"Mind the Gap": Dosimetry Modeling to Aid Experimental Design, Evidence Integration, and Inferences

Annie M. Jarabek
Deputy National Program Director and Senior Toxicologist
Human Health Risk Assessment (HHRA)
National Center for Environmental Assessment (NCEA)
Office of Research and Development (ORD)

Design of an *in vitro* System to Assess the Inhalation of Nanomaterials
February 24 & 25, 2015
Washington D.C.

Outline

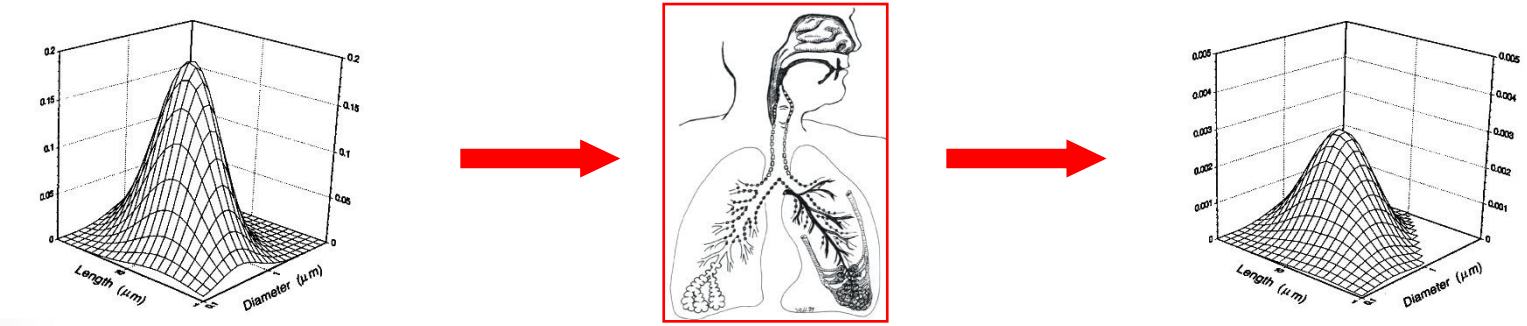
- **Conceptual basis and background: Mechanistic modeling**
  - **Dosimetry as bridge between exposure and response**
  - **Modeling mechanisms of deposition and retention of inhaled particles in the respiratory tract**
- **Context: Applications in risk assessment**
  - **Data integration and inferences**
  - **Dose metrics to describe mode of action**
- **Challenges and considerations for an approach to *in vitro* inhaled nanomaterials**

Disclaimer: These views are those of the author and do not represent US EPA policy.

Mechanistic Modeling

- Qualitative agreement with biological understanding of a process
- Quantitative agreement with existing data describing the process
- Validation through prediction of experimental data not used in model construction and novel to the construction process
- Comparisons quantitatively characterized by differences in critical parameters
- Consistent with contemporary toxicology: Comprehensive descriptions of pathogenesis and key events coupled with enhanced computational capacity

Motivation: Dosimetry to "Mind the Gap"



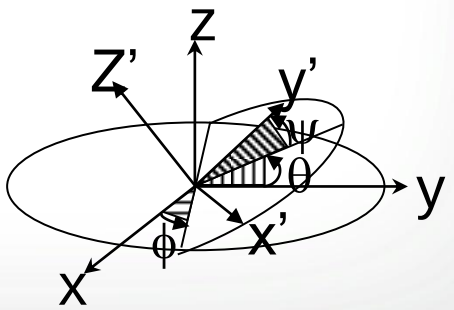
- **External exposure ≠ Internal dose (i.e., tissue burden)**
- **Incorporates current biological understanding and testing measures**
- **Provides insights on important properties of different particles or fibers and their associated toxicity**
- **Translates dose across various experimental designs to improve data integration**
- **Addresses differences between test species and humans to refine inferences**
- **Quantifies and explores properties systematically and consistently!**

Precedent: Particle Model Applications

- **Data rich: Particle dosimetry began with radionuclide efforts of 1940's**
- **National Ambient Air Quality Standard (NAAQS) for particulate matter (PM): PM10 and PM2.5 criteria based on dosimetry models**
- **Basis of dosimetric adjustment factor (DAF) used for interspecies extrapolation in development of inhalation reference concentration (RfC) risk estimates of air toxics**
- **Strategy of "size-selective" exposure sampling: "nasal" or "thoracic" or "respirable" samples**
- **Evaluation criteria for refractory ceramic fibers (RCF) and man-made vitreous fibers (MMVF)**
- **Targets pharmaceutical drug delivery**
- ***Now extending approaches to nanoparticles and in vitro systems***

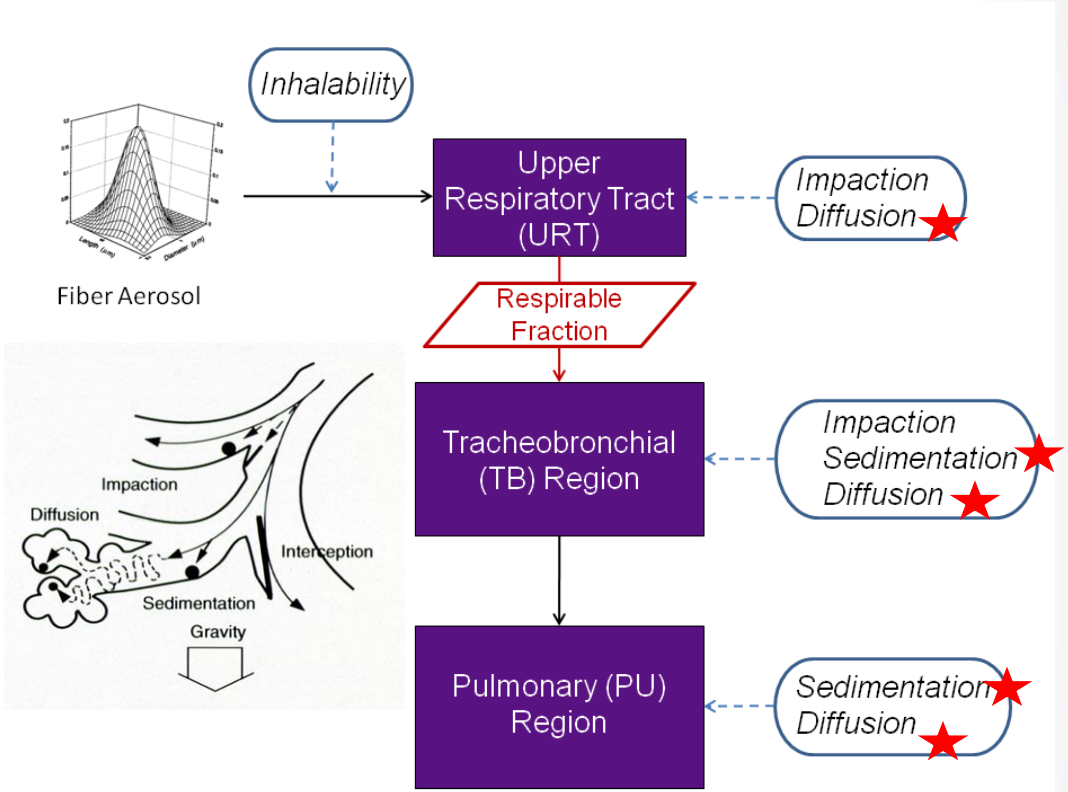
Anatomy, Airflow, Aerodynamics and Physics of Particle or Fiber Deposition

