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Is there sufficient evidence of adverse effects of nanoparticles on neuro-degenerative diseases when compared to their cardiovascular and respiratory health effects?

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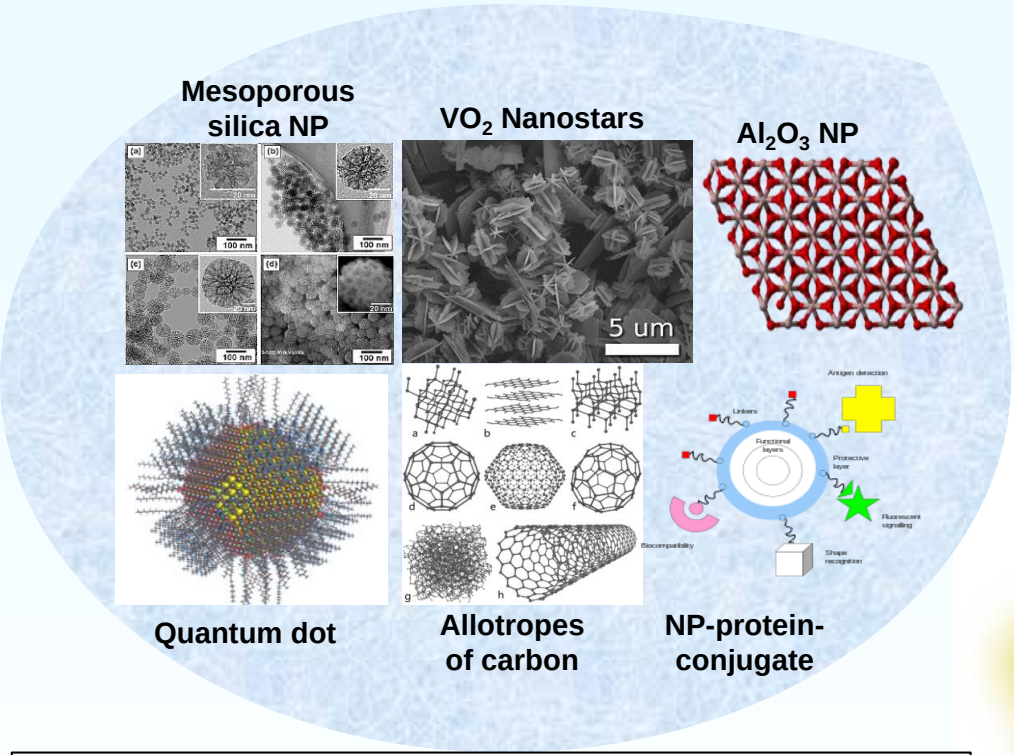
Outline

- Particles < 100 nm (NP): engineered nanoparticles (ENP) & ambient ultrafine particles (UFP)
- Differences between nano-sized and larger particles (submicrometer & micron-sized particles, µP) and their interactions with the organism:
  - Physicochemical properties
  - Total and regional lung deposition during inhalation
  - Rapid relocation from the alveolar epithelium to interstitial spaces
  - Long-term re-entrainment from interstitium on top of lung epithelium for machrophage-mediated clearance towards the larynx into the gastro-intestinal-tract
  - Translocation across the air-blood-barrier into blood followed by accumulation in secondary organs and tissues including the brain
  - Transport from blood across the placental membrane into fetuses
  - Neuronal transport into the central nervous system (CNS, brain), e.g. from nasal mucosa via olfactory nerves
- Evidence for respiratory & cardio-vascular effects of PM_{10}, $PM_{2.5}$ (and NP?)
- Evidence for neuro-degenerative effects of NP in the brain (CNS)
- Conclusions

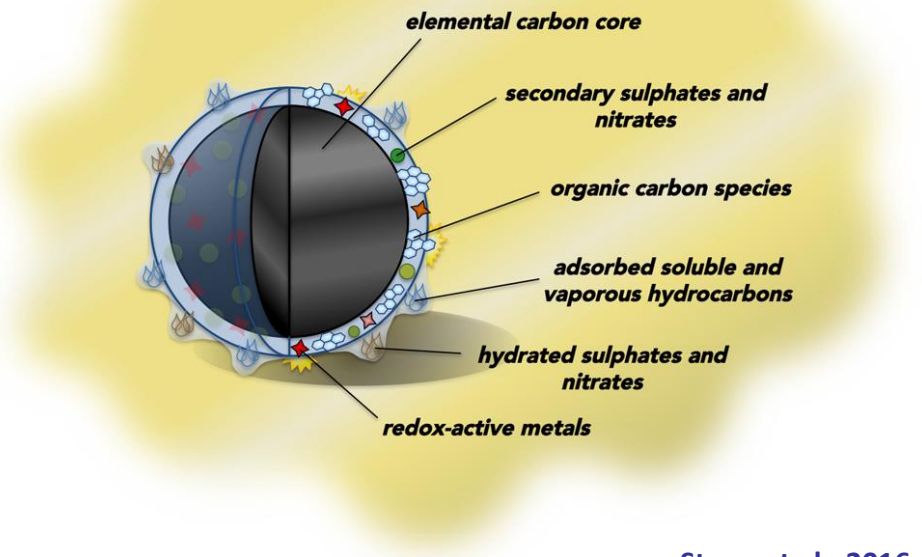
Review - Database

- **Particulate Matter Air Pollution and Cardiovascular Disease – An Updated Scientific Statement from the American Heart Association.** Brook, R. D.; Rajagopalan, S.; Pope, C. A.; Brook, J. R.; Bhatnagar, A.; Diez-Roux, A. V.; Holguin, F.; Hong, Y. L.; Luepker, R. V.; Mittleman, M. A.; Peters, A.; Siscovick, D.; Smith, S. C.; Whitsel, L.; Kaufman, J. D. *Circulation 2010, 121, 2331-2378.*
- **Review of evidence on health aspects of air pollution – REVIHAAP.** Scientific Advisory Committee: H.R. Anderson, B. Brunekreef, A. Cohen, K. Katsouyanni, D. Krewski, W.G. Kreyling, N. Künzli , X. Querol & 29 Expert Authors. WHO Regional Office for Europe & European Commission (2013).
- **Understanding the Health Effects of Ambient Ultrafine Particles. HEI Perspectives 3.** Review Panel: M.W. Frampton (Chair), M. Brauer, M. Kleeman, W.G. Kreyling, L. Ntziachristos, S. Ebelt Sarnat & HEI Science Staff. Health Effects Institute, Boston, MA. 2013. http://pubs.healtheffects.org/view.php?id=394
- **Nanomaterials vs Ambient Ultrafine Particles: an Opportunity to Exchange Toxicology Knowledge.** Stone, V.; Miller, M. R.; Clift, M. J.; Elder, A.; Mills, N. L.; Moller, P.; Schins, R. P.; Vogel, U.; Kreyling, W. G.; Jensen, K. A.; Kuhlbusch, T. A.; Schwarze, P. E.; Hoet, P.; Pietroiusti, A.; De Vizcaya-Ruiz, A.; Baeza-Squiban, A.; Tran, C. L.; Cassee, F. R., *Environ Health Perspect 2016, 106002-1 - 17.*
- **Neurodegenerative and neurological disorders by small inhaled particles.** Heusinkveld, H. J.; Wahle, T.; Campbell, A.; Westerink, R. H.; Tran, L.; Johnston, H.; Stone, V.; Cassee, F. R.; Schins, R. P., *Neurotoxicology 2016, 56, 94-106.*
- **Neurotoxicity of traffic-related air pollution.** Costa, L. G.; Cole, T. B.; Coburn, J.; Chang, Y.-C.; Dao, K.; Roqué, P. J., *Neurotoxicology* 2017, 59, 133-139

