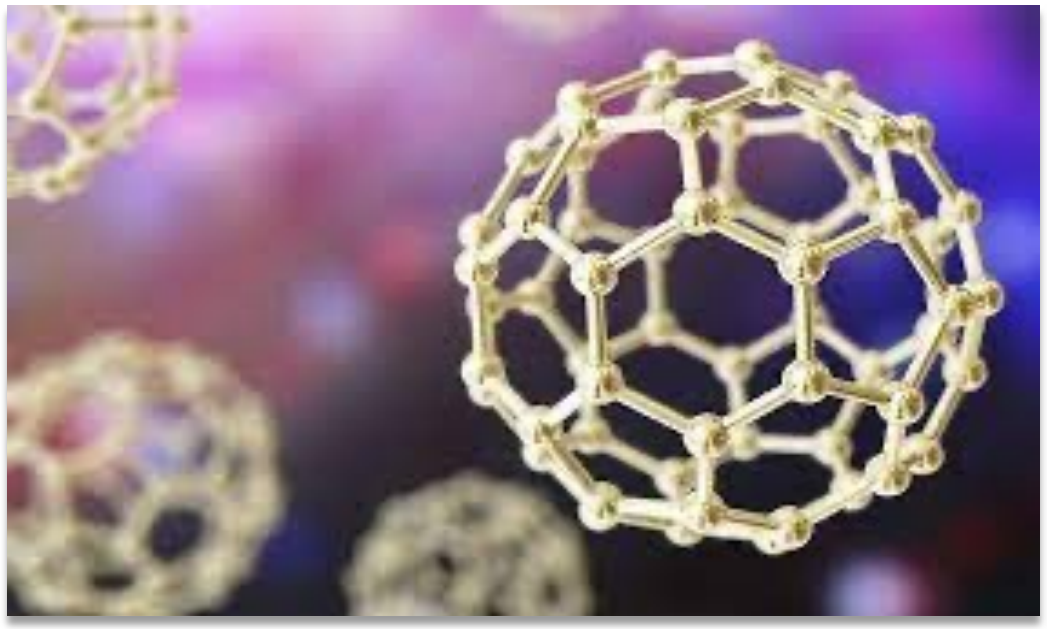


20th & 21st October 2021 - Pullman Hotel London, UK

Particles & Health 2021

An International conference addressing
issues in science and regulation

Dose- and Dosi-Metric Aspects and Biokinetics of Inhaled Poorly Soluble Low Toxicity (PSLT) Particles in Lung Overload

Günter Oberdörster

University of Rochester, School Of Medicine

Rochester, NY, USA

Some Background Notes:

LUNG PARTICLE OVERLOAD-INDUCED EFFECTS IN RATS

PSLT particles → if lung deposition rate > lung clearance rate → lung overload → impaired clearance → → lung tumors in rats, not in mice and hamsters
Extrapolatable to Humans?

Volumetric overloading of Alveolar Macrophage (AM) as cause for retarded particle clearance:
Morrow, 1988: If 60% of AM volume is occupied by phagocytized particles: Stasis of clearance

Retained particle surface area in AM as plausible alternative dose-metric in particle Overload

Whether volume or surface area, key is when extrapolating to humans: In addition to using the retained whole lung burden, consider the retained burden per AM as the appropriate dose-metric

Reason: Pulmonary retention kinetics of PSLT particles are different between rats and humans:
Primarily alveoli (AMs, rats) vs. interstitial sequestration (humans): Shift of inflammation from alveoli to interstitium?

PULMONARY EFFECTS IN DIFFERENT SPECIES RELATED TO HIGH PARTICLE LOAD

	Rat	Mouse	Hamster	Evidence in Coal Miners
Prolonged particle clearance	++	++	+	(yes)
Inflammation	++	+	(+)	yes
Cell proliferation	++	+	(+)	yes
Fibrosis	++	+/–	(+)	yes
Mutations	+	–	–	no
Tumors	++	–	–	no

Overall response to poorly soluble low toxicity particles:

rats > mice > hamsters

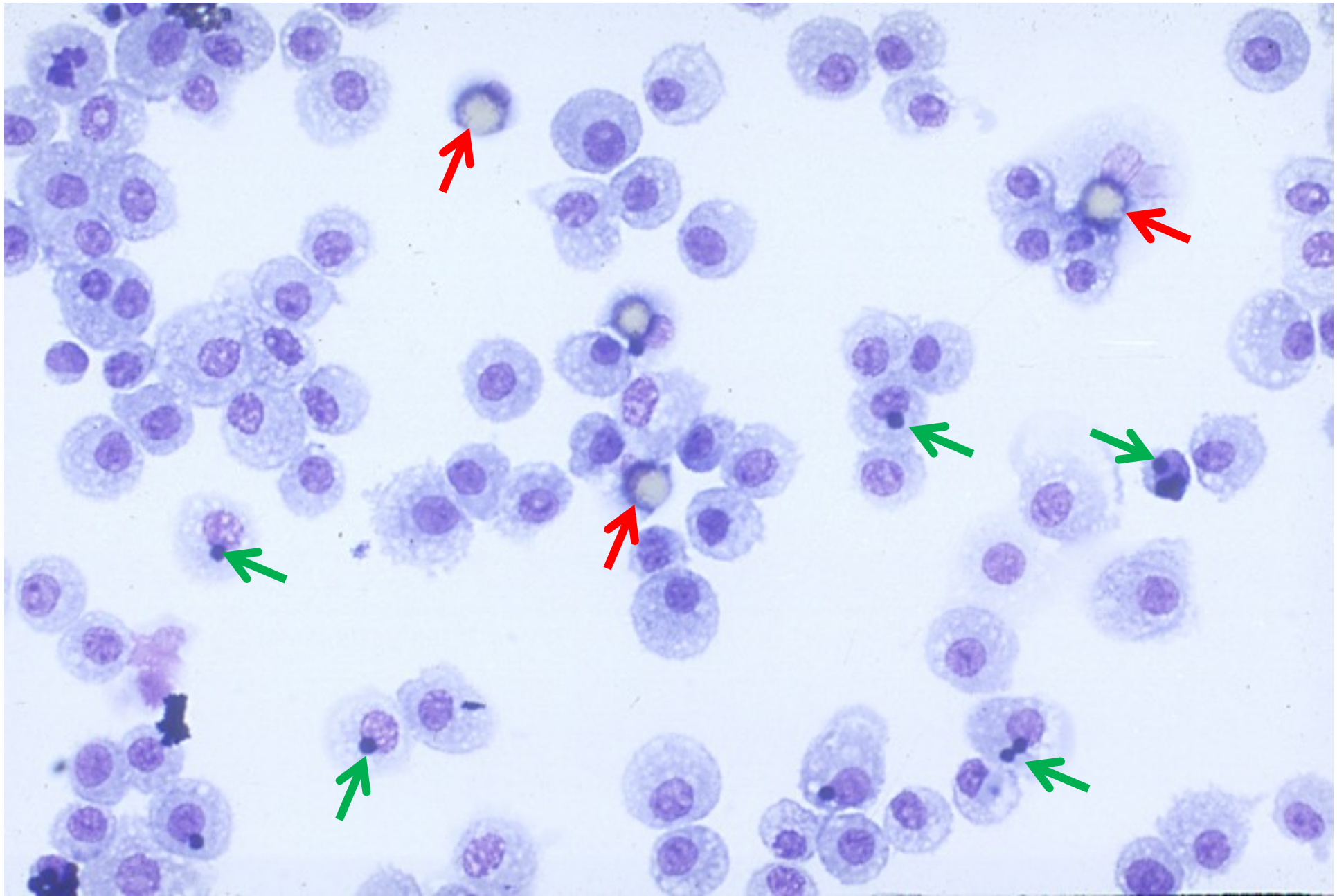
particle overload based secondary genotoxicity only in rats

