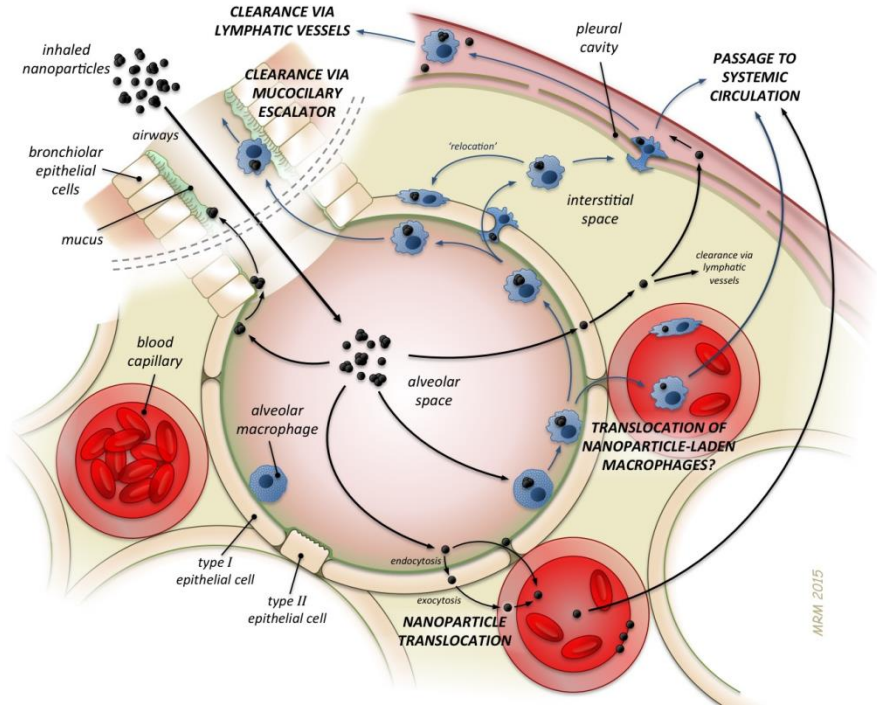
National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport



Nanotoxicology progress for urban and manufactured nanoparticles

Flemming R. Cassee

PARTICLE AND FIBRE TOXICOLOGY

Utrecht University

FP7 → H2020 self-review/achievements

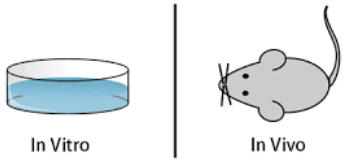
Derk *Brouwer, Flemming Cassee, Harald Krug, Iseult Lynch, Jérôme Rose, and Socorro Vacquez*

- Issues with nanoparticle **interference** with tests to assess toxicity
- Issues with lack of **batch-to-batch** reproducibility of nanoparticles
- Limited **characterisation** of nanoparticles in the actual exposure medium,
  - no understanding of agglomeration or actual dose presented in experiments;
- Early understanding of the **interactions** with biomolecules as playing a major role in determining nanoparticle uptake in cells – relevance to in vivo not yet established

FP7 → H2020

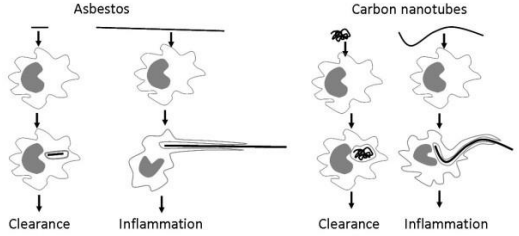
- Belief that direct correlations between simple descriptors and toxicity could be determined;

- Focus on simple cytotoxicity with acute time points only,
  - no correlation with actual dose of nanoparticles (i.e. agglomeration not fully considered).
  - Poor in vitro – in vivo correlation



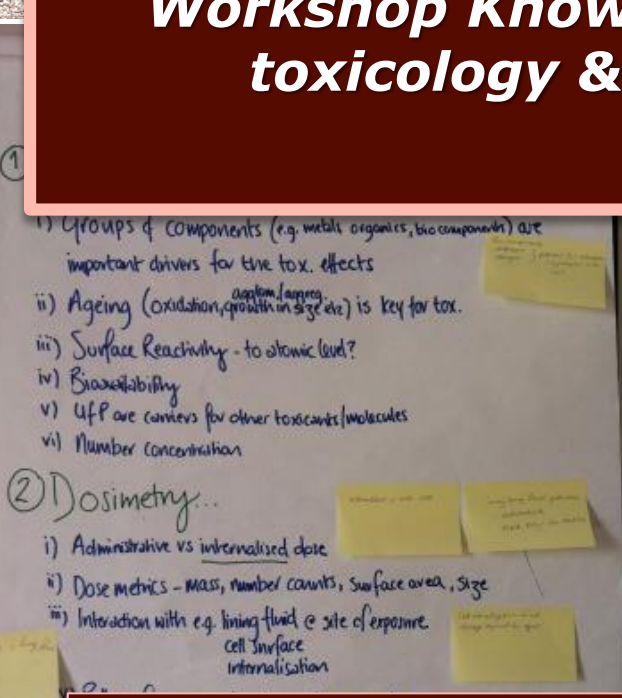
- Almost non-existent research in ecotoxicology l

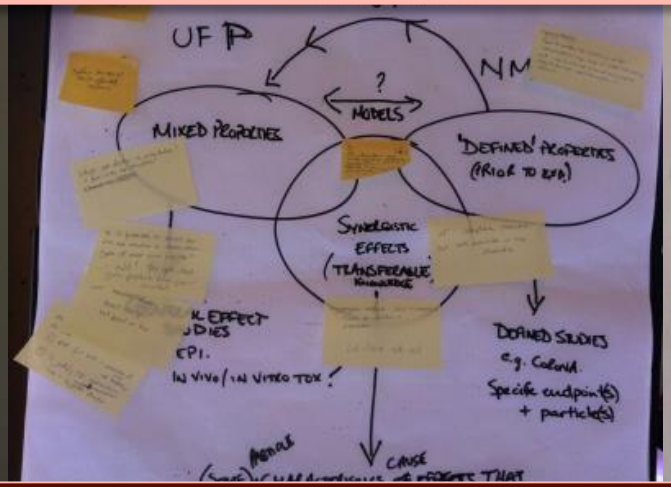
- Lack of generic models or paradigms for nano-activity.

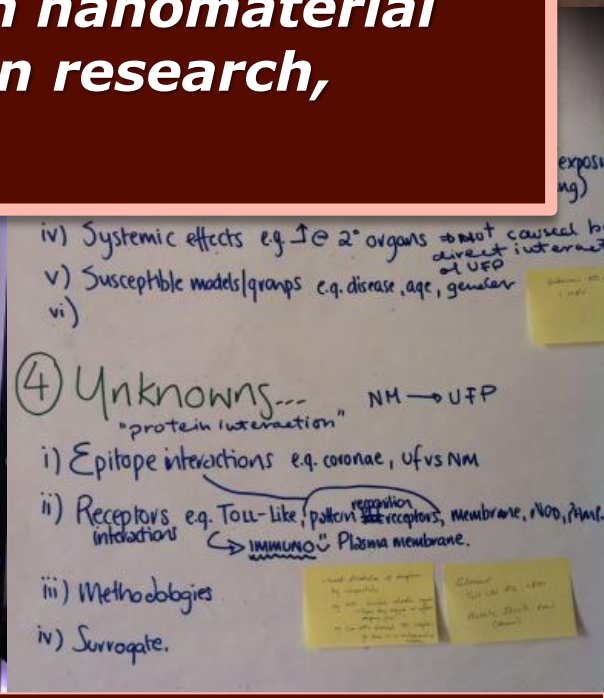


Donaldson et al, Part Fibre Toxicol. 2010; 7: 5

Workshop Knowledge transfer between nanomaterial toxicology & particulate air pollution research, Rome, 5-6 May 2015.