Engineered Nanoparticles (ENP) & Ambient Ultrafine Particles (UFP)



- UFP are formed coincidentally by natural and/or man-made processes and are subject to ageing;
- Matrix and surface of UFP are complex mixtures, e.g. inorganic and organic carbon, metals, salts, biologic materials;
- Not present as a single entity but always together with ambient PM_{10}, $PM_{2.5}$, gases



- ENP matrix + surface are thoroughly engineered re. chemical compounds, physical structure
- The design of ENP becomes more and more complex with the emerging development of nanotechnologies

Stone et al., 2016

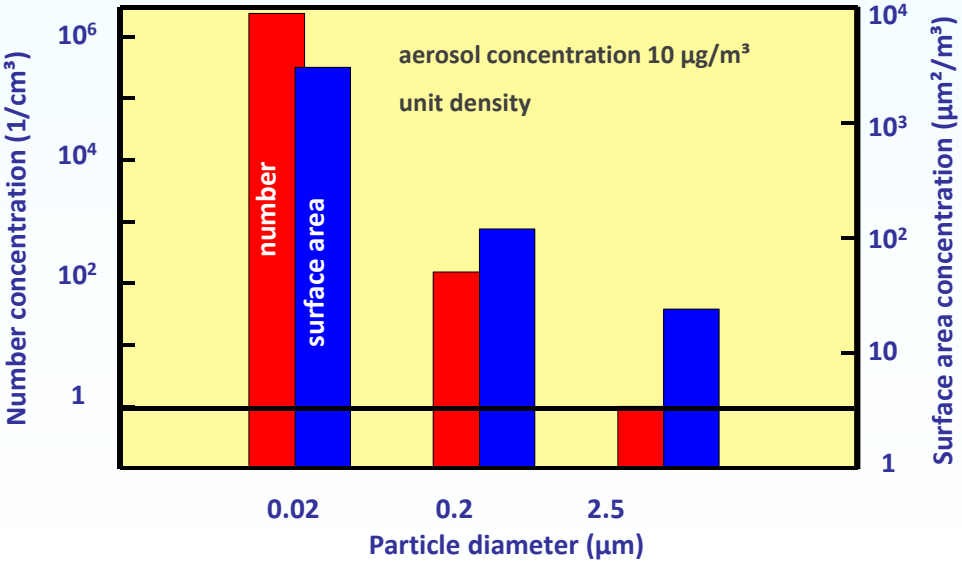
Physico-chemical Properties Specific for NP

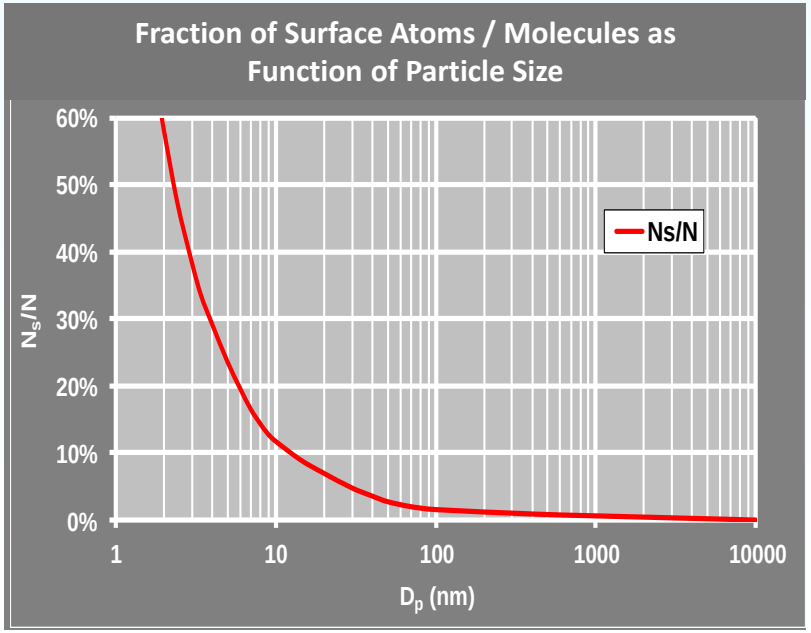
- The volume of one 2.5 µm particle corresponds to that of 2.5 10^6 NP of 20 nm size;
- And the surface area increases 1000-fold for 20 nm NP

➔ The surface-related reactivity of NP is much higher than that of µP

➔ i.e. many more binding sites for proteins / biomolecules

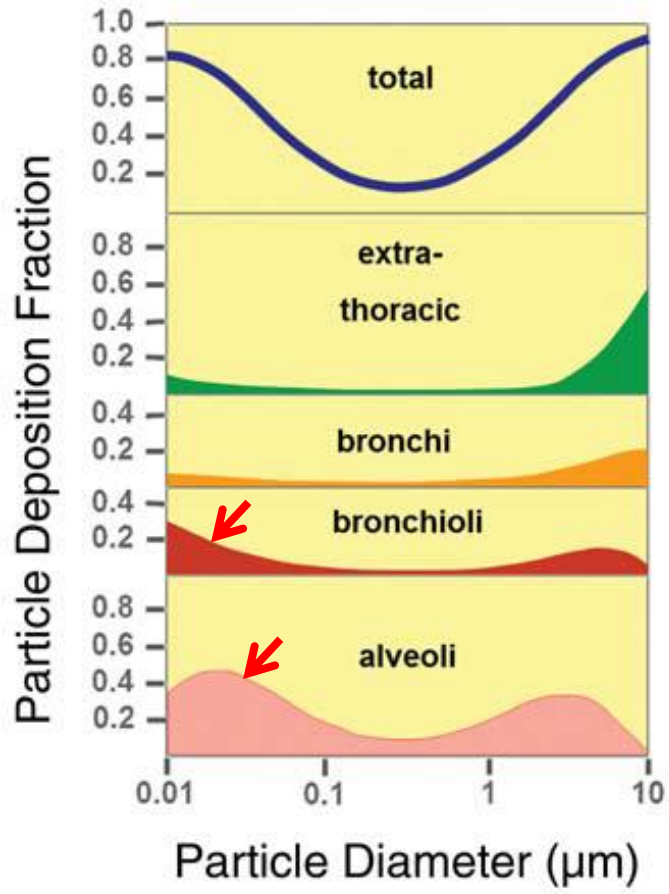
Relative to the total number of atoms / molecules of a NP the fraction of atoms / molecules at the surface increase steeply with decreasing NP size