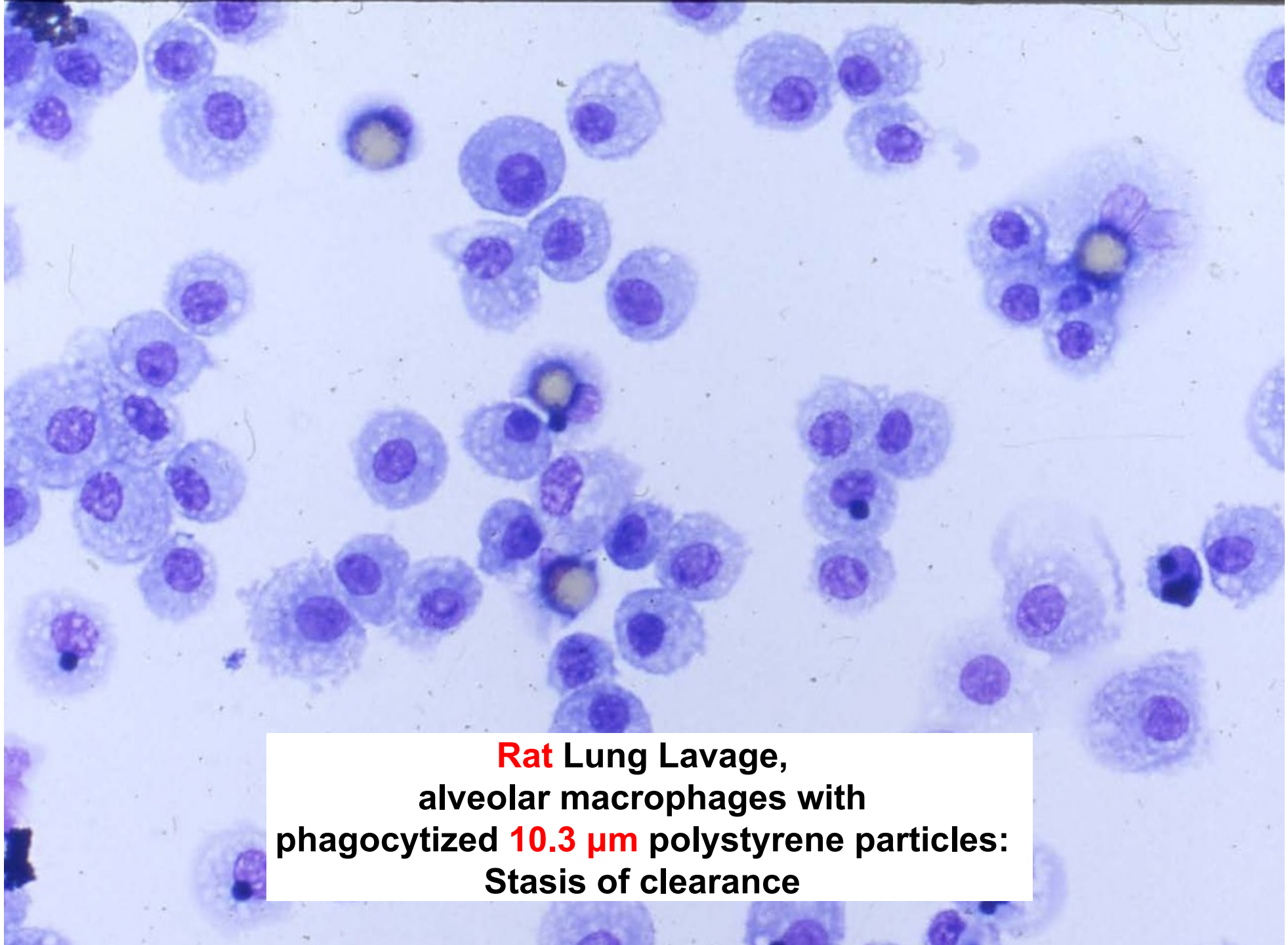
PULMONARY PARTICLE RETENTION HALFTIMES IN RATS AND MICE

(T1/2 in Days)

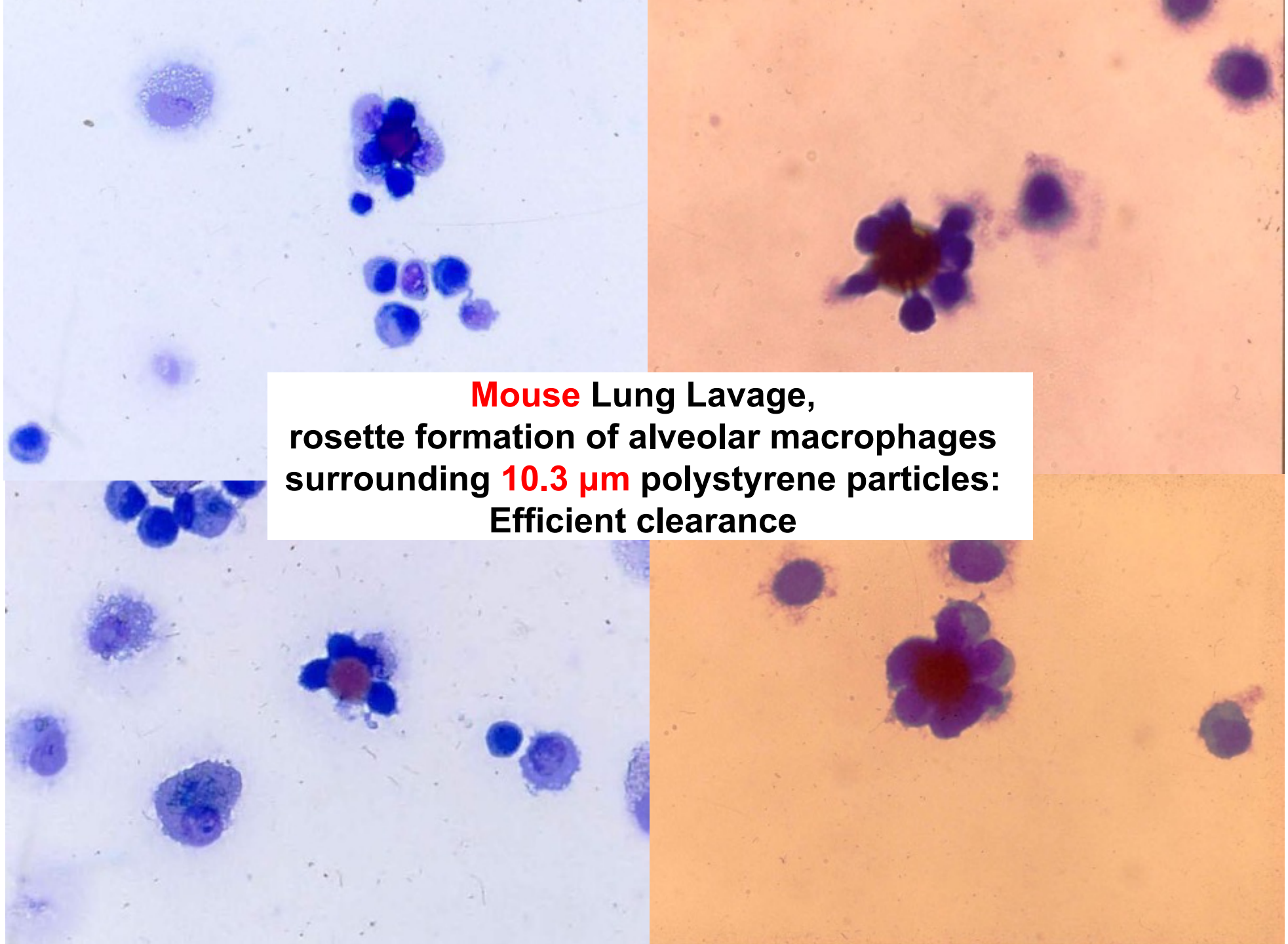
	Normal Undisturbed	Particle "Overload" Studies (TiO_2; Talc)	10.3 µm Polystyrene Particles
Rats:	**55 – 80**	**~120 – >1000**	**~1000**
Mice:	**30 – 40**	**~250 – 1000**	**108**

