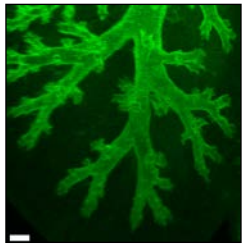

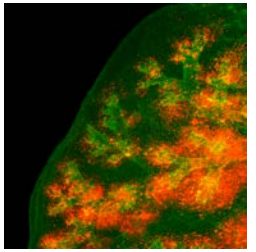

# The Role of Tissue-Delivered Dose and Dose Metric for Hazard Grouping and Risk Assessment of Nanomaterials (NMs)

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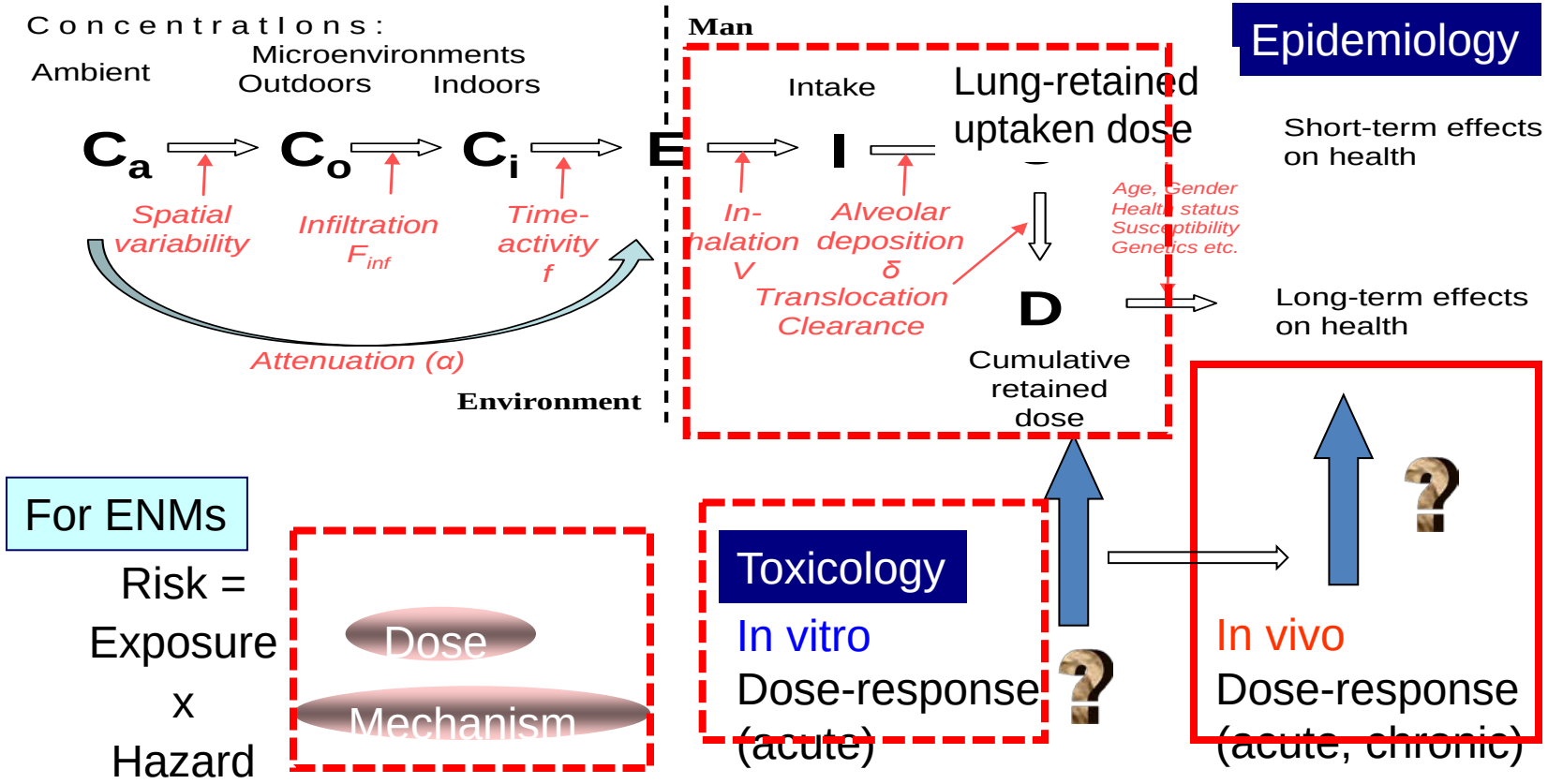
SmartNanoTox Projcet Online Conference, 24 June 2020

# From exposure to inhaled NMs to health effects



# Pivotal Role of Tissue Delivered Dose

Acceptable/desired health effect

*Schmid, Cassee, Part. Fibre Tox., 14(1):52, 2017*

Animal/cell models

Exposure level

Delivered concentration

**Animal ≠ Human exposure level**

**Change in aerosol size distribution & composition**
- Coagulation, condensation, evaporation,
- Photoprocessing, gas/medium-particle interaction
- Wall deposition losses, dilution, filtration,

**Relating concentration to tissue dose**
- Inhaled air/medium volume
- Tissue-deposited aerosol/particle fraction (~size, density, shape)
- Clearance from tissue (~size, shape)

