2nd Annual nano

BRUSSELS, 2nd 3rd October 2008

SAFETY FOR SUCCESS DIALOGUE

Health & Consumer Protection Directorate-General

Session 1: Science and Risk Assessment

Developments in Nanotoxicology:
Some Old and New Concepts

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Topics:

Respiratory Tract as Portal of Entry for Airborne Nanoparticles

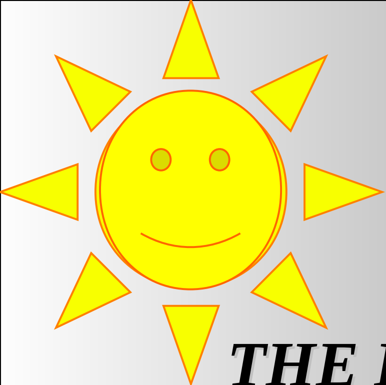
Predictive toxicity testing/extrapolation

Inhalation/Deposition in Respiratory Tract

Biokinetics, Translocation to Secondary Organs

- Central Nervous System

Elimination/Clearance



NANOTECHNOLOGY

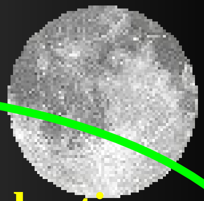
THE BRIGHT! *and* *THE DARK?*

Multiple Applications/Benefits

- **Structural Engineering**
- **Electronics, Optics**
- **Consumer Products**
- **Alternative Energy**
- **Soil/Water Remediation**
- **Nanomedicine:**
  - ***therapeutic***
  - ***diagnostic***
  - ***drug delivery***
  - ***cancer***
  - ***nanosensors***
  - ***nanorobotics***

Consumer Fears/Perceived Risks

- **Safety: Potential adverse effects**
- **Environmental Contamination**
- **Inadvertent Exposure** *(inhalation, dermal, ingestion)*
- **Susceptible Subpopulation**
- **Societal Implications**



- **Nanotoxicology/Safety Evaluation:** ***supplies information for risk***
  -assessment,
  -management,
  -communication

Safety Evaluation of Nanomaterials through Characterizing Nanoparticle Hazard and Risk:

Nanoparticle "X" is cytotoxic, nanoparticle "Y" is not.

At what dose? What assay?

Even benign nanoTiO_2 at high enough doses is fibrogenic, and in rats carcinogenic

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Need:

Developing and Validating simple non *in vivo* method(s)

for predicting in vivo responses

- **cell-free assays** ***(ROS generating potential; chem-reactivity; solubility)***
- **cellular assays** ***(primary cells; cell-lines; co-cultures [from primary and secondary organs])***
- **Future: in silico models?**

Predictive Toxicity Testing/Extrapolation

Controversy:

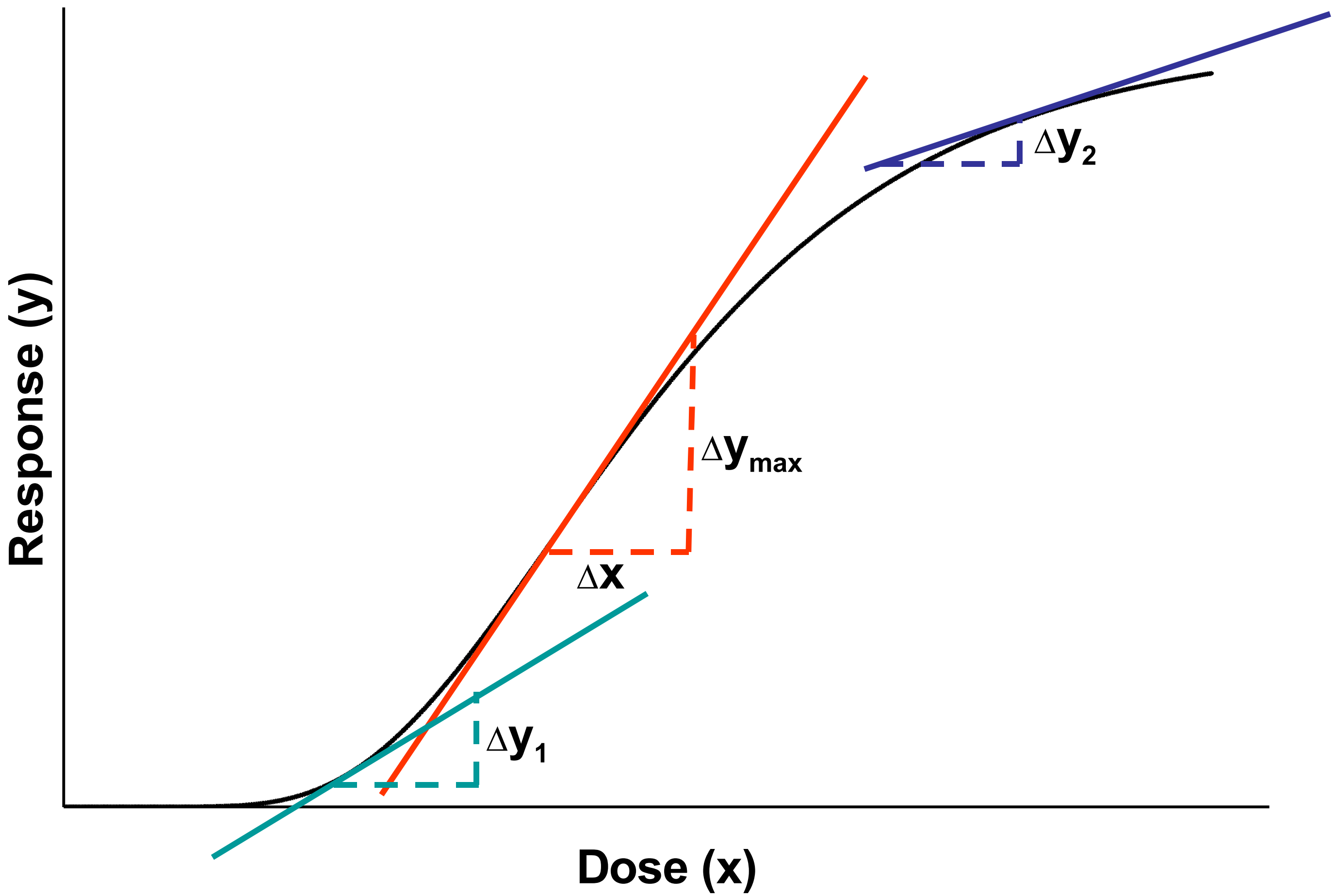
— **in vitro studies do not predict in vivo response**

— **in vitro studies can predict in vivo response**

— ***how to compare doses and responses between in vivo and in vitro studies?***

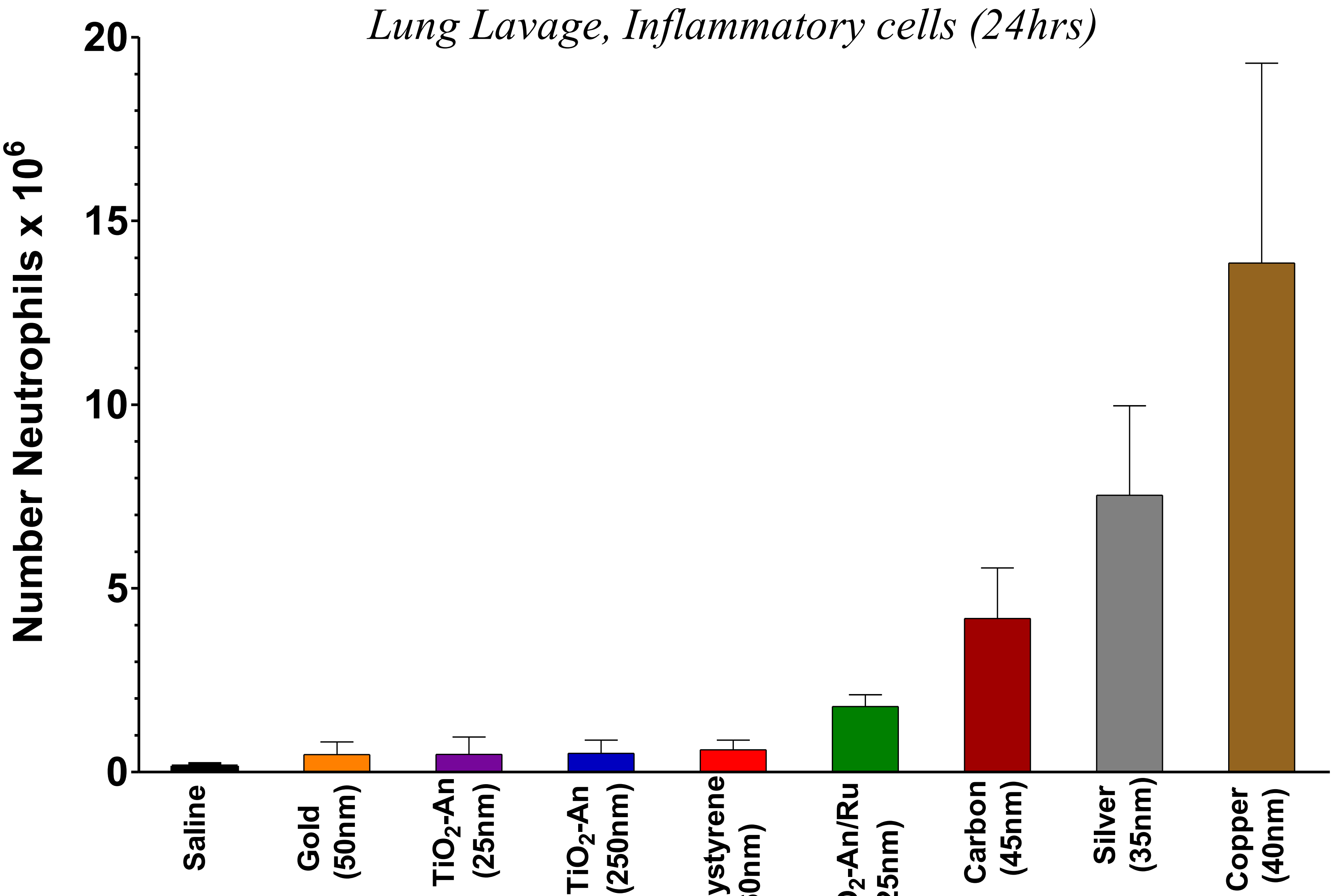
concept of equivalent doses and response metric

