5-Я МЕЖДУНАРОДНАЯ НАУЧНО-ПРАКТИЧЕСКАЯ КОНФЕРЕНЦИЯ

«НАНОМАТЕРИАЛЫ И ЖИВЫЕ СИСТЕМЫ»

NANOMATERIALS AND LIVING SYSTEMS

NLS-2018

# Assessing the Pulmonary Toxicity of Nanoparticles: Contemporary Approaches and Models

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# Outline

- Nanoparticles (NP) – why they are special.
- Pulmonary exposure to NP.
- ~~Modern approaches and models.~~
- Foundations of the quality toxicological study:
  - Material characterization;
  - Model relevance;
  - Dosing relevance;
  - Descriptive -> hypothesis-driven science.

# Disclaimer

- Going to omit a lot of stuff intentionally/unintentionally, e.g.:
  - Some trivial nanotechnology things;
  - Detailed mechanisms, translocation to other organs/tissues etc.;
  - Detailed exposure scenarios;
  - Recent presidential elections;
  - …

# Nanoparticles – Why So Special?

- Nanotechnology – manipulations at the nanometer-scale.
  - Nanomaterials vs. nanoparticles?

- By 2020 - $1 trillion impact and 2-5 million exposed workers. General consumers?

From Antonini J. 2018

# Nanoparticles – Why So Special?

- Types of NPs:
  - Engineered – usually specific properties.
  - Incidental – mostly heterogenous properties.

- Toxicity of micro- vs. nano- could be quite(!) different if on the mass basis.

# Nanoparticles – Why So Special?

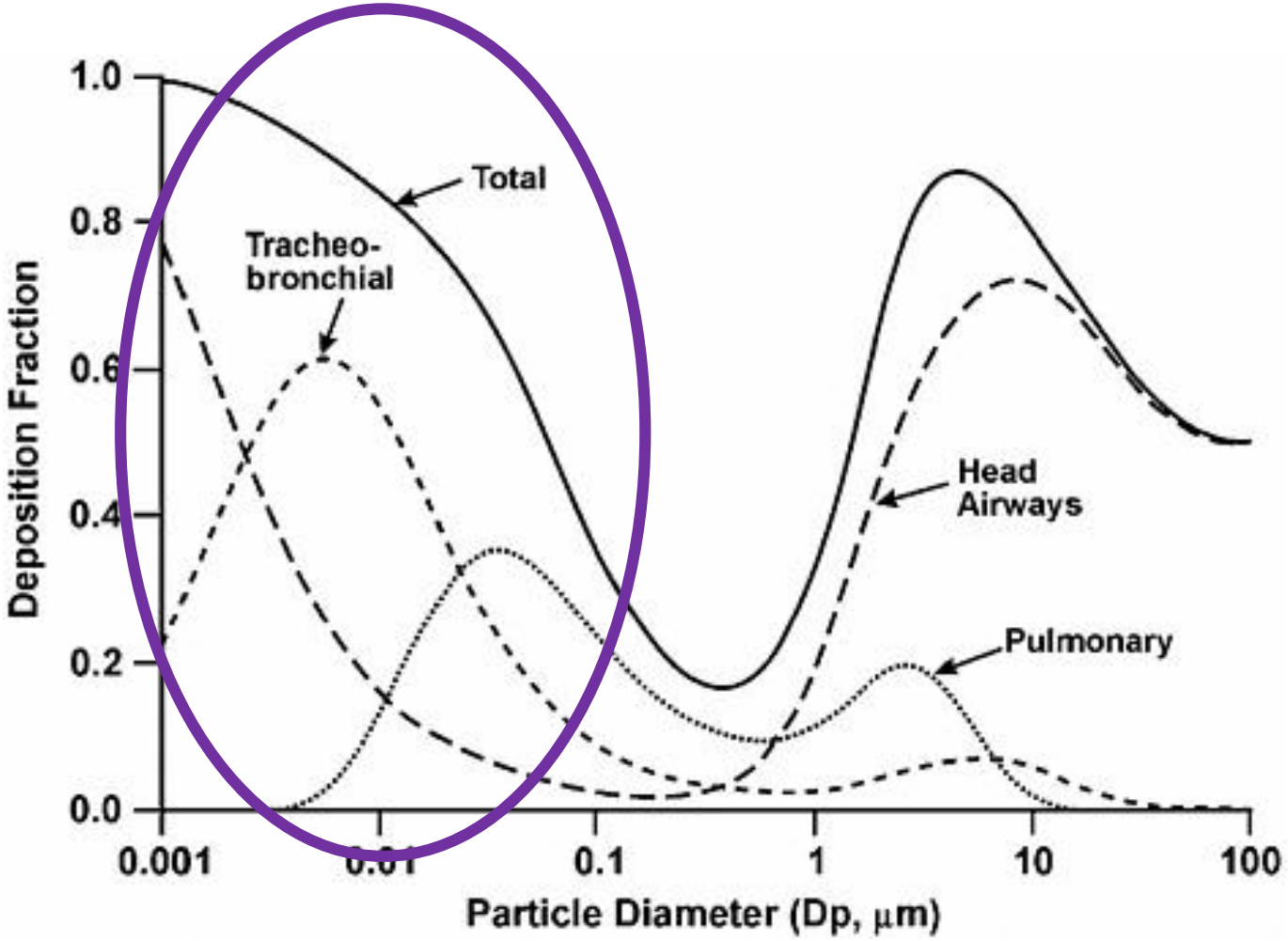
- Research challenges:
  - Sampling – needs special instruments.
  - Dose metrics – mass? Surface area? Number? Charge?
  - Particles above 100 nm size – should we not treat them as NPs?
  - Agglomerated particles.

# Pulmonary exposure to NPs

- Workers in:
  - Manufacturing;
  - Handling;
  - Incidental contact.