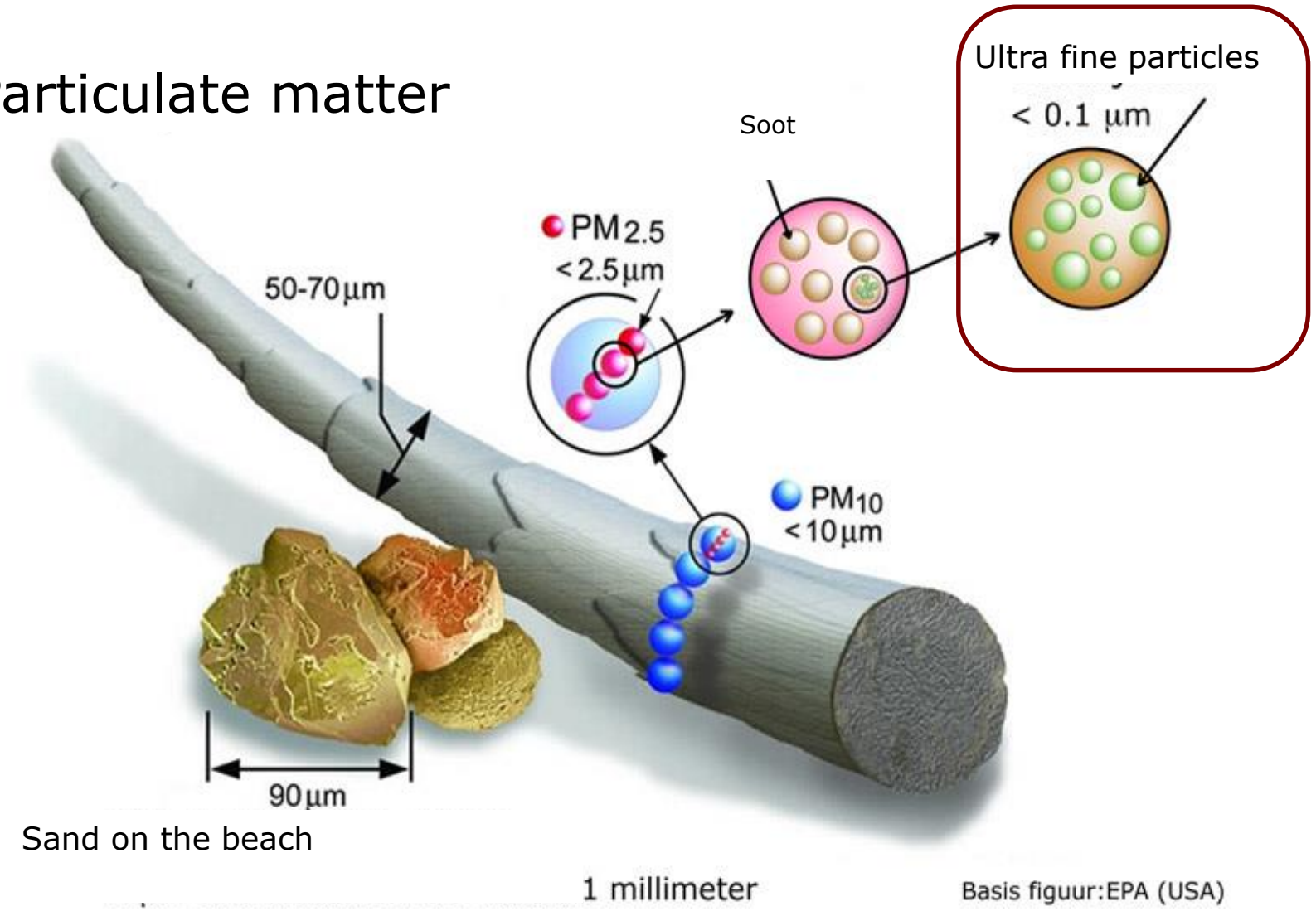




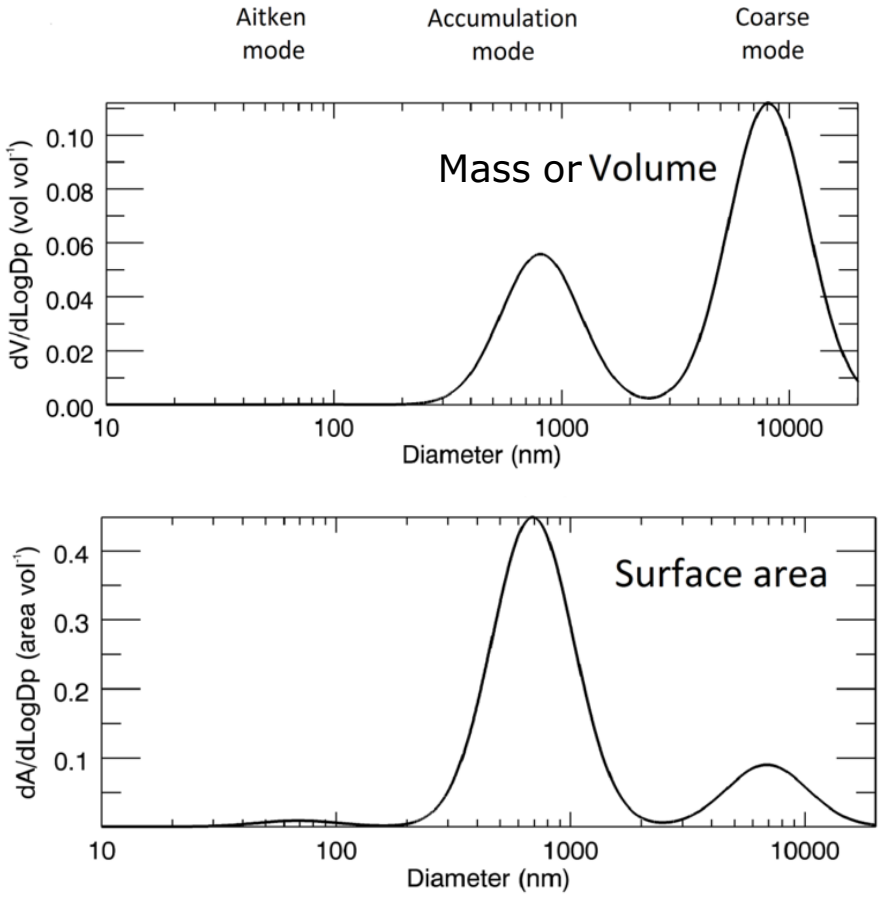


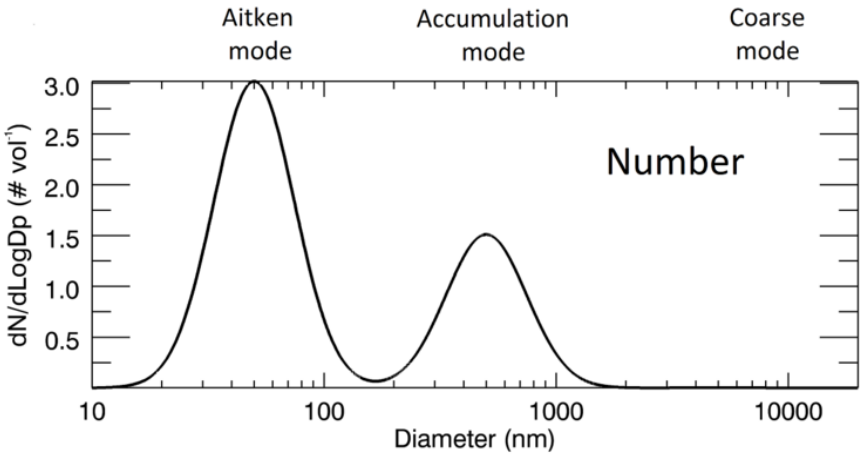
Vicki Stone, Mark Miller, Martin Clift, Alison Elder, Nicholas Mills, Peter Møller, Roel Schins, Ulla Vogel, Wolfgang Kreyling, Keld Alstrup Jensen, Thomas Kuhlbusch, Per Schwarze, Peter Hoet, Antonio Pietroiusti, Andrea De Vizcaya-Ruiz, Armelle Baeza-Squiban, Bryony Ross, Dominique Balharry Lang Tran Flemming Cassee

