Willkommen
Welcome
Bienvenue

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Toxic effects from nanoparticles: limits and gaps in the assessment

Savvina Chortarea
Laboratory for Particles-Biology Interactions

65th LCA Discussion Forum,
May 24th 2017

Nanomaterials - Applications

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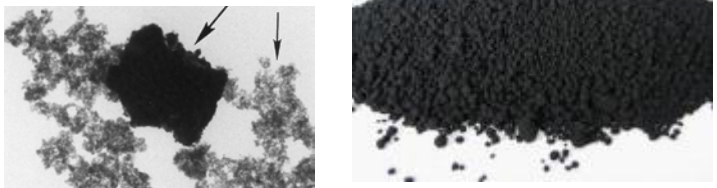
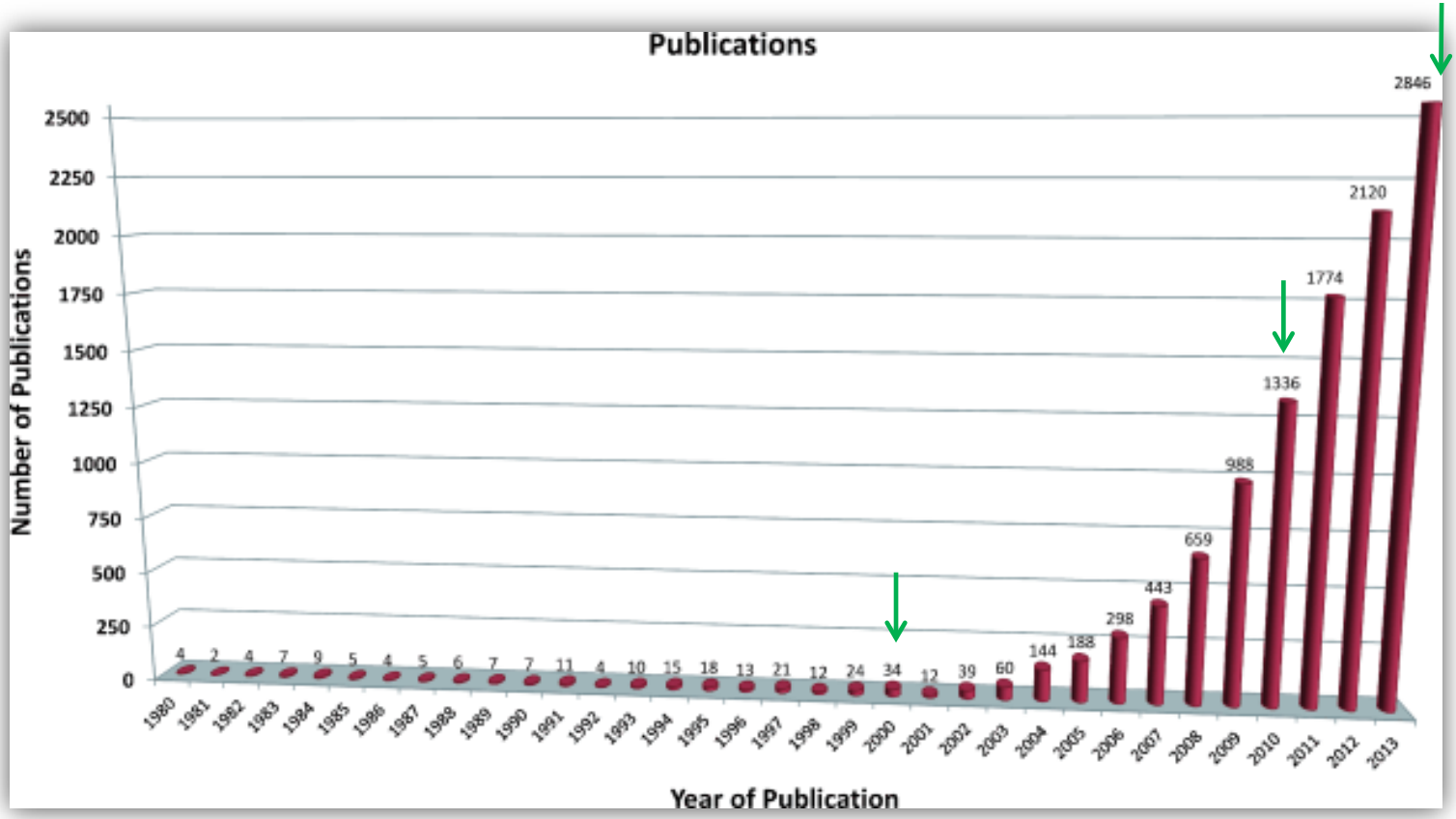
Engineered NPs



Toxicity of nanomaterials-Publications

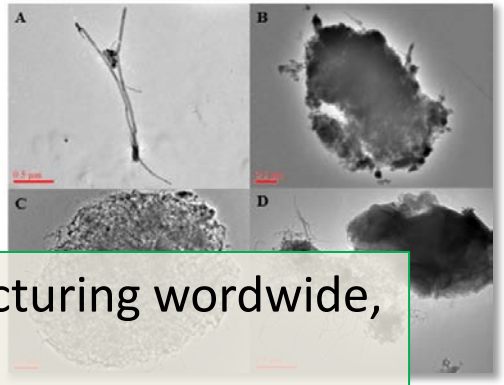


Krug et al., Angewadte Rev, **2014**

Human exposure

- Occupational

Samples collected in CNTs production facilities

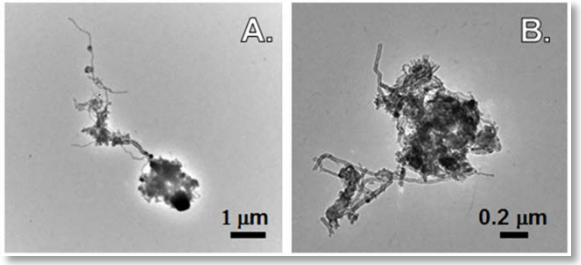


Erdely et al., Part Fibre Toxic, 2014.

6 million of workers in nanoscience and manufacturing wordwide, by the year 2020.

