2006 CRITICAL REVIEW

ISSN 1047-3289 *J. Air & Waste Manage. Assoc.* 56:709–742

C. Arden Pope III

Douglas W. Dockery

Health Effects of Fine Particulate Air Pollution: Lines that Connect

C. Arden Pope III
Department of Economics, Brigham Young University, Provo, UT

Douglas W. Dockery
Department of Environmental Health, Harvard School of Public Health, Boston, MA

No safe level of exposure

Recent empirical evidence about the shape of the PM concentration-response function is not consistent with a well-defined no-effects threshold. Concentration-re-

Little or no knowledge of the toxicity of particulate effluvia

Relative Toxicity and Role of Sources and Copollutants

One of the biggest gaps in our knowledge relates to what specific air pollutants, combination of pollutants, sources of pollutants, and characteristics of pollutants are most responsible for the observed health effects. Although the literature provides little evidence that a single major or trace component of PM is responsible for the observed health effects,[473] various general characteristics may affect the relative toxicity of PM pollution. For example, with regard to particle size, the epidemiological, physiological, and toxicological evidence suggests that fine particles (indicated by $PM_{2.5}$) play a substantial role in affecting human health. These fine particles can be breathed

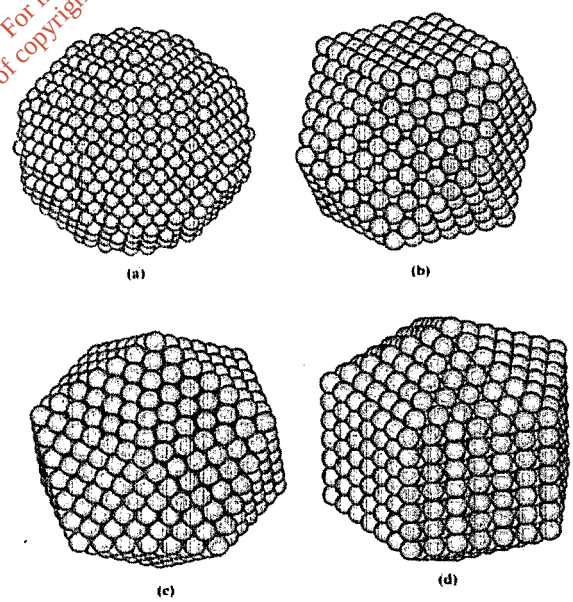


Figure 5.2. (a) Schematic model of a spherical metal nanoparticle with the fcc structure, showing atoms at surface steps. (b) A model of a cuboctahedral particle of similar size, containing 777 atoms. (c) Icosahedron with the same number of atoms, constructed using the homogeneous strain model. (d) decahedron, also with 777 atoms.

David Jefferson
University of Cambridge

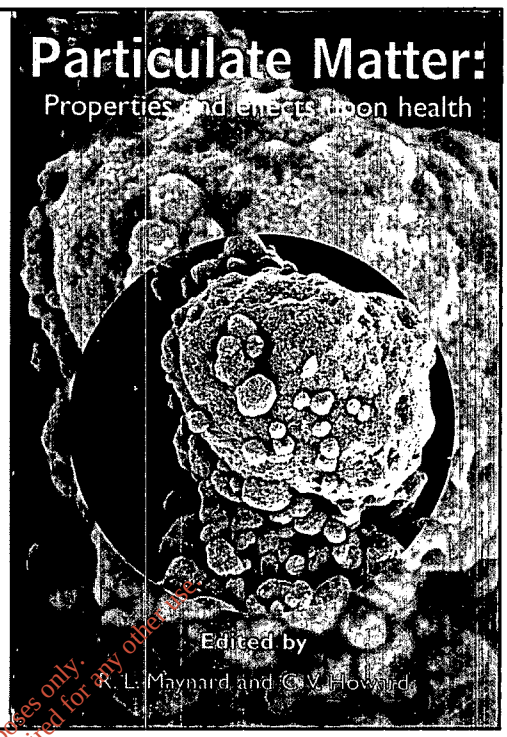
Micro '98 – London
Wed 8th July 1998

"Particulate Aerosols – Physical, Chemical & Bio-pathological Properties"

organised by the

Royal Microscopical Society

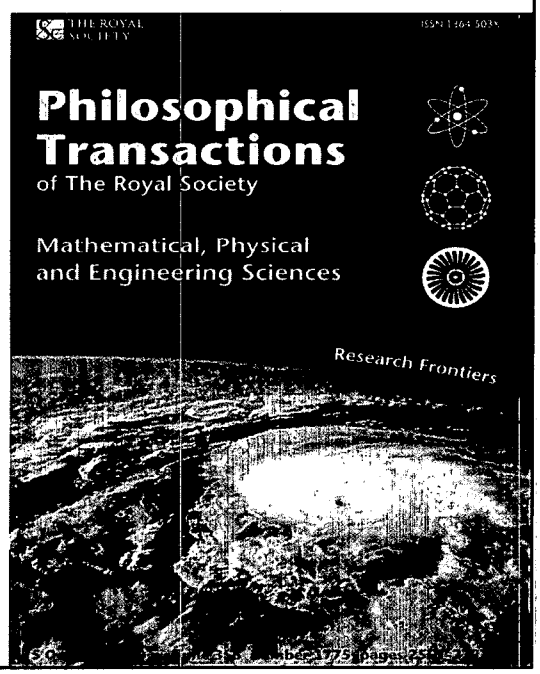
ISBN 1-85996-172-X



Royal Society Discussion Meeting 15th & 16th March 2000.