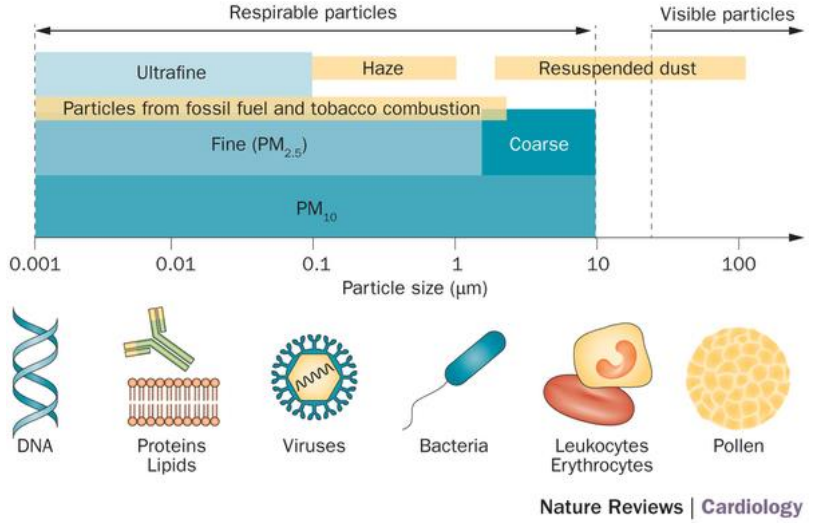
Particulate matter



Typical outdoor profile







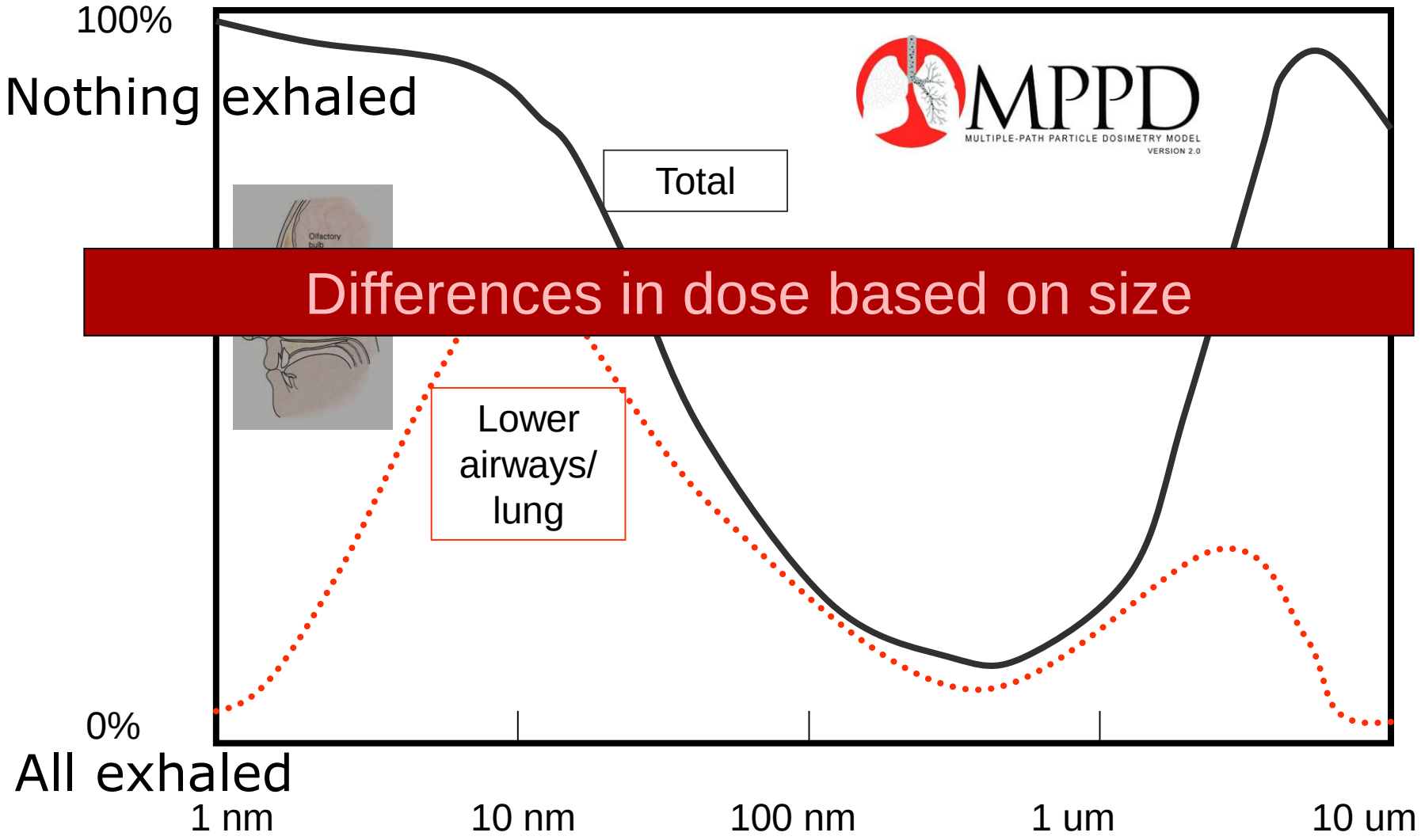
Author: Niall Robinson

Proposed EU definition of nanomaterials

- 'Nanomaterial' means
  - a natural, incidental or manufactured material containing particles, in an **unbound** state or as an **aggregate** or as an **agglomerate** and
  - where, for **50 % or more (majority)** of the particles in the number size distribution, **one or more external dimensions** is in the size range **1 nm – 100 nm (arbitrary!)**.

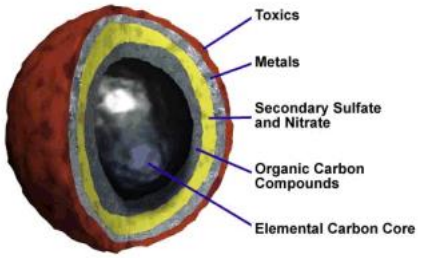
- Alternatively, a material should be considered as falling under the definition where the **specific surface area** by volume of the material is greater than **60 m^2/cm^3**.

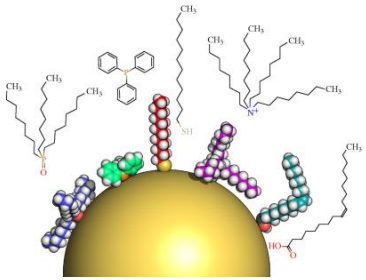
Mass based deposition after inhalation



Ambient UFP versus engineered NM (1)

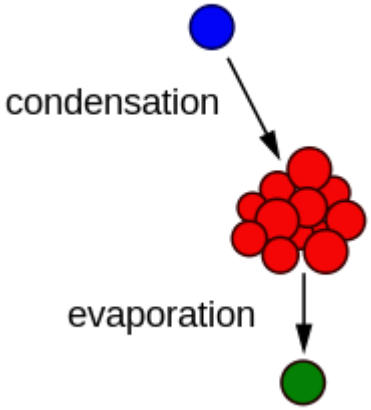
Ambient UFP	Nanomaterials
• Ambient air PM composition is complex, including coarse (2.5-10 um), fine (<2.5 um) and UF (<100 nm) particles.	• A number of definitions exist which usually stipulate that at least one dimension is in the nano-scale (1-100 nm).

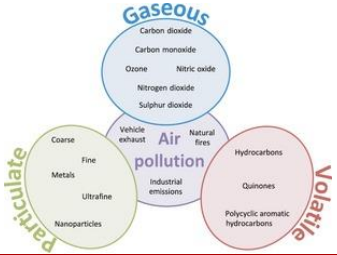




Ambient UFP versus engineered NM (2)

Ambient UFP	Nanomaterials
• Mixture of **insoluble to soluble particles and droplet**s, possibly leading to the release of several (semi-volitile) constituents from one particle in lungs.	• Can vary significantly in particle morphology, chemical composition but are well defined at production and close to production levels. Solid particles only and can also include fibres





Associations health effects & ultrafine particles

- Rehospitalisation with myocardial infarction
- Acute asthma
- Increased systolic blood pressure
- Ischaemic stroke
- Impaired lung function
- Allergic inflammation
- Myocardial ischemia and infarction
- Arrhythmia
- Lung cancer
- Bronchitis
- Deep vein thrombosis
- Cognitive and behavioural changes
- Neuropathy & neurodegenerative diseases
- Low birth weight, pre-term birth and small gestational age

✓ Not exclusively

✓ Causality ?

✓ Often exacerbation of existing disease

Toxicological mechanisms linked with UFP

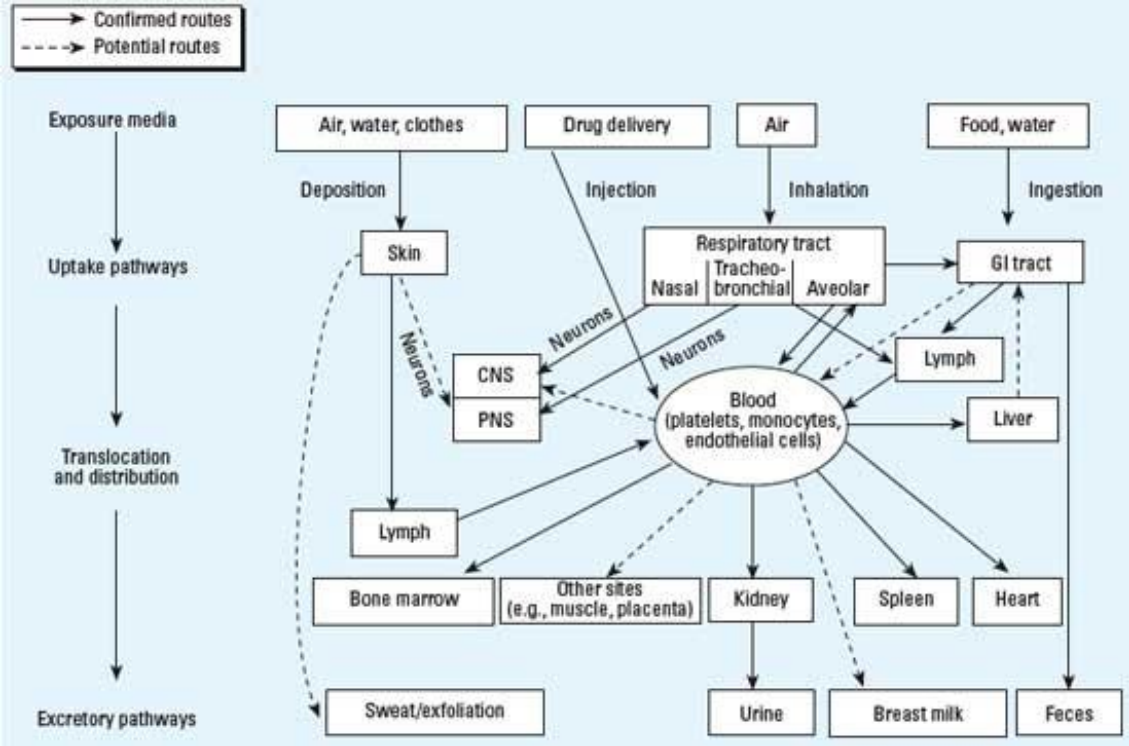
Examples

- Oxidative stress
- Pulmonary and systemic inflammation
- Genotoxicity
- Blood
  - Changes in fibrinogen & prothrombin level
  - Platelet activation
  - Von Willebrand factor induction
- Reduced heart rate variability
- Increased blood pressure
- Vasomotor dysfunction
- Disturbed Lipid metabolism

- Effects seen beyond the lung: brain, cardiovascular.

