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FIFTH NANOSAFE
INTERNATIONAL
CONFERENCE
HEALTH AND SAFETY
ISSUES RELATED
TO NANOMATERIALS
FOR A SOCIALLY RESPONSIBLE APPROACH
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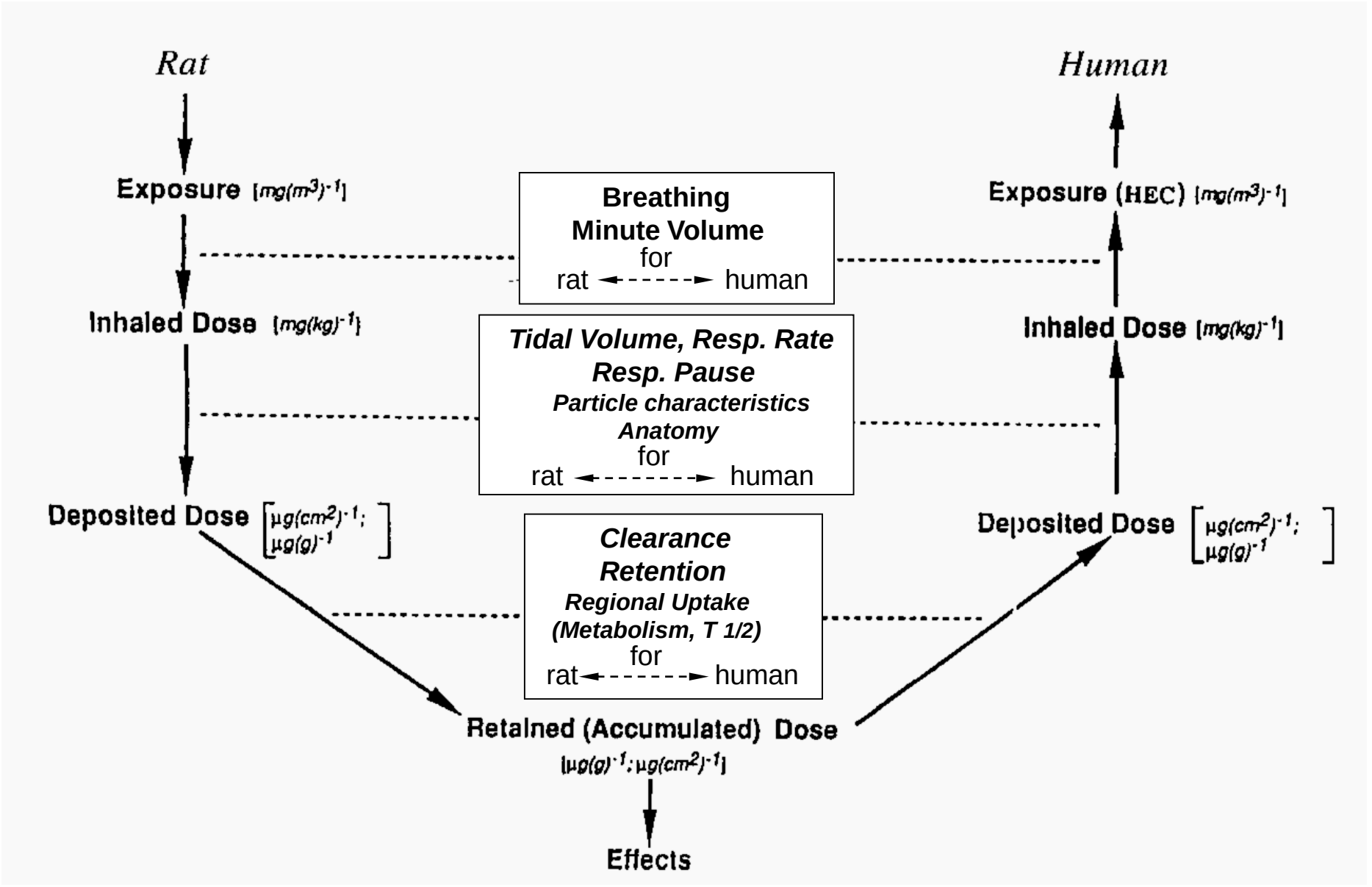
Refining Dosimetric Extrapolation Modeling of Inhaled Nanoparticles for Deriving a Human Equivalent Concentration

Günter Oberdörster
University of Rochester, NY
and
Uschi Graham
University of Kentucky, KY

November 9, 2016

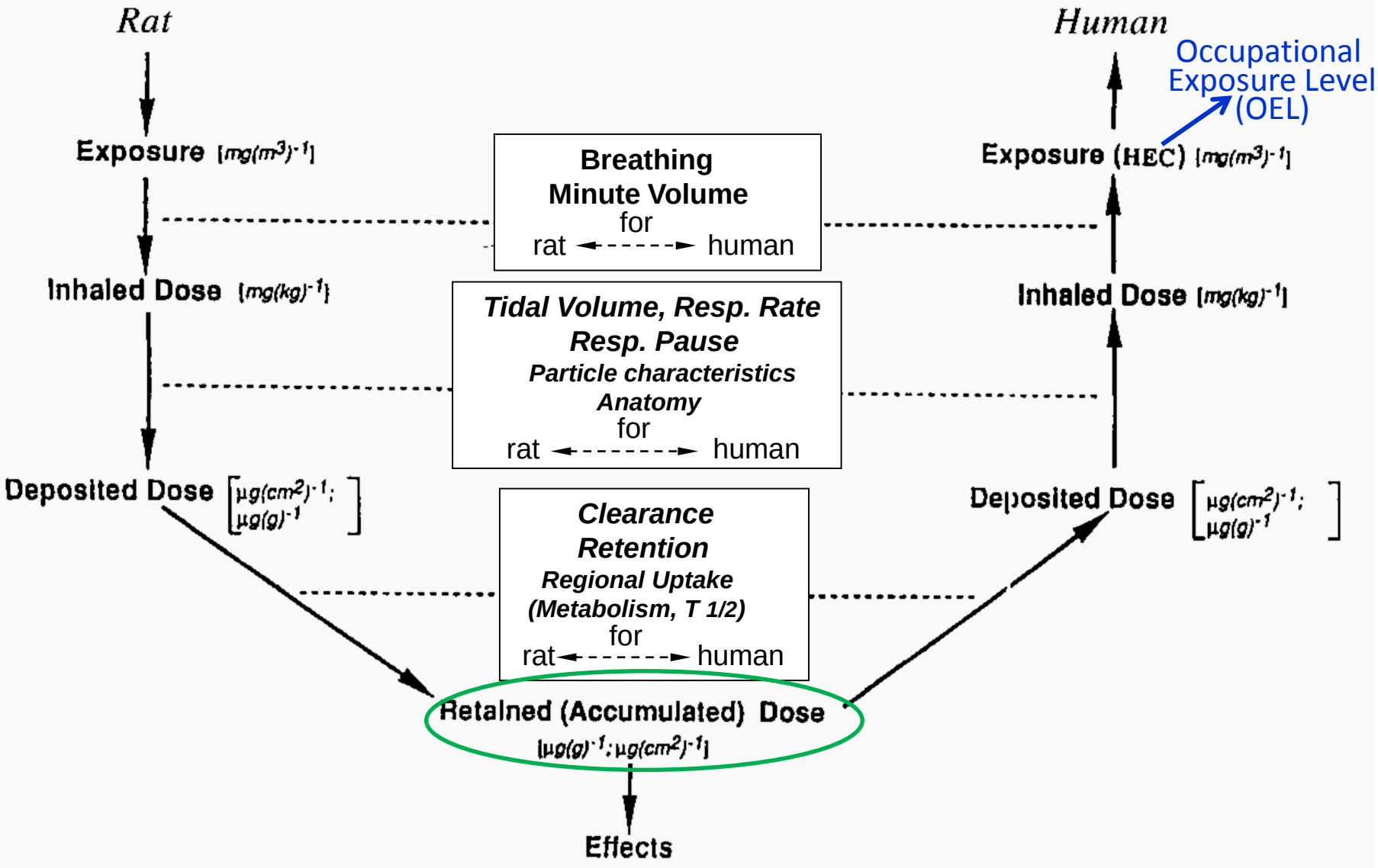
Dosimetric Extrapolation of Particle Exposures from Rats to Humans



Modified from: Oberdörster, 1998

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

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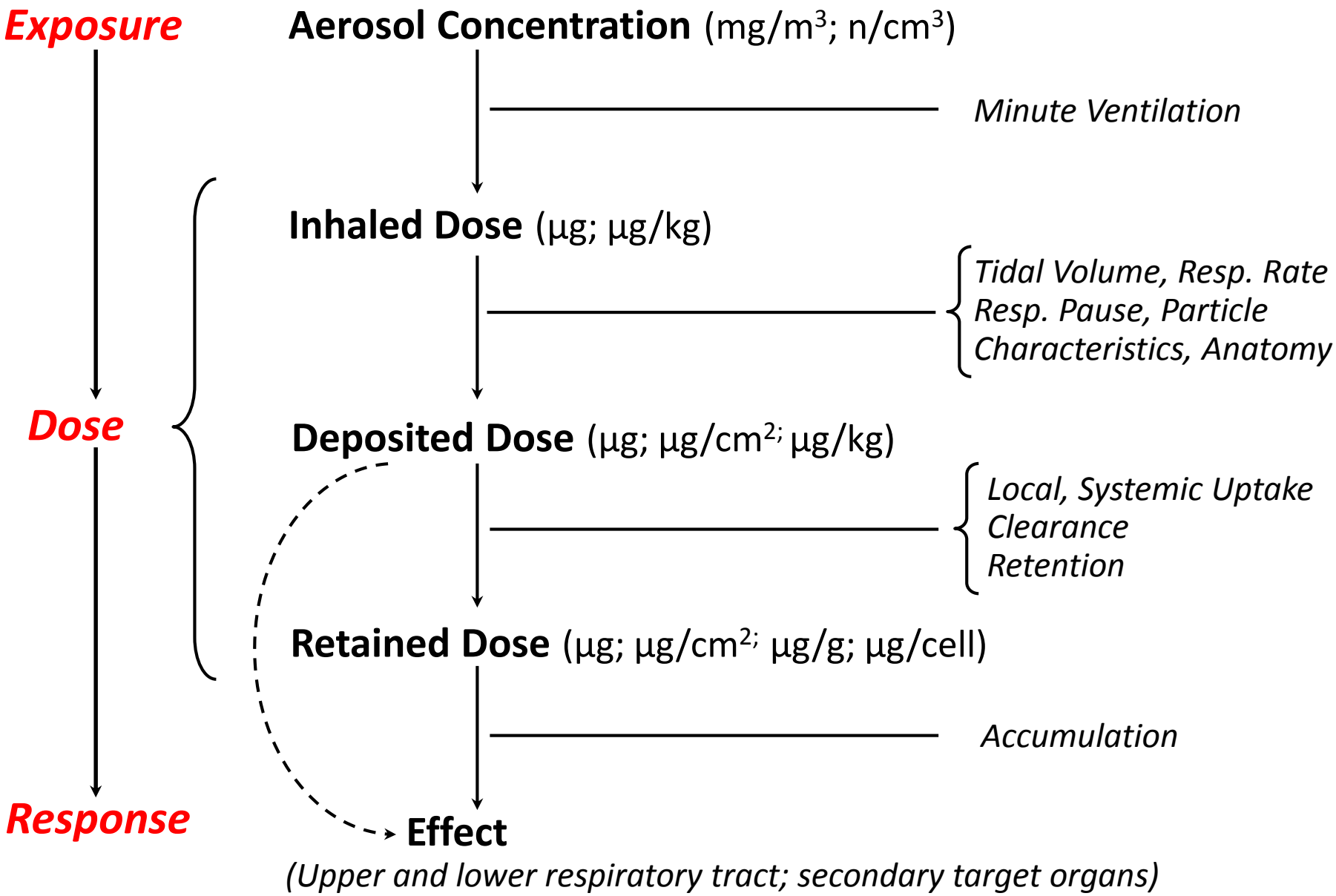
Factors Involved in Respiratory Tract Dosimetry



Information Needed to Analyze Exposure-Dose-Response of Inhaled Nanomaerials For Risk Extrapolation Modeling

- ***Aerosol specifics*** (*agglomerate/aggregate; MMAD; GSD*)
- ***Resp. tract geometry*** (*species specific branching pattern*)
- ***Resp. tract physiology*** (*species specific breathing parameters*)
- ***NM properties*** (*physico-chemical [intrinsic]; functional [extrinsic]*)
- ***Exposure duration***

Physico-Chemical and Functional NP Properties of Relevance for Toxicology

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)*

Properties can change

-with: method of production
preparation process
storage

-when introduced into physiol. media, organism

Key parameter: Dose!

