Nanoparticle toxicology

Scientific state-of-the-art

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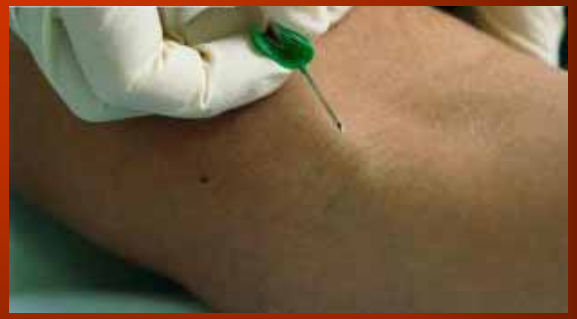
combustion-derived nanoparticles

bulk manufactured nanoparticles

engineered manufactured nanoparticles



medical nanoparticles

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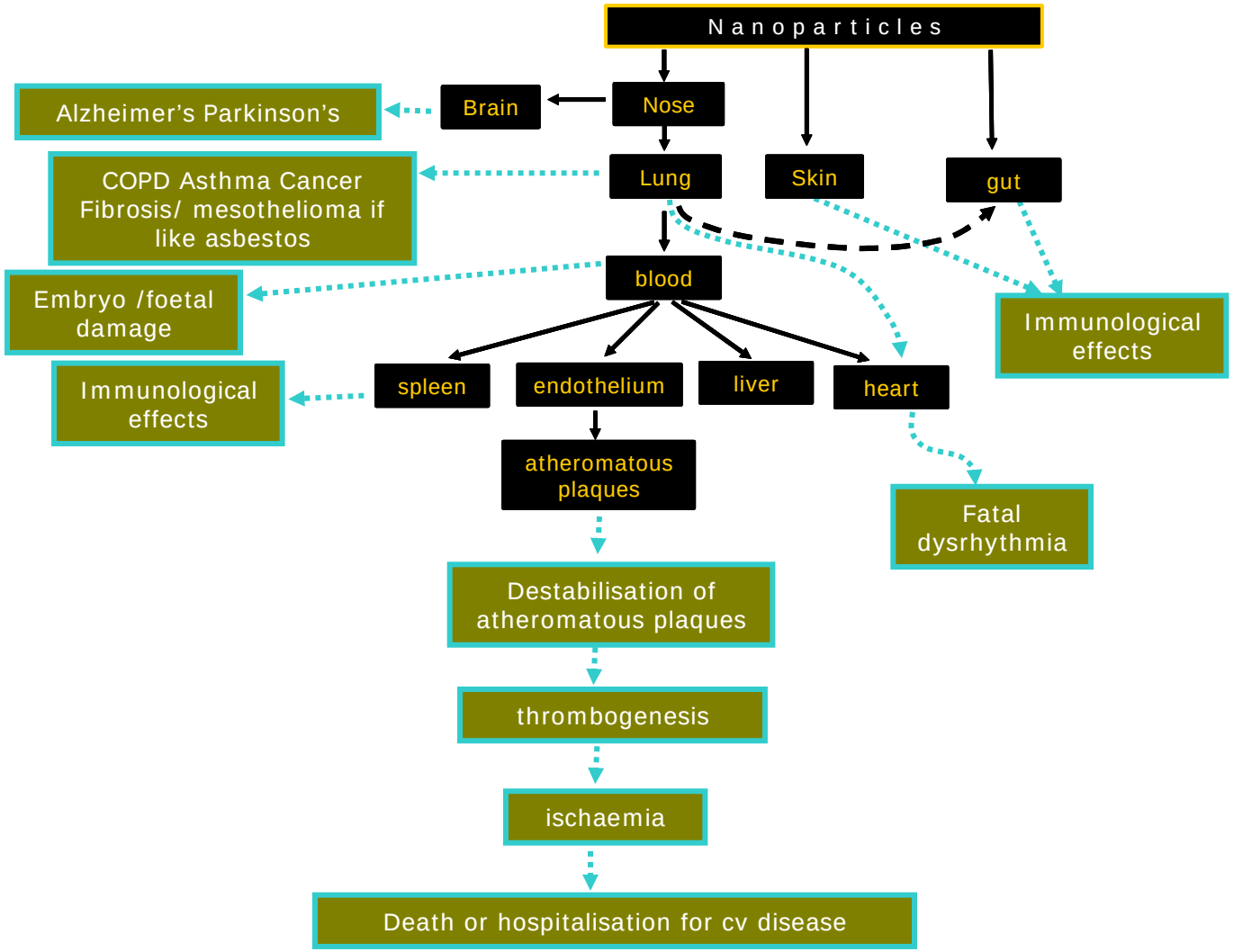
The current model for the hypothetical toxicokinetics and harmful effects of manufactured nanoparticles



Based on CDNP in PM10 in epi studies and a few bulk-produced manufactured NP in animals and intuition

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Likely pathogenic processes in adverse effect of NP at portals of entry and target tissues

Translocation

Portal of entry

↓

oxidative stress

Inflammation

genotoxicity

↓

COPD

Asthma

Cancer

Gut/skin

Target organs/tissues

↓

Inflammation

oxidative stress

genotoxicity

other

↓

Atherothrombosis

Brain degeneration

Placenta/foetal damage

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State of the art in:-

1. The oxidative stress hypothesis
2. Toxicokinetics and translocation
3. The large numbers of particles that require testing – in vitro models and structure activity paradigms
4. The effects on the cv system
5. Long thin nanoparticles and the asbestos issue

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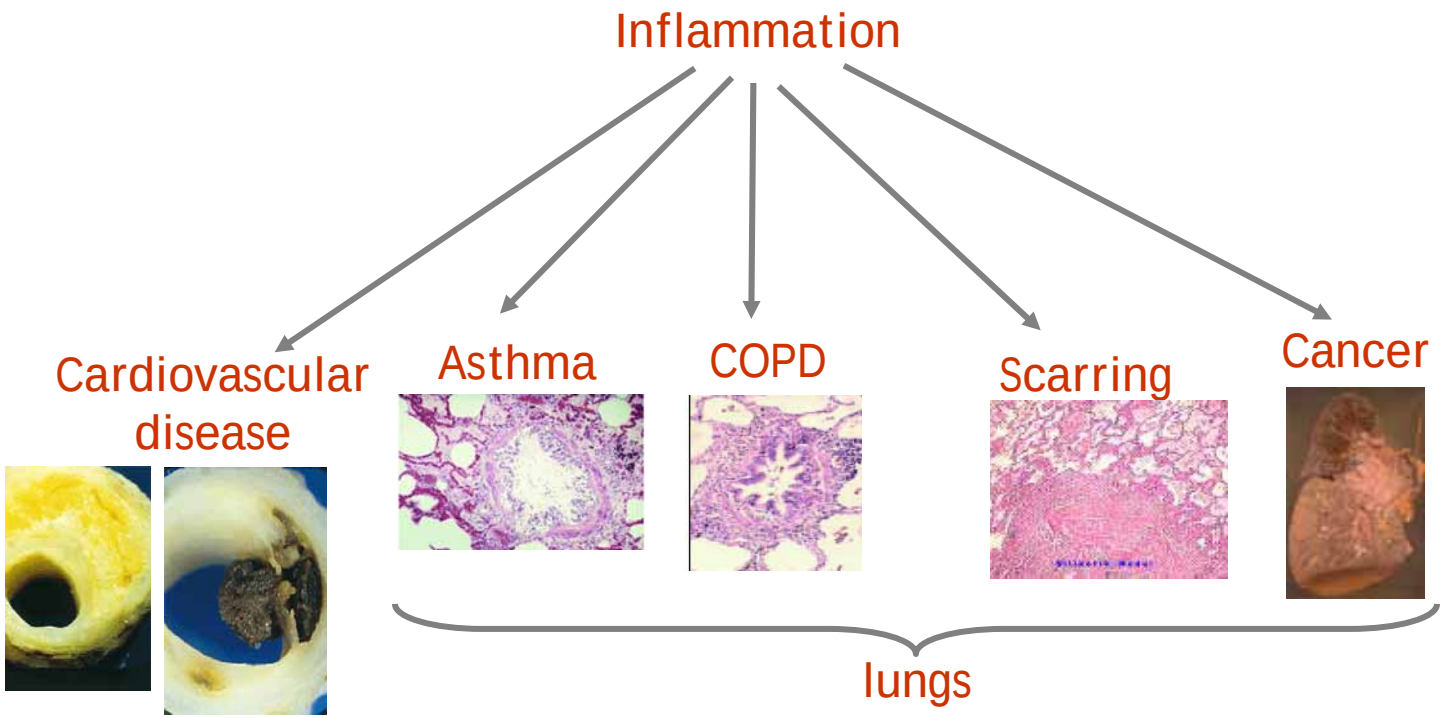
The central role of inflammation in diseases especially those associated with particle exposure



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State of the art:- The oxidative stress hypothesis

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Table 2. NM effects as the basis for pathophysiology and toxicity. Effects supported by limited experimental evidence are marked with asterisks; effects supported by limited clinical evidence are marked with daggers.

Experimental NM effects	Possible pathophysiological outcomes
ROS generation*	Protein, DNA and membrane injury,* oxidative stress†
Oxidative stress*	Phase II enzyme induction, inflammation,† mitochondrial perturbation*
Mitochondrial perturbation*	Inner membrane damage,* permeability transition (PT) pore opening,* energy failure,* apoptosis,* apo-necrosis, cytotoxicity
Inflammation*	Tissue infiltration with inflammatory cells,† fibrosis,† granulomas,† atherogenesis,† acute phase protein expression (e.g., C-reactive protein)
Uptake by reticulo-endothelial system*	Asymptomatic sequestration and storage in liver,* spleen, lymph nodes,† possible organ enlargement and dysfunction
Protein denaturation, degradation*	Loss of enzyme activity,* auto-antigenicity
Nuclear uptake*	DNA damage, nucleoprotein clumping,* autoantigens
Uptake in neuronal tissue*	Brain and peripheral nervous system injury
Perturbation of phagocytic function,* "particle overload," mediator release*	Chronic inflammation,† fibrosis,† granulomas,† interference in clearance of infectious agents†
Endothelial dysfunction, effects on blood clotting*	Atherogenesis,* thrombosis,* stroke, myocardial infarction
Generation of neoantigens, breakdown in immune tolerance	Autoimmunity, adjuvant effects
Altered cell cycle regulation	Proliferation, cell cycle arrest, senescence
DNA damage	Mutagenesis, metaplasia, carcinogenesis

Nel, A., T. Xia, L. Madler, and N. Li. 2006. Toxic potential of materials at the nanolevel *Science* 311:622-627.

Key questions-

1. Is oxidative stress a generic mechanism for the adverse effects of nanoparticles- at Portals of Entry, at target sites?
2. Can it form a basis for predictive testing?

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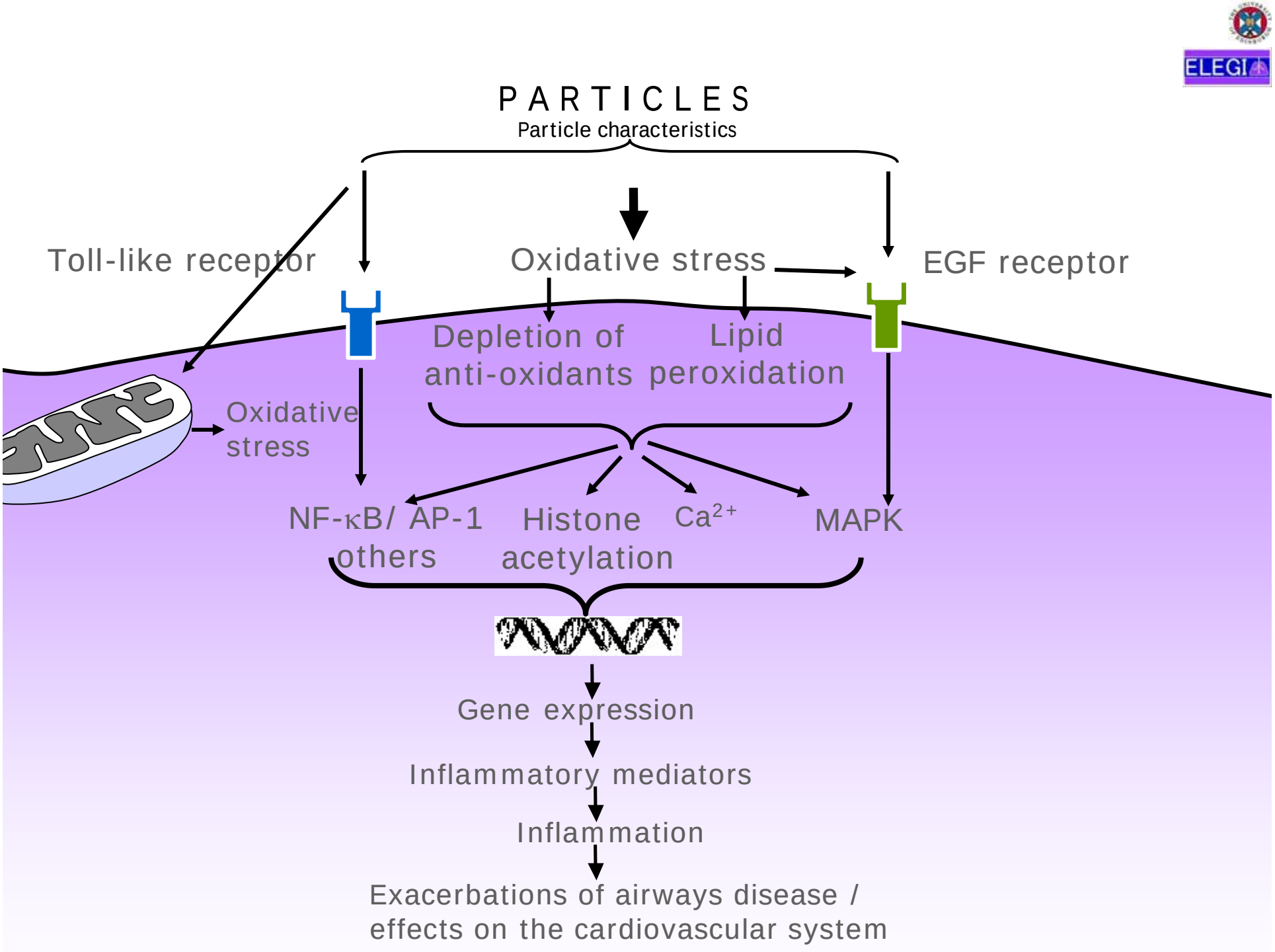
PARTICLES
Particle characteristics
Toll-like receptor
Oxidative stress
EGF receptor
Depletion of anti-oxidants
Lipid peroxidation
Oxidative stress
NF-κB/ AP-1 others
Histone acetylation
Ca2+
MAPK
Gene expression
Inflammatory mediators
Inflammation
Exacerbations of airways disease / effects on the cardiovascular system

Is oxidative stress a generic mechanism for nanoparticles?

- Oxidative stress is an attractive mechanism due to:-
- its ability to kick-off inflammation
- To be caused by inflammation
- Its known role in the adverse effects of all previously identified pathogenic particles and in all the diseases associated with particle exposures
- Is oxidative stress a generic mechanism for the adverse effects of nanoparticles- at POE, at target sites?
- Piecemeal data still emerging that NP cause oxidative stress:-

C60, cerium oxide, TiO2, carbon black, carbon nanotubes, silica, etc.

- Other data suggests that NP are anti-oxidant:–

cerium oxide, C60, platinum

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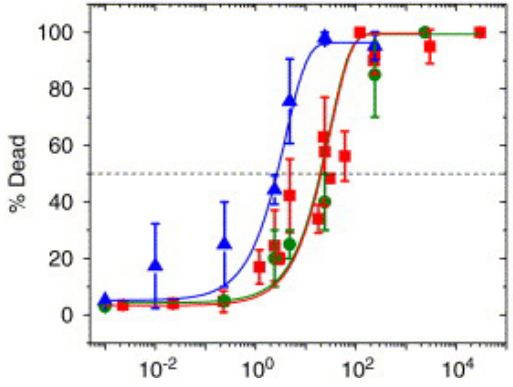
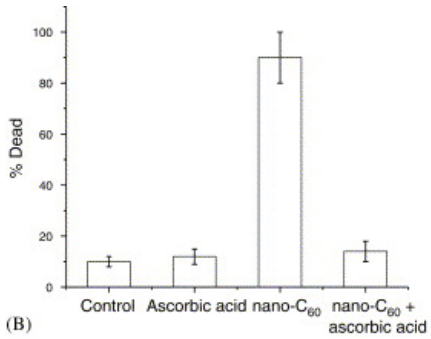
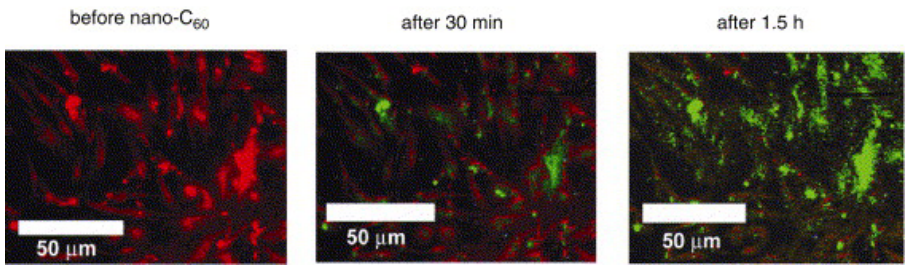
Contrasting findings for oxidant/antioxidant status of C60

C60 Causes oxidative stress and lipid peroxidation in cells

Sayes, C. M., A. M. Gobin, K. D. Ausman, J. Mendez, J. L. West, and V. L. Colvin. 2005. Nano-C60 cytotoxicity is due to lipid peroxidation. Biomaterials 26:7587-7595.

