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Engineered nanoparticles versus ambient ultrafine particles: how comparable are their interactions with the organism and what do we know and where are the gaps?

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Outline

Part 1: Interactions of engineered nanoparticles (ENP) vs. ambient ultra fine particles (UFP) with the organism

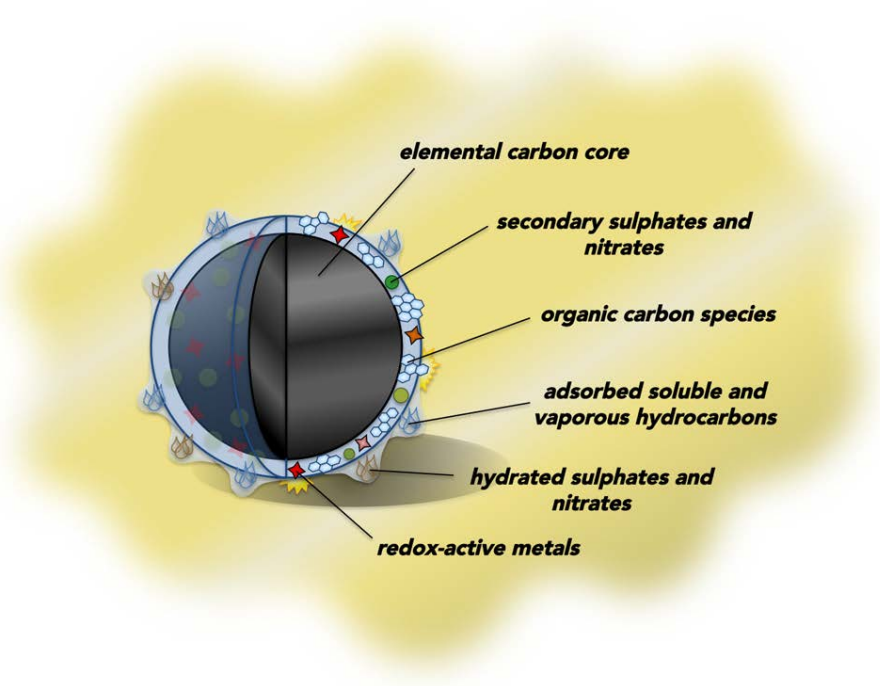
- Characteristics of ENP vs. UFP
- Development of ENM or UFP publications over the last 30 years
- Three major pathways linking possible adverse health effects of inhaled UFP or ENM
- Lessons learnt from UFP research vs. that from ENM research (Stone et al. 2016)

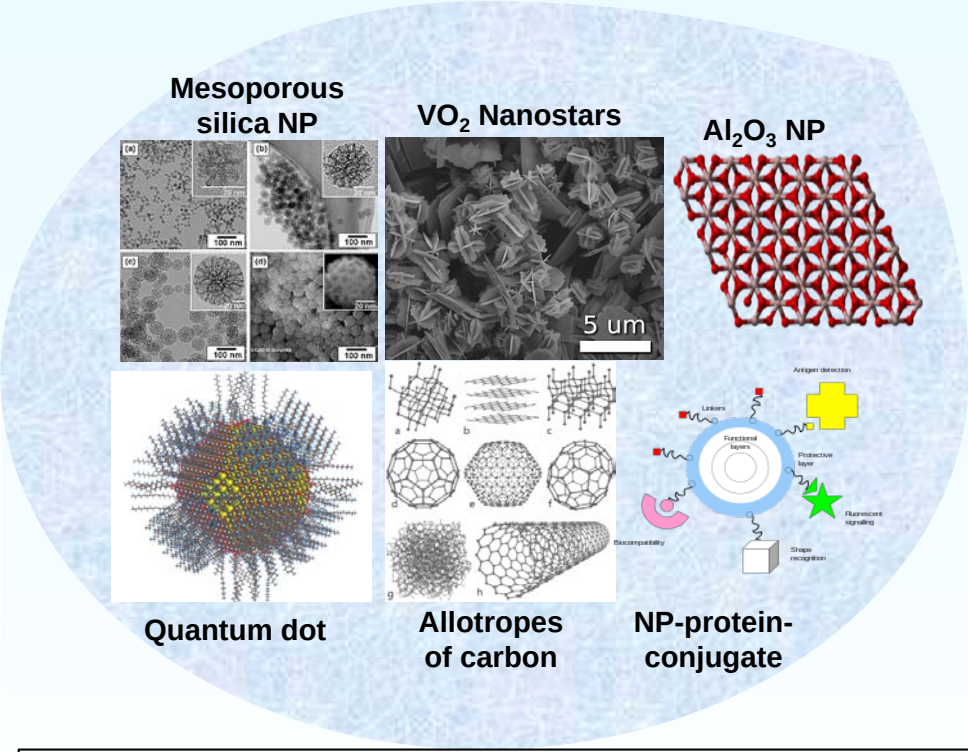
Part 2: Recent scientific biokinetics highlights after NP aerosol inhalation

- Rapid relocation from rat alveolar epithelium into interstitial spaces and back onto the epithelium for macrophage-mediated clearance to larynx and gastro-intestinal-tract
- Disagglomeration of inhaled AuNP agglomerates in rat lungs
- Translocation of inhaled 20 nm AuNP across the air-blood-barrier is similar in man and rat

Ambient Ultrafine Particles (UFP) vs. Engineered Nanoparticles (ENP)

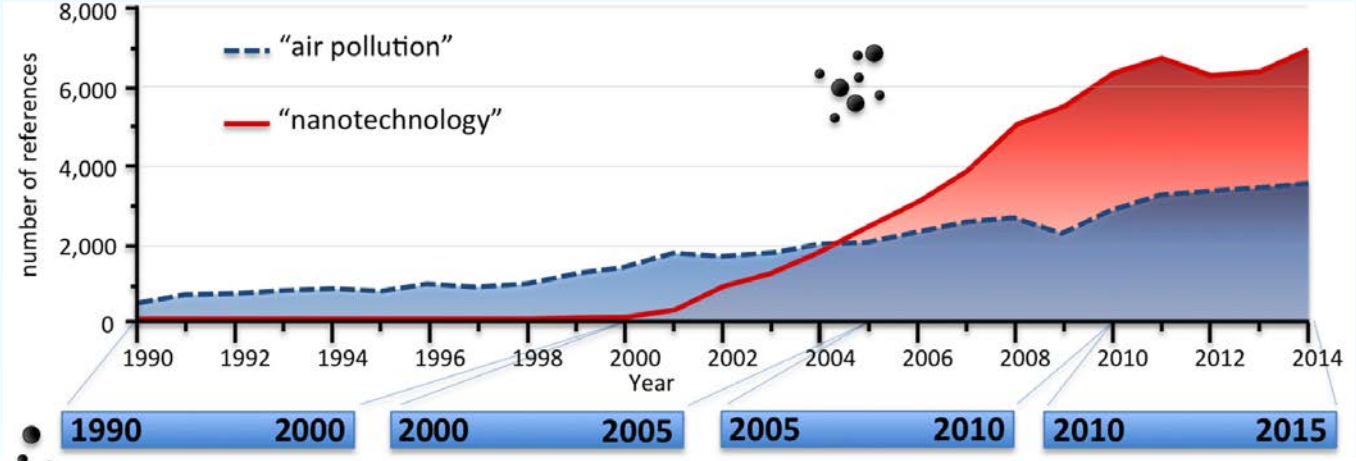
- UFP originate from diverse natural and/or man-made processes resulting in complex chemistry and physical structure
- Matrix and surface of UFP are complex mixtures, e.g. inorganic and organic carbon, metals, salts, biologic mat.