Slope (or response per dose) is dose dependent



Intracheal Instillation of 100μ g Particles in Rats

Copper = 25ug



Rushton et al, 2008

<u>Conclusions: For Assessing NP Toxicity</u>

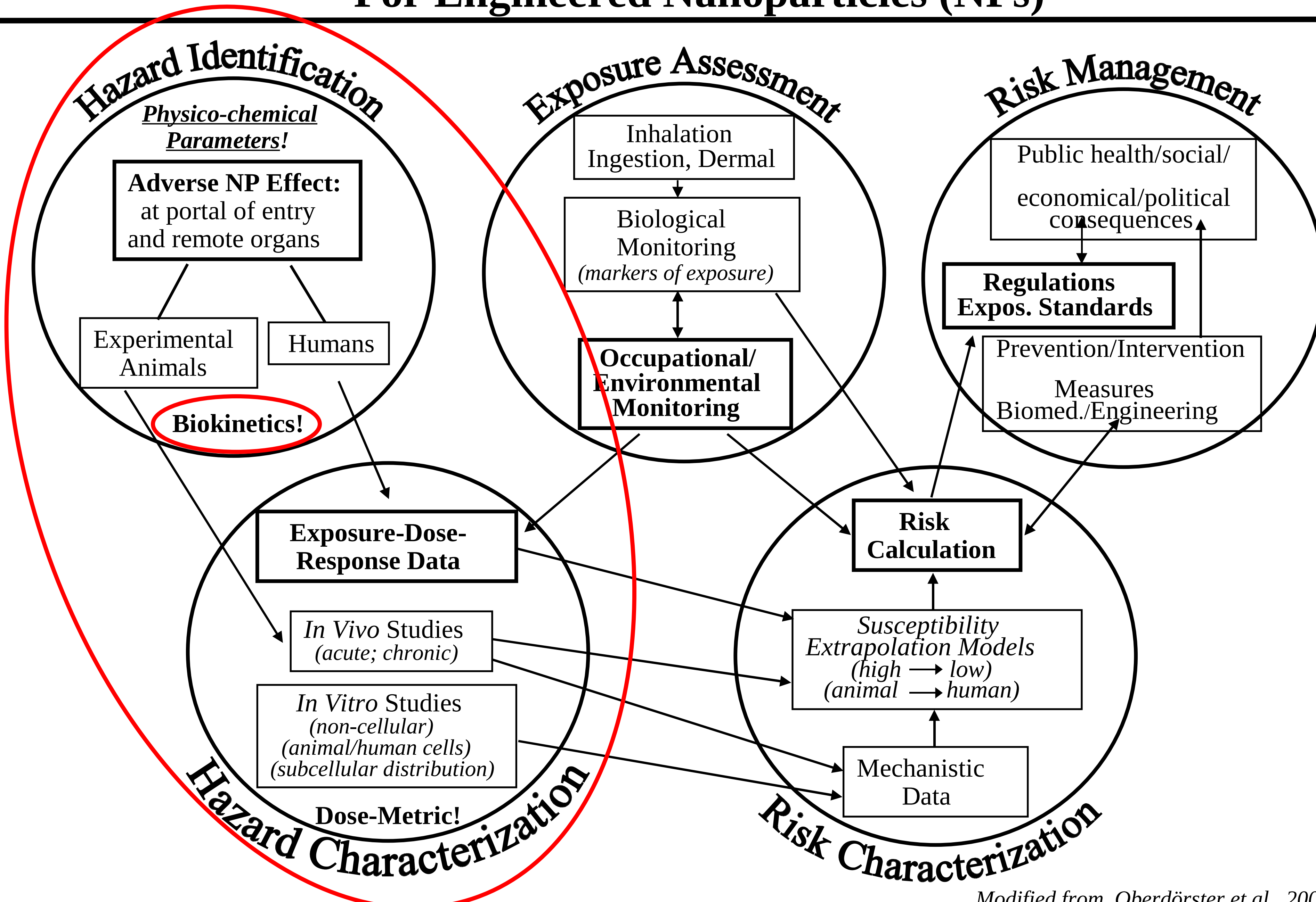
- **Dose-Metric as well as Response-Metric should be considered**

- **Expressing NP chemical or biological activity per unit surface area provides additional information for NP characterization**

- **This concept allows selection of an appropriate dose for comparing responses among different assays, with predictive value**

- ***Limitations*:** Only Hazard Identification and Characterization,
  not complete Risk Assessment
  Only acute toxicity

Risk Assessment and Risk Management Paradigm For Engineered Nanoparticles (NPs)



Modified from Oberdörster et al., 2005

Inhalation of Nanoparticles

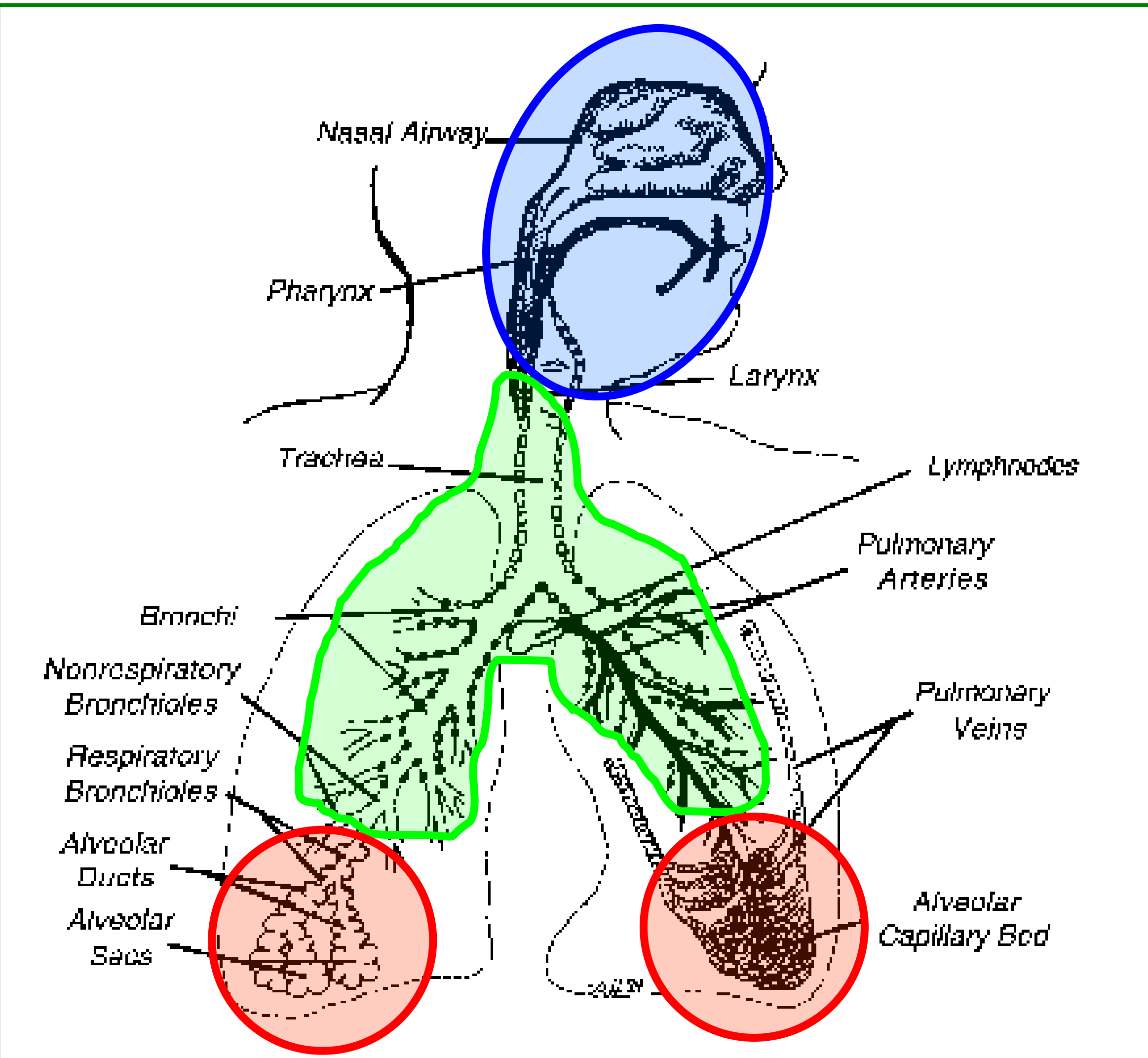
Misunderstanding:

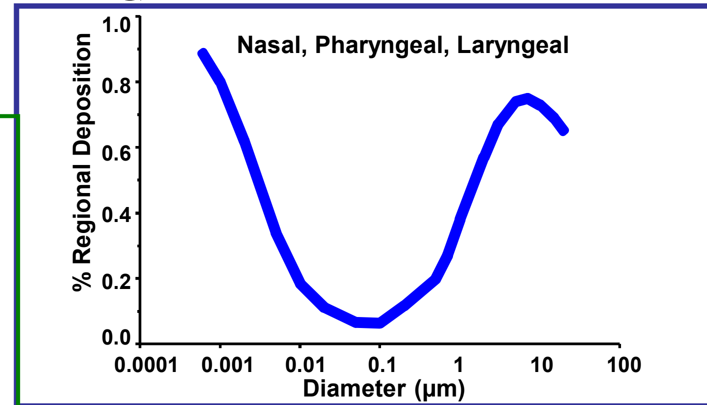
— **the smallest NPs (<10 nm) will not be deposited in the respiratory tract but are exhaled again**

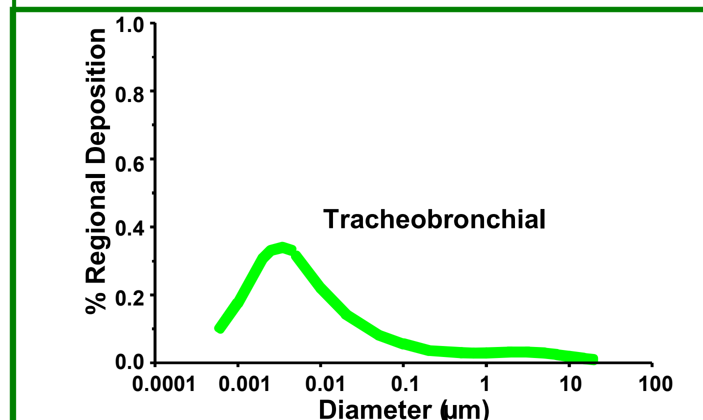
— **the smaller the NPs are, the deeper they penetrate into the peripheral lung**

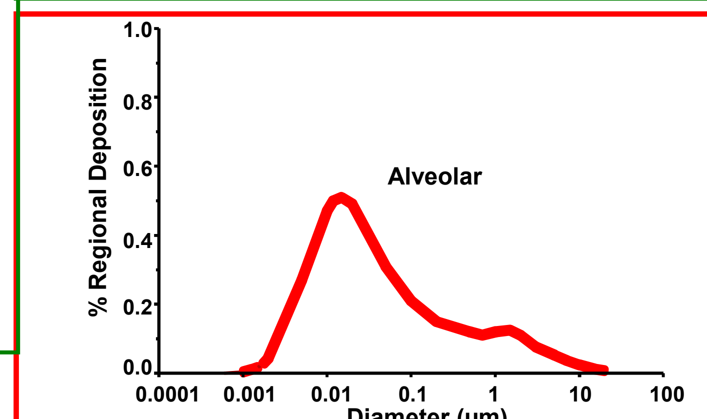
Fractional Deposition of Inhaled Particles in the Human Respiratory Tract

(ICRP Model, 1994; Nose-breathing)



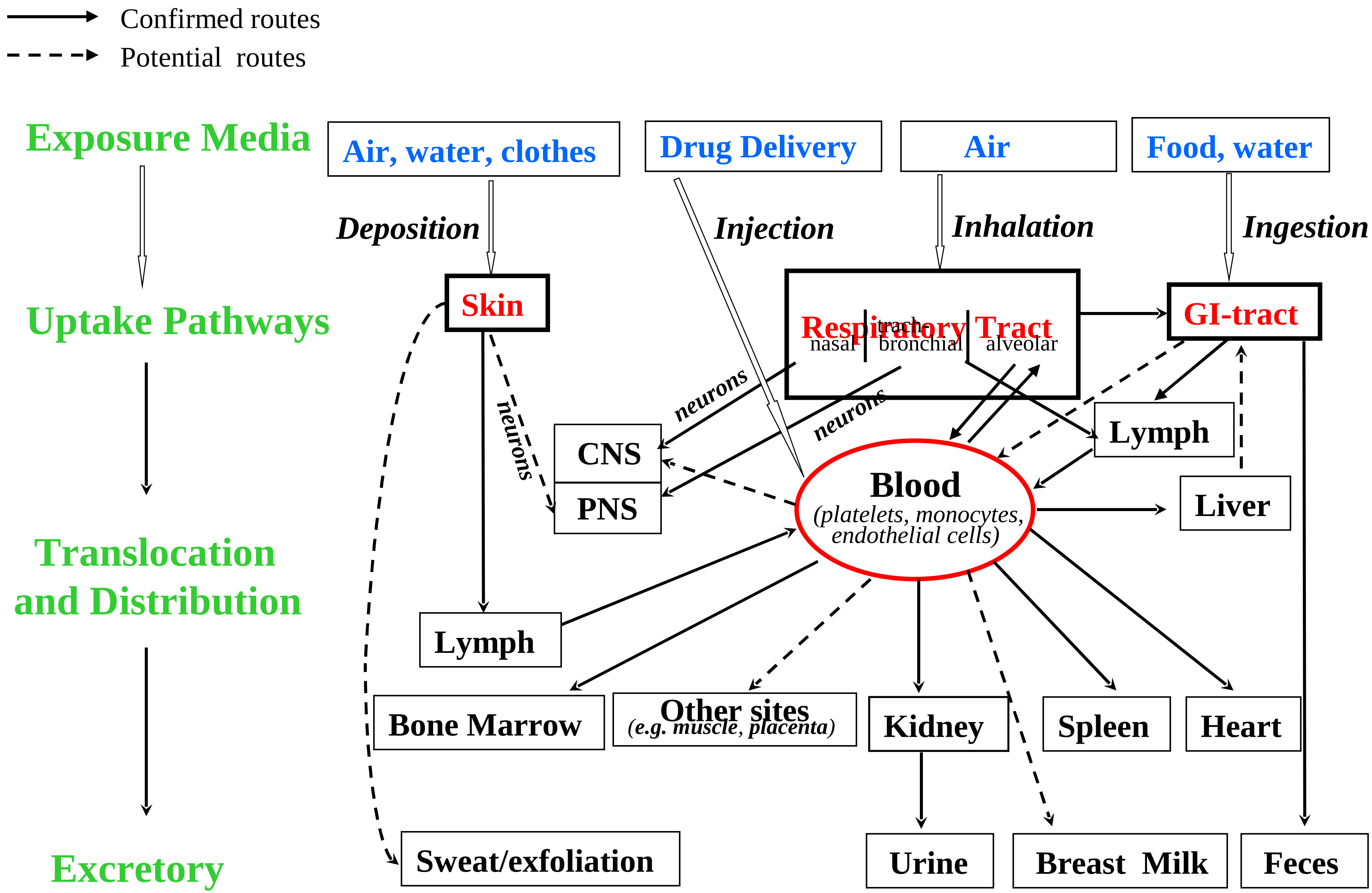






Oberdörster et al, 2005

Exposure and Biokinetics of Nanosized Particles



Translocation rates are largely unknown!