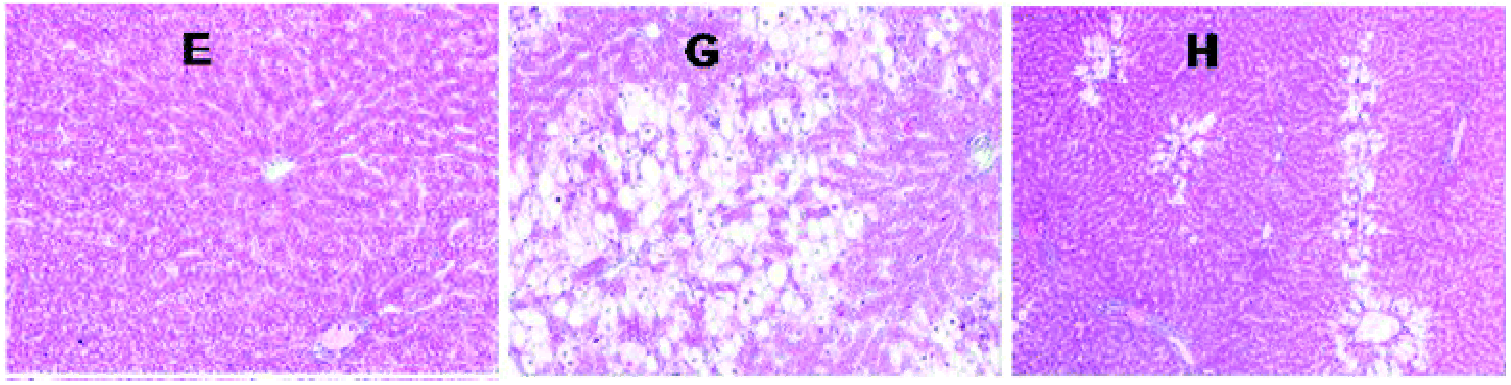






C60 is an antioxidant and protects against CCl4 injury in rats

Gharbi, N., M. Pressac, M. Hadchouel, H. Szwarc, S. R. Wilson, and F. Moussa. 2005. [60]fullerene is a powerful antioxidant in vivo with no acute or subacute toxicity. *Nano Lett.* 5:2578-2585.



C60 CCl4 CCl4 + C60

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Can free radical generation by NP form the basis of a screening strategy for detecting pro-inflammatory particles

Nanoparticle	SA(M^2/g)	Mass (mg)		
		300 cm^2	1500 cm^2	3000 cm^2
CeO2	24	1250	6250	12500
NiO	92	326	1630	3261
TiO_2 (Rutile)	28	1071	5357	10714
TiO_2(Anatase)	259	116	579	1158
SiO_2	523	57	287	574
MgO	42	714	3572	7143
Co3O4	35	857	4285	8571
ZnO	50	600	3000	6000
Al_2O^3(nanowhiskers)	250	120	600	1200
Al_2O_3(Taimincron)	221	136	678	1357
Al_2O_3(GE30)	25	1200	6000	12000
Al_2O_3(A125)	103	291	1456	2913
Carbon black	254	118	591	1182
Fine Carbon black	7.9	3798	18940	37880

Data courtesy of Dr Sen Lin Lu

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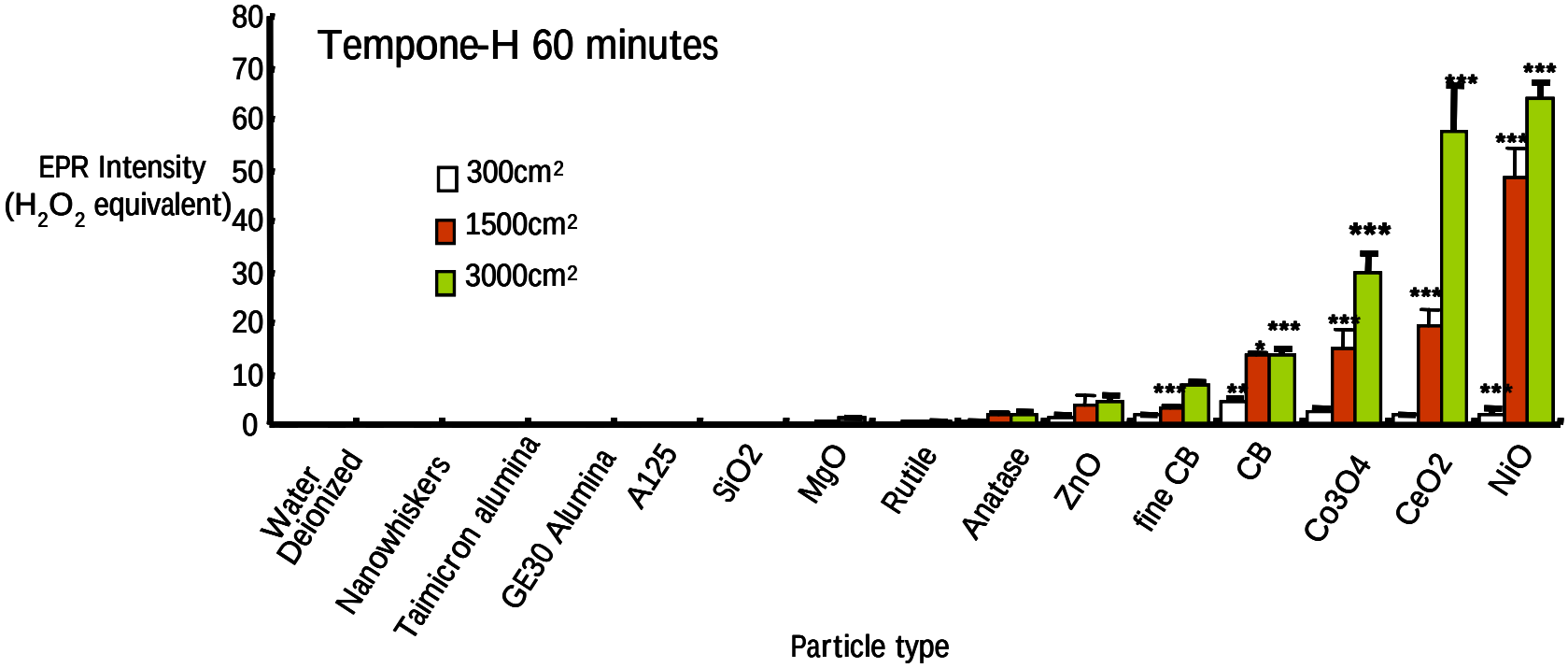
Free radical generation by a panel of metal oxide nanoparticles at equal surface area dose



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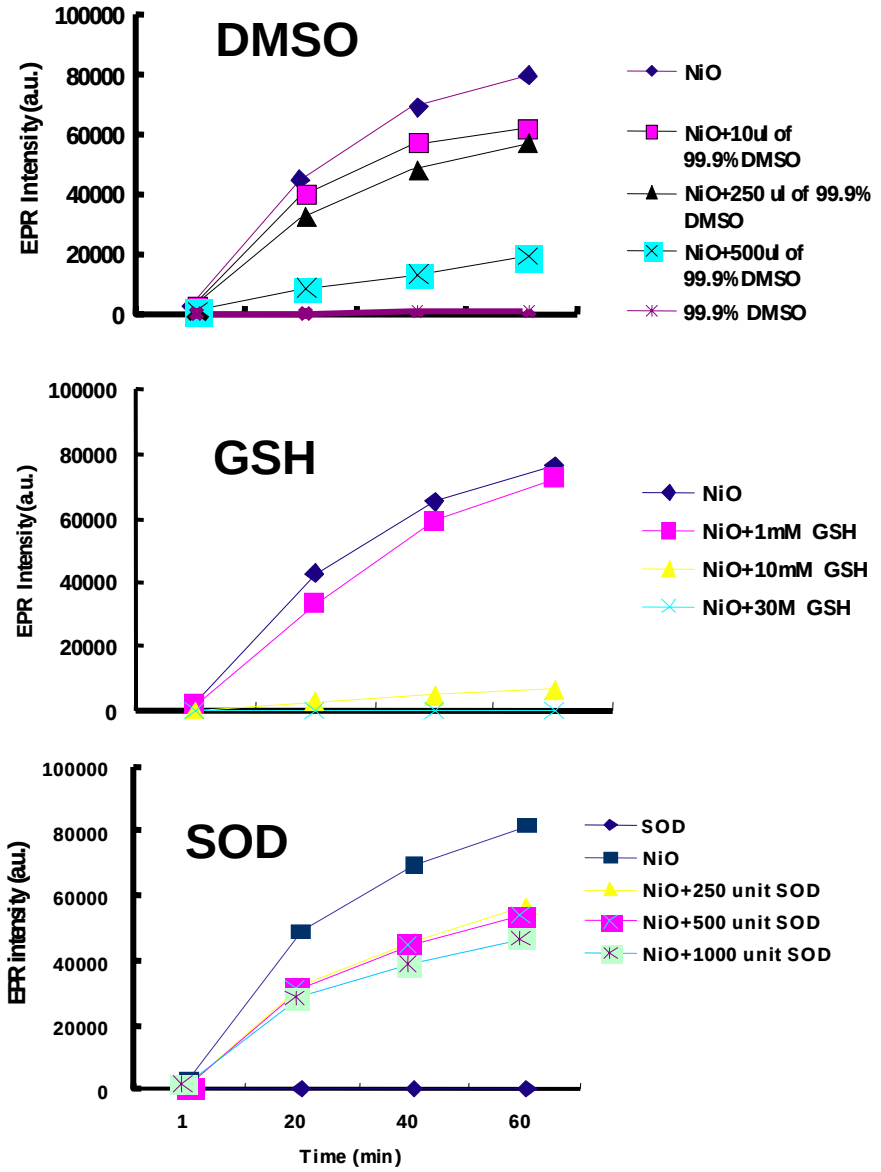
Free radical intensity is ameliorated by antioxidants



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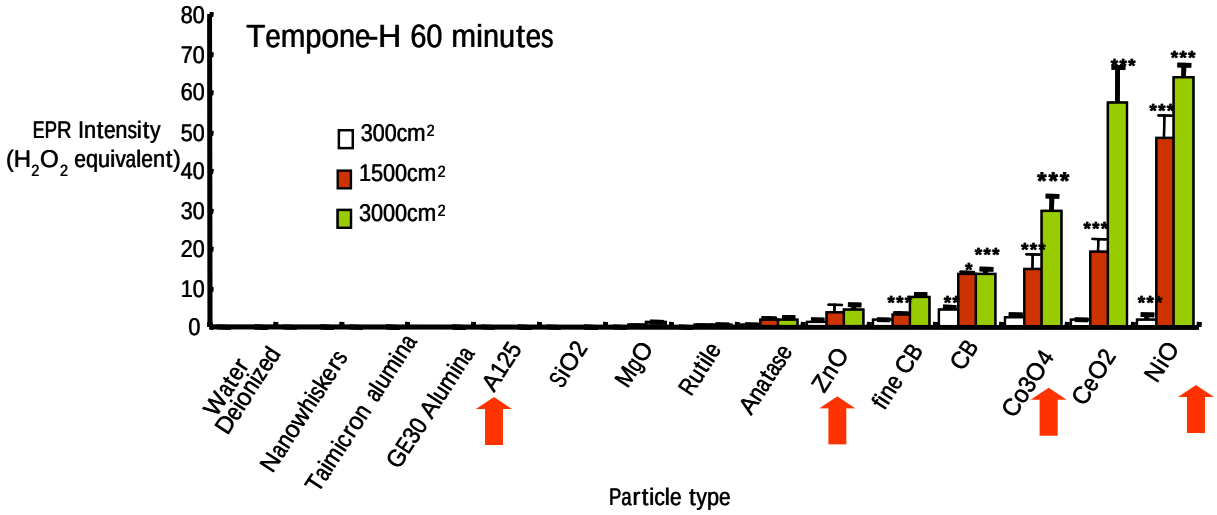
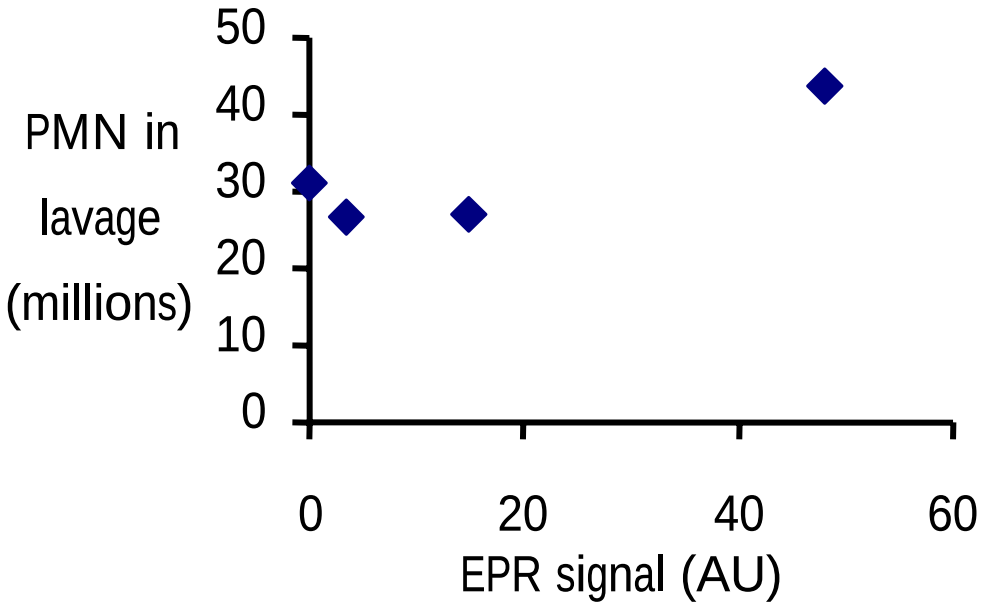
Selected NP tested for ability to cause inflammation in rat lungs at equal surface area dose





A larger inflammation study needs to be carried out for more particles

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State of the art:-Toxicokinetics and dosimetry