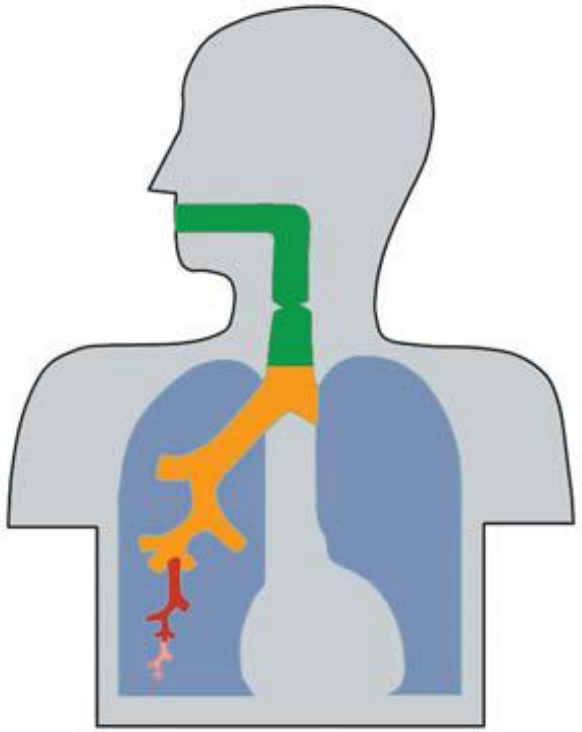




Regional deposition of inhaled nanoparticles (NP < 0.1 µm) vs. larger particles (µP > 0.1 µm)

➔Preferential deposition of NP in bronchioli and alveoli in contrast to µP



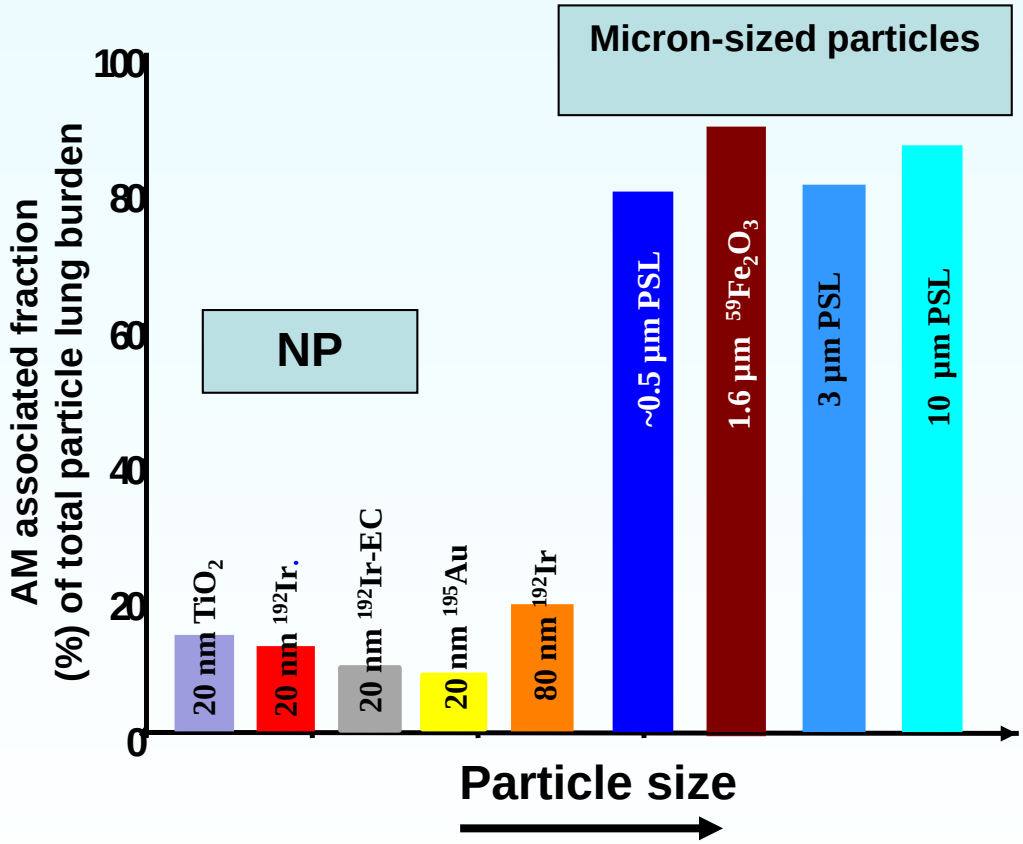


Particle density: 1 g cm^{-3}
Respiratory flow rate: 300 cm^3 s^{-1}
Mouth breathing at rest, cycle period: 5 s

Heyder et al. 1995
Kreyling et al. 2006

BAL 24 hours after particle administration

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



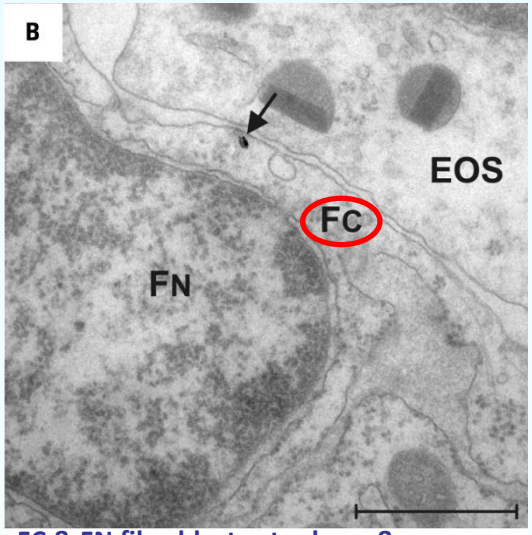
Oberdörster et al., 2001; Kreyling et al., 2002

- 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 less accessible for AM phagocytosis (Geiser et al. 2005, Mühlfeld et al. 2007)

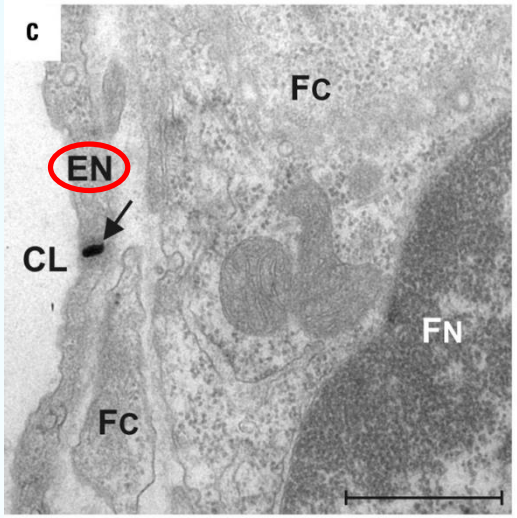
Retention of TiO_2 & Gold NP in Lungs & Liver of Rats