- Aerodynamics dependent on particle size, distribution, and density
- Material transport is dictated by dimensions of airway architecture and ventilation rate in each species
  - Inhalability
  - Breathing mode (nose or mouth) and ventilation activity pattern
- "Slip correction" factors for objects (e.g., particles or fibers) transported in a fluid (i.e., air)
- Deposition based on fundamental first principles of physics: Laws of conservation of mass and momentum for both airflow and particles
- Fiber orientation: Based on statistics and deterministic description (e.g., parallel or perpendicular) to airflow
- Characterization of aerodynamics for fibers requires bivariate distribution (i.e., length *and* width) and density



Deposition: Mechanisms and Dosimetry Modeling

- **Semi-empirical: Structure based on fit to data and theory**
- **Species-specific architecture and airflows or activity patterns**
- **Fundamental first principles of physics (Laws of conservation of mass and momentum for both airflow and fibers)**
- **Equivalent aerodynamic fiber diameters derived based on dimensions and density for each deposition mechanism**

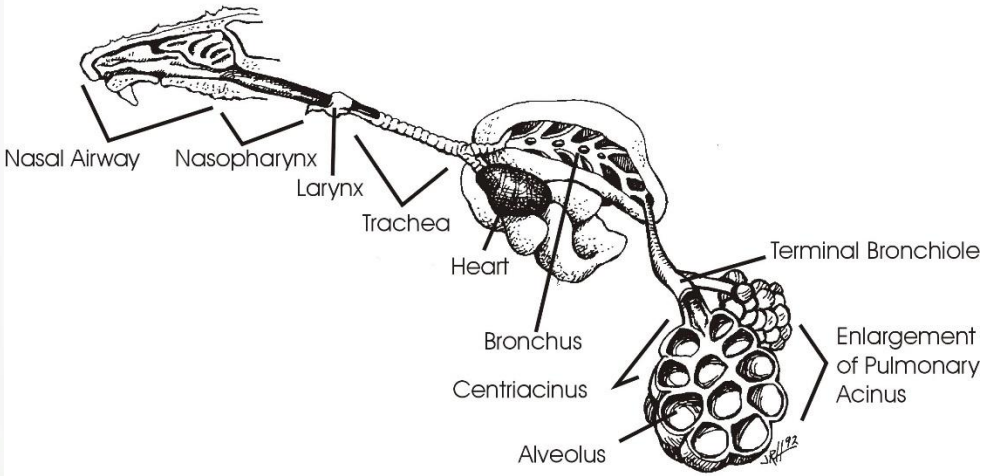


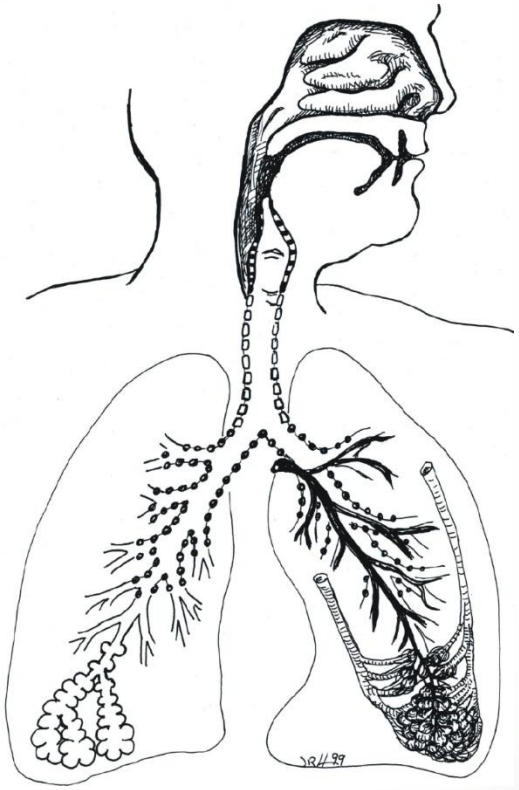
Retained burden = (Inhalability + Deposition) - Clearance

Note: Relative contribution of each mechanism is different in each region of respiratory tract

Airway Anatomy

- Nasal or URT
- Tracheobronchial
- Pulmonary
- Other (e.g., pleura?)