Human

Exposure limit

Delivered concentration

**(Tissue-delivered) Dose - Response Relationship**

Lung/Tissue-delivered dose ⟷ Toxicological response

Acceptable/desired response or health effect

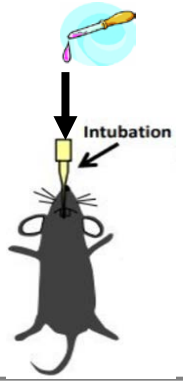
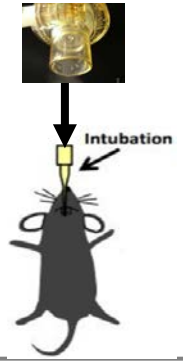
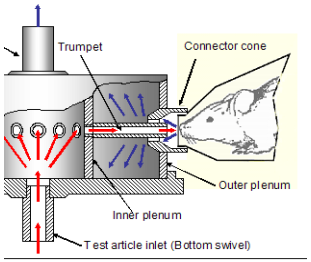
Maximum allowed tissue-specific dose in model organism **(NOAEL/LOAEL)**

**Translation from model to human**
- Model-human susceptibility factor
- Safety factor

**NOAEL/LOAEL** in humans

# Tissue-delivered dose

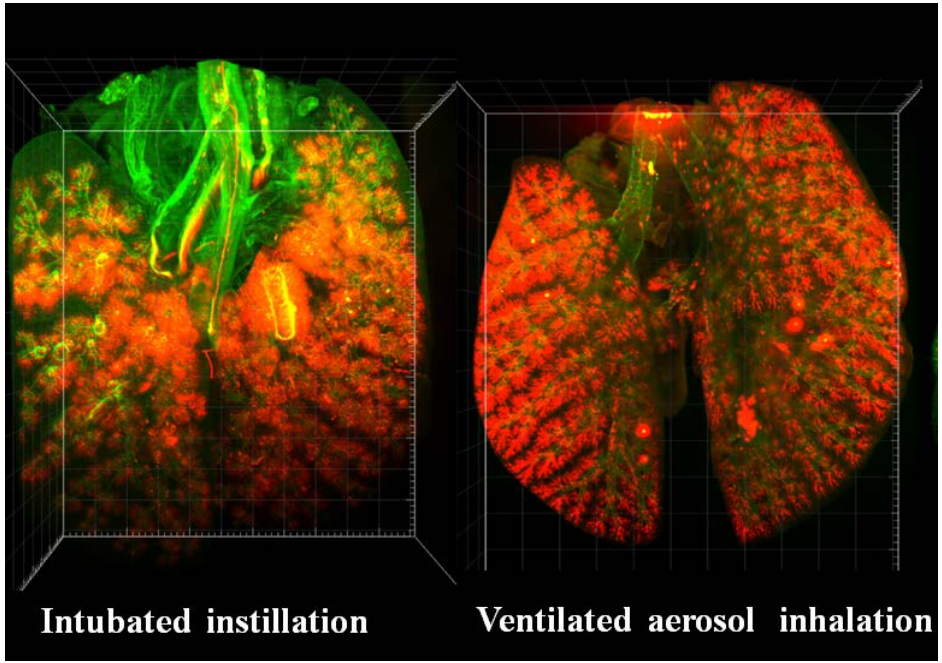
# *In vivo* – Pulmonary Exposure Technologies and Models

| | Mouse (C57BL/6)<br>Intratracheal<br>**Instillation**<br>Bulk liquid | Mouse (C57BL/6)<br>Ventilator-assisted<br>**Inhalation**<br>Liquid aerosol | Rat (Wistar)<br>Nose-only<br>**Inhalation**<br>Liquid/dry aerosol |
|---|---|---|---|
| |  |  |  |
| Dose rate/delivery period | High / ~ sec | **Medium / ~ min** | Low / ~ hours to months |
| Substance efficiency | High / ca. 80% | **Medium/ ca. 5%** | low / <0.1% |
| Degree of physol. Relevance | Low | **Medium** | High |
| Dosimetry method | 80% - applied dose | 5% - applied dose (account for retainment of NM in nebulizer) | - From inhaled conc. & lung deposition model<br>- ICP-MS, themogravimetry in lung homgenates |