Cell-free (a-cellular) Functional Assays

SURFACE SPECIFIC REACTIVITY OF NANOSIZED PARTICLES

- **High specific surface area: m^2/g**

Surface Reactivity as Dose-Metric,

***e.g.*, ROS inducing potential expessed per unit particle surface area**

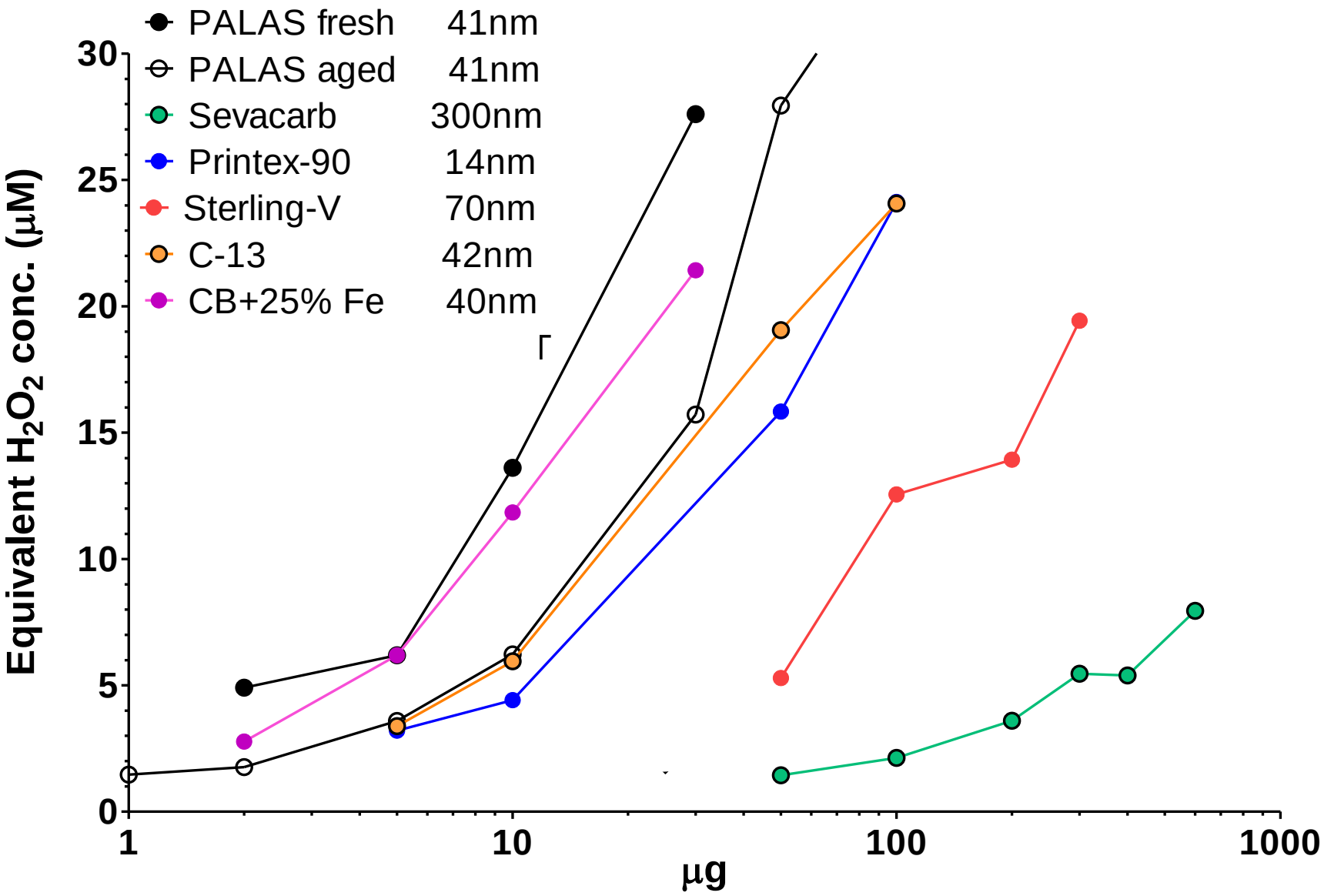
DTT *(dithiothreitol)* assay
DCFH-DA *(2'-7' dichlorofluorescin-diacetate)* assay
FRAS *(ferric reducing ability of serum)* assay
Vit C assay
ESR
others…

as screening tool for hazard ranking of NPs based on their reactivity in cell free or cellular assays

[Bello et al., 2009; Rushton et al., 2010]

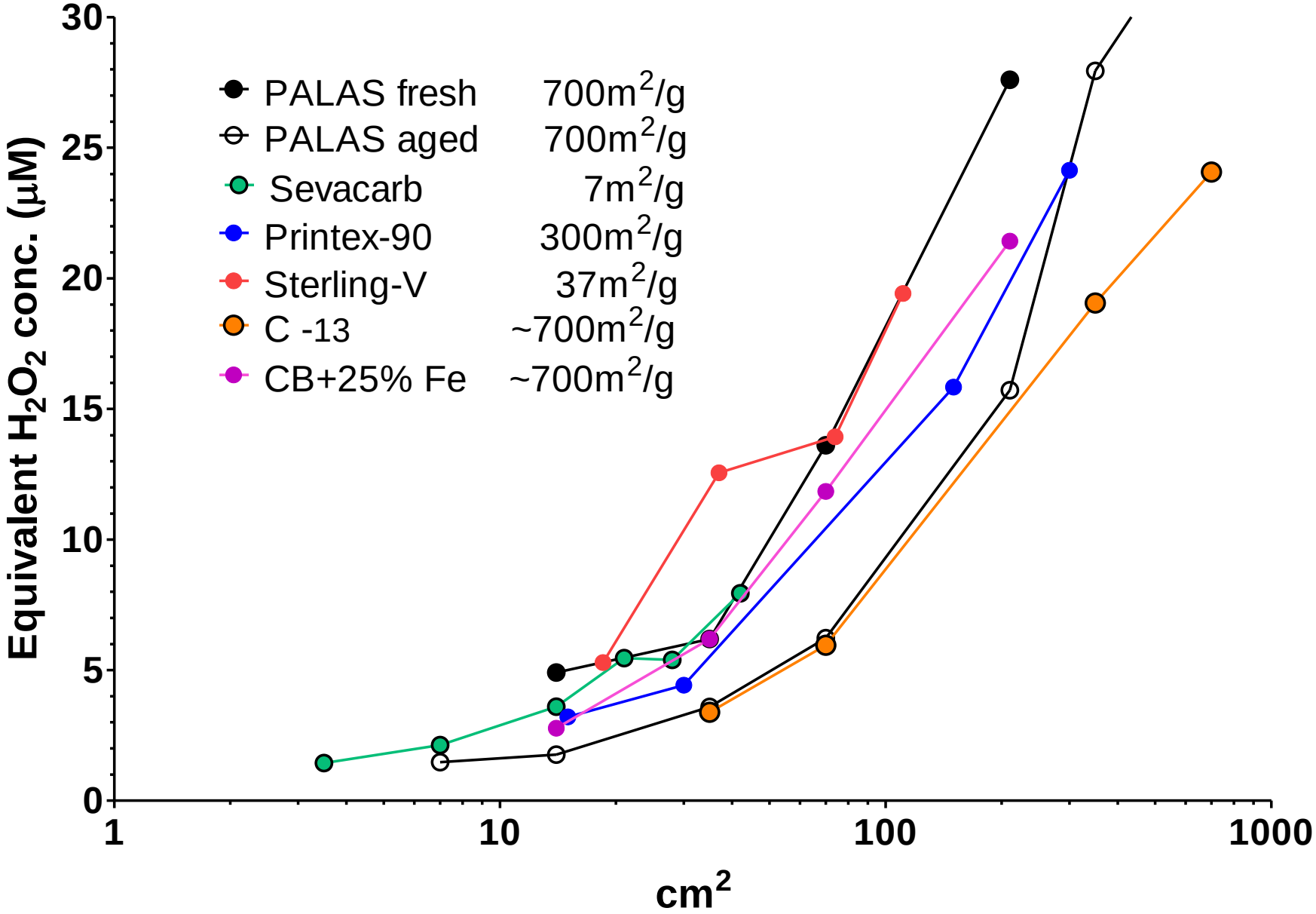
Cell-free Assay, NP bound ROS, Summary *(Carbon Particles)*

Particle Mass Correlation



Cell-free Assay, NP bound ROS, Summary *(Carbon Particles)*

Particle Surface Area Correlation



Dissolution as one Determinant of Pulmonary Biopersistence of Inhaled Particles

Physiological Clearance Processes → **Particle Biopersistence** ← **Physicochemical Processes**

AM mediated; Translocation

↓

Larynx
Interstitium
Pleura

↓

Species Differences

↓

Retention $T_{1/2}$ Effects

Biodurability: dissolution; leaching; bioprocessing
(intra-, extra-cellular)

↓

No Species Differences

Biopersistence = f (Physiological Clearance; Biodurability)

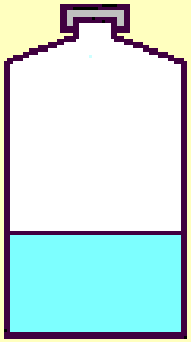
Overall clearance rate = AM-mediated clearance rate + dissolution* rate