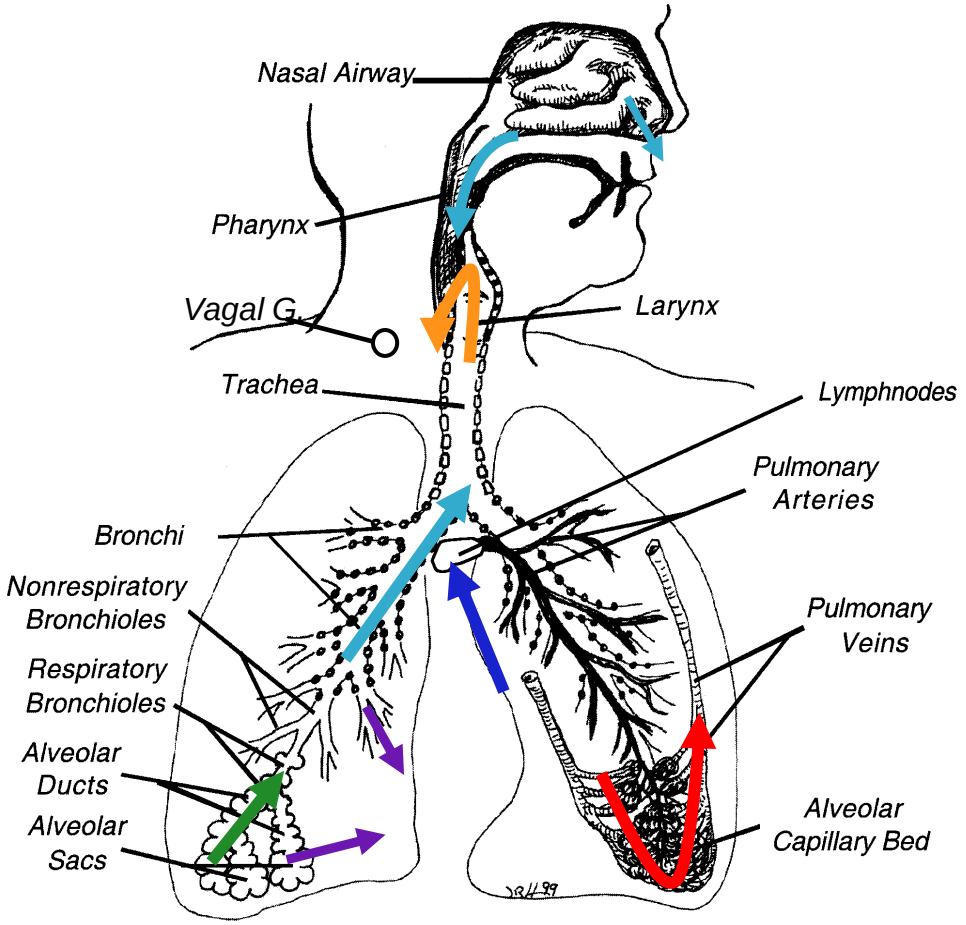




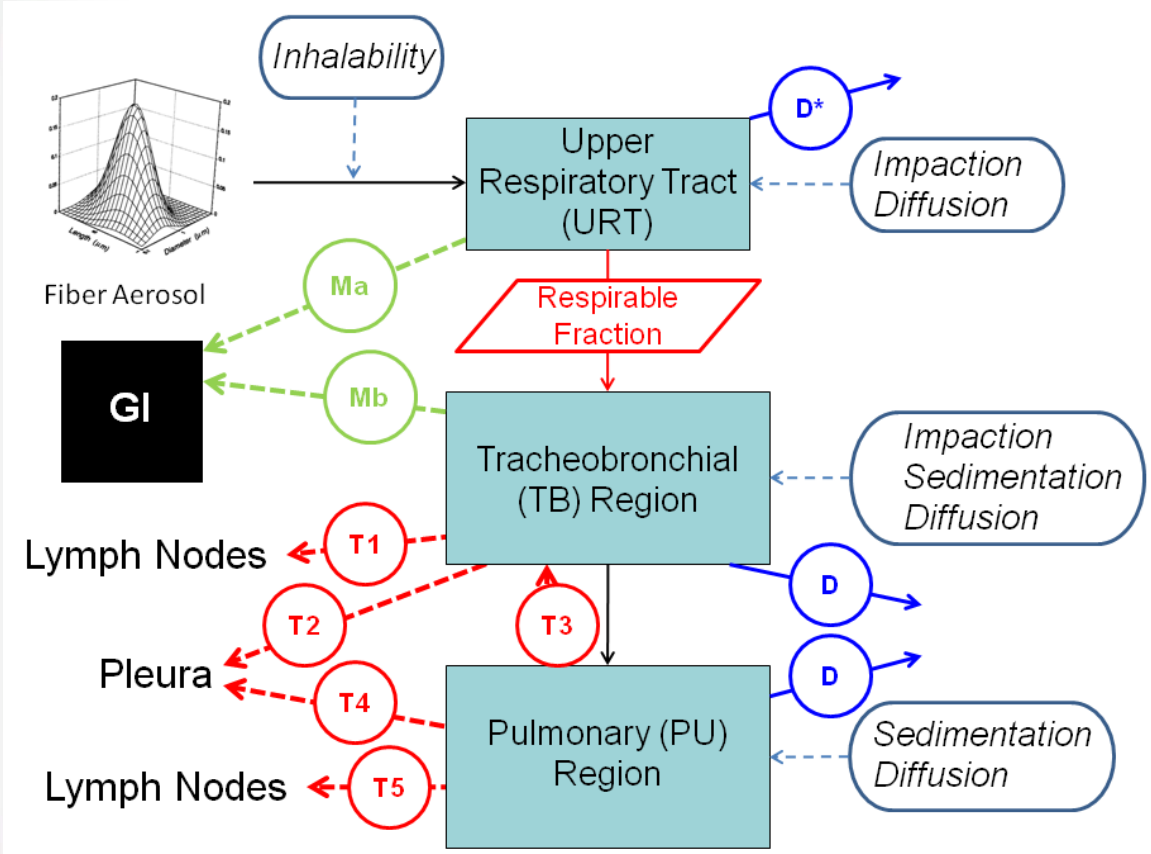
Illustrations courtesy of Dr. Jack R. Harkema, Professor of Comparative Pathology, Michigan State University.

Clearance Mechanisms

- Mucociliary Escalator
- GI Tract
- AM-mediated Clearance
- Interstitium *(via Epithelium)*
- Lymphat. Circulation
- Blood Circulation



Clearance Model: Fibers



- **Based on MPPD Model**
- **Compartmental structure to address 3 major components**
  1) **Mucociliary clearance (M)**
  2) **Translocation (T)**
  3) **Dissolution (D)**
- **Derived from time-course data for fiber burdens in each tissue**

Comprehensive Dosimetry Model for Libby Amphibole Asbestos: Inhalability, Deposition, and Retention in the Respiratory Tract of F344 Rats and Humans

A.M. Jarabek, O.T. Price, S.H. Gavett, and B. Asgharian. Accepted for SOT 2015 in San Diego. (SOT Poster 615; Abstract No. 733).