# 3D Co-Mapping of Morphology and Aerosol Deposition in Murine Lung

## 3D Light sheet fluorescence microscopy after optical clearing of tissue

Mouse lung after application of fluorescent nanoparticles

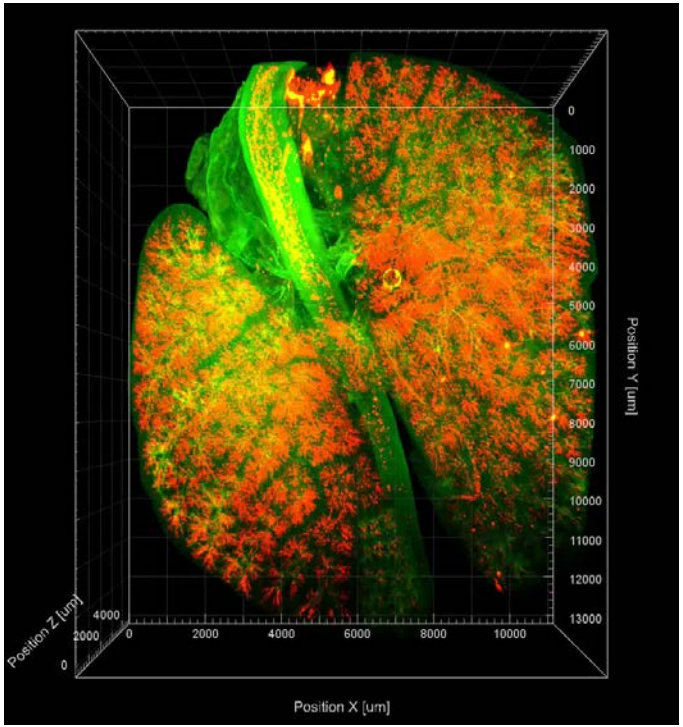



Green – autofluorecence of lung tissue

Red - Fluorescent melamine 600 nm particles

# 3D Co-Mapping of Morphology and Aerosol Deposition in Murine Lung

Ventilator-assisted aerosol inhalation

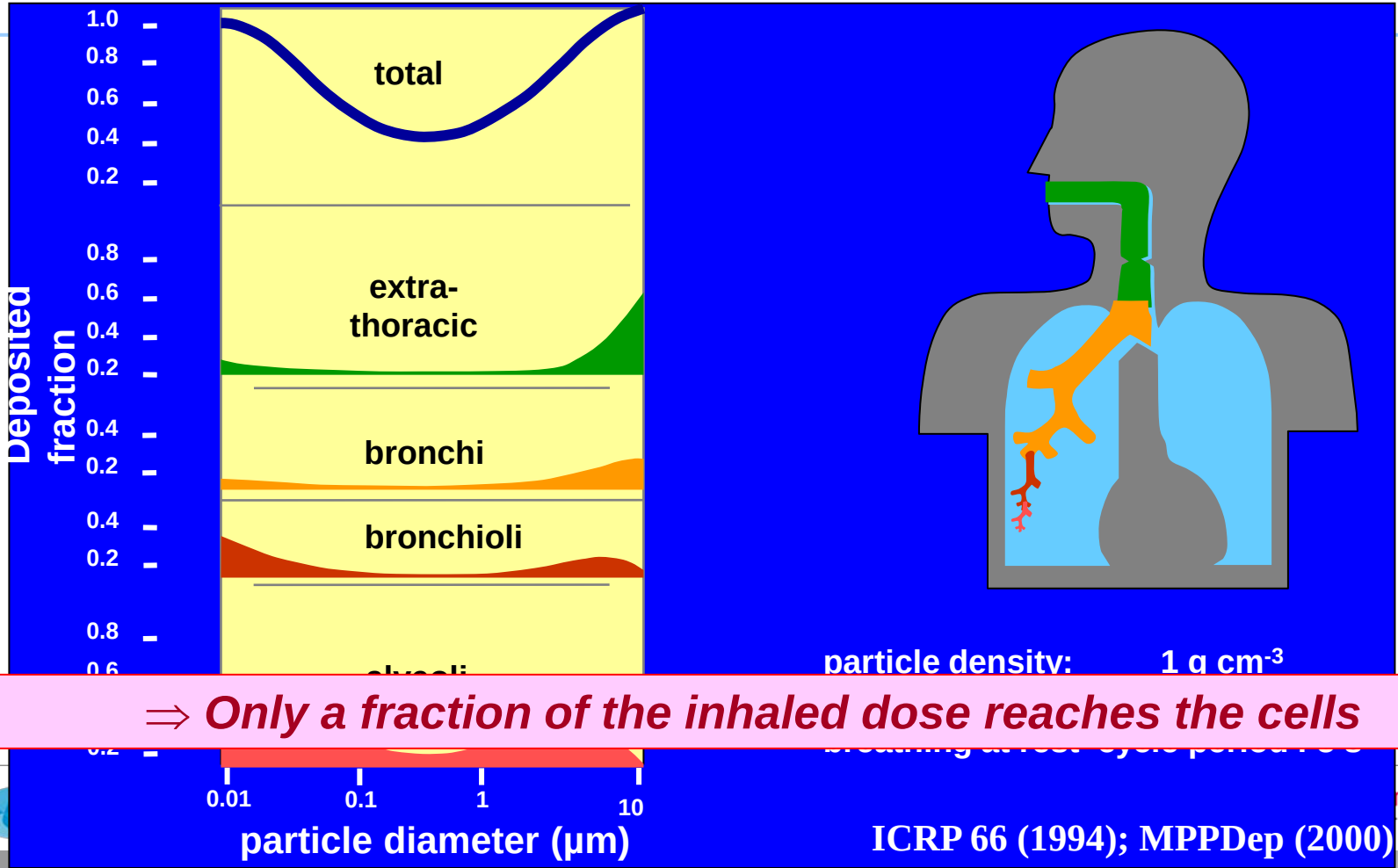



Green – autofluorecence of lung tissue

Red - Fluorescent melamine 600 nm particles
(delivered as suspension in 3 µm droplets)

Yang, …,Stoeger, Schmid, ACS Nano, 51, 4, 526-535, 2019

# Aerosol Deposition in Human Lung




⇒ *Only a fraction of the inhaled dose reaches the cells*

# *In vitro* models: Cells, exposure types, assays

**Pulmonary cell types/assays**

**Mouse:** LA4 - epithel cells
MHS - macrophages
co-culture: LA4 + MHS

**Rat:** N8383 - macrophages

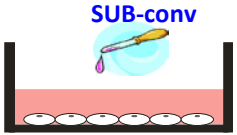
**Endpoints (24h)**

- Viability (WST-1)