- Use of consumer products/disposal

www.nanotechproject.org.



Shvedova et al., Plos One, 2016.

Risks of Occupational Exposure

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PLOS ONE

RESEARCH ARTICLE

Integrated Analysis of Dysregulated ncRNA and mRNA Expression Profiles in Humans Exposed to Carbon Nanotubes

Anna A. Shvedova[1,2]*, Naveena Yanamala[1], Elena R. Kisin[1], Timur O. Khailullin[2,4], M. Eileen Birch[3], Liliya M. Fatkhutdinova[4]

1 Exposure Assessment Branch/HELD/NIOSH/CDC, Morgantown, WV - 26505, United States of America, **2** Department of Physiology and Pharmacology, West Virginia University, Morgantown, WV -26505, United States of America, **3** NIOSH/CDC, 4676 Columbia Parkway, Cincinnati, OH - 45226, United States of America, **4** Department of Hygiene and Occupational Health, Kazan State Medical University, ul. Butlerova 49, Kazan, 420012 Russia

CrossMark

- **785 genes** were **differentially expressed** in MWCNT-exposed workers
- MWCNT have the potential to trigger **pulmonary, cardiovascular and carcinogenic** effects in humans

potential human risk => chronic occupational exposure?

Nanomaterials: routes of exposure

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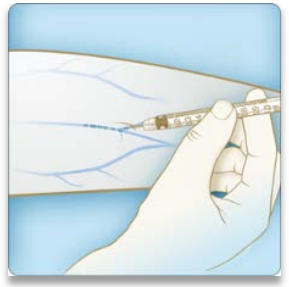
skin:
1.8 m^2
barrier **very thick**,
epidermis, dermis and subcutis

injection:
efficient distribution in the body (4 - 5l cardiac output per minute)



gastrointestinal tract:
surface: 2000m^2 incl Microvilli,
intestinal mucosa thick;
distance to blood vessels **big**