Multi-path Particle Dosimetry Model (MPPD)

- Established in regulatory practice
  - Flexible and friendly GUI
  - Publicly available and supported by Applied Research Associates, Inc.
- Updated deposition efficiencies verified with experimental data
- Enhanced algorithms
  - Inhalability
  - More explicit mechanisms
- Capable of stochastically predicting deposition and retained dose as a function of various physicochemical (size, distribution, density, shape, solubility) and physiological factors (age, ventilation rates, breathing mode and activity patterns)
- Comprehensive range of particle sizes:
  - EPA to release fiber version
  - *NIOSH contract has extended coverage to nanoparticles: Version 3 soon to be released*

Defining Dose: Operational Dosimetry Modeling in Risk Assessment

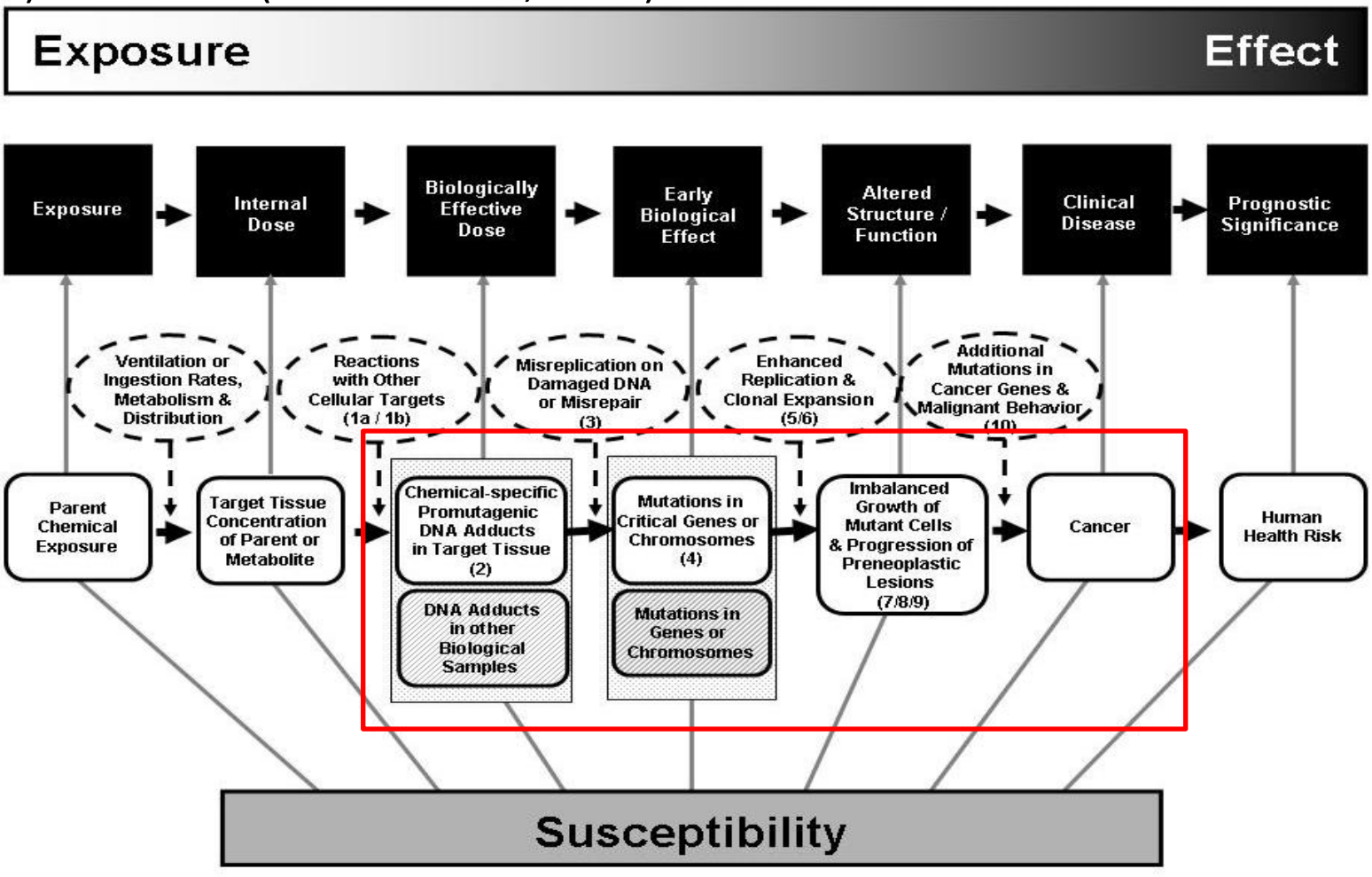
- **"Dose"**
  - **Exposure versus internal amount (deposited or retained)**
  - ***Defined best as causal or at least a metric best associated (correlated) with toxicity or key event / endpoint used to evaluate "dose-response" relationship***
- **"Metric"**
  - **Measurement: mass, surface area (SA), number (#)**
  - **Scale of metric should be same as observation or response endpoint (e.g., lung region versus local, specific cell type)**
- **"Model"**
  - **Conceptual or quantitative description of important processes**
  - **Simulate different exposure scenarios and experimental designs**

Risk Assessment: Mode of Action (MOA) and Adverse Outcome Pathways (AOP)

- **The term *"mode of action" (MOA)* is defined as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation (US EPA, 2005).**
- **A *"key event"* is an empirically observable precursor step that is itself a necessary element of the mode of action or is a biologically based marker for such an element.**
- **An Adverse Outcome Pathway (AOP) is a conceptual framework that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome, at a level of biological organization relevant to risk assessment. (Ankley et al., 2010)**

Sequence of Key Events

Revised NAS Biomarker Scheme: DNA Adducts in DNA-reactive Mode of Action (MOA) for Cancer (Jarabek et al., 2009)



Improving Measures of Dose

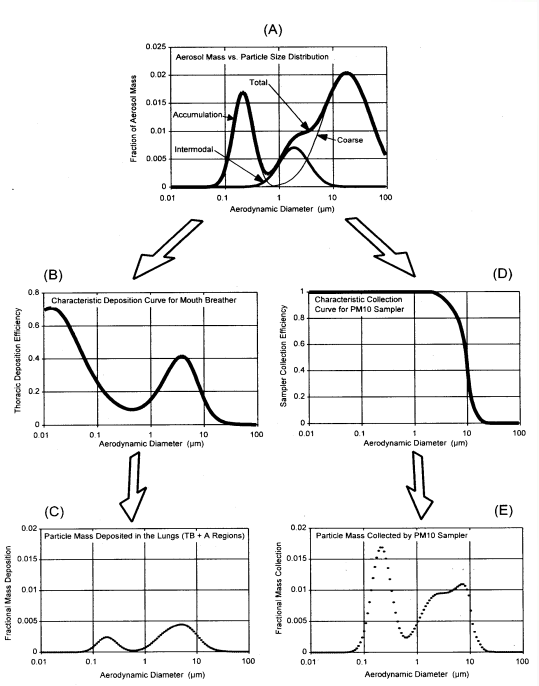
- **Mass administered**
- **Media mass, surface area (SA) or number (#) concentration**
- **Deposited mass, surface area or #**
- **Deposited mass, SA or # / cell or cm^2**
- **Retained mass, SA or #**
- **Internalized mass, SA or # per cell or cm^2**
- **Target site mass, SA or #**

Adapted from Teeguarden et al. (2007).