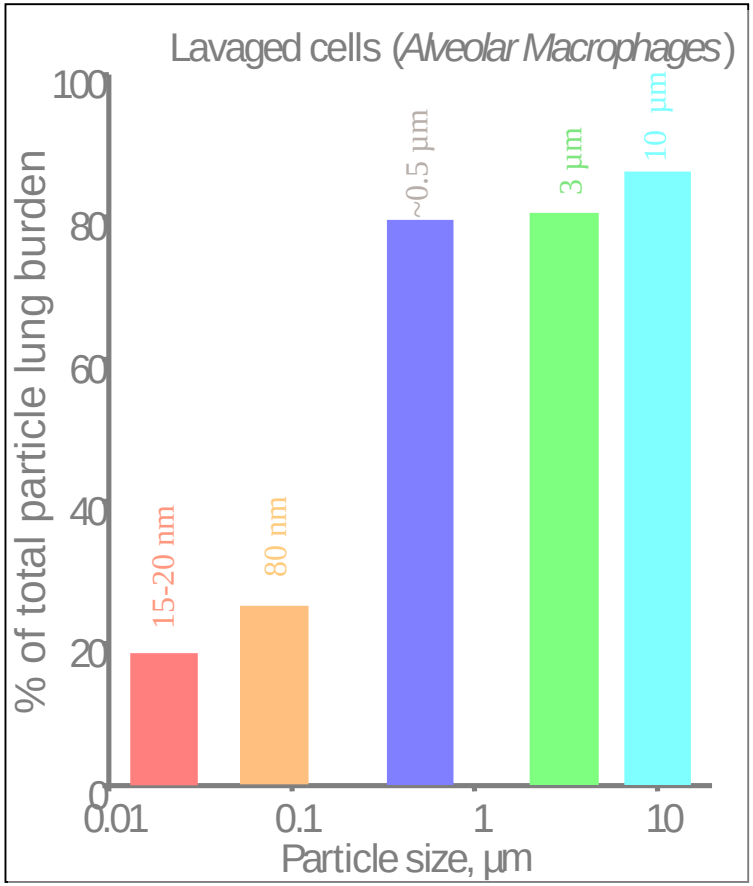
What did we learn from nanomaterial toxicology?

- NM translocation studies provide clear evidence of the potential for UFP to translocate from the lung surface into blood and to distribute around the body, accumulating in a range of secondary organs.