lung:
140 m^2
air / blood barrier
very thin < 2 µm

Primary route of exposure

Oberdorster et al., Nanotox, **2007.**

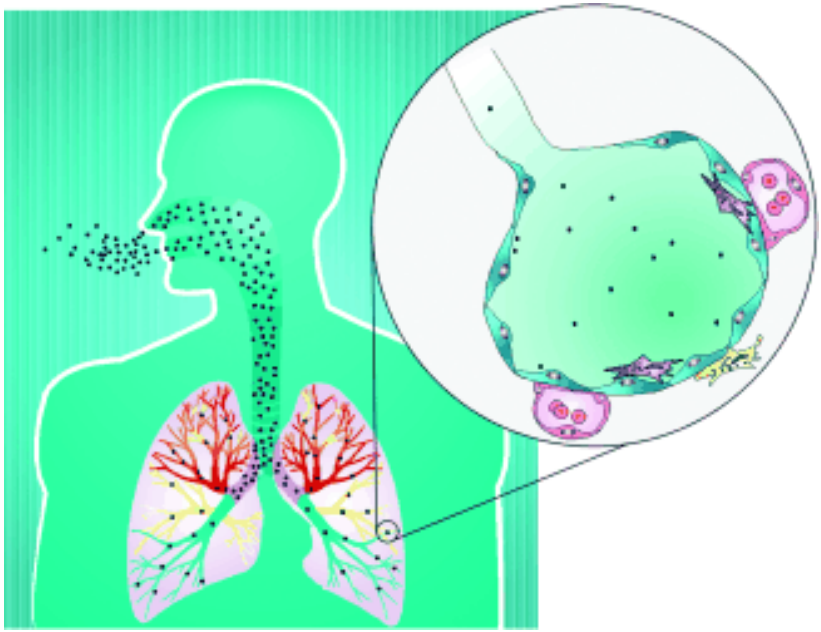
Images: www.cdc.gov

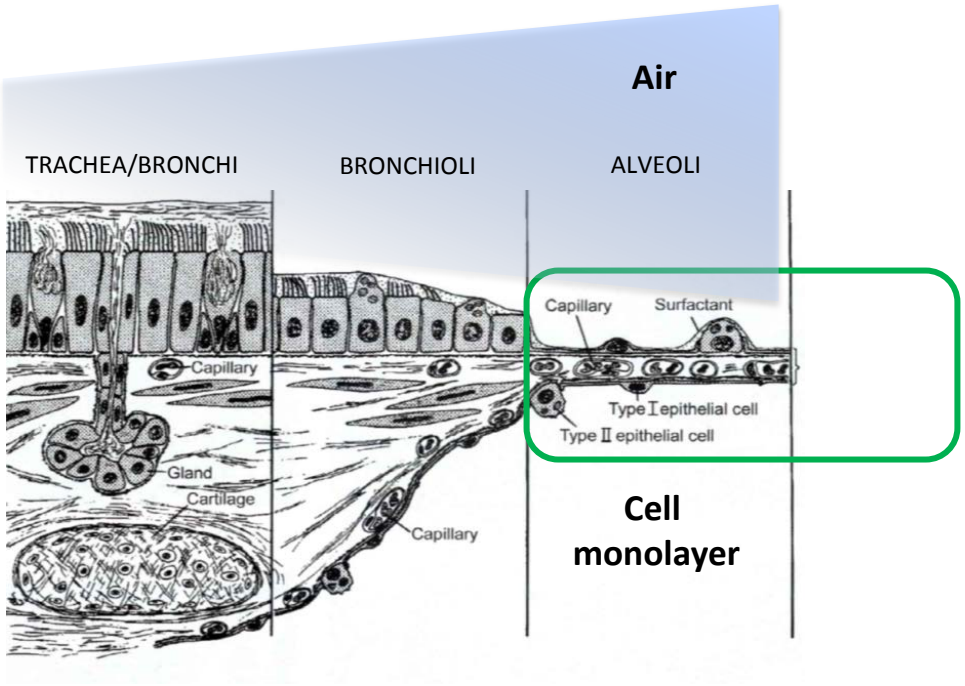
Human respiratory tract

Fast clearance

Slow clearance

Gas exchange



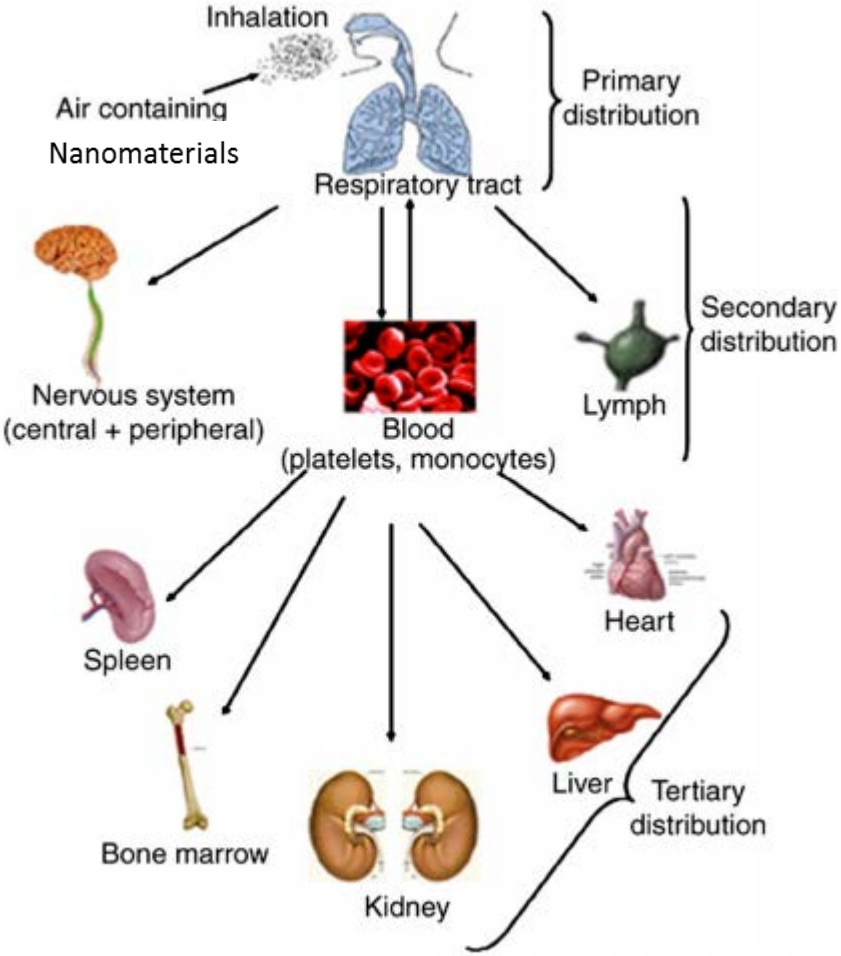


Presence of immune cells

Krug and Wick et al., Angewandte chemie, **2011**

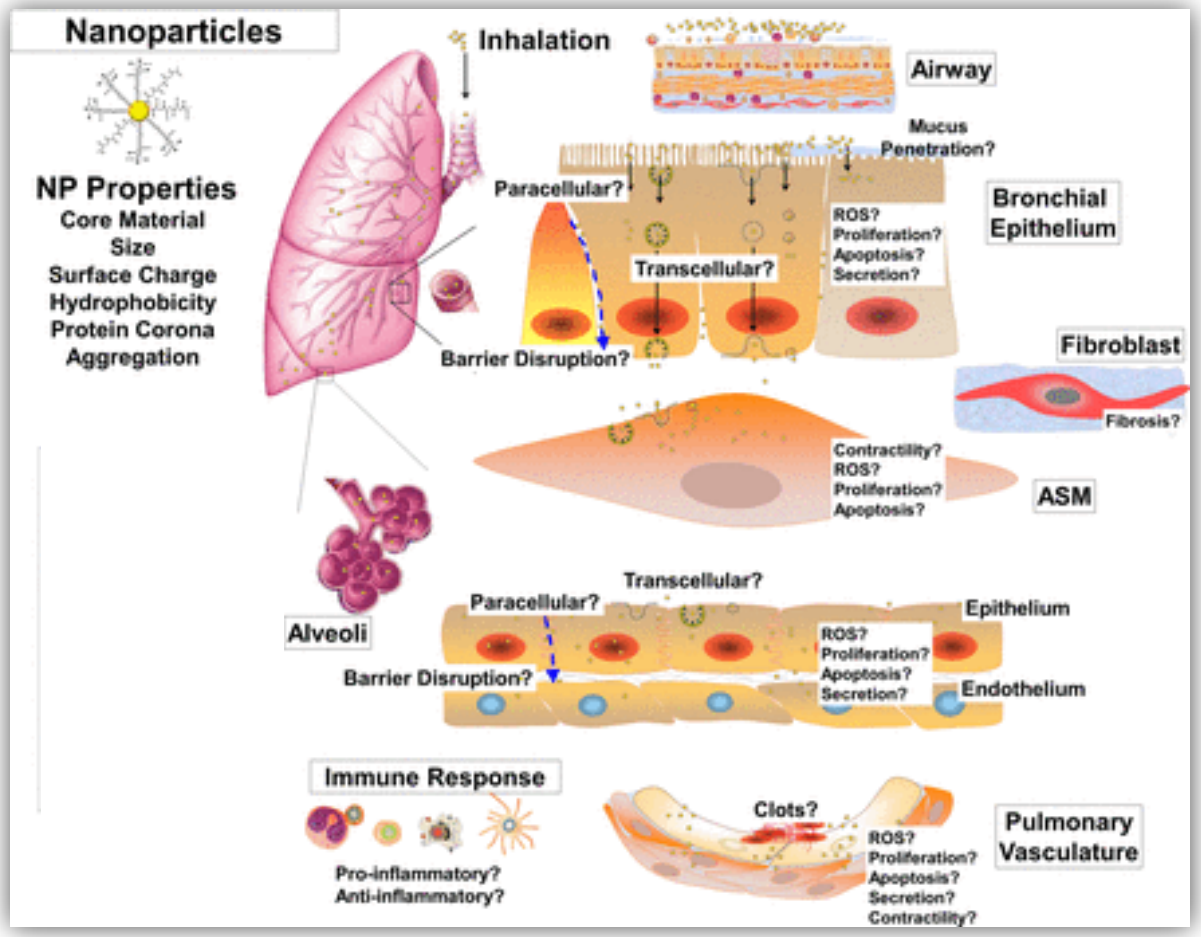
Ochs and Weibel. In: *Fishman´s Pulmonary Diseases and Disorders*, New York, **2008**