"Ultrafine Particles in the Atmosphere"

Organised by L.M.Brown, N. Collings, R.M.Harrison, A.D.Maynard & R.L.Maynard



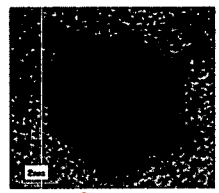
Royal Microscopical Society
37/38 St Clements
Oxford OX4 1AJ
Telephone: +44 (0) 1865 248768 Fax: +44 (0) 1865 791237
email: clare@rms.org.uk
Contact name: Clare Oxenbury

Royal Microscopical Society

NANOTOX 2004

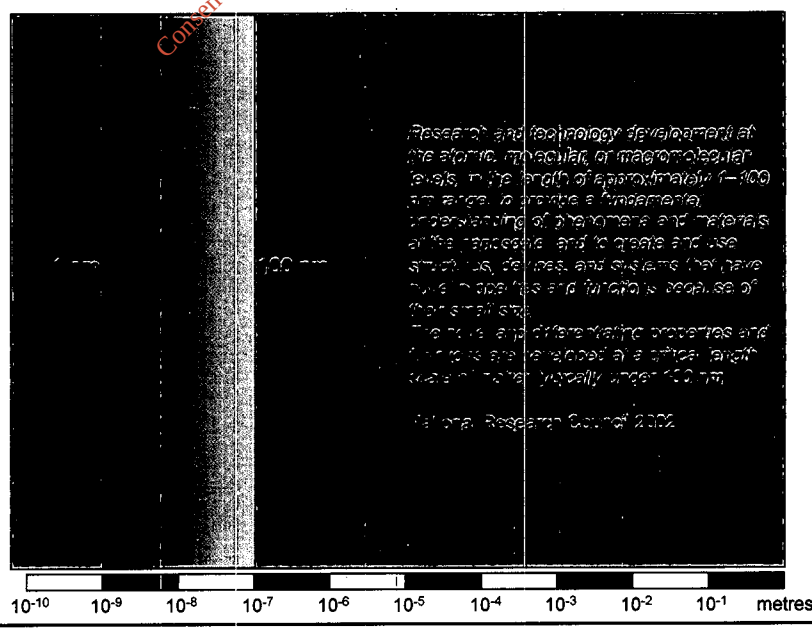
Nano Particles and Nanostructured Materials: *Implications for Health*

13 and 14th January 2004
Daresbury Laboratories
Warrington, Cheshire, U.K.



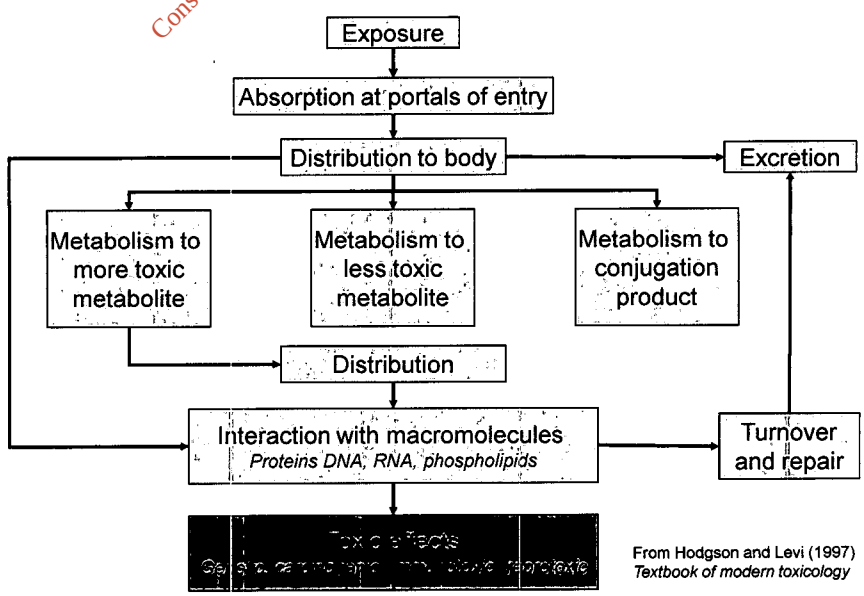
Co-sponsors : EMAG, Institute of Physics
CME Accreditation is being sought

Where is nanoscale?

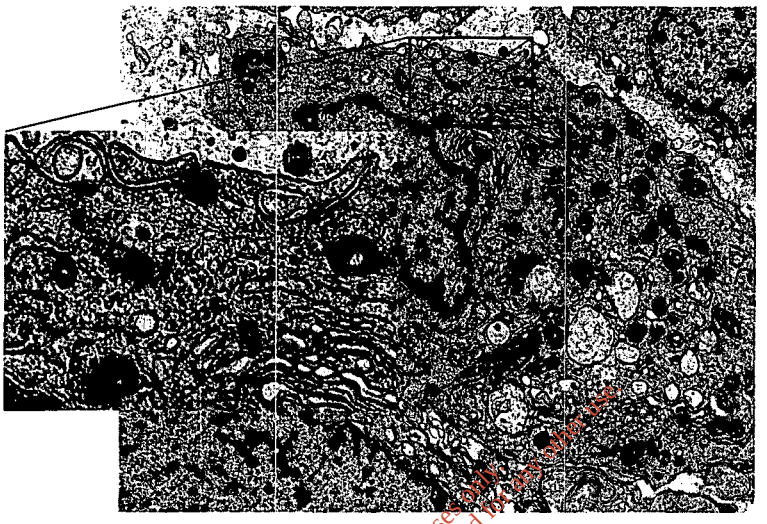


Where is nanoscale?