Rat AM's with phagocytized 10 µm and 3 µm particles





Rat Lung Lavage,
alveolar macrophages with
phagocytized 10.3 µm polystyrene particles:
Stasis of clearance



Mouse Lung Lavage,
rosette formation of alveolar macrophages surrounding 10.3 µm polystyrene particles:
Efficient clearance

Physico-Chemical and Functional Particle Properties of Relevance for InhalationToxicity

Size *(aerodynamic, hydrodynamic)*

Size distribution

Shape

Agglomeration/aggregation

Density *(material, bulk)*

Surface properties:

- area *(porosity)*
- charge
- chemistry *(coatings, contaminants)*
- defects

Crystallinity

Biol. contaminants (e.g. endotoxin)

Solubility/dissol-rate (*physiol. fluid,* ***in vivo***)

Surface reactivity *(ROS inducing capacity)*

Biotransformation *(intracellular breakdown)*

Properties can change

-with: method of production
preparation process
storage

-when introduced into
physiol. media, organism

Key parameter: Dose!

Physico-Chemical and Functional Particle Properties of Relevance for InhalationToxicity

Size *(aerodynamic, hydrodynamic)*

Size distribution

Shape

Agglomeration/aggregation

→ **Density** *(material, bulk)*

→ **Surface properties:**

- area *(porosity)*
- charge
- chemistry *(coatings, contaminants)*
- defects

Crystallinity

Biol. contaminants (e.g. endotoxin)

Solubility/dissol-rate (*physiol. fluid,* ***in vivo***)