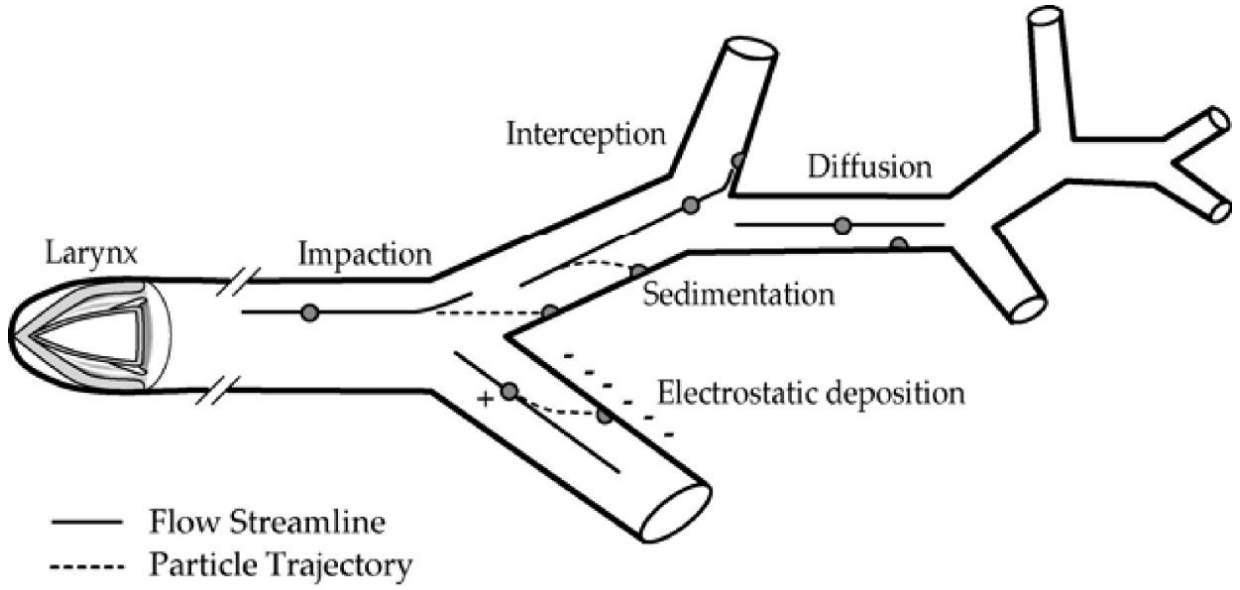
- End-users/consumers.

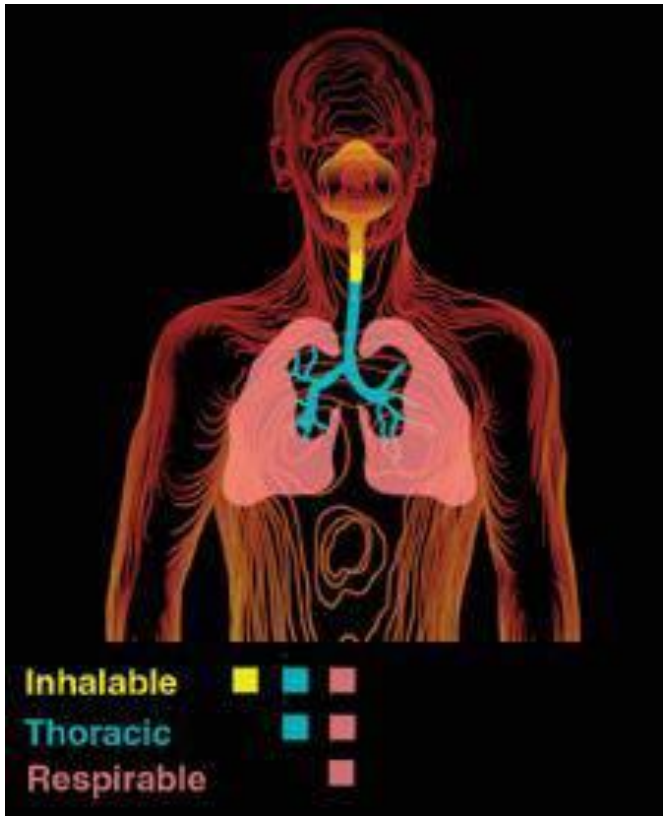
# Fate of the Inhaled Particles




Snipes, MB. 1994. Biokinetics of inhaled radionuclides. In: Internal Radiation Dosimetry. OG Raabe, ed. Madison, WI: Health Physics Society.

# Particle Deposition Respirable vs. Inhalable



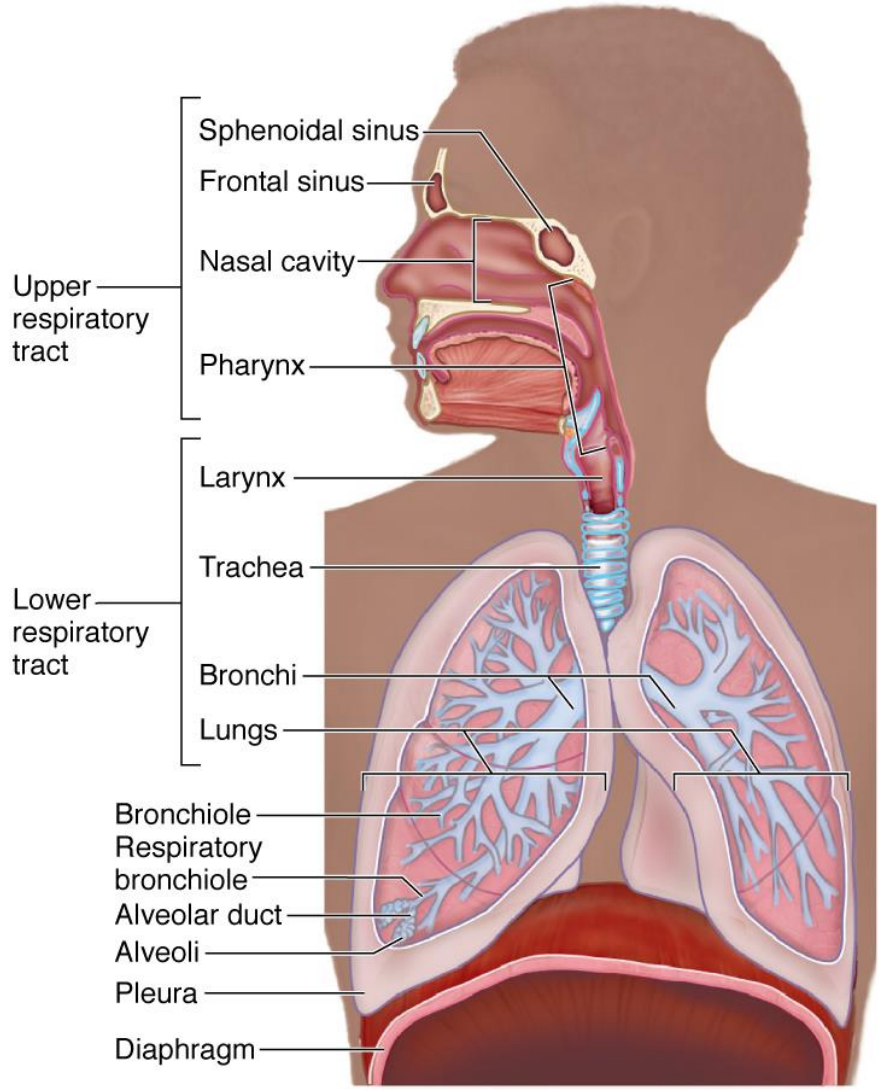

Mossman et al. 2011. Pulmonary Endpoints (Lung Carcinomas and Asbestosis) Following Inhalation Exposure to Asbestos, Journal of Toxicology and Environmental Health, Part B, 14:1-4, 76-121,

# Physical Characteristics that Affect Deposition

- Aerodynamic size:
  - Diffusion at smaller sizes.
  - But! Remember the agglomeration issues.
- Particle shape:
  - Interception vs. Impaction.
- Hygroscopicity.
- Electrical charge:
  - Electrostatic deposition.