**Cell free Oxidative Potency of NMs**

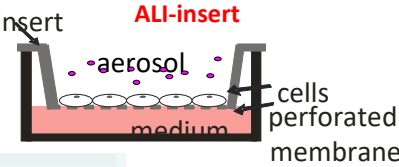
- DCFH assay: Reactive oxygen species (ROS)

**Submerged cell culture**




**Air-liquid interface (ALI)**

co-culture: LA4 + MHS
(with whole surfactant




**VitroCell® Cloud**

**Exact real-time dosimetry**
Quartz Crystal Microbalance (QCM)

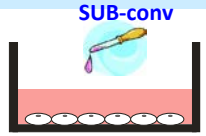
# *In vitro* particokinetics affects cellular dose

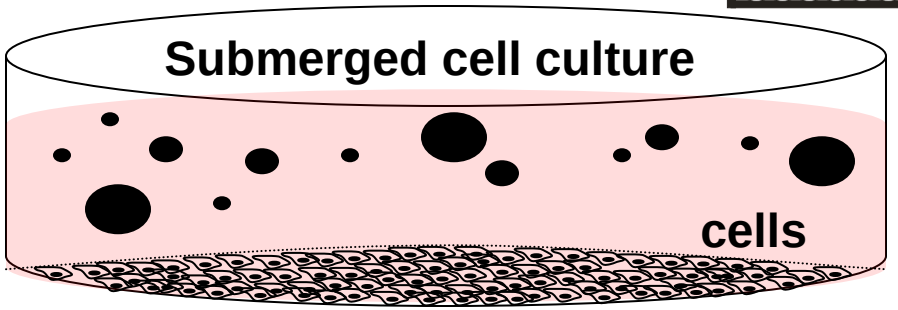
**Media "dose" is different than dose to the cell**

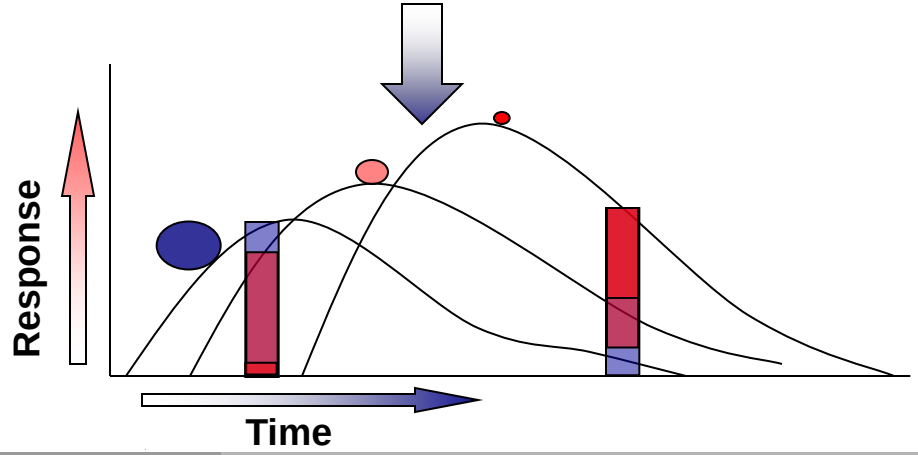
**Shape, size, and density affect**
- **delivered dose**
- **delivery rate**

**Delivery rate / dose impacts timing / magnitude of response**

Courtesy J. Teeguarden



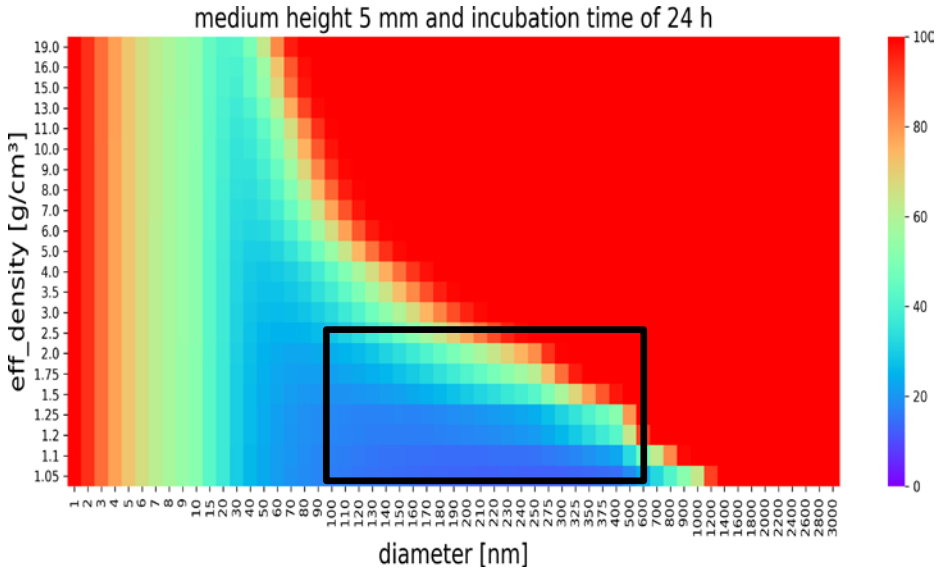



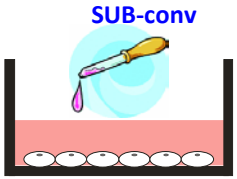



# Delivered dose fraction in submered cell cultures systems

## ISDD particokinetics model

(Hinderliter et al., Part Fibre Tox., 7:36, 2010)




Typical cell culture conditions:

- Medium height ~ 5 mm
- Incubation time 24h

Typical NM parameters:

DLS volume diameter: 100 – 600 nm

Agglomerate density: 1.05 – 2 g/cm$^3$

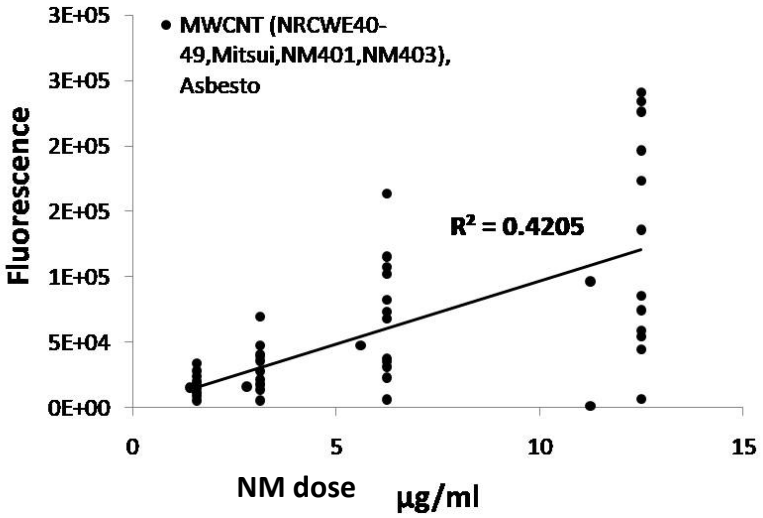
**Typical cell-delivered dose fraction: <5 – 100%**

***⇒ Only a fraction of the nominal dose reaches the cells***

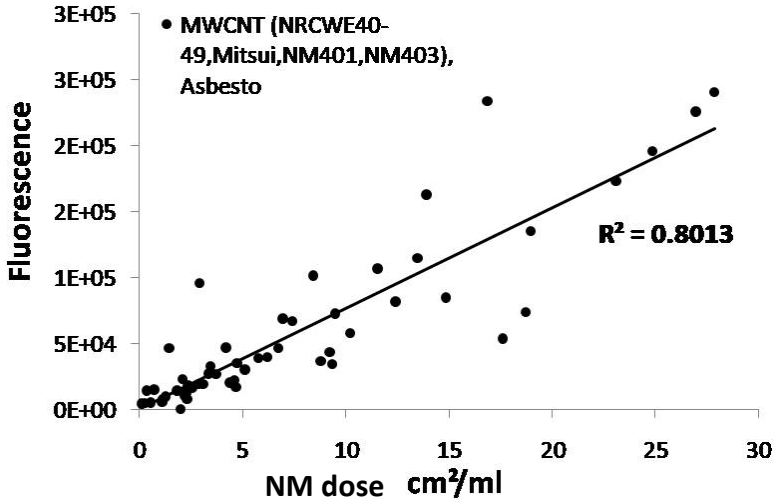
# Dose Metric

# Cell free ROS assay (DCFH) for 13 different fiber-like NMs (12 MWCNTs & asbestos)

## Mass



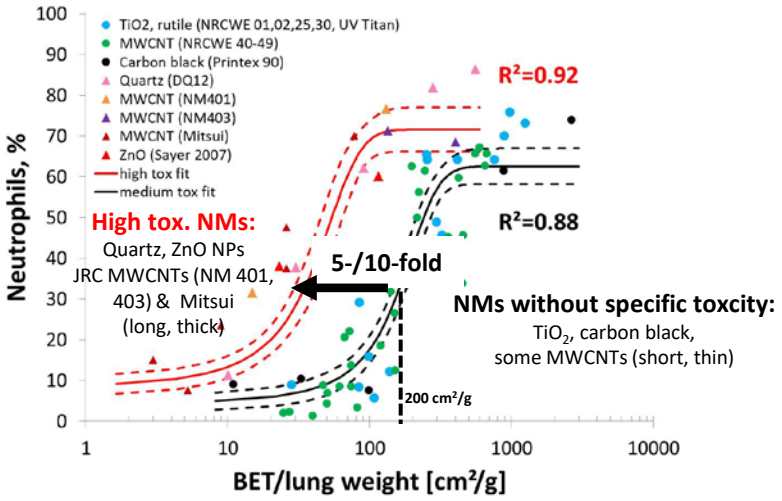

## Surface area

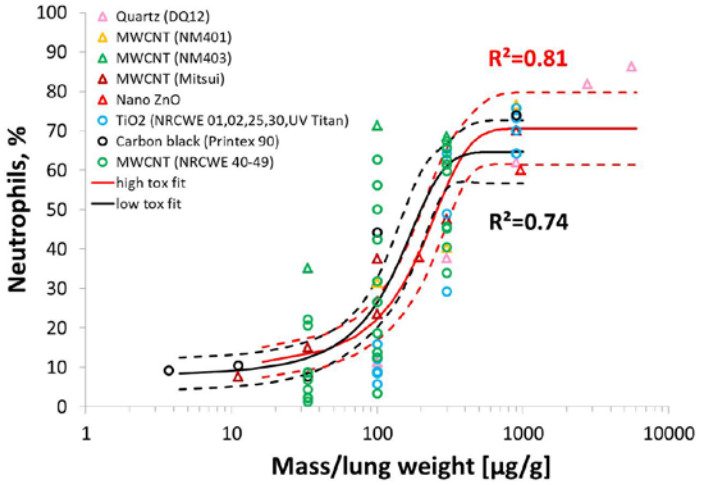


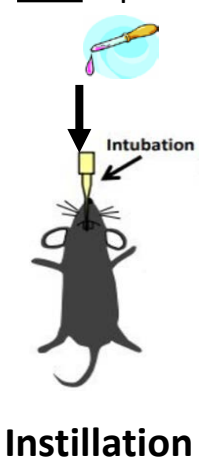

**⇒ *Surface area is more predictive of ROS than mass***

# Acute lung inflammation in mice – Instillation of NMs

Number of neutrophils/total cell count in Bronchoalveolar lavage (BAL) 1d after instillation