Bio-distribution of Nanomaterials



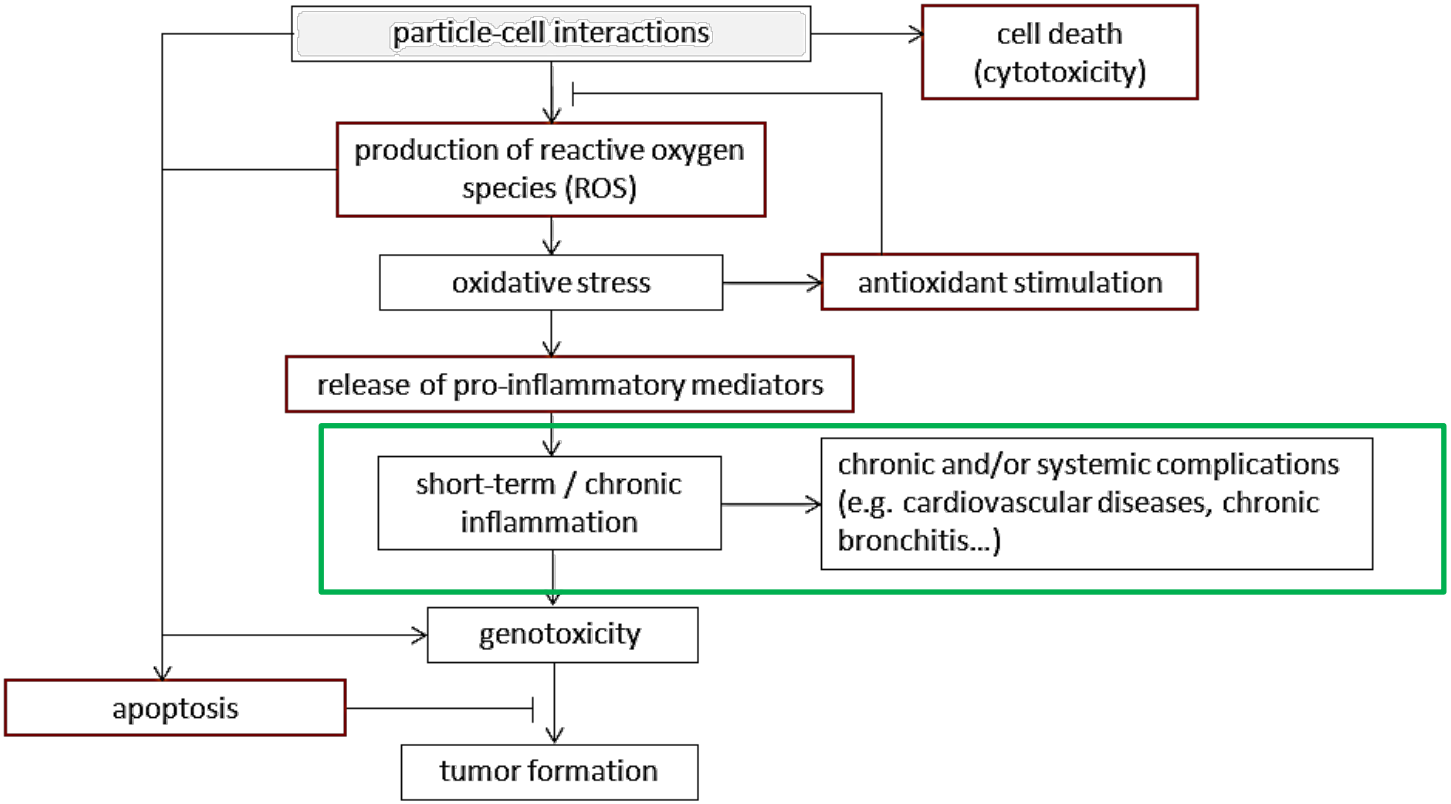
Modified from Gajewicz et al., Advanced Drug Delivery Reviews, **2012**

Nanoparticle potential effects



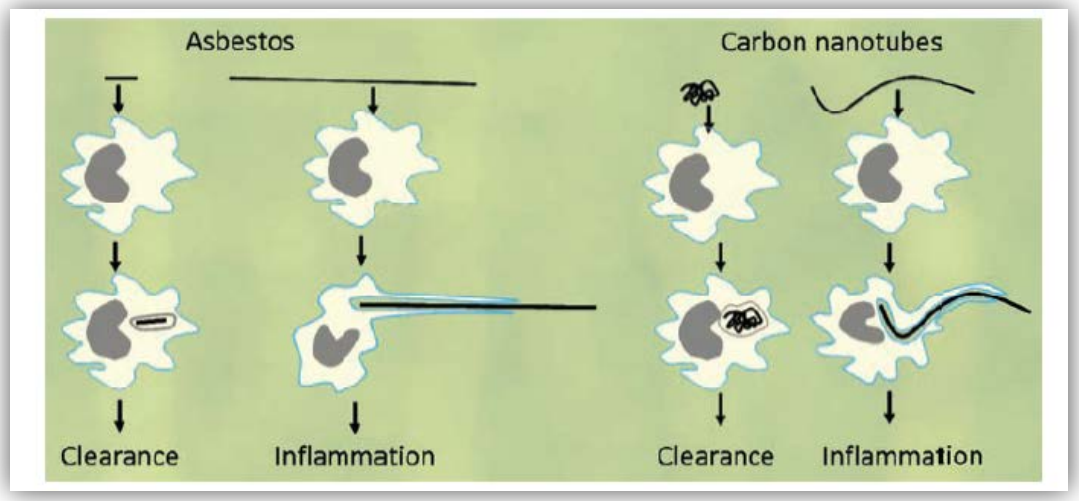
Prakash et al., American Journal of Physiology, 2014.