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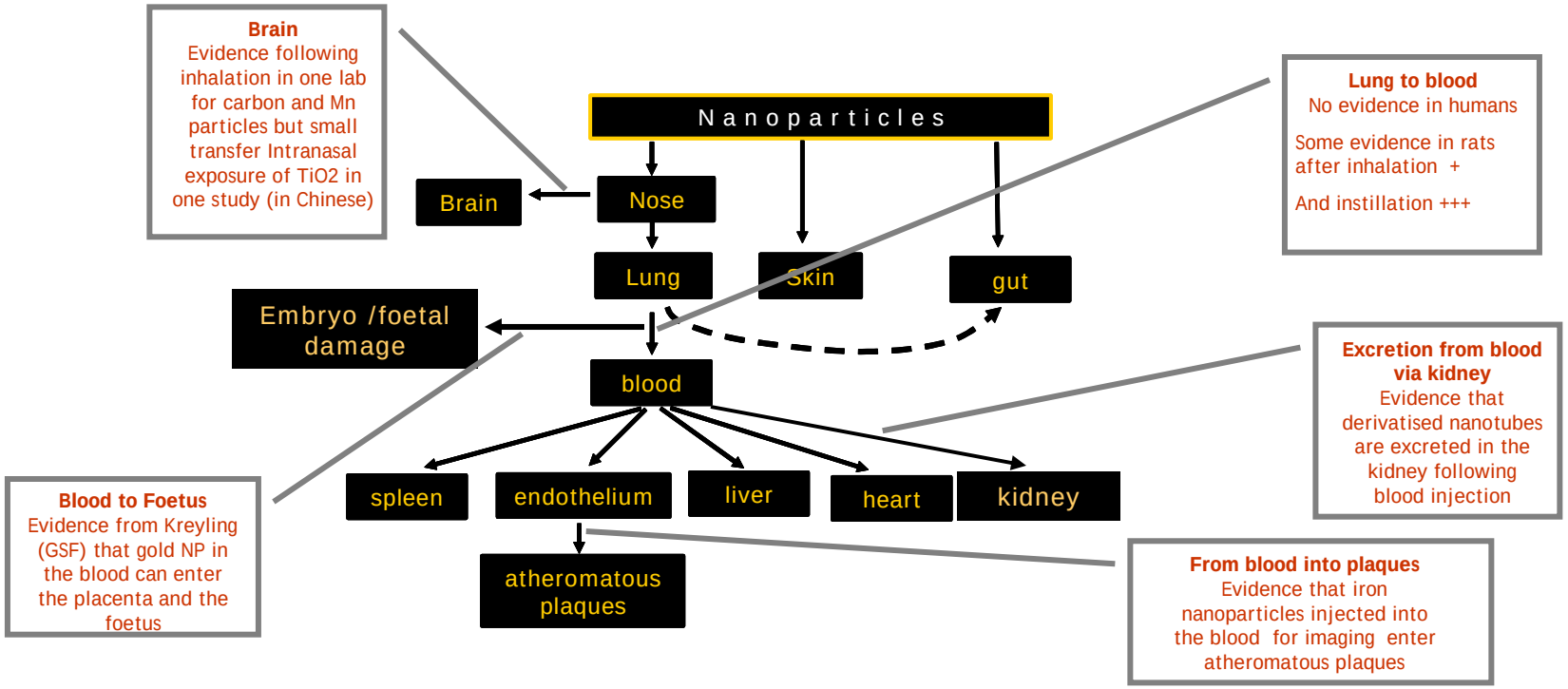
Toxicokinetics



- All of the translocation data is weak and piecemeal
- No mass balance toxicokinetics for any nanoparticle
- So no proper dosimetry for target organ toxicity

More good translocation data urgently required

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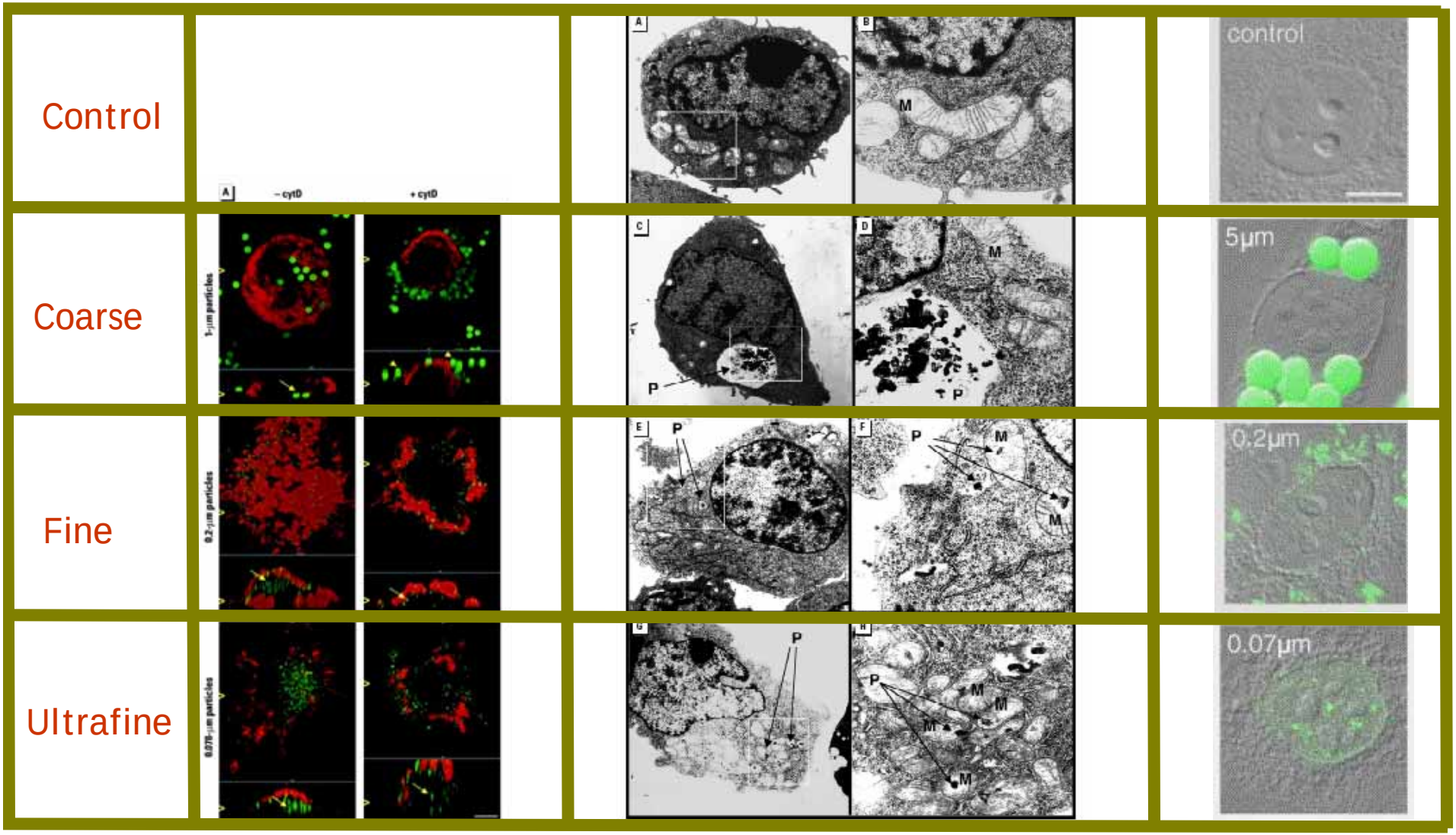
Micro-toxicokinetics – size-related compartmentalisation?



Geiser, M., B. Rothen-Rutlshauser, N. Kapp, S. Schurch, W. Kreyling, H. Schulz, Semmler M, H. Im, V, J. Heyder, and P. Gehr. 2005. Ultrafine particles cross cellular membranes by non-phagocytic mechanisms in lungs and in cultured cells. *Environ Health Perspect*. 113:1555-1560

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.

Chen, M. and A. von Mikecz. 2005. Formation of nucleoplasmic protein aggregates impairs nuclear function in response to SiO2 nanoparticles. *Experimental Cell Research* 305:51-62.

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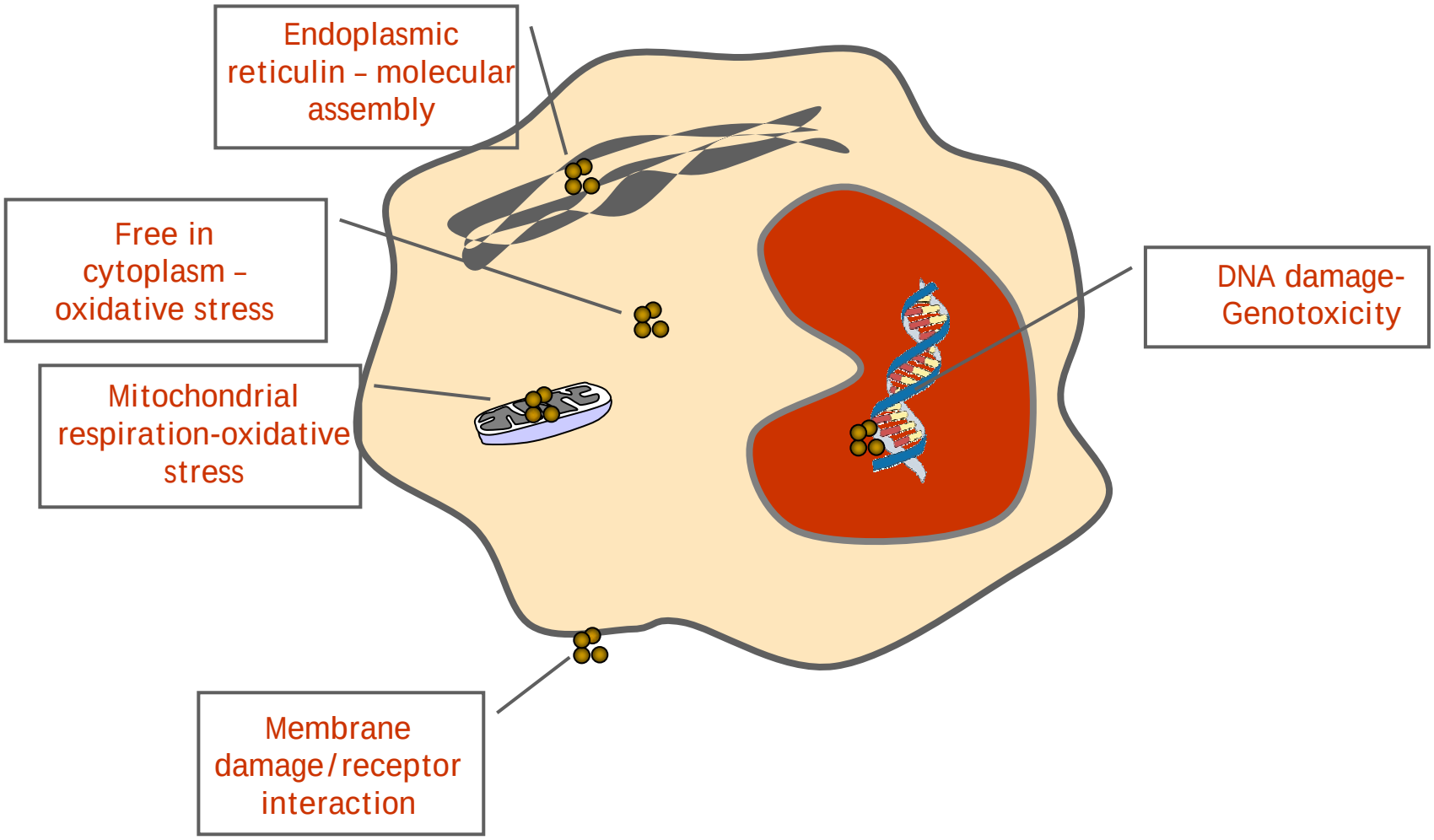
Compartment-related toxicity?



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State-of-the-art:- The problem of testing large numbers of particles – in vitro models and structure activity paradigms

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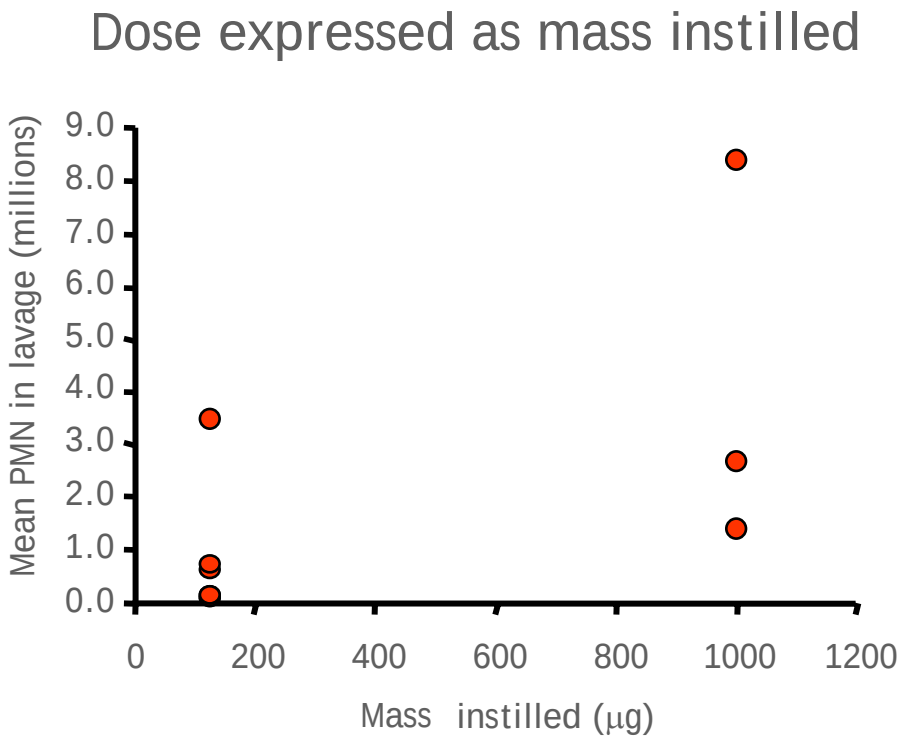
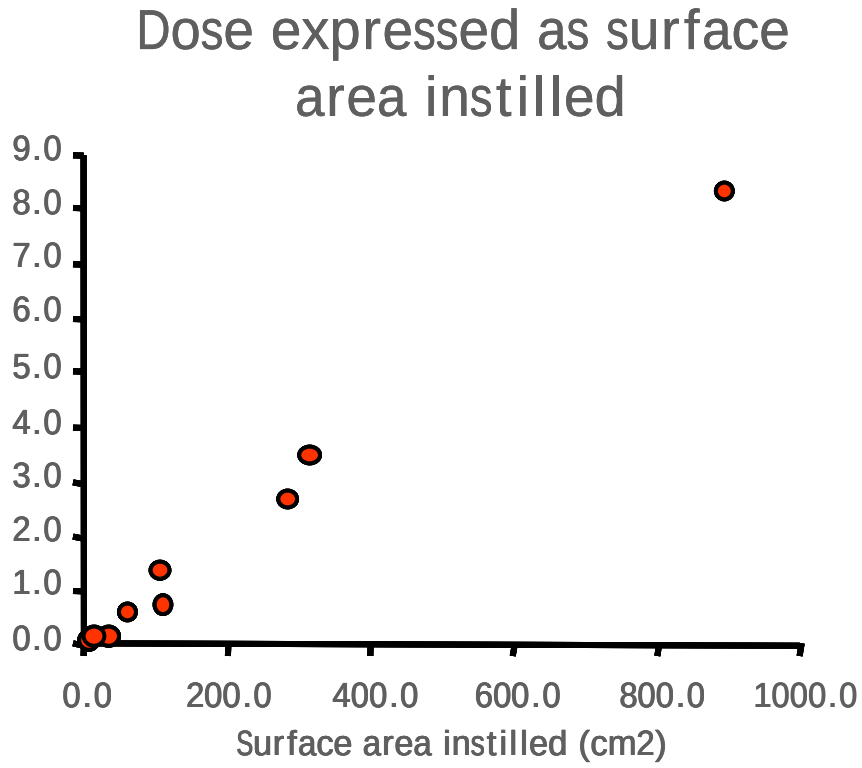
A range of fine and nanoparticle-sized low toxicity, low solubility particles cause inflammation in relation to surface area dose, not mass dose





Duffin R, Clouter A, Brown DM, C. L. Tran, MacNee W, Stone V, and Donaldson K. 2002. The importance of surface area and specific reactivity in the acute pulmonary inflammatory response to particles. *Ann Occup.Hyg* 46 Suppl 1:242-245.