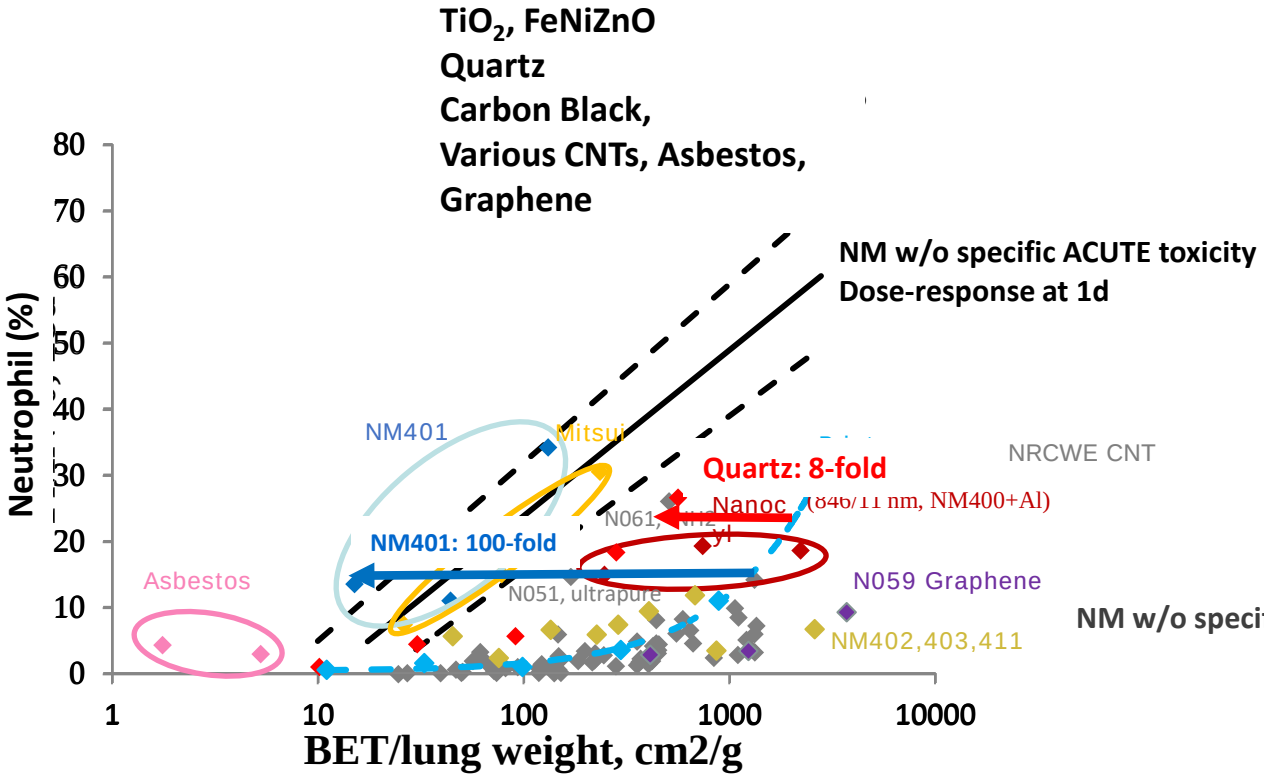




⇒ ***Surface area: Allows identification of Hazard Classes***

⇒ ***Hazard factors (relative to NMs w/o spec. tox): 5 – 10-fold***

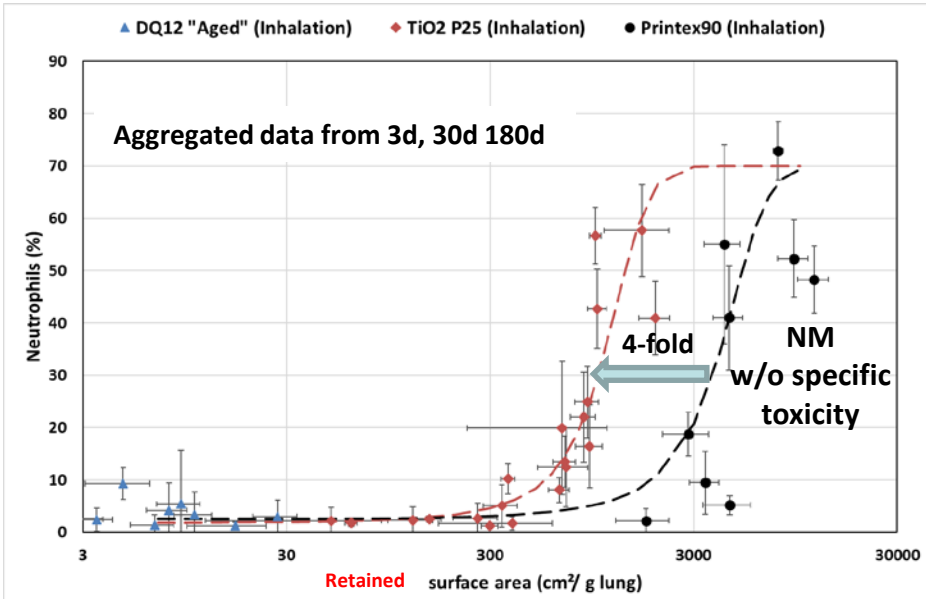
# Sustained inflammation in mice – 28d



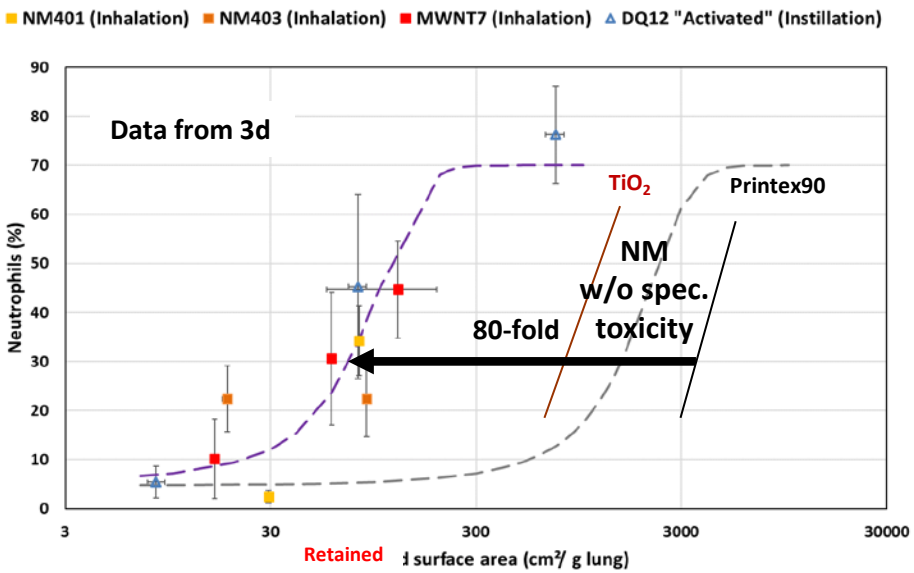

⇒ *Similar hazard classes as for acute inflammation (1d)*