# Clearance After Deposition

- Physical clearance
  - **Extrathoracic**:
    - Mostly swallowed,
    - Some by blowing the nose.
  - **Tracheobronchial**:
    - Mucociliary “elevator” → swallowed;
    - 24 – 48 h clearance time.
  - **Pulmonary**:
    - Collection by macrophages;
    - Slow clearance; months…
- Dissolution-absorption
  - Dependent on physical and chemical properties.




From: Chapter 17. The Respiratory System. In: Mescher AL. eds. Junqueira's Basic Histology: Text & Atlas, 13e New York, NY: McGraw-Hill; 2013.

# Foundations of the Quality Study

- **Material characterization**
- Model relevance
- Dosing relevance
- Descriptive -> hypothesis-driven science.

# Data from the Field Exposure Assessments

- Particle-size information, depending on type of instrument used:
  - MMAD and GSD,
  - Type of particle-sizing instrument used, including manufacturer name and location,
  - Sampling time and flow rate,
  - Calibration method for the particle sizing instrument,
  - …

# Material Characterization and Preparation

- The more you know, the better:
  - Morphology,
  - Structural make-up,
  - Chemical composition,
  - Solubility,
  - Surface properties,
  - Size distribution,
  - Biopersistence (!),
  - …

# Material Characterization and Preparation

- Dispersion medium is extremely important:
  - pH – increased/decreased dissolution
  - Chemical composition:
    - Corona formation
    - Particle agglomeration
  - Acellular ROS production
  - Etc…

# Nanoclays *in vitro* toxicity study

- Chemical composition:
  - Fourier Transform Infrared Spectroscopy (FTIR, Digilab FTS 7000) equipped with diamond Attenuated Total Reflection (ATR).
- Surface morphology and elemental composition:
  - Hitachi S-4700 Field Emission Scanning Electron Microscope (Hitachi High-Technologies Corporation) with an energy dispersive X-ray spectroscopy (EDX).
- Size distribution:
  - Dynamic light scattering (DLS) via the Mastersizer 2000 with a Hydro 2000S accessory (Malvern Instruments).
- Sedimentation studies:
  - Changes in absorbance upon different incubation times when using an Evolution 300 UV–VIS spectrophotometer (Thermo Scientific).

Wagner, Alixandra et al. “Toxicity Evaluations of Nanoclays and Thermally Degraded Byproducts through Spectroscopical and Microscopical Approaches.” *Biochimica et biophysica acta* 1861.1 Pt A (2017): 3406–3415. *PMC*. Web. 21 Mar. 2018.

# Nanoclays *in vitro* toxicity study

- Thermal degradation confirmed the loss of organic modifiers.
- All of the samples formed agglomerates with size distribution a function of chemical signature, cell culture media favoring larger agglomerates than PBS.
- All of the samples had around 85% or more particles settled by 6 h, greatest sedimentation within the first 3 h.

Wagner, Alixandra et al. “Toxicity Evaluations of Nanoclays and Thermally Degraded Byproducts through Spectroscopical and Microscopical Approaches.” *Biochimica et biophysica acta* 1861.1 Pt A (2017): 3406–3415. *PMC*. Web. 21 Mar. 2018.

# Know Thy Limits!

- Dynamic Light Scattering (DLS)
  - Accurate estimate for monodisperse nanoparticle solutions,
  - For polydisperse populations DLS is biased towards larger particles.
- Light Microscopy
  - Can’t see the actual nanoparticles, but agglomerates can be seen,
  - Advanced methods (darkfield microscopy) may provide good source of data,
  - Confocal microscopy for the cell uptake studies.
- Electron Microscopy
  - Not always a good option for biological particles,
  - Cannot image the surface groups without the use of cryogenic methods,
  - Requires high vacuum, thin sample sections and sample drying.