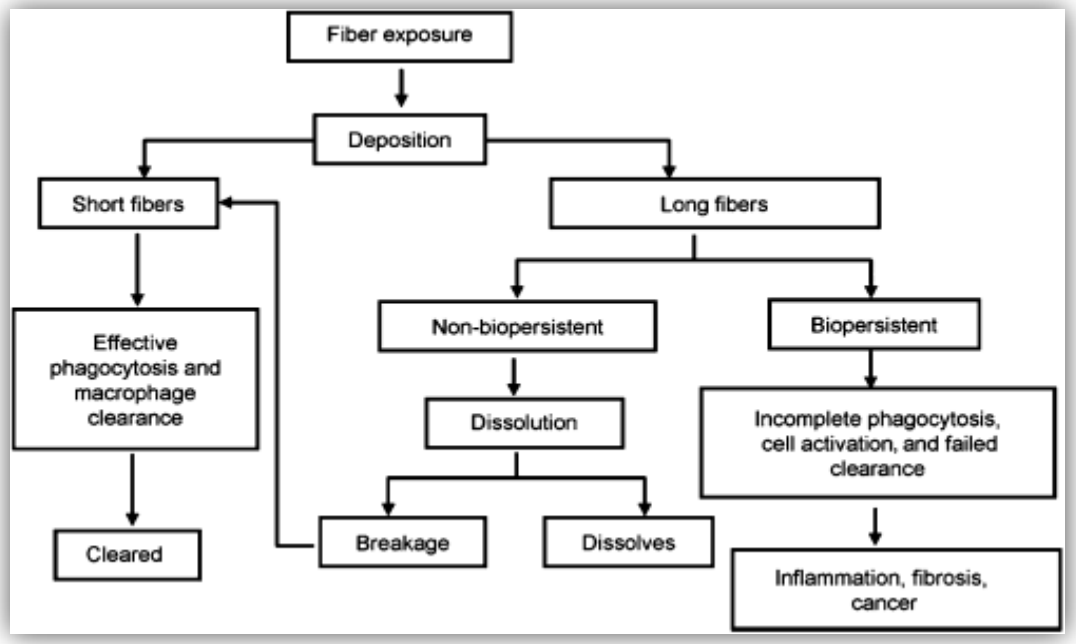
Nanosafety assessment- Oxidative stress paradigm



Scheme adapted from: Donaldson et al., Occup Environ Med **2004**; Schins, Knaapen,Inhal Toxicol, **2007**

Nanosafety assessment – fibre paradigm

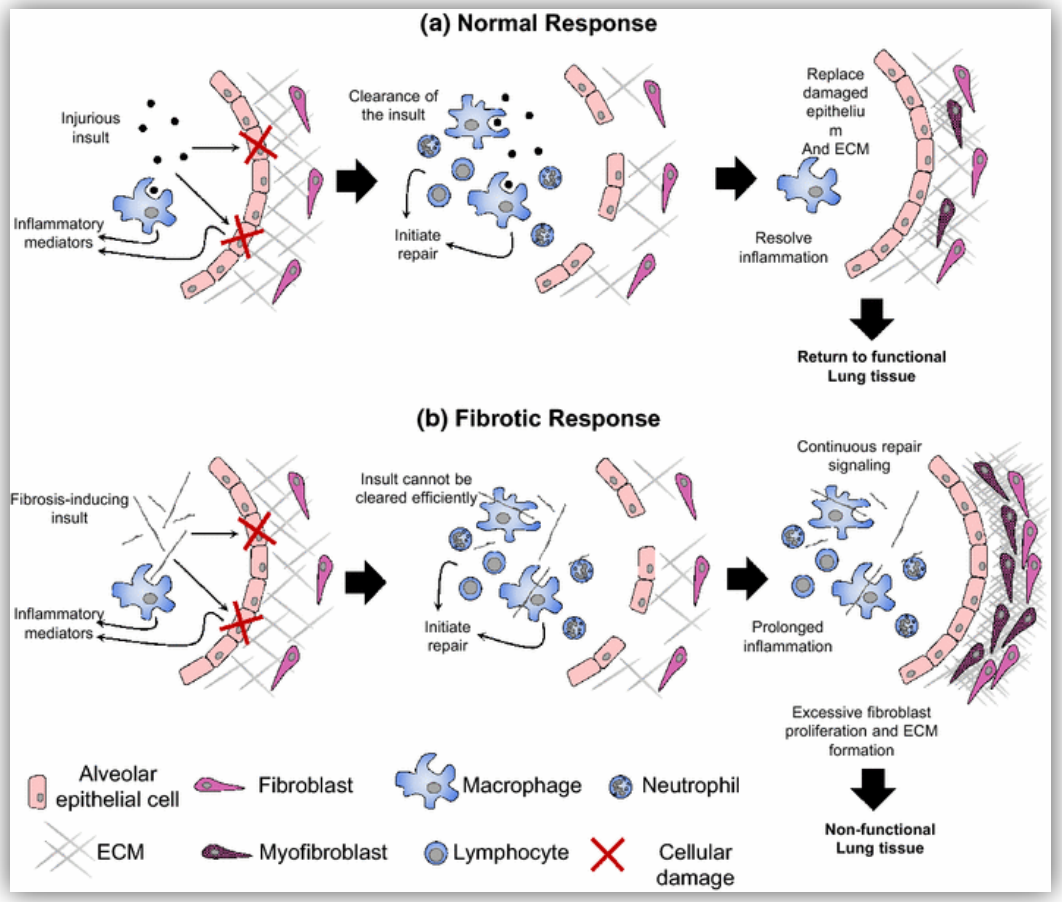




Donaldson et al., Toxic. Sci., **2006.**

Donaldson et al., PFT, **2010**.

Nanosafety assessment- Lung fibrotic effects



Sharma et al., Arch Toxic, **2016**.