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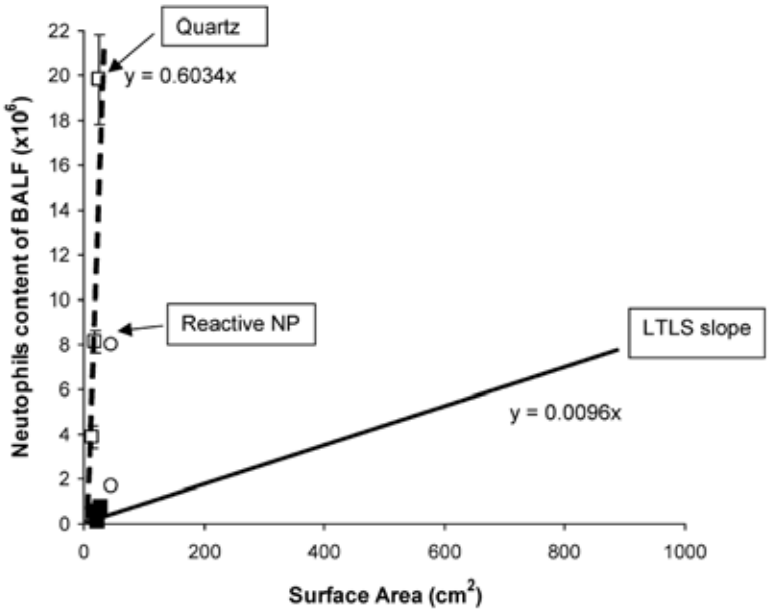
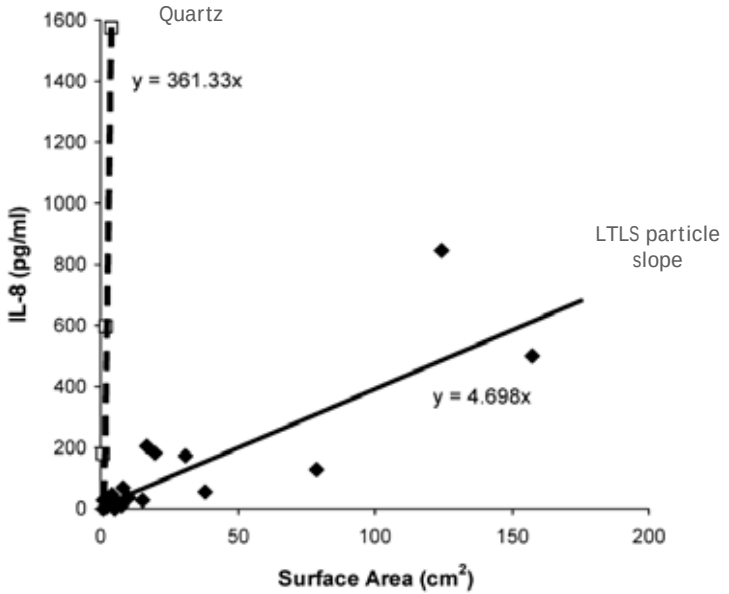
In vitro systems such as the A549 epithelial cell line can model the lung inflammatory response and detect differences in surface reactivity





Duffin, R., L. Tran, D. Brown, V. Stone, and K. Donaldson. 2007. Proinflammogenic effects of low-toxicity and metal nanoparticles in vivo and in vitro: highlighting the role of particle surface area and surface reactivity *Inhal.Toxicol.* 19:849-856.

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3) State-of-the-art:- The prpblem of testing large numbers of particle – in vitro models and structure activity paradigms

Definition

Quantitative correlation of the biological (toxicological) activity to the structure of chemical compounds (particles)

Which structures?	Which activities?	
Size/Surface area	Oxidative stress	Generic toxicity
Free radical generation	Pro-inflammatory	
Charge	Translocation	
Adsorption	Genotoxicity	
Crystallinity	Neuronal action potential	Target cell-specific' toxicity
Coatings	Platelet aggregation	
Metals	Etc.	
Surface derivatisation		
etc.		

We need a QSAR to deal with the huge numbers of NP types and their variants

Everyone who carries out good characterisation could be adding to the QSAR model

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		intensity, known as static light scattering (SLS) and of time dependent scattered intensity due to density or/and concentration fluctuations, often known photon correlation spectroscopy (PCS) or dynamic light scattering (DLS).	[3]
	Acoustic spectroscopy	Ultrasound spectroscopy calculates particle size distribution based on the attenuation of sound waves when passing through the medium. It can measure particle size from a few nanometers to several hundred μm. No sample preparation is needed.	[4]
	Electron microscopy	Transmission Electron Microscopy (TEM) is often used for the direct examination of particles in the size range 0.001 to 5 μm. The TEM produces an image on a fluorescent screen or a photographic plate by means of an electron beam. Analysis is often carried out on the images recorded photographically. Scanning Electro Microscopy (SEM) is caused to scan across the sample in a series of parallel tracks. The SEM is considerably faster and gives more 3-D details than the TEM. Resolutions of SEM are between 15 – 20 nm, while TEM can reach 0.l3-0.5 nm. Sample preparation is very important for both SEM and TEM. SEM and TEM can provide individual particle size information as well as PSD.	[5]
Specific surface area	BET	The theory developed in 1938 by Brunauer, Emmett, and Teller (BET theory) about the physical adsorption of gas molecules on a solid surface can be used to estimate specific surface area based on experimental adsorption data.	[6] [3]
Shape/ Dimensions	Electron microscopy	It is widely accepted that size along often based on a volumetric equivalent diameter is not sufficient for describing the particle size and geometrical dimensions. Electron microscopic imaging and image analysis can be used to determine the shape of particles. Shape can be described by descriptors that have physical meanings such as maximum length and roundness. Such descriptors cannot reconstruct the original shape. Latent shape descriptors such as Fourier descriptors and principal component analysis can be used to reconstruct the original shape because they lose less original shape information.	[3] [7]
Surface chemistry Surface Elements	X-ray photon spectroscopy / / Raman/ potentiometric titrations/ Isoelectric point / polymer adsorption / Atomic Force Microscopy-based scanning probe techniques	Surface chemistry / Surface elements can be characterised by X-ray photo spectroscopy [8] [9], Raman spectroscopy [10][11], potentiometric titrations, Isoelectric point and polymer adsorption [12] AFM can be used to observe the nucleation at surface.	[8] [9] [10] [11] [12]
Bulk chemical composition	energy dispersive X-ray spectroscopy / ICP-MS / SIMS	Chemical composition can be measured by energy dispersive X-ray spectroscopy [13,114,15], Inductively Coupled Plasma Mass Spectrometry (ICP-MS) [16] and secondary ion mass spectroscopy (SIMS) [17]	[13] [14] [15] [16] [17]
Surface porosity	N_2 gas adsorption / Hg porosimetry	Mercury intrusion porosimetry can be used for determination of the average pore size, pore volume, pore size and void distribution and bulk density (pore diameter size range 8 nm – 15 μm). N_2 gas adsorption can also be used for the same purpose. (0,5 nm – 100 nm)	[18] [3]
Roughness	N_2 gas adsorption / SEM/TEM/AFM	The roughness factor r is used as an index which shows the complexity of the solid surface, $r = S_{N2} / S_d$. is the surface area measured by the N_2 adsorption, and is the apparent surface area estimated from particle diameter. When r >1, it is presumed that roughness on a molecular order exists in the surface. More recently, fractal dimensions (i.e. descriptors) are used to describe roughness which can be estimated from SEM / TEM / AFM image analysis.	[3] [19] [20]

Morphology	Electron microscopy	For crystalline particles, for the same polymorph, there may have different morphology or shape. Electron microscopy imaging and image analysis can be used to determine the morphology.	
Agglomeration status	Electroacoustic spectrometry/Acoustic attenuation spectrometry/ Light microscopy/ ranulometry	Agglomeration is a mass-conserving, but number-reducing process that shifts the particle distribution towards larger sizes. Agglomeration can be characetrised by a variety of instruments e.g. dynamic light scateering , AFM, acoustic spectrometry and electroacoustic spectrometry.	[4] [21] [22] [23]
Crystalinity (better called Crystal polymorph or crystalline form)	X-ray diffraction / Raman	Identical chemicals can exist in different polymorphs due to the varied molecular arrangements in the solid state phase. Different polymorphs can have very different physical and chemical properties such as melting point, solubility, density, stability, and bioavailability for drug molecules. X-ray diffraction (XRD) is the most widely accepted technique for determination of polymorphs of a compound, A polymorph has a unique three-dimensional internal structure arrangement (but regular), therefore provides a particular diffraction pattern. Other techniques such as Raman spectroscopy were also investigated for polymorph identification.	[24]
~~Crystal structure~~			
Charge/ zeta potential	Light scattering / electro acoustic spectroscopy	Zeta potential refers to the electrostatic potential generated by the accumulation of ions at the surface of a colloidal particle that is key to define the stability of a colloid system. Zeta potential can be measured by dynamic light scattering and ultrasound	[24] [25][26] [4]
Magnetic properties	photoelectron spectroscopy and x-ray absorption spectroscopy.	Electronic properties of magnetic nano-sized systems can be studied by photoelectron spectroscopy and x-ray absorption spectroscopy. [3, Cpater 3.4]	[3]
Chemical stability /biopersistence	Dissolution rate measurement in vitro	Biopersistence / chemical stability can be assessed by measuring dissolution rate in vitro	[27,[28] [29]
Acidity		Surface acidity of the oxide surface is predictable to some degree based on the chemical property of the atom composition [3, p71]. Surface acidity can me beasured using microcalorimeter [30]	[3] [30]
Redox potential	Electrodes	Redox potential (also known as standard reduction /oxidation potential) indicates the tendency of a chemical species to acquire electrons and thereby be reduced. Each species has its own intrinsic reduction potential. Redox potential is measured in volts (V) or millivolts (mV), and is often defined relative to the standard hydrogen electrode (potential of 0.00 volts). In practice, Ag/AgCl and saturated calomel reference electrodes are commonly used. Redox potential measurement is inexpensive, straightforward and requires little maintenance.	[31]
Oxidative stress	Electron paramagnetic	Electron paramagnetic resonance (EPR, also named electron spin resonance, ESR) is a spectroscopic technique in which free	[32]

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State of the art:- Cardiovascular effects of particles

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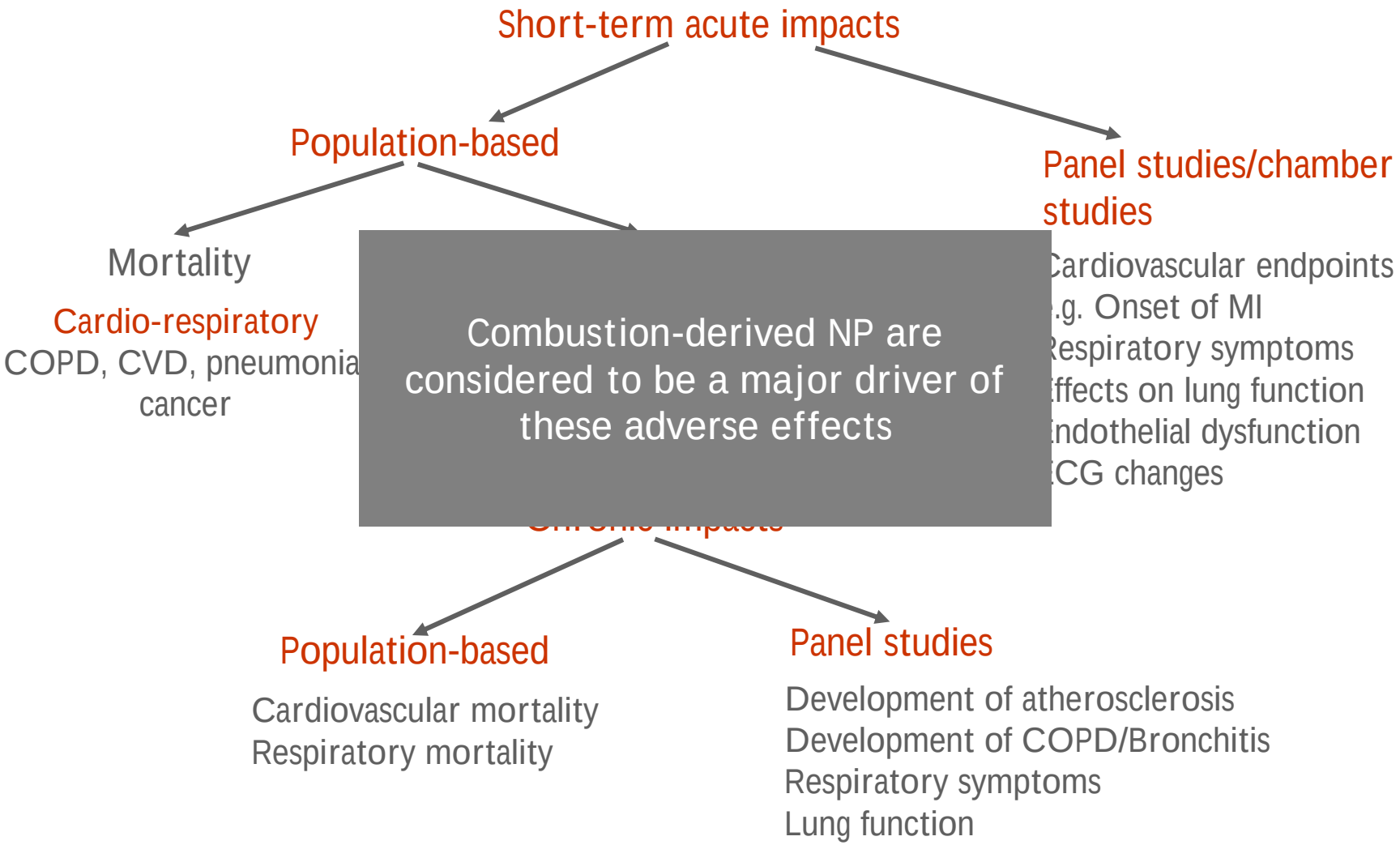
Why the cardiovascular system :- adverse health effects of PM



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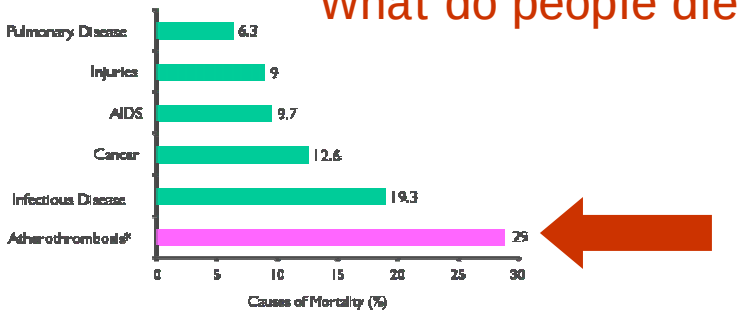
4) State of the art Cardiovascular effects of particles –

The main target of concern if the PM10 data is telling us about combustion derived nanoparticle effects

What do people die of when the particle levels increase?

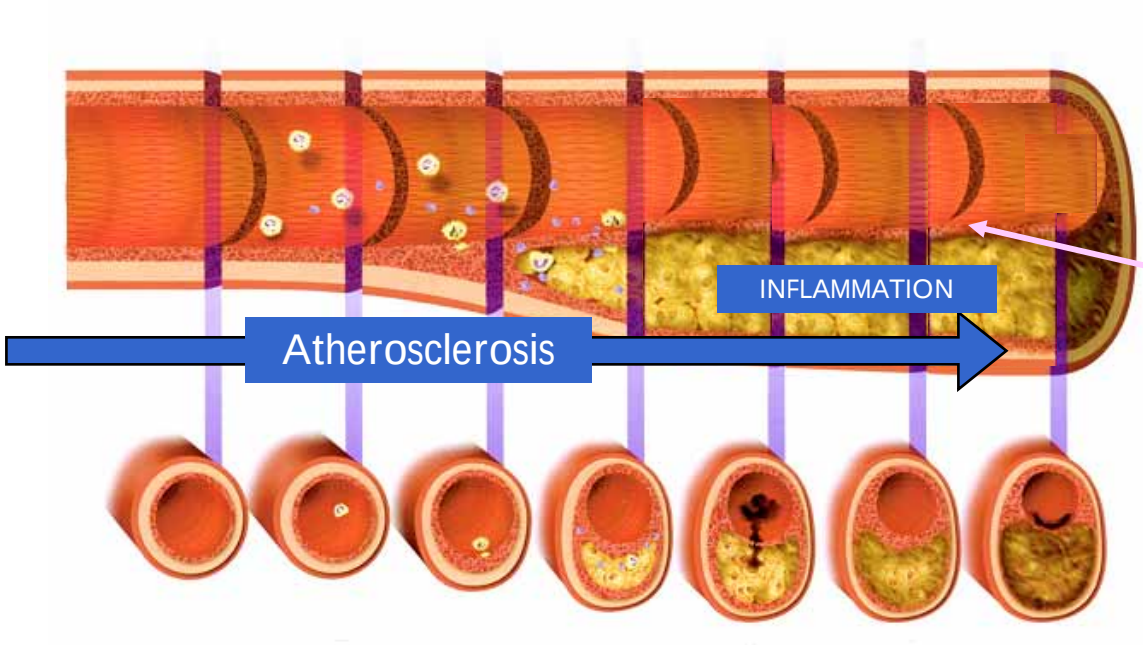


Plaque rupture and Thrombosis

Unstable angina

Myocardial infarction

CV death



Adapted from Libby P. *Circulation.* 2001;104:365-372.