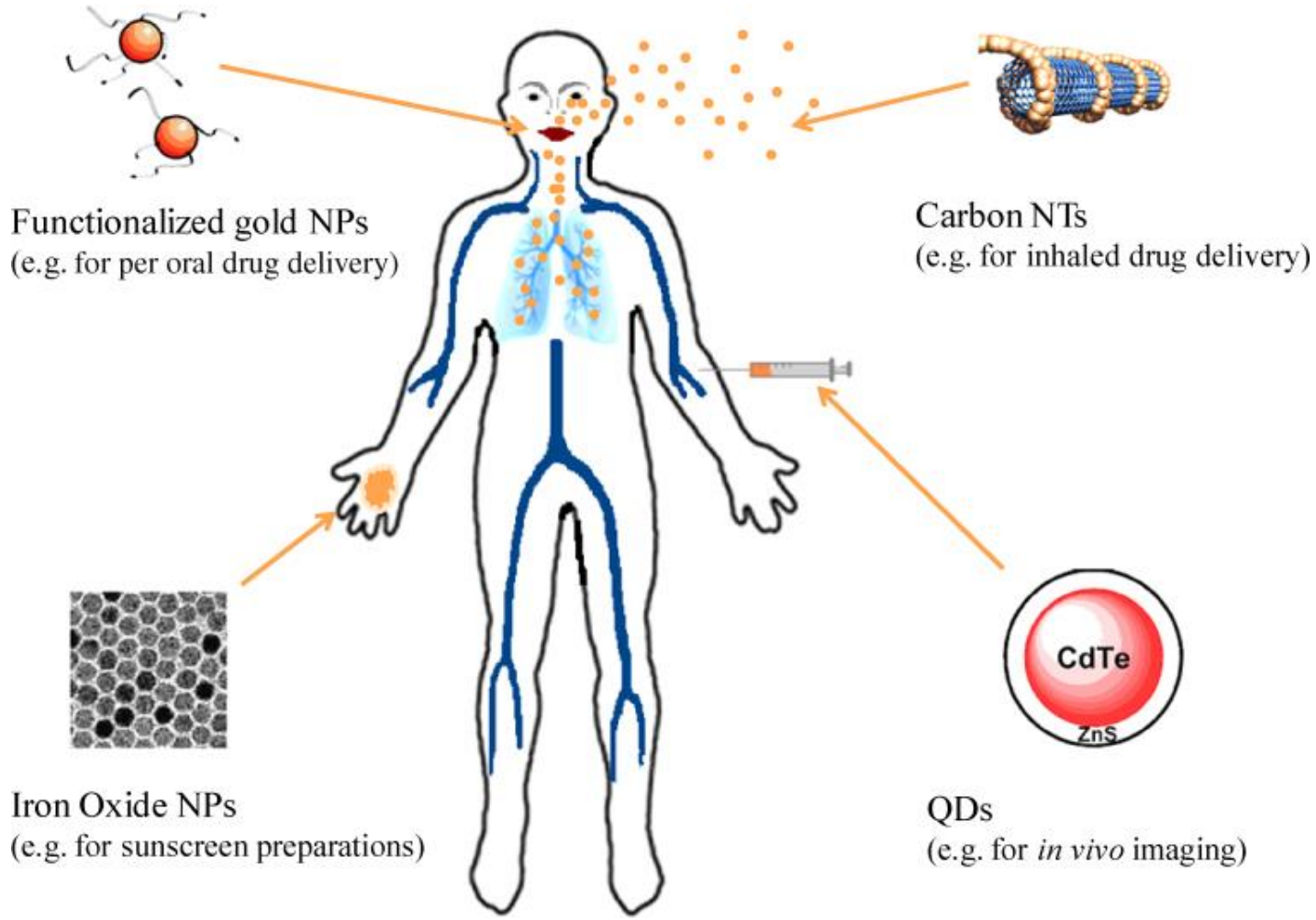
# Further Reading


- Sharma, A., Madhunapantula, S. V., & Robertson, G. P. (2012). Toxicological considerations when creating nanoparticle based drugs and drug delivery systems? *Expert Opinion on Drug Metabolism & Toxicology*, *8*(1), 47–69.

# Foundations of the Quality Study

- Material characterization
- **Model relevance**
- Dosing relevance
- Descriptive -> hypothesis-driven science.

# Human Model – Perfect!




From: Yildirimer L, Thanh NT, Loizidou M, Seifalian AM. Toxicology and clinical potential of nanoparticles. Nano Today. 2011 Dec;6(6):585-607.

# *In Vivo* Models First thing First

- Investigators must show how they have considered alternative methods and procedures that address animal welfare concerns.

- A consideration of alternatives is usually addressed by responding to “the Three Rs” (**Refinement**, **Replacement**, and **Reduction**).

# Three Rs

- Refinement - decreasing pain or distress by modifying the husbandry or experimental procedures.
- Replacement - using methods that avoid or replace the use of animals by substitution with non-animal systems or less sentient animals.
- Reduction - using strategies that result in fewer animals being used or maximizing the information obtained from the number of animals being used.
- Some links to help with the concepts:
  - http://awic.nal.usda.gov/alternatives
  - http://altweb.jhsph.edu/

# *In Vivo* Models

- Financially and technically it is not possible to assess all currently known NPs *in vivo*.

- According to estimates, comprehensive long-term testing would cause costs of $1.18 billion and require 34–53 years (2009 paper).

The impact of toxicity testing costs on nanomaterial regulation. Choi JY, Ramachandran G, Kandlikar M Environ Sci Technol. 2009 May 1; 43(9):3030-4.

# *In Vivo* Models

- Mostly rodents. Poorly representative models for human inhalation exposure -> instillation / aspiration exposures
- Respiratory tract in animals vs. human:
  - Nasal vs. oronasal breathers
  - Bronchial tree structure, bronchial wall thickness
  - Size of alveolar macrophages and their number per alveolus
  - Kinetics and efficacy,  sensitivity to toxicants
  - Lining fluid chemical composition
  - Etc.

# Inhalation Exposure Systems

- Pros:
  - More physiological.
  - More even inhaled particles distribution.
  - Highly relevant for particle deposition/translocation/clearance.
- Cons:
  - Costly. For nanomaterials especially.
  - Safety concerns.
  - Need technical staff.
  - Uncertain exposure for individual animals.

# Instillation / Aspiration

- Pros:
  - Delivered dose is more accurate.
  - Simple/Safe/Cheap.
  - No skin/pelt exposure.
  - Can test components of the mixture (i.e. welding fumes)
- Cons:
  - Non physiological, overload issues, clearance compromised.
  - Upper respiratory tract bypass and uneven distribution .
  - Vehicle may affect the responses.

# What to use?

- Inhalation:
  - All types of pulmonary response studies if resources and technical support allow it.

- Instillation / Aspiration:
  - Comparative pulmonary toxicity,
  - Component analysis.

# What to use?

- A number of specific rodent strains exist to accommodate various study design and hypotheses:
  - Cancer-prone or resistant animals,
  - Knock-out and knock-in animals,
  - Humanized strains,
  - …

# *Ex Vivo* models

- Isolated perfused lung models from rats, guinea pigs, and rabbits (Byron and Patton 1994)
- Precision-cut lung slices (Ressmeyer et al. 2006)
- Whole excised tissues (bronchial/tracheal epithelium)
- Isolated cells (alveolar macrophages, lymphocytes etc.)

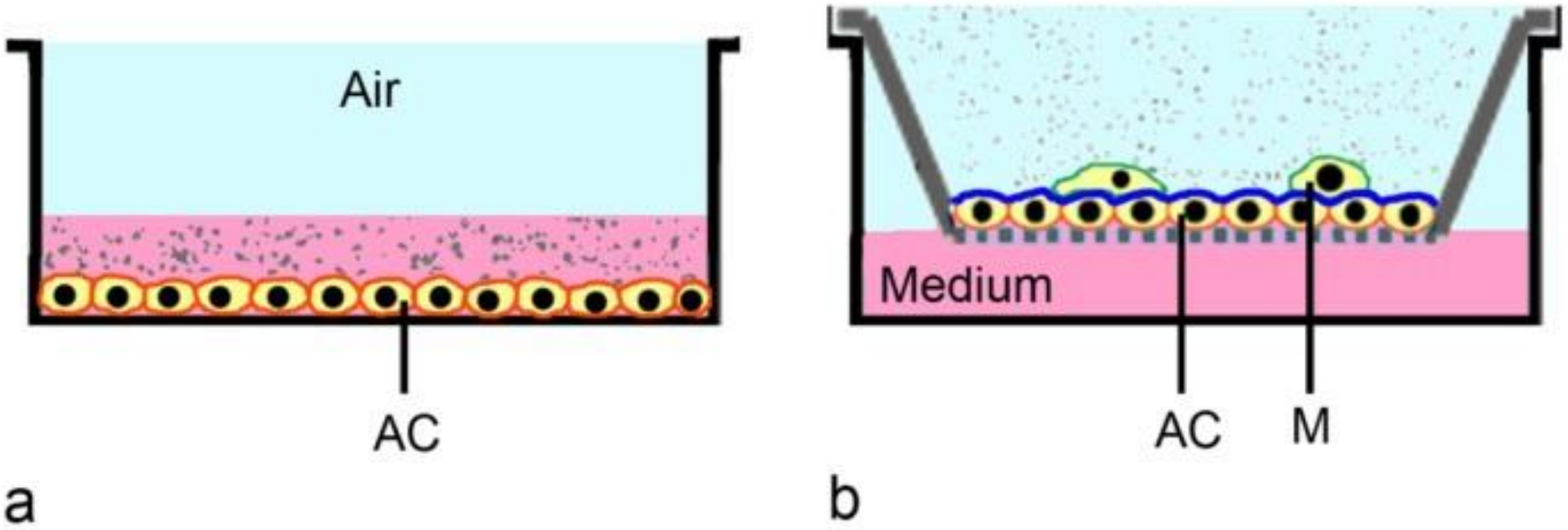
Characterisation of guinea pig precision-cut lung slices: comparison with human tissues. *Ressmeyer AR, Larsson AK, Vollmer E, Dahlèn SE, Uhlig S, Martin C Eur Respir J. 2006 Sep; 28(3):603-11.*