→ **Surface reactivity** *(ROS inducing capacity)*

Biotransformation *(intracellular breakdown)*

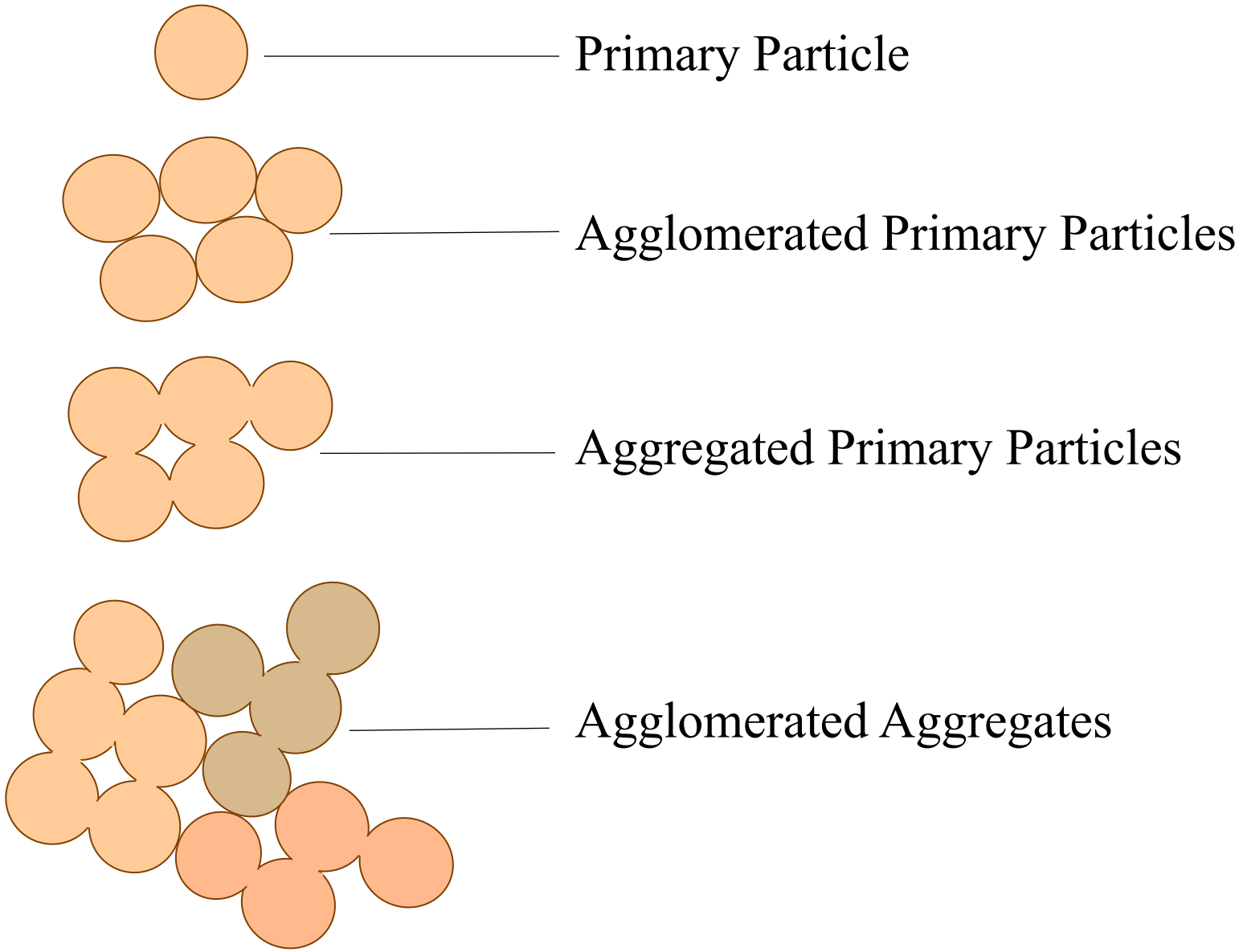
Properties can change

-with: method of production
preparation process
storage

-when introduced into
physiol. media, organism

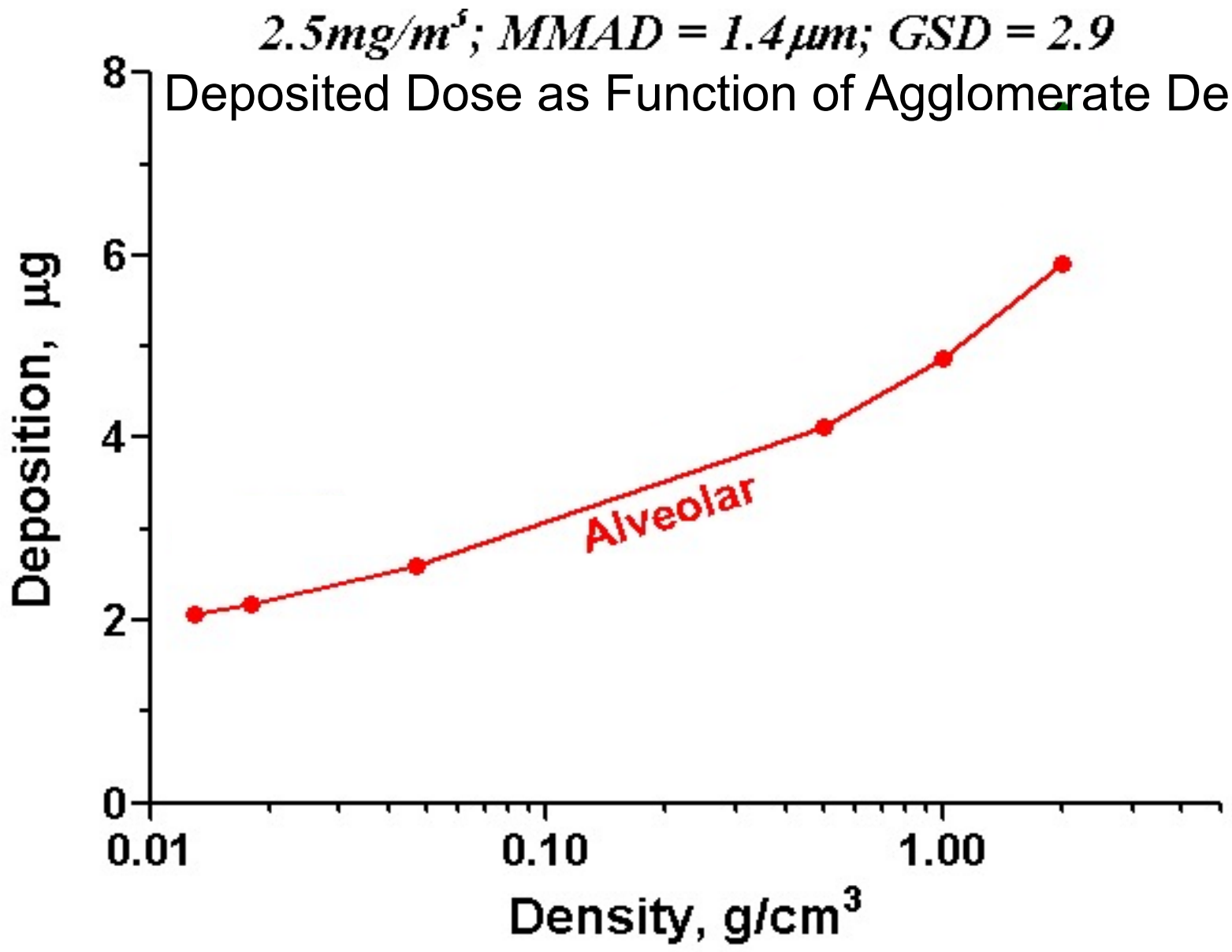
Key parameter: Dose!

Changing Density of Particle Clusters: Primary *vs.* Agglomerate *vs.* Aggregate Nanoparticles

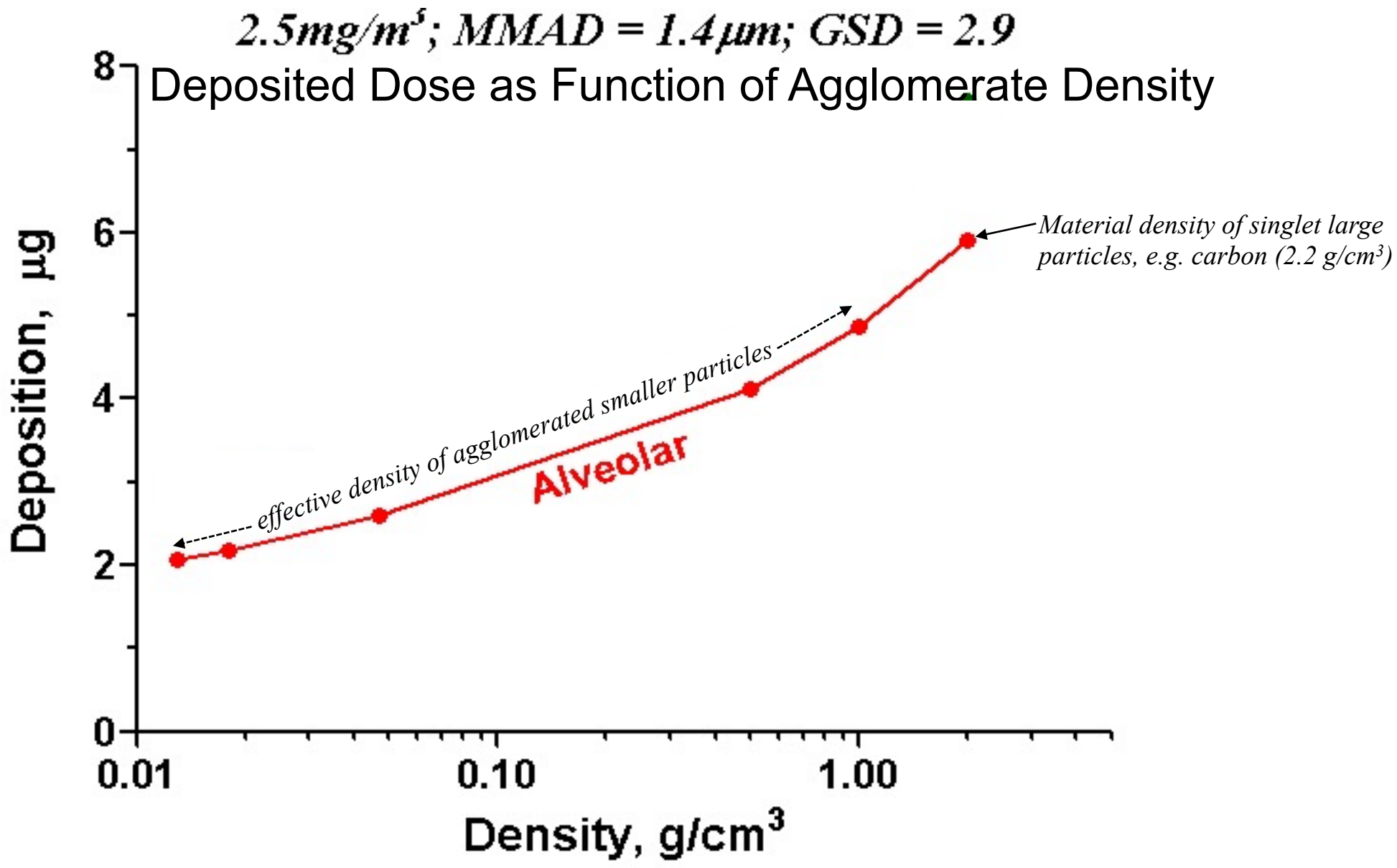


Impact of Aerosol Density on Lung Deposition of Inhaled Agglomerated Particles:

MPPD Prediction, Rat, 4 hour Inhalation



Impact of Aerosol Density on Lung Deposition of Inhaled Agglomerated Particles:
MPPD Prediction, Rat, 4 hour Inhalation



What is different about airborne nano-sized particles?