*(*may be masked due to prolonged retention of bioprocessed particles/ions)*

Acellular solubility/dissolution assays with simulated lung fluids

Static

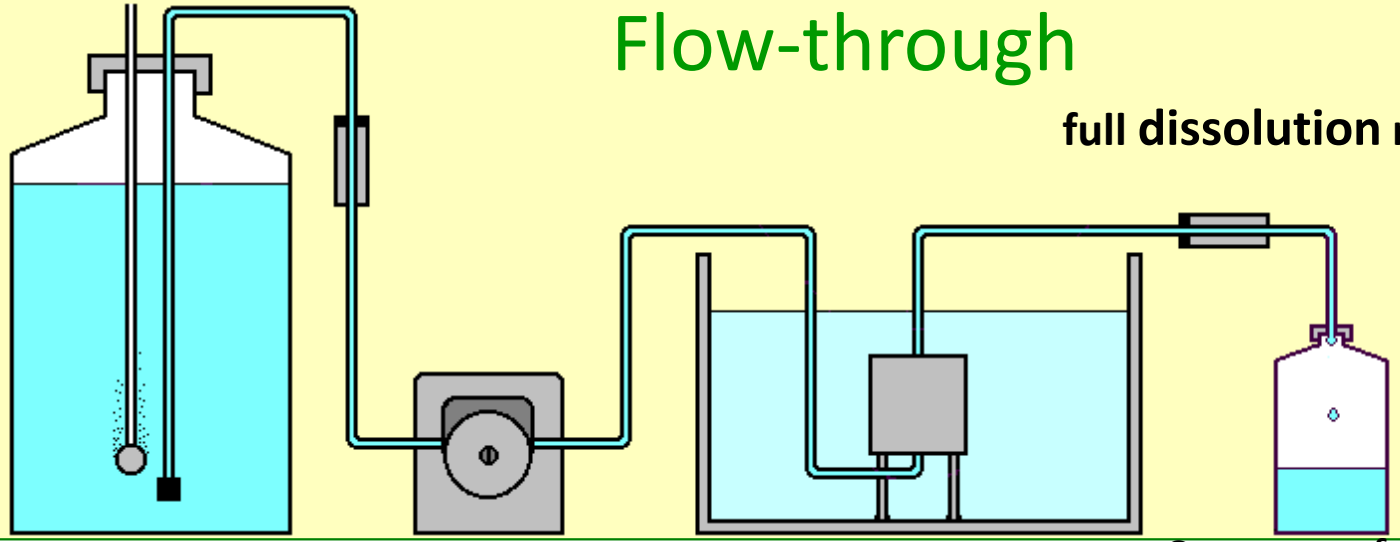
equilibrium solubility (g/L)



+ agitation

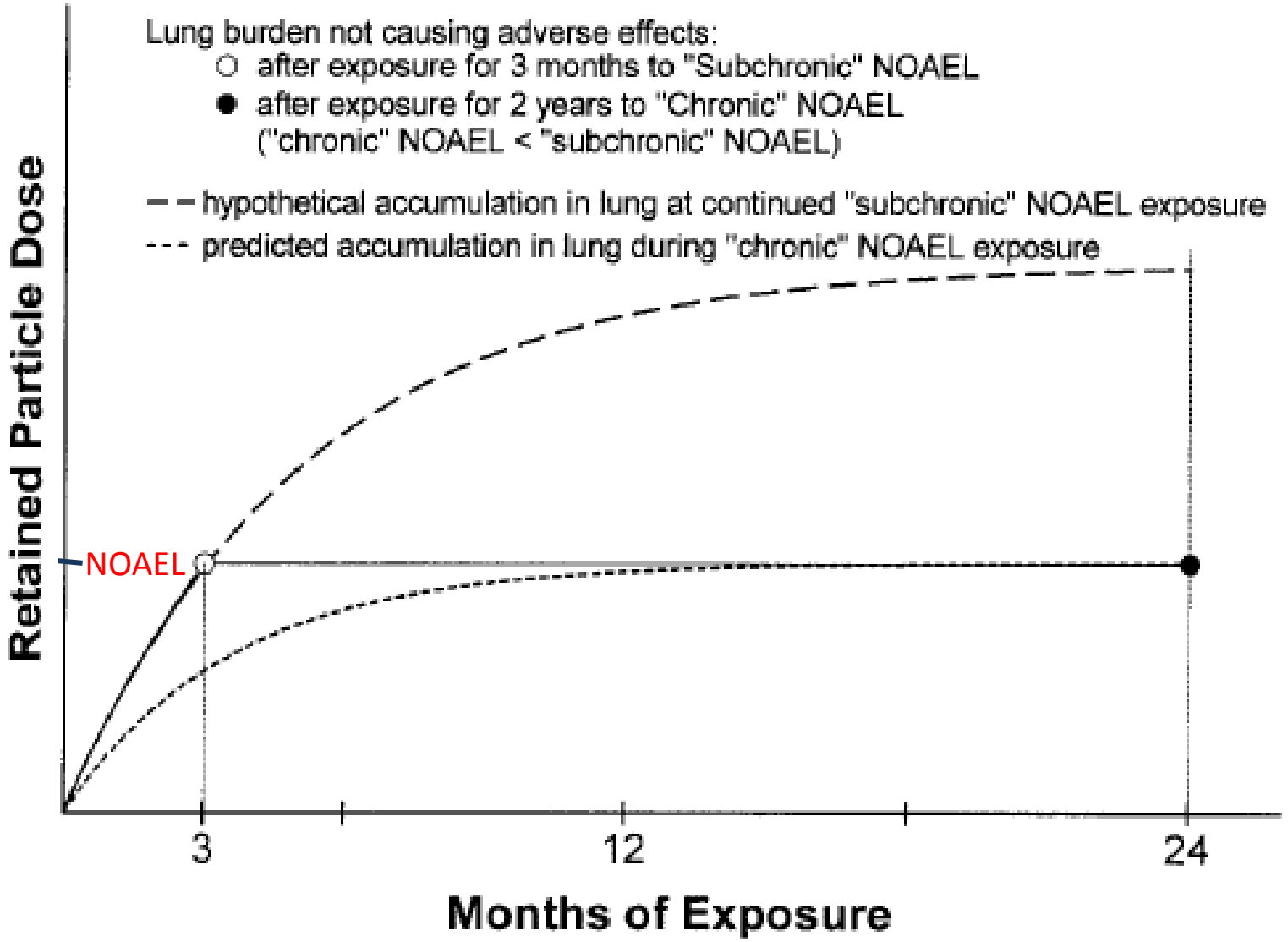
Flow-through

full dissolution rate ng/cm^2/day



Courtesy of Potter, 2015

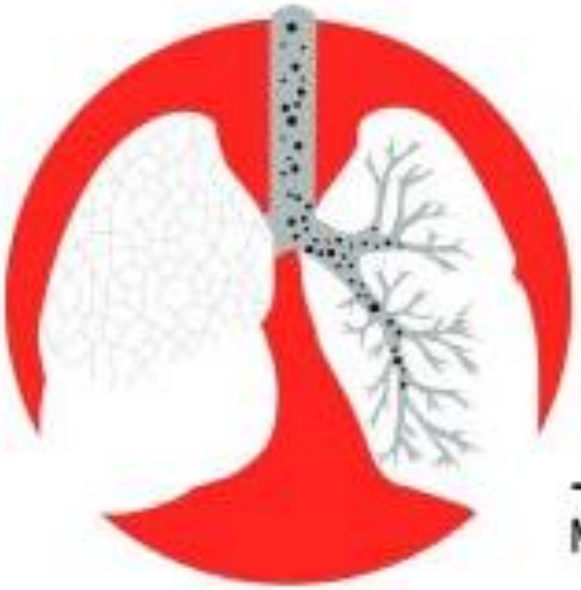
Estimation of Chronic NOAEL from Subchronic Rodent Study using MPPD Model



From: Oberdörster, 2002

MPPD v3.04

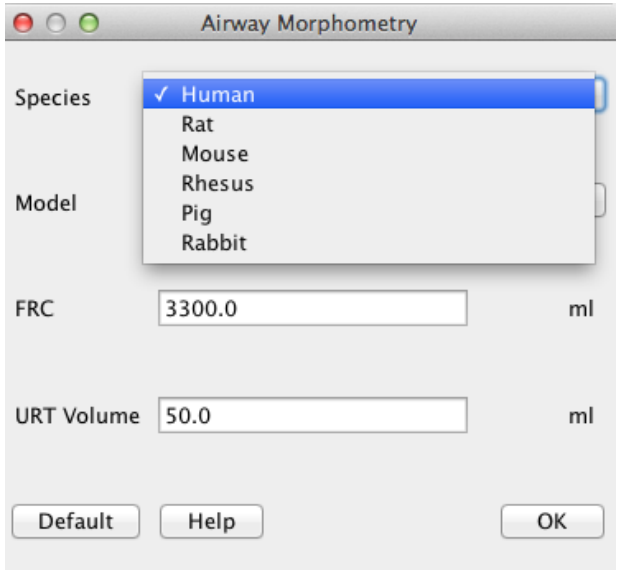
File Input Data Calculations Report Results Plot Results Help Get Started