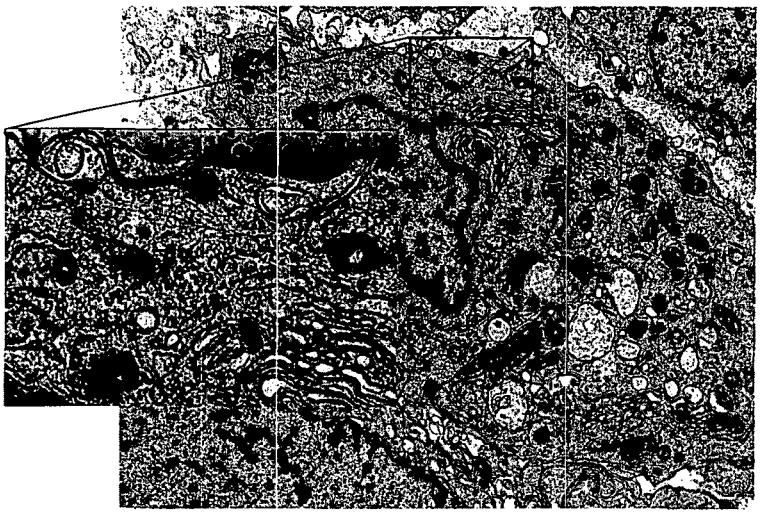
Routes to nanoparticle cytotoxicity



Nanoparticle view of human nasal epithelium



Nanoparticle view of human nasal epithelium



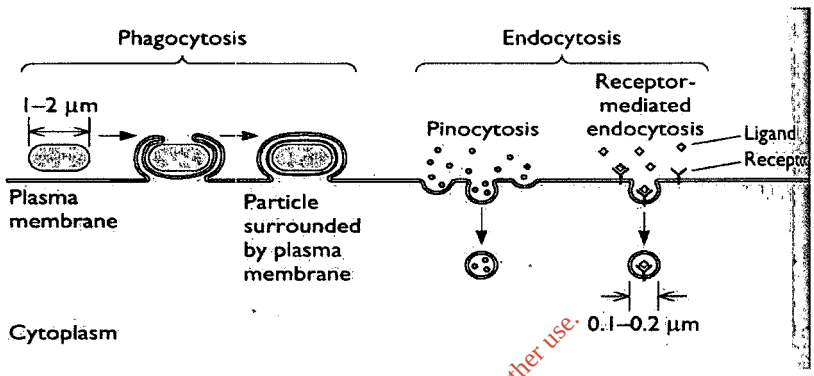
Pulmonary defence mechanisms

- **Nose, pharynx & larynx**: impaction
- **Trachea & bronchi**: muco-ciliary escalator and ingestion
- **Terminal bronchi and alveolar air space**: alveolar macrophages, engulf particles and transport to lymphatics. They do not easily recognise particles of < 65 nm and are easily overwhelmed by large numbers

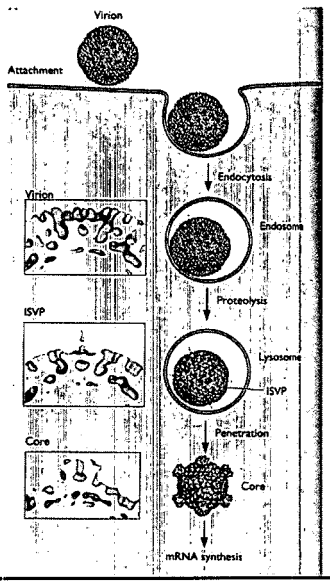
Temporal perspectives

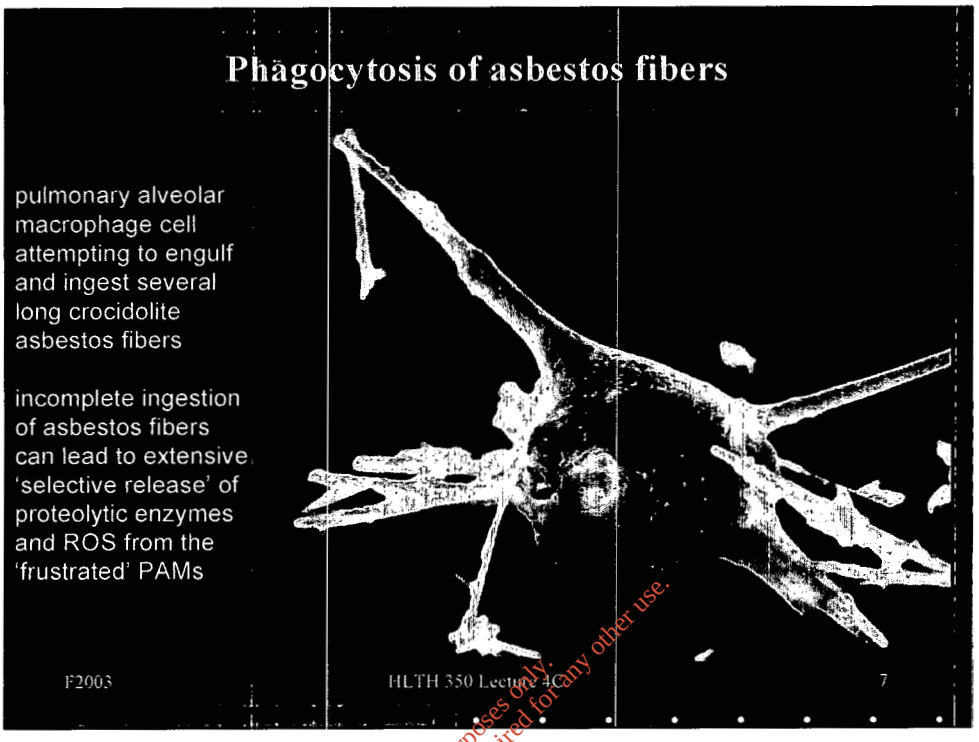
- Life started about 3.5 billion years ago
- Unicellular organisms primarily internalise larger objects by pinocytosis
- Multicellular organisms appeared about 450 million years ago.
- Although multicellular organisms have complex digestive systems, they rely totally on the ability of cells to engulf particles

Engulfing mechanisms



Caveolar mechanism





Definitions of particle size

- **Coarse particles** = PM_{10} = particles with average diameter less than 10 μm
- **Fine particles** = $PM_{2.5}$ = particles with average diameter less than 2.5 μm
- **Ultrafine particles** = $PM_{0.1}$ = particles with average diameter less than 100 nm

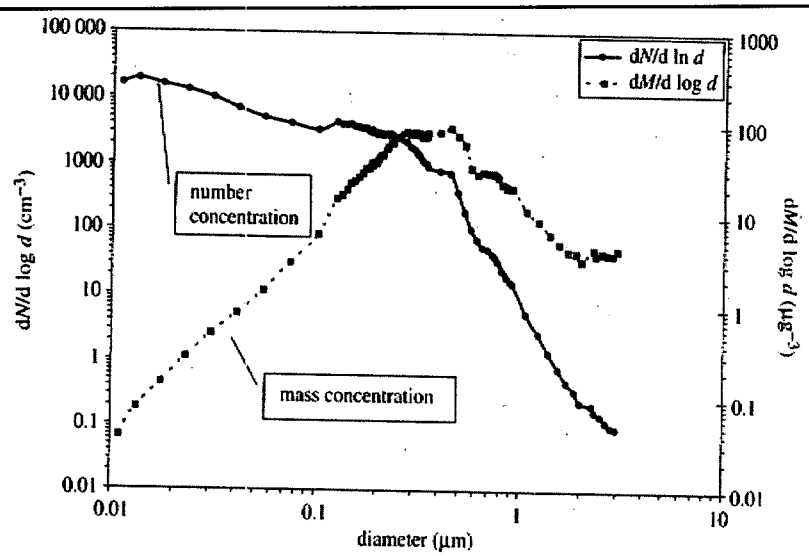


Figure 1. Typical particle number and mass distribution averages from approximately 10 000 single measurements, Erfurt. From Wichmann *et al.* (2000*a*).