Interstitial retention of inhaled 20 nm TiO_2 NP in rat lungs

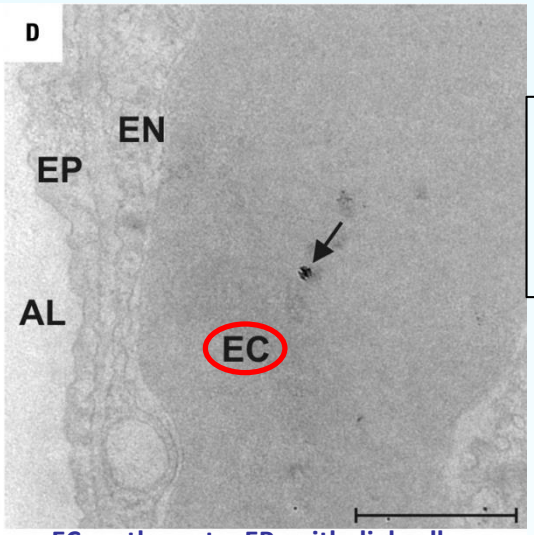
Geiser et al. EHP 2005



FC & FN fibroblast cytoplasm & nucleus, respectively



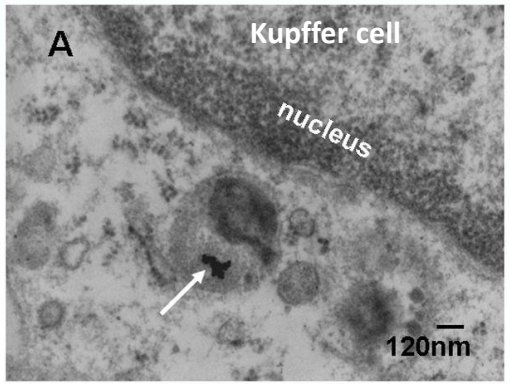
EN endothelial cell, CL capillary lumen

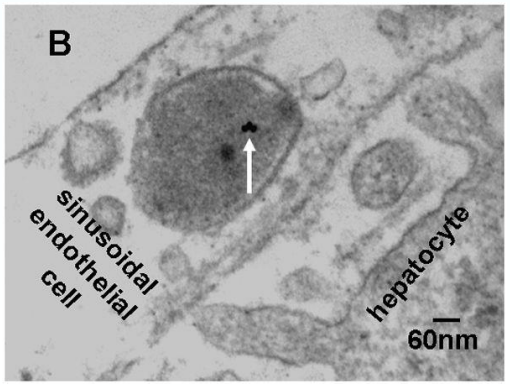


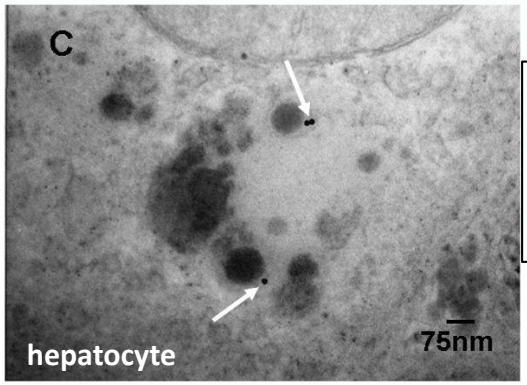
EC erythrocyte, EP epithelial cell, AL alveolar lumen

No interstitially retained micron-sized TiO_2 particles found in lungs!

retention of IV-injected 18 nm gold NP in rat liver



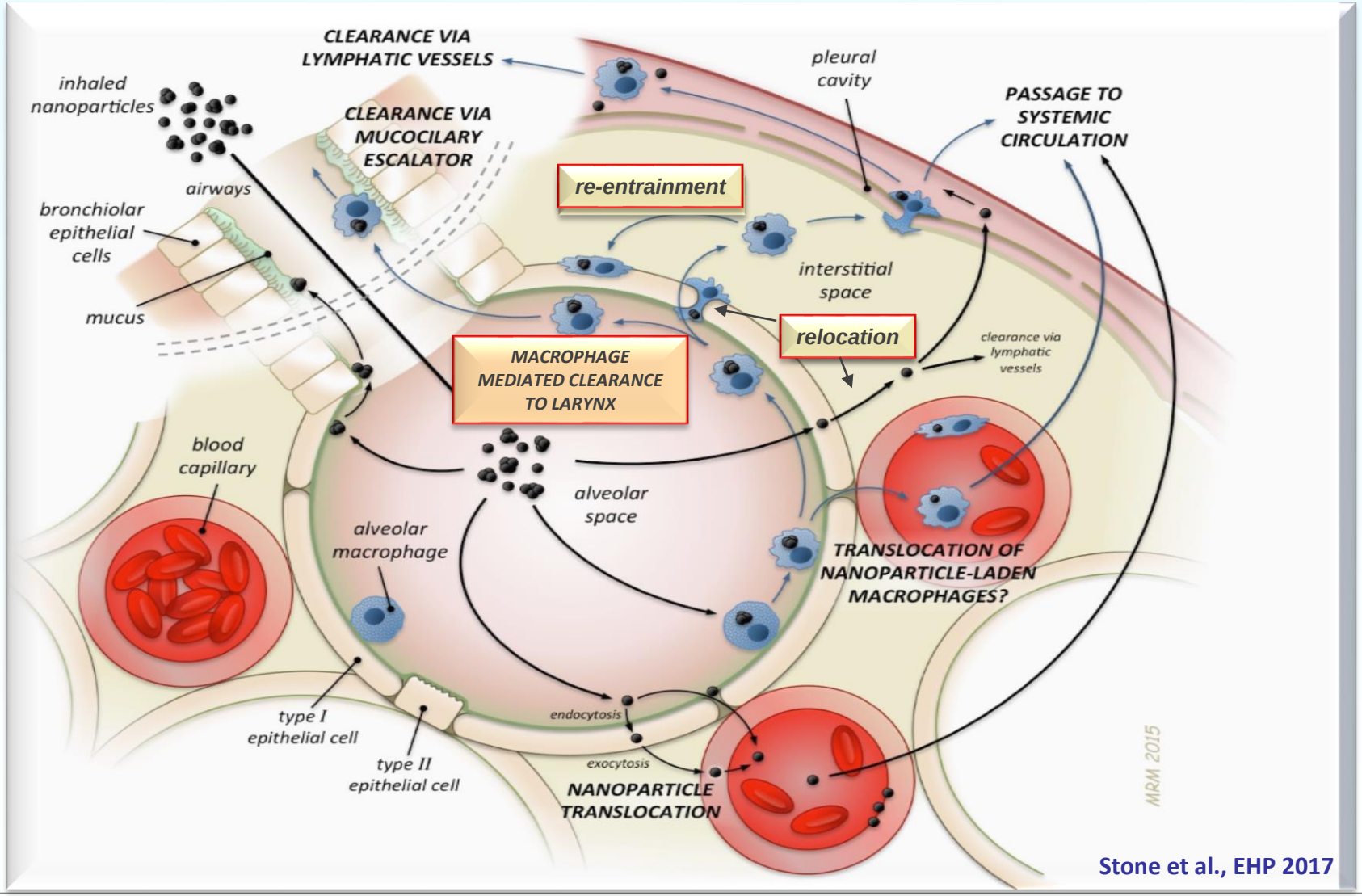




No retained micron-sized Gold particles found in the liver!

Hirn et al. EJPB 2011

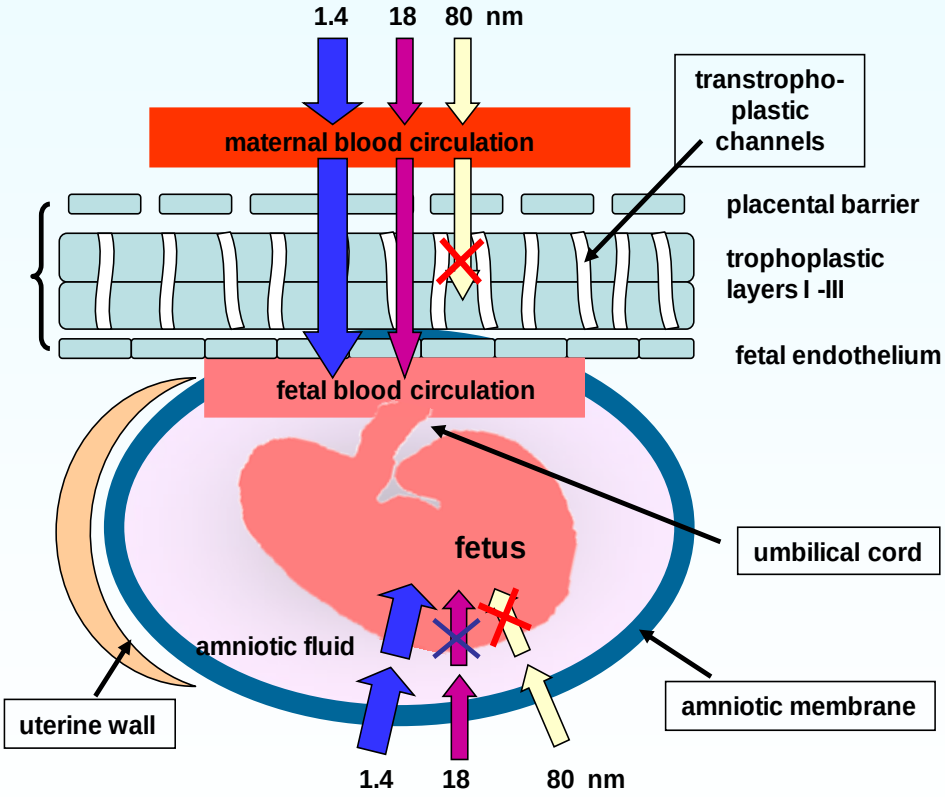
Transport pathways of inhaled NP within + out of the lungs