(Oberdorster et all, 2005)

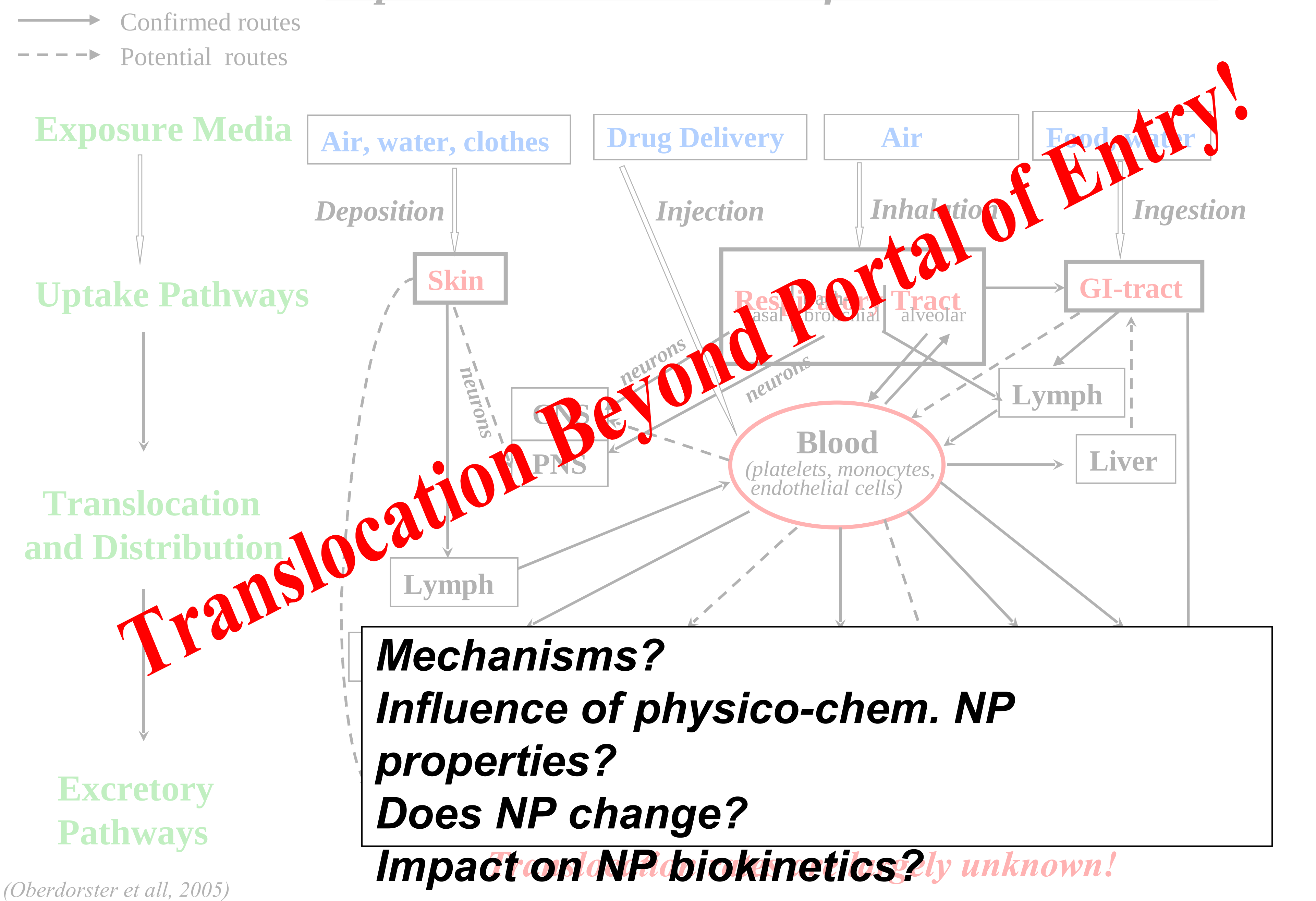
Translocation to Secondary Organs

Misconception:

— **NPs depositing in the respiratory tract translocate easily and to a high degree to extrapulmonary organs, e.g. the brain**

— **the biodistribution of NPs entering the blood circulation from lung deposits is the same as that of iv. administered NPs**

Exposure and Biokinetics of Nanosized Particles



Mechanisms?
Influence of physico-chem. NP properties?
Does NP change?
Impact on NP biokinetics?

“Concept of Differential Adsorption”

Modified from Müller and Heinemann, 1989

NP physicochemical properties:

- Size
- Surface Area
- Charge
- Surface hydrophobicity
- Redox activity
- Others

Body compartment media:

- Respiratory tract lining fluid
- Gastrointestinal milieu
- Plasma proteins
- Other mucosal membranes
- Intra/extracellular fluid

↓ ***determine*** ↓

protein/lipid adsorption patterns

↓ ***determine***

localisation in target tissues/cells

Altered plasma composition in disease state is likely to change adsorption pattern

Hypothesis:

NP size, surface coating and portal of entry

affects particle biodistribution

Testing Hypothesis: Administration of Nanogold to Rats

2 Portals of Entry

LUNG MICROSPRAY –or– **IV INJECTION**

50 µg Au | **15 µg Au**

Lung Lavage Analysis

Respiratory Tract
Epithelial Lining Fluid
Lipids/Proteins

Blood
Plasma Proteins

Secondary Organs
Biodistribution/Uptake

3 Sizes:
5, 50, 200 nm

3 Coatings:
Citrate
Serum albumin
PEG 5kD, 20kD

2 Time-points:
1 and 24 hours

Analysis:
NAA or ICP-MS

Conclusions from translocation studies of gold NPs delivered to lower respiratory tract:

Minimal translocation into blood ciculation and extrapulmonary organs:

< 3-20 ngAu/mL blood (after 50 µg i.t.)

To be considered for design of in-vitro studies when assessing impact of nanoparticles originating from deposits in the respiratory tract:

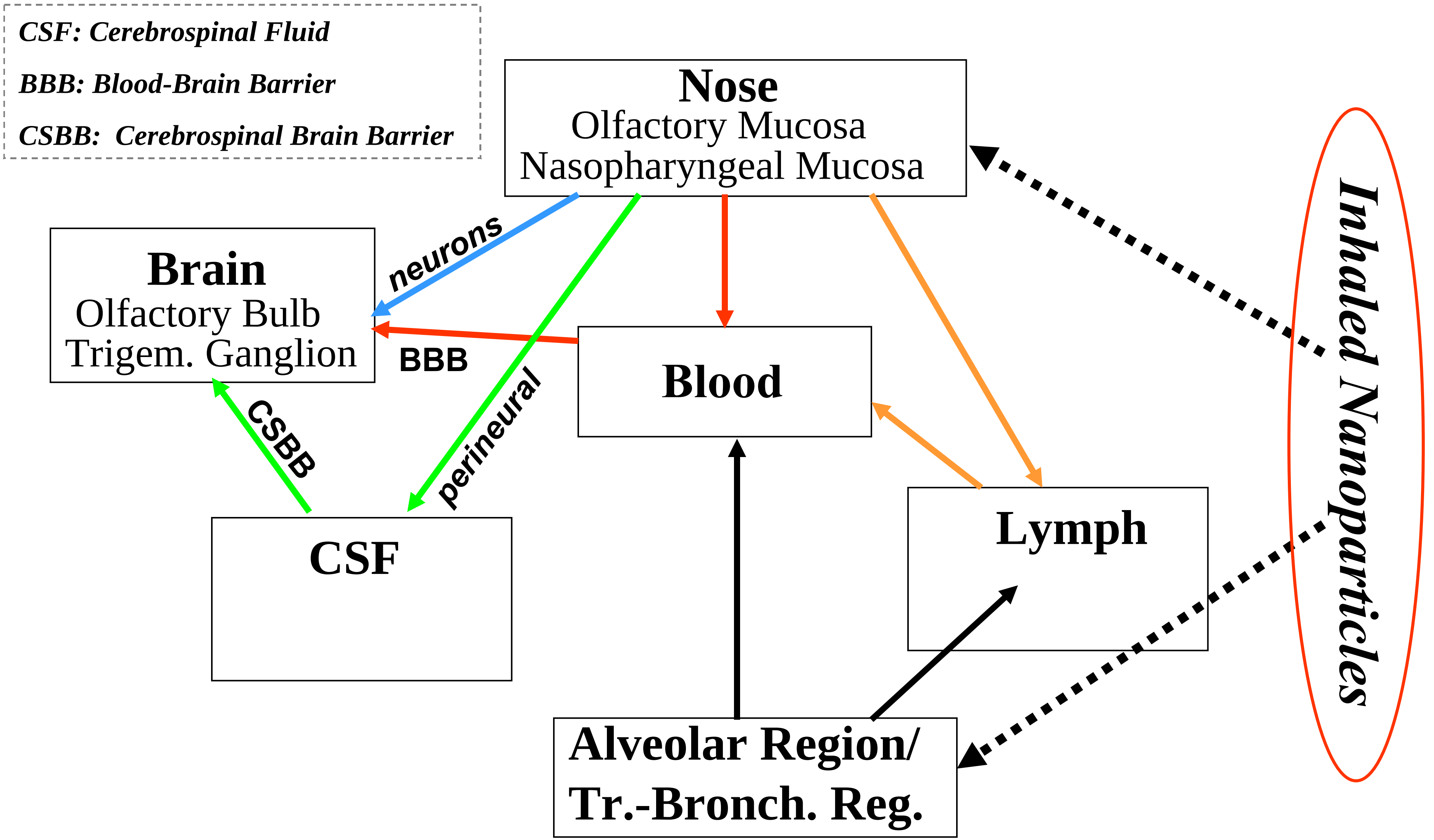
Consider relevance of doses and dose rates!