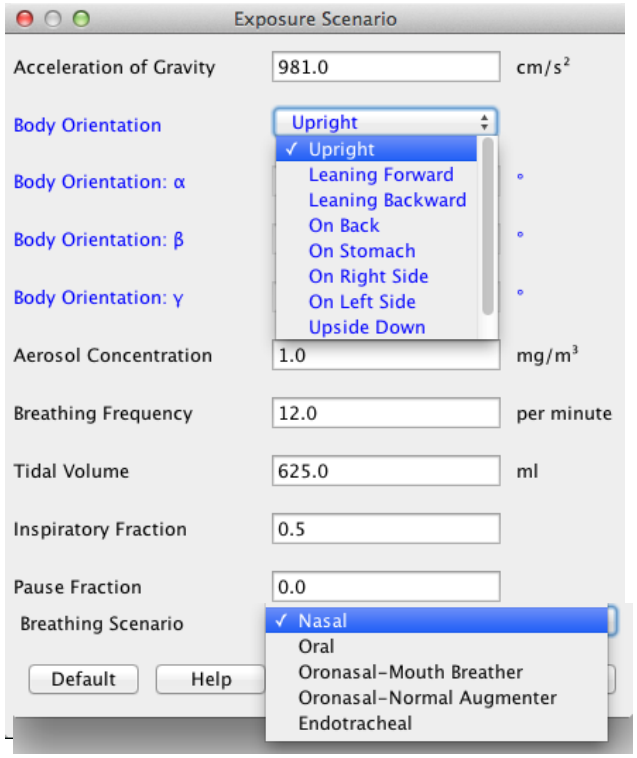


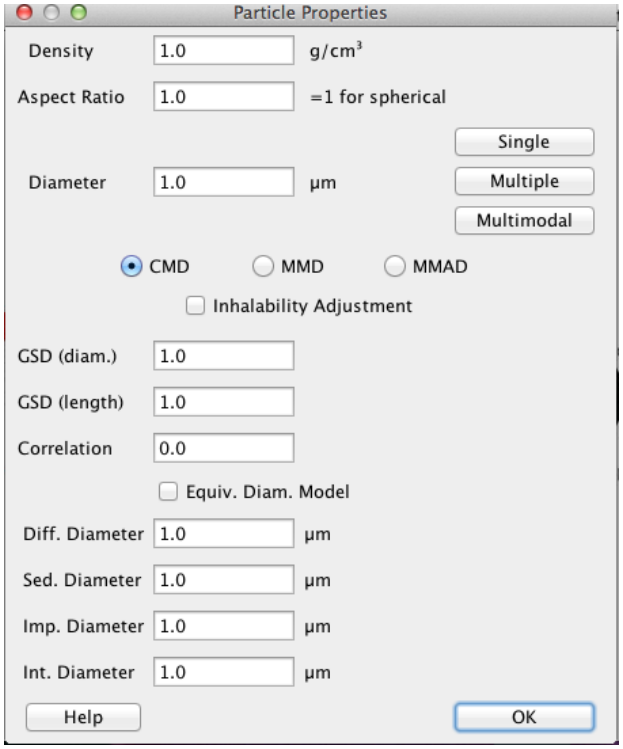
MPPD©

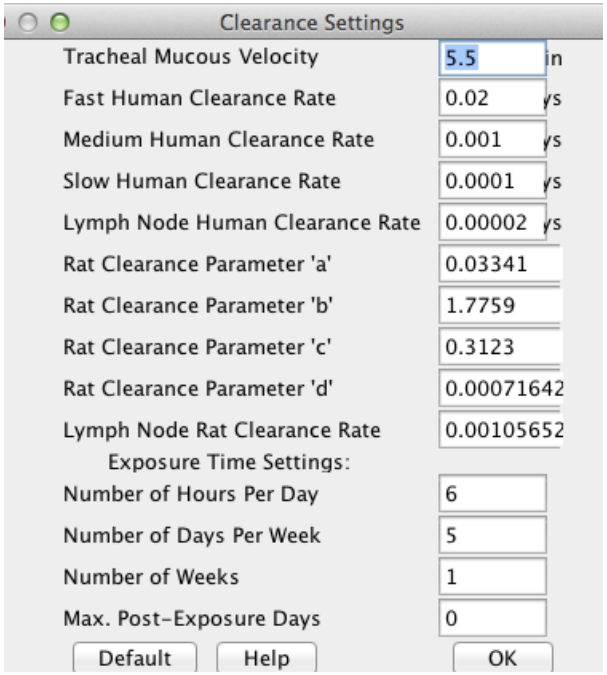
MULTIPLE-PATH PARTICLE DOSIMETRY MODEL

MPPD Model Input Choices

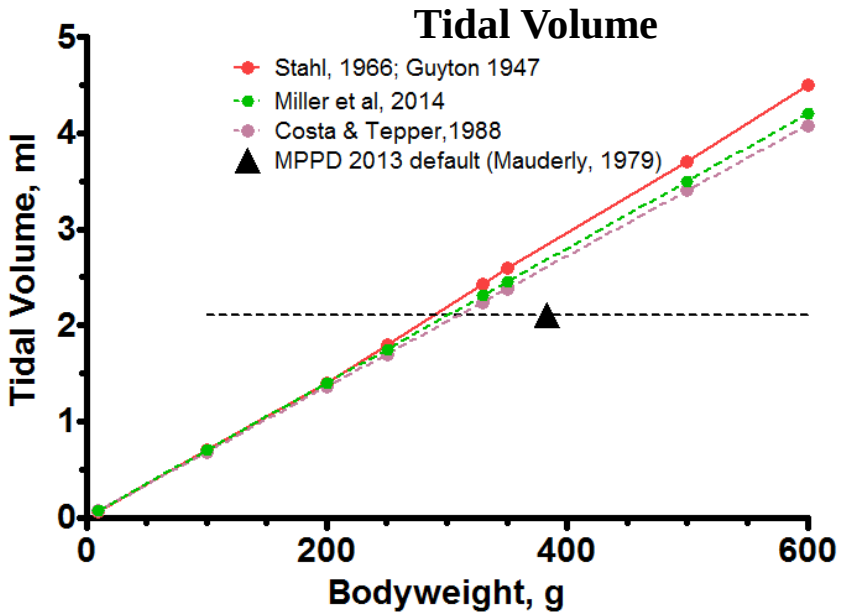


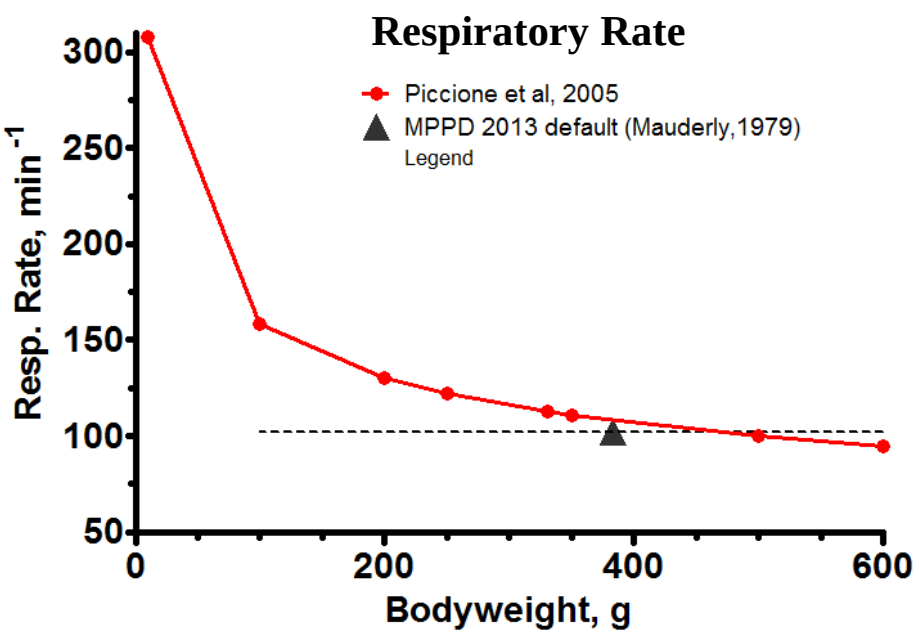


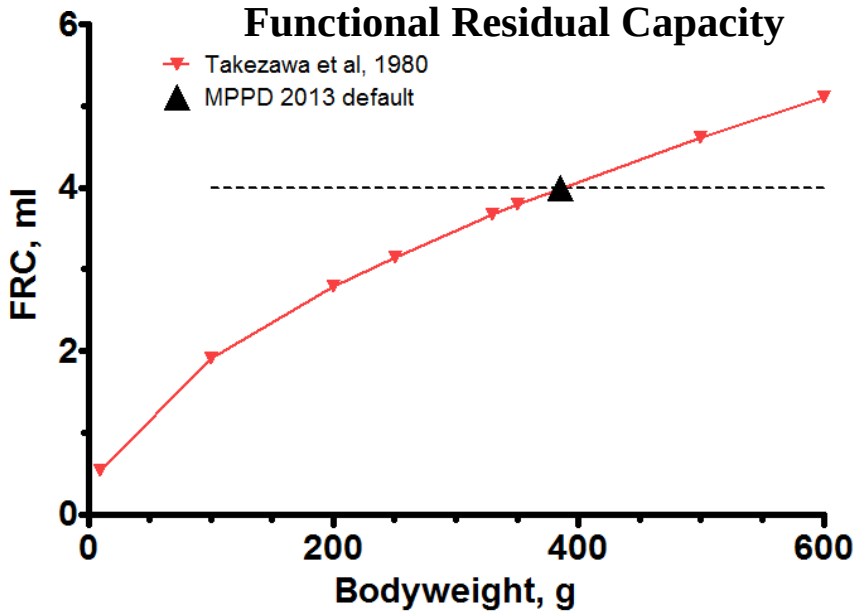


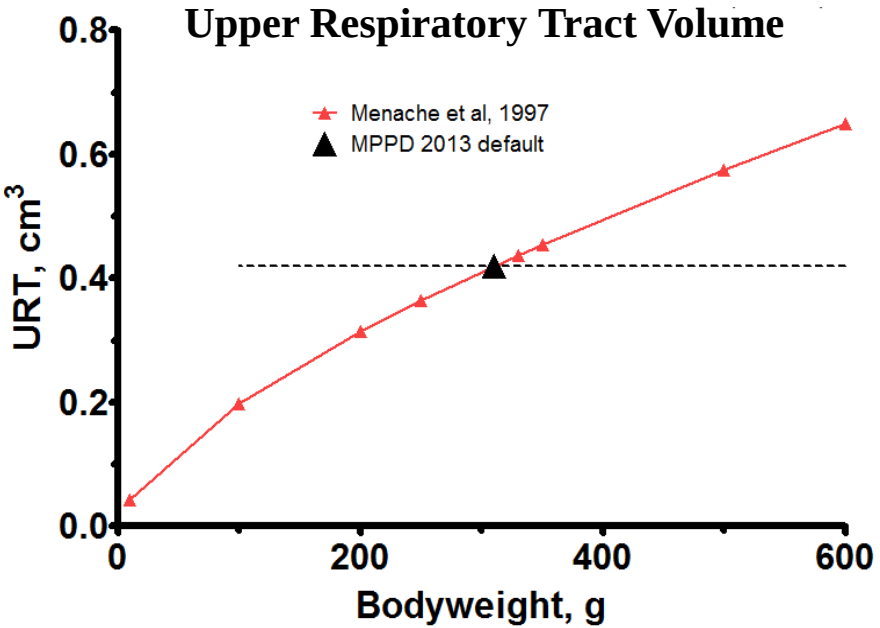


Allometric Scaling of Respiratory Parameters to Bodyweight of Rat, for Input into MPPD