- ENP matrix + surface are thoroughly engineered re. chemical compounds, physical structure

Landmarks + trends in ambient UFP research



Landmarks and Trends in **ultrafine pollution** research

1990 – 2000

- **1993-5.** Large scale studies on particulate air pollution and mortality
  - Dockery et al. 1993
  - Pope et al. 1995
- **1995.** Ultrafine hypothesis proposed to account for the systemic actions of UFP
  - Seaton et al. 1995
- **1997.** Epidemiological data sets observed to support the ultrafine hypothesis
  - Peters et al. 1997

2000 – 2005

- **2001.** Acute exposure to PM can trigger a myocardial infarction
  - Peters et al. 2001
- **2002.** Hierarchical Oxidative Stress Model for susceptibility to air pollutants
  - Li et al. 2002
  - Li et al. 2003
- **2004.** AHA statement on the cardiovascular effects of air pollution
  - Brook et al. 2004

2005 – 2010

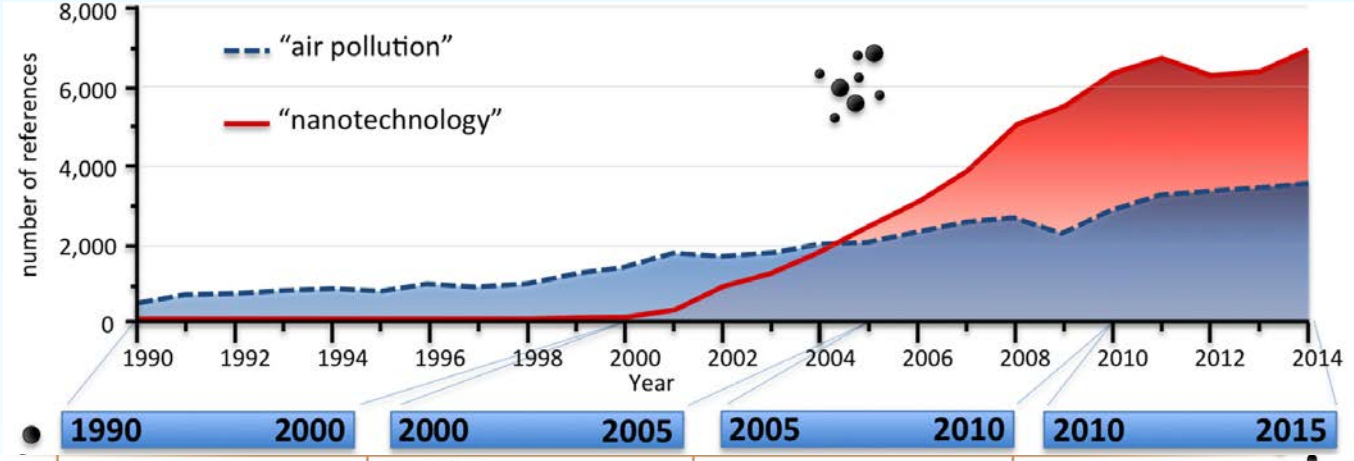
- **2005-7.** Air pollution promotes atherosclerosis (in man)
  - Kunzli et al. 2005
  - Hoffmann et al. 2007
- **2005-9.** Controlled exposures to diesel exhaust impair multiple aspects of cardiovascular health.
  - Mills et al. 2005
  - Mills et al. 2007,
  - Lucking et al. 2008

2010 – 2015

- **2010-15.** Growing evidence for pre-natal exposure to air pollution affecting birth outcomes and future health
  - Volk et al. 2013; Pedersen et al. 2013
- **2011-15.** Revised estimates suggest several million people die of air pollution per year.
  - WHO statistics 2011, 2014; Lim et al. 2012; Lelieveld et al. 2015
- **2012.** IARC classify diesel exhaust as a carcinogen.
  - IARC Monographs
  - Benbrahim-Tallaa et al. 2012
- **2012-15.** Estimates of the effects of long-term exposure to air pollution (ESCAPE project).
  - Beelen et al. 2014

Stone et al., 2016

Landmarks + trends in nanotoxicology research



Landmarks and Trends in **nanotoxicology** research

1990 – 2000

- **1990-1992.** First studies showing toxicology of nano-sized particles highlights concept of size, surface area, etc.
  - Oberdorster et al. 1990
  - Ferin et al. 1992
- **1995-1999.** Increased laboratory based studies coincided with epidemiological studies (see above) assessing the impact of nanoparticles upon cellular machinery
  - Li et al. 1996
  - Stone et al. 1998

2000 – 2005

- **2000-2005.** Progress into the cellular mechanisms of nanoparticles
  - Stone et al. 2000a,b;
  - Brown et al. 2001, 2004a,b
- **2002.** Studies demonstrating the possibility of NP translocation from lung to circulation
  - Oberdorster et al. 2002
- **2004.** Coining of the phrase 'Nanotoxicology'
  - Donaldson et al. 2004

2005 – 2010

- **2005-7.** Governmental reports on 'Nanoscience' and why we must conduct more health-effects based research
  - The Royal Society & Royal Academy of Engineering 2004
  - SCENIHR 2005, 2007