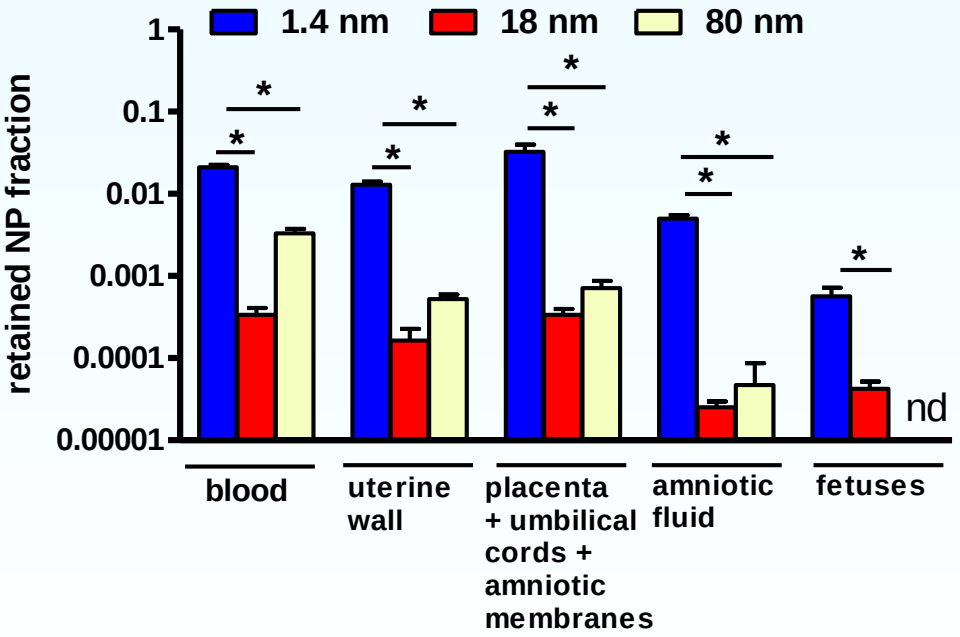
Stone et al., EHP 2017

Translocation of NP across the Placenta into Fetuses - but not for µP

Size dependent AuNP pathways from the maternal blood towards the fetus



Retained fractions of IV-injected 1.4 nm, 18 nm or 80 nm monodisperse AuNP found after 24 hours in blood, uterus, placenta and fetuses of pregnant rats.

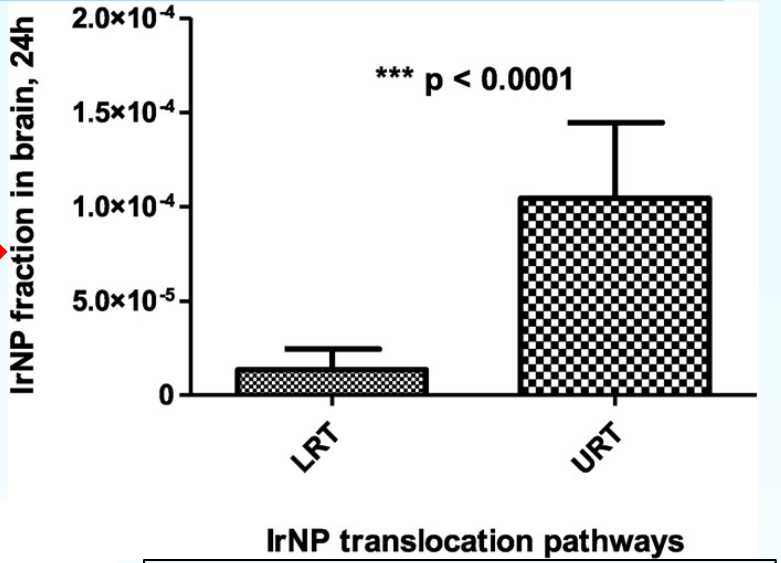


Note, 80 nm AuNP were not detected in fetuses

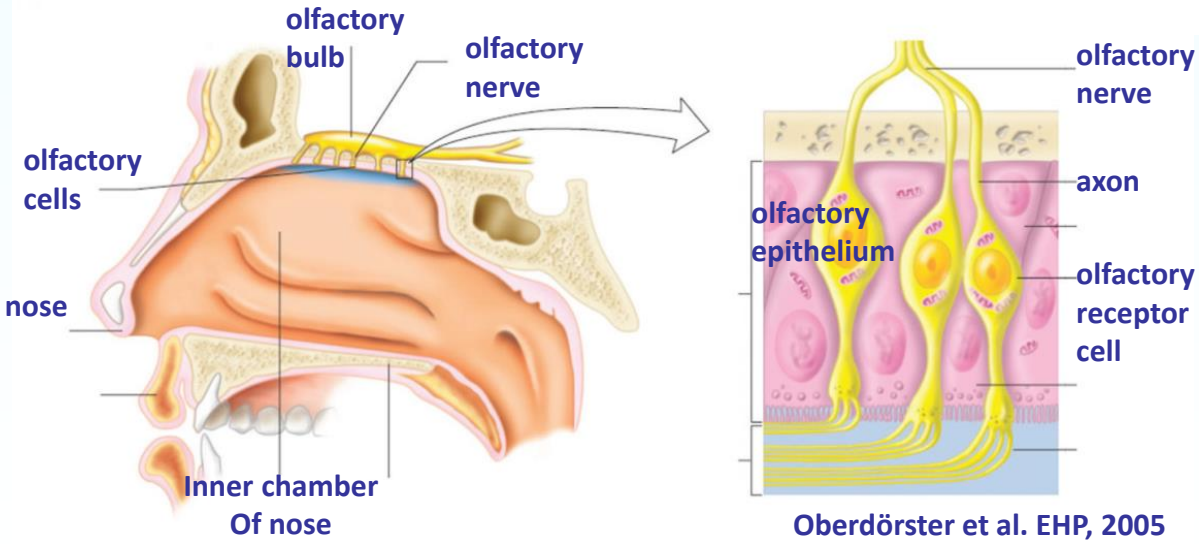
Semmler-Behnke et al. PNAS 2014

Translocation of Inhaled NP to the Brain - but not for µP

- Inhaled NP can travel via the olfactory nerves from the nose to the brain; in contrast to larger µP.
- Using two exposure methods - nose only inhalation & intratracheal inhalation - we differentiated IrNP translocation from the lungs via blood to brain vs. IrNP transport from the nose via neuronal pathways to the brain;
- ➔ in rats the latter is predominant.