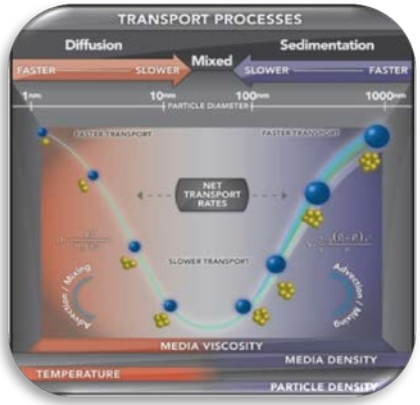
Challenges in nanomaterial safety assessment

Unrealistic exposure conditions

Suspension exposures

Air-Liquid Interface

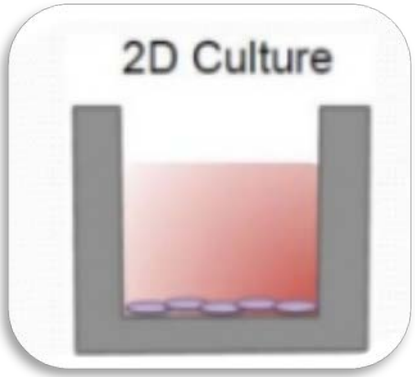
Doses



Hinderliter et al., PFT, **2010**

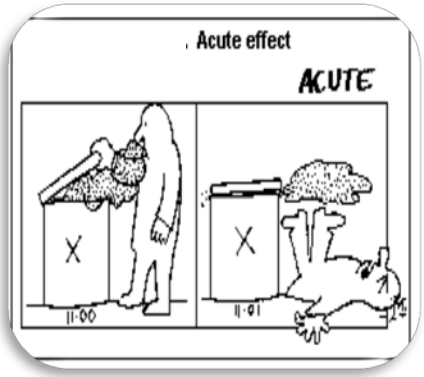
Realistic doses

2D cultures



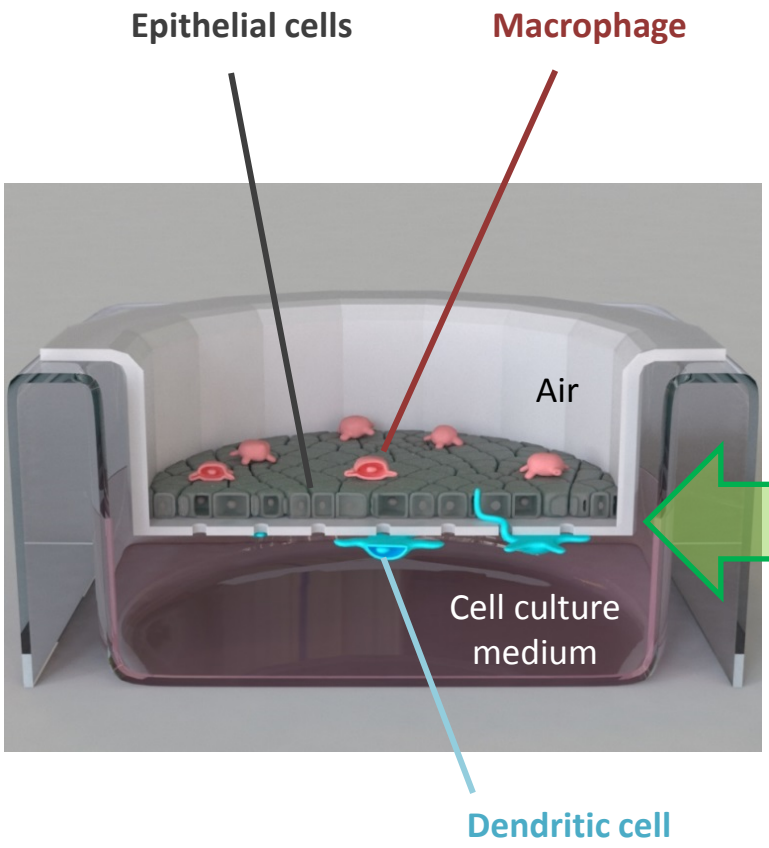
3D advanced cultures

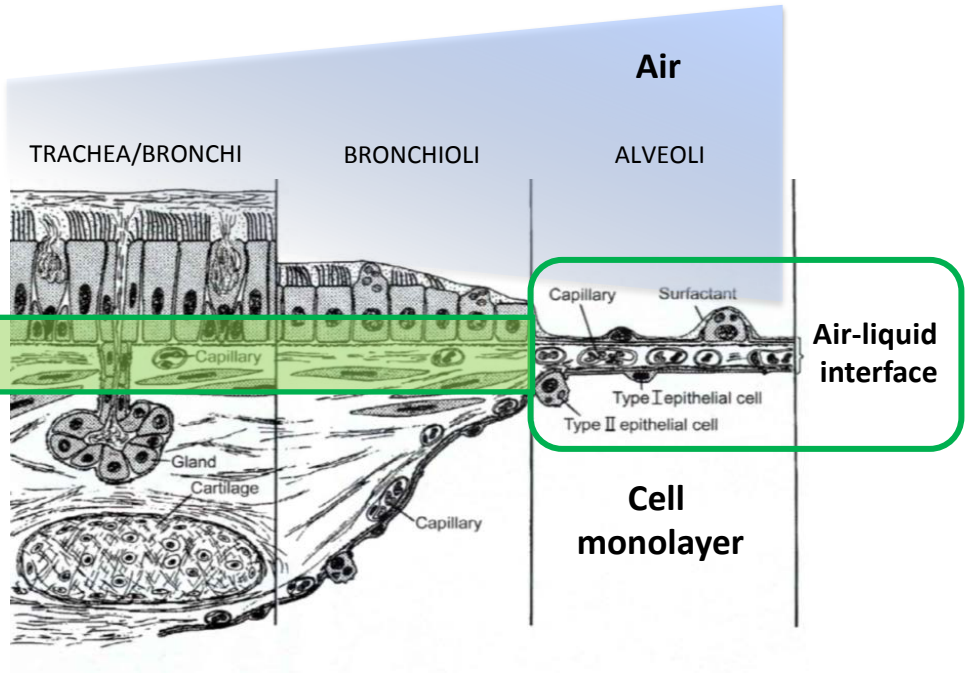
Acute exposures



Short-, long-term exposures

3D advanced human alveolar epithelium model





3D lung model: Rothen-Rutishauser et al. *Am J Respir Cell Mol Biol,* **2005**
Scheme: Fytianos and Drasler et al. Nanomedicine (Lond), **2016**,