Wichmann HE & Peters A (2000). *Phil. Trans. R. Soc. Lond. A* **358**: 2751-2769

Figure 4.4. TEM image of a pioloform-coated grid supporting 'plaques' of CB R250 aggregated particles. Scale bar = 300 nm.

BéruBé KA et al (1999). In: *Particulate Matter*, (Eds. Maynard RL & Howard CV), Bios, Oxford. ISBN 185996172X

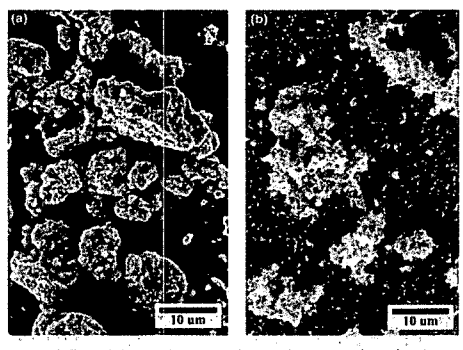


Figure 4.5. Field emission scanning electron microscope images comparing sonicated versus impacted diesel exhaust particulates (DEP). (a) Sonicated DEP exhibiting a compact arrangement of spherulites. Note reduced number of ultrafine/fine particles in the background. (b) Impacted DEP revealing flocculated arrangement of spherulites. Note significant number of ultrafine/fine particles in the background. Scale bars = 10 μm.

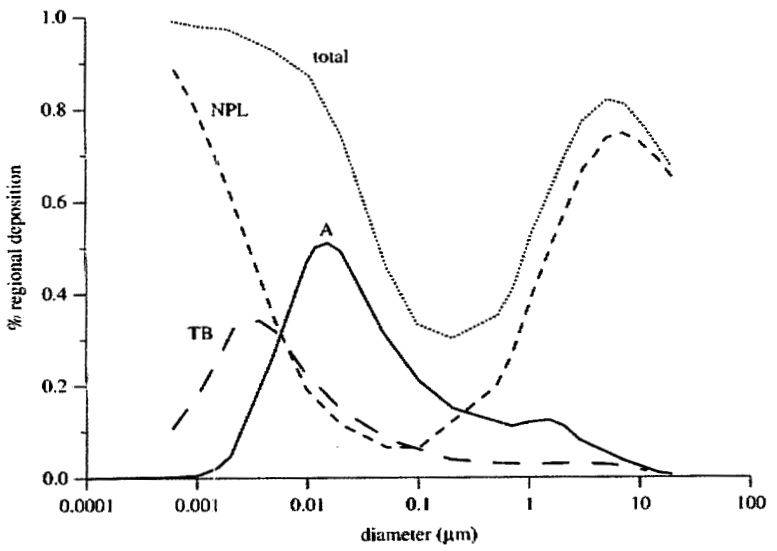


Figure 6. Predicted deposition of inhaled particles of different sizes of unit density in the human respiratory tract during nose breathing, light exercise (ICRP 1994). 'NPL' denotes nasopharyngolaryngeal deposition; 'TB' denotes tracheobronchial deposition; 'A' denotes alveolar deposition; 'total' denotes the sum of particle depositions in the respiratory tract for all three compartments.

Kelly Berube
University of Wales at Cardiff

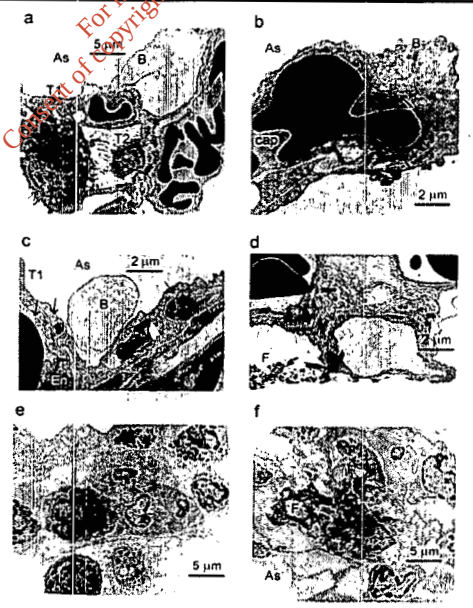


Figure 7.2. Sequential stages of alveolar damage and inflammation following instillation of zinc chloride. Key as for *Figure 7.1*. (a) Extensive blebbing (B) to epithelial type I cells. (b) Blebbing in type I cells and evidence of an increase in plasmalemmal vesicles in endothelial cells (arrows). (c) Both endothelial and type I epithelial cells show an increase in plasmalemmal vesicles (arrows). (d) Accumulation of fibrin (F) in the alveolar space. (e) Interstitial and alveolar surface accumulation of inflammatory cells. (f) Extensive damage to alveolar architecture with accumulation of cell debris, fibrin and monocytic cells

Table 8.5. Percentage PMN in lavage 6 h following instillation of 125 mg of carbon black of various primary particle sizes (from Li *et al.*, in press)

Carbon black primary particle size (nm)	Neutrophils in the bronchoalveolar lavage (%) (mean (SEM))
14	39.9 (4.2)
50	13.7 (2.6)
260	4.2 (0.73)

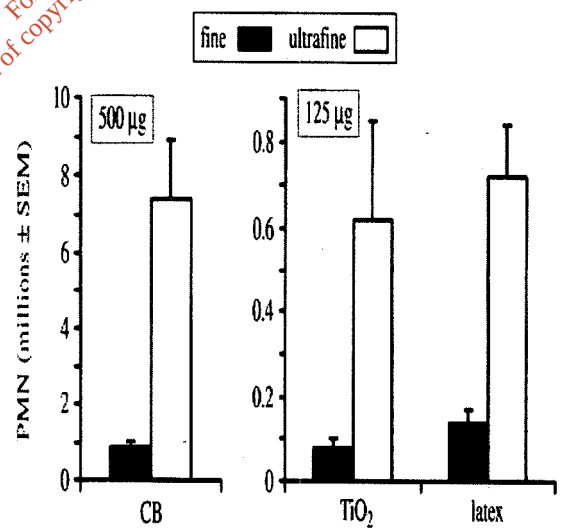


Figure 1. Inflammation, measured as the number (mean ± SEM of three rats) of neutrophils (PMN) in the lavage of rats instilled with either 125 or 500 µg of fine or ultrafine carbon black (CB), titanium dioxide (TiO_2) or latex 24 h previously.