Drug delivery via the respiratory tract. *Byron PR, Patton JS J Aerosol Med. 1994; 7(1):49-75.*

# *In Vitro* Models

- Single cell type models
  - Mechanistic studies.
- Co-culture models
  - Usually immortalized cell lines, since primary cells have limited life span and variations in their quality due to donor and preparation differences.
  - Contain mainly respiratory cells and cells of the immune system.
  - Disease-relevant co-culture models (obstructive lung diseases) may also include fibroblasts.

# *In Vitro* Models




(**a**) Exposure of alveolar cells (AC) in submerged culture.
(**b**) AC cultured in air-liquid interface exposed to nanoparticle-loaded aerosol.

From Fröhlich, E., & Salar-Behzadi, S. (2014)

# *In Vitro* Models

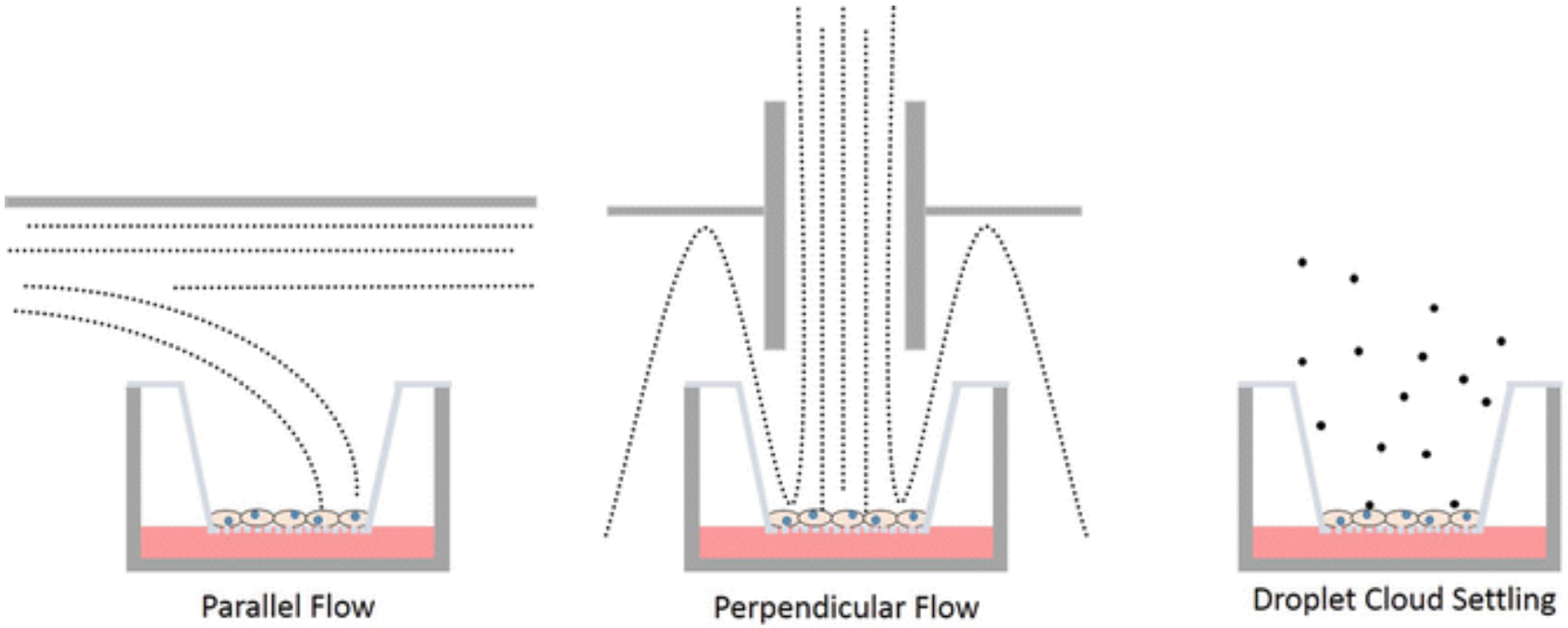
- Commercial co-culture models are based on bronchial epithelial cells obtained from hospitals or patients donations and are optimized for specific applications.

- Cells are well differentiated, produce mucus, show beating cilia, and maintain differentiation over a period of several months

Human primary bronchial lung cell constructs: the new respiratory models. Bérubé K, Prytherch Z, Job C, Hughes T Toxicology. 2010 Dec 30; 278(3):311-8.

# *In Vitro* Models

- Air-Liquid Interface (ALI)-Based Exposure Systems




Lewinski N.A., Liu N.J., Asimakopoulou A., Papaioannou E., Konstandopoulos A., Riediker M. (2017) Air–Liquid Interface Cell Exposures to Nanoparticle Aerosols. In: Petrosko S., Day E. (eds) Biomedical Nanotechnology. Methods in Molecular Biology, vol 1570. Humana Press, New York

# Further Reading


- Fröhlich, E., & Salar-Behzadi, S. (2014). Toxicological Assessment of Inhaled Nanoparticles: Role of *in Vivo*, *ex Vivo*, *in Vitro*, and *in Silico* Studies. *International Journal of Molecular Sciences*, *15*(3), 4795–4822.