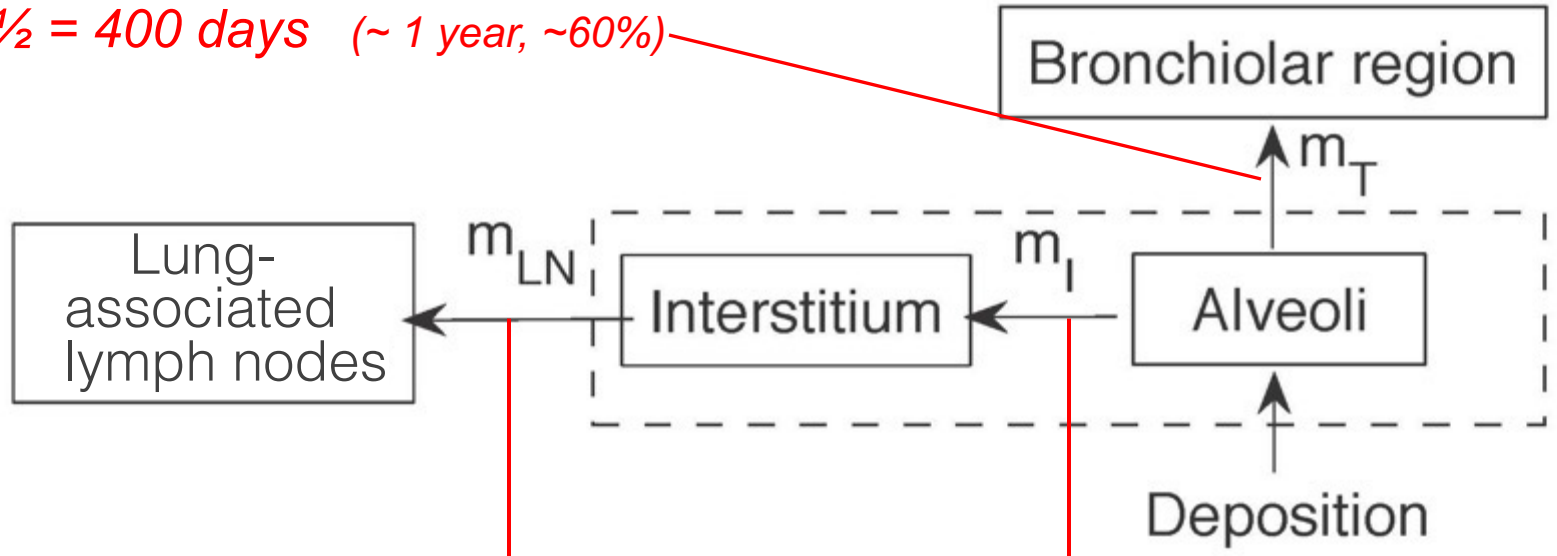
- **Large Number and Surface Area per Volume/Mass**
  – *potential for greater reactivity*
  (ROS inducing capacity; more surface atoms or molecules per mass)
- **Deposition in Respiratory Tract**
  – *by diffusion*
  – *all regions of the respiratory tract are targeted*
- **Disposition/Biokinetics**
  – *translocation: across cell barriers into cells (subcell. structures)*
  along axons/dendrites

Gregoratto et al (2010) particle clearance model for the gas exchange region of the human respiratory tract

(based on Kuempel et al, 2001, model)

Clear. Rate = 0.0017/day

T½ = 400 days (~ 1 year, ~60%)



Clear. Rate = 0.00003/day

T½ = 23,000 days *(~ 63 years)*

Clear. Rate = 0.001/day

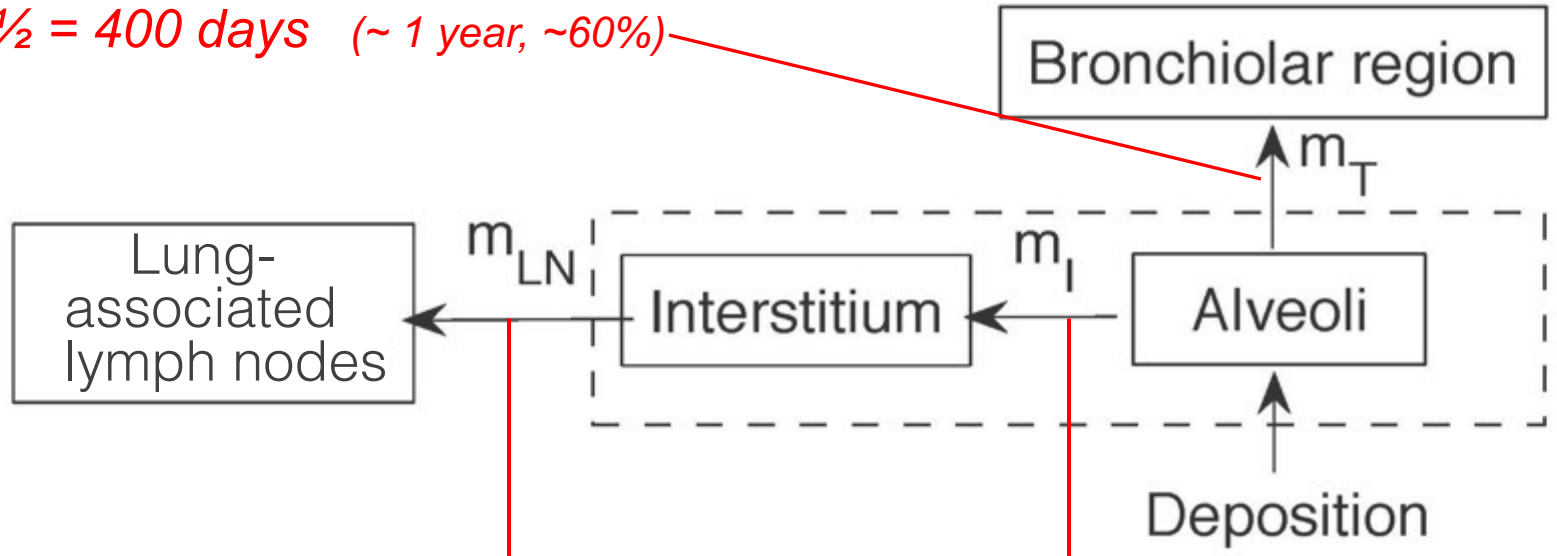
T½ = 700 days (~ 2 years, ~40%)

Gregoratto et al (2010) particle clearance model for the gas exchange region of the human respiratory tract

(based on Kuempel et al, 2001, model)

Clear. Rate = 0.0017/day

T½ = 400 days (~ 1 year, ~60%)



Clear. Rate = 0.00003/day

T½ = 23,000 days *(~ 63 years)*

Clear. Rate = 0.001/day

T½ = 700 days (~ 2 years, ~40%)

Combined alveolar clearance: rate = 0.0027/day

T½ = 250 days (~ 0.7 years, 100%)

Which Dose-Metric related to retained lung burden is appropriate for defining Lung Overload by PSLT Particles in a rodent inhalation study?

mg/g dry lung weight

mg/g wet lung weight

mg/g control lung

mg per whole lung

μm^3 per whole lung *(retained particle volume)*

μm^2 per whole lung *(retained particle surface)*

μm^3 per 10^6 alveolar macrophages *(volume)*

μm^2 per10^6 alveolar macrophages *(surface)*

Which Dose-Metric related to retained lung burden is appropriate for defining Lung Overload by PSLT Particles in a rodent inhalation study?

mg/g dry lung weight

mg/g wet lung weight

mg/g control lung

mg per whole lung

µm^3 per whole lung *(retained particle volume)*

µm^2 per whole lung *(retained particle surface)*

µm^3 per 10^6 alveolar macrophages *(volume)*

µm^2 per10^6 alveolar macrophages *(surface)*

All of the above have been used; however, the last two metrics denote mechanistic information

Comparing Volume and Surface Area Dose-Metric in three months rat inhalation study

Design: *Three particle types: TiO_2 fine (0.250μm); TiO_2 ultrafine (25nm); cristobalite (0.8 μm) (**PSHT** particle).*

At end of exposure: *15 minute inhalation of ^{85}Sr labelled test particles to measure **in vivo** lung clearance function over 200 days.*

Extensive lung lavage *(10 times) at end of exposure and 41 and 64 weeks after exposure.*

Measurements: *Retained dose expressed as mass, volume and surface area in lungs and in 10^6 **alveolar macrophages***

This is the only study to measure the dose-metric of retained particle burden per AM

Lung Particle Overload, Nanoparticles and AM mediated Particle Clearance:

Does volumetric overload concept apply to nanoparticles?