Ochs and Weibel. In: *Fishman´s Pulmonary Diseases and Disorders*, New York, **2008**

Advanced 3D cell culture models

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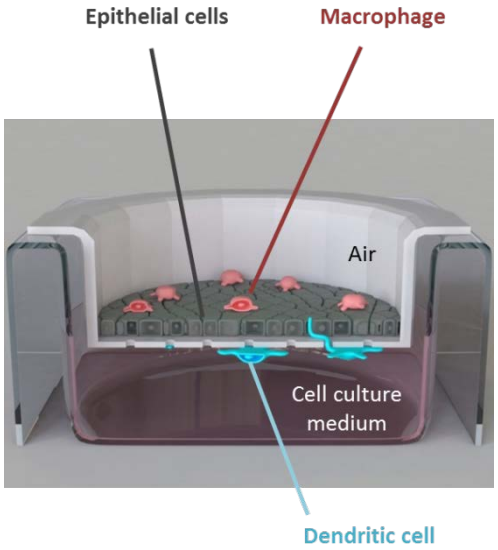
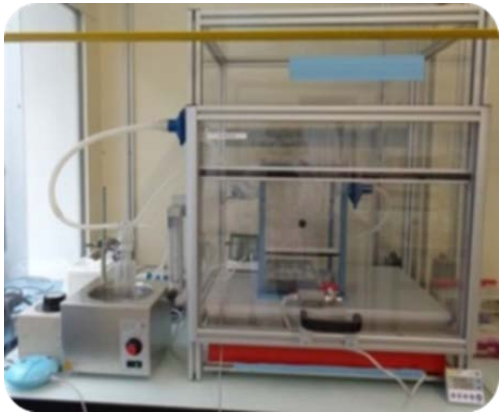
In vitro system								
Nanofibre	SWCNTs	MWCNTs	SWCNTs	MWCNTs	SWCNTs	MWCNTs	SWCNTs	MWCNTs
Cytotoxicity (LDH release)	-	-	-	-	-	-	-	-
TNF-α ELISA	++	++	++	++	N/A	N/A	++ (Upper+Lower)	++ (Upper+Lower)
IL-8 ELISA	N/A	N/A	N/A	N/A	++	++	++ (Upper) - (Lower)	++ (Upper) - (Lower)
GSH content	-	++	-	++	++	++	++ (Upper+Lower)	++ (Upper+Lower)

Clift et al., Tox Sci, **2013**.

Short-term *in vitro* MWCNT exposure

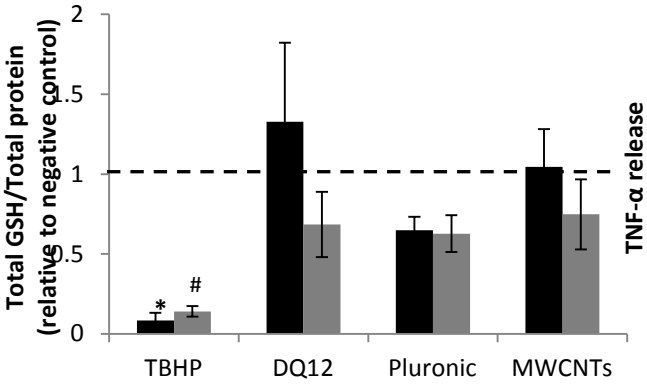


ALICE Exposure System

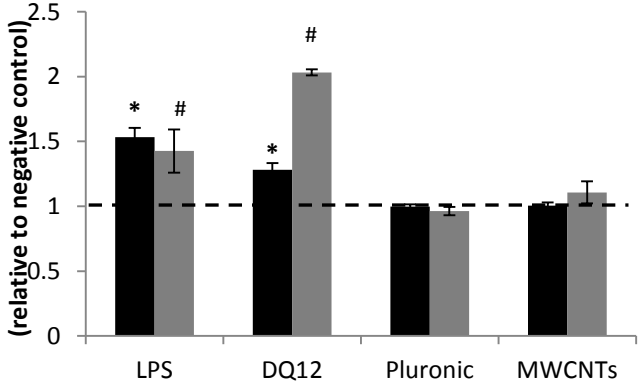


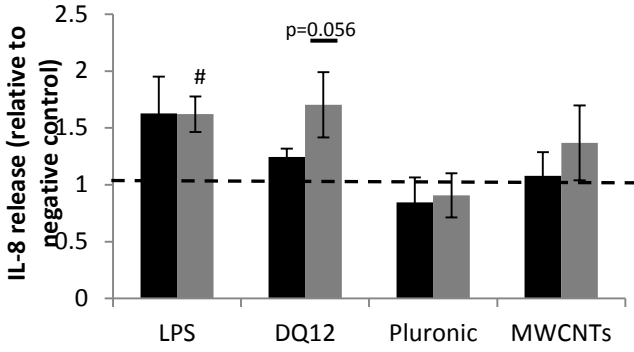
1 Day repeated exposure
3 Day repeated exposure

Oxidative Stress



(Pro-)inflammatory Response



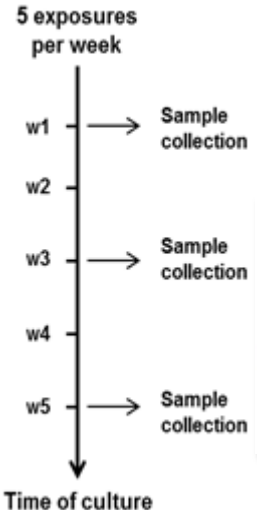


Repeated MWCNT exposures to lung cell cultures at the ALI elicit a **limited biological impact** over a three day period.