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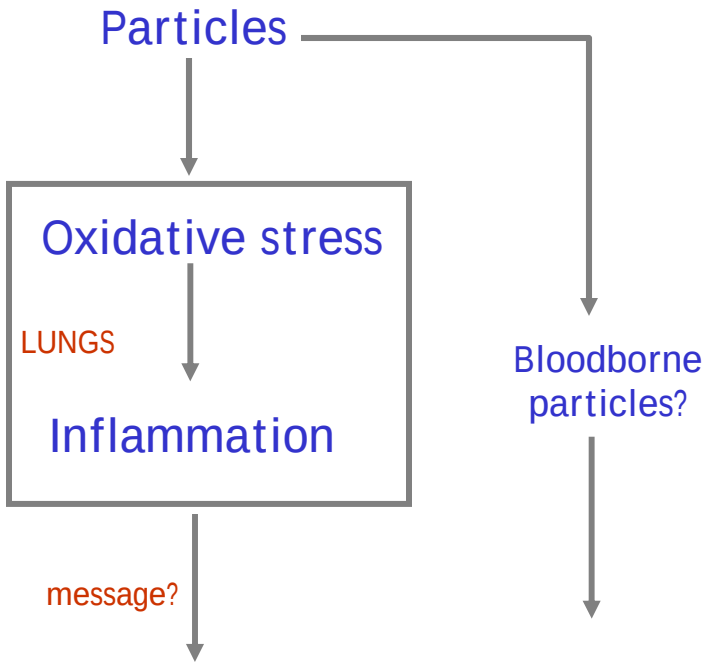
Two pathways for the cv effects of inhaled nanoparticles



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Studying atherothrombotic effects in toxicology studies

Human chamber studies	
Endothelial dysfunction	Forearm plethysmography
Coagulability of the blood	Badimon chamber
Cardiac blood flow/electrical activity	ECG
Mediators/oxidative tress	Fibrinogen, tPA
Rodent models	
Atherogenesis	ApoE mice
Thrombogenesis	Thrombosis models
Blood vessel function	Blood vessel myography
Mediators	Fibrinogen etc

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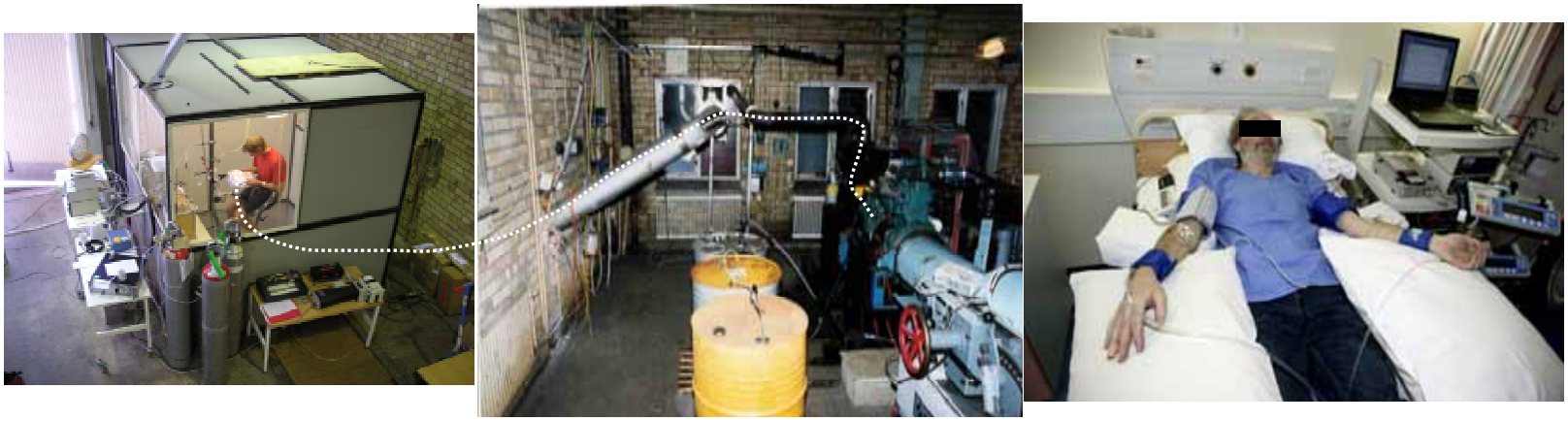
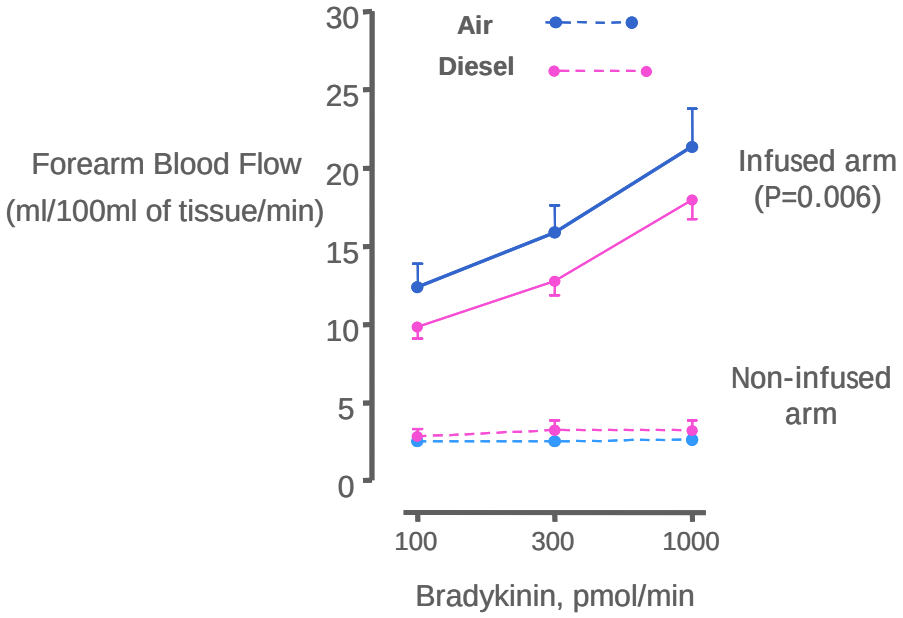
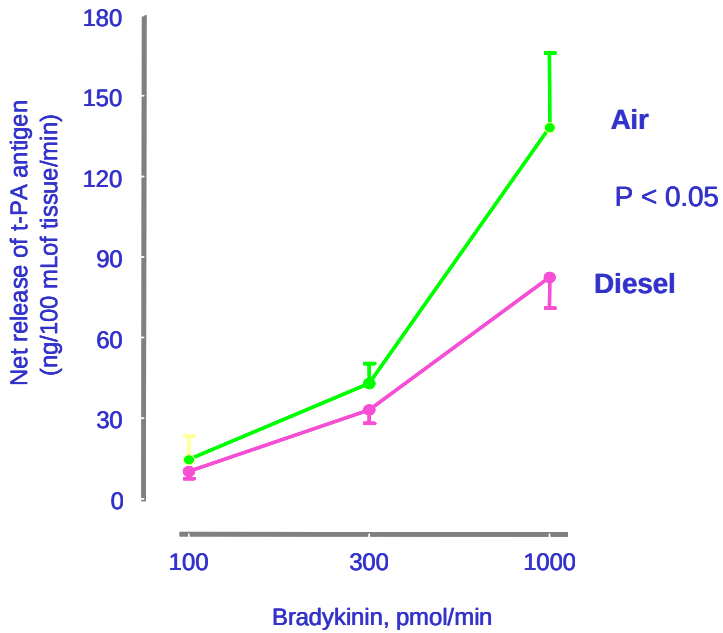
Endothelial dysfunction after diesel exhaust inhalation



Diesel exhaust nanoparticles (300ug/m^3 for 1 hour)





Mills, N. L., H. Tornqvist, S. D. Robinson, M. Gonzalez, K. Darnley, W. MacNee, N. A. Boon, K. Donaldson, A. Blomberg, T. Sandstrom, and D. E. Newby. 2005. Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis *Circulation* 112:3930-3936.

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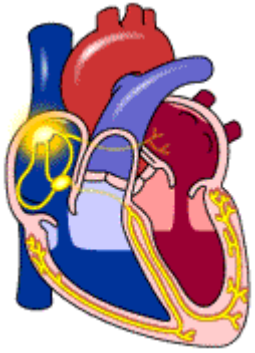
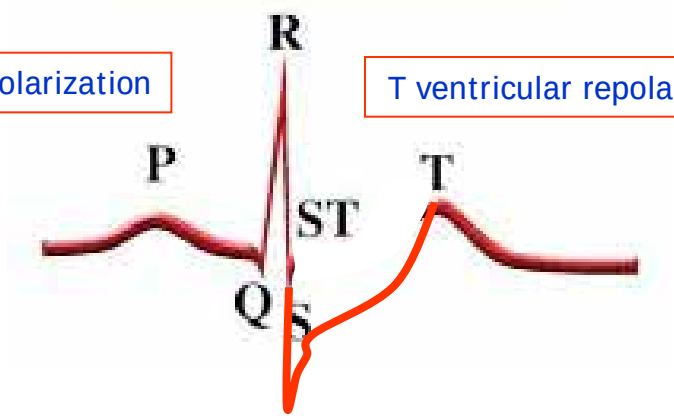
The sequential electrical activation of the heart P-, QRS- and T-waves in the ECG.

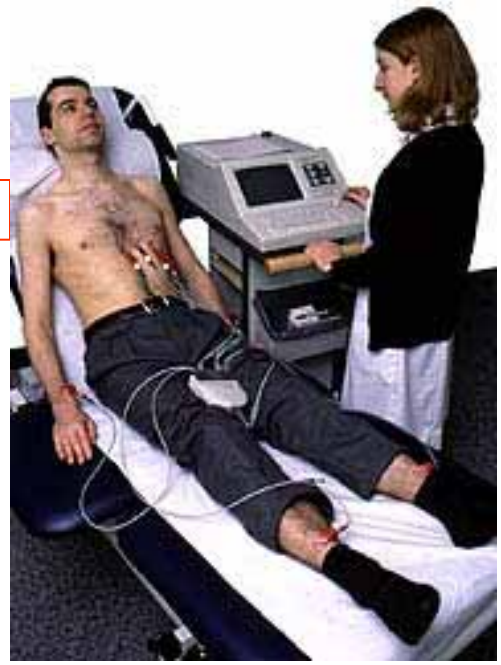


QRS ventricular depolarization

P atrial depolarization

T ventricular repolarization.





- ST usually slanted slightly upward
- In coronary artery disease the blood flow to the heart is compromised.
- If oxygen delivery is not sufficient, ischemia results in the ventricular myocardium.
- The ischemic tissue cannot maintain the membrane potential.
- This is seen as displacement of ST segment downwards
- The larger the ischemic area, the greater this deviation.

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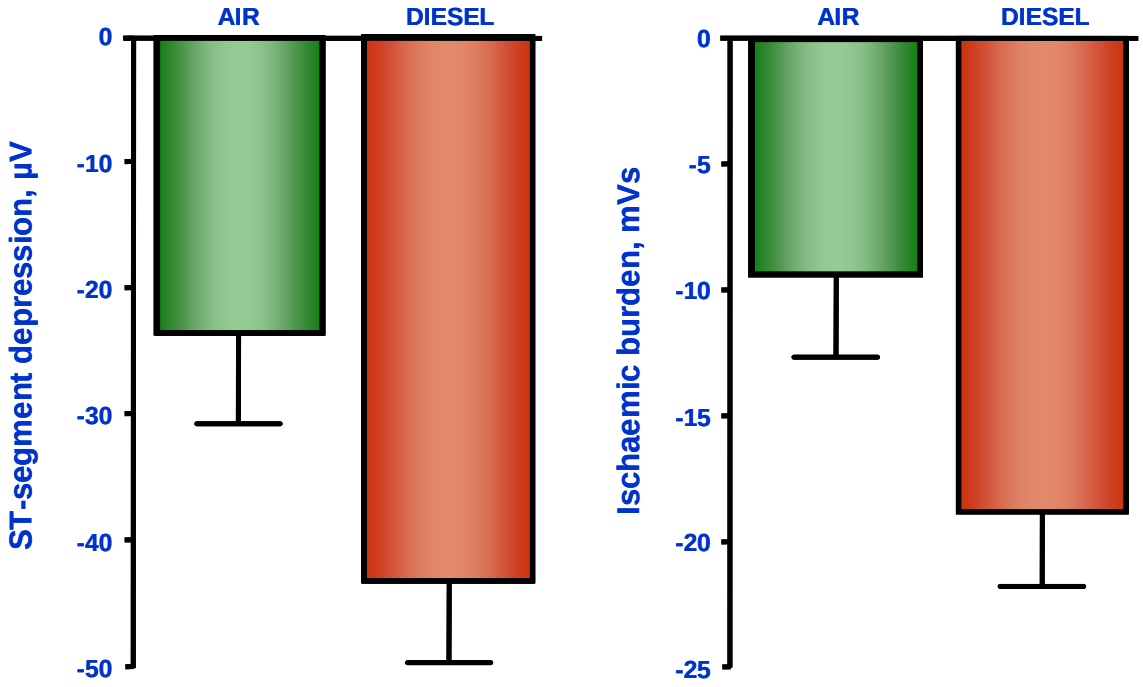
S-T segment depression in patients with stable Coronary Heart Disease exercising in air or dilute diesel exhaust



Inschaemic burden =Duration of exercise x change in ST segment depression

Mills, N. L., H. Tornqvist, M. C. Gonzalez, E. Vink, S. D. Robinson, S. Soderberg, N. A. Boon, K. Donaldson, T. Sandstrom, A. Blomberg, and D. E. Newby. 2007. Ischemic and thrombotic effects of dilute diesel-exhaust inhalation in men with coronary heart disease. *N.Engl.J Med* 357:1075-1082.

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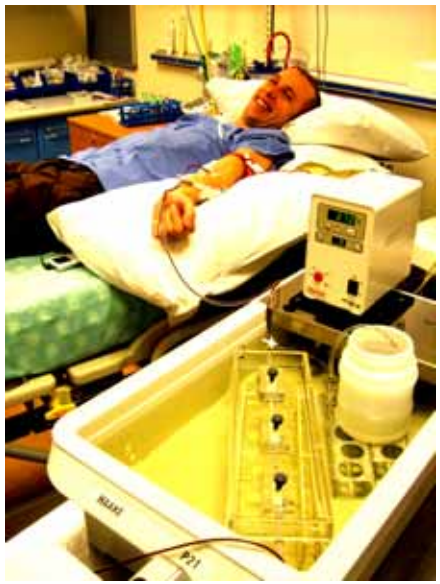
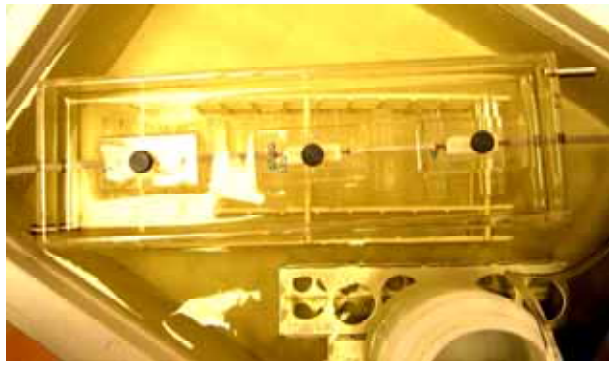
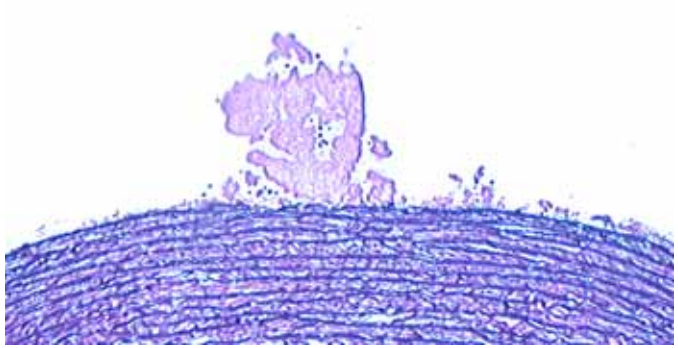
Studying thrombosis in humans after diesel exhaust inhalation
The Badimon Chamber

Expose to Diesel exhaust nanoparticles
(300ug/m3 for 1 hour)