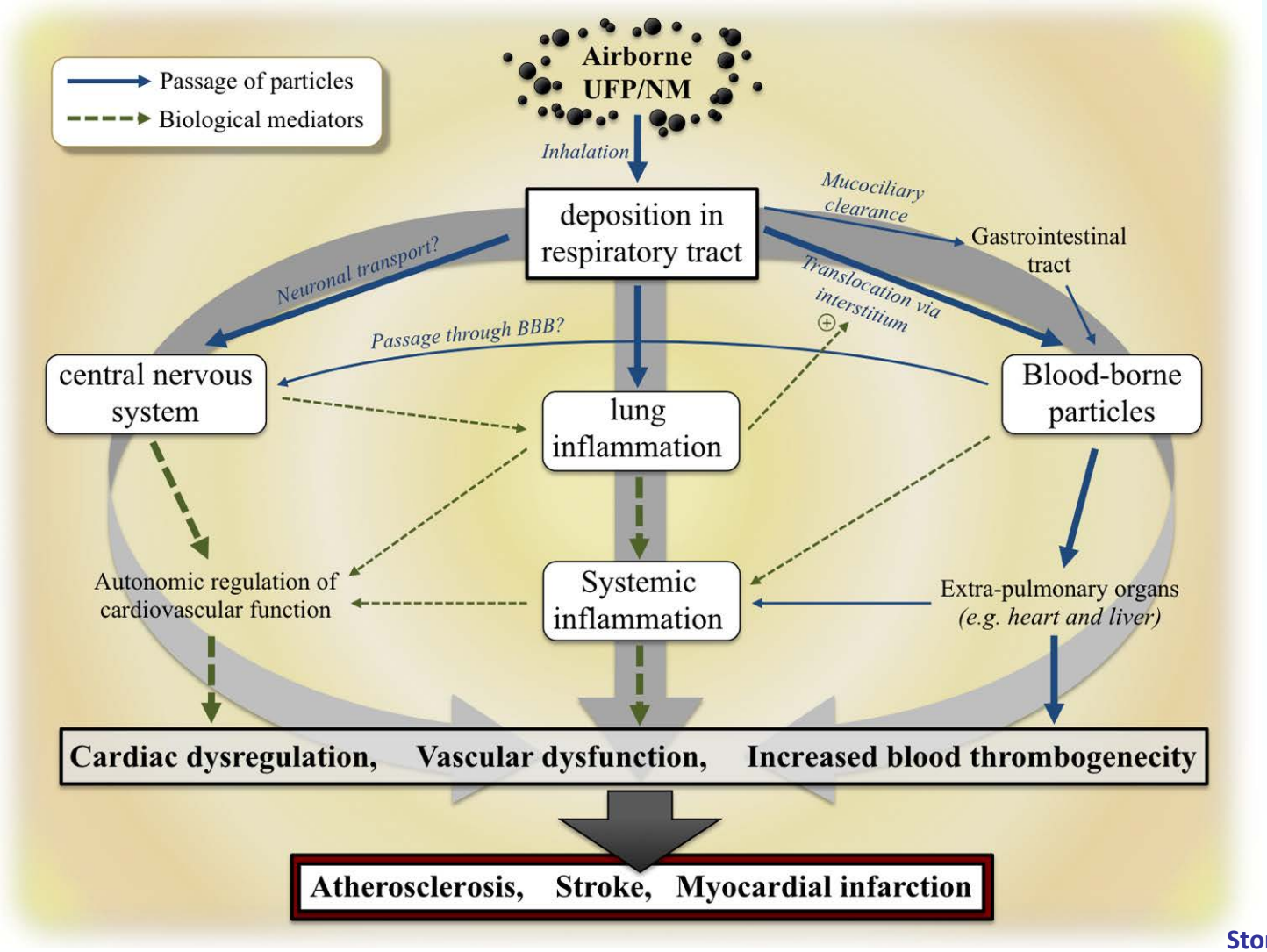
  2005. Start of FP6 (Particle Risk Project) continuing on to FP7
- **2006-8.** Recognition of nanoparticle-epitope (protein) binding and protein coronas (leading to a sub-discipline within the field)
  - Lynch et al. 2007; Lynch & Dawson 2008
- **2007.** Important research on the influence of the nanoparticle-cell interaction
  - Unfried et al. 2007
- **2008.** Specific characteristics of CNTs can produce asbestos-like health effects.
  - Poland et al. 2008

2010 – 2015

- **2010.** Emphasis on dose-response metrics
  - Oberdorster 2010
- **2010 & 2013.** NIOSH recommendations for occupational exposure levels for CNTs
- **2011.** Detailed characterisation of nanoparticles increasingly required for publication
  - Bouwmeester et al. 2011
- **2013.** EU directives on *in vivo* research increases desire for *in vitro* systems to predict nanoparticle hazard.
- **2013.** The first nano-legislation: under EU cosmetic regulation 1223/2009
- **2014.** IARC classification of Mitsui-7 MWCNTs as possible human carcinogens (2B)

Stone et al., 2016

Three pathways linking possible effects of inhaled UFP or ENM to the pulmonary and cardiovascular systems



Stone et al., 2016

Effects of engineered nanoparticles versus ambient ultrafine particles

Ambient particulate matter (PM) including UFP

HEALTH EFFECTS

Rehospitalisation with myocardial infarction
Acute asthma
Increased systolic blood pressure
Ischaemic stroke
Impaired lung function
Allergic inflammation
Myocardial ischaemia 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

A rich body of epidemiology-based literature exists that has demonstrated adverse human health effects following exposure to PM, with a proportion of that literature providing support for UFP involvement.

TOXICOLOGICAL MECHANISMS

Engineered nanomaterials (ENM) including ENP

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

Stone et al., 2016

ENM research provides an opportunity to better under-stand the (mechanistic) role of UFP in the genotoxicity, mutagenicity, and carcinogenicity of PM in clinical human studies and toxicological animal and in vitro studies.

Lessons learned from studies on inhaled ENP vs. ambient UFP

Interactions between UFP / ENP and the organism depend on the physicochemical properties of UFP / ENP and on the plethora of responses and reactions of the organism. However, while ENP are produced on purpose, UFP occur incidentally. This has consequences for the investigation of their interactions with the organism:

Adverse effects of inhaled ambient UFP cannot easily be separated because UFP always come as part of ambient PM_{10}, $PM_{2.5}$, including gases

- ➔ **therefore, they have been subject to epidemiological studies so far;**
- ➔ **attempts to explore mechanisms and/or modes of actions in clinical and animal studies are challenging and have not been performed properly yet;**
- ➔ **however, long-term epidemiological studies are required which select distinct exposure conditions for the different exposure groups in order to eventually obtain insight into possible mechanisms triggered solely by UFP and not by ambient PM_{10}, $PM_{2.5}$, including gases.**

On the other hand, interactions of inhaled ENP and the organism have been successfully studied to determine toxicological mechanisms and modes of actions because of the well-defined, physicochemical properties of ENP