IrNP translocation pathways



Oberdörster et al. EHP, 2005

LRT from lower resp. tract via blood to brain

URT from upper resp. tract via neurons to brain

Kreyling TAAP, 2016

The Fate of inhaled ENP and UFP differs from that of inhaled micron-sized particles (µP)

When compared to µP inhaled NP differ in:

- **Higher surface to mass ratio suggesting higher reactivity**
- **Higher peripheral lung deposition**
- **Relocation within the lungs from the epithelium to the interstitium**
- **Re-entrainment from interstitial sites back to the epithelium for macrophage-mediated clearance to larynx**
- **Translocation across the air-blood barrier into circulation with subsequent accumulation in 2nd organs + tissues**
- **Transport from blood across the placental membrane into the fetus**
- **Neuronal transport into the central nervous system**

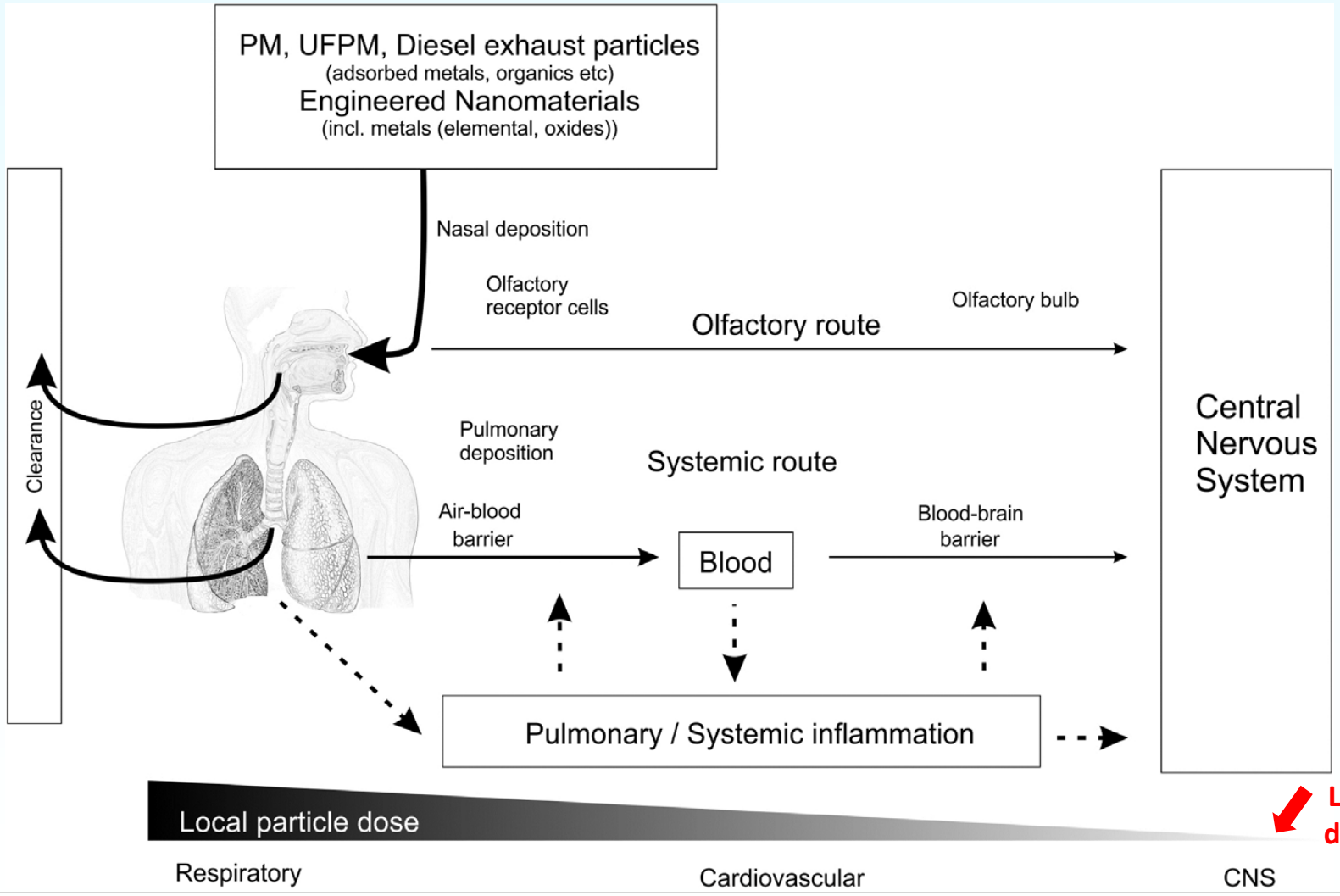
➔ **However, does this mean that ENP or UFP can cause different adverse health effects in the lungs, in the cardio-vascular system or in the central nervous system?**

Conclusions from the database of the cited reviews

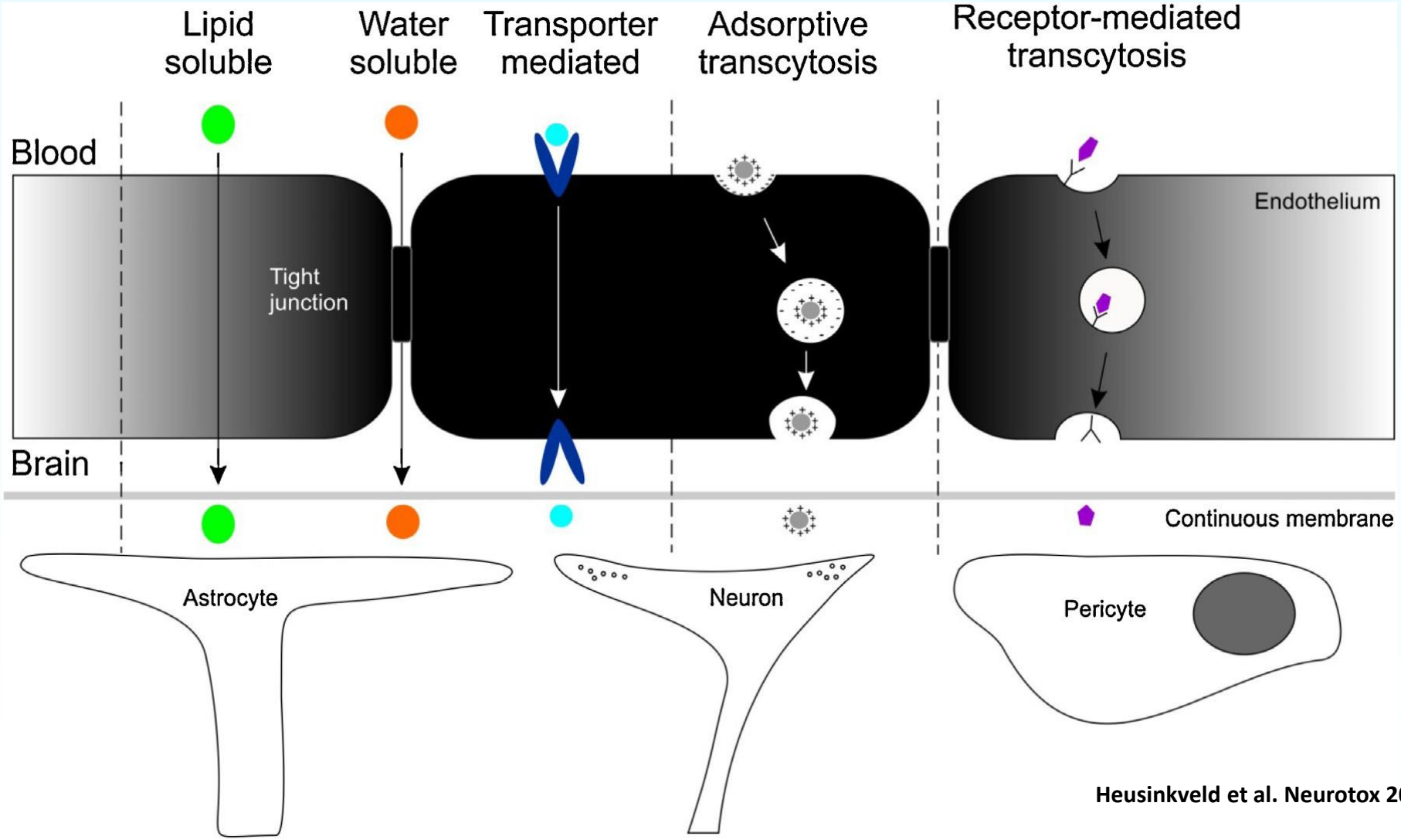
Summary of conclusions and possible concepts presented in the cited reviews how PM and UFP may possibly mediate or cause adverse health effects in the brain