Application: Aid Experimental Design and Impact on Inferences

- **Context for comparisons**
  - **Epidemiological studies: Exposure**
  - ***In vivo* studies: Inhalation or instilled**
  - ***In vitro* studies: Applied to media or at cell level**
- **Impact on inferences**
  - **Biases introduced based on**
    - **Exposure sampling methods**
    - **Analytical methods**
    - **Sample or tissue preparation**
  - **Poor correlation due to failure to account for determinants of dose and causative events of response**

Ambient Aerosol



Thoracic deposition

PM10 sample

Selecting the Relevant Dose Metric

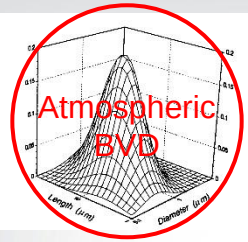
- Appropriate selection depends on describing the hypothesized mode of action
  - Corresponding to key event (e.g., cytotoxicity, inflammation, proliferation)
  - At the level of organization for observation (e.g., genomic, cellular, tissue)
  - Accounts for temporality of disease dimension (e.g., deposited for acute, retained for chronic endpoints)
- Accounts for key characteristics of
  - Exposure
    - Concentration, duration
    - Periodic, ambient constant, workplace
  - Individual physiological parameters
    - Age-specific anatomy and ventilation rate
    - Activity pattern (e.g., rest, exertion)
    - Breathing mode (nasal, oronasal or mouth)
  - Particle properties – more dynamic and complicated than chemical only
    - Size, distribution
    - Density

Engineered Nanomaterial (ENM) Properties

- **Unique properties for their application also are likely essential to characterize to understand their potential toxicity**
- **Consider dynamics of test system to understand spatial and temporal impacts**
- **Critical properties to characterize:**
  - **Particle size and distribution**
  - **Density (*)**
  - **Agglomeration state**
  - **Shape**
  - **Crystal structure**
  - **Chemical composition (spatially averaged (bulk) and heterogenous)**
    - **Physiosorption or chemisorption of biomolecules (e.g., proteins)**
    - **Biochemically-induced changes in surface chemistry**
  - **Surface area**
  - **Surface chemistry**
  - **Surface charge (Zeta potential)**
  - **Porosity**

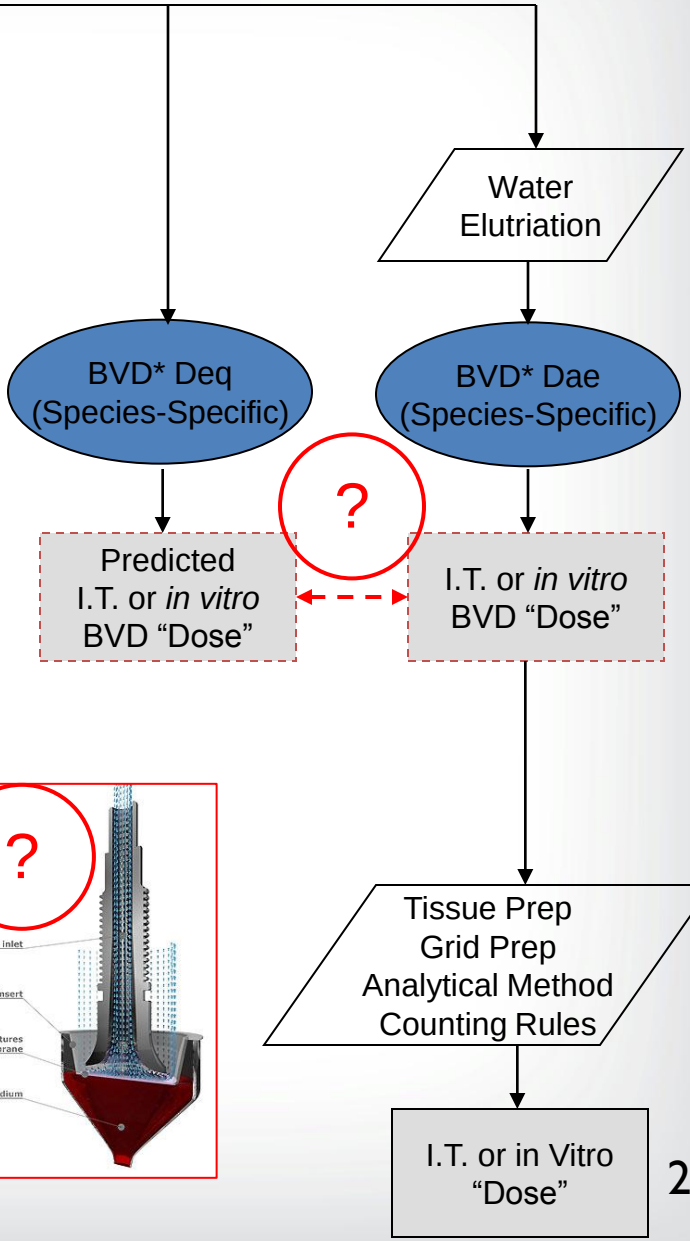
Recommended Elements of Screening Strategy for ENM

- **ILSI Research Foundation / Risk Science Institute Nanomaterial Toxicity Screening Working Group report (Oberdorster et al., 2005)**
- **Multidisciplinary testing strategy – setting characterization criteria would be premature**
- **Collect sufficient information on potentially significant properties to enable quantitative interpretation of data; notably characterize critical physical metrics of**
  - **Mass**
  - **Surface area**
  - **Number**
- **Context for screening of toxicity testing includes:**
  - **Human exposure characterization**
  - **Material following administration**
  - **Administered material**
  - **As-produced or supplied material**