# Choosing Relevant Methods

- Sample collection *in vivo*:
  - Broncho-alveolar lavage or fixation or digestion or all together(!);
  - Whole blood/serum/plasma;
  - Harvesting other relevant organs.
- Sample collection *in vitro*:
  - Live/fixed cells;
  - Cell supernatants: (volume!);
  - Cell lysates;
  - Post-processing (organelle fractionation etc.).

# Comparative *Ex Vivo* and *In Vitro* Study Design

C57BL6/J mouse

AM isolation

Isolated AMs

Cytokine/chemokine response

NPs

Cytotoxicity

RNA sequencing

THP-1 human monocytic cells

rPMA differentiation

Macrophage-like THP-1 cells

Cytokine/chemokine response

# *In Silico* Models

- Complex:
  - Quantitative structure nanotoxicity relationships (QSNR),
  - Nano-read-across model,
  - Physiologically based pharmacokinetic (PBPK) models.

- Less complex but still:
  - Virtual cell based assay estimates  time-dependent concentration of a test chemical in the cell and cell culture for a given *in vitro* system.

# *In Silico* Models

- Special interest - Deposition models:
  - Single-Path
  - Multi-Path - more realistic description of lung morphology and its asymmetric geometry. Get it for free from the website listed below!

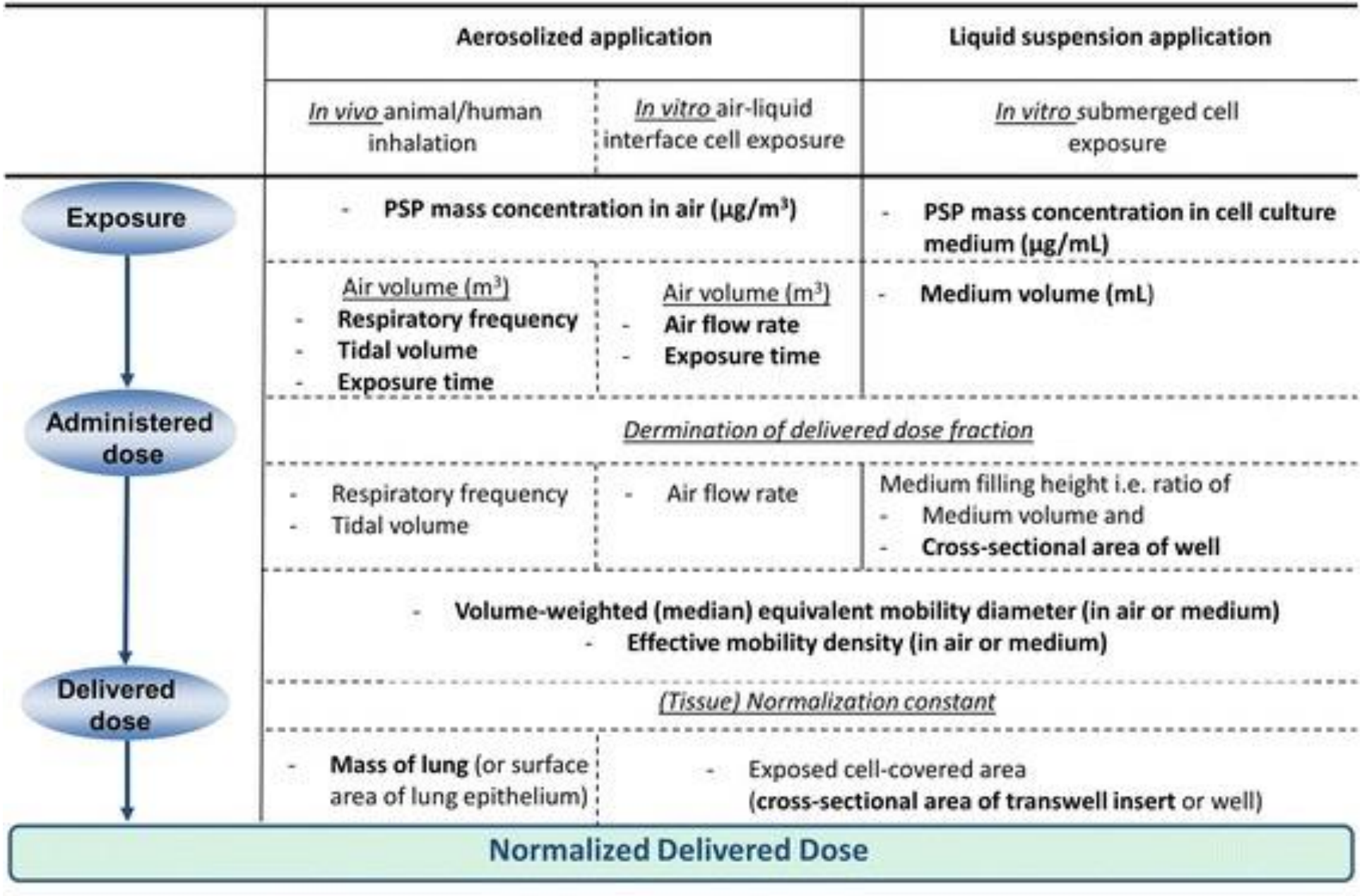

https://www.ara.com/products/multiple-path-particle-dosimetry-model-mppd-v-304

# Further reading


- Winkler DA, Mombelli E, Pietroiusti A, Tran L, Worth A, Fadeel B, McCall MJ. Applying quantitative structure–activity relationship approaches to nanotoxicology: current status and future potential. Toxicology 2013, 313:15–23.
- Gajewicz A, Cronin MTD, Rasulev B, Leszczynski J, Puzyn T. Novel approach for efficient predictions properties of large pool of nanomaterials based on limited set of species: nano-readacross. Nanotechnology 2015, 26:015701.
- Li M, Reineke J. Physiologically based pharmacokinetic modeling for nanoparticle toxicity study In: Reineke J, editor. , ed. Nanotoxicity. Humana Press: New York; 2012, 369–382.
- Graepel, R., Lamon, L., Asturiol, D., Berggren, E., Joossens, E., Paini, A., … Worth, A. (2017). The virtual cell based assay: Current status and future perspectives. Toxicology in Vitro, 45, 258–267.
- Moskal A., Sosnowski T.R., Gradoń L. (2010) Inhalation and Deposition of Nanoparticles: Fundamentals, Phenomenology and Practical Aspects. In: Marijnissen J., Gradon L. (eds) Nanoparticles in medicine and environment. Springer, Dordrecht