Courtesy of Dr Andy Lucking

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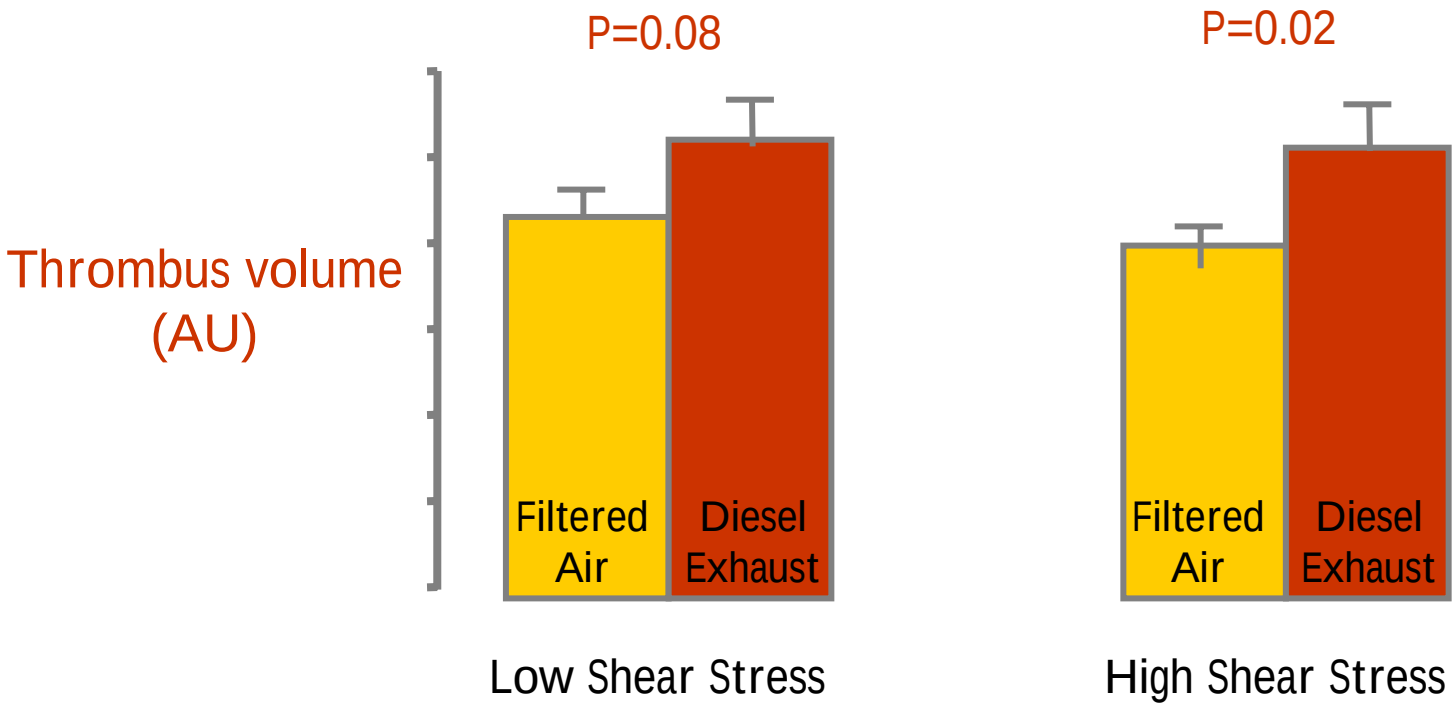
Effect of Diesel Exhaust on Thrombus Formation in the Badimon Chamber

Diesel exhaust nanoparticles (300ug/m^3 for 1 hour)



Lucking *et al*. in press AJRCCM

Courtesy of Dr Andy Lucking

Data are mean + SEM (n=7)

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Stat of the art:-Long thin nanoparticles and asbestos

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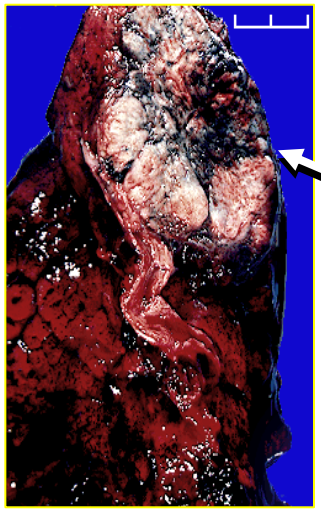
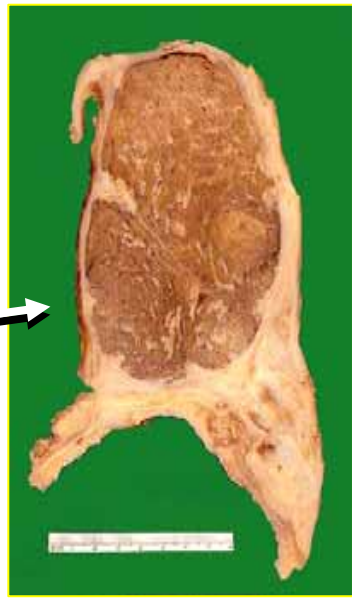
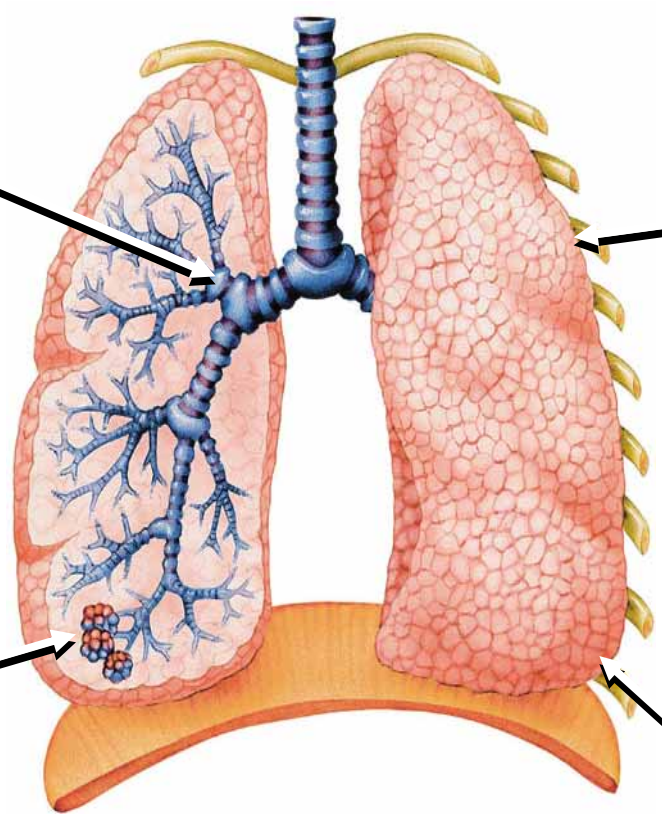
Asbestos -related lung diseases

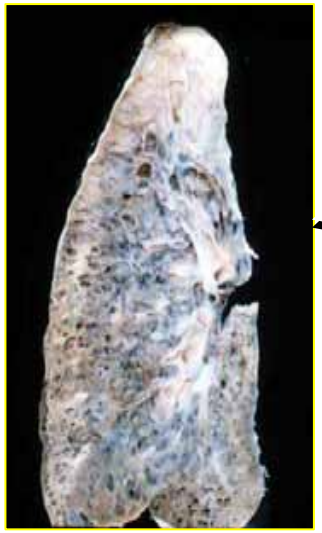
Bronchogenic carcinoma



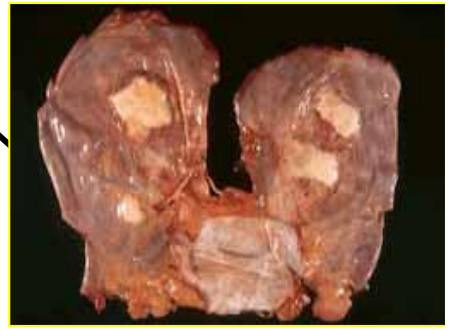
Pleural mesothelioma







Asbestosis Honeycomb lung



Pleural plaque

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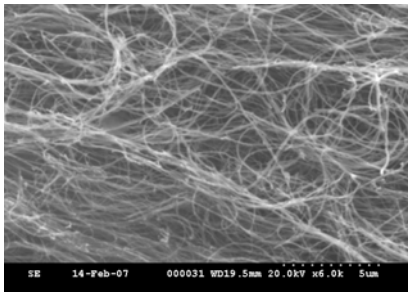
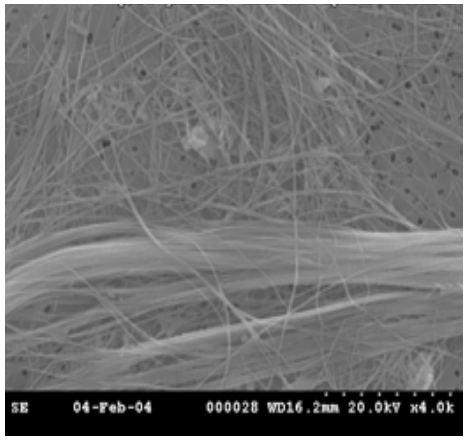
Are nanotubes likely to cause adverse health effects on the basis of being fibres: i.e. could they be the new asbestos?





Asbestos or nanotubes?

Are NTs biopersistent?

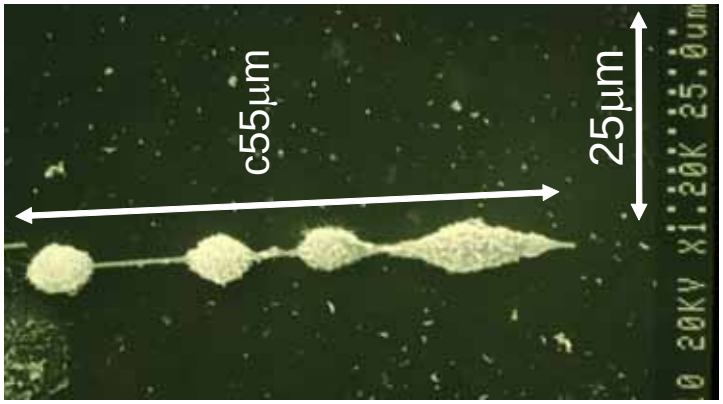
Nanotubes are essentially graphene and so likely to be highly biopersistent in the lungs

Are NTs Long?

How long is long?

Longer than about 15μm poses problems for alveolar macrophages that ingest and remove them

But only if the fibres are rigid – if they roll up into balls then they are less potentially harmful



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Are carbon nanotubes the new asbestos?

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Asbestos and the mesothelium

To determine if fibres act like asbestos it is necessary to assay for unique pathogenic effects of asbestos

Of the diseases that particles cause, the ones unique to asbestos are affects on the mesothelium – mesothelioma, pleural plaques etc

	Inflammation	Fibrosis	Bronchogenic carcinoma	Mesothelioma	Pleural plaques
Quartz	yes	yes	yes	no	no
PM10	yes	yes	yes	no	no
Asbestos	yes	yes	yes	yes	yes

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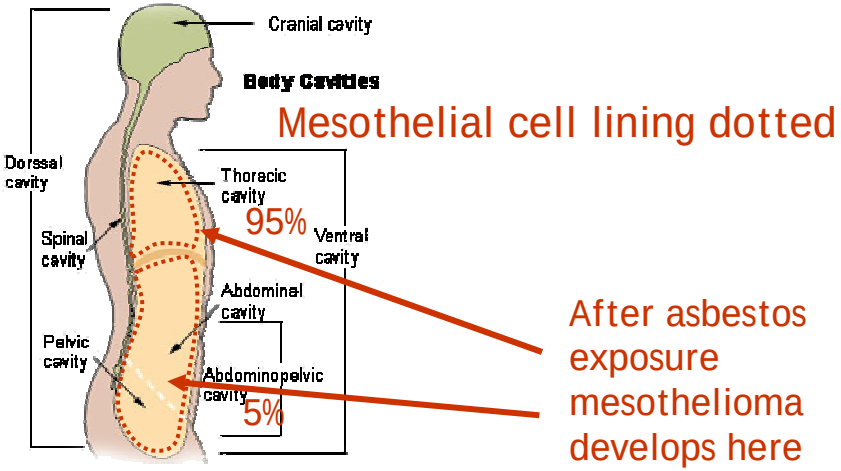
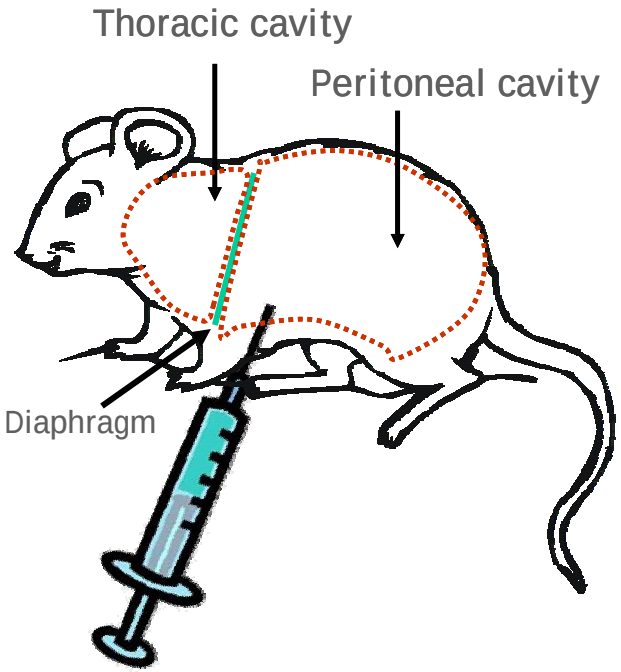
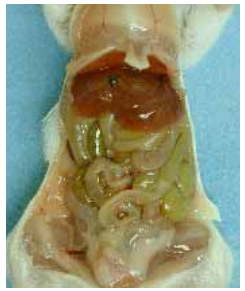
A mesothelial exposure model for assessing the potential toxicity of MWCNT to the mesothelium



Mesothelial cell lining dotted

After asbestos exposure mesothelioma develops here





Inject particles and expose the peritoneal cavity and directly expose the mesothelium and assess the response

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Not a new assay!

Ann. occup. Hyg., Vol. 32, pp. 299–306 Supplement 1, 1988.
Printed in Great Britain
Inhaled Particles VI

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Pergamon Press plc

INFLAMMATORY CELL RECRUITMENT AS A MEASURE OF MINERAL DUST TOXICITY

K. DONALDSON, R. E. BOLTON and D. BROWN

Pathology Branch, Institute of Occupational Medicine, Roxburgh Place, Edinburgh

Abstract—The cellular inflammatory response has been assessed following intra-peritoneal injection of mineral dusts in mice. To calibrate the system, dusts of known pathogenicities were injected at 50, 500 or 2500μg/animal, and the inflammatory response assessed at 2, 4 and 8 days; inflammation was measured as total cells recovered by peritoneal lavage, differential cell count and macrophage activation status. The cellular response was found to be highly sensitive, since a transient response could be detected following injection of saline alone. Using standard dust preparations, titanium dioxide was found to be relatively inactive while quartz and chrysotile asbestos induced a marked inflammatory response. There was some evidence that the cell yield was influenced by macrophage adherence and possibly direct cytotoxicity, and the dose response was consequently not linear. Further work is planned to systematically examine this aspect. Two other particulates were examined in the test system — a respirable coal mine dust and an aramid fibre preparation. The response to the coal mine dust was small while aramid fibre induced a considerable sustained inflammatory reaction. We conclude that the mouse peritoneal cavity can provide a rapid, simple and reliable *in vivo* test of the potential pathogenicity of respirable material.

Ann. occup. Hyg., Vol. 32, pp. 299–306 Supplement 1, 1988.

INTRODUCTION

THE INFLAMMATORY response is a concerted reaction of the host to tissue injury and is now well characterised (RYAN and MAJNO, 1977). One of the earliest manifestations of inflammation is the accumulation at the site of injury of leukocytic inflammatory cells; in the case of a non-antigenic injurious agent such as mineral dust these comprise principally activated macrophages and neutrophils.

Recruitment of both neutrophils and macrophages has been demonstrated following experimental pulmonary deposition of several mineral dusts including quartz (SYKES *et al.*, 1983) and asbestos (LEMAIRE, 1985) and different aspects of dust induced inflammatory reactions have been described (HAMILTON, 1980; DONALDSON *et al.*, 1982). The peritoneal cavity of experimental animals is a site which can be quickly and accurately dosed with known amounts of dust and this has led to its use in studies on the response to particulates. Using this approach it has been possible to obtain large numbers of inflammatory cells recruited in response to dust (HAMILTON, 1980; DONALDSON *et al.*, 1982; MILLER, 1978) and to study the tumorigenicity of mineral dusts (BOLTON *et al.*, 1982).

In the course of our studies into the inflammatory response we noted that a highly repeatable cellular reaction occurred in the peritoneal cavity and we set out to systematically examine this reaction in the hope that it might provide a means for accurately assessing the inflammatory potential of dust. We report here the preliminary results of these experiments on the magnitude and duration of the inflammatory response to two dusts of known pathogenic potential, quartz and chrysotile asbestos; and a relatively inactive control dust, titanium dioxide. We also

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Demonstrating the sensitivity of the inflammatory response in the mouse peritoneal cavity to long fibres and insensitivity to particles

British Journal of Industrial Medicine 1989;46:271–276

Inflammation generating potential of long and short fibre amosite asbestos samples

K DONALDSON, G M BROWN, D M BROWN, R E BOLTON, J M G DAVIS
From the Institute of Occupational Medicine, Edinburgh EH8 9SU, UK

ABSTRACT Previous studies have shown that long thin asbestos fibres are more pathogenic in in vivo and more active in in vitro assays than short fibre samples. In the present study a long fibre amosite asbestos sample and a short fibre sample prepared from it were tested for ability to cause inflammation in the peritoneal cavity of the mouse; a UICC sample intermediate in fibre size and an inert compact dust, TiO_2, were also tested. The ability of the dust samples to cause inflammation, as judged by macrophage and neutrophil recruitment, was ranked in the order long fibre > UICC > short fibre > TiO_2. Ability of amosite samples to cause inflammation was therefore related to the proportion of long fibres. The enhanced ability of long fibres to cause inflammation and cause macrophage activation is probably a key factor in the ability of long fibres to cause pulmonary fibrosis and may also be important in fibre carcinogenesis.