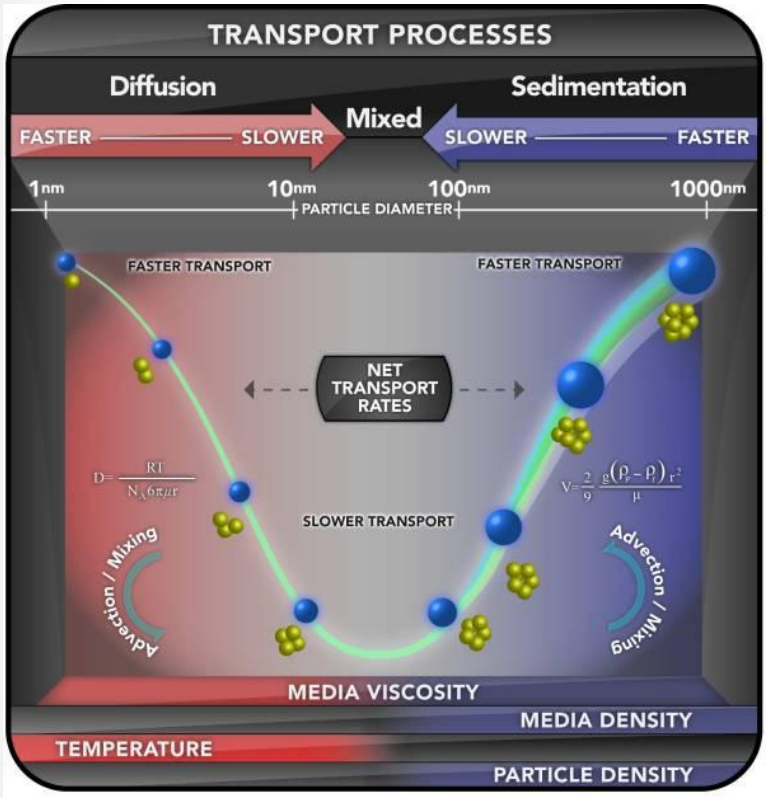


Example: Respirable Fraction (RF) Defined by fiber equivalent diameter (d_{eq}) or Particle aerodynamic diameter (d_{ae})

- Preparation of a respirable fiber sample is a critical and challenging first step of toxicological studies. The respirable fraction (RF) is defined as the amount of aerosol that will penetrate to the lower respiratory tract (LRT).
- Water elutriation method assumes spherical particles and sedimentation, but impaction is most important deposition mechanism for fibers.
- *Consider operating specifications and dose definitions of in vitro system!*



Dosimetry in the Dish

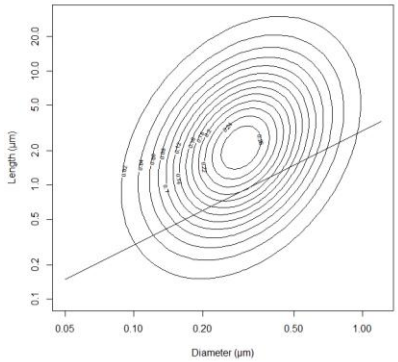


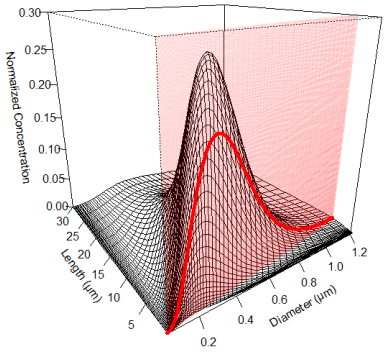
- **Considerations of transport mechanisms for particles in an *in vitro* system shown to be a major factor in delivered dose to cells.**
- **These considerations should be interfaced with predicted doses to respiratory tract of test species in question to best estimate dose range for realistic testing**

Hinderliter et al. (2010). ISDD: A computational model of particle sedimentation, diffusion, and target cell dosimetry for *in vitro* toxicity studies. Part Fibre Toxicol. Nov 30;7(1):36.

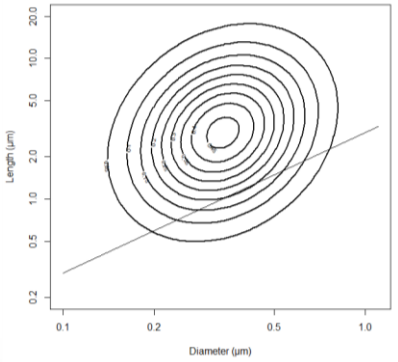
Characterization by Bivariate Distribution versus β: Truncation of Exposure and Internal Fiber Burdens

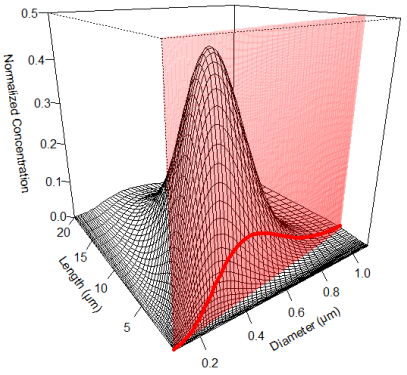
- **Exposure isopleth and bivariate distribution of 3.5 mg/m³ (1-day with 0-hr recovery)**
- **Red line indicates truncation of exposure distribution by definition of fiber using aspect ratio (β) of 3:1**