Table 1: Identified plasma proteins bound to 50:50 NIPAM–BAM copolymer particles after centrifugation. *(Cedervall et al., 2007)*

Protein	MW [kDa]	Peptides (#)	P[a]	Lipoprotein[b]
apolipoprotein AI[c,d]	28	29/8	1	yes
apolipoprotein AII[c]	9	3/1	0.9	yes
apolipoprotein AIV[c]	43	18/15	1	yes
apolipoprotein E[c]	34	12/15	1	yes
HSA[c]	69	10/25	1	
fibrinogen, alpha	66	10	1	
orosomucoid 1	22	9	1	
paraoxonase 1	40	8	1	yes
C4BP α-chain	67	6	1	
apolipoprotein D	19	4	1	yes
IgM heavy chain	50	3	1	
CETP[e]	53	2	1	yes
galectin-3-binding protein	63	2	1	yes
Ig kappa chain	12	1	1	
LCAT[f]	47	1	1	yes

[a] Protein prophet score. [b] Protein known to associate with lipoproteins. [c] Identified in two independent experiments. [d] Previously identified.[11] [e] Cholesteryl ester transfer protein. [f] Lecithin–cholesterol acyltransferase.

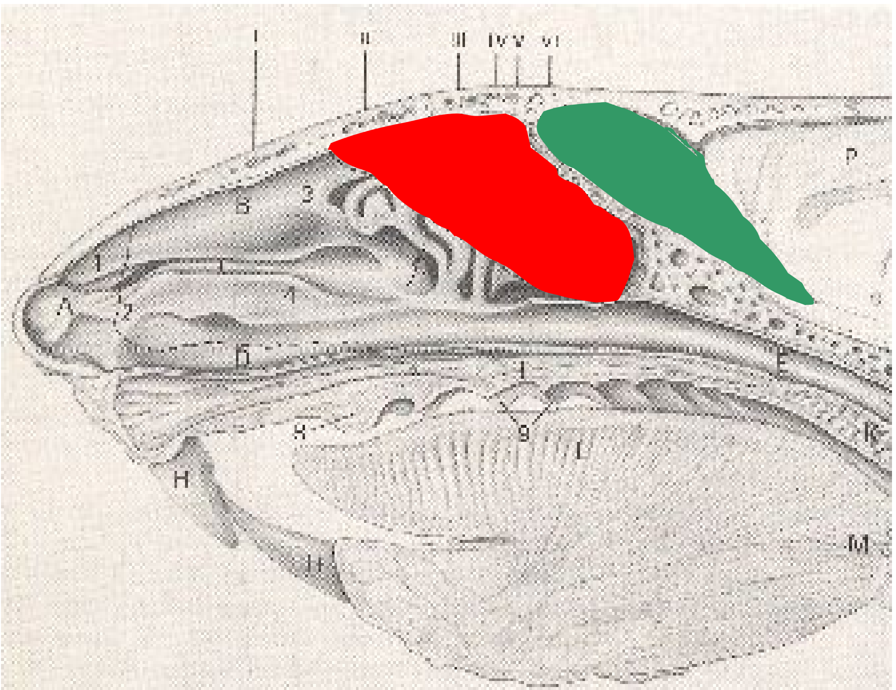
FROM RESPIRATORY TRACT TO BRAIN: POTENTIAL TRANSLOCATION PATHWAYS OF NANOPARTICLES

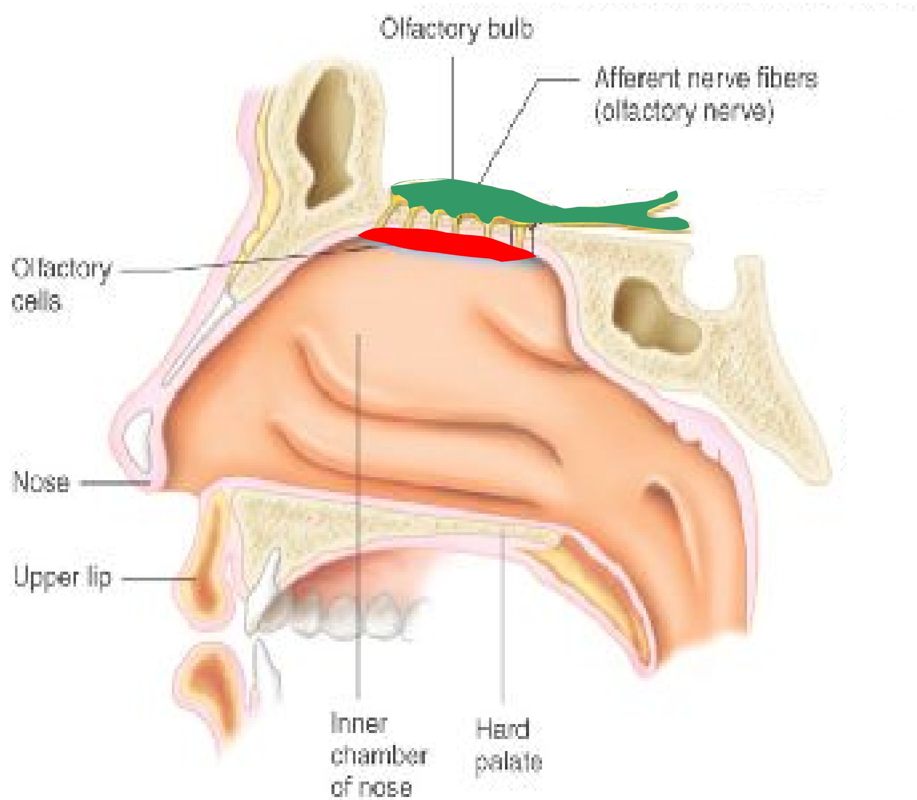


Olfactory Nerve Translocation Pathway:

Comparison of Rat and Human Olfactory Region

Olfactory mucosa **Olfactory bulb**

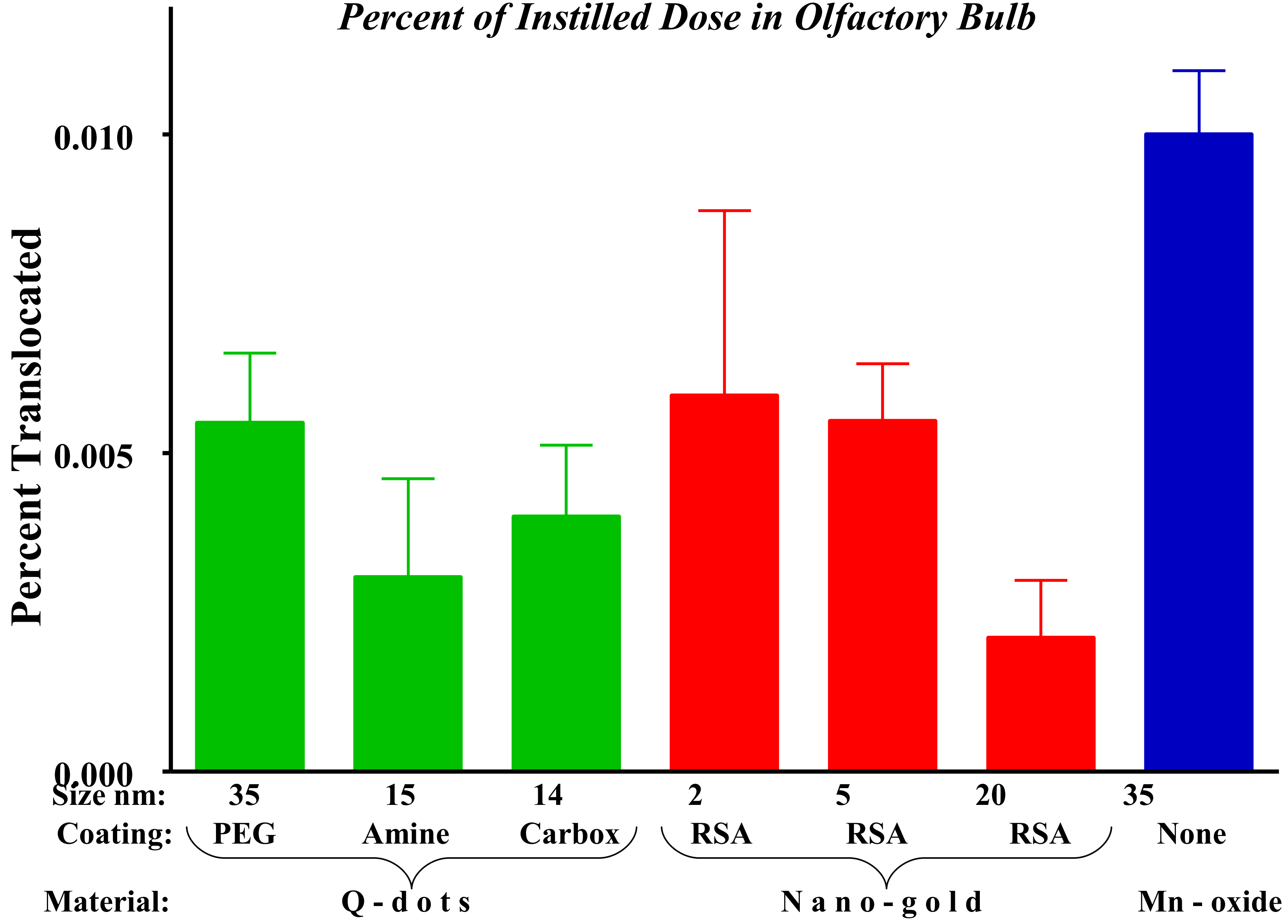




From Hebel & Stromberg, 1976

McGraw-Hill Co., Inc.

Nanoparticle Translocation to Olfactory Bulb
Left Intranasal Instillation, Rats
Percent of Instilled Dose in Olfactory Bulb

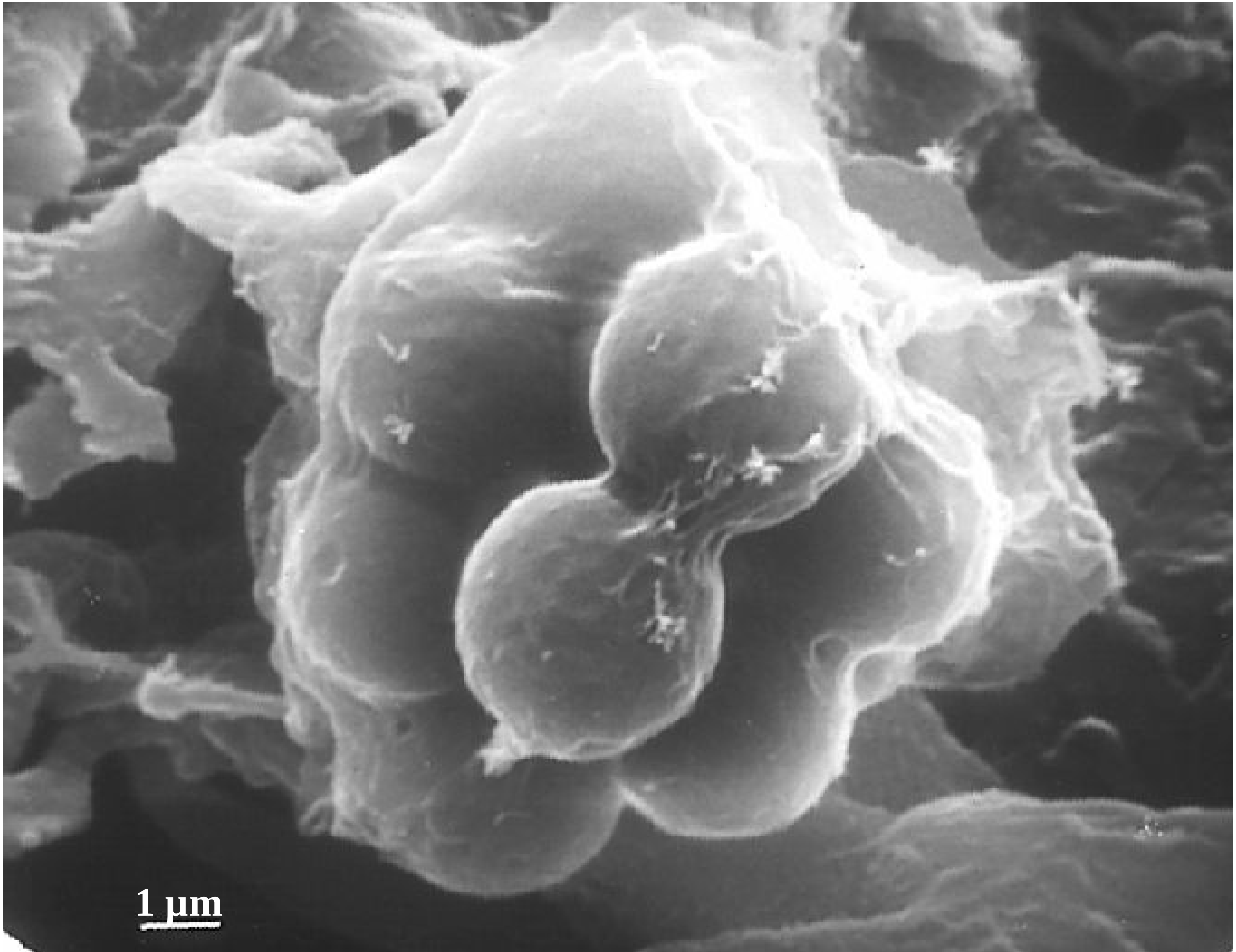


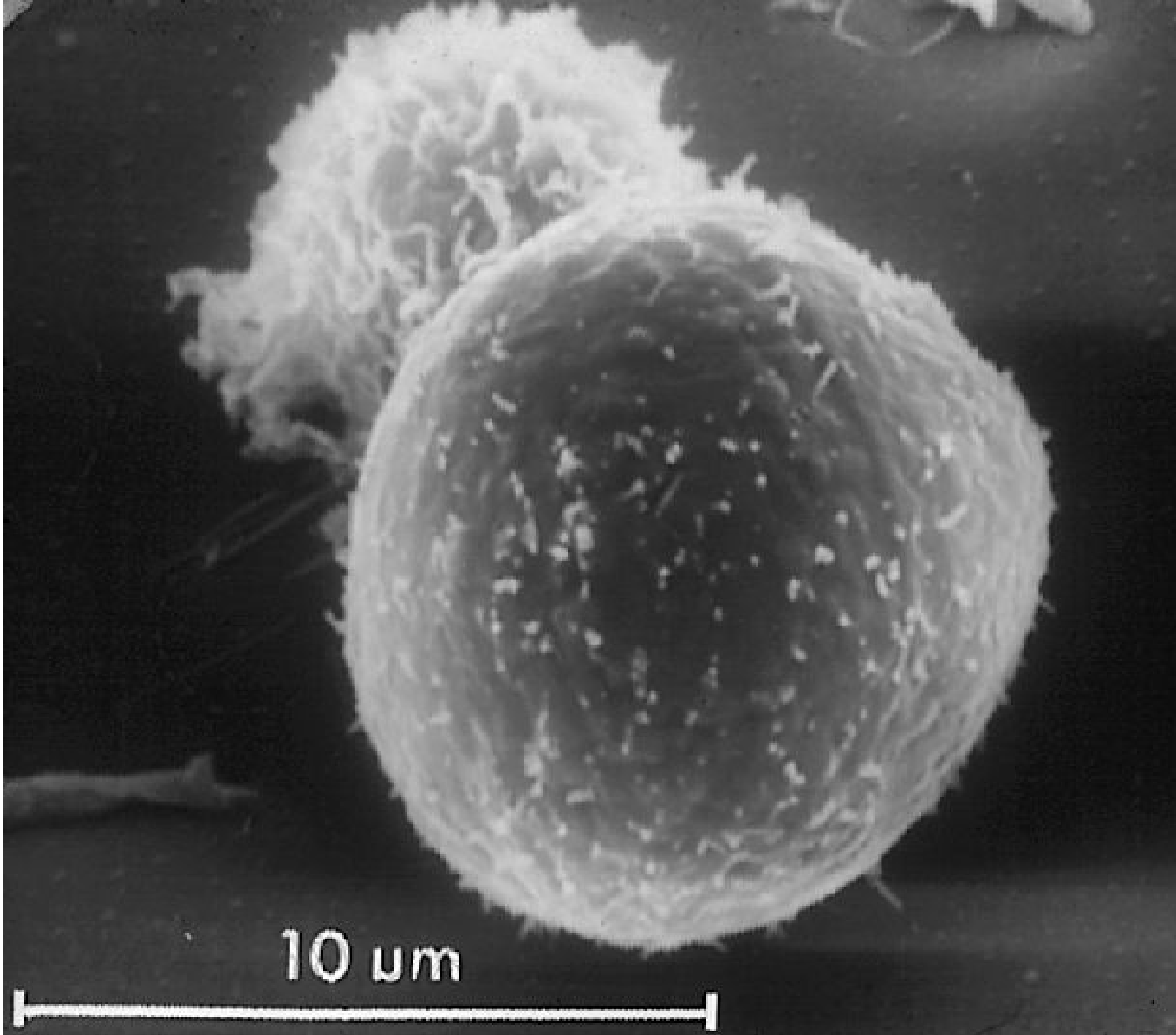
Elimination of Nanoparticles

Belief:

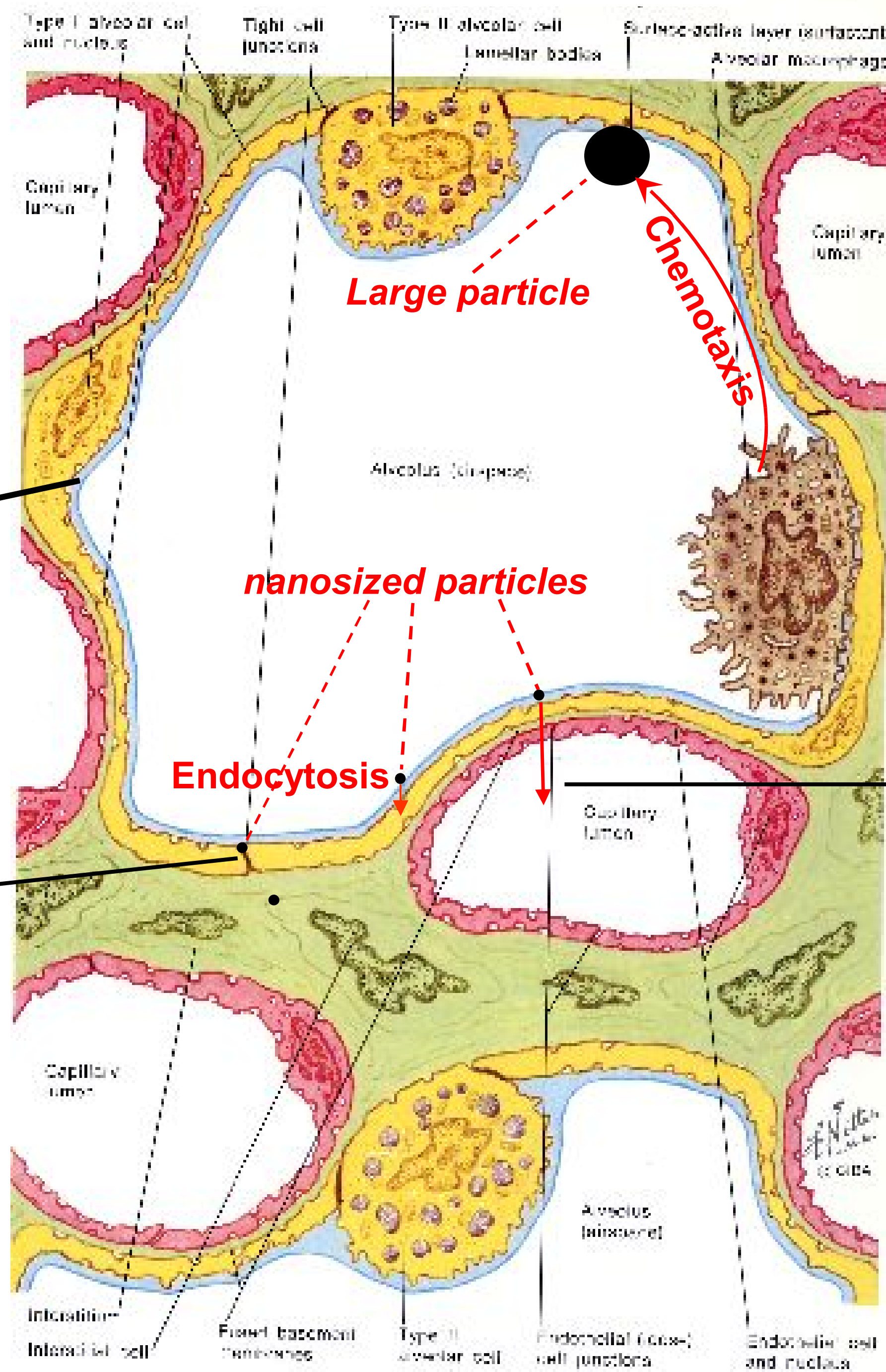
- **alveolar macrophages effectively phagocytize NPs and remove them from the lung**