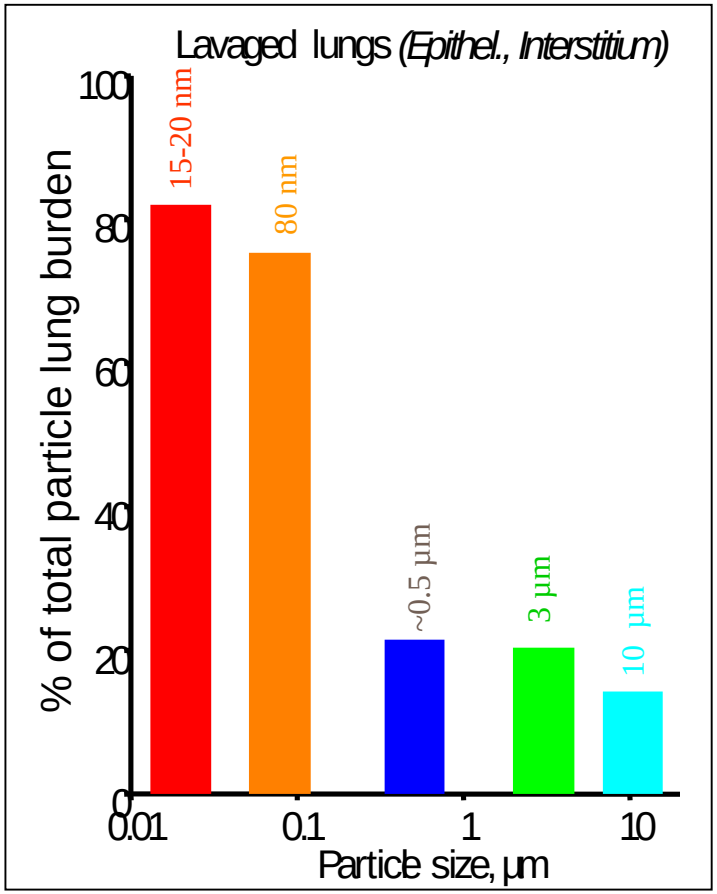


Particle-size dependent retention sites in lungs

Retention iridium particles 24 hrs after 2 hr exposure

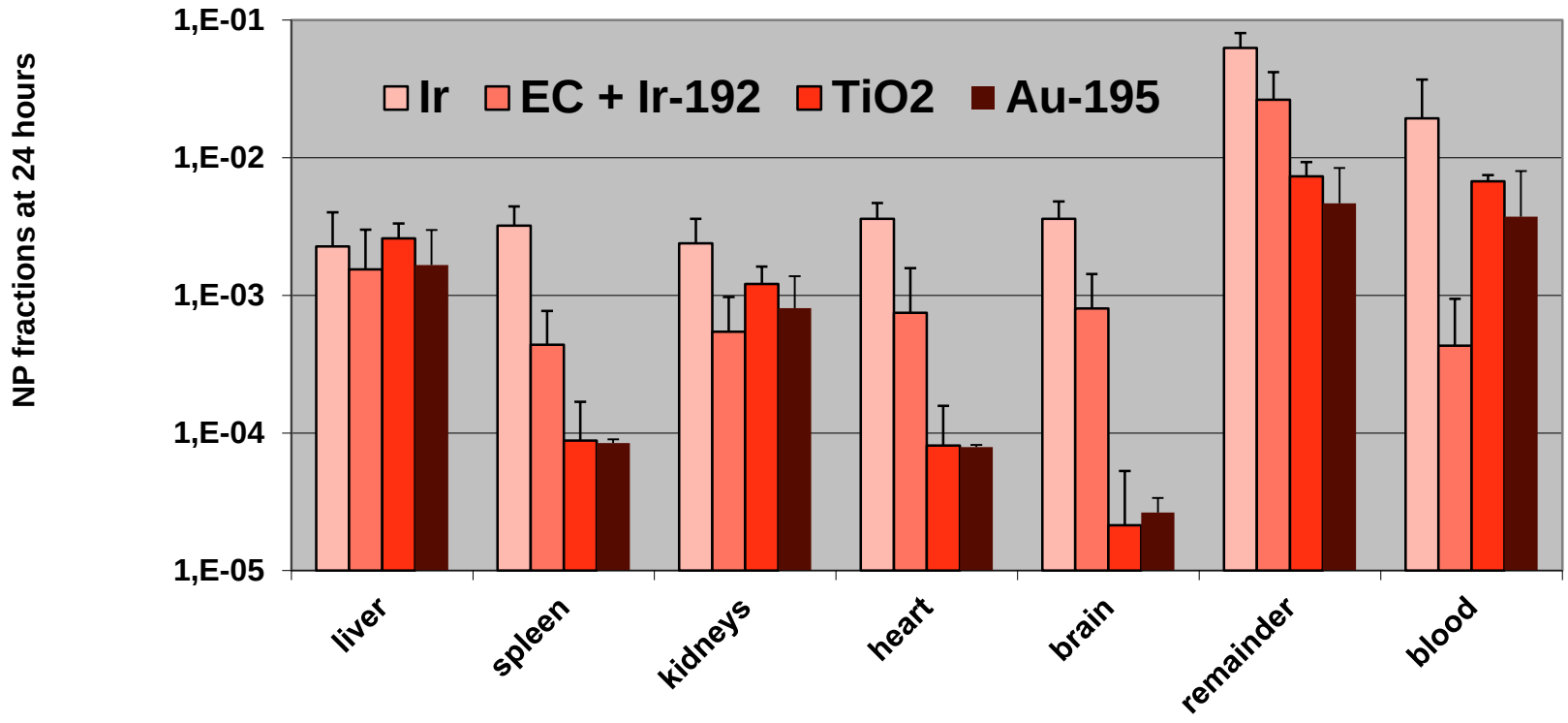


Oberdörster et al., 2001; Kreyling et al., 2002



Translocation of various 50 nm particles

Endotracheal inhalation - rats

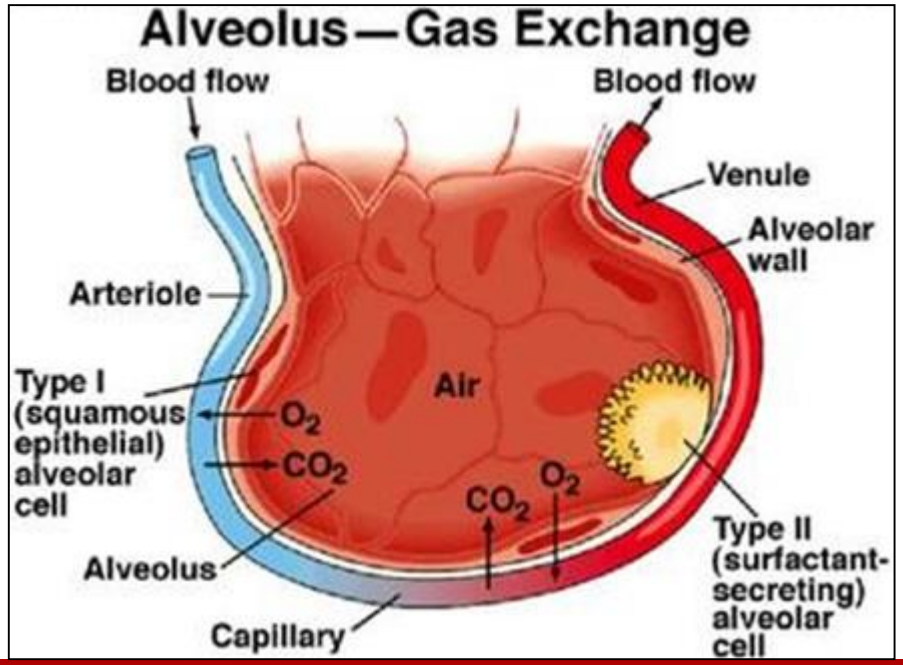


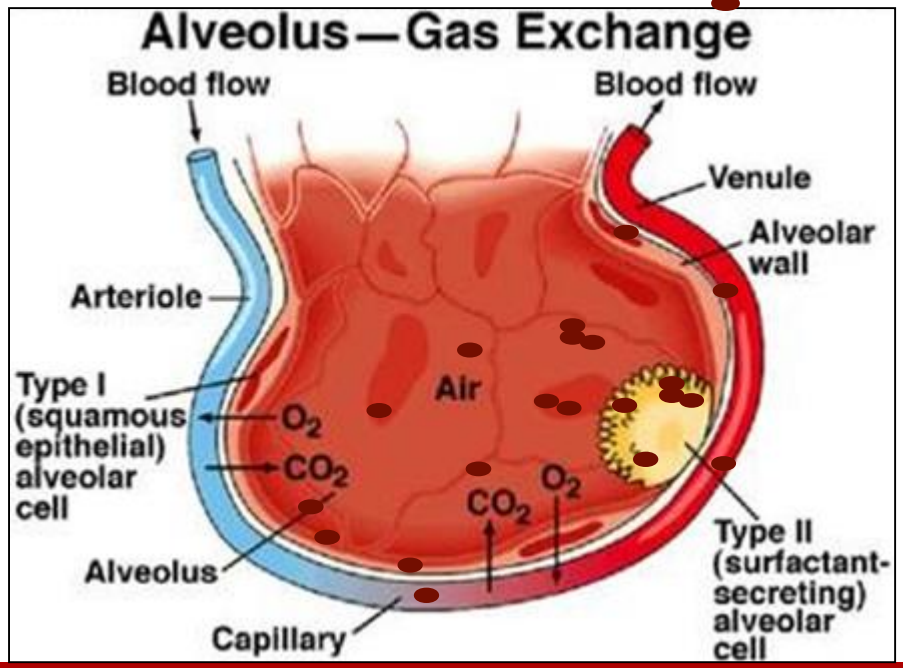
Courtesy Wolfgang Kreyling

What did we learn from nanomaterial toxicology?

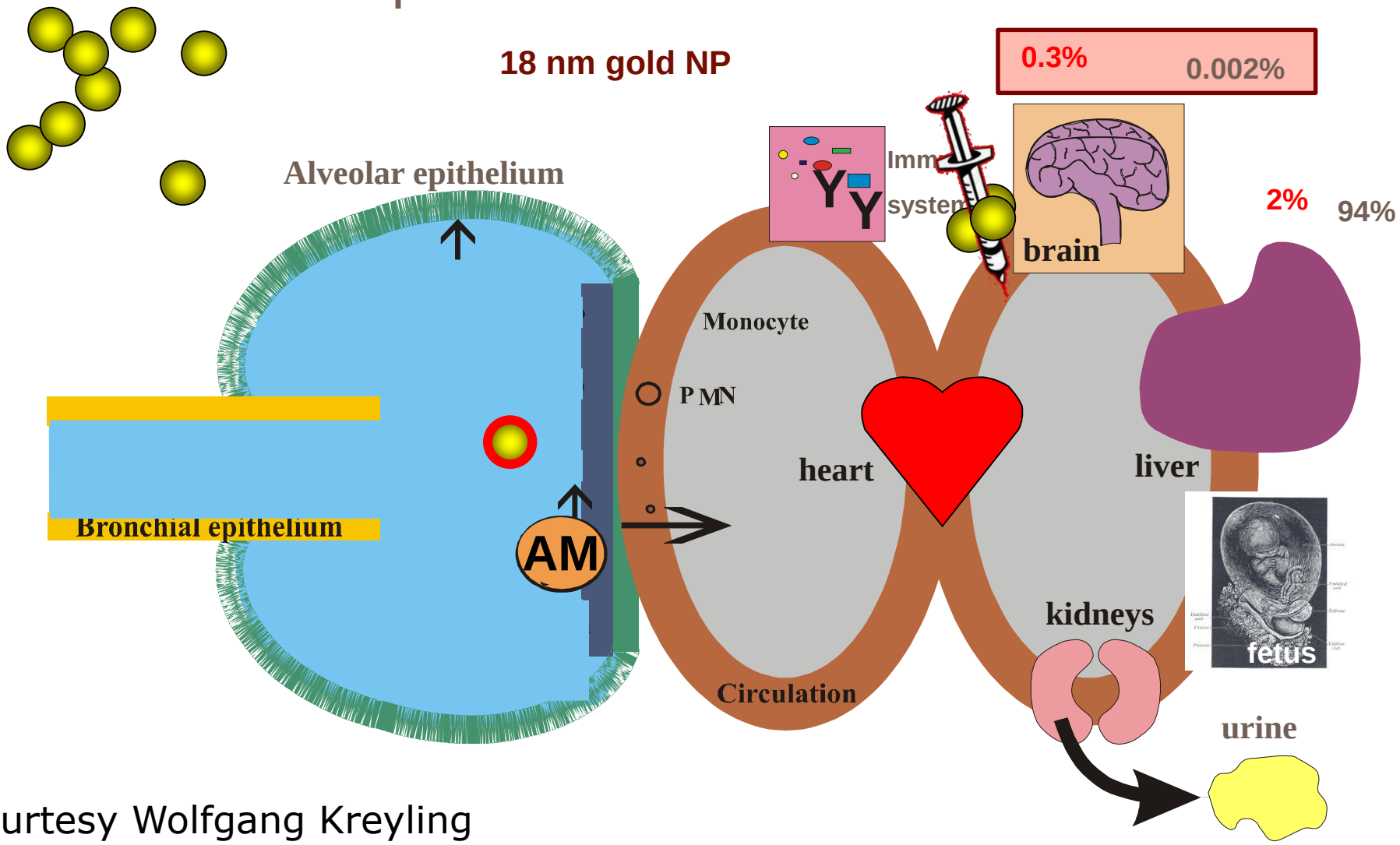
- The differential clearance and uptake by NM and micron-sized particles could also apply to the varied size fractions of outdoor PM, adding to the plausibility of a difference in their toxicity between fine (aggregates) and UFP.