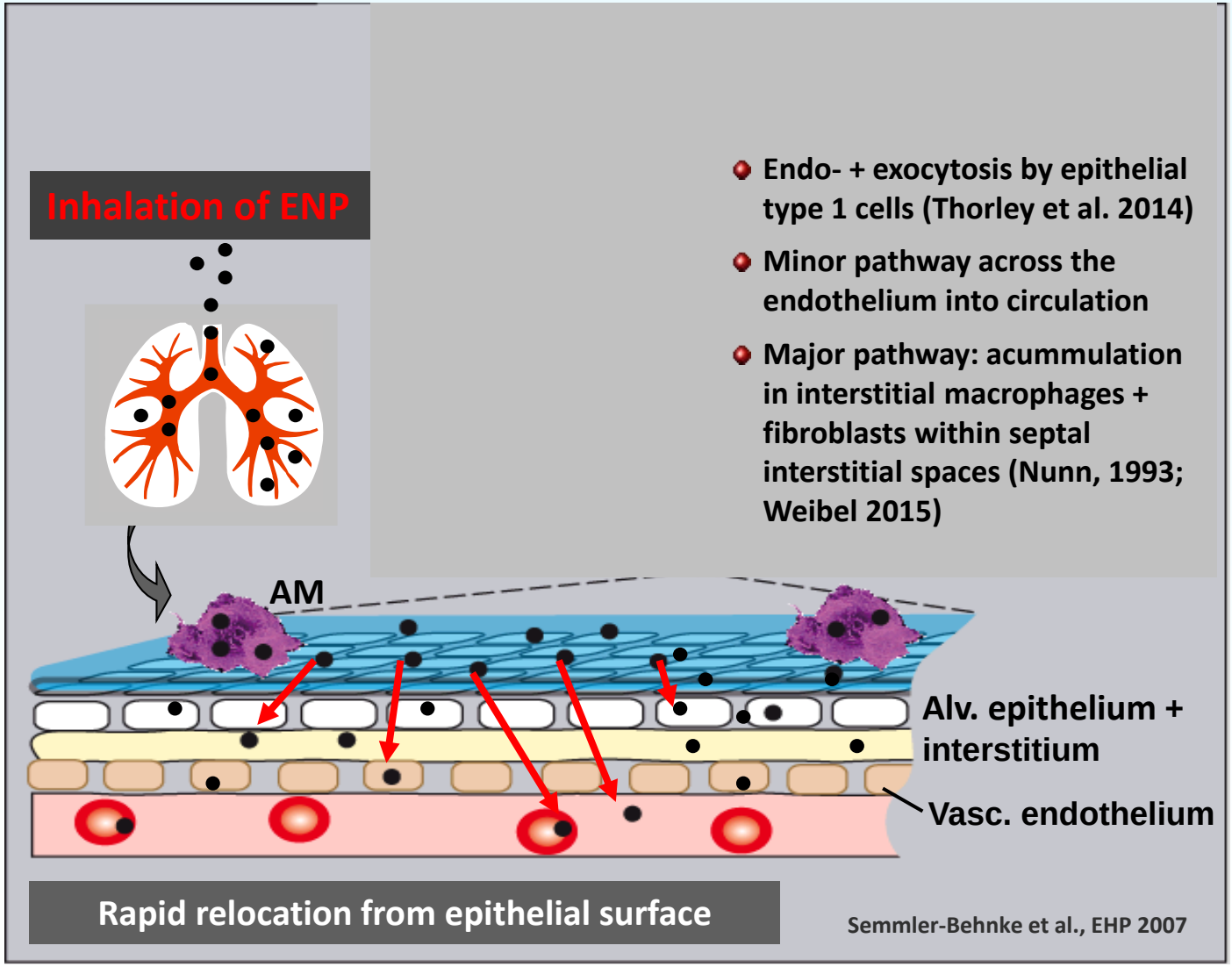
- ➔ **but usually at high doses in short-term studies and often under non-physiological exposure conditions (e.g. instillation);**
- ➔ **in fact, long-term toxicological animal studies are required using well-defined ENP at relevant doses under physiological conditions.**

Currently we can make the best out of UFP and ENP research when we compare their results and cautiously extrapolate in between both.

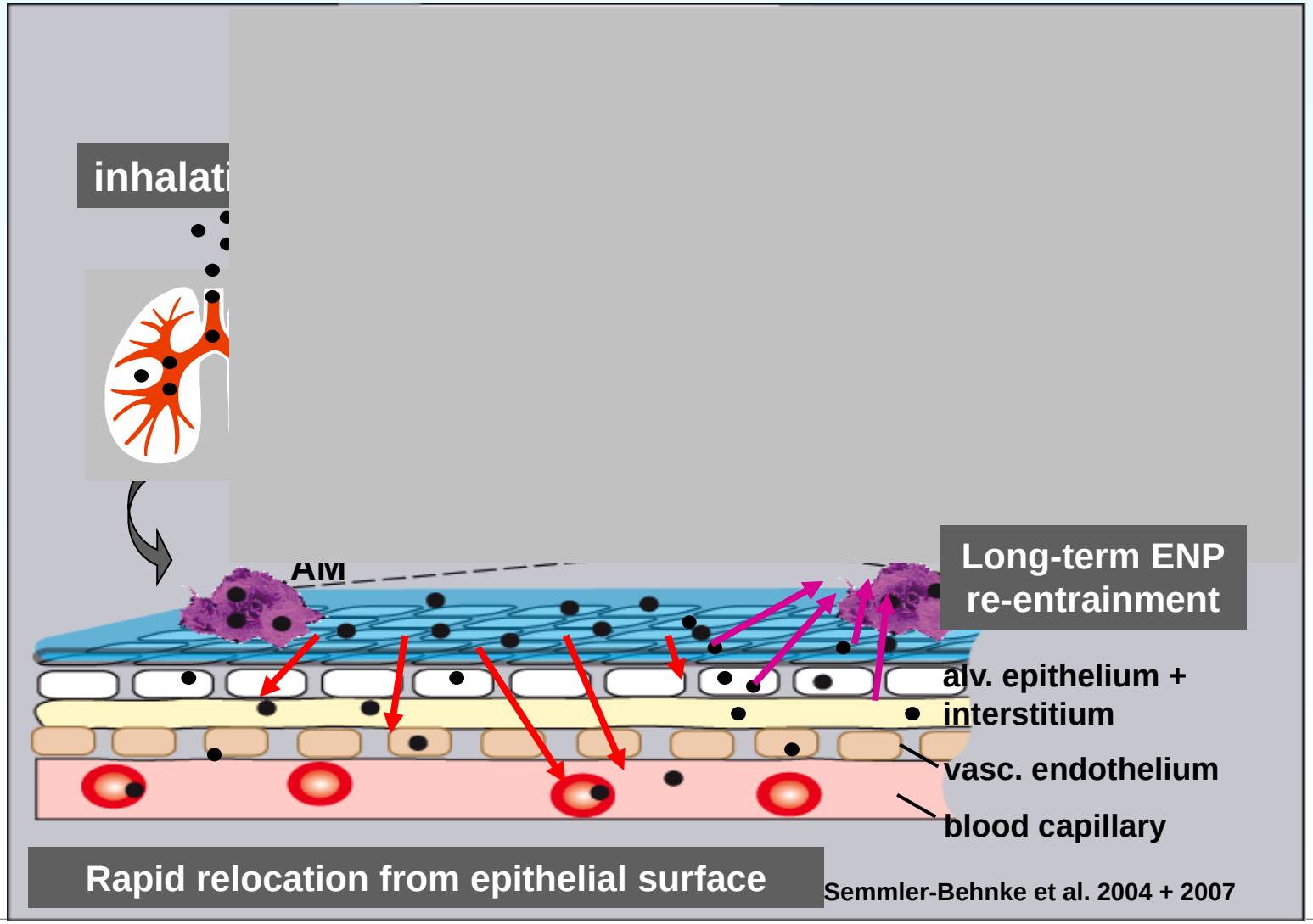
Part 2: Recent scientific biokinetics highlights after NP aerosol inhalation