# Inflammation after 28d nose-only inhalation (rat)

4 week inhalation (sub-chronic): 6h/d, 5d/w, **4w**




**Carbon Nanotubes** **and Quartz**



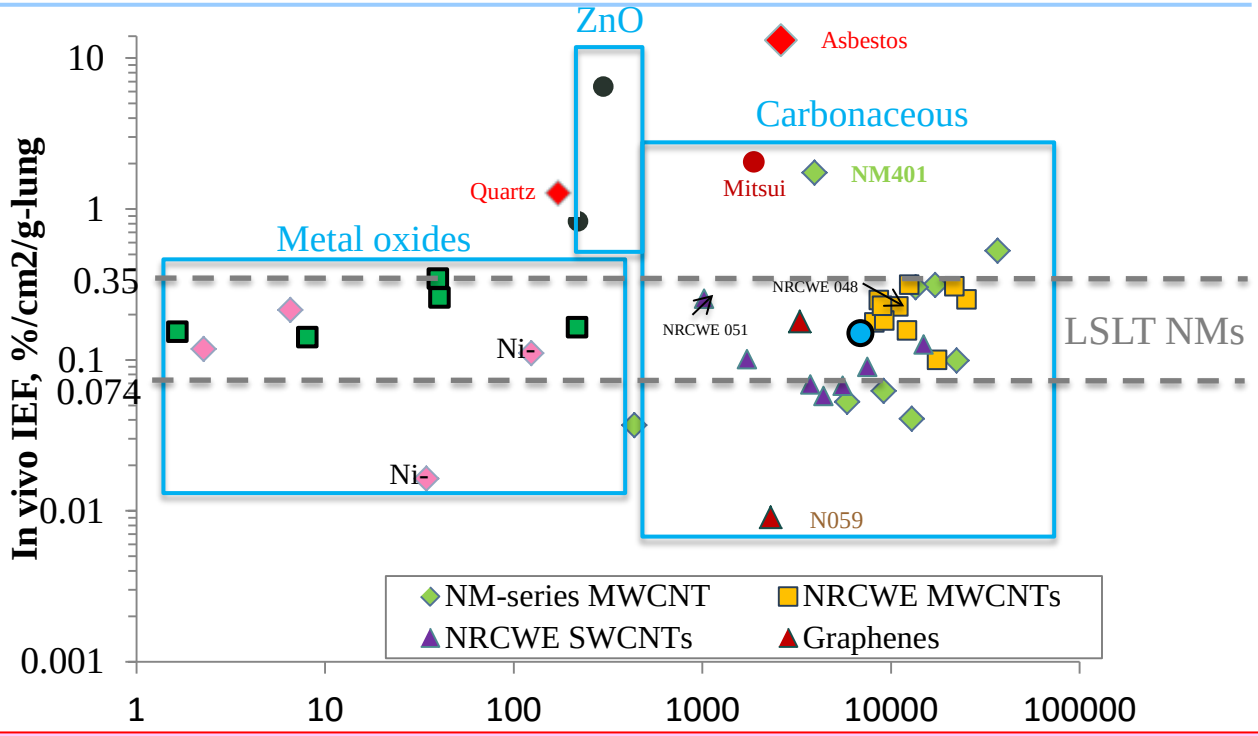

⇒ ***28d hazard factor after instillation is similar (MWCNT (NM401) or smaller (DQ12) than after nose-only inhalation***