Same mass, different numbers





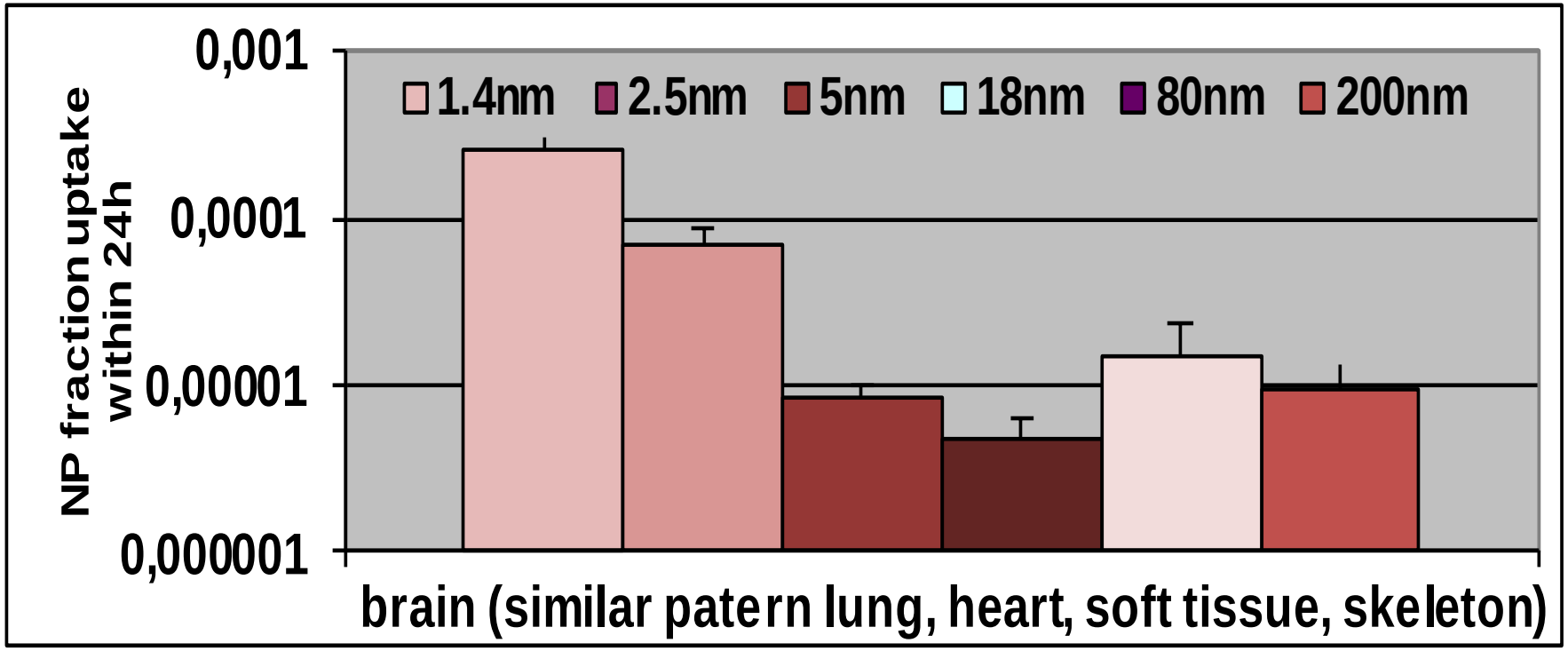
Route of exposure affects NP translocation



Courtesy Wolfgang Kreyling

Translocation as function of size after

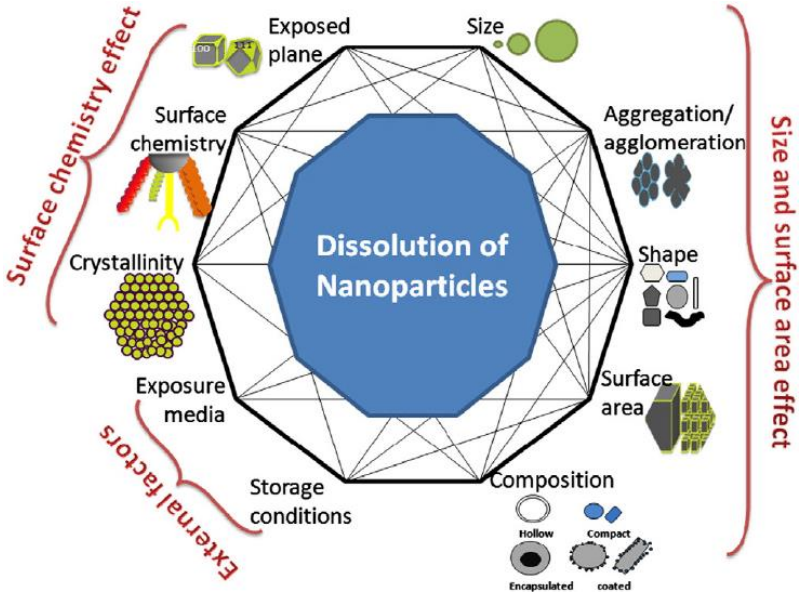
Single IV injection gold particles in rats



Semmler-Behnke, Small, 2008

What did we learn from nanomaterial toxicology?

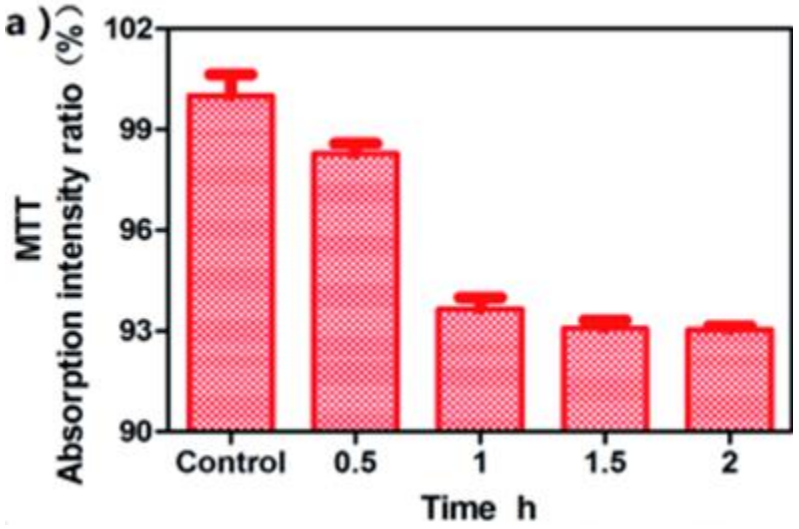
- Understanding of the composition of the molecular corona of NMs as well as several other influential factors can be applied to UFP as this is likely to influence their uptake, fate and effects within the body.→ Total mass not good predictor for UFP toxicity



Misra et al Science of The Total Environment 438:225-232 · November 2012

What did we learn from nanomaterial toxicology?

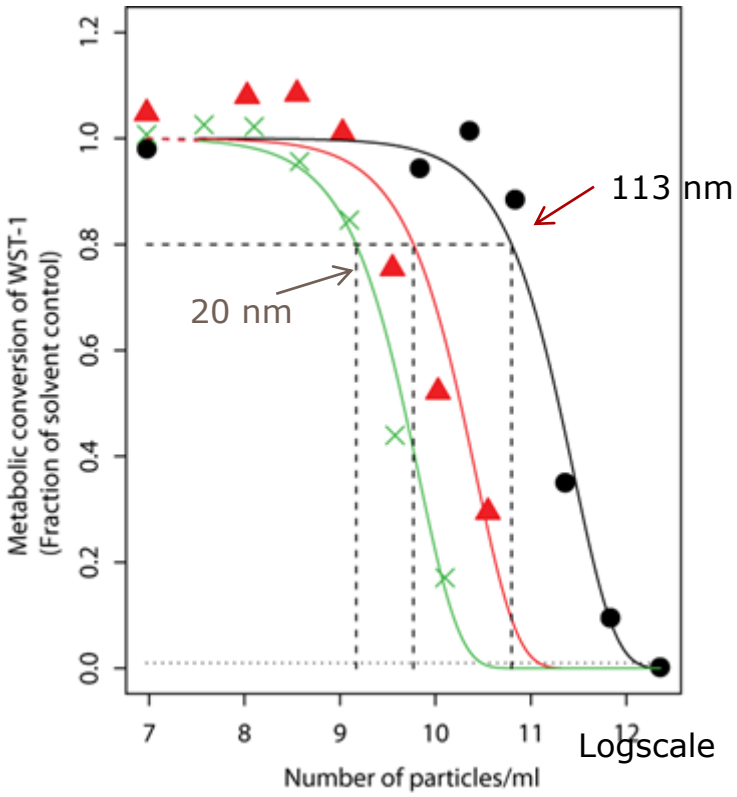
- **Standardised protocols** for assessing biological responses to NMs, once wholly available, could be applied to both UFP and PM.
- Evidence for the ability of NM to **interfere in various assays** means that study designs for NM and UFP require consideration of control procedures to limit the potential to confound result interpretation.