- Rapid relocation from rat alveolar epithelium into interstitial spaces and re-entrainment back onto the epithelium for macrophage-mediated clearance to larynx and gastro-intestinal-tract
- Disagglomeration of inhaled AuNP agglomerates in rat lungs
- Translocation of inhaled 20 nm AuNP across the air-blood-barrier is similar in man and rat

Rapid ENP Relocation from Rodent Alveolar Epithelium Towards Interstitium



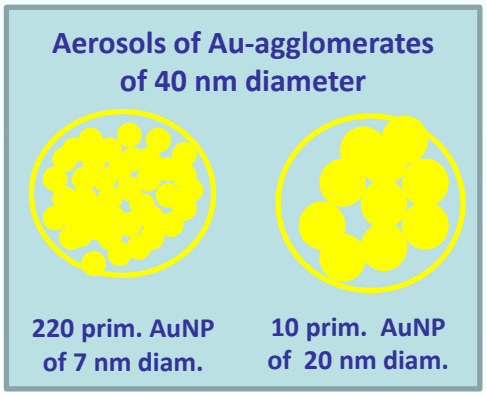
ENP Trafficking across Rat Lung Membrane

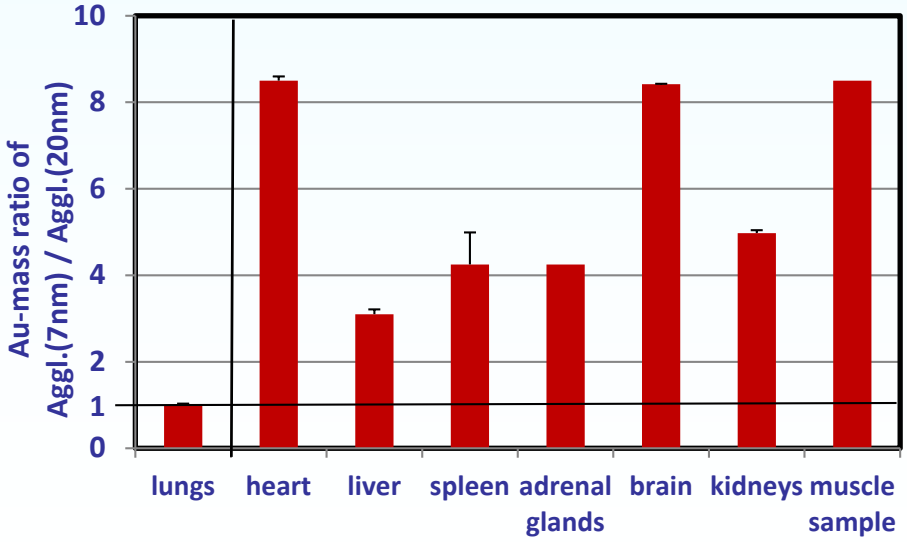


Disagglomeration of inhaled AuNP agglomerates in rat lungs

Study design:
Healthy adult Wistar rats inhaled 40 nm-sized AuNP-agglomerates at a concentration of $1*10^6$ cm^{-3} consisting either of 7nm primary AuNP or 20nm primary AuNP. Exposure: 3-weeks inhalation at 6h/d, 5d/wk; whole-body exposure.
Two days after the final inhalation Au-contents in lungs and 2nd organs were determined by ICP-MS.

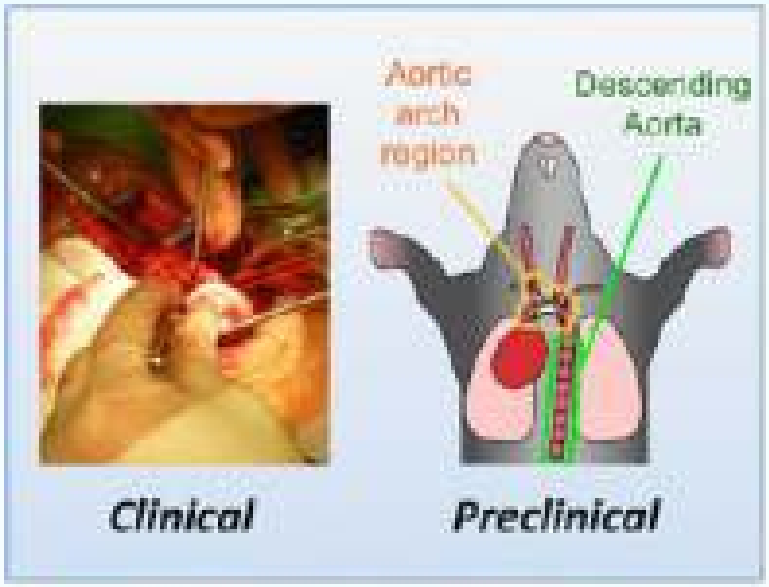
- ➔ **Deposition distribution was equal for both aerosols of 40 nm-sized AuNP agglomerates;**
- ➔ **After inhalation of the AuNP-agglomerates containing 7nm primary AuNP all 2nd organs contained more Au than after the inhalation of AuNP-agglomerates containing 20nm primary AuNP;**
- ➔ **The 7nm AuNP containing AuNP-agglomerates disagglomerated and translocated across the air-blood-barrier more than the 20nm AuNP containing AuNP-agglomerates**
- ➔ **The graph shows Au-mass ratios of 7nm agglomerates / 20nm agglomerates**

