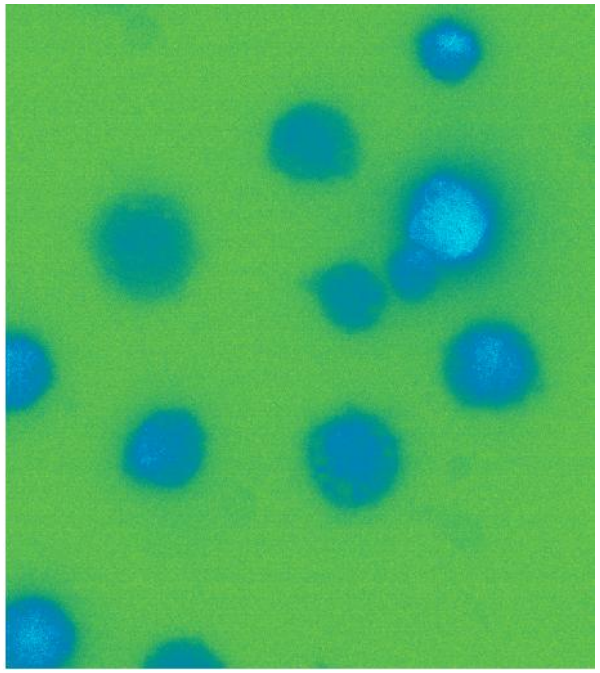
Wilson *et al.* 2002 TAP 184: 172-179.



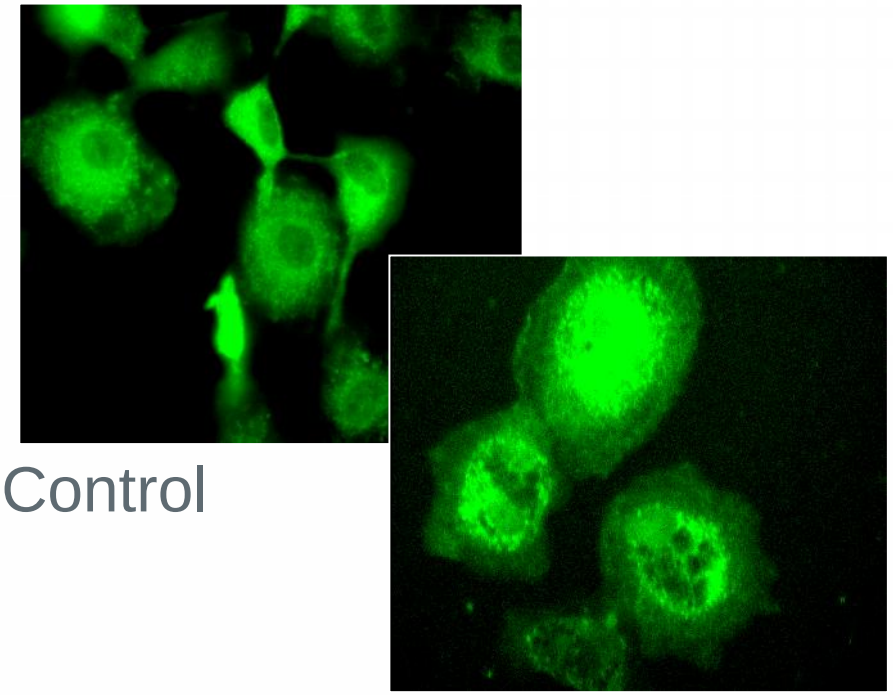
Brown *et al.* 2001 TAP 175: 191-199.

Surface and cell reactivity

Calcium signalling



NF-kB (p65) immunofluorescence



Control

14 nm CB

Brown *et al.* 2004 AJP 286; L344-L353

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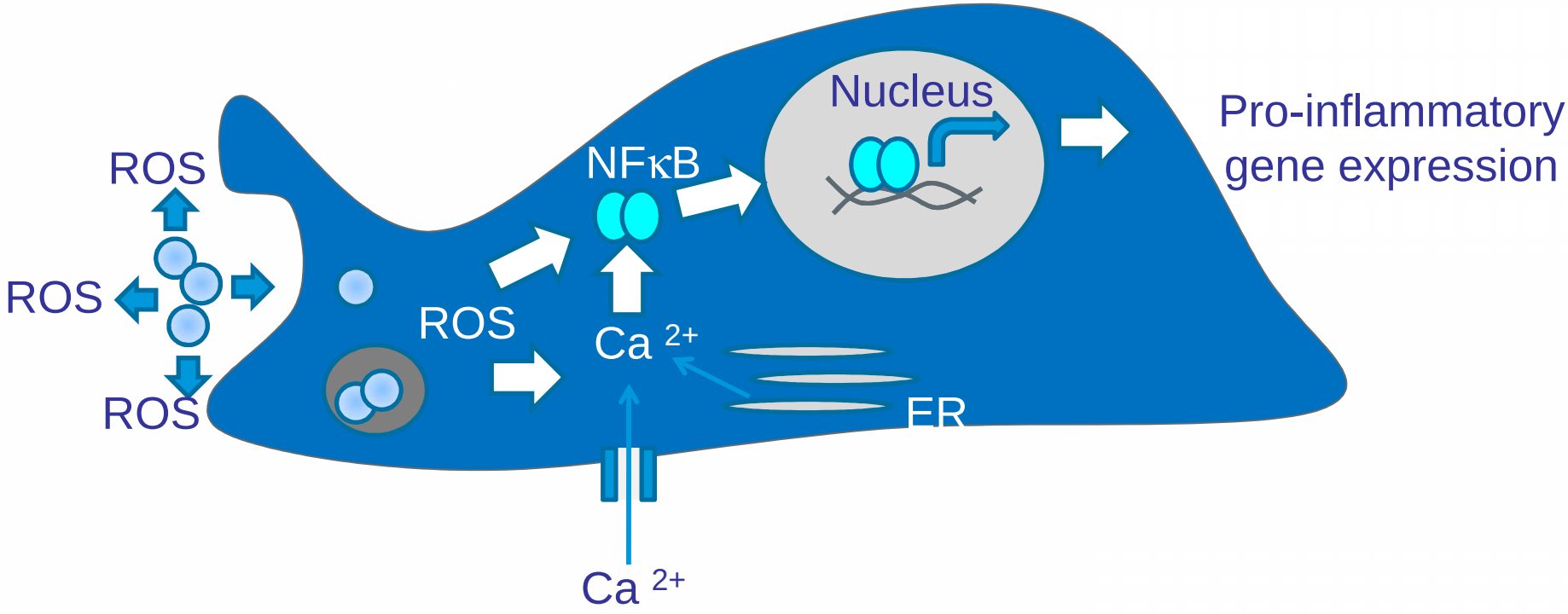
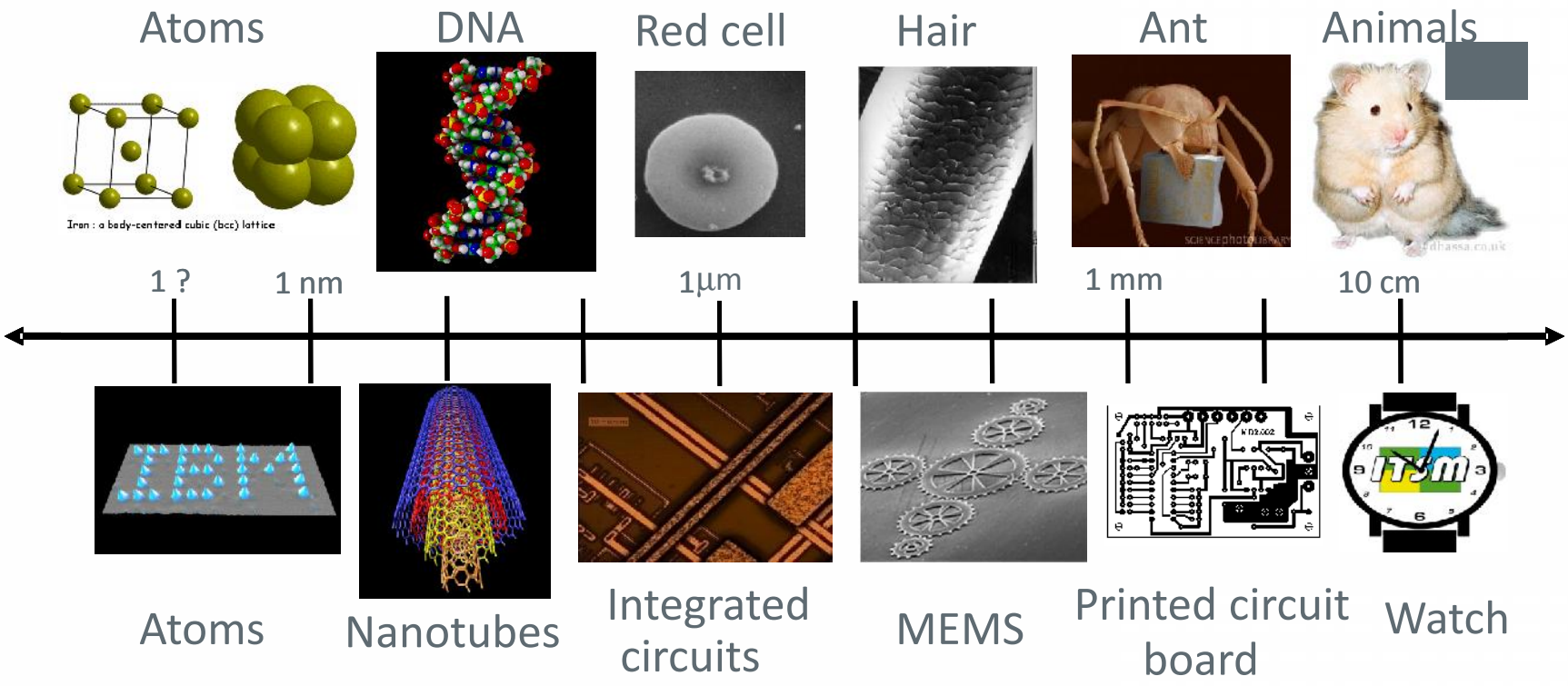
Mechanisms



How small is nano?

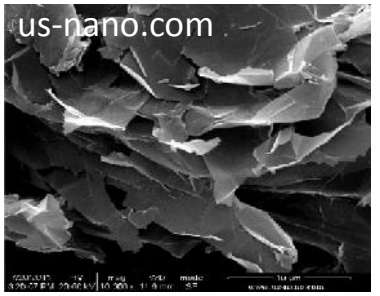
Natural structures

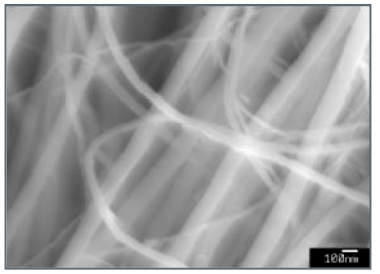


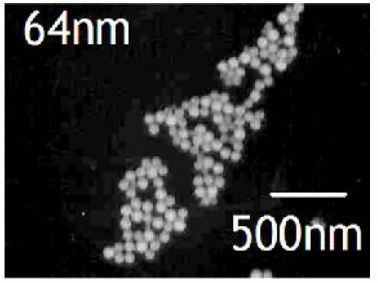
Artificial structures

Definitions

- Nanoscale 1-100nm
- Nano object at least 1 dimension 1-100nm
  - E.g. disc, graphene sheet
- Nanomaterial 2 dimensions 1-100nm
  - E.g. fibre, carbon nanotube
- Nanoparticle all 3 dimensions 1-100nm
  - E.g. sphere or cube





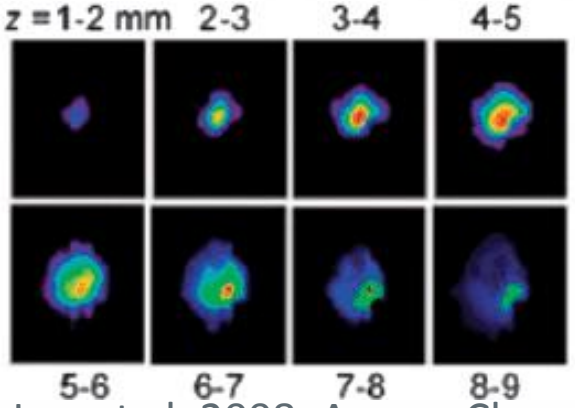


Nano-scale properties

Altered physicochemical characteristics compared to larger forms or the bulk chemical

goldstriker.co.uk

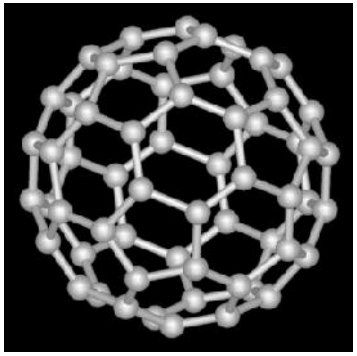
sigmaaldrich.com



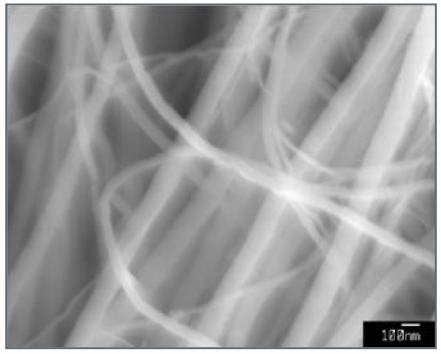
Lee et al. 2008. Angew Chem Int Ed 47; 2804-7

Eustis and El-Sayed 2006 Chem Soc Rev 35; 209-17
Boisselier and Astruc 2009 Chem Soc Rev 38; 1759-82

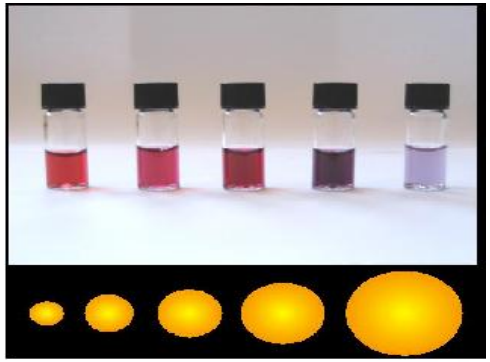
The diversity of nanomaterials



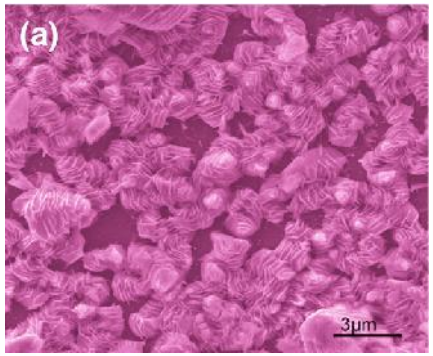
C60 Fullerenes



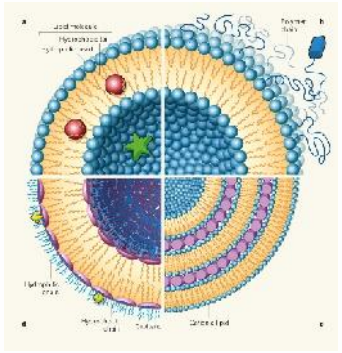
Carbon nanotubes



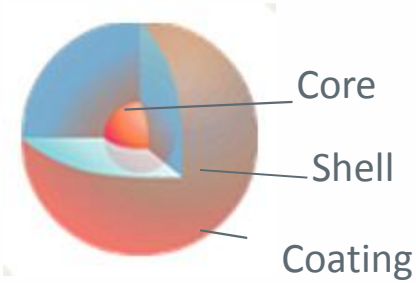
Nanoparticle gold



ZnO nano flowers



Liposomes



Quantum Dots

- Size, shape, charge…
- Matrices…
- Aged, weathered…
- Released…

Exposure to nanomaterials

Human Exposure

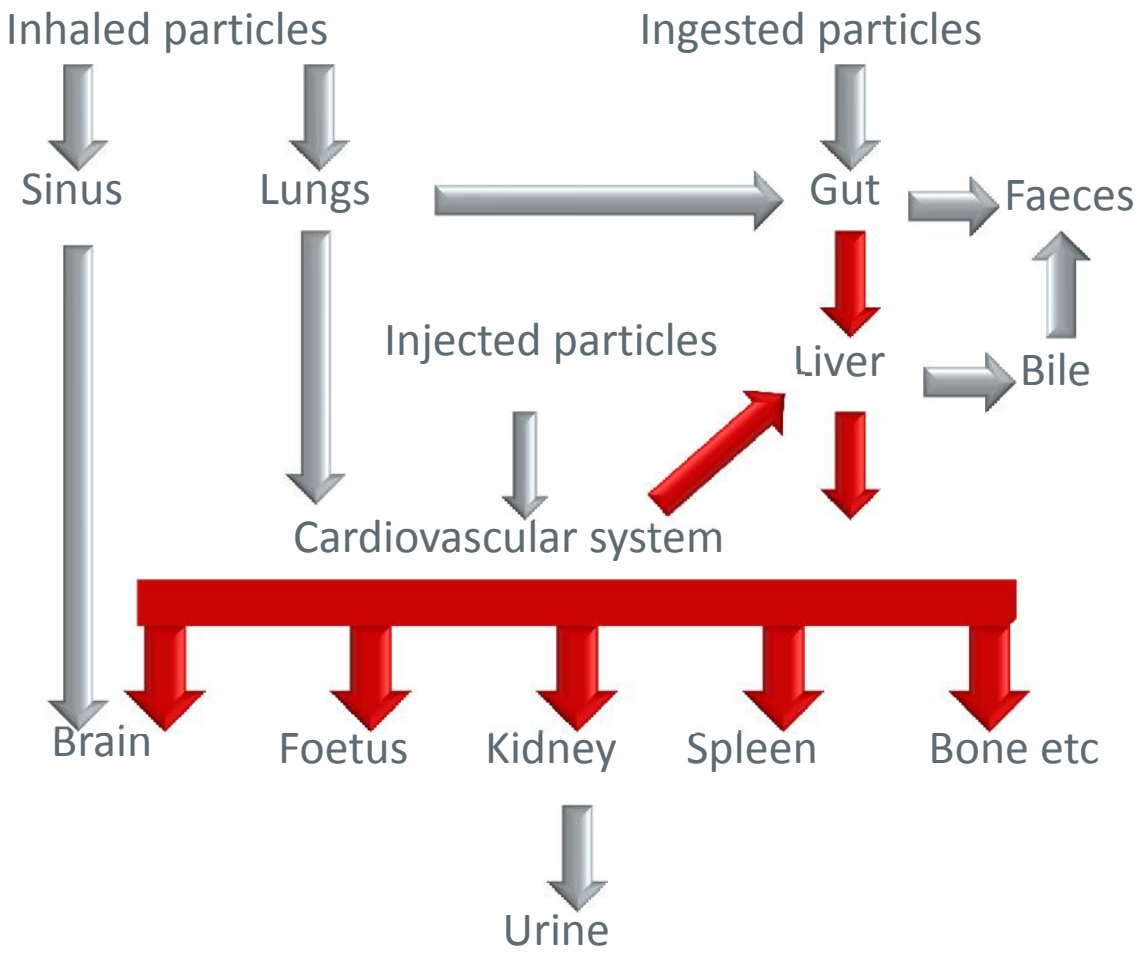
Incidental ↑ Purposeful

Incidental ↓ Purposeful

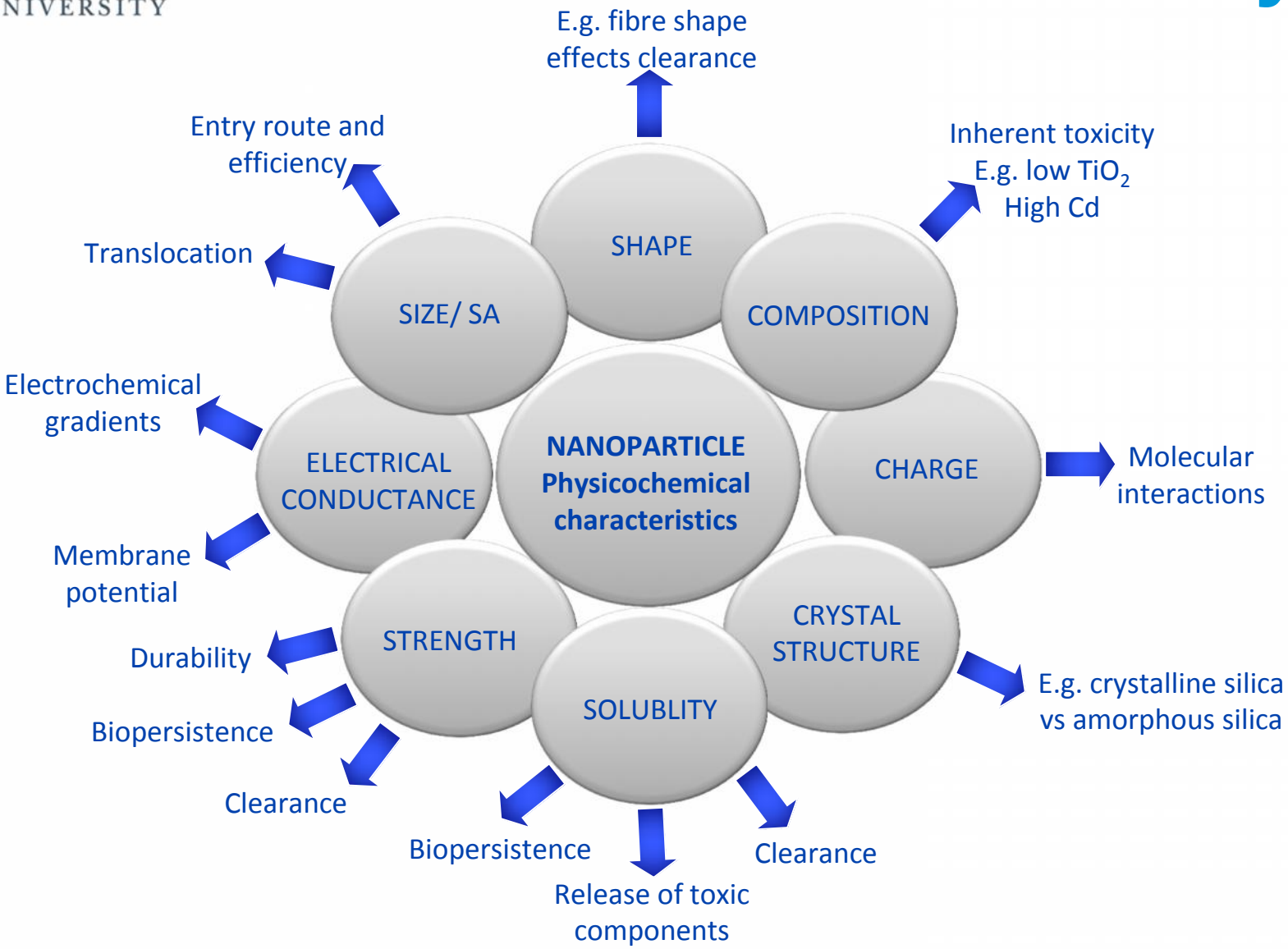
Environmental Exposure

- Size, shape, charge…
- Matrices…
- Aged, weathered…
- Released…

Widening the routes and targets



Characteristics vs Toxicity



Stone and Kinloch Nanotoxicology (book), 2007, Ed Monteiro and Tran.

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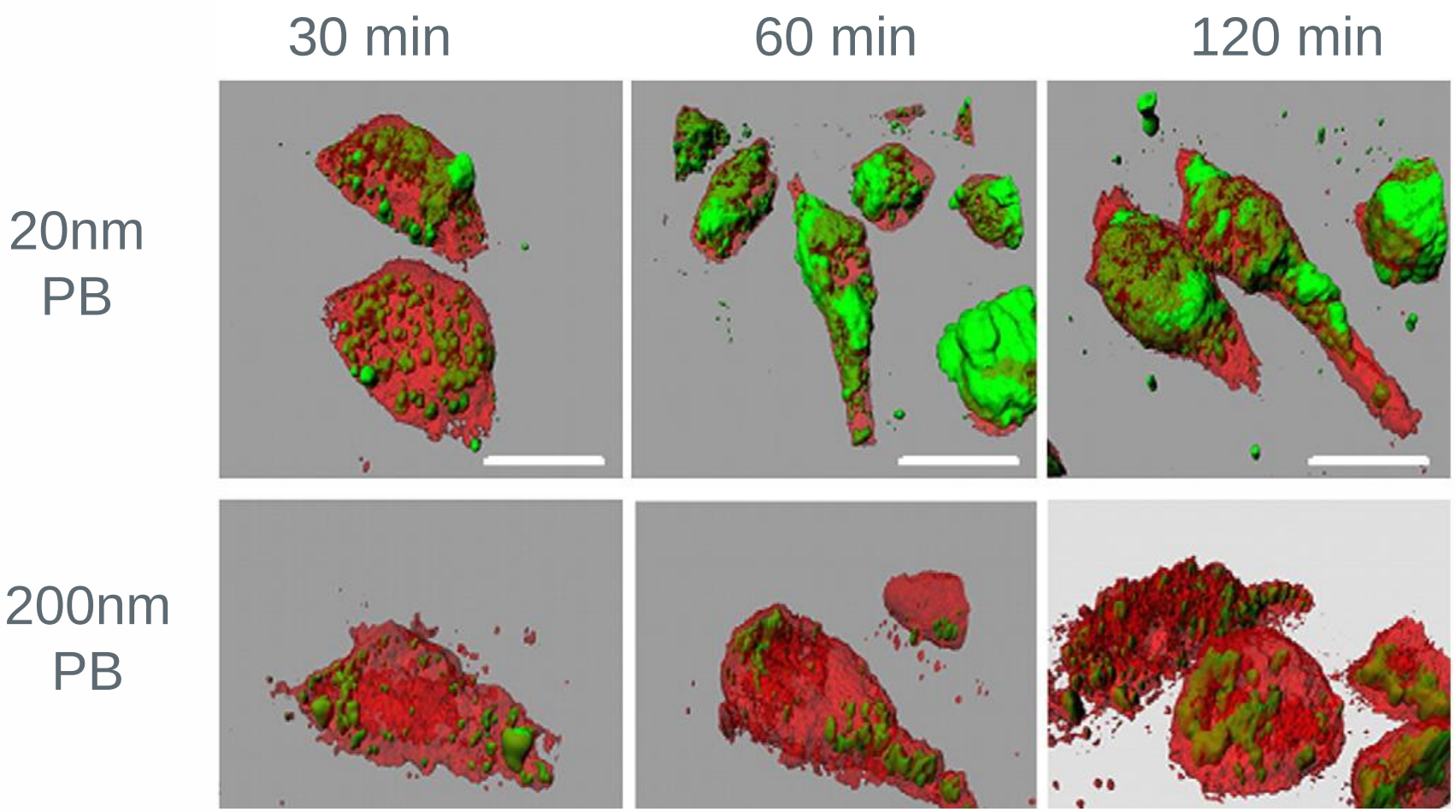
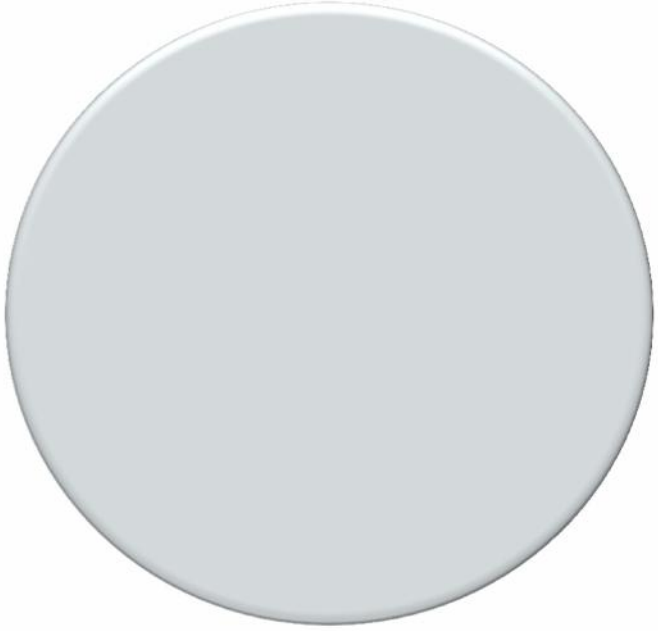
Size and uptake



Clift *et al*. 2008 TAAP 232; 418-427.

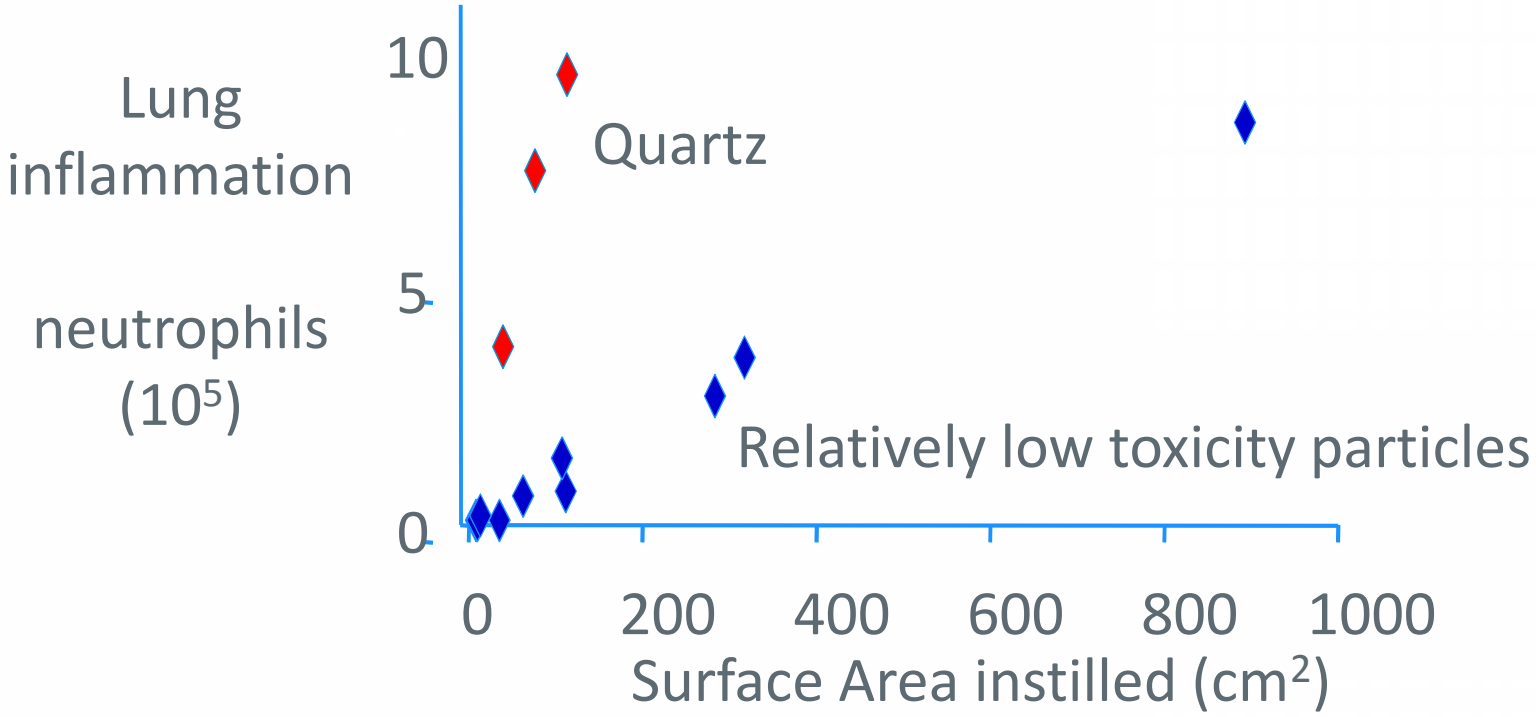
Surface area

Stone and Kinloch Nanotoxicology (book), 2007, Ed Monteiro and Tran.



Diameter	100 μm	10 nm
Surface area	0.03 m^2/g	286 m^2/g
% atoms at surface	0.001 %	10.5 %

Surface area and reactivity



Duffin *et al*. 2002 Ann Occup Hyg 46 [suppl 1]; 242-245.

Fibre induced disease

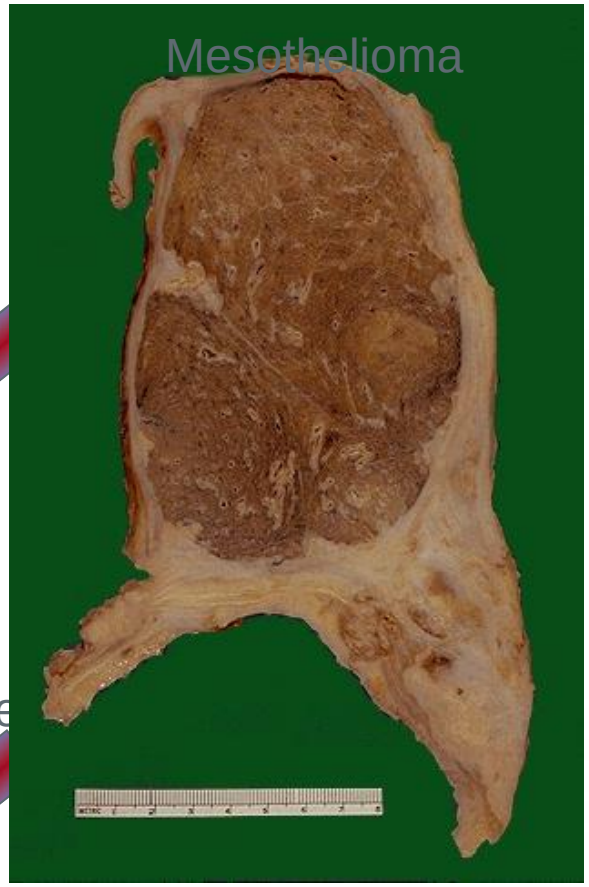
Property Durable Long and thin (High aspect ratio) Iron

Consequence Biopersistent >15um too long to be cleared by macrophages

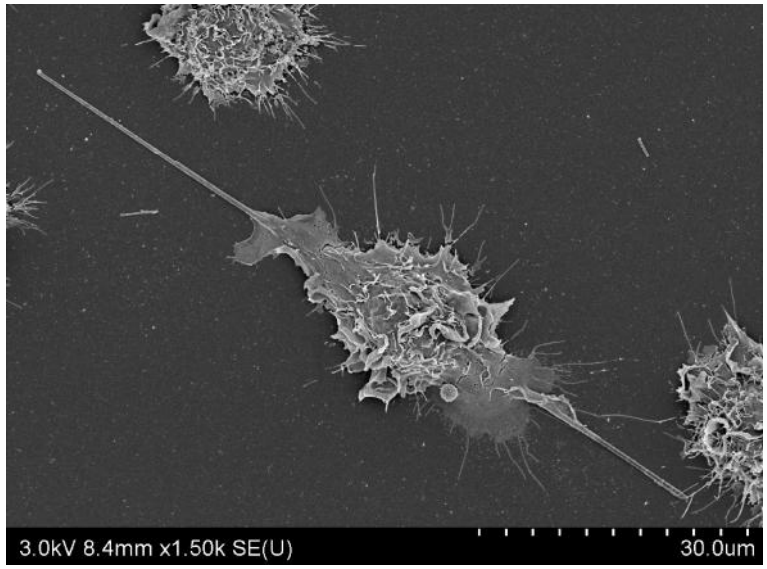
Biological impact Inflammation

Increased cell proliferation Ge

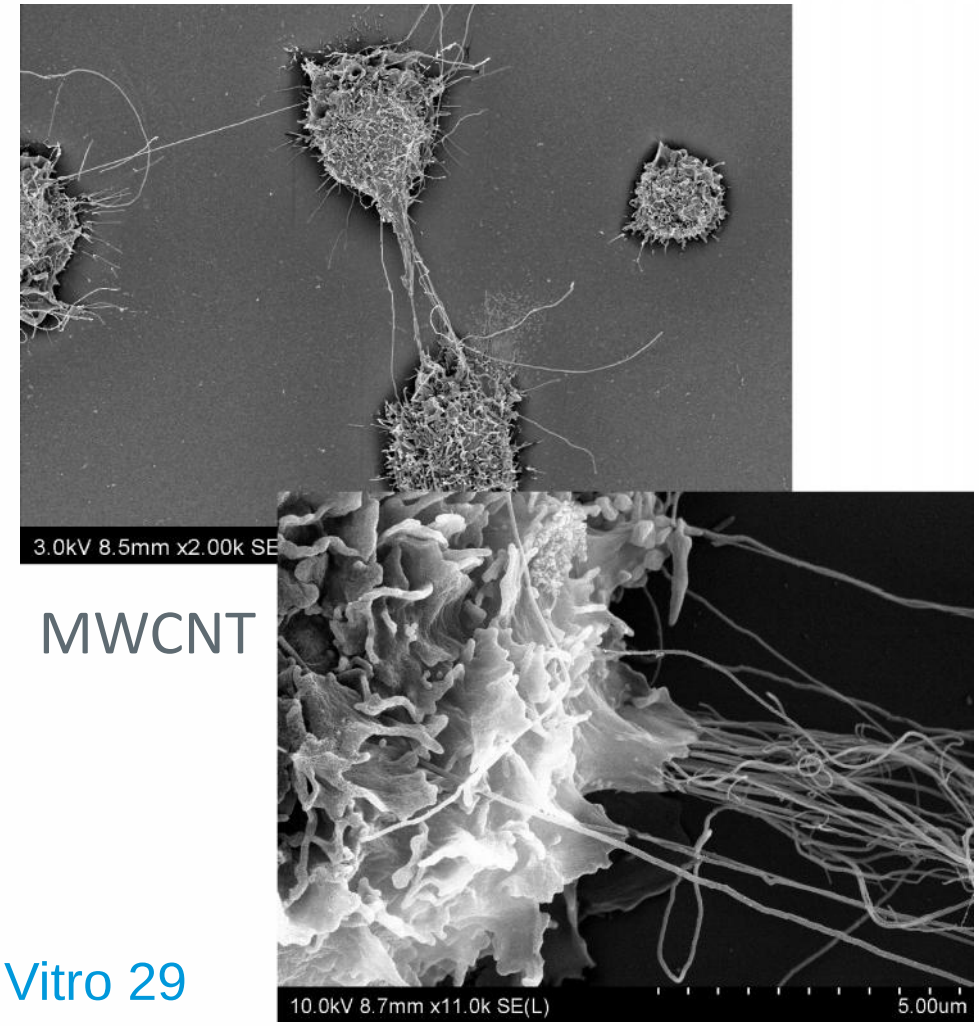
Fibrosis & Cancer



Carbon nanotube frustrated phagocytosis



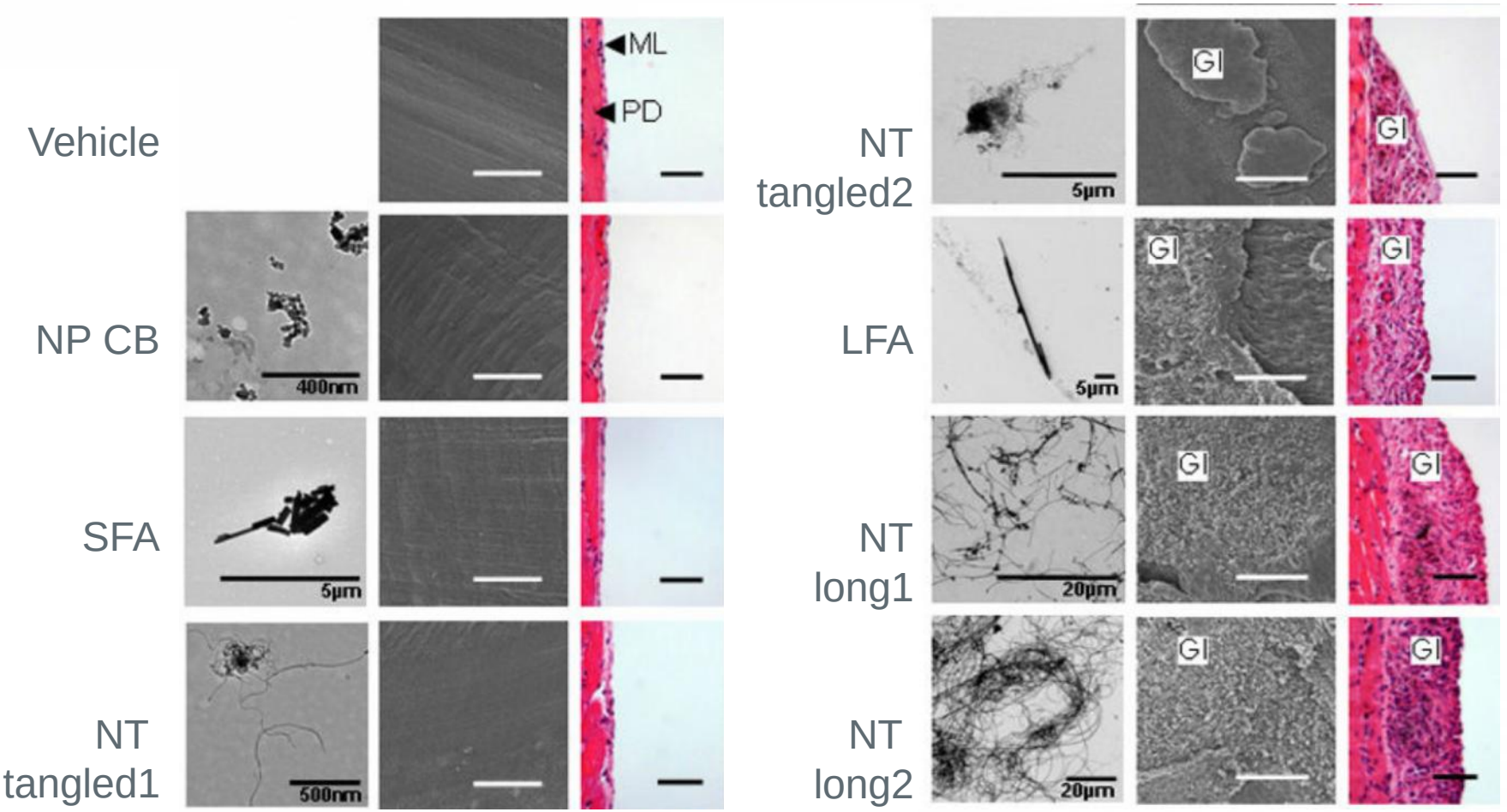
Asbestos



MWCNT

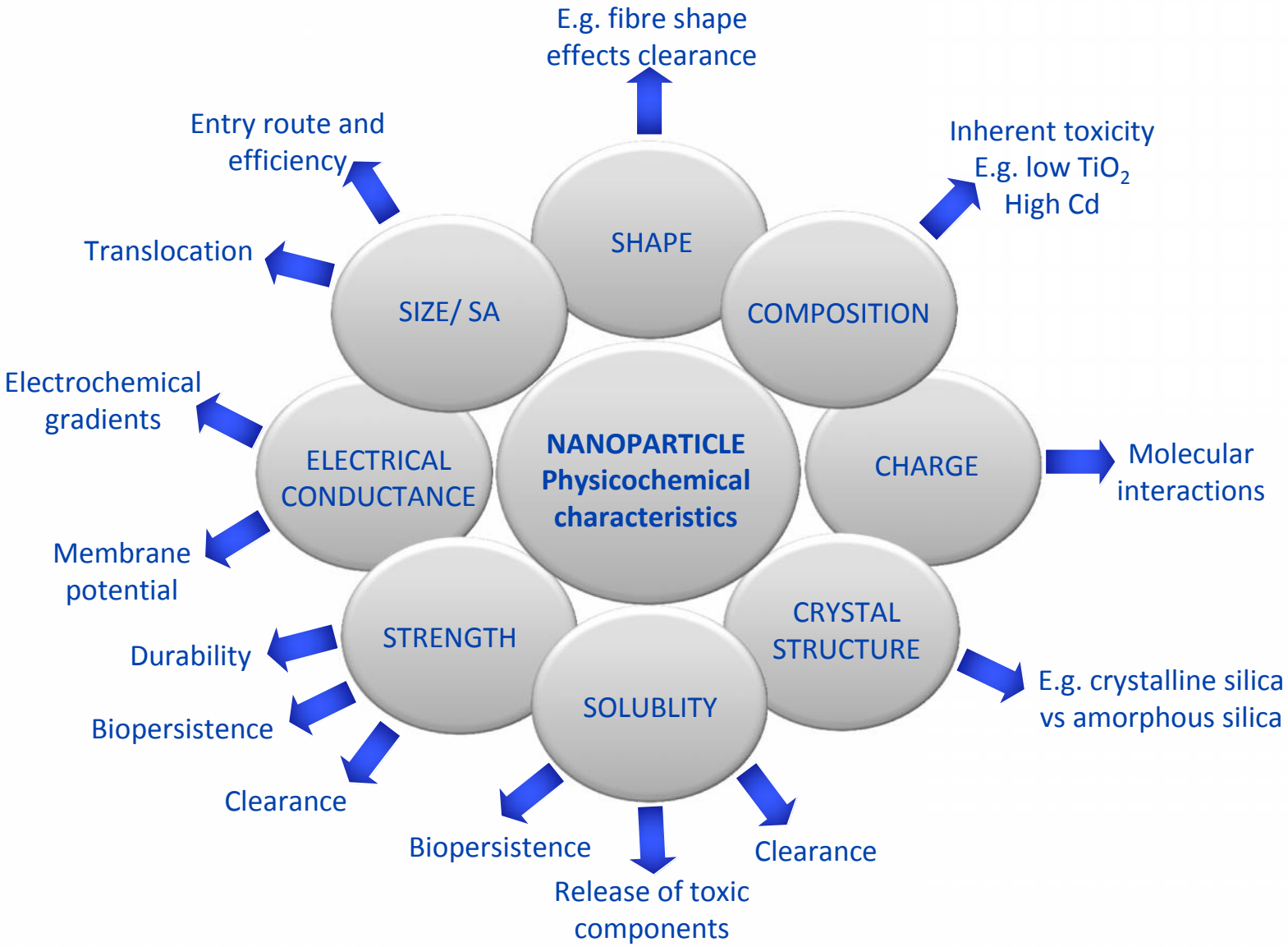
Boyles et al. 2015 Toxicology In Vitro 29

MWCNT induced pathology



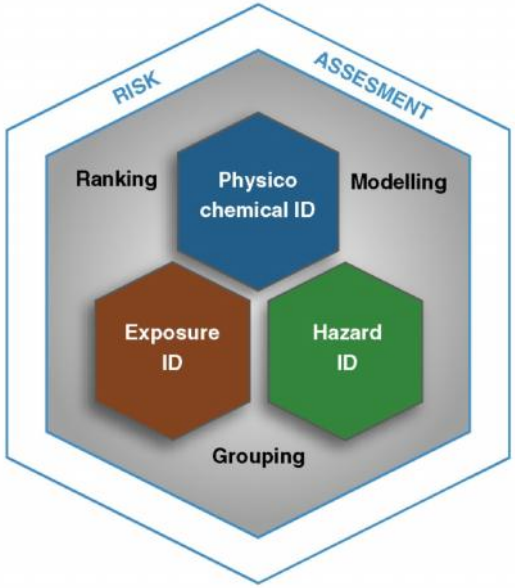
Poland *et al*. 2008 Nature Nanotech 3, 423-428.

Characteristics vs Toxicity



Research prioritization to develop an Intelligent Testing Strategy

- **Short term** - understanding of the connections between physicochemical, exposure and hazard IDs to enable **grouping/ ranking**.
- **Longer term** - development of **modelling** approaches for RA with a continual reduction in testing.
- In the **distant future** RA based on modelling and extrapolations and only if additional information is required with focused **physicochemical, exposure and hazard testing**.



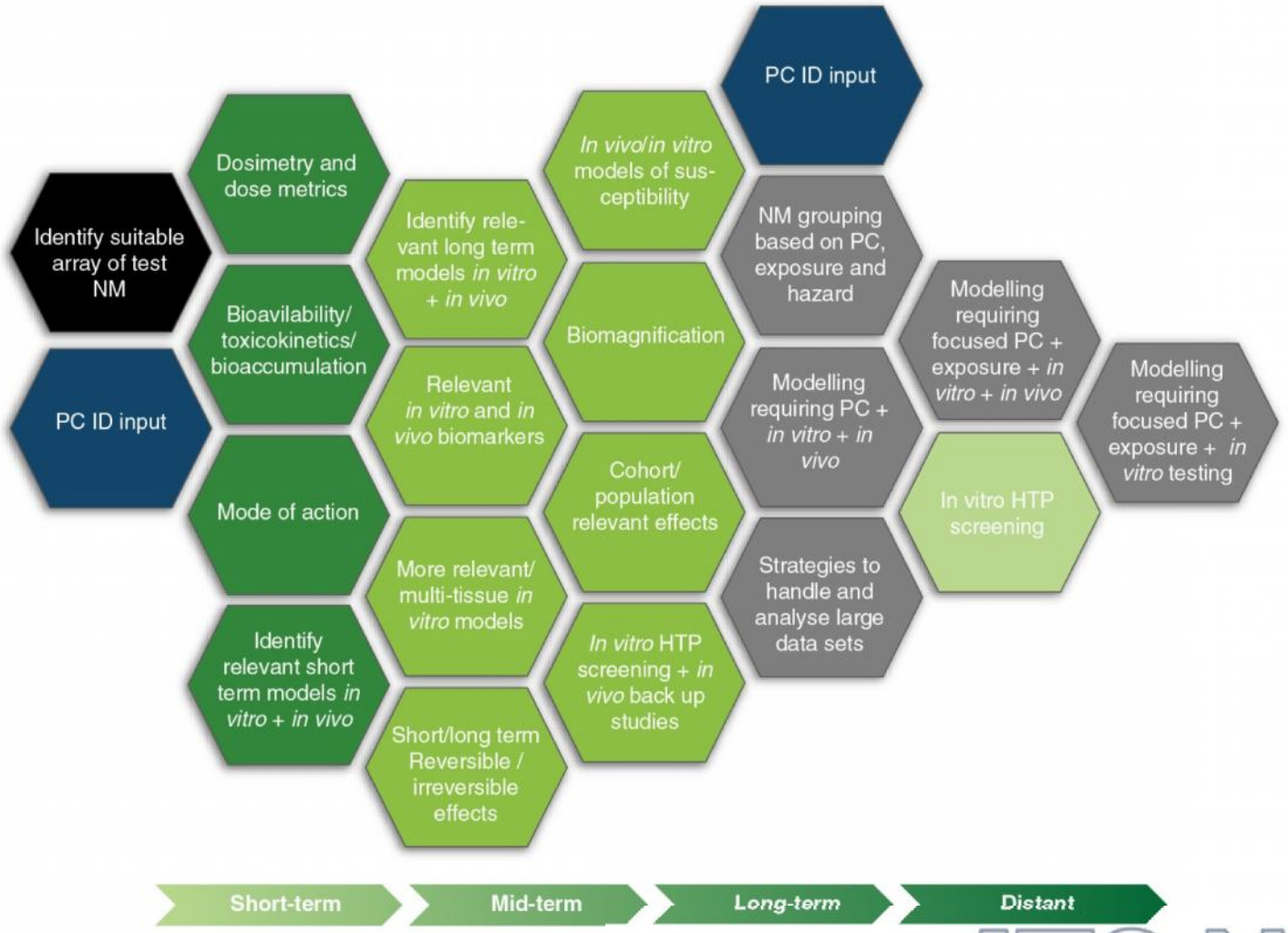
Stone et al. 2014
Part Fibre Toxicol 11, 9.

Hazard - final recommendations

- Develop more appropriate **dose metrics** for **dosimetry** studies.
- Determine the **mode of action** and identify reliable **biomarkers**.
- Assess the **bioavailability** and **toxicokinetics**.
- **Standard protocols; *in vitro* and *in vivo, s*hort and long term**
- Additional **time course** knowledge; **rapid/delayed, reversible/irreversible**
- **Population effects** and **biomagnification**.
- *In vitro* and *in vivo* **models of susceptibility**
- **New tests should**, become **HTP** and eventually ***in silico***.
- Future Hazard ID's will become focused as ***in silico*** approaches develop.
- The *in silico* models should aim to **extrapolate knowledge** across **NMs, target tissues and taxa**.

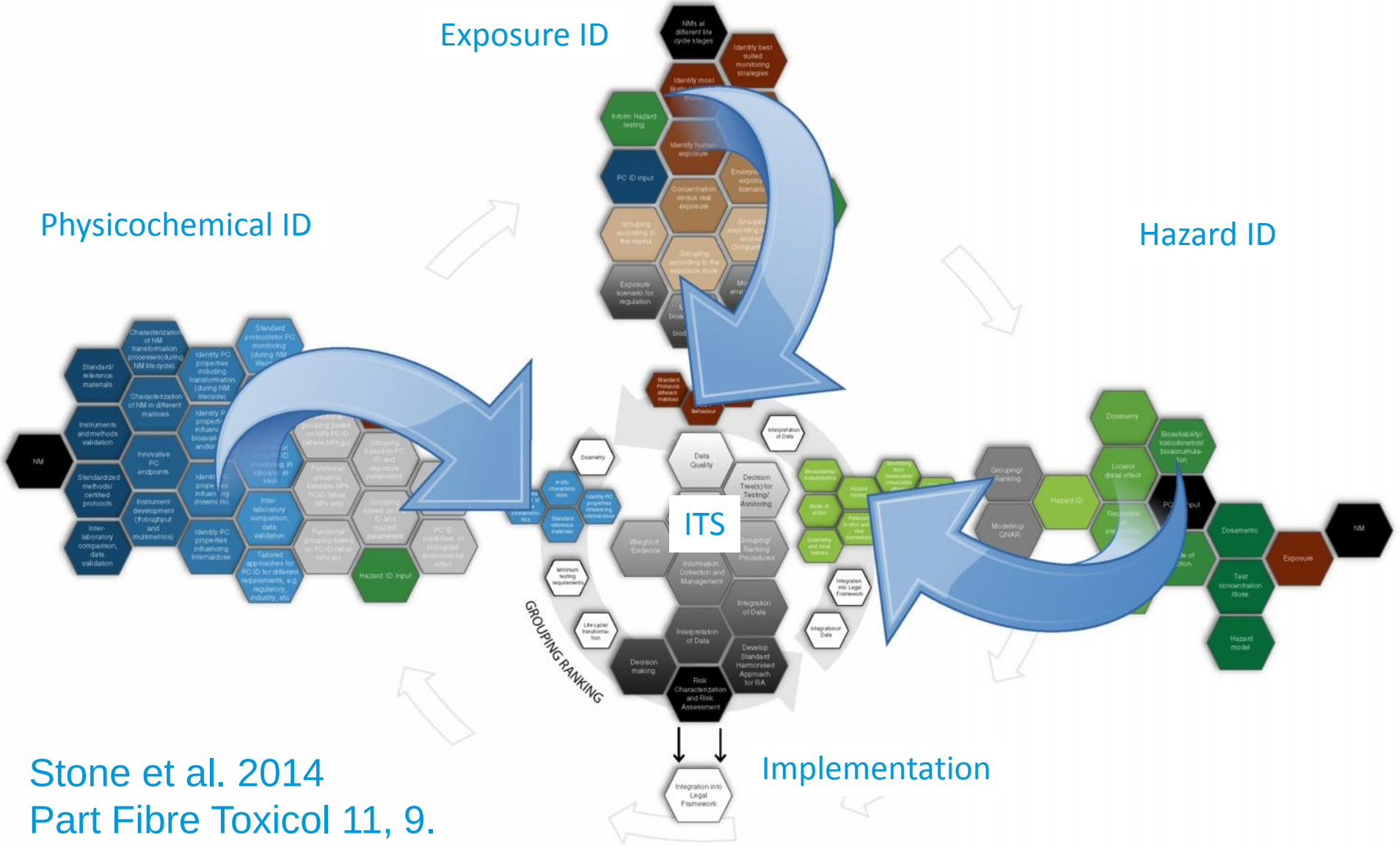
Stone et al. 2014
Part Fibre Toxicol 11, 9.

Hazard Priorities



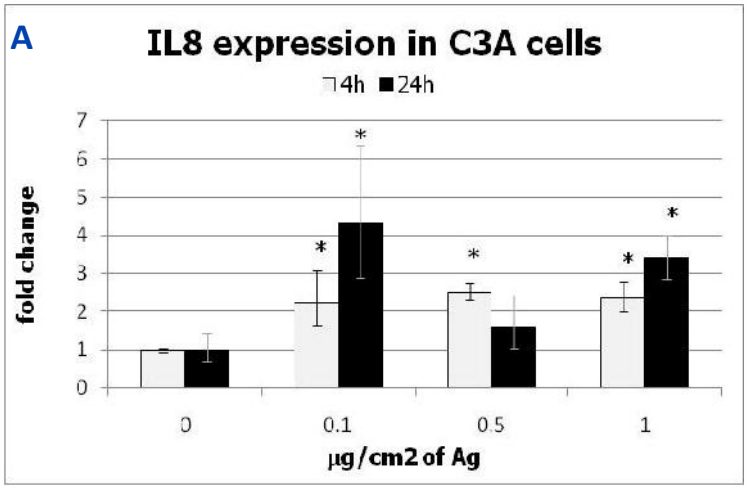
Stone et al. 2014
Part Fibre Toxicol 11, 9.

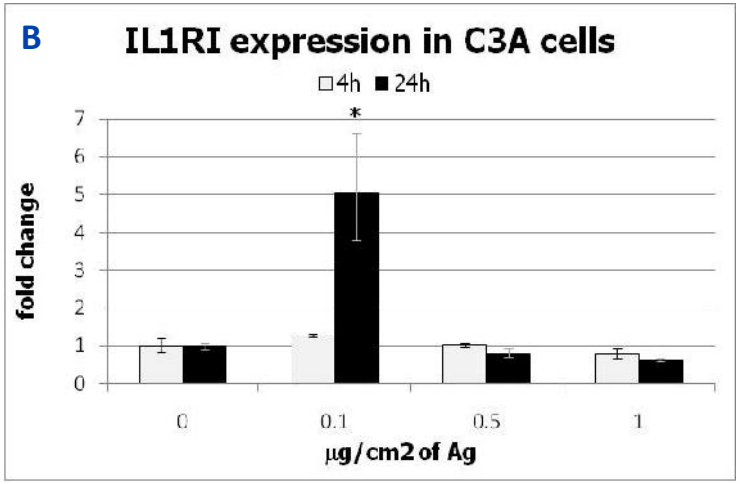
ITS-NANO Research Prioritisation

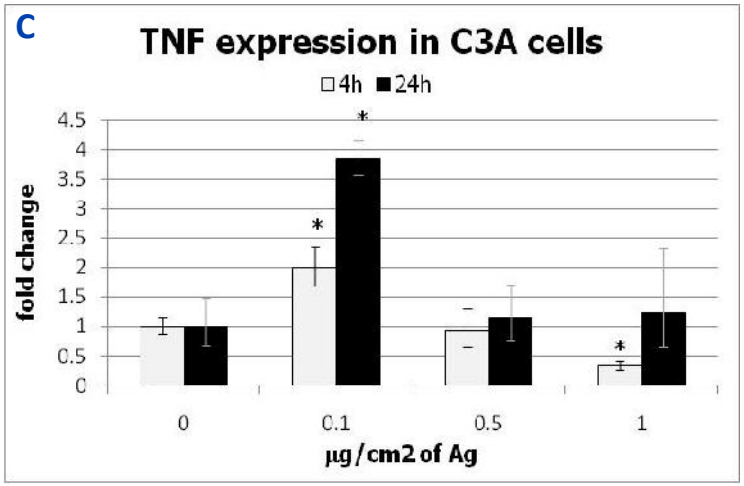


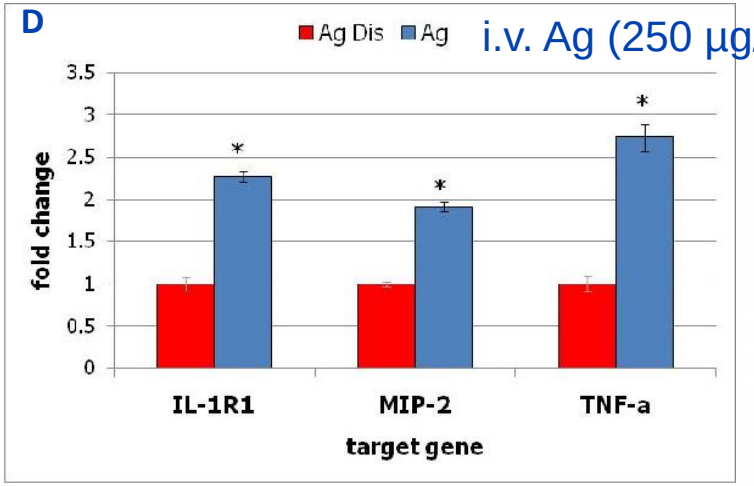
Stone et al. 2014
Part Fibre Toxicol 11, 9.

How relevant are *in vitro* models?







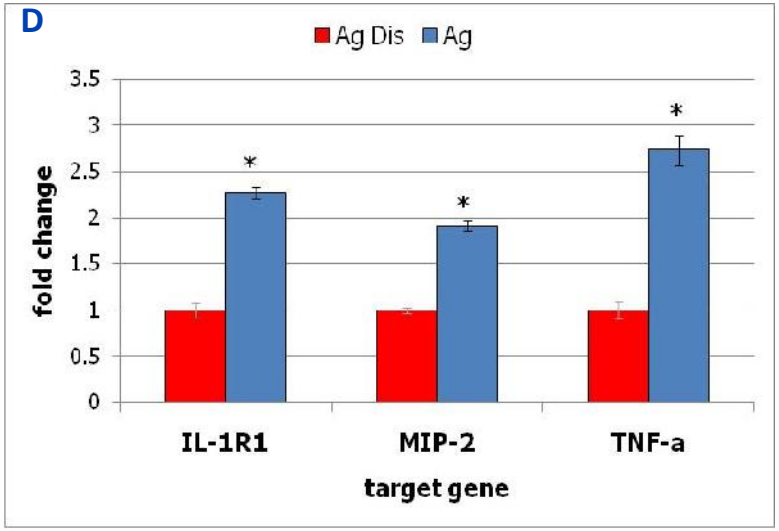


i.v. Ag (250 µg/kg)

Gaiser, et al., 2013 Toxicol Sci. **131**, 537-547

Liver inflammatory gene expression I.V. vs Gavage

i.v. Ag (250 µg/kg)



Gavage Ag 250 µg/kg

Ag 250

tissue	liver	intestine	PPs
gene	gavage	gavage	gavage
FasL			
GSH Pox			
IL-1b			
IL-1R1			
IL-10			
MCP-1			
MIP-2			
TNF			

increase

decrease

no change

Gaiser, et al., Toxicol Sci. **131**, 537-547

Understanding Limitations

- Can a culture of hepatoma cells *in vitro* be used to predict liver responses to nanomaterials?
  - Very good at predicting liver responses to injected Ag, TiO_2 and Au
  - OK at predicting liver responses to pulmonary delivered Ag, TiO_2, MWCNT and ZnO
  - Poor at predicting liver responses to ingested NP

- Intravenous– particles direct into plasma,
- Inhaled – particles become coated in lung lining fluid
- Ingested – particles modified by gut contents
- *In vitro* – particles become coated in medium components

Toxicology model development

Barbara Rothen-Rutishauser

Hans Bouwmeester

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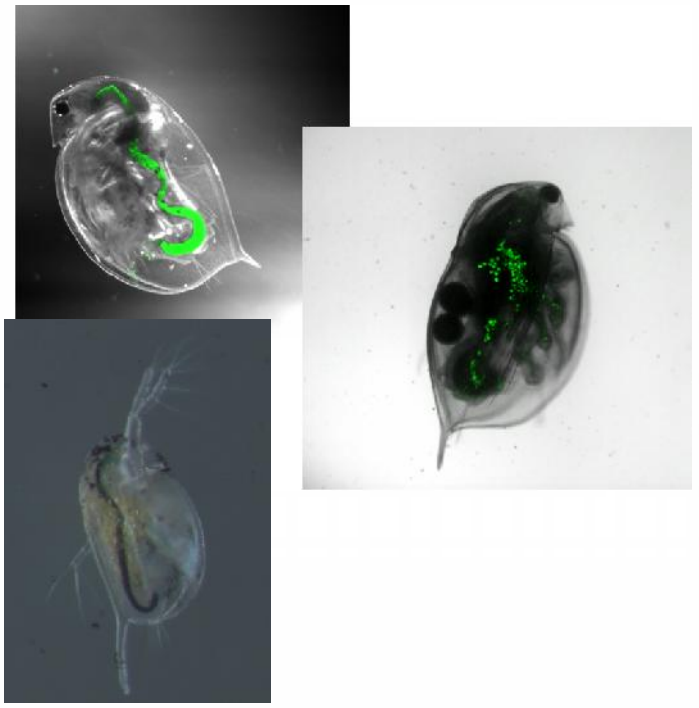
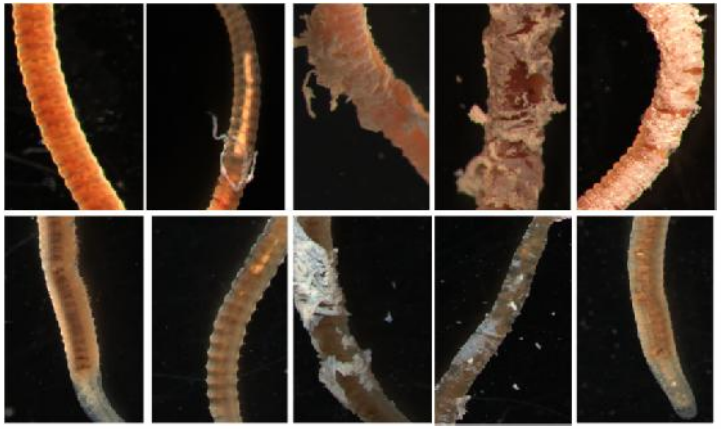
Ecotoxicology of nanomaterials

Teresa Fernandes





CuO nanoparticle – Industrial collaboration

Tiered strategy

Stage 1: Identify existing data

Stage 2: Prioritise NM

lacking data,

likely to be toxicity due to chemistry,

potential for exposure and

high volume of use

Stage 3: Assess toxicity *in vitro* with macrophages and hepatocytes, compared to other nanomaterials and relevant salts.

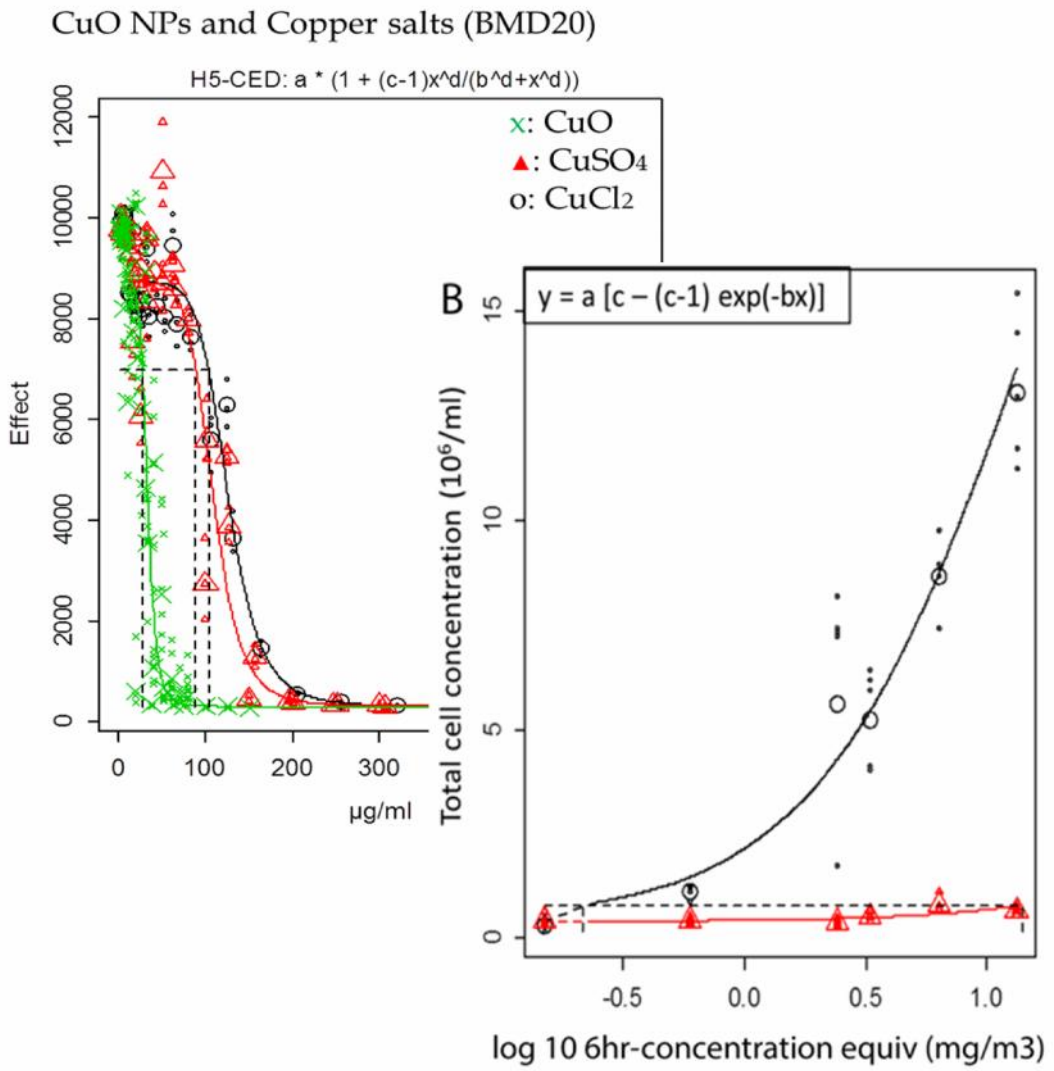
Stage 4. Use *in vitro* data to design dose range for rodent inhalation study

CuO

CuO nanoparticle – Industrial collaboration

Tiered strategy results pristine CuO

- Relatively toxic *in vitro* compared to other NM and Cu salts
- Produced inflammation in lung at day 6
- Inflammation largely resolved by day 28, but small elevation of macrophages remains



CuO nanoparticle – in polymer matrix

- Within product, CuO NPs are embedded in a polymer matrix
- Impossible to generate fragmented particles at room temperature.
- Only possible to generate fragmented particles in very cold conditions while wet.
- This suggests exposure to fragmented particles containing CuO NPs is likely to be low or unlikely
- Therefore risk to consumers/workers once incorporated should be low
- Currently no plans to assess toxicity of fragmented particles.

Safety by Design

Details	Label	BMD_{20}
Pristine	CuO	33.2
Buffer	101	18.7
Citrate	102	25.7
PVP	103	16.6
PEI	104	20.4
Ascorbate	105	64.8

Phosphate buffer

Surface modification

BMD_{20} Bench mark dose 20 %

20% increase in toxicity over the control value

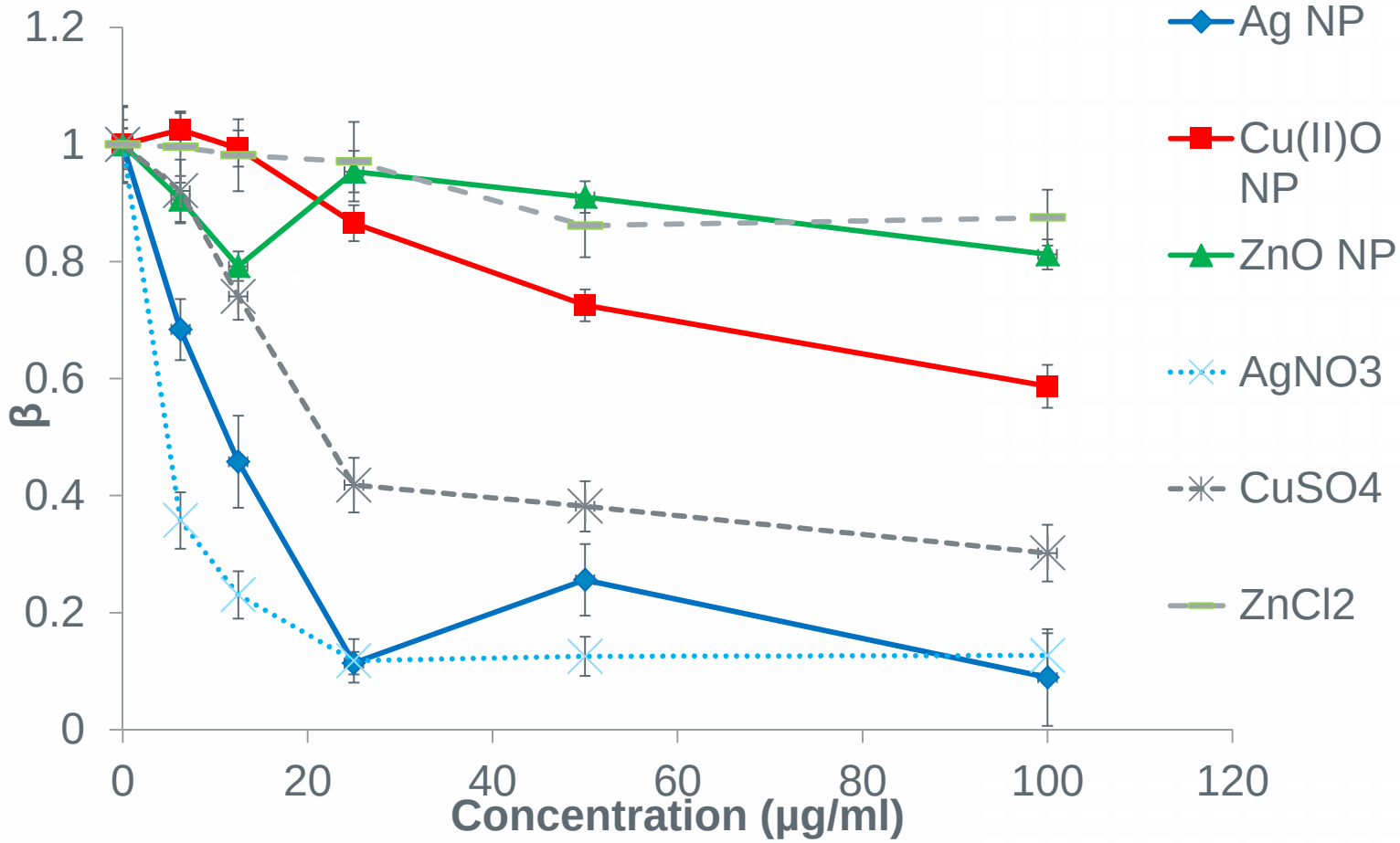
PVP Polyvinylpyrrolidone

PEI Polyethylenimmine

Using nanomaterials to target TB

- Pathogenic *Mycobacterium* species are slow growing, difficult and expensive to culture *in vitro.*
- Surrogate organism: *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) which causes paratuberculosis or Johnes disease in ruminants.
- Use a *K10 Map* GFP organism to generate fast (7 day vs 7 weeks) fluorescence based assay for assessing efficacy of antibiotics.
  - Donnellan et al. 2015 Nanotoxicology

Using nanomaterials to target TB



*β represents the change in fluorescence (representing mycobacterial growth) per day

**Each point displaying 2* the SEM (n=min of 3)

(Donnellan et al., Nanotoxicology 2015)

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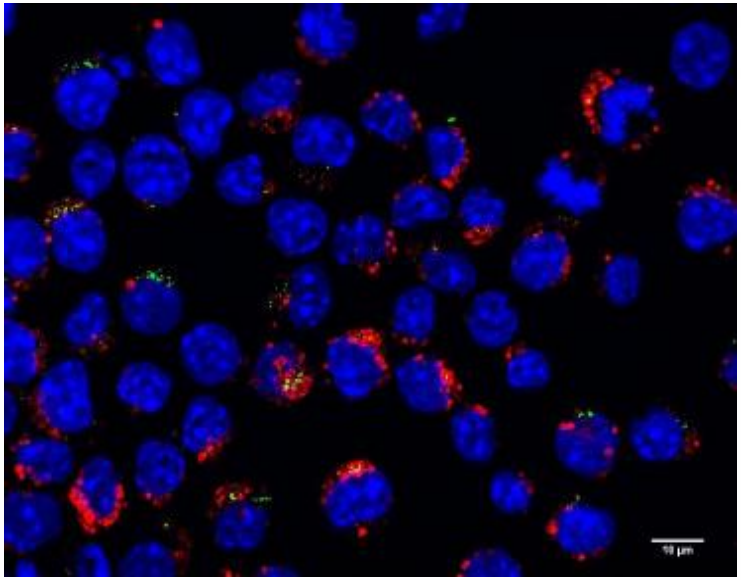
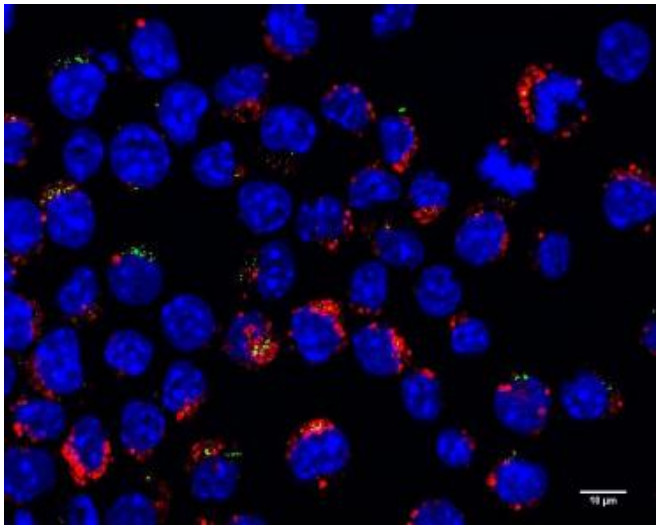
Solid Drug Nanoparticles

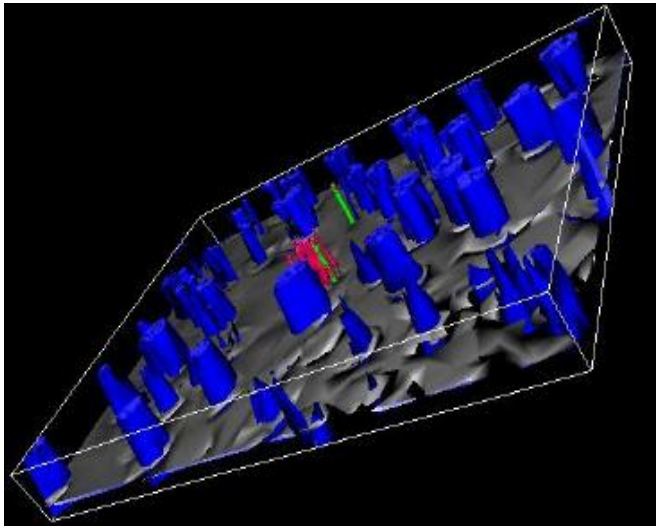
Andrew Owen

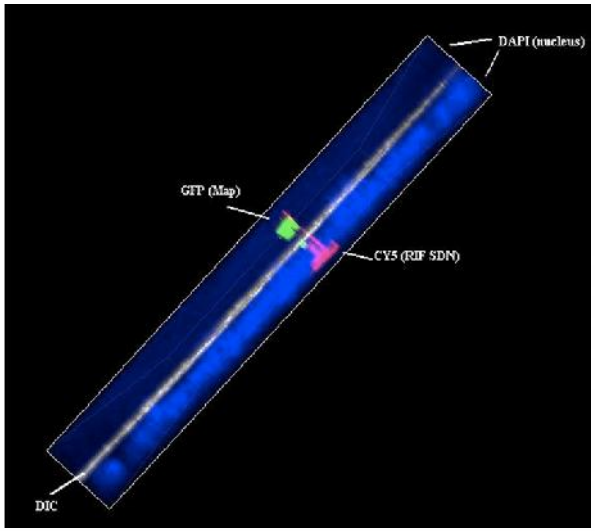


Colocalisation of Mtb and Solid Drug Nanoparticles

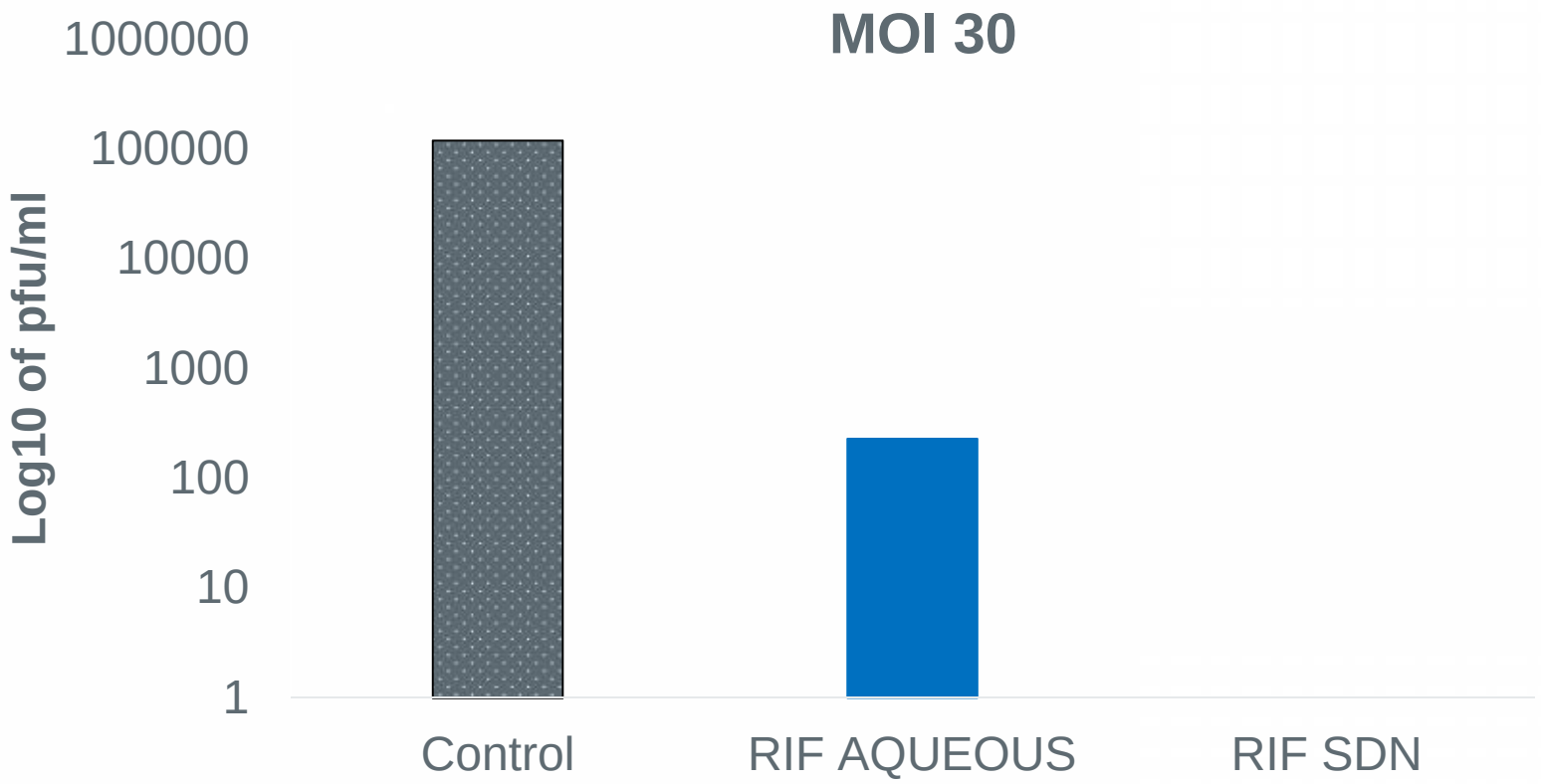


J774 murine macrophage cell line
Blue – DAPI stain of DNA
Green – K10 Map GFP
Red – SDN rifampicin





Intracellular killing of *K10* *Map* GFP by SDN Rifampicin



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Summary

- Factors influencing toxic potential include size, shape, composition, charge, dissolution….
- Toxicity involves promoting inflammation via oxidative stress and intracellular signalling via calcium
- Intelligent testing strategies are needed to reduce testing
- Toxicity information can be used to prioritise decision making for produce us and for design
- Nanomaterials can be used to target mycobacterial

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THANK YOU!


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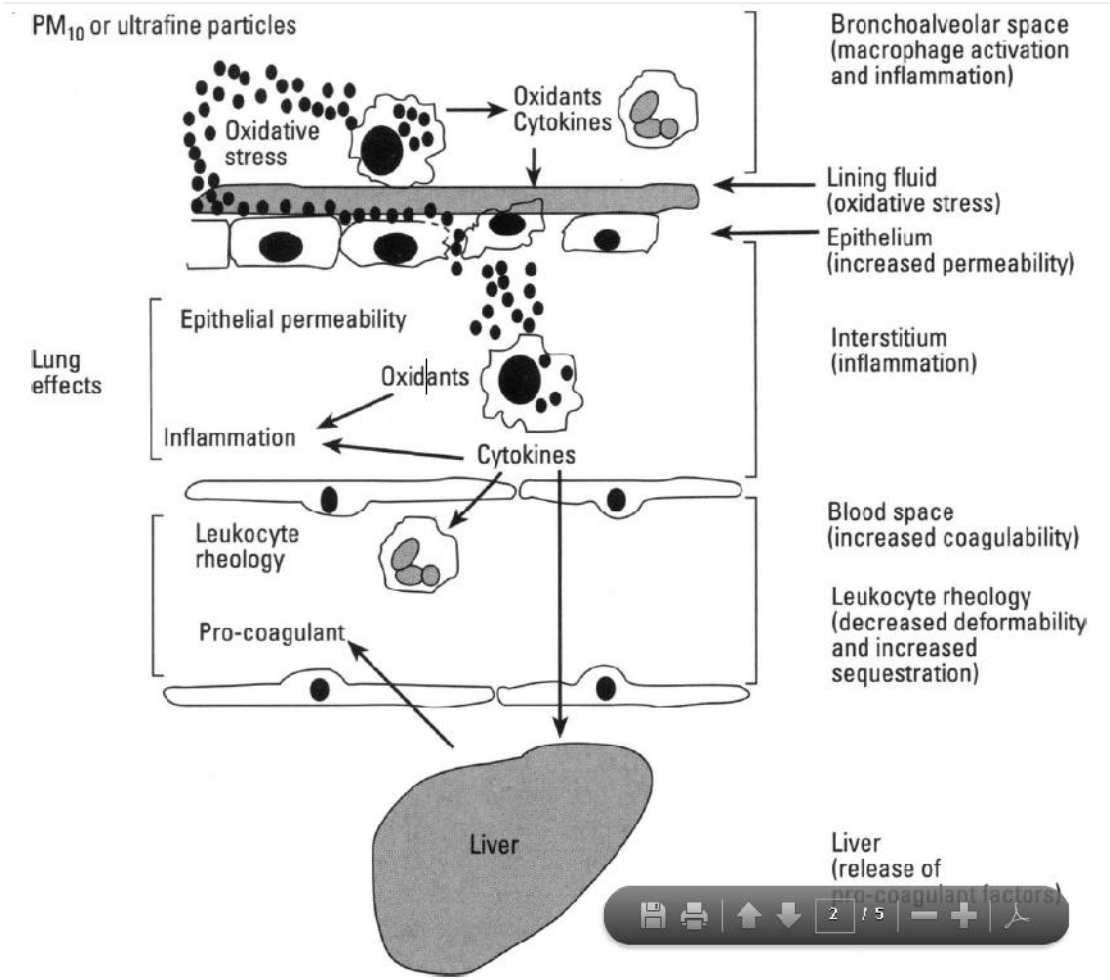
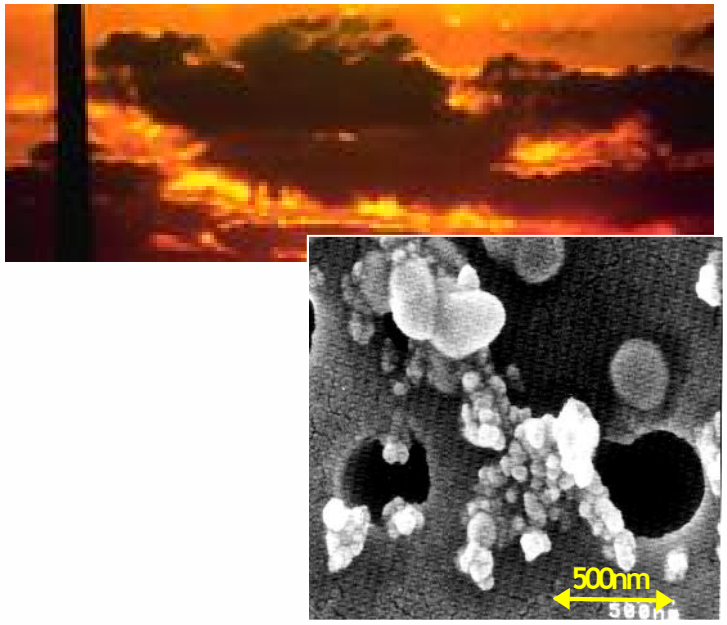
The Ultrafine Hypothesis + oxidants

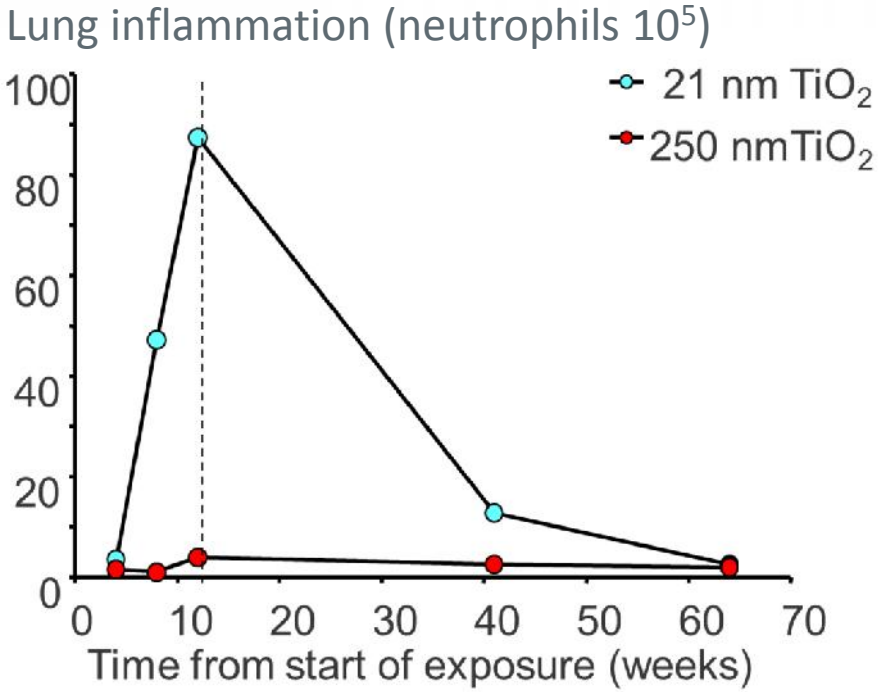


Li *et al*. Environmental Health Perspectives1997, 105 (suppl 5); 1279-1283.

The ultrafine particle hypothesis

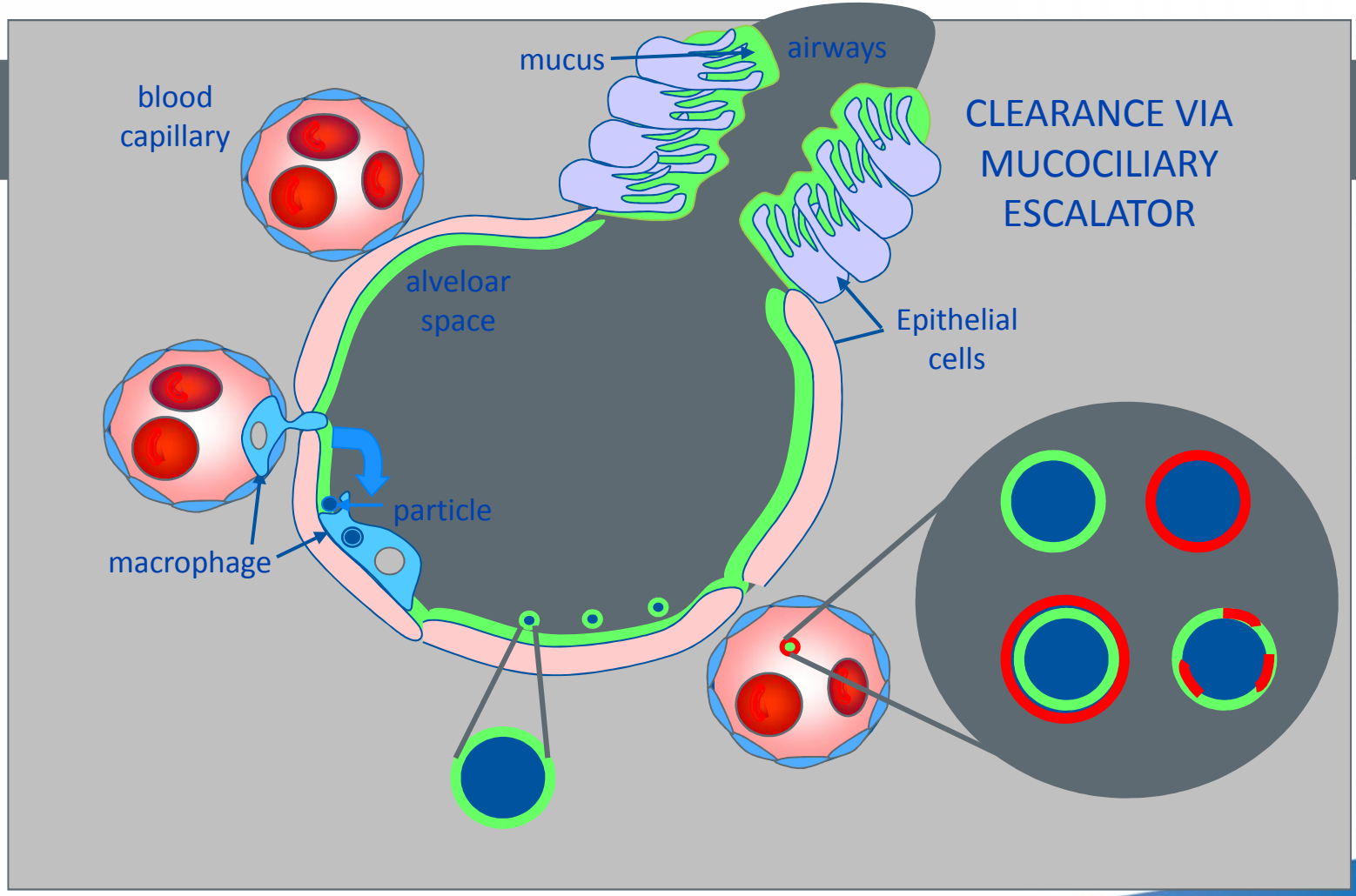


Seaton A., MacNee W., Donaldson K., Godden D. Particulate air pollution and acute health effects. Lancet 1995. 345; 176-178.



Ferin et al. 1992 Am.J.Respir.Cell Mol. Biol. 6: 535-542.

Manipulate NM to reflect route of entry



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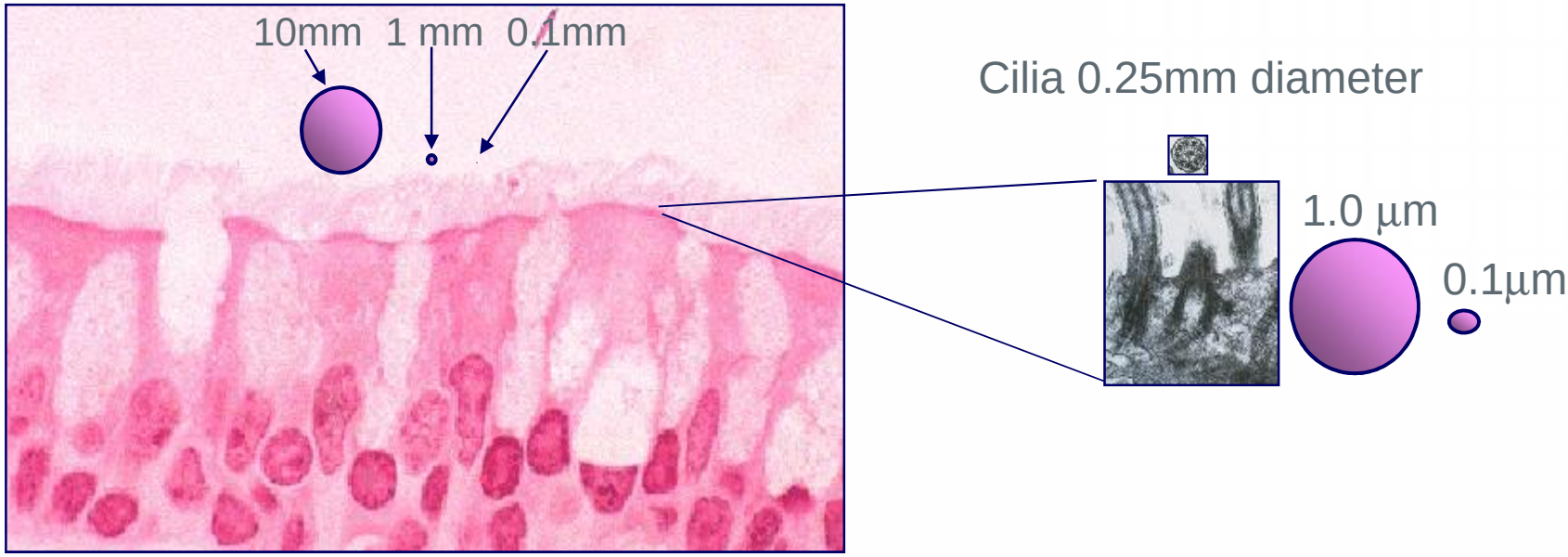
Trying to visualise the size of ultrafine particles



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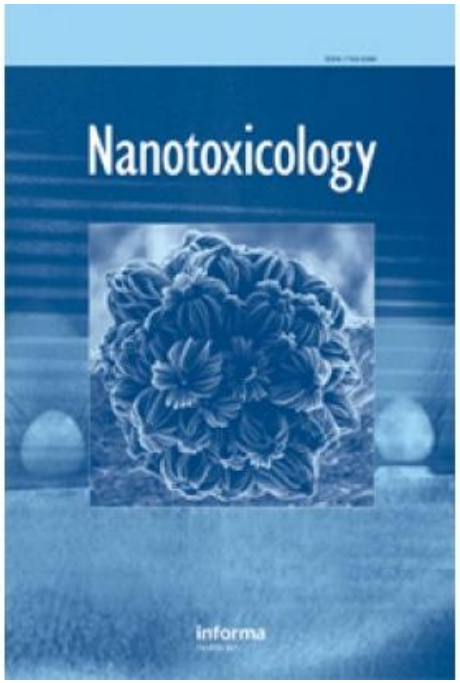
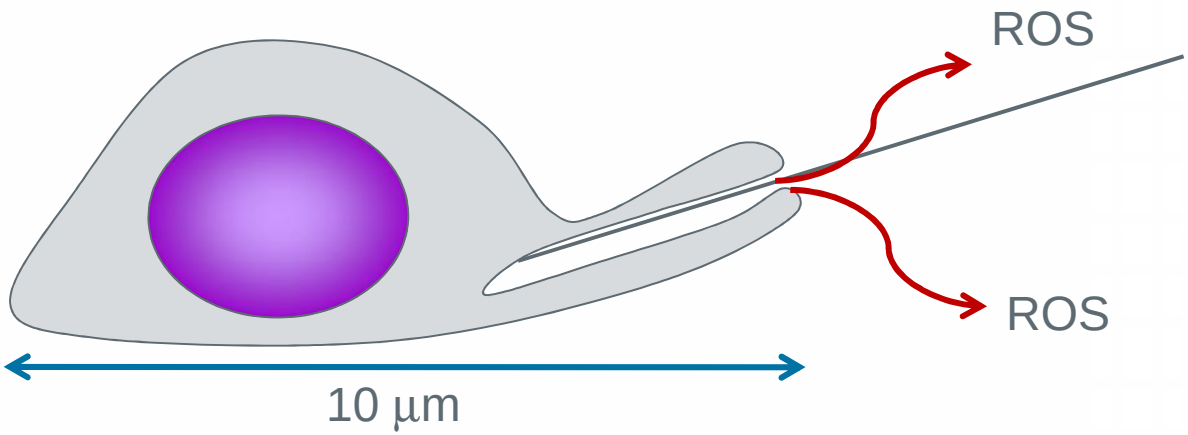


NANOTOX 2008, Zurich

Frustrated phagocytosis



Enhanced ROS production

- Antimicrobial function
- Continued production leads to cell activation and tissue damage
- Cell activation leads to cytokine/chemokine production and the recruitment of more inflammatory cells

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Adverse health effects of PM_{10}