Ken Donaldson
Napier
University

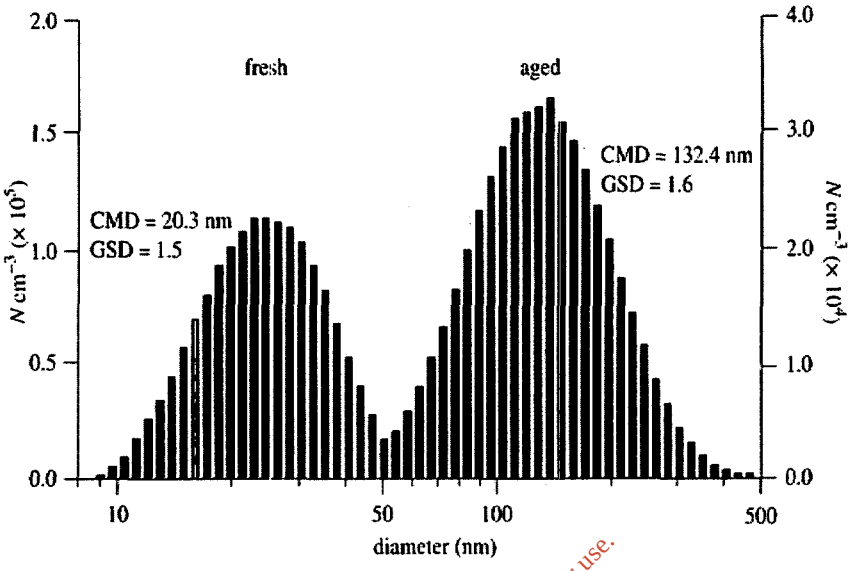


Figure 1. Particle size distribution of freshly generated and aged PTFE fumes showing the shift from ultrafine particle distribution to a size distribution with a median greater than 100 nm due to coagulation after 3.5 min of ageing.

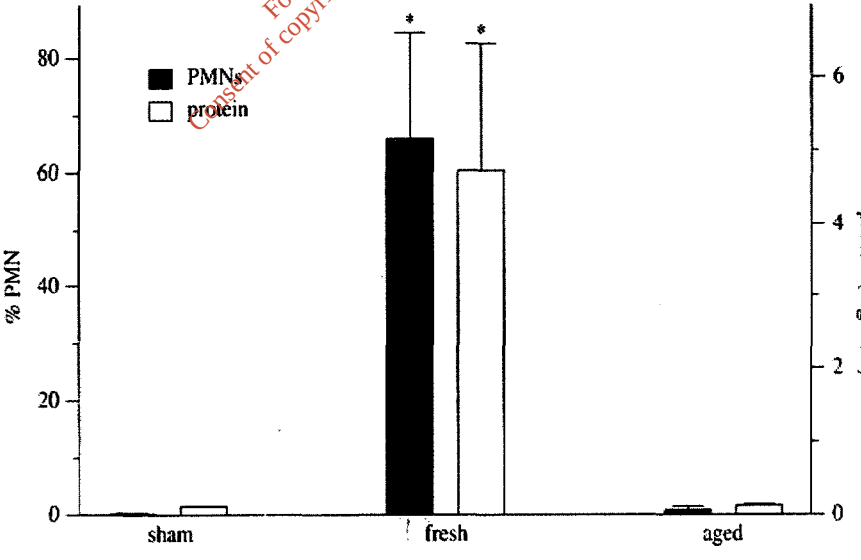


Figure 2. Lung lavage parameters of rats 4 h after a 50 min exposure to fresh and aged PTFE fumes. The percentage of neutrophils of the total lavage cells and the lavage protein content are shown. ($N = 4$ per group; mean ±SD; *, significantly different from sham and aged (ANOVA, $p < 0.05$).)

Wichmann and Peters. *Phil. Trans. Roy. Soc. Lond.*

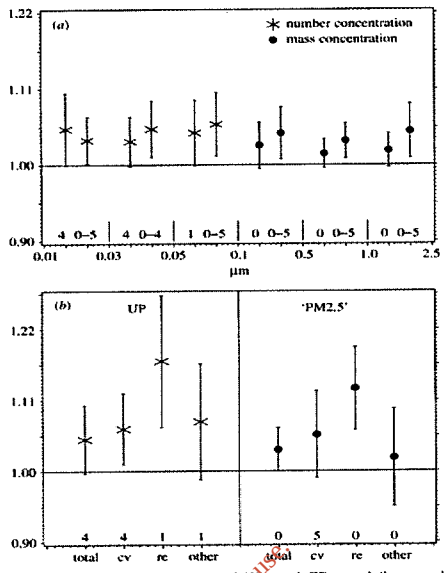


Figure 3. (*a*) Effects of different size classes of UPs and FPs on daily mortality in Erfurt, 1995–1998. Left, best one-day lag; right, distributed lag model. The lags (days) are given at the bottom. (*b*) Effects of UPs and FPs on mortality for prevalent diseases (total, cardiovascular, respiratory, others). Best day-lag model. There seems to be a stronger immediate effect (lag 0 or 1 days) on respiratory causes and a stronger delayed effect (lag 4 or 5 days) on cardiovascular causes. Modified from Wichmann *et al.* (2000*a*).