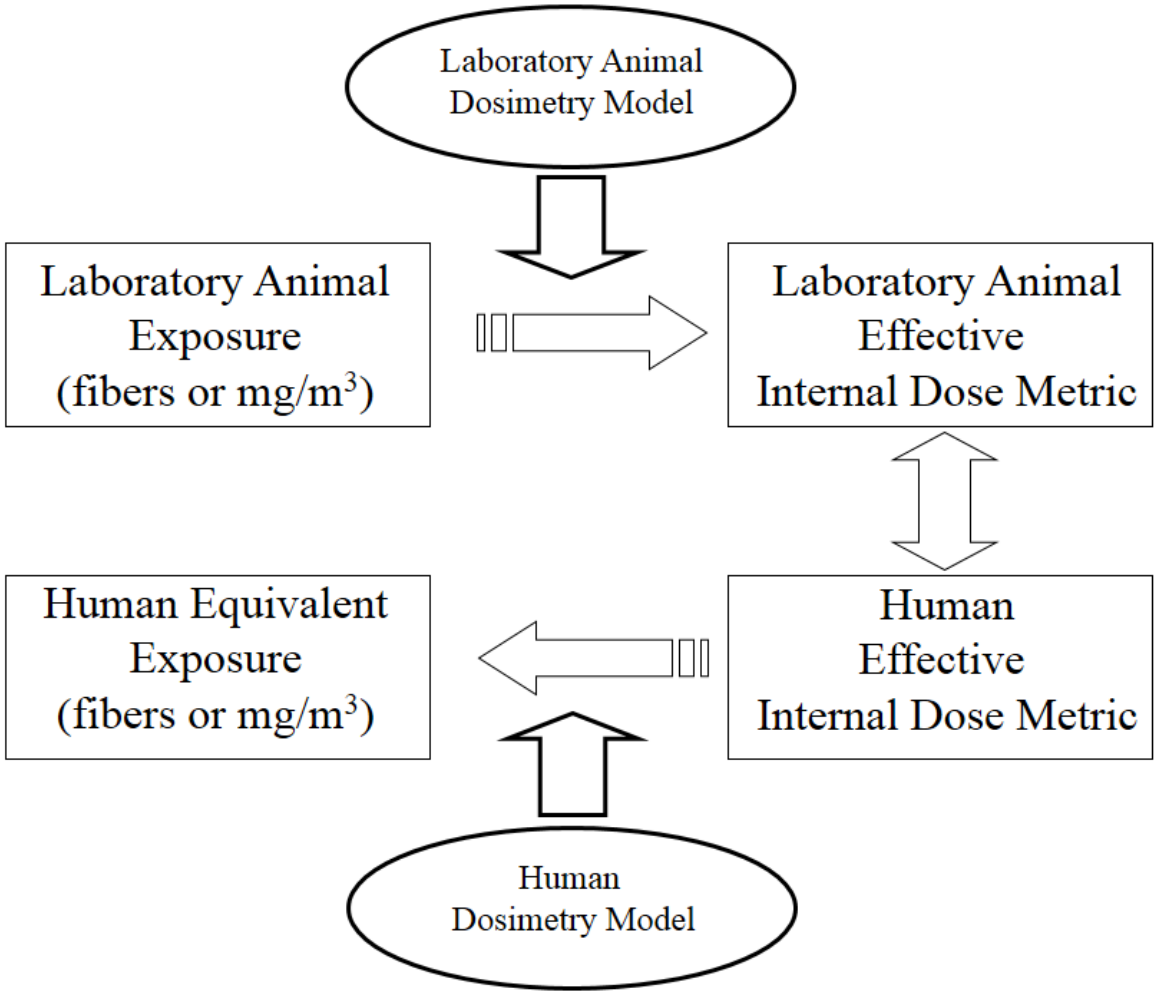


- **Isopleth and bivariate distribution of resultant LRT fiber burden**
- **Red line indicates truncation of fiber burdens by definition of fiber using aspect ratio (β) of 3:1**





Interspecies Extrapolation



Human Equivalent Concentration (HEC) Calculation

- **Illustrated for deposited but can be calculated for any other dose metric (SA, #) or normalizing factor (# epithelial cells, # alveolar macrophages)**
- **Minute volume can be age-specific and incorporate a ventilatory activity pattern reflecting breathing mode (nasal, mouth, oronasal)**

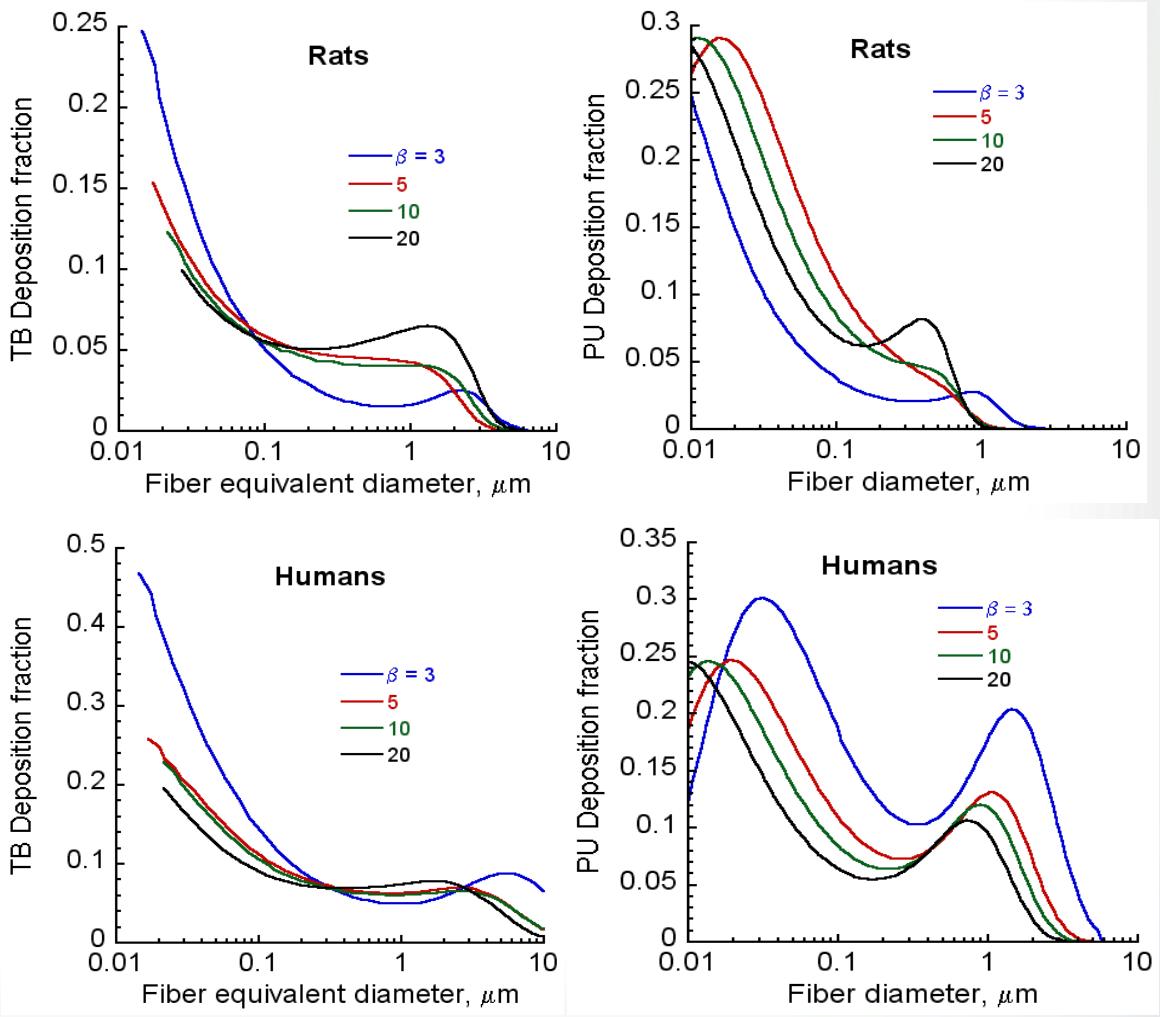
$$\begin{pmatrix}\text{deposited}\\ \text{mass}\end{pmatrix}=\begin{pmatrix}\text{deposition}\\ \text{fraction}\end{pmatrix}\times\begin{pmatrix}\text{exposure}\\ \text{concentration}\end{pmatrix}\times\begin{pmatrix}\text{minute}\\ \text{volume}\end{pmatrix}\times\begin{pmatrix}\text{exposure}\\ \text{time}\end{pmatrix}$$