n=4, Error Bars: SEM
(– – .): Negative Control

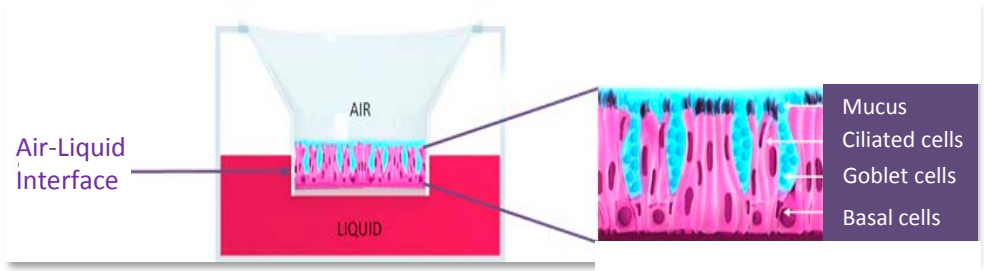
Chortarea et al., Nanotoxicology, **2015.**

Long-term effects of MWCNTs at occupationally relevant doses

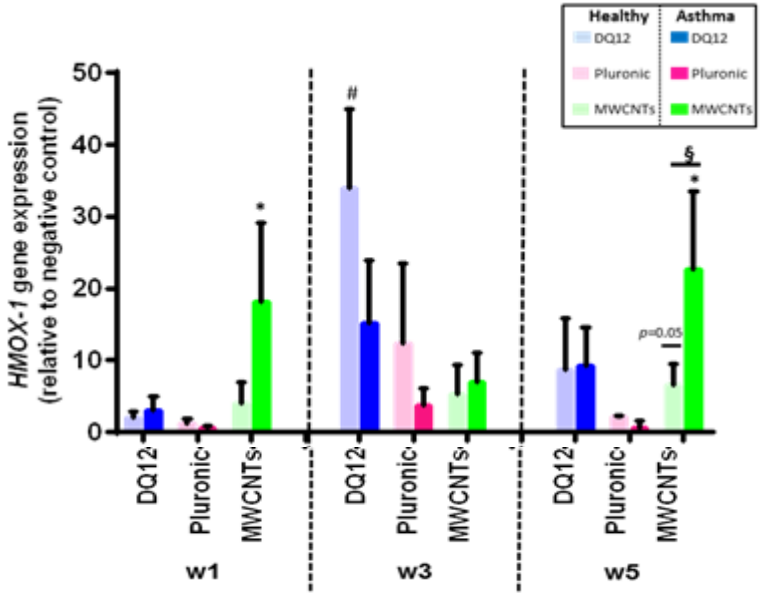


Primary bronchial epithelial cells

- Healthy donors
- Asthma donors



http://www.epithelix.com/products/mucilair



- **Alterations** in mucociliary clearance
- **Substantial** expression of oxidative stress and (pro-)inflammatory markers
- **Asthma cells** more **susceptible** than healthy cultures

Chortarea et al., ACS Nano, **2017**.

Challenges in nanomaterial safety assessment

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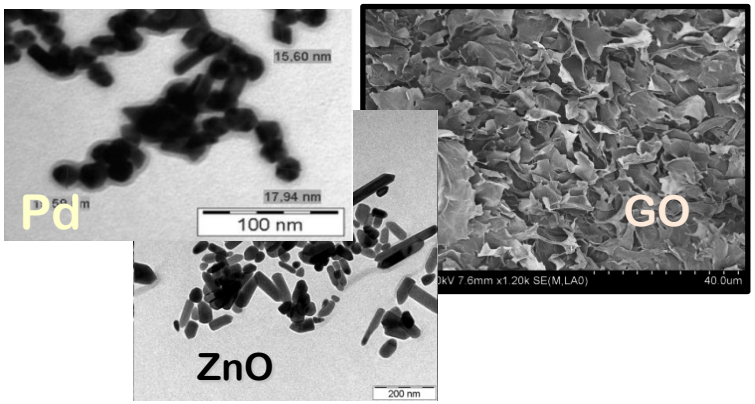
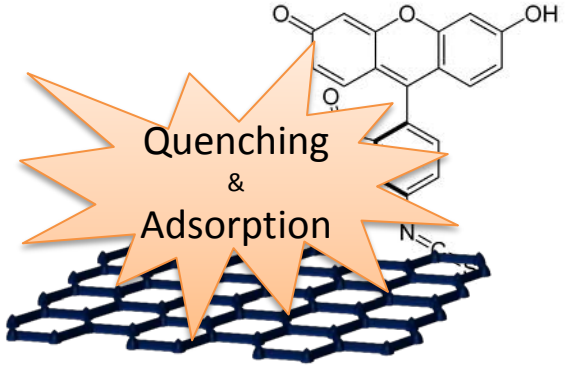
Insufficient material characterization



Interference with biological assays

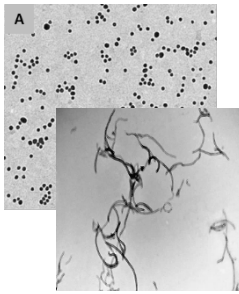


Lu et al. 2009 Angew Chem

Non-standarized protocols and no appropriate reference materials

Where to go from here…

- Obligatory and **sufficient material characterisation** (before and after exposure)

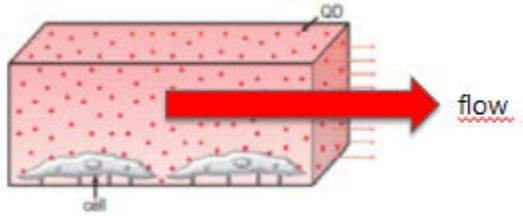


- **Standarization** of protocols based on SOPs
- Use of **reference materials** for comparability

- **Realistic** doses and conditions
- Appropriate choice of **biological models**

from phenomenological analysis towards mechanistically-based *in vitro* testing with reliable and relevant cell models

Hartung T, 2009 Nature



More **complex models** : breath pattern, blood flow

Acknowledgements

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adolphe merkle institute
excellence in pure and applied nanoscience

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AMI BioNanomaterials Group

Particles-Biology Interactions Group

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Thank you for your attention!

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