# In vitro / in vivo comparison

# Acute Inflammatory Efficacy (in vivo) - Oxidative potency (in vitro)

In vivo: Mouse
NM instillation
Neutrophil influx (1d)
Slope of Dose-Response

In vitro:
Cell-free ROS (DCFH)
Slope of Dose-Response




⇒ ***Cell-free ROS is a good predictor for MetOx vs Carbonaceous NM induced inflammation***

⇒ ***Cell-free ROS is NOT a good predictor for acute in vivo inflammation***

## In vitro / in vivo comparison: $IC_{50}$ ($cm^2/cm^2$) for *in vitro* viability (WST1, 1d)) and *in* vivo inflammation (neutro.; 1d & 28d)

| Material | BET (m2/g) | Murine (in vitro; submerged) | | | Rat (in vitro, subm.) | Mouse (in vivo) Instillation (applied dose) | | Rat (in vivo) Inhalation |
|---|---|---|---|---|---|---|---|---|
| | | LA4 (epithel) | MHS (macs) | LA4+ MHS (ALI) | N8383 (mac) | 1d | 28d | 28d |
| Printex 90 | 272 | 25 | 54 | >20 | 400* | 0.067 | 1 | 1 |
| MWCNT Mitsui-7 | 26 | 0.84* | 0.38 | 0.3 | 63* | 0.005 | 0.05 | 0.02 |
| MWCNT NM401 | 18 | 2.4* | 0.25 | 0.12 | 44* | 0.006 | 0.03 | 0.02 |
| ZnO (NM110) | 12 | 0.24 | 0.18 | 0.3 | 0.6* | 0.008 | --- | --- |
| Quartz (DQ12) | 10 | 0.16* | 3 | >0.9 | 0.2* | 0.01 | 0.14 | 0.02 |

**Printex90: $f_{haz}$ = 1**

**Low Tox - $f_{haz}$ = 1 - 5**

**Med. Tox: $f_{haz}$ = 5 - 10**

**High Tox: $f_{haz}$ = 10 - 100**

* $IC_{50}$ ~ 4*$IC_{12.5}$)

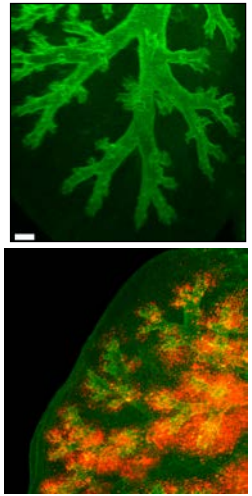
⇒ ***Mouse instill. (1d) is good qualitative predictor of rat inhalation (28d + 3d)***

⇒ ***Mouse instill. (28d) is good quantitative predictor of rat inhalation (28d + 3d) (3 out of 4)***

⇒ ***In vitro cell viability is a good predictor for 28d (+3d) inflammation (MHS; LA4+MHS: 4 out of 4/5)***

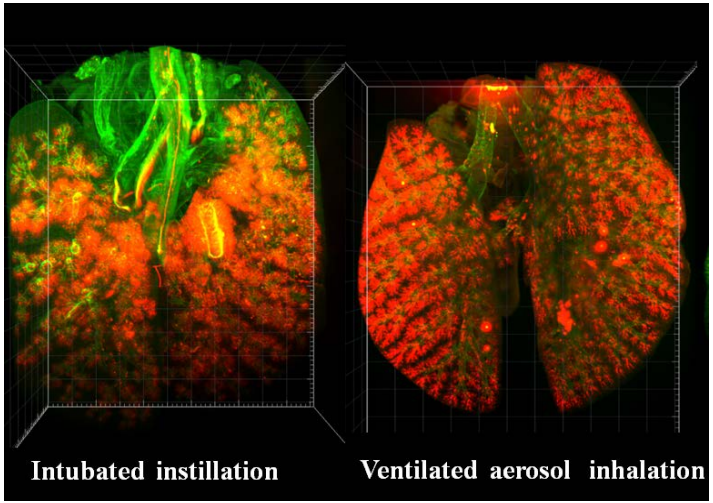
⇒ ***In vitro cell viability requires higher dose than in vivo inflam. (~ 100-fold (1d) ~ 10-fold (28d))***

# Summary/Conclusions



- Surface area is a powerful dose metric not only for spherical, but also for fiber-like NMs
  - Clear classification into NMs with and without specific toxicity
- Tissue-delivered surface area dose (not exposure concentration) facilitates
  - Experimental determination of hazard factors
  - *In vitro* / *in vivo* translation ---- prediction of *in vivo* onset dose from *in vitro* data
- Instillation (in mice) is a good predictor for inhalation
  - 1d instillation ~ 1d acute inhalation (ventilator-assisted; in mice)
  - 28d instillation ~ Subchronic inhalation in rats (28d + 3d)
- *In vitro* cell viability is powerful predictor of acute (1d) and subchronic *in vivo* inflammation (28d)
- *In vitro* cell viability requires about 100-fold higher dose than acute inflammation (instillation)
  - For simples cell culture models (viability assay) we have to accept „non-realistic“ high doses for hazard testing

# Questions/Comments?

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