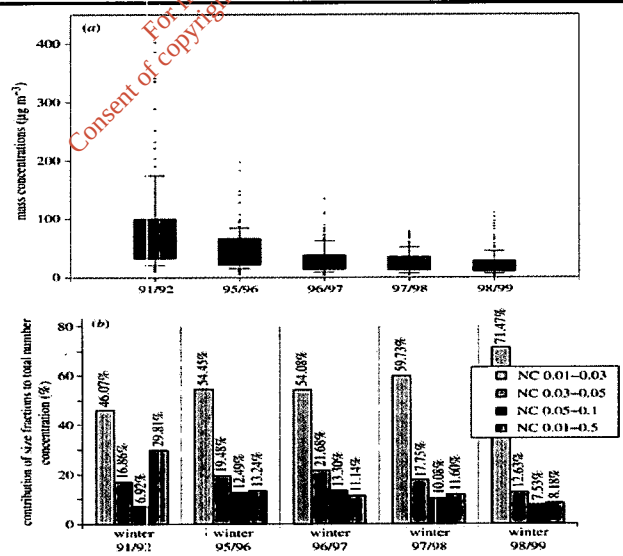


Figure 4. (*a*) Seven years trend of the mass concentration (MC 0.01–2.5 = $PM_{2.5}$ of FPs in Erfurt, winters 1991/2 to 1998/9. From Wichmann *et al.* (2000*a*). (*b*) Seven years trend of the relative particle number concentration (in %); different size ranges (0.01–0.03, 0.03–0.05, 0.05–0.1, 0.1–0.5 µm diameter). The concentration of UPs is approximately constant (see table 1) and the fraction in the smallest size fraction increases steadily. From Wichmann *et al.* (2000*a*).

Wichmann HE & Peters A (2000). *Phil. Trans. R. Soc. Lond. A* **358**: 2751-2769

Anderson HR (2000). *Phil. Trans. R. Soc. Lond. A* **358**: 2771-2785

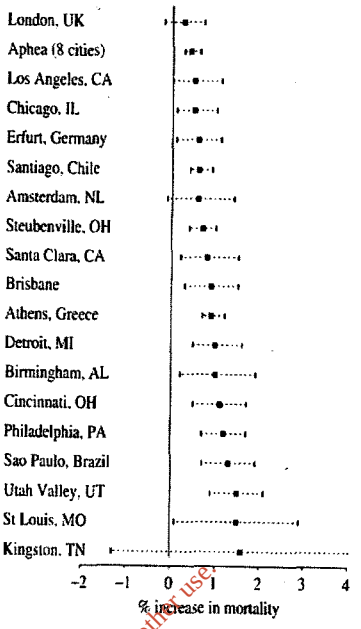
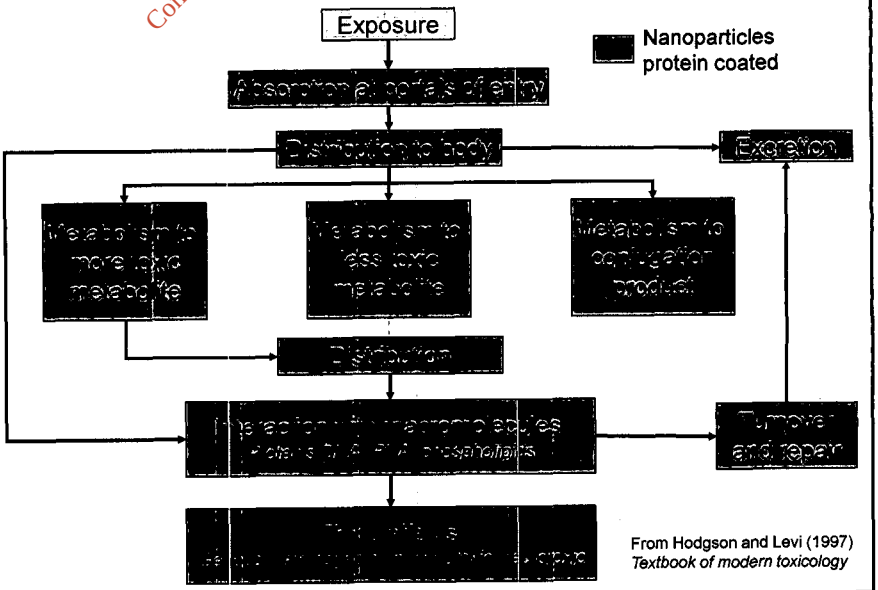


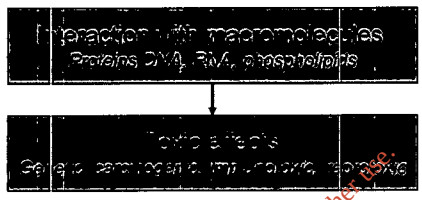
Figure 1. Particulate matter with aerodynamic diameter less than 10 μm (PM_{10}) and daily mortality from cities around the world. Expressed as a percentage change in daily mortality associated with a 10 μg m^{-3} increase in PM_{10}.

Routes to nanoparticle cytotoxicity



From Hodgson and Levi (1997) *Textbook of modern toxicology*

What are the sites of cellular vulnerability?



Sites of especial cellular vulnerability

- Maintenance of the integrity of cellular membranes
  - Critical for ionic and osmotic homeostasis
- Aerobic respiration
  - Mitochondrial oxidative phosphorylation and ATP production
- Protein synthesis
  - Structural integrity of cellular compartments
  - Preservation of intermediary metabolism
- Preservation of the integrity of the genetic apparatus of the cell
  - Prevention of alterations to genetic material
  - Repair of damage to DNA

Mechanisms of nanoparticle cytotoxicity

- Catalysis
  - Often oxidative damage
- Membrane perturbation
  - Lipid peroxidation
  - Surfactant effects
- Chaperone effects on proteins
  - Pathological effects on folding
- Physical damage
  - Accumulation at extracellular or intracellular sites

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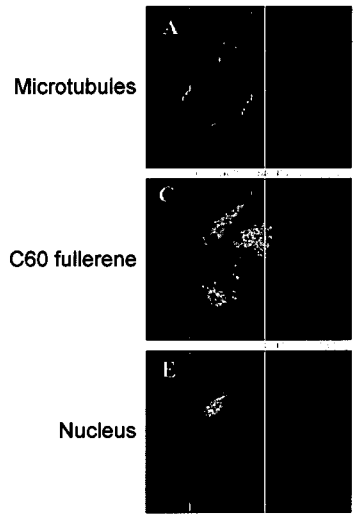
Mechanisms of nanoparticle cytotxicity



Microtubules

C60 fullerene

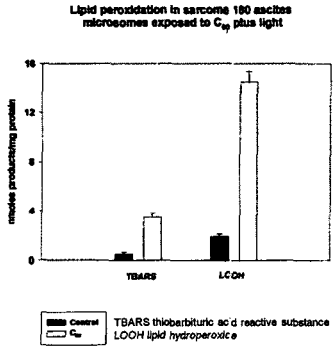
Nucleus

Intracellular distribution of a modified C60 fullerene, C61 carboxy-fullerene detected using an antibody