Potential particle translocation routes and pathways which can lead to particle accumulation the central nervous system

Heusinkveld et al. Neurotox 2016



Possible transport routes of particles across the blood-brain barrier



Heusinkveld et al. Neurotox 2016

Epidemiological evidence of the cardio-vascular effects of traffic- or combustion-related $PM_{2.5}$ exposure at ambient levels

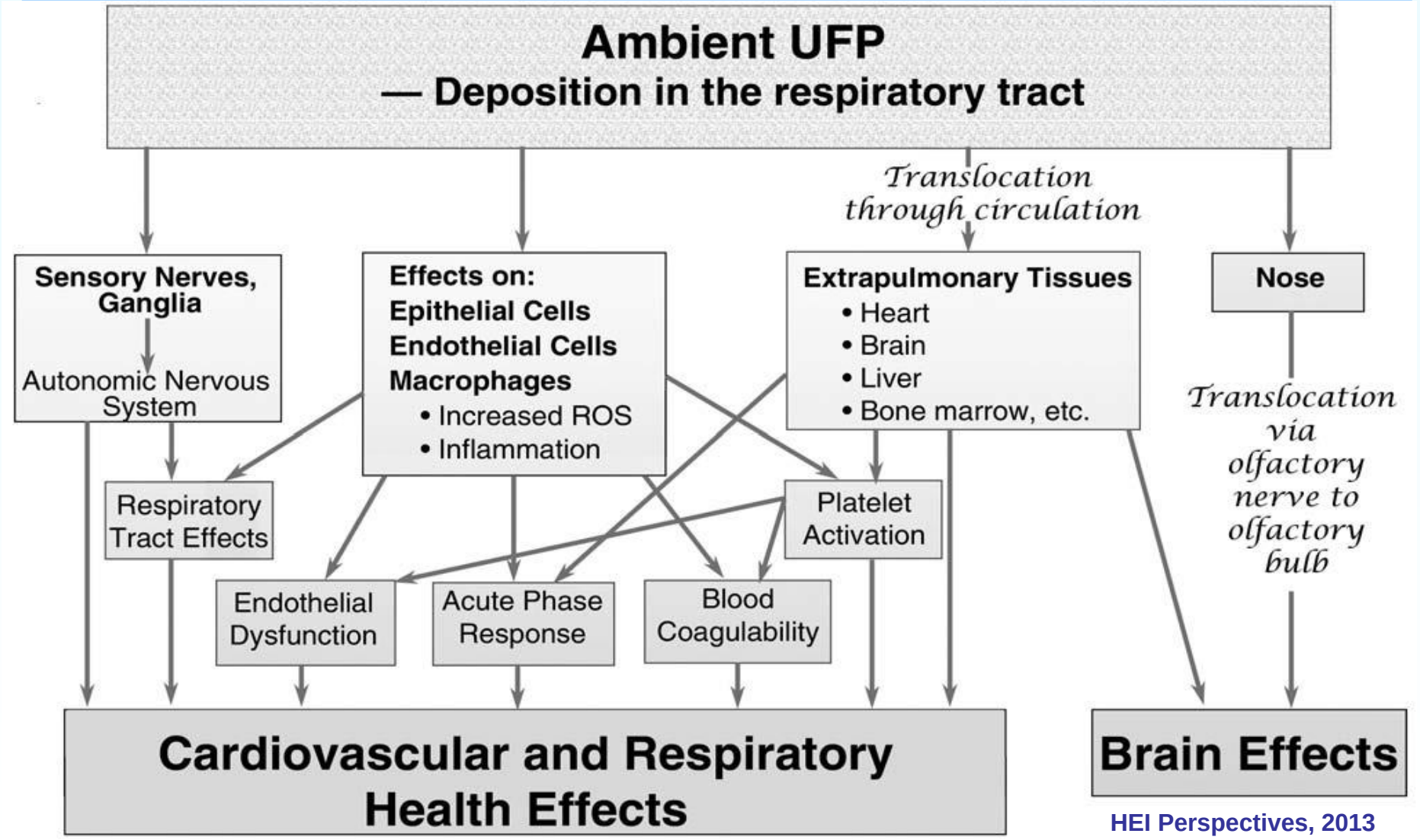
Health Outcomes	Short-Term Exposure (Days)	Longer-Term Exposure (Months to Years)
Clinical cardiovascular end points from epidemiological studies at ambient pollution concentrations		
Cardiovascular mortality	↑ ↑ ↑	↑ ↑ ↑
Cardiovascular hospitalizations	↑ ↑ ↑	↑
Ischemic heart disease*	↑ ↑ ↑	↑ ↑ ↑
Heart failure*	↑ ↑	↑
Ischemic stroke*	↑ ↑	↑
Vascular diseases	↑	↑†
Cardiac arrhythmia/cardiac arrest	↑	↑
Subclinical cardiovascular end points and/or surrogate measures in human studies		
Surrogate markers of atherosclerosis	N/A	↑
Systemic inflammation	↑ ↑	↑
Systemic oxidative stress	↑	
Endothelial cell activation/ blood coagulation	↑ ↑	↑
Vascular/endothelial dysfunction	↑ ↑	
BP	↑ ↑	
Altered HRV	↑ ↑ ↑	↑
Cardiac ischemia	↑	
Arrhythmias	↑	

Evidence supporting biological pathways + specific mechanisms whereby traffic- or combustion-related $PM_{2.5}$ exposure can affect the cardiovascular system

	Animal Studies	Human Studies
General "intermediary" pathways whereby PM inhalation can instigate extrapulmonary effects on the cardiovascular system		
Pathway 1: Instigation of systemic proinflammatory responses	↑ ↑ ↑	↑ ↑ ↑
Pathway 2: Alterations in systemic ANS balance/activity	↑	↑ ↑
Pathway 3: PM and/or associated constituents directly reaching the systemic circulation	↑	↑
Specific biological mechanisms directly responsible for triggering cardiovascular events		
Vascular dysfunction or vasoconstriction	↑ ↑ ↑	↑ ↑
Enhanced thrombosis or coagulation potential	↑ ↑	↑ ↑
Elevated arterial BP	↑ ↑	↑ ↑
Enhanced atherosclerosis or plaque vulnerability	↑ ↑	↑
Arrhythmias	↑	↑