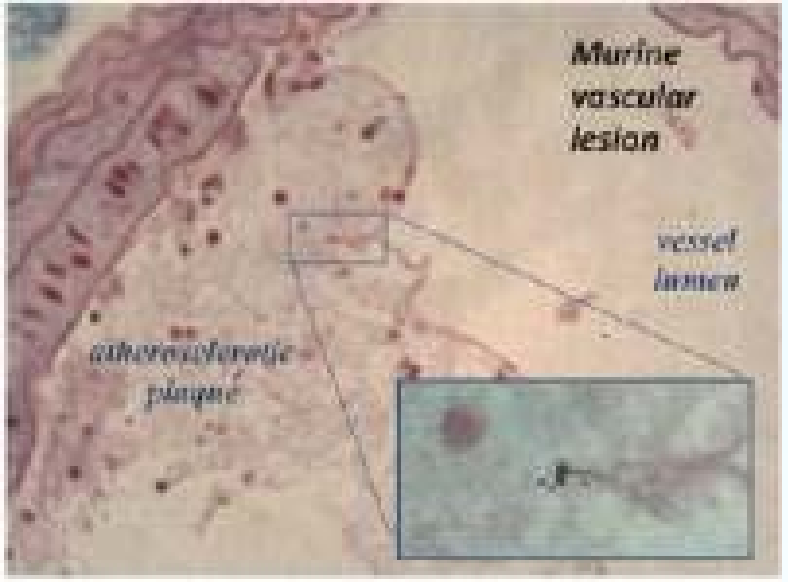


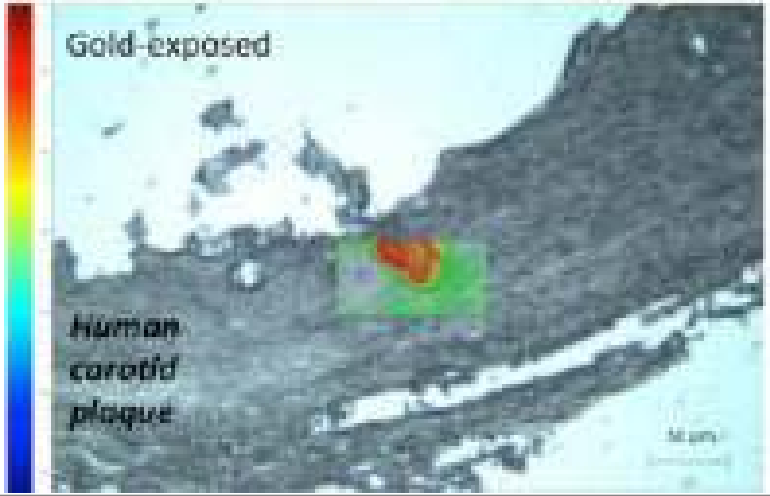


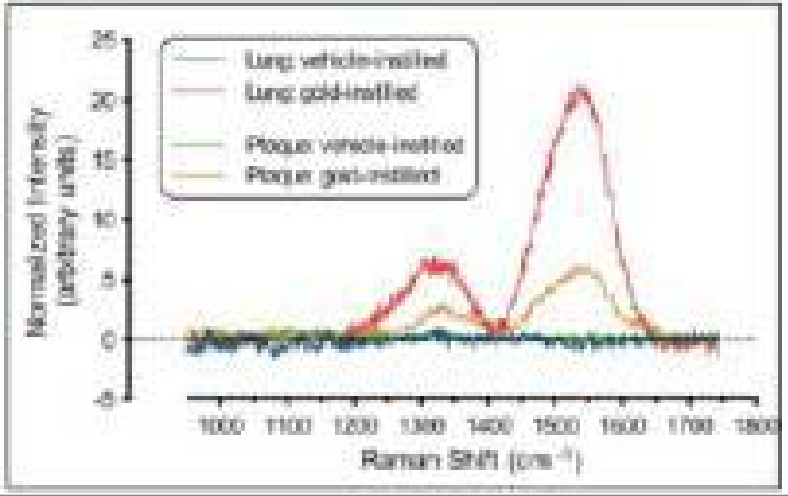
Balasubramanian et al., 2013

Miller *et al.*, Inhaled Nanoparticles Accumulate at Sites of Vascular Disease. ACS Nano 2017





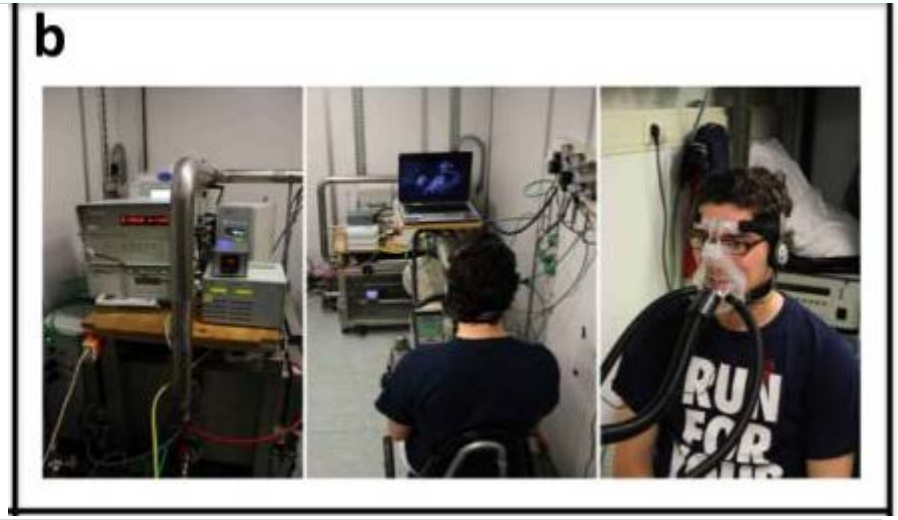




Human inhalation study using 18 nm gold NP (AuNP) is directly comparable with our quantitative biokinetics assay in rats