# Foundations of the Quality Study

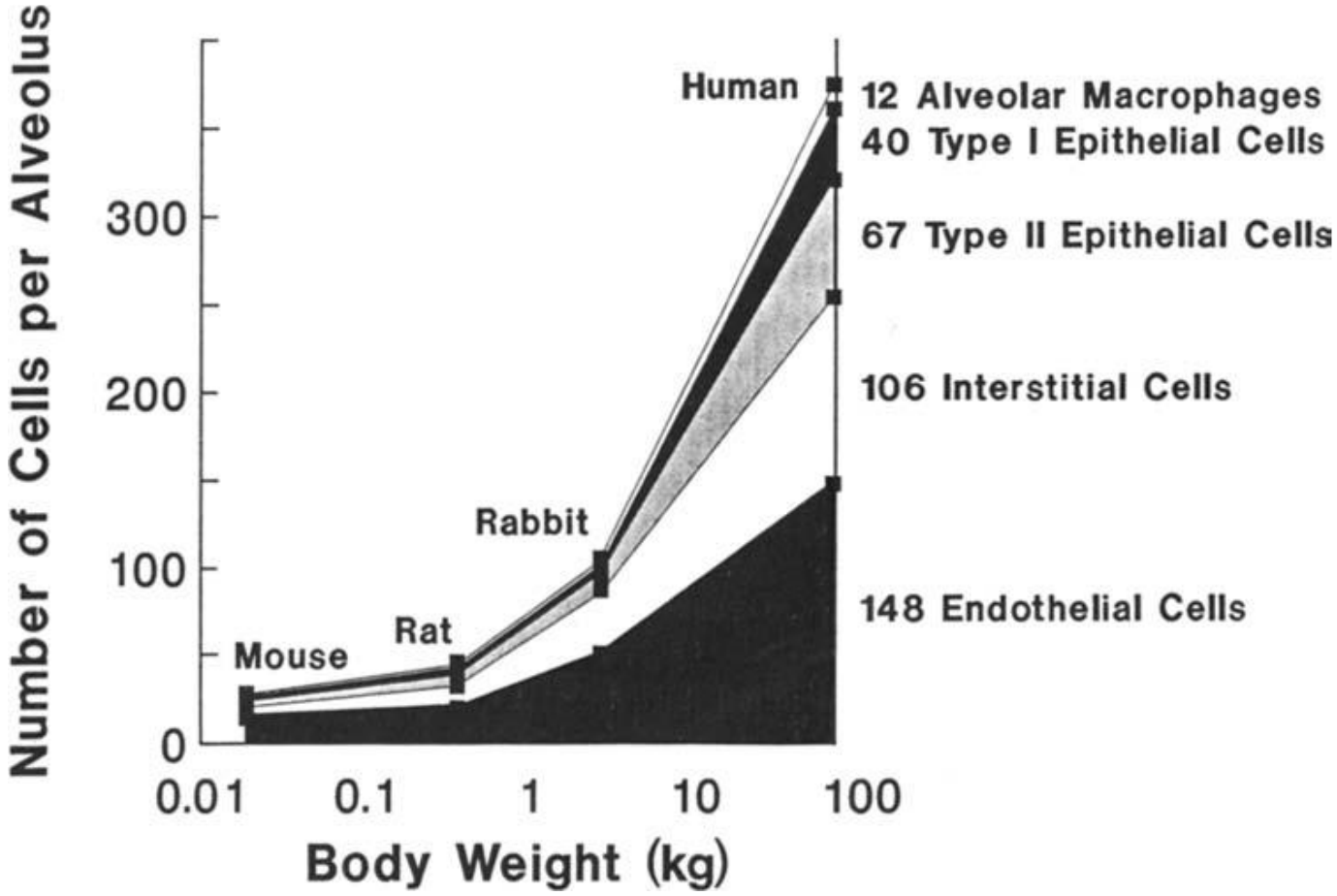
- Material characterization
- Model relevance
- **Dosing relevance**
- Descriptive -> hypothesis-driven science.




From Schmid, O., & Cassee, F. R. (2017).

# Again, Know the Species Differences!

Average number of cells per alveolus.




Stone KC, Mercer RR, Gehr P, Stockstill B, Crapo JD. Allometric relationships of cell numbers and size in the mammalian lung. Am J Respir Cell Mol Biol. 1992 Feb;6(2):235-43.

TABLE 1

*Characteristics of the species studied**

| Species | *n* | Body Weight (*kg*) | Lung Volume (*ml*) | Surface Area for Alveolar Region ($m^2$/*both lungs*) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Alveolar Epithelium | Type I Epithelium | Type II Epithelium† | Capillary Endothelium |
| Shrew (*Suncus etruscus*) | 2 | 0.0027 ± 0.0002 | 0.10 ± 0.02 | 0.013 ± 0.002 | 0.013 ± 0.002 | 0.000079 ± 0.000057 | 0.010 ± 0.001 |
| Mouse | 2 | 0.0192 ± 0.0008 | 0.65 ± 0.19 | 0.05 ± 0.02 | 0.05 ± 0.02 | 0.001 ± 0.0002 | 0.04 ± 0.01 |
| Hamster | 2 | 0.106 ± 0.002 | 4.36 ± 0.35 | 0.214 ± 0.004 | 0.210 ± 0.004 | 0.0036 ± 0.0004 | 0.220 ± 0.003 |
| Fisher 344 rat | 4 | 0.29 ± 0.01 | 8.60 ± 0.31 | 0.41 ± 0.04 | 0.39 ± 0.04 | 0.015 ± 0.005 | 0.38 ± 0.04 |
| Sprague-Dawley rat | 8 | 0.363 ± 0.004 | 10.55 ± 0.37 | 0.40 ± 0.03 | 0.39 ± 0.03 | 0.015 ± 0.002 | 0.452 ± 0.03 |
| Guinea pig | 2 | 0.335 ± 0.002 | 13.63 ± 0.76 | 0.68 ± 0.05 | 0.66 ± 0.05 | 0.029 ± 0.009 | 0.67 ± 0.08 |
| Rabbit | 2 | 2.72 ± 0.00 | 78.2 ± 3.3 | 3.43 ± 0.24 | 3.36 ± 0.23 | 0.070 ± 0.011 | 3.10 ± 0.12 |
| Dog | 4 | 16.0 ± 0.7 | 1,322 ± 64 | 52.0 ± 1.2 | 50.6 ± 1.0 | 1.4 ± 0.2 | 57.0 ± 2.3 |
| Human | 4 | 68.5 ± 4.6 | 4,777 ± 750 | 102.2 ± 20.5 | 96.0 ± 19.1 | 6.2 ± 1.5 | 72.3 ± 16.5 |
| Horse | 2 | 513 ± 3 | 45,075 ± 74 | 1,536 ± 54 | 1,505 ± 43 | 30.6 ± 11.3 | 1,259 ± 70 |

* All data are mean ± SEM.