$$\text{Mass}=(\text{DF})\times(\text{C})\times(\dot{V}_E)\times(\delta t)$$

$$\left(\frac{\text{Mass}}{\text{SA}}\right)_H=\left(\frac{\text{Mass}}{\text{SA}}\right)_A \quad \Rightarrow \quad \frac{\text{HEC}}{C_A}=\frac{(\dot{V}_E)_A}{(\dot{V}_E)_H}\times\frac{(\text{DF/SA})_A}{(\text{DF/SA})_H}$$

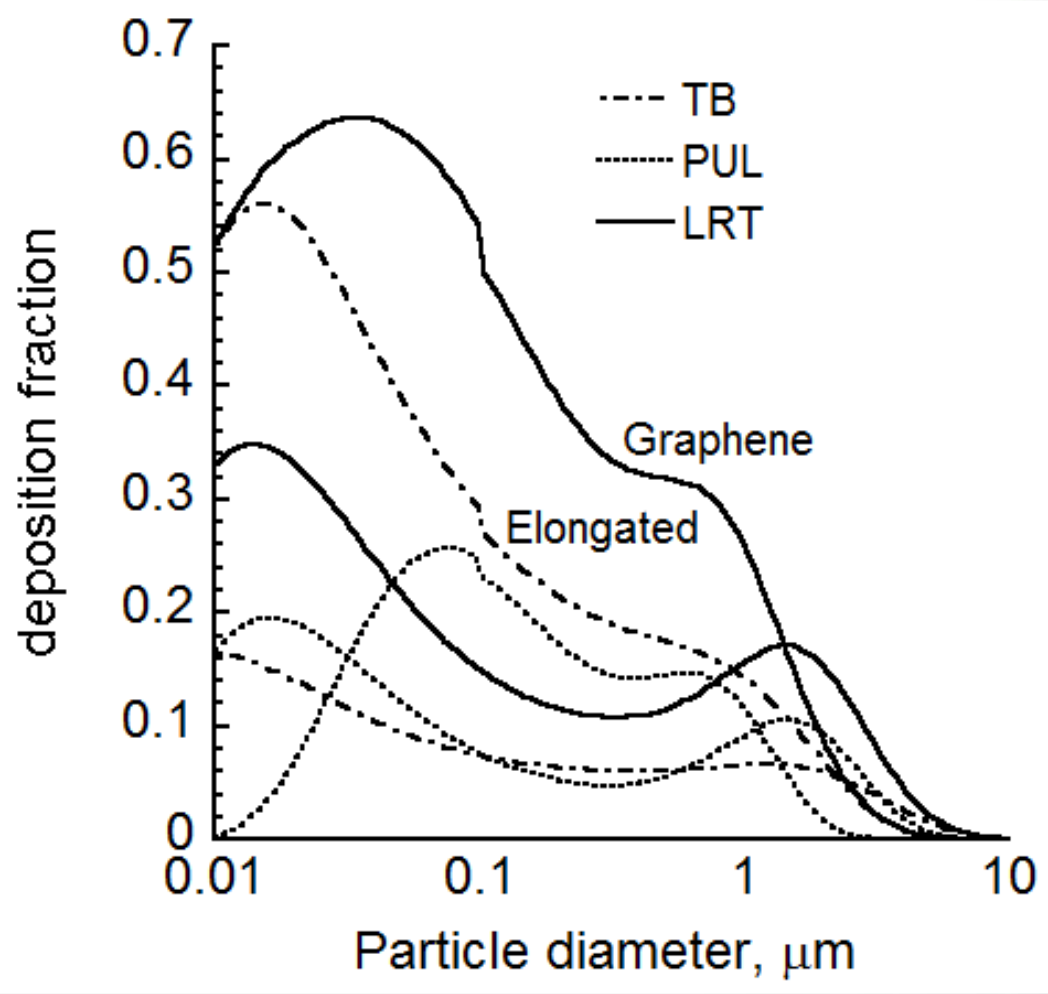
Predicted Interspecies Differences in Fiber Mass Deposition

- **TB (Left) and PU (right) deposition in rats (top) and humans (bottom) for different aspect ratios**



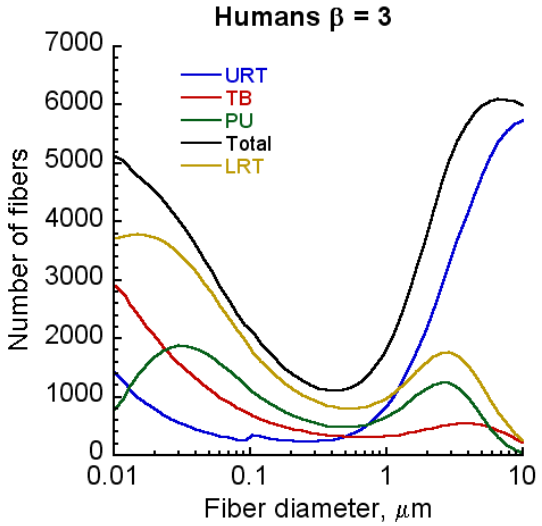
Predicted ENM Mass Deposition Fraction in Humans