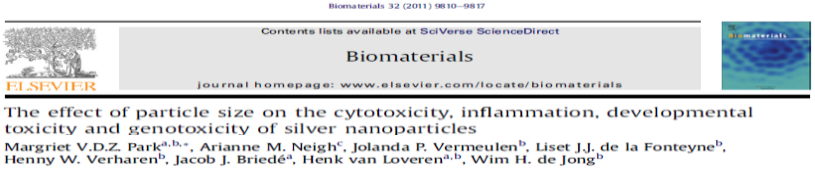


- ***Jiao et al., RSC Adv.***, 2015,**5**, 53240-53244

Size dependent cytotoxicity silver particles

- Test system: Metabolic activity fibroblasts after 24 hr exposure to mono disperse silver (20, 80 of 113 nm)




Biomaterials 32 (2011) 9810–9817

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

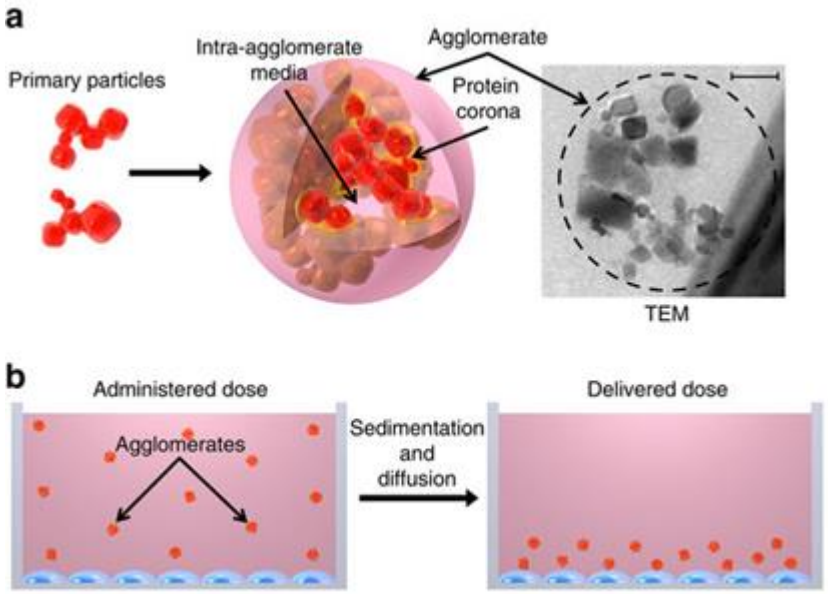
The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles

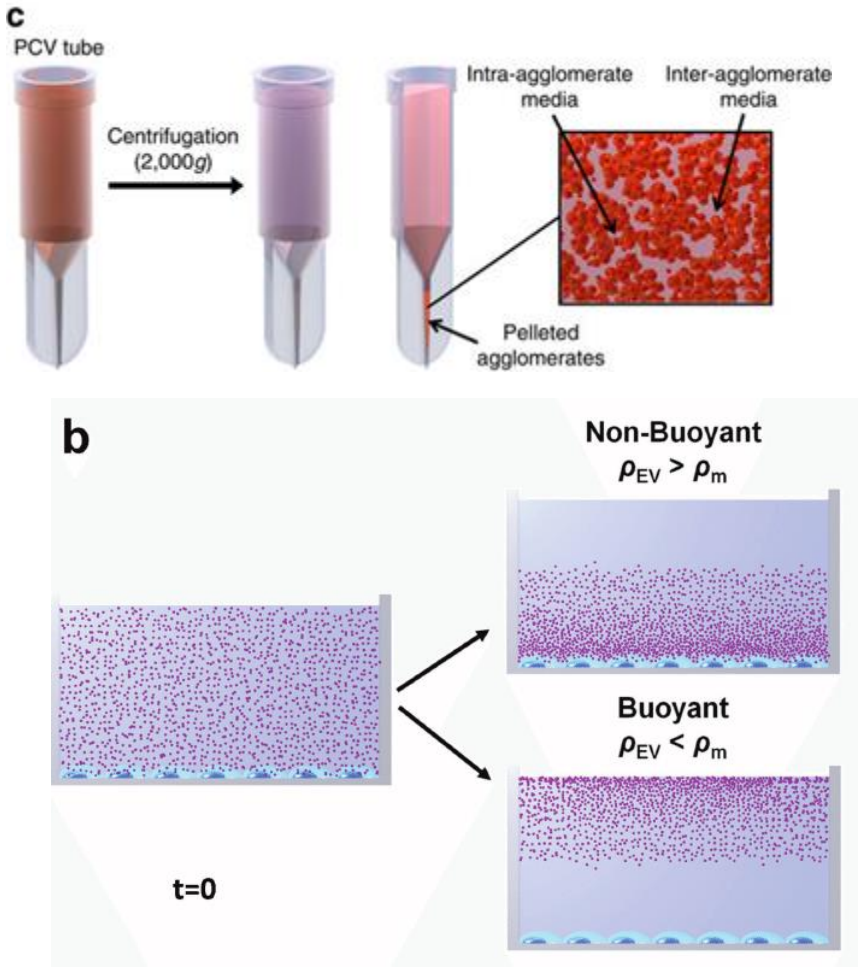
Margriet V.D.Z. Park[a,b,*], Arianne M. Neigh[c], Jolanda P. Vermeulen[b], Liset J.J. de la Fonteyne[b], Henny W. Verharen[b], Jacob J. Briedé[a], Henk van Loveren[a,b], Wim H. de Jong[b]

EC_{20} (#particles/ml)