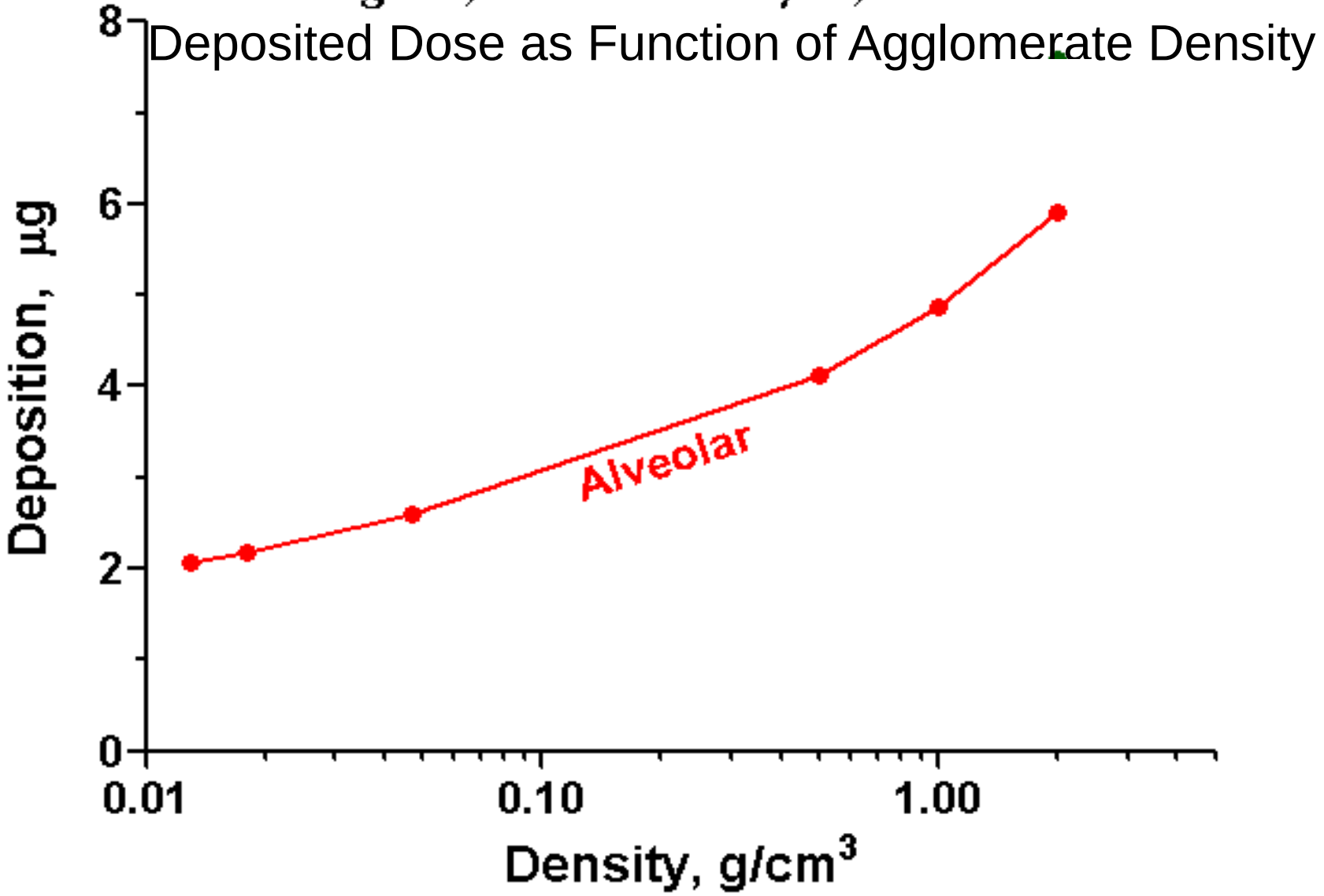






Impact of Aerosol Density on Lung Deposition of Inhaled Agglomerated Particles: MPPD Prediction, Rat, 4 hour Inhalation

$2.5 mg/m^3$; $MMAD = 1.4 \mu m$; $GSD = 2.9$



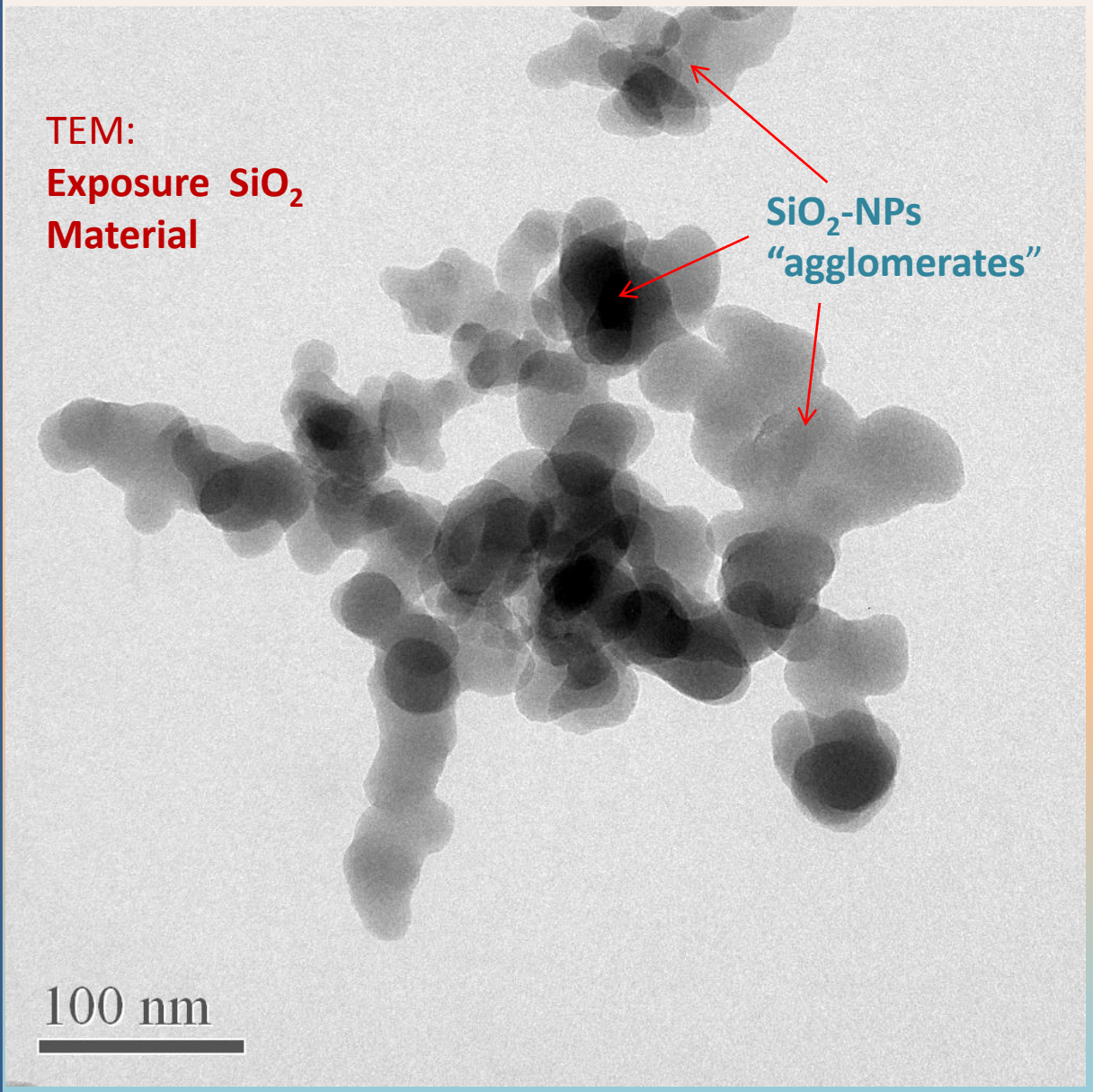
Case Study: 28 Day Nano-Silica Rat Inhalation Study as basis for human extrapolation modeling

Objective/Questions:

Determine aerosol characteristics, effects and fate of amorphous SiO_2 NPs in a short (4 hr) and a repeat (4-wk) rat inhalation study:
(suspended as slurry used for CMP in electronics industry)

- ***What are results telling us in terms of hazard extrapolation to humans?***
- ***Is a 4-week exposure duration sufficient for risk characterization?***
- ***What is a safe level for worker exposure?***

Silica/SiO_2 Starting Materials

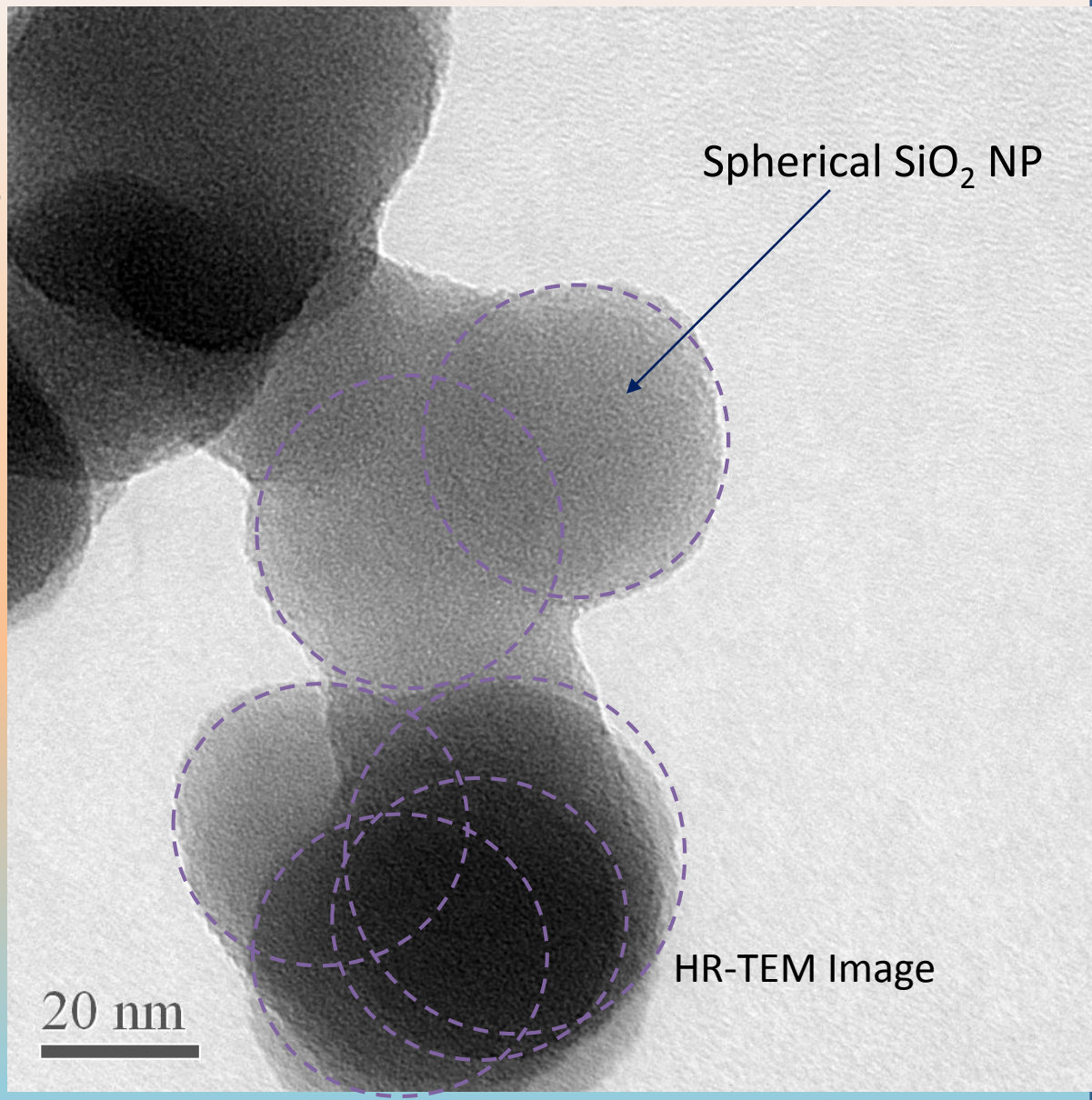


- Majority of the NPs are spherical or semi-spherical and ~ 20-40 nm in size.
- Nanoparticles tend to form dense agglomerated aggregates.
- Nanoparticles have smooth surfaces without etching or dissolution patterns.
- Particles are not zoned or show different densities (core to surface).
- Particles are amorphous

SiO_2 Starting Nanoparticles

This HR-TEM shows the amorphous nature of the supplied SiO_2 NPs.

Aggregation and Agglomeration is part of NPs Formation.