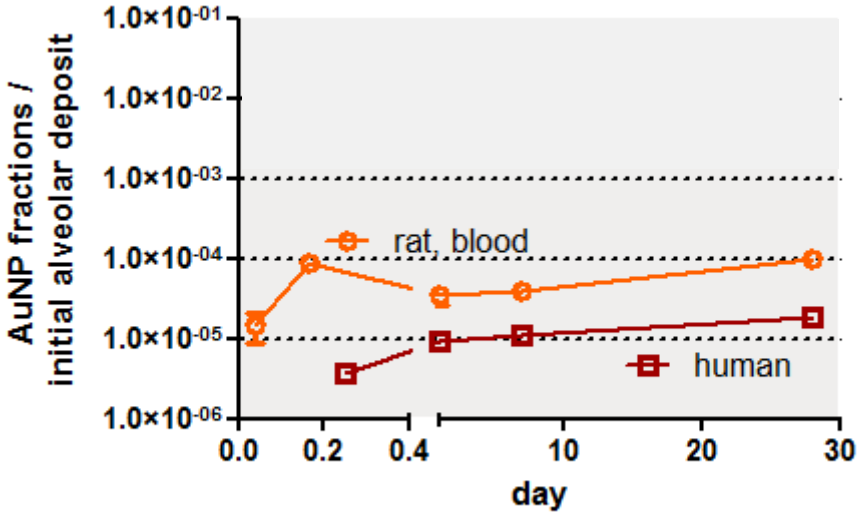
- Here I will focus only on the human inhalation study since this is the first controlled human study showing AuNP translocation across the ABB and providing biokinetic data which are directly comparable to our rat biokinetics data:
- AuNP aerosols: freshly generated by spark ignition aerosol generation like in our rat studies
- 1. Study: 14 healthy volunteers inhaled an AuNP aerosol, 18 nm median diameter; GSD 1.4; primary gold particles 4 nm
- 2. Study: Two more groups (10 + 9 volunteers) inhaled AuNP aerosols, either 18 nm or 52 nm median diameter
  - All exposures lasted for 2 h with intermittent exercise followed by
  - serial blood samples up to 28 days
  - and 24-hours urine samples at day 1, 28 and 60 after exposure
- 3. Study: 3 patients (+ 7 controls) at risk of stroke inhaled the same 18 nm AuNP aerosol, 24h prior to vascular plaque removal in the carotid artery to prove retention of inhaled AuNP

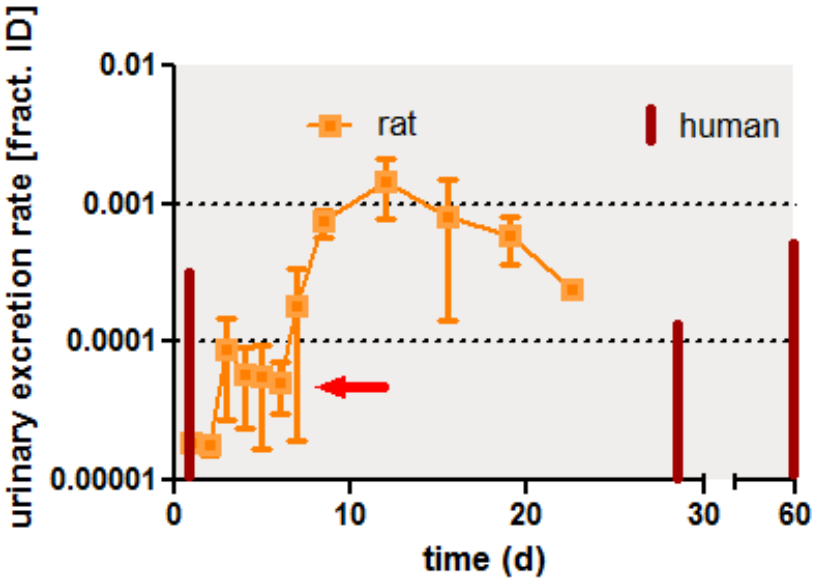
Miller et al. 2017 a+b



Similar biokinetics of blood + urine fractions in man and rat after inhalation of 18 nm AuNP

Measured Au contents in total blood and 24-hour urine samples are normalized to the initial AuNP deposit in the alveolar region of human subjects and rats





- Kinetics of human AuNP fractions in blood is rather similar to fractions in rat blood
- Total translocated AuNP fractions across the rat ABB are 100-fold higher than those in blood due to rapid uptake + accumulation in 2nd organs and tissues
- Similar in man????

- The limited data of human AuNP fractions in urine are in the same range as fractions in rat urine
- Note the delayed onset of AuNP excretion after one week; reasons might be: (i) clearance in 2nd organs + tissues, (ii) kidney functions, (iii) AuNP disagglomeration in lungs + 2nd organs and tissues
- Similar patterns in man????

Conclusions

Part 1: Effects of engineered nanoparticles versus ambient ultrafine particles: how comparable are their interactions with the organism?

- Currently we can make the best out of UFP and ENP research when we compare their results and cautiously extrapolate in between both.

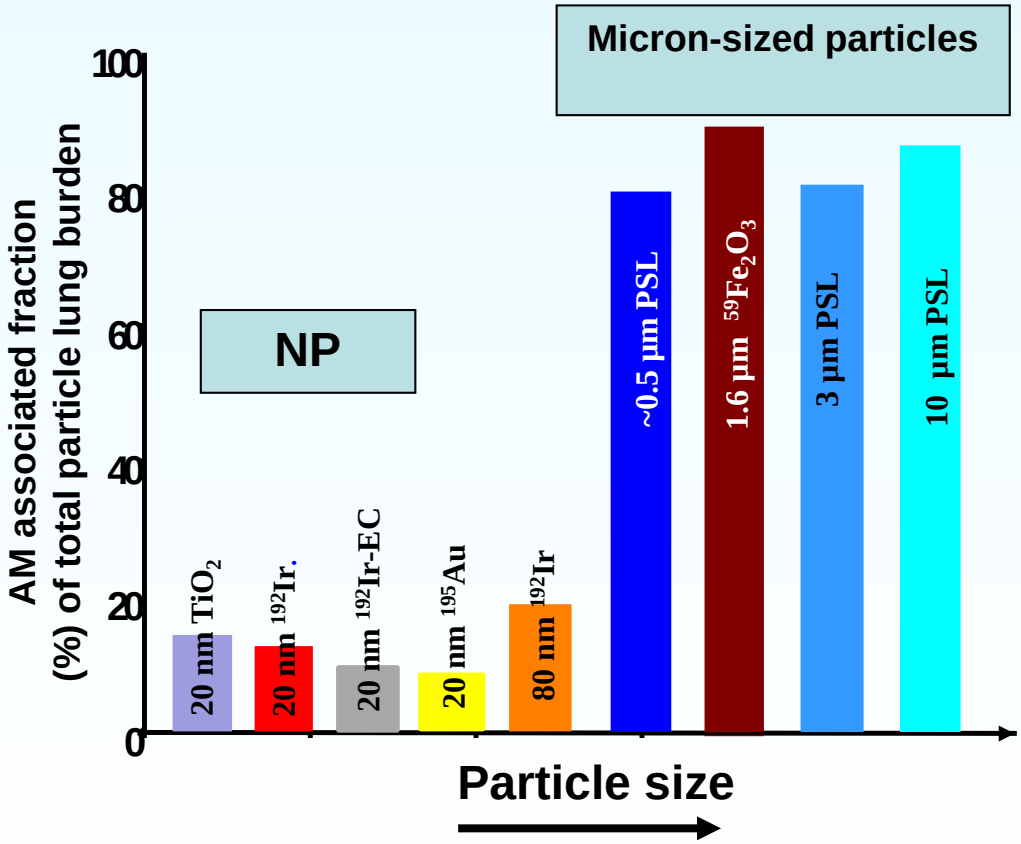
Part 2: Recent scientific biokinetics highlights after NP aerosol inhalation