The fullerene derivative gains access to the interior of the living cell and accumulates in association with the mitochondria

Foley et al (2002) **BBRC** 294 116-119

Mechanisms of nanoparticle cytotoxicity

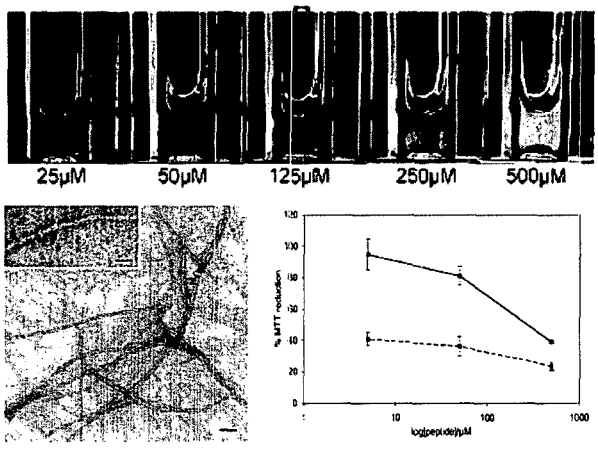


Kamat et al (2000) **Toxicology** 155 55-61

Internal membrane fractions from tumour cells are damaged by peroxidation of membrane lipids on exposure to fullerenes, which act as photosensitisers

The lipid peroxidation eventually leads to loss of membrane flexibility, a drop in trans-membrane potential, progressively increased permeability to ions and eventually cell death

Mechanisms of nanoparticle cytotoxicity

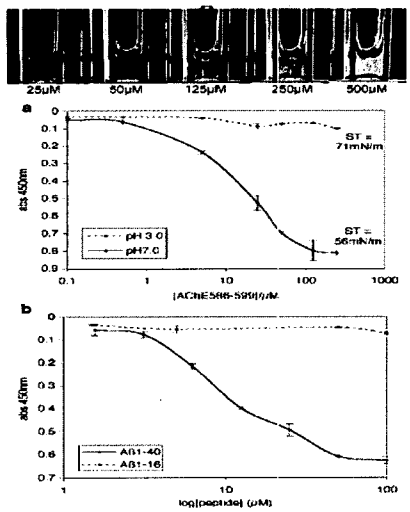


Cottingham et al (2002) **Biochem** 41 13539-47

Self-assembling biological nanoparticles, in this case oligomers and fibrils of an amyloid-forming peptide, exhibit cytotoxicity to cultured neuronal cells

The mechanism was initially obscure, but became more apparent when the surfactant properties of the nanoparticles was examined

Mechanisms of nanoparticle cytotoxicity



Cottingham et al (2004) **Lab Invest** 84 523-9

Self-assembling biological nanoparticles, in this case oligomers and fibrils of an amyloid-forming peptide, were found to be strongly surface active

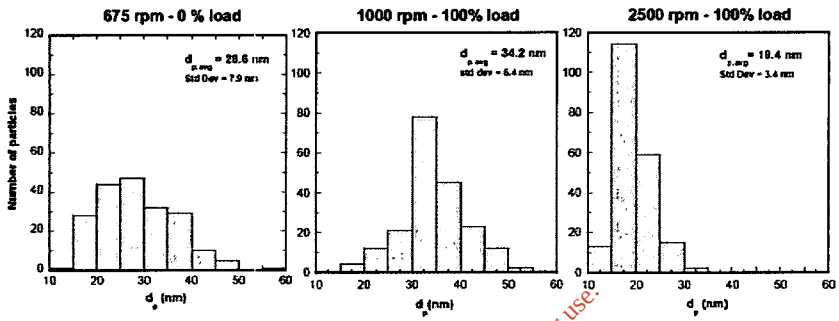
Moreover, other examples of self-assembling toxic amyloid species, such as the A-beta peptide associated with Alzheimer's Disease, are also highly surface active

The conditions under which cytotoxicity is detected closely parallel conditions under which oligomer assembly takes place and surfactant properties emerge

Mechanisms of nanoparticle cytotoxicity

- Catalysis
  - Often oxidative damage
- Membrane perturbation
  - Lipid peroxidation
  - Surfactant effects
- Chaperone effects on proteins
  - Pathological effects on folding
- Physical damage
  - Accumulation at extracellular or intracellular sites

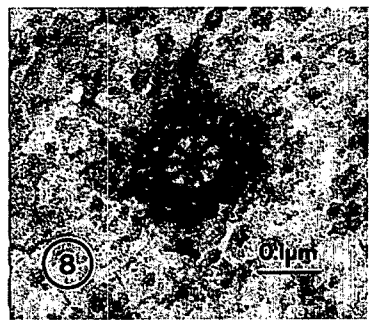
Engine emission particulates may be similar in scale to subcellular structures



Characterization of Particulate Sizes, Microstructures and Fractal Geometry of a Light Duty Diesel Engine via Thermophoretic Sampling
Kyoung Lee, Jinyu Zhu and Raj Sekar (2003) Center for Transportation Research, Argonne National Laboratory

Engine emission particulates may be similar in scale to subcellular structures

Diesel particulates



Centriole
a subcellular protein assembly critical in cytoskeletal regulation

Outcomes of protein-nanoparticle interaction

- Nanoparticles stabilised as monodisperse suspension
- Aggregation of nanoparticles minimised
- Protein adsorbed onto nanoparticle surface
  - Protein conformation and function unaltered (e.g. antibody label)
  - Protein conformation altered with loss of function (molten globule)
  - Protein conformation altered with altered function (chaperone)

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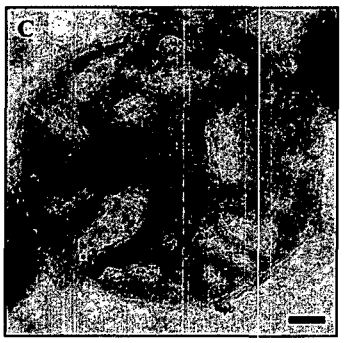

Colloidal gold nanoparticles (10nm diameter) have been stabilised with a protein, in this case an antibody that recognises a protein found in the inner membrane of mitochondria

The antibody adsorbed to the nanoparticles retains normal folding and function, targeting the gold particles to the inner membrane of the mitochondria