12-Week Inhalation Exposure, Ultrafine and Fine TiO_2 and Cristobalite (SiO_2)

Retained dose/10^6 AM at end of exposure

	Mass	Volume		Surface	Number	Test Particle Retention
	µg	nL	% of AM volume	cm^2	x 10^{-9}	control = 1
Control	**0**	**0**	**0**	**0**	**0**	**1**
TiO_2 fine (250 nm)	**340**	**90**	**9**	**21.9**	**10.9**	**1.8***
TiO_2 ultrafine (25 nm)	**99.8**	**26**	**2.6**	**49.9**	**5420**	**8.2***
Cristobalite	**~20**	**7.6**	**0.76**	**2.4**		**28.8***

Material Density

***Significantly different from control**

Oberdörster, Ferin and Morrow, 1994

Lung Particle Overload, Nanoparticles and AM mediated Particle Clearance:

Does volumetric overload concept apply to nanoparticles?

12-Week Inhalation Exposure, Ultrafine and Fine TiO_2 and Cristobalite (SiO_2)

Retained dose/10^6 AM at end of exposure

	Mass	Volume		Surface	Number	Test Particle Retention
	µg	nL	% of AM volume	cm^2	x 10^{-9}	control = 1
Control	**0**	**0**	**0**	**0**	**0**	**1**
TiO_2 fine (250 nm)	**340**	**90**	**9**	**21.9**	**10.9**	**1.8***
TiO_2 ultrafine (25 nm)	**99.8**	**26**	**2.6**	**49.9**	**5420**	**8.2***
Cristobalite	**~20**	**7.6**	**0.76**	**2.4**		**28.8***

Material Density

***Significantly different from control**

Oberdörster, Ferin and Morrow, 1994

Need for using agglomerate density in fluid!

Measuring effective particle density in physiological fluid simulants

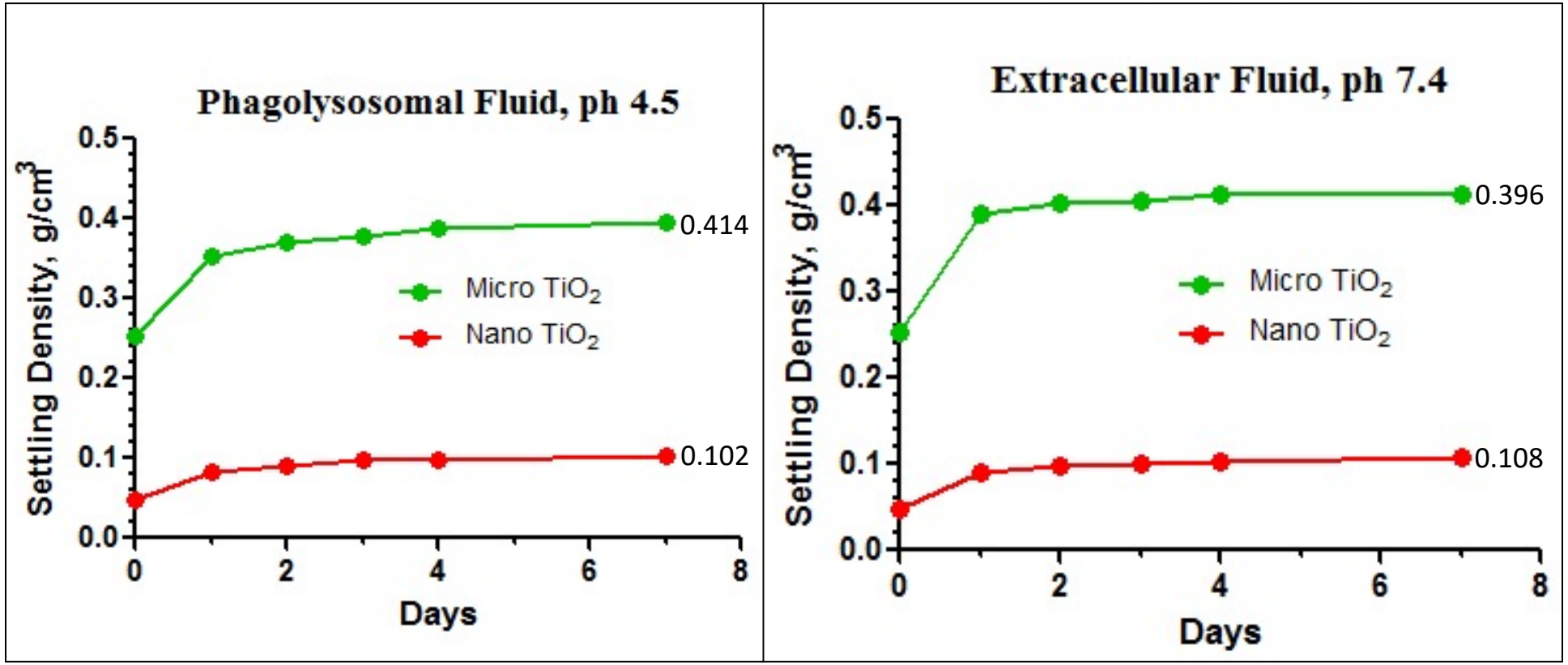
(phagolysosomal [**PFS, pH 4.5**] and extracellular [**EFS, pH 7.4**])

Nano-TiO_2 (P-25 anatase/rutile) **and micro-TiO_2** (Fisher, ~250 nm, anatase)

Settling Density of ultrasonically dispersed TiO_2:

settling, 1-7 days at 1 g_n after 25 sec cuphorn sonication, in 15 ml conical tubes

Settling Density of TiO_2 in:



Lung Particle Overload, Nanoparticles and AM mediated Particle Clearance:

Does volumetric overload concept apply to nanoparticles?

12-Week Rat Inhalation Exposure, Ultrafine and Fine TiO_2 and Cristobalite (SiO_2)

Retained dose/10^6 AM at end of exposure

	Mass	Volume *(settling)*		Surface	Number	Test Particle Retention
	µg	nL	% of AM volume	cm^2	x 10^{-9}	control = 1
Control	**0**	**0**	**0**	**0**	**0**	**1**
TiO_2 fine (250 nm)	**340**	**850**	**85**	**21.9**	**10.9**	**1.8***
TiO_2 ultrafine (25 nm)	**99.8**	**998**	**100**	**49.9**	**5420**	**8.2***
Cristobalite	**~20**	**7.6** *22*	**0.76** *2.2*	**2.4**		**28.8***

***Significantly different from control**

settling density volume, g/cm^3: 0.4 (fine); 0.1 (ultrafine); 0.9 (SiO_2)

Updated from:

Oberdörster, Ferin and Morrow, 1994

Analysis of retained lung burden in 2 year rat inhalation study by Lee et al *(1985/86)* and

Extrapolation to human workers considering species differences for interstitial access of particles

Lee et al. TiO_2 Inhalation Study, 1985; 1986 :

2-year rat inhalation at 10; 50; 250 mg/m³

Measured vs Predicted Retained TiO_2 Lung Burden at 2 Year Exposure:

Exposure Conc mg/m³	Measured in study mg/lung	Predicted by model mg/lung	Lung Tumors
10	*26.5*	*3.8*	*No*
50	*124*	*19*	*No*
250	*665*	*95*	Yes
Clearance:	***impaired***	***non-impaired***	

Lee et al. TiO_2 Inhalation Study, 1985; 1986 :