Inhalation of asbestos dust is associated with the development of interstitial pulmonary fibrosis and pulmonary neoplasia.[1] Experimental studies have shown, however, that not all asbestos samples are imbued with the same potential to cause lung disease. The shape of the fibres is one factor of major importance in this phenomenon and has been extensively studied (see review[2]) showing that long thin fibres are more pathogenic than short fibres. Studies from our own laboratory have recently shown that long amosite asbestos fibres, administered by inhalation, are substantially more pathogenic than a short fibre sample prepared from the long fibres and having essentially the same crystallinity and elemental composition.[3] The in vivo findings on the pathogenicity of long fires have been, in general, supported by in vitro studies showing that long fibres are most active in short term assays.[4]

Inflammatory responses in the lung parenchyma, while an important defence mechanism in normal circumstances, is considered to be an important arbiter of tissue damage,[5] leading to alveolar destruction or fibrosis if the inflammation persists.[6,7] The inflammatory potential of mineral dusts is therefore likely to be an important correlate of their pathogenic potential.[8]

Recent work from our laboratory has described an assay of the ability of mineral dust to cause inflammation in vivo[9] and differences in pathogenicity of long and short fibre amosite samples.[3]

The present study brings these together and seeks to examine the inflammation generating potential of the long and short fibre amosite samples to determine whether this correlates with the ability to cause damage to rat lungs as previously described.[3]

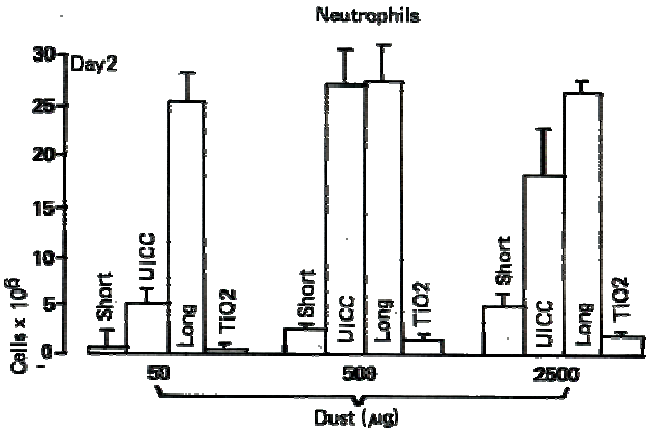
Accepted 21 March 1988

Materials and methods

DUSTS

The titanium dioxide was the rutile form supplied by Tioxide Limited (Stockton-on-Tees).

The long fibre amosite comprised a batch of commercially available, milled South African amosite. This sample was generated as a cloud in an exposure chamber (for details see ref 3) and the airborne fibres were found to have a size distribution substantially longer than that of the standard UICC amosite which we have used previously.[10] The long fibre sample used in the present study was collected from the chamber air on to filters. Details of preparation of the short fibre amosite samples have also been given previously.[3] Briefly, a quantity of the bulk long fibre sample described above was ground in a ceramic ball mill and sedimented in water; comprehensive analysis of the final sample showed no loss of crystallinity and an elemental composition close to the parent long amosite sample. Details of preparation of the UICC amosite sample have been given previously.[11]



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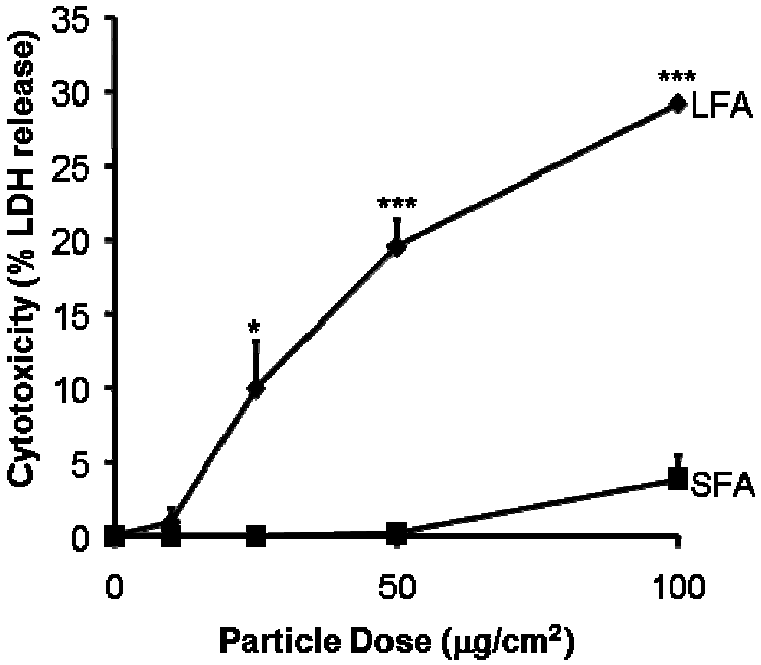
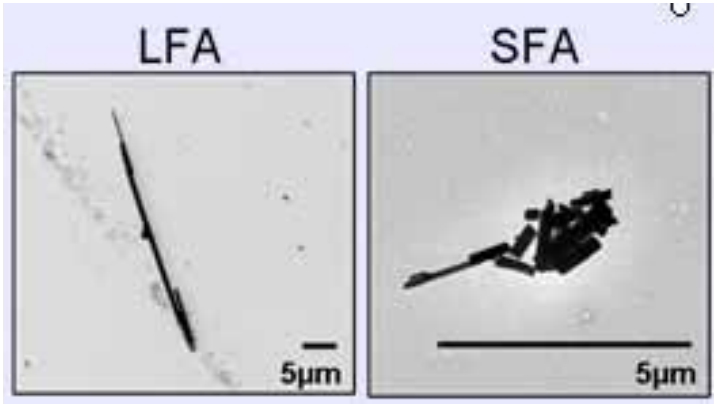
Mesothelial cells in culture are exquisitely sensitive to the toxic effects of long fibres





Long fibre amosite Short fibre amosite

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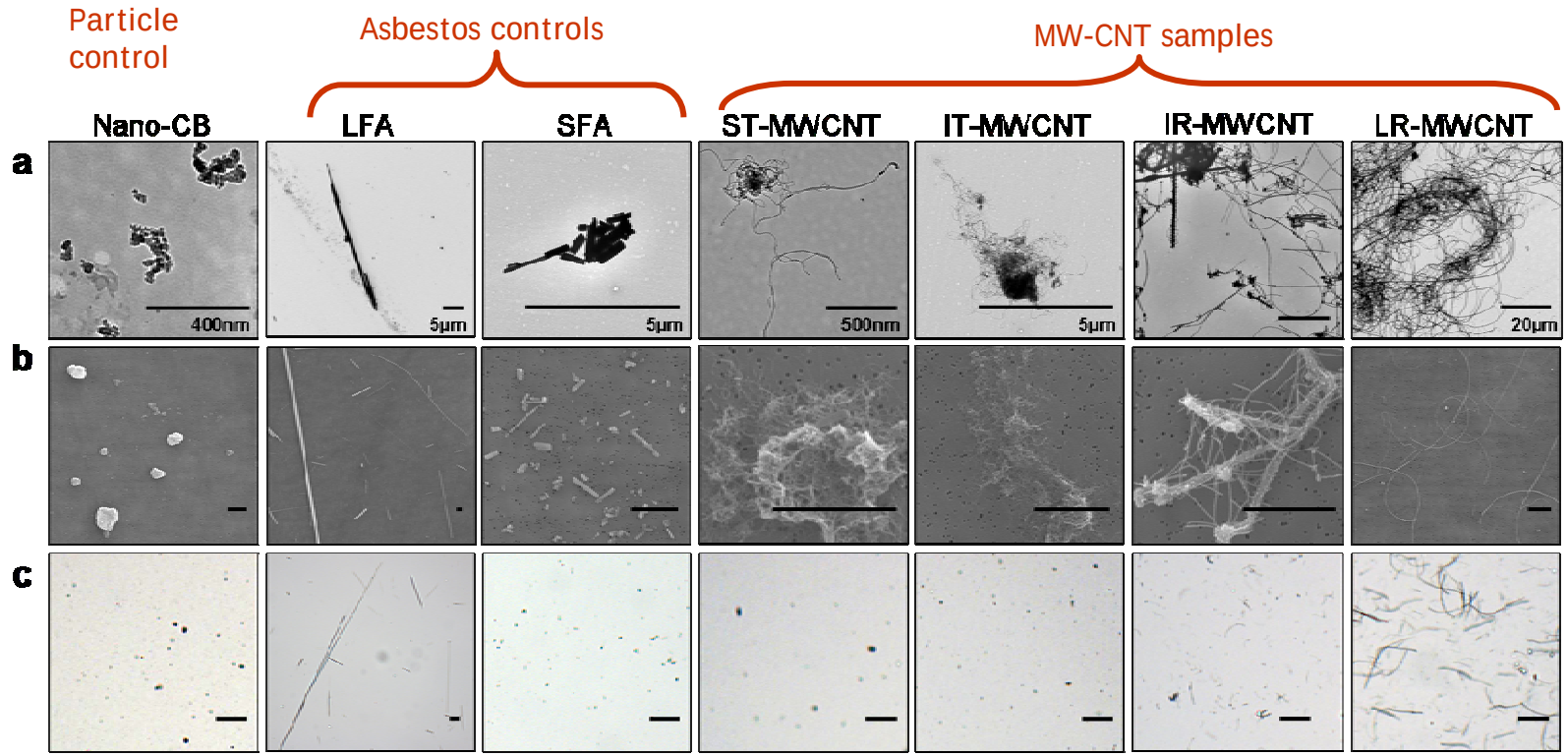
The controls and the hypothesis and the test samples of MWCNT



Long fibres visible	no	+++	no	no	no	++	+++++

If the model responds to long fibres these will be pathogenic

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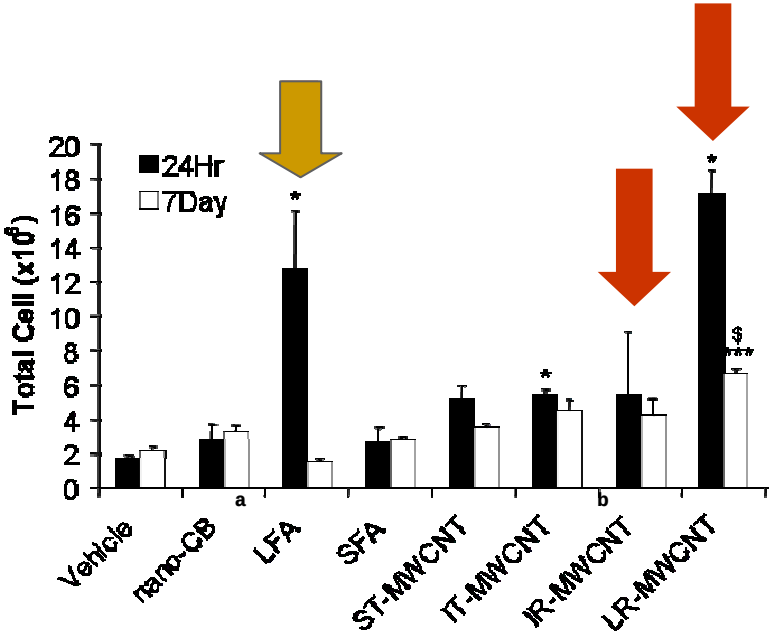
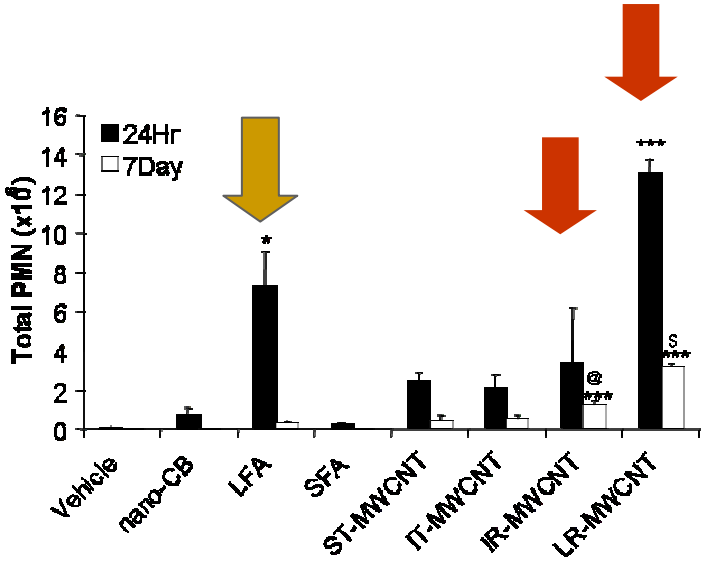
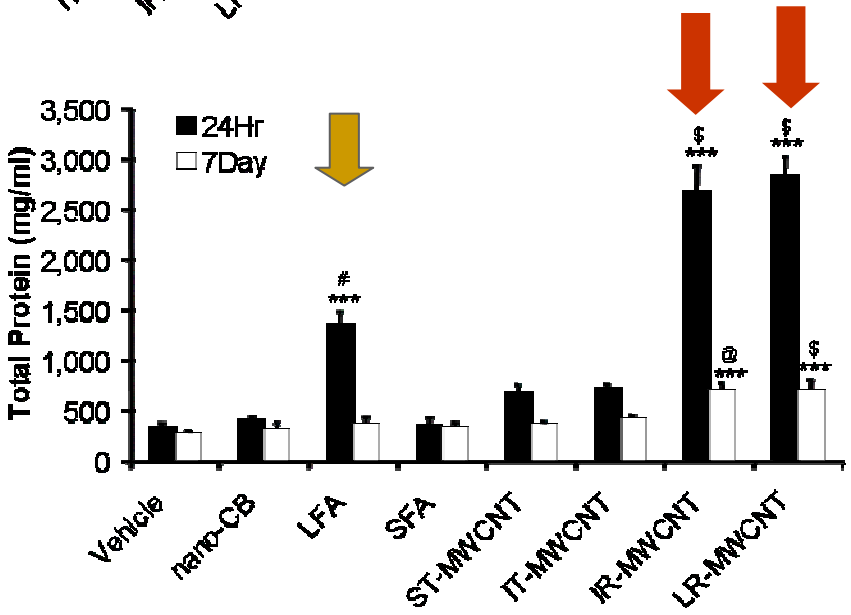
The inflammatory response at 1 and 7 days







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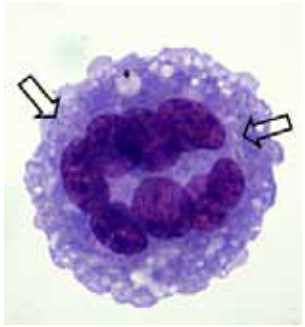
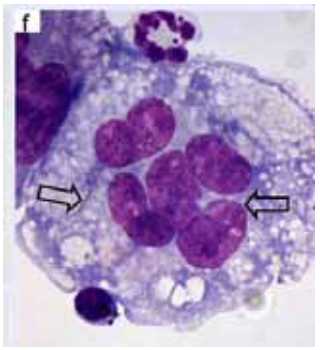
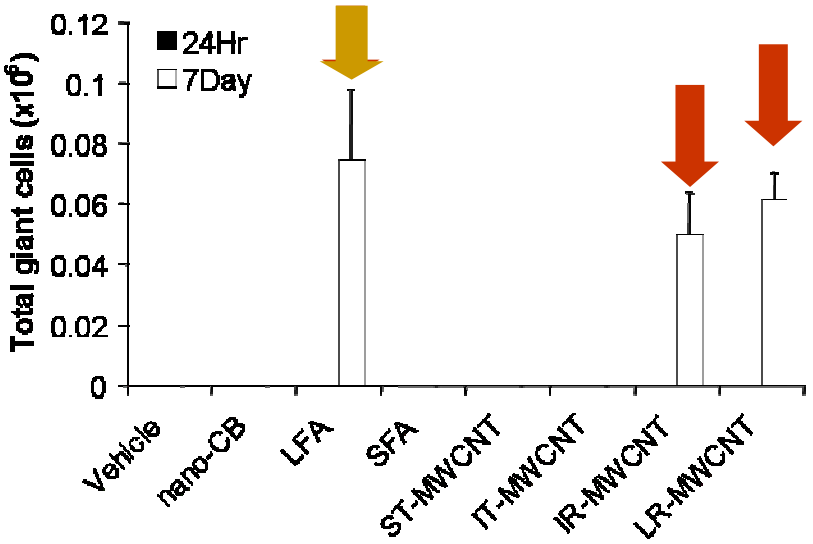
The foreign body giant cell response at 1 and 7 days