Outline, study plan of SiO_2 NP inhalation:

- **4 hour acute inhalation in rats:**
  - to determine effective density ρ_{eff} of SiO_2 aerosol "in vivo"

- **4 week inhalation in rats:**
  - three concentrations to determine NOAEC
  - estimate overall lung clearance rate (b_{tot}) of SiO_2 NPs
  - compare to normal clearance rate (b_{mech}) for insoluble particles
  - derive in vivo dissolution rate (b_{diss}) of SiO_2 NPs: $b_{tot} = b_{mech} + b_{diss}$
  - dosimetric extrapolation to human exposure

- **verify in vivo dissolution by HRTEM analysis**
  - bioprocessing in phagolysosome of macrophages
  - analyzing chemistry of subcellular interactions of NPs

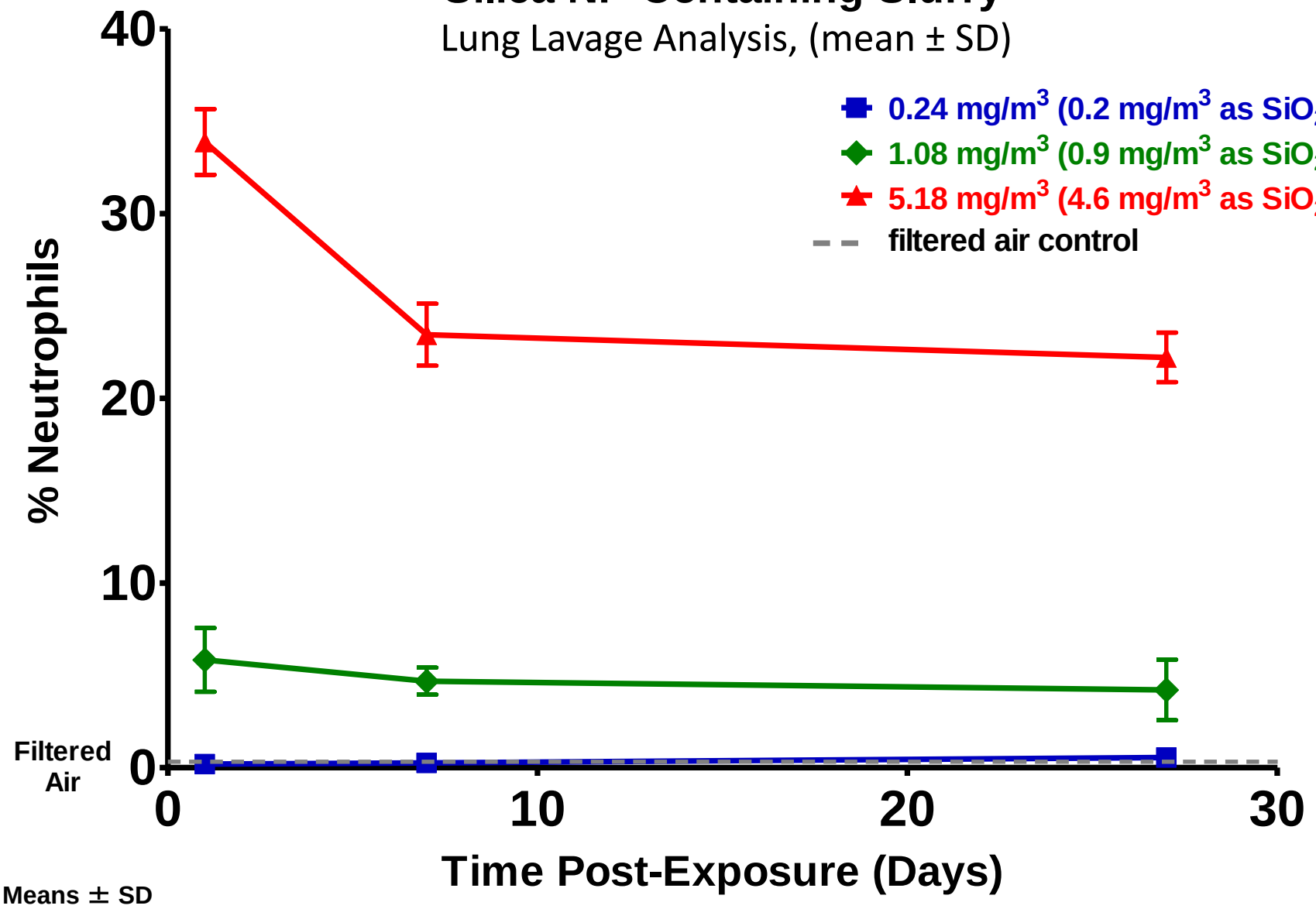
4 Week Study:
Exposure Characteristics and Retained Doses (µg) Using Silica Nanoparticle-Containing CMP Slurries
Fisher-344 Male Rats

	High Dose	Mid Dose	Low Dose
SiO_2 Aerosol (mg/m^3)	**4.66**	**0.98**	**0.22**
Lung Retained Dose (µg, as SiO_2, at 4 wks expos)	**196** ± 7	**47** ±9	**13.6** ±3.3

NOAEL

Pulmonary Inflammation in Rats After 4 Weeks of Exposure to Silica NP-Containing Slurry

Lung Lavage Analysis, (mean ± SD)



Means ± SD

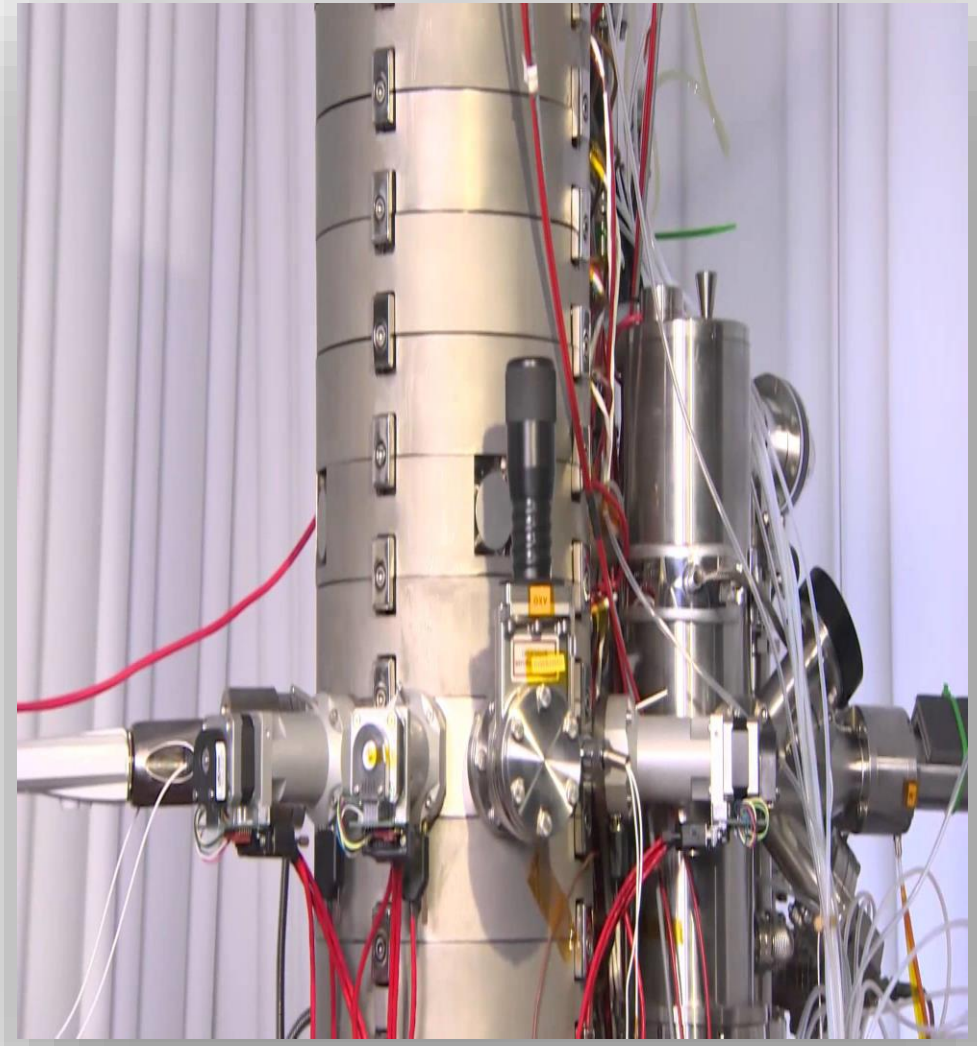
Effective Density of SiO_2 Aerosols

Result of MPPD derived ρ_{eff} for SiO_2 slurry aerosols using data of 4-hr. rat inhalation study:

$$\rho_{eff} = 0.165\ g/cm^3$$

Compare to SiO_2 material density of 2.65 g/cm^3 !

Verifying in vivo dissolution of SiO_2 NPs by HR-TEM/STEM/EELS analysis



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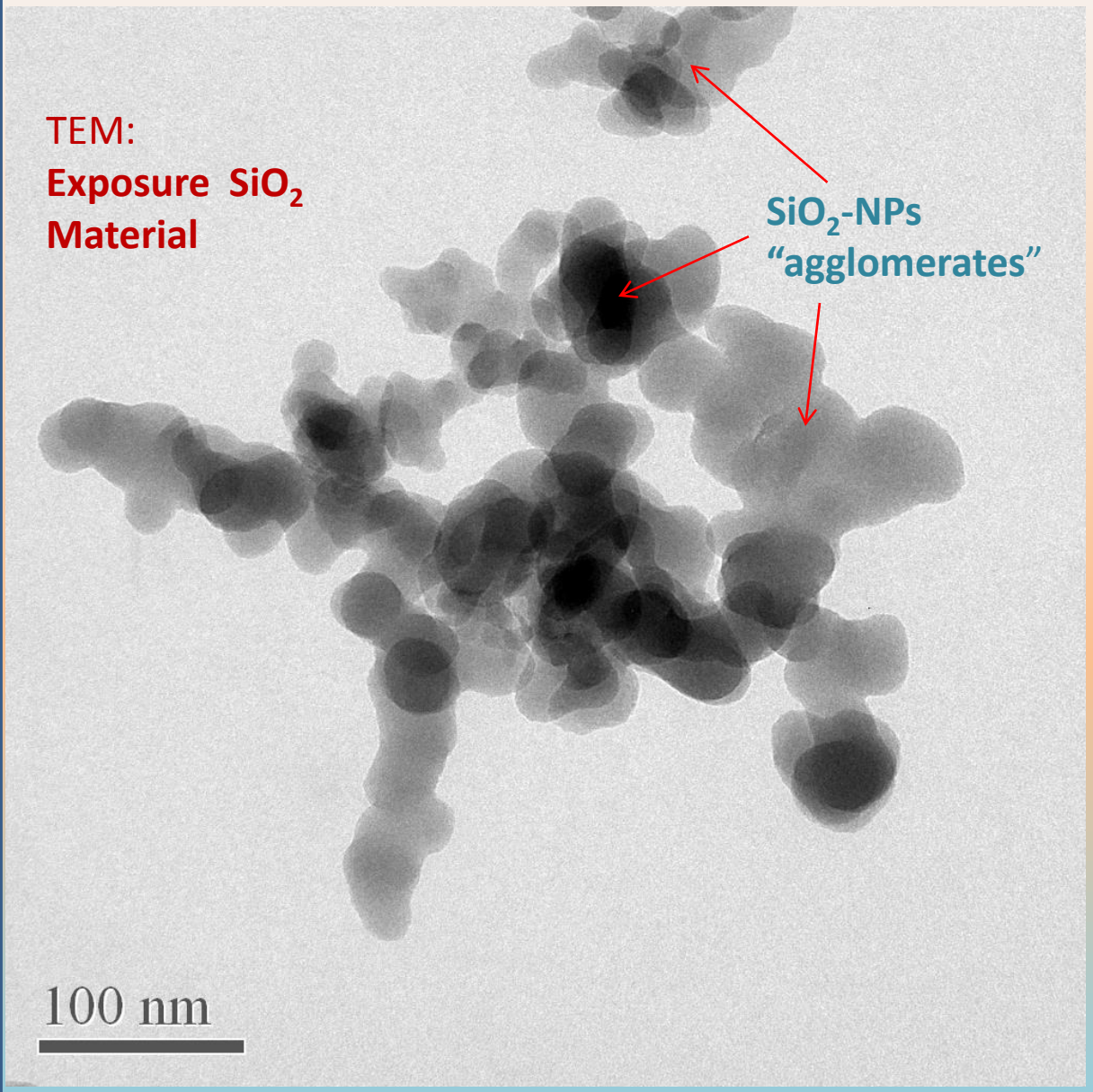