† Type II cell surface area is the air surface of the cell excluding additional surface area contributed by microvilli.

Stone KC, Mercer RR, Gehr P, Stockstill B, Crapo JD. Allometric relationships of cell numbers and size in the mammalian lung. Am J Respir Cell Mol Biol. 1992 Feb;6(2):235-43.

# Further Reading

- Schmid, O., & Cassee, F. R. (2017). On the pivotal role of dose for particle toxicology and risk assessment: exposure is a poor surrogate for delivered dose. *Particle and Fibre Toxicology*, *14*, 52.
- Stone KC, Mercer RR, Gehr P, Stockstill B, Crapo JD. Allometric relationships of cell numbers and size in the mammalian lung. Am J Respir Cell Mol Biol. 1992 Feb;6(2):235-43.
- Mercer RR, Russell ML, Roggli VL, Crapo JD. Cell number and distribution in human and rat airways. Am J Respir Cell Mol Biol. 1994 Jun;10(6):613-24. PubMed
- *In vitro* Protocol paper:
  - DeLoid, G. M., et al. (2017). "Preparation, characterization, and in vitro dosimetry of dispersed, engineered nanomaterials." Nature Protocols **12**: 355

# Foundations of the Quality Study

- Material characterization
- Model relevance
- Dosing relevance
- **Descriptive -> hypothesis-driven science.**

# Descriptive -> Hypothesis-Driven Science

- "…The **%Journalname%** will not consider papers that are… purely descriptive…"

# Descriptive -> Hypothesis-Driven Science

- Descriptive - referring to, constituting or grounded in matters of observation or experience.*

- Example: a survey of changes in gene expression or cytokine production under a given condition.

*Merriam-Webster online dictionary

# Powerful Descriptive Scientific Tools

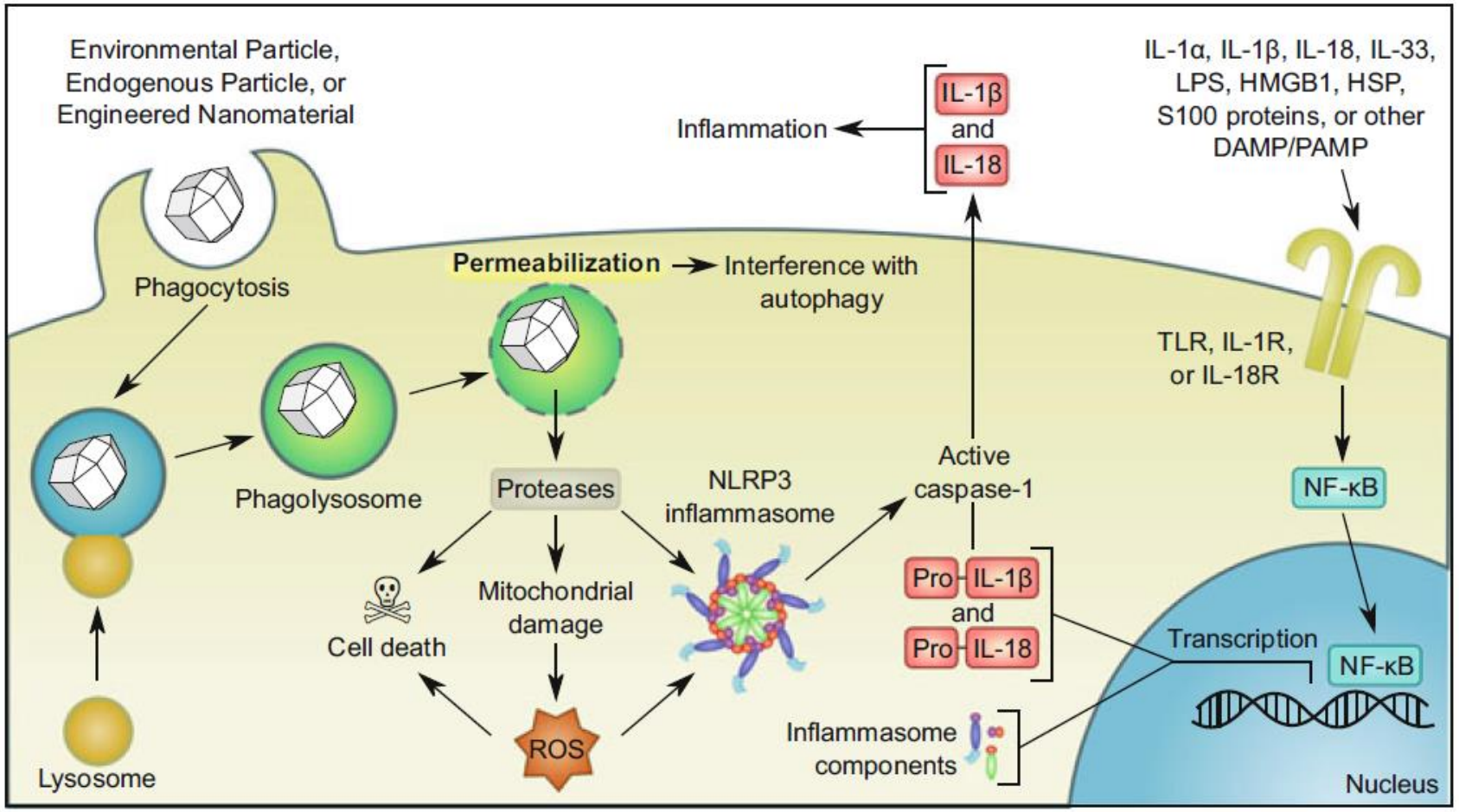
- Data acquisition for Nanoparticles toxicity studies:
  - High Throughput Screening (HTS)
  - Plethora of  -omics approaches,
  - All kinds of gene sequencing,
  - …
- Data analysis for Nanoparticles toxicity studies:
  - Ingenuity Pathway Analysis (IPA),
  - Decision-tree making, cluster analysis.
  - …

# Descriptive -> Hypothesis-Driven Science

- Descriptive observations play a vital role in scientific progress. However, by itself it is seldom conclusive.

- Solution?
  - Avoid collecting the information without a particular question in mind;
  - Move to “hypothesis-driven research,” designed to test a specific explanation for a phenomenon.

# Example: You've got Some IL-1β in the Lavage




M. Bunderson-Schelvan et al. 2016

# Spasibo za vnimanie!

# Reproducibility Crisis Very Recent Paper

- **Opinion: Is science really facing a reproducibility crisis, and do we need it to?** Daniele Fanelli Proceedings of the National Academy of Sciences Mar 2018, 201708272; DOI:10.1073/pnas.1708272114

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