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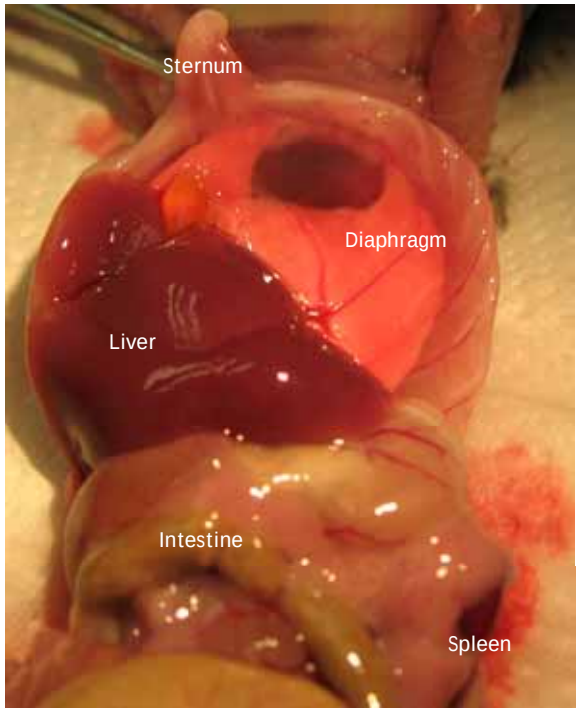
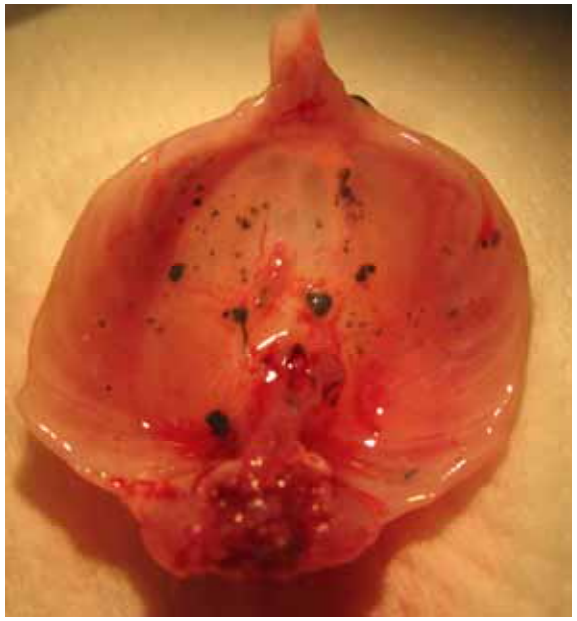
Harvesting diaphragms for histology





Diaphragm after LR-MWCNT

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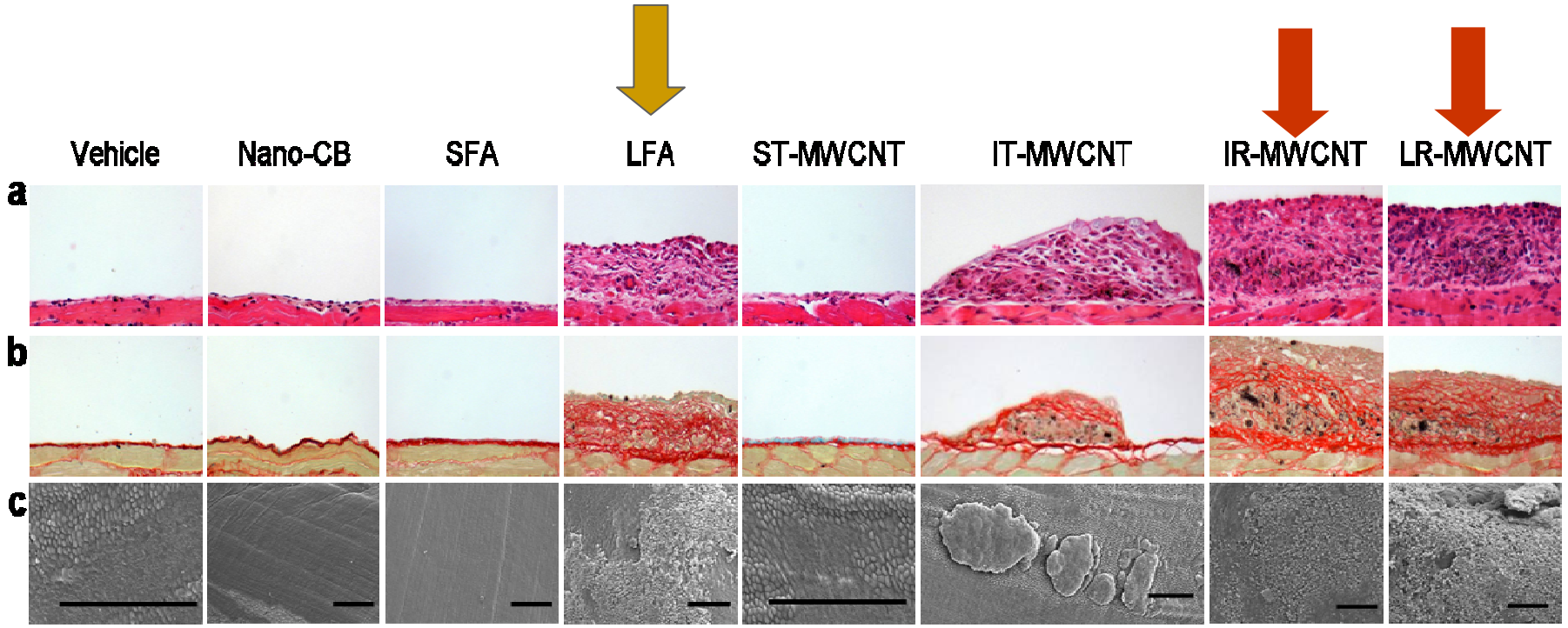
Granulomatous lesions on the diaphragm



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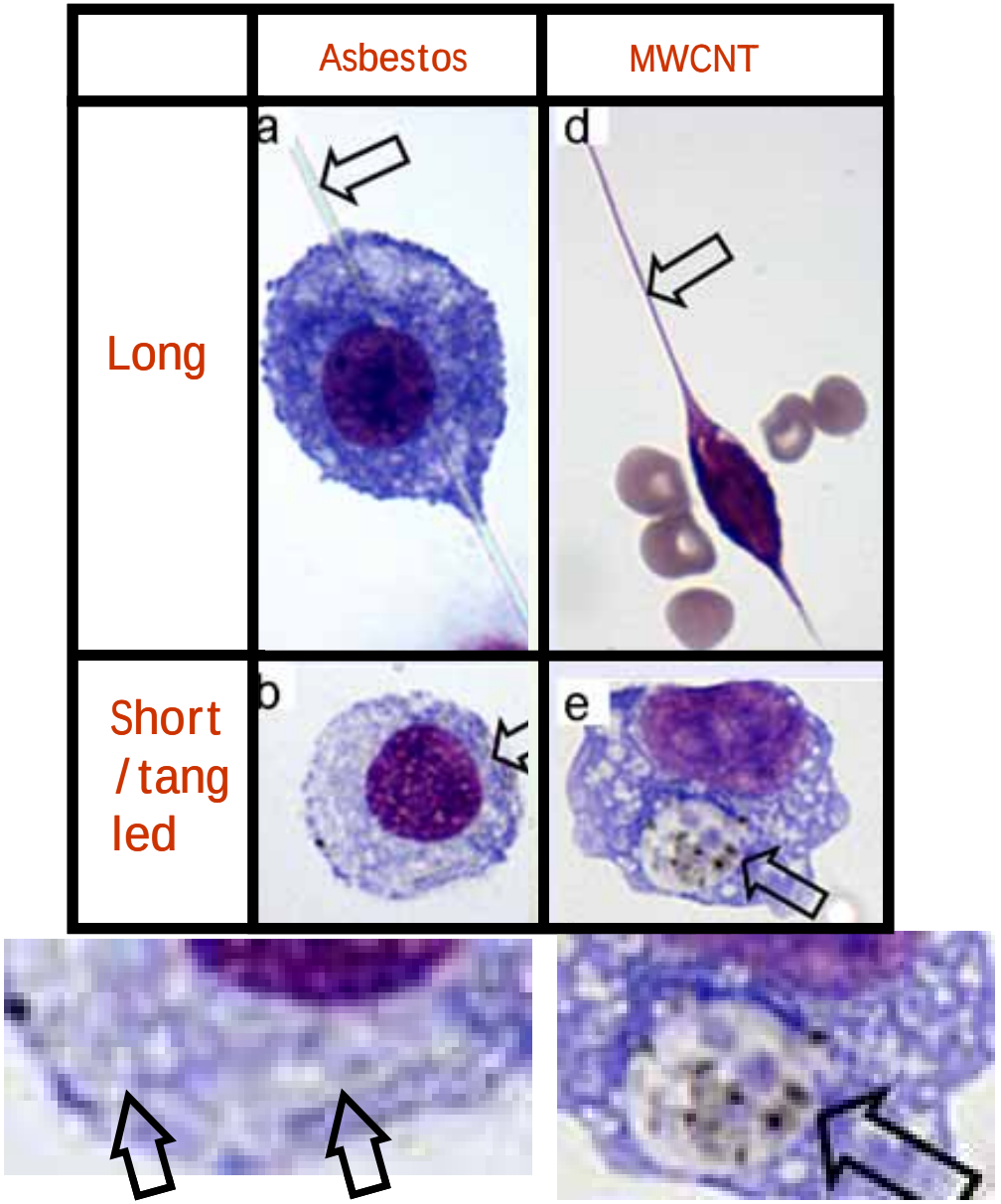
Mechanism – frustrated phagocytosis of long fibres- complete phagocytosis of the short/tangled



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Conclusion

1. Not all nanotubes are created equal
2. Some samples seem to act like asbestos and some don't
3. The more long ,rigid and fibre-like the CNT sample, the more likely it is to behave like asbestos -unsurprising when asbestos has its effects via its length and rigidity

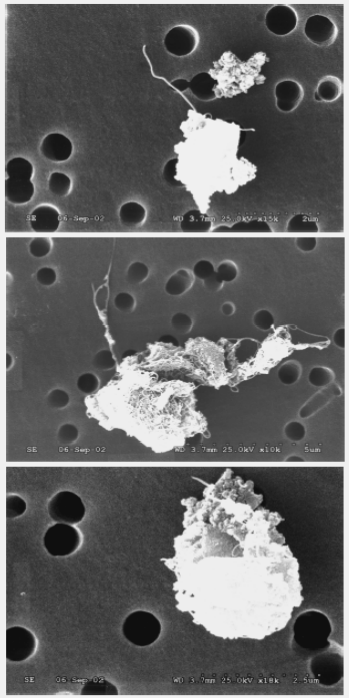

FIGURE 5. SEM images of typical micrometer-sized aerosol particles collected during handling laser ablation nanotube material.

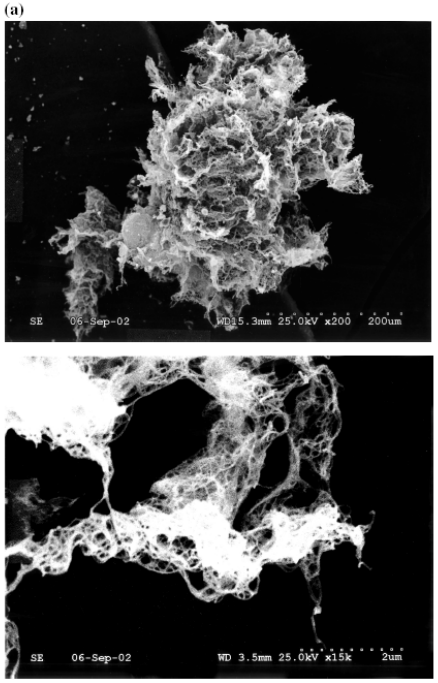

FIGURE 4. SEM images of aerosol particles generated from handling HiPCO SWCNT. (a) Particles lager than ~100 μm in diameter. (b) Particles of the order of μm in diameter.

Maynard, A. D., P. A. Baron, M. Foley, A. A. Shvedova, E. R. Kisin, and V. Castranova. 2004. Exposure to carbon nanotube material: aerosol release during the handling of unrefined single-walled carbon nanotube J.To*xicol.Environ.Health A* 67:87-107.

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State of the art – summing up

- The oxidative stress hypothesis

Many NP appear to cause oxidative stress- some are antioxidant too- choice of assay-work in progress

- Toxicokinetics and the problem of hazard identification

Hardly any data in the last few years – difficult and expensive to do but badly needed

- The large numbers of particles that require testing – in vitro models and structure activity paradigms

Pro-inflammatory effects in the lungs - in vitro might be predictive – more work needed

Non-pulmonary targets - in the absence of toxicokinetic data there will be false positive data due to high dose effects

- The effects on the cv system

Atherothrombosis is a potential target for NP if the PM_{10} literature (Combustion-derived nanoparticles) can be extrapolated to manufactured NP – models are available for the key effects (endothelial dysfunction, atherogenesis, thrombosis)

- Long thin nanoparticles and the asbestos paradigm

The more long and thin the CNT (by definition biopersistent) the more likely it is to behave like asbestos

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Toxicologists study hazard

It doesn't make sense for a toxicologist to use a low dose, not get an effect, stop and go home

We increase the dose till we get an effect (dose response) and then that needs to be interpreted by risk assessors using data from exposure to contextualise the toxicology data to plausible doses

There is a desperate need for more exposure/dosimetry research to get from hazard to risk

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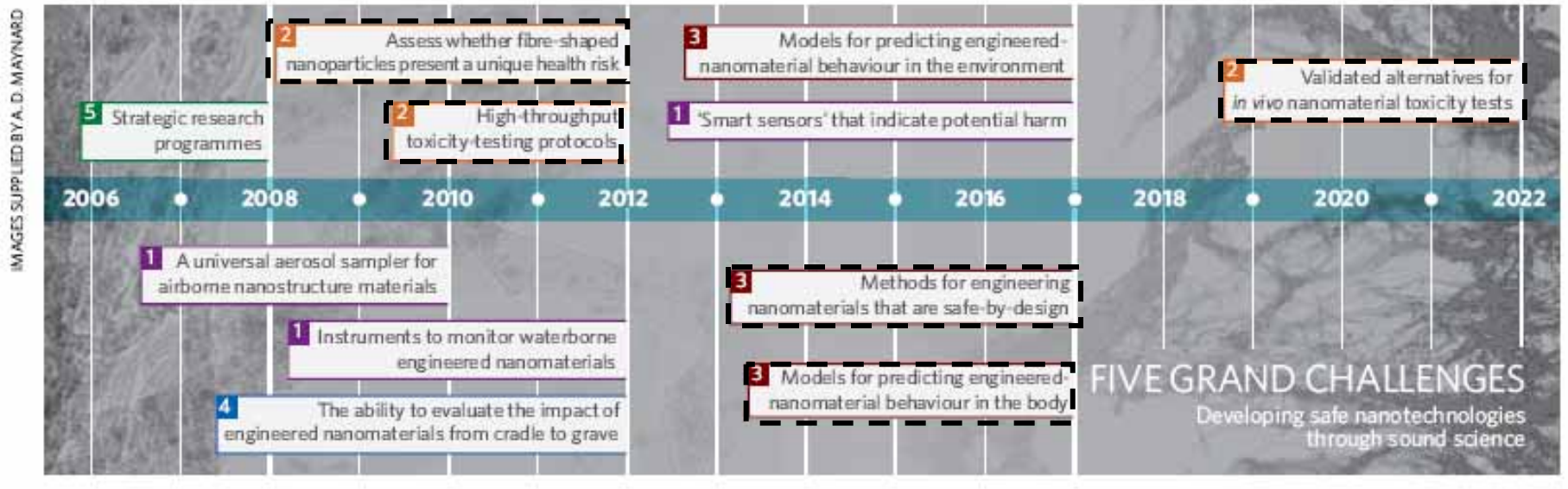
A way forward for safe nanotechnology industry



Maynard, A. D., Aitken, R. J., Butz, T., Colvin, V., Donaldson, K., Oberdorster, G., Philbert, M. A., Ryan, J., Seaton, A., Stone, V., Tinkle, S. S., Tran, L., Walker, N. J., and Warheit, D. B. (2006). Safe handling of nanotechnology
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