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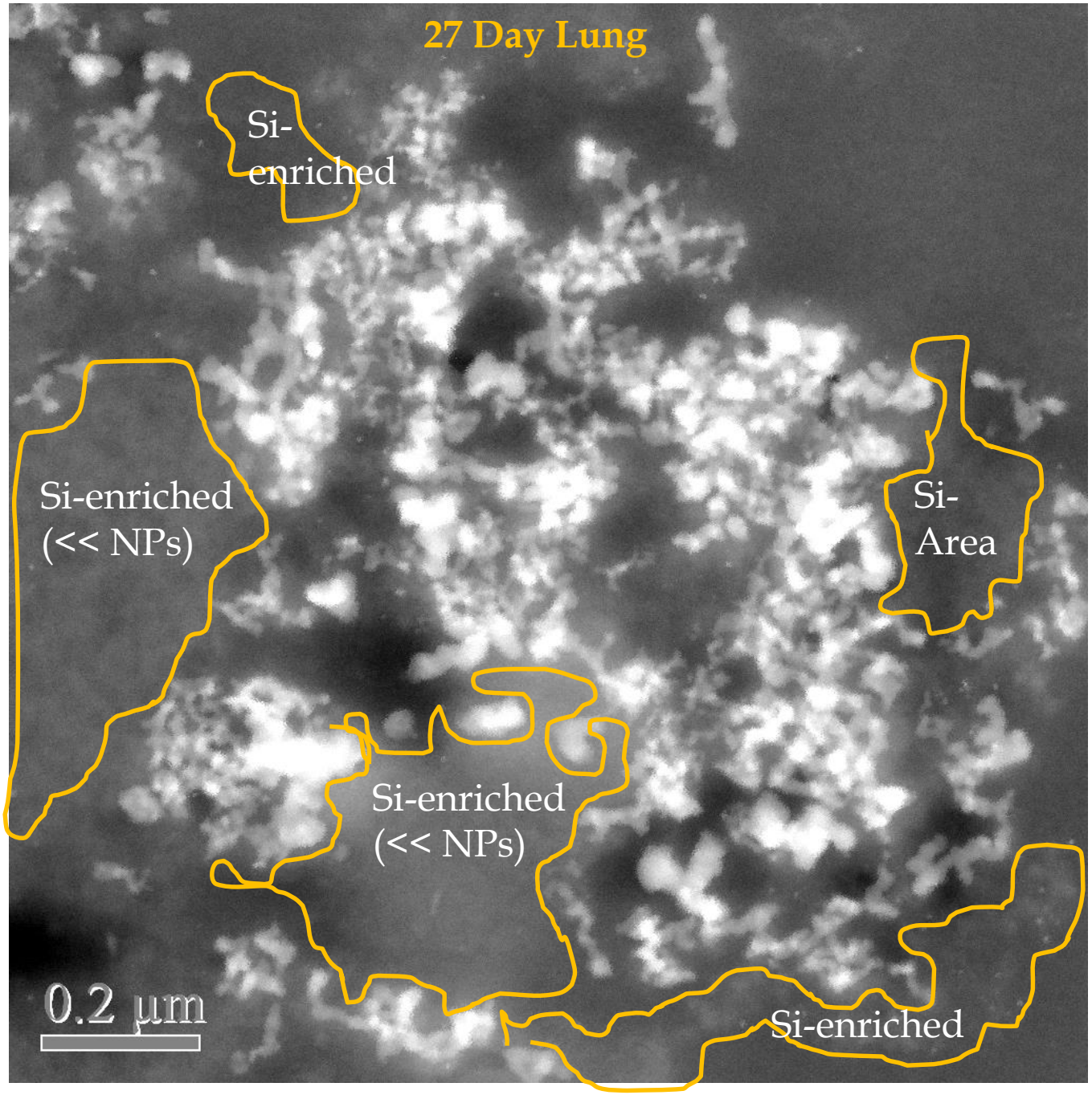
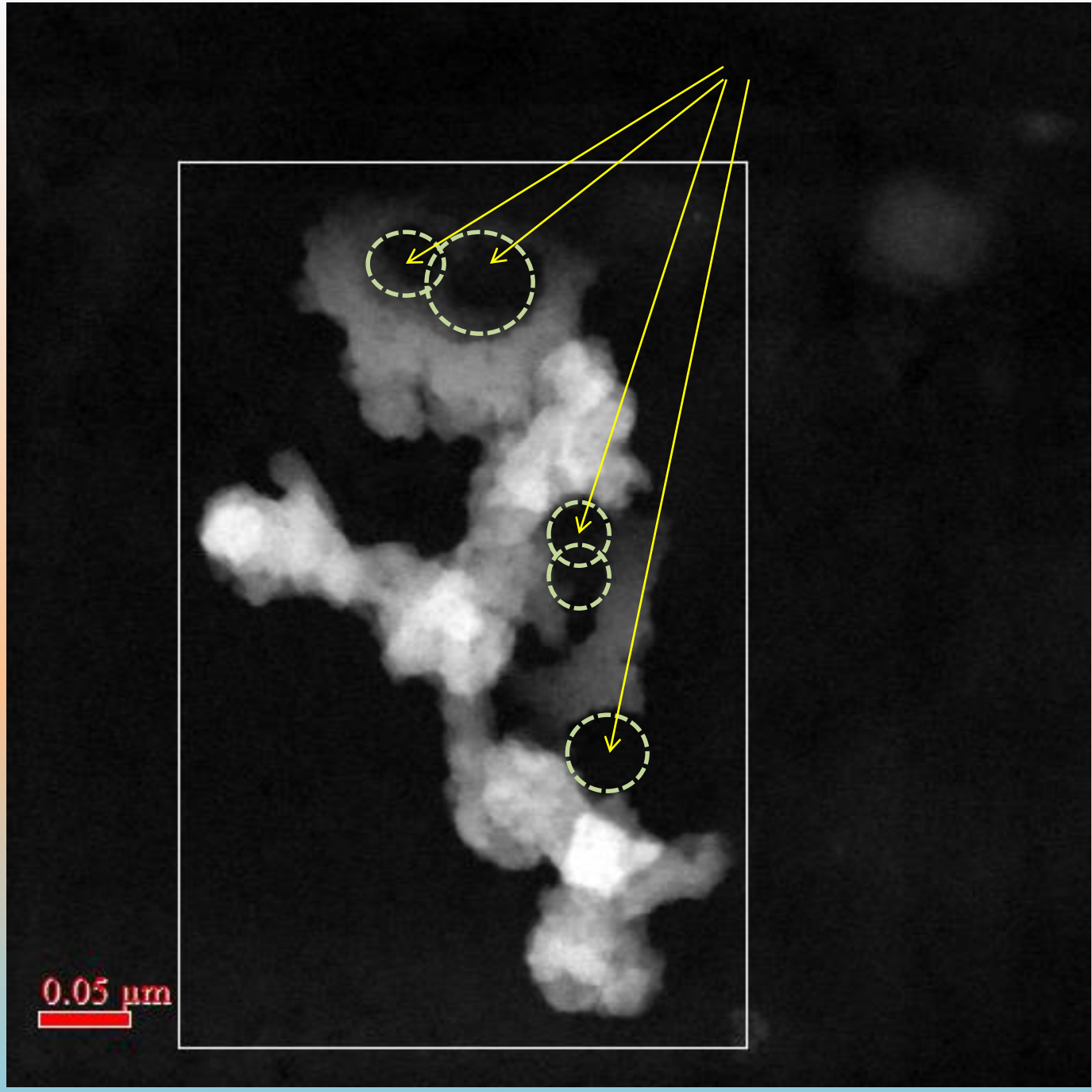

27 Day Lung
Si-
enriched
Si-enriched
(<< NPs)
Si-
Area
Si-enriched
(<< NPs)
Si-enriched
0.2 µm

Agglomerate 27 Days p.E.

- SiO_2 NPs show significant in vivo processing.

Most SiO_2 NPs lost original spherical morphology. NPs show dissolution patterns, void/pore formation and outward growth (secondary growth)