2-year rat inhalation at 10; 50; 250 mg/m³

Measured vs Predicted Retained TiO_2 Lung Burden at 2 Year Exposure:

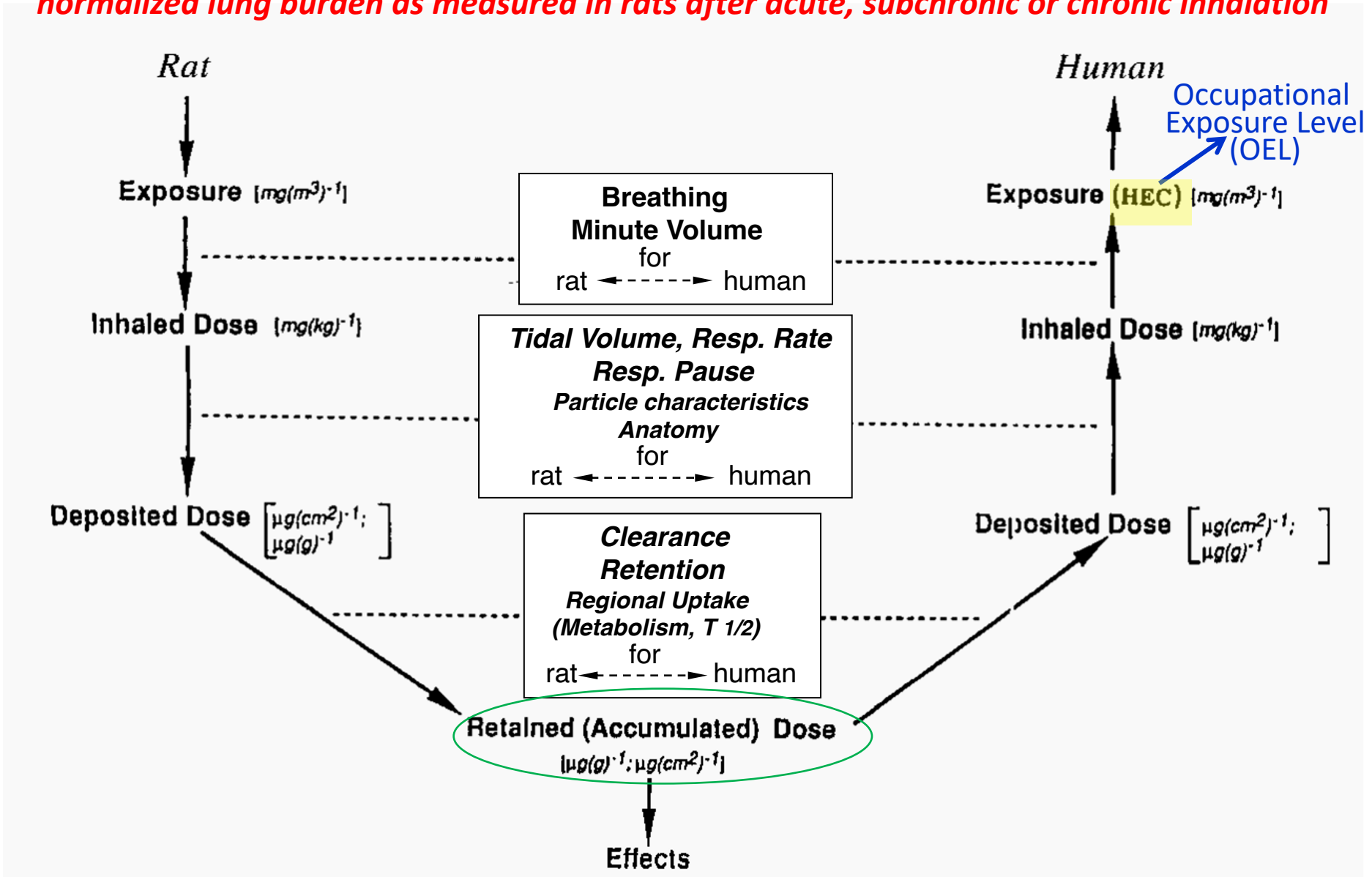
Exposure Conc mg/m³	Measured in study mg/lung	Predicted by model mg/lung	Lung Tumors
10	*26.5*	*3.8*	*No*
50	*124*	*19*	*No*
250	*665*	*95*	Yes
Clearance:	*impaired*	*non-impaired*	

Only excessively overloaded lungs in rats resulted in lung tumors

***But:* Prolongation of particle clearance was present at all three concentrations**

Dosimetric **Extrapolation of Particle Exposures from Rats to Humans**

Concept: ***HEC is defined as the Exposure Concentration resulting in Humans in the same normalized lung burden as measured in rats after acute, subchronic or chronic inhalation***



Effects may be different for both species

Modified from Oberdörster, 1989

Using the highly TiO_2 overloaded rat lung with no tumor induction *(Lee et al, 1985/1986)*
for comparing rat lung burdens (2 yrs, 50 mg/m^3)
with those of exposed workers (40 years, 50 mg/m^3):

MPPD (Version 3.04) Model results:

Workers are predicted to accumulate **15 mg/g lung** (assuming normal clearance)

Applying finding in coal workers that 80% of total lung burden is in interstitium at end-of-working life,(*)

that extrapolates to only **3 mg/g lung** *in the alveolar lumen phagocytized by AM*

*(*Kuempel et al, 2001; Nikula et al, 1997; 2001; Tran and Buchanan, 2001; Gregoratto et al, 2010; 2011)*

Rats retained in the 2-year study **39 mg/g control lung** (with impaired clearance)

If no impaired clearance had occurred: **5.9 mg/g control lung**

In order for workers to accumulate the same normalized rat lung burden of 39 mg/g,

the HEC would have to be: **130 mg/m^3**

(obviously, that would result in severe pathology and disease)

Summing Up:

1. Important differences in pulmonary retention kinetics of PSLTs between rats and humans:
   clearance rates and T½
   ***interstitial lung sequestration** compartment in humans*
   ***very long T½** (63 years) in interstitium and hilar lymphnodes in humans*
2. Total retained lung burden of chronically high exposed **workers** exceeds tumor inducing Overload of rats

   But: Alveolar lumen (AM) particle burden in humans does not reach these overload levels

   due to efficient AM mediated clearance along the muco-ciliary escalator
3. No data on phagocytized particle levels in human alveolar macrophages could be found
4. Impairment of AM mediate particle clearance in rats due to Overload does **not necessarily** result

   in induction of lung tumors in rats, **only when excessive**
5. For both the volumetric and the surface area Overload concept the **retained dose per AM**

   is the most appropriate dose-metric
6. The surface area concept considers the existing Specific Surface Reactivity (ROS/cm^2) of PSLT particles

   which is unlikely to be the same for all PSLT particles.
7. The volumetric Overload concept assumes the same threshold for all PSLT particles,