- NP are rapidly relocated from the rat lung epithelium into interstitial sites for long-term retention which has not been observed for µP
- Only a modest NP fraction translocates across the rat air-blood-barrier into blood circulation which has not been observed for µP
- From the interstitium the major fraction of NP gradually re-entrain onto the rat lung epithelium for macrophage-mediated transport to the larynx and into the GIT
- Agglomerates of 7-nm-sized AuNP disagglomerate, translocate the ABB and accumulate in 2nd organs + tissues to a larger extent than agglomerates of 20-nm-sized AuNP
- Remarkable similarities in the biokinetics of blood + urine fractions in man and rat after inhalation of 18 nm AuNP

Thank you for your attention!

BAL 24 hours after particle administration

Retention of particles in alveolar macrophages (AM) 24h post exposure

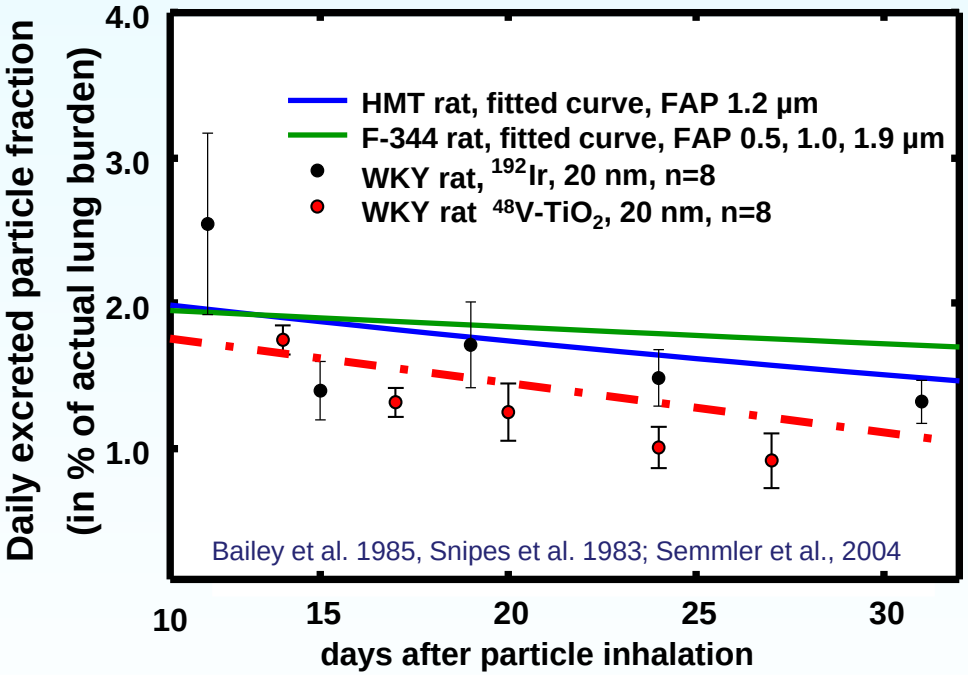


Oberdörster et al., 2001; Kreyling et al., 2002

- within 24h most of the micron-sized particles are phagocytized by AM and accessible to BAL
- in contrast AM play a minor role in nanoparticle uptake within 24h
- NP are rapidly taken up in the lung tissue (epithelial, interstitial and endothelial cells) and are not accessible for AM phagocytosis (Geiser et al. 2005, Mühlfeld et al. 2007)

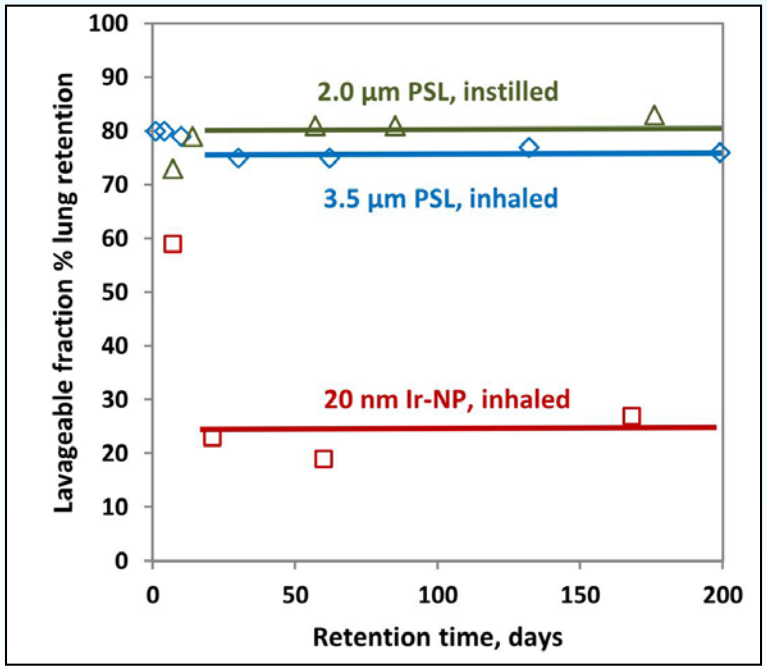
Long-term Clearance Fate: NP versus µP in Rodent Lungs

Comparison of daily excreted particle fraction: NP versus µP



✘ long-term kinetics of AM-mediated NP clearance rate is basically the same as for µP (micron-sized particles)

BAL fraction of µP versus ENP:



Lehnert et al. 1989; Oberdörster et al., 1994; Kreyling et al., 2002

✘ Different to µP retained on the epithelium, NP are not accessible to BAL and are long-term retained in the interstitium

➔ NP need to be re-entrained from the interstitium onto the epithelium prior to AM-mediated clearance towards larynx

ENP Trafficking across Rat Lung Membrane