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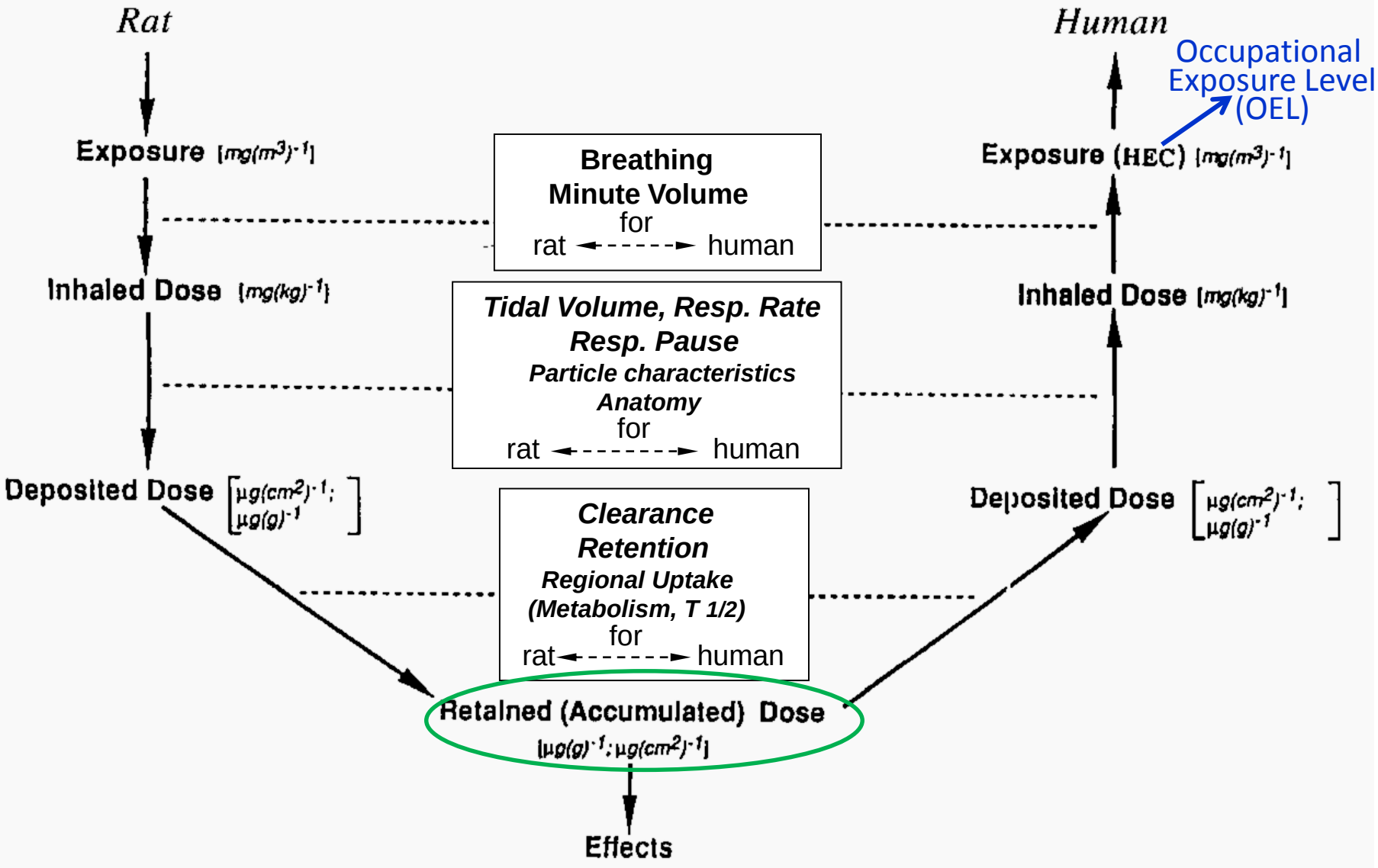


Effects may be different for both species

Modified from: Oberdörster, 1998

HEC Calculation from 4 week rat inhalation study with SiO_2 slurry aerosol for Occupational Exposure:

Deposition in **human lung** of inhaled SiO_2 aerosol of same particle size as in rat study, predicted by MPPD model with MMAD = 0.38 µm, GSD = 2.0, ρ = 0.165:
5.5 % deposition in alveolar region, **3.75 %** in tracheo-bronchial region
occupational setting: TV 1025 ml; BF 20 min^{-1} (light exercise)

Normalizing per Unit Alveolar Surface Area of Human and Rat

Surface Areas of Respiratory Tract Regions at FRC

	Rat		Human	
	cm^2	% of total	cm^2	% of total
Nasal	*18.5*	*0.75*	*210*	*0.03*
Trach-bronch	*24*	*1.00*	*4149*	*0.65*
Alveolar	*2422*	*98.25*	*634620*	*99.32*

Keyhani et al., 1997; Kimbell et al., 1997; Miller et al., 2011

CONCLUSIONs, re:
REFINING DOSIMETRIC EXTRAPOLATION MODELING

— **Appropriate allometric adjustment of respiratory parameters as input into MPPD is critical for**

- ***determining effective aerosol density during exposure***
- ***separating biosoluble from biopersistent NPs (non-inflammatory conditions)***

— **NP *in vivo* dissolution rate is important for NP characterization**

- ***dynamic "in vitro" dissolution as surrogate?***
- ***contrast with static solubility***
- ***desirable: retention/clearance kinetic in post-exposure period***
- ***need to study composition and fate of newly found secondary NPs***

— **When to use alv. surface area *vs*. lung weight for extrapolation of retained lung burden?**

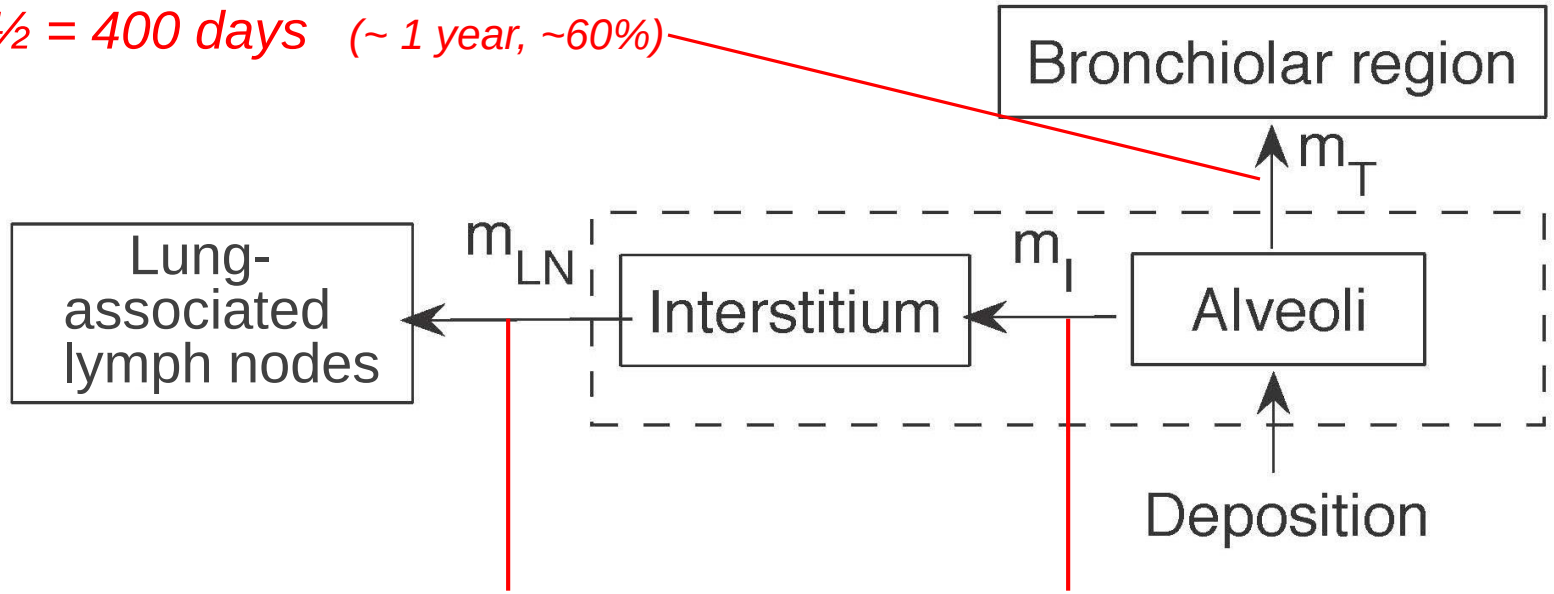
— **A well-designed 4-week inhalation study may be sufficient for risk characterization**

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)*

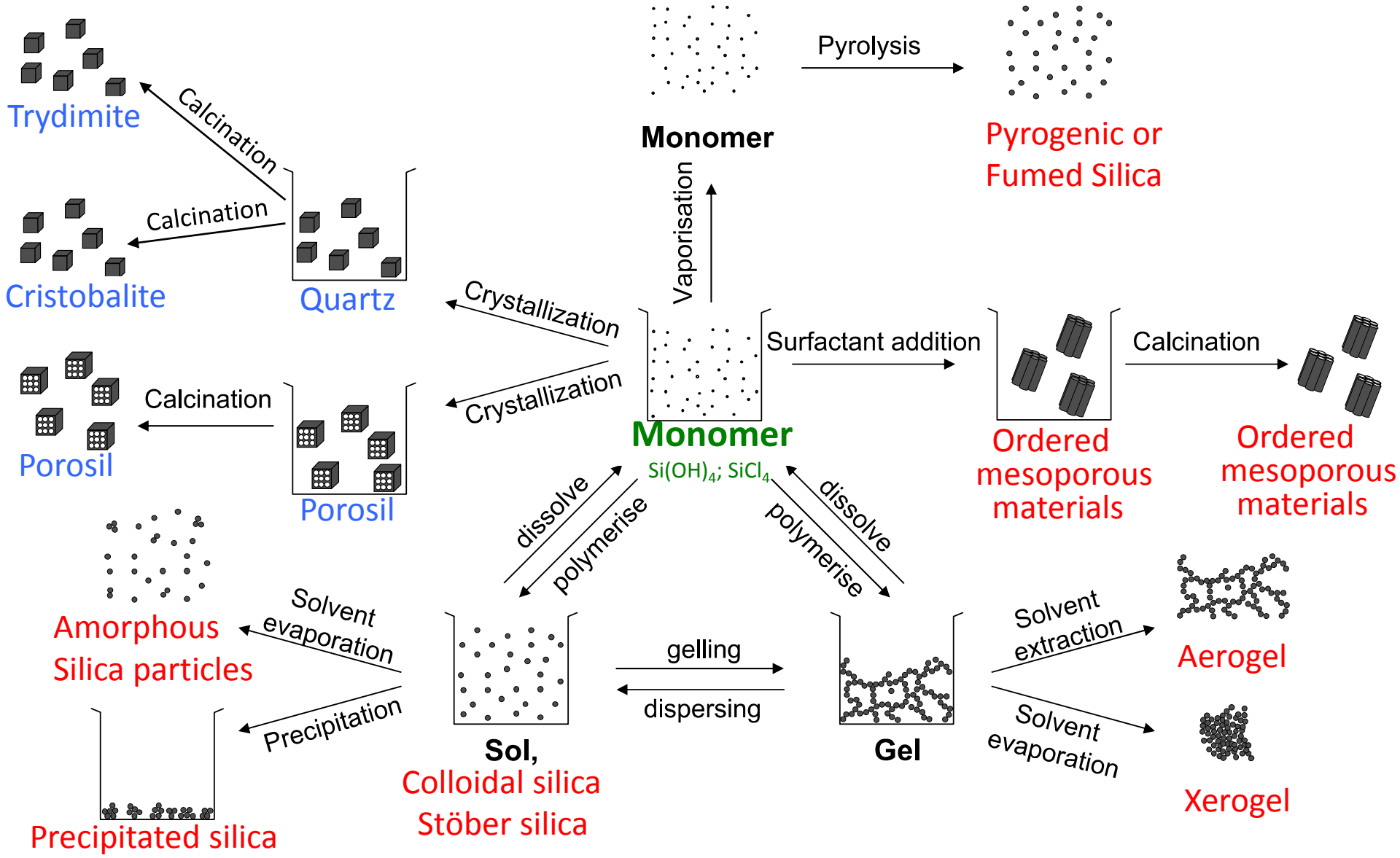
Clear. Rate = 0.001/day

T½ = 700 days (~ 2 years, ~40%)

Combined alveolar clearance: rate = 0.0027/day

T½ = 250 days (~ 0.7 years, 100%)

Amorphous and Crystalline Silica Types



Modified from Napierska et al., 2010