- **there is no effective elimination of nanoparticles retained in body organs**


1 µm


10 um

Ultrastructure of Pulmonary
<u>Alveoli and Capillaries</u>

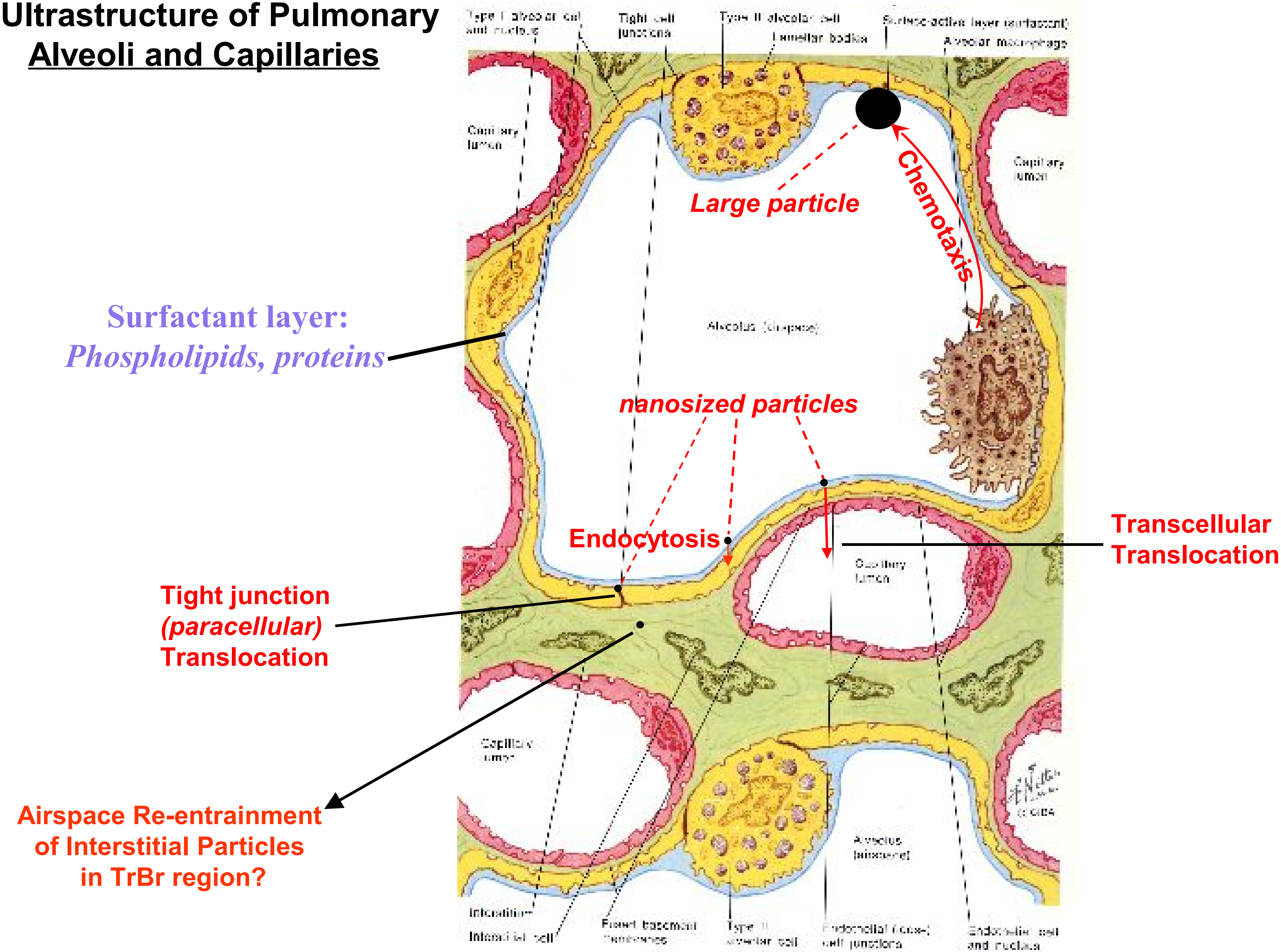


Surfactant layer:
Phospholipids, proteins

Tight junction
(paracellular)
Translocation

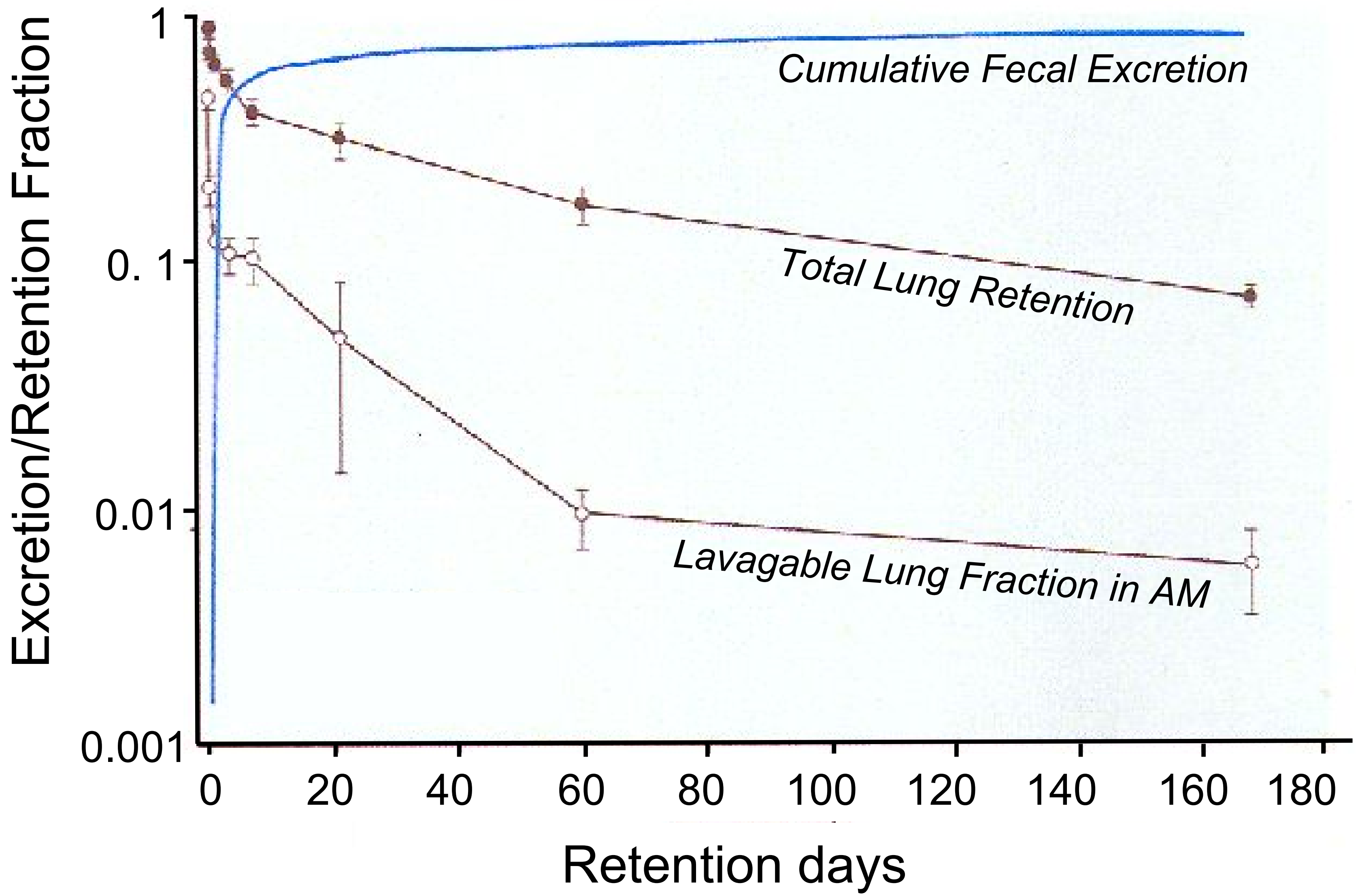
Transcellular
Translocation

Ultrastructure of Pulmonary <u>Alveoli and Capillaries</u>



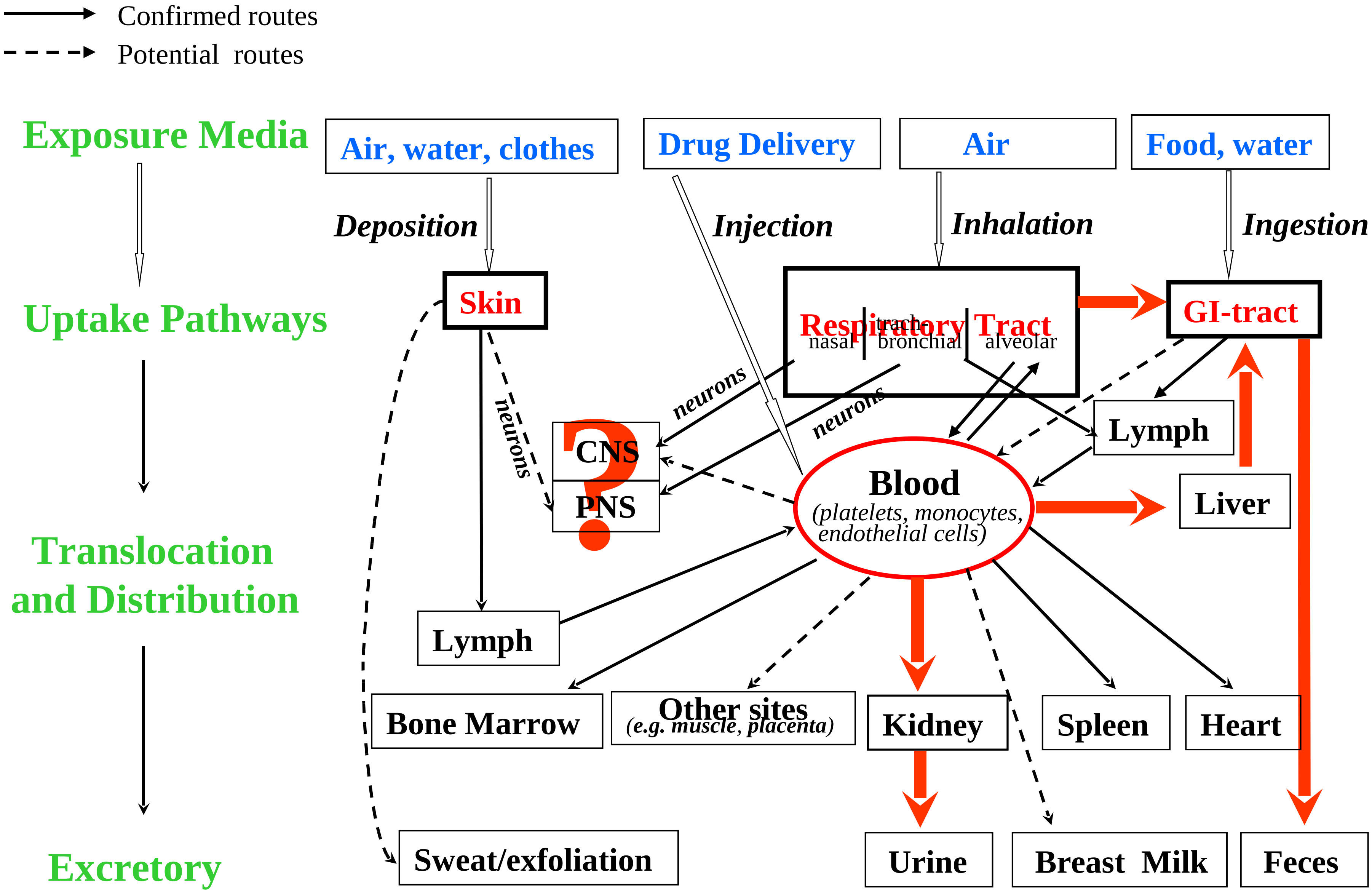
Long-term retention and excretion of ^{192}Ir NPs *(17 to 20nm)*

after a single 1 to 1.5 hr. inhalation in rats



From Semmler-Behnke et al., 2007

Elimination Pathways of Nanosized Particles



GI-tract and kidney as major excretory organs?

(Oberdörster et all, 2005)

Multidisciplinary University Research Initiative Team *[Sponsor: DoD (Airforce)]*

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• • • Collaborations • • •

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