Brook et al. (American Heart Association) 2010

Hypothesized Effects of Inhaled Ambient UFP on Cardiovascular, Respiratory and Central Nervous systems



HEI Perspectives, 2013

Conclusions Found in Literature (1)

Conclusions (Heusinkveld et al. Neurotox 2016 and Costa et al. Neurotox 2017):

- **Accumulating evidence indicates that it is plausible that (chronic) exposure to inhalable PM plays a role in the pathogenesis/pathophysiology of neurodegenerative diseases.**
- **As transport of inhalable material to the brain parenchyma has been demonstrated, direct effects on the brain are plausible.**
- **However, considering exposure levels, current paucity on particle specific translocation kinetics, and the plethora of systemic effects that are demonstrated to influence CNS pathology, it is hard to tell which route provides the strongest link.**
- **Moreover, species differences in respiratory tract morphology and physiology require careful consideration when assessing the potential effects of inhalable particles on the CNS.**
- **It is especially intriguing to consider that direct and indirect mechanisms could act together in an additive or even synergistic manner.**
- **Future experiments will need to unravel the mechanism (s) of particle-induced neurotoxicity and identify which components of inhalable PM contribute to CNS pathology.**

Conclusions Found in Literature (2)

Conclusions (Stone et al., EHP, 2017):

Neurotoxicological health outcomes could be explained by findings that

- **inhaled particles can travel to the brain via the blood following alveolar deposition;**
- **in addition via nose – brain transport following olfactory mucosa deposition;**
- **or by the spillover of systemic inflammation to the CNS;**
- **a combination of these processes is also possible;**
- **it is interesting to note that neurodegenerative diseases are on the rise and that there is a well established — albeit mechanistically murky — link between inflammation and neuro degeneration**

Summary

In other words: The currently existing literature describes an association between air pollution and neurotoxicity and shows bits and pieces but it lacks to sufficiently identify:

- **which fraction (UFP, $PM_{2.5}$, gases or which constituents or a combination of all of them) causes neurotoxicity?**
- **by which mechanisms and by which modes of action?**

That means, we cannot lay back and claim "UFP are not a problem for the brain" but we need to be alerted and vigorously search for a comprehensive understanding of how air pollution may affect the central nervous system.

Wishing you a successful and enjoyable conference

and

thanks for your attention!

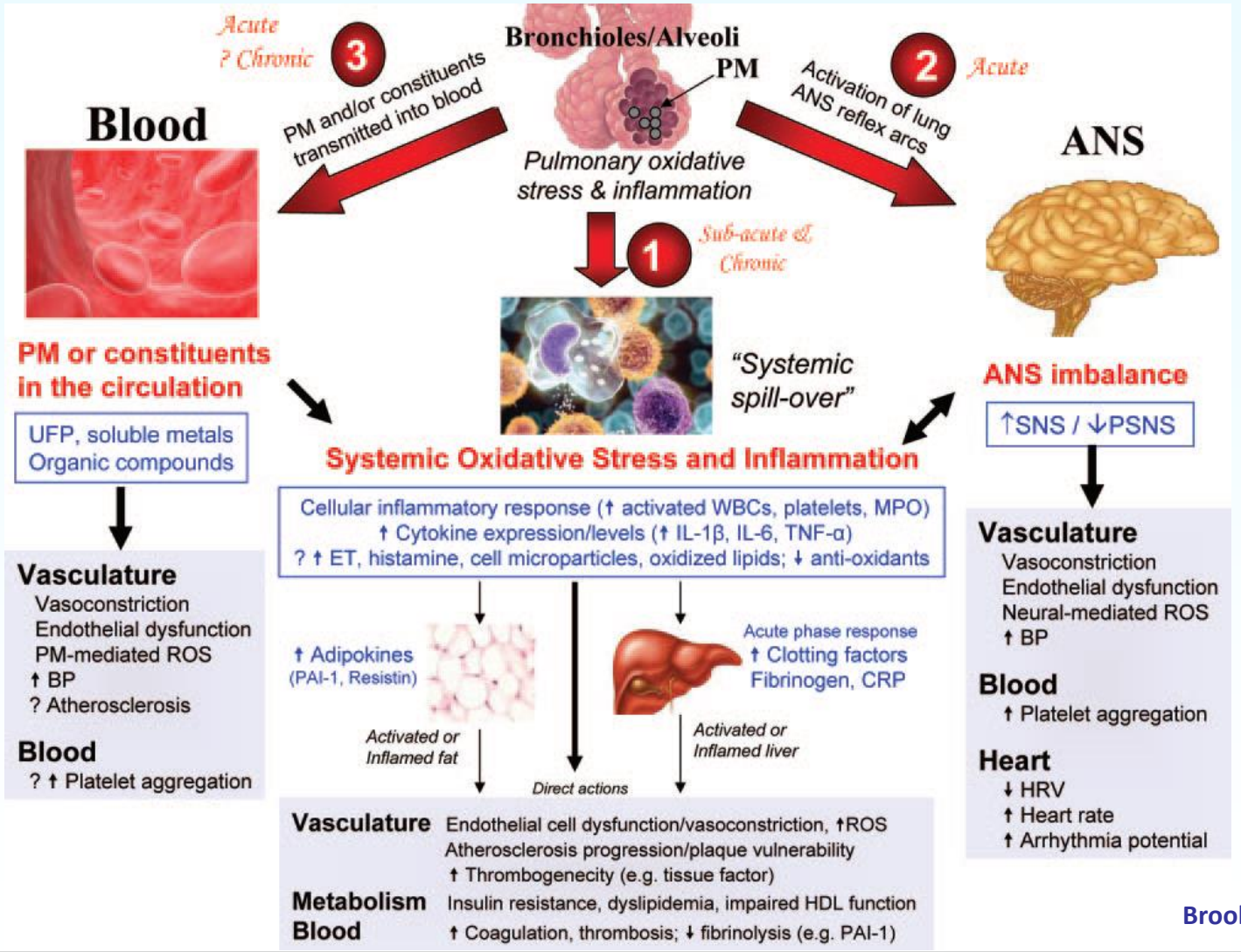
Neurotoxicity of air pollution: epidemiological and experimental evidence found in numerous papers:

- Inhaled PM2.5 and UFP may enter the circulation and reach the brain
- Decreased cognitive function, olfactory dysfunction, auditory deficits, depressive symptoms have been reported in humans
- controlled acute exposure of humans and rodents to Diesel Exhaust:
  - induce EEG changes in human subjects
  - alter motor activity, spatial learning and memory
  - cause oxidative stress and neuro-inflammation in the human CNS
  - in adult & developing mice oxidative stress was increased in brain regions by measuring lipid peroxidation, and a number of pro-inflammatory cytokines

Costa et al. write: "*Air pollution is a risk factor for neurodevelopmental and neurodegenerative diseases".* But they add: "*Further studies aimed at better characterizing the effects of air pollution on the CNS, and its underlying mechanisms are certainly warranted.".*

In other words: This literature describes an association between air pollution and neurotoxicity but it remains to be resolved which fraction (UFP, PM2.5 or gases or all of them) causes neurotoxicity by what mechanism or which mode of action.

Biological pathways linking PM exposure with cardio-vascular diseases



Brook et al., Circ. 2010