120 nm	6.27E+10
80 nm	5.89E+09
20 nm	1.48E+09

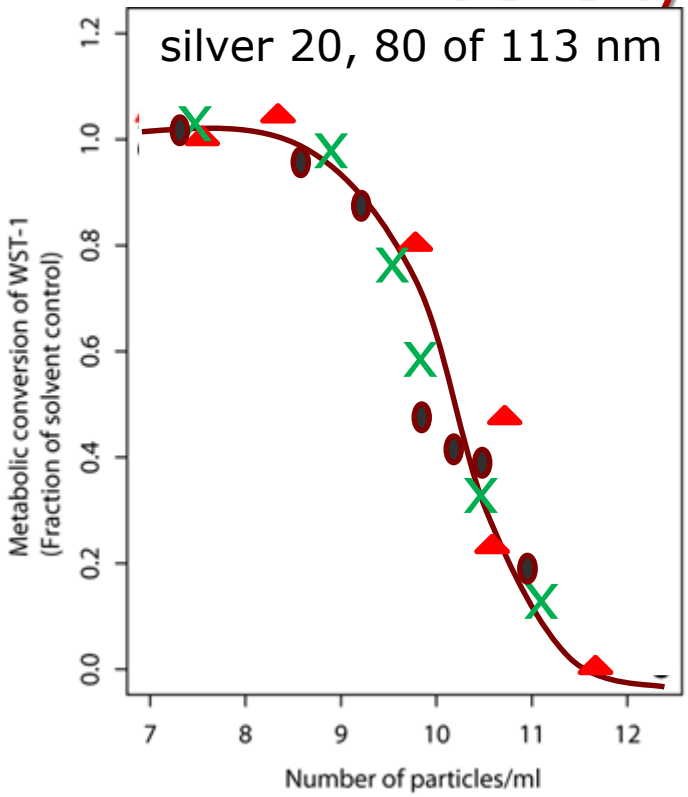
Impact on dispersion of NMs

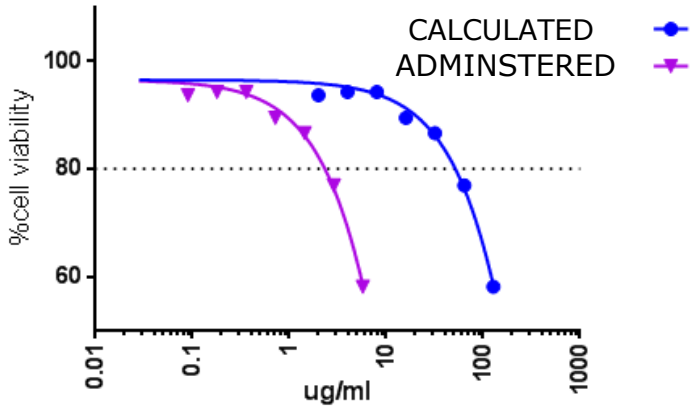




- DeLoid et al, doi:10.1038/ncomms4514

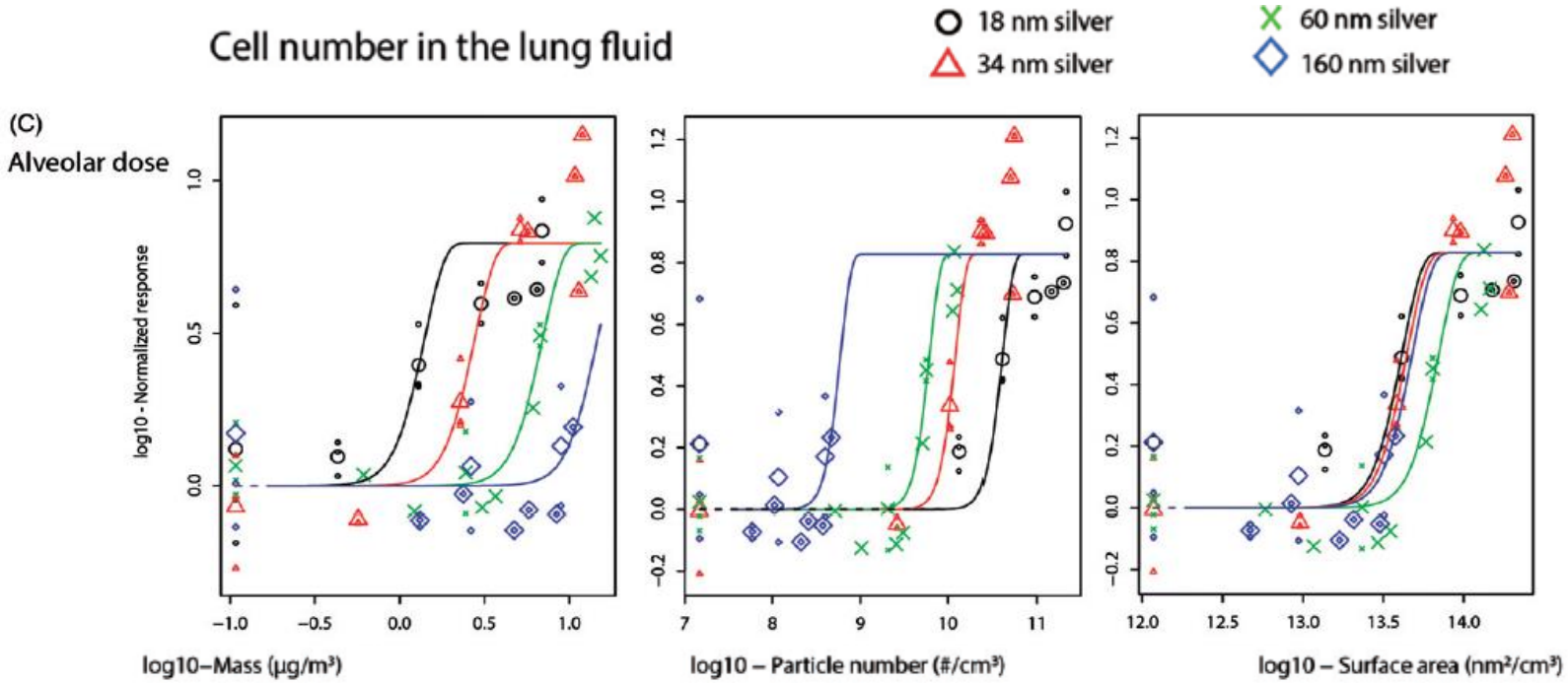
Different dose-response based on density and viscosity





Implication? Retrospectively assess the biological effective dose in order to make a lot of in vitro studies meaningful!

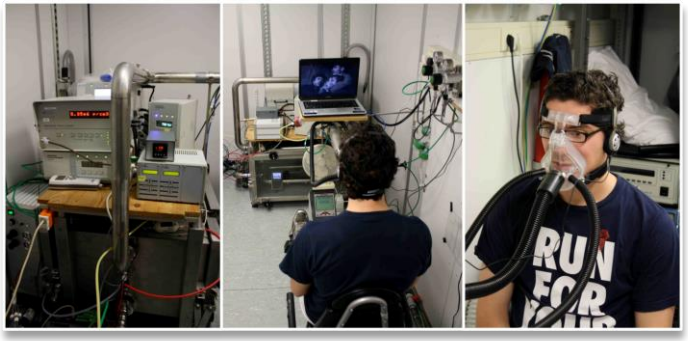
Total cell number in the rat lung after inhalation of different sizes of silver nanoparticles

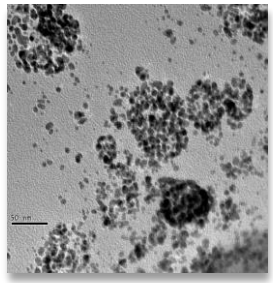


Mass Number Surface area

Adjusted for modelled ALVEOLAR content silver

2 hr exposure to nanogold by inhalation





Rapid translocation and effects, also very slow clearance

Nanogold 5-15 nm
5. 10^6 particle/cc,

Fibre paradigm applies also to NM fibres: frustrated phagocytosis

Thin

Results in **small aerodynamic diameter** enables deposition beyond the ciliated airways

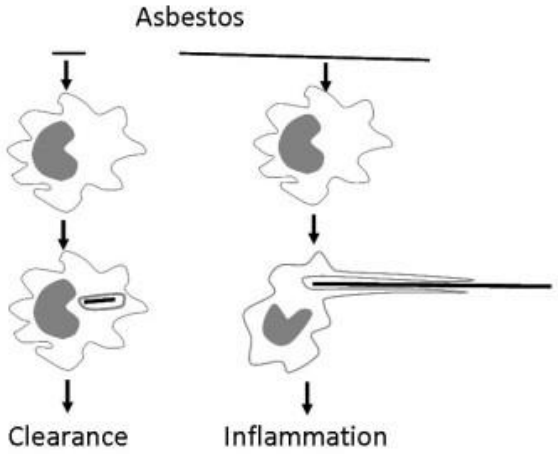
Bio-persistent

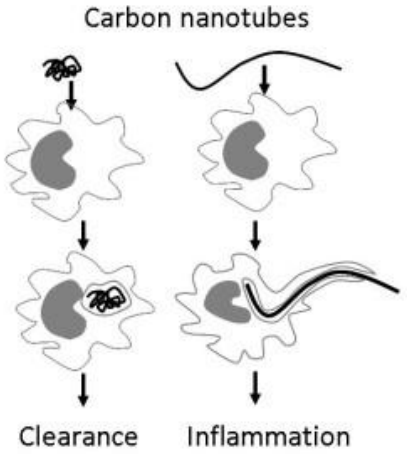
Retains its long term shape over long-term residence in the lungs

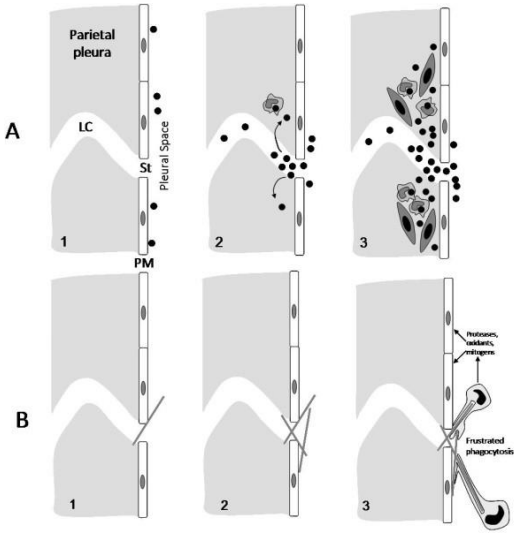
Long

Cannot be completely enclosed by a macrophages producing frustrated phagocytosis

Longer than 15-20µm







Donaldson et al, Part Fibre Toxicol. 2010; 7: 5.

Lessons from both UFP and nanomaterials

- Ambient PM research provided evidence of potential **health impacts** for UFP, whilst NM toxicology has largely provided essential evidence of the **mechanistic plausibility** of these health effects

- PM research provides indications of, at least in part, the potential **disease effects** to consider,
  - early initial human health studies involving workers suggest this may also be true and other materials

- UFP and NM share the same general biological mechanisms of adverse effects, such as oxidative stress and inflammation, where much of the evidence on the role of the **physicochemical characteristics** is derived from nanomaterial toxicology.

Still missing / areas for further investment include

- Understanding of how engineered nanomaterials **interact** with living system is so far incomplete and, thus, the reliable assessment of nanomaterials toxicity is not yet thoroughly possible;
- **Predictive** toxicology
  - understanding of the characteristics of nanomaterials, an their relationships with ensuring harmful effects.
- **Risk** = hazard x exposure. Need for (long term) exposure (and dose) assessment
- The definition of dose **metrics** (characteristics) is an open issue troubling NM safety assessment.

Size and size distribution	Surface chemistry
Aggregation/agglomeration state	Composition including coating and surface modifications
Shape	Surface charge
Surface area	Solubility/dispersibility

Ready for NM risk assessment?

Regulatory Toxicology and Pharmacology 80 (2016) 46–59

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Towards a nanospecific approach for risk assessment

Susan Dekkers [a, *], Agnes G. Oomen [a], Eric A.J. Bleeker [a], Rob J. Vandebriel [a], Christian Micheletti [b], Joan Cabellos [c], Gemma Janer [c], Natalia Fuentes [c], Socorro Vázquez-Campos [c], Teresa Borges [d], Maria João Silva [e], Adriele Prina-Mello [f], Dania Movia [f], Fabrice Nesslany [g], Ana R. Ribeiro [h], Paulo Emílio Leite [h], Monique Groenewold [a], Flemming R. Cassee [a], Adrienne J.A.M. Sips [a], Aart Dijkzeul [i], Tom van Teunenbroek [i], Susan W.P. Wijnhoven [a]

Nanomaterials vs Ambient Ultrafine Particles: an Opportunity to Exchange Toxicology Knowledge.

Environmental Health Perspectives, 2016

Vicki Stone, Mark Miller, Martin Clift, Alison Elder, Nicholas Mills, Peter Møller, Roel Schins, Ulla Vogel, Wolfgang Kreyling, Keld Alstrup Jensen, Thomas Kuhlbusch, Per Schwarze, Peter Hoet, Antonio Pietroiusti, Andrea De Vizcaya-Ruiz, Armelle Baeza-Squiban, Lang Tran and Flemming Cassee