   particle volume alone is considered

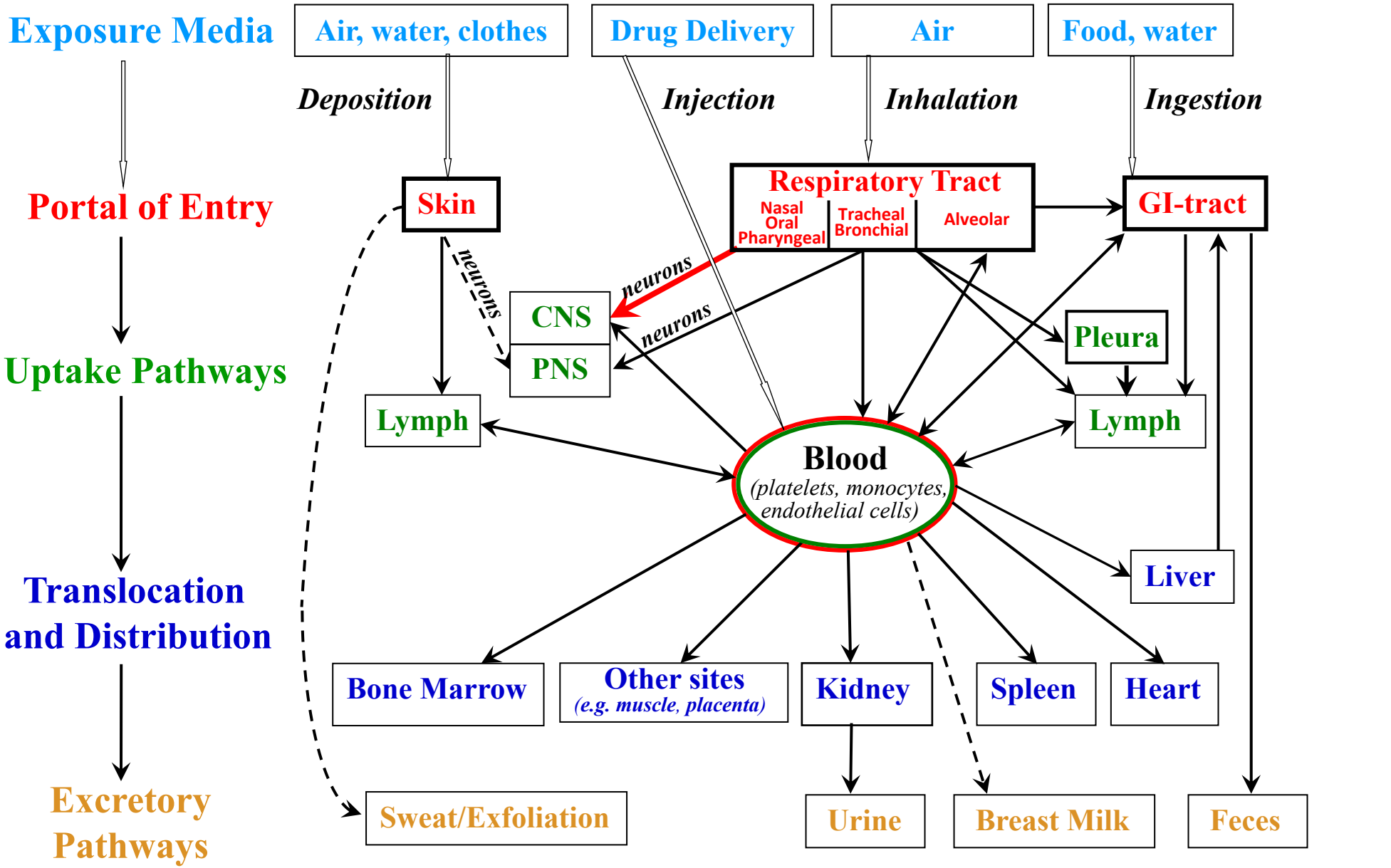
Outlook: Moving on to new discoveries:

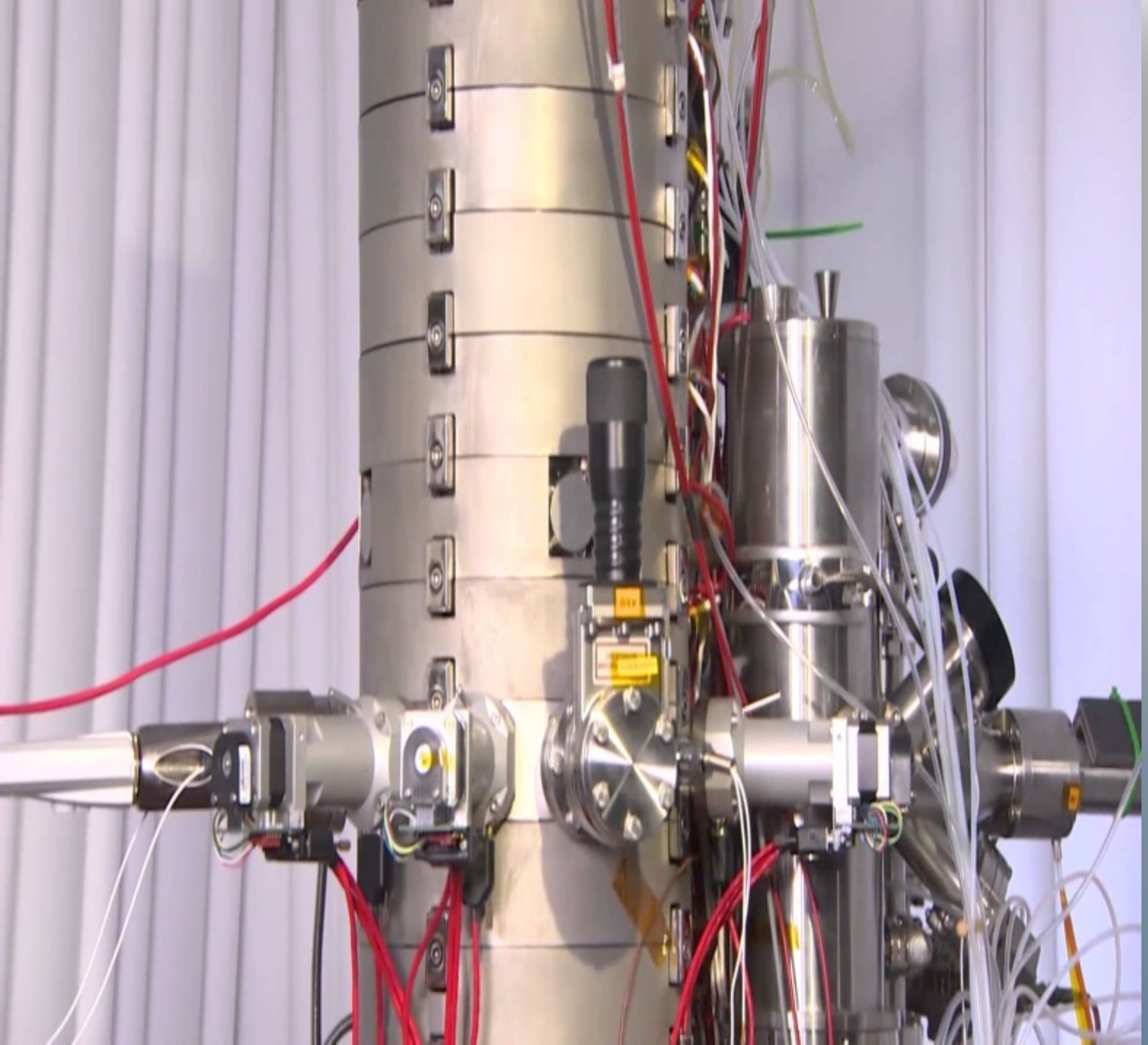
*Continuing from: "The Respiratory Tract as **Target** for **Inducing** Effects of Inhaled Particles"*

*To: "The Respiratory Tract as **Portal of Entry** of Inhaled Particles for **Translocating** to Secondary Organs"*

with Focus on the Fate of Nanosized Particles in the Central Nervous System

Exposure and Biokinetics of Nanoparticles





High Resolution Analytical Imaging

(Dr. Uschi Graham)

- ***Sub-nanometer Resolution***
- ***In situ Analysis***
- ***Oxidation States***
- ***3D- reconstruction***
- ***Solubility***