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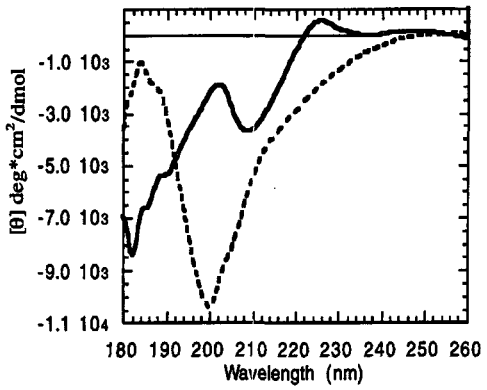


Silica nanoparticles (9nm diameter) have been stabilised with a protein, in this case a human enzyme, carbonic anhydrase *II*

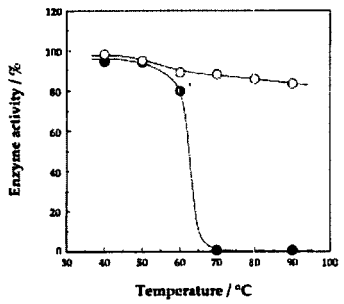
The circular dichroism spectrum shown reveals the secondary structure of the enzyme in solution (solid line; predominantly beta-sheet) and after exposure to the nanoparticles for 24 hours (dotted line; most beta structure lost)

Billsten et al (1997) **FEBS Lett** 402 67-72

Outcomes of protein-nanoparticle interaction

- Nanoparticles stabilised as monodisperse suspension
- Aggregation of nanoparticles minimised
- Protein adsorbed onto nanoparticle surface
  - Protein conformation and function unaltered (e.g. antibody label)
  - Protein conformation altered with loss of function (molten globule)
  - Protein conformation altered with altered function (chaperone)

Outcomes of protein-nanoparticle interaction

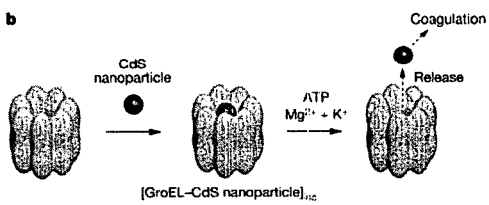


Akiyoshi et al (1999) **Bioconj Chem** 10 321-324

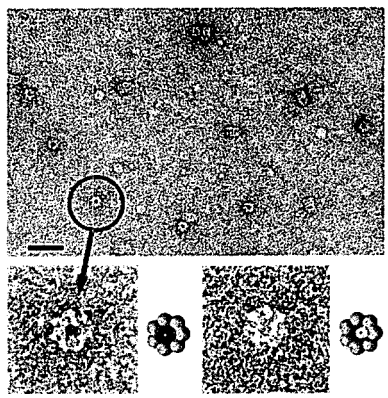
Amphiphilic hydrogel nanoparticles (18nm diameter) can act as chaperone to prevent heat damage to an enzyme

In this experiment, carbonic anhydrase was heated to different temperatures for 10 minutes, cooled and assayed for activity. The black dots show permanent heat damage to the enzyme; the open dots shows the protective chaperone effect of the nanoparticles

Cellular chaperone machinery can interact with nanoparticles



Chaperonin-mediated stabilization and ATP-triggered release of semi-conductor nanoparticles
Ishii et al (2003) **Nature** 423 628-32

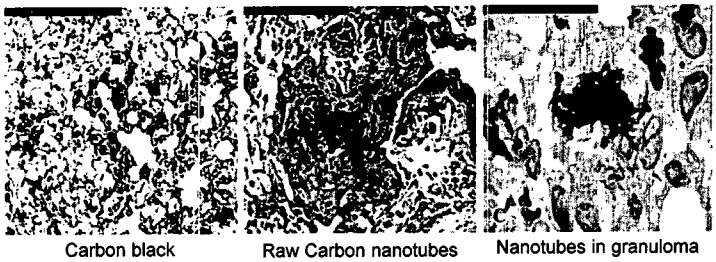


Mechanisms of nanoparticle cytotoxicity

- Catalysis
  - Often oxidative damage
- Membrane perturbation
  - Lipid peroxidation
  - Surfactant effects
- Chaperone effects on proteins
  - Pathological effects on folding
- Physical damage
  - Accumulation at extracellular or intracellular sites

Outcomes of protein-nanoparticle interaction

Lam et al (2004) **Tox Sci** 77 126-134



Carbon black | Raw Carbon nanotubes | Nanotubes in granuloma

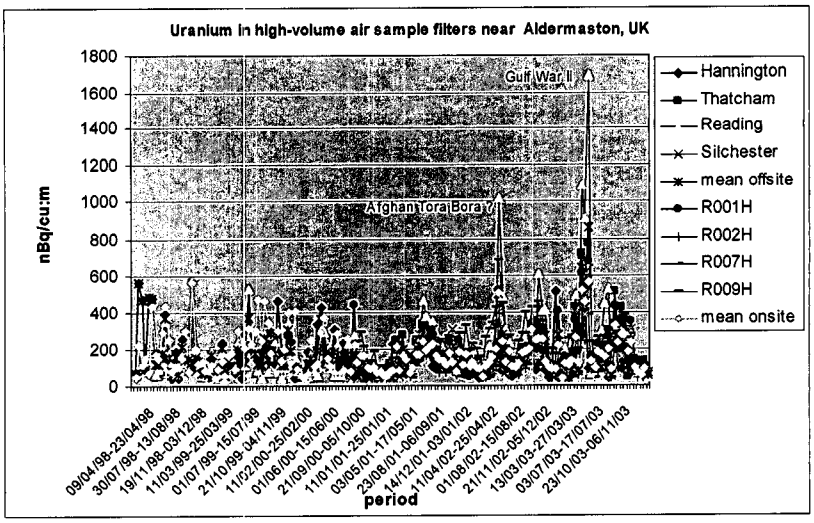
Rats were challenged by intratracheal instillation of carbon as carbon black (a low toxicity dust) or as single walled nanotubes and the lungs were examined after 90 days

Although the carbon black has been taken up by alveolar macrophages, these cells have remained dispersed in the lung. In contrast, macrophages that have taken up nanotubes have migrated, become activated and have proliferated to form granulomas

Can ultrafine particles travel?

High Volume Air Samplers

Yes, thousands of miles



Gulf War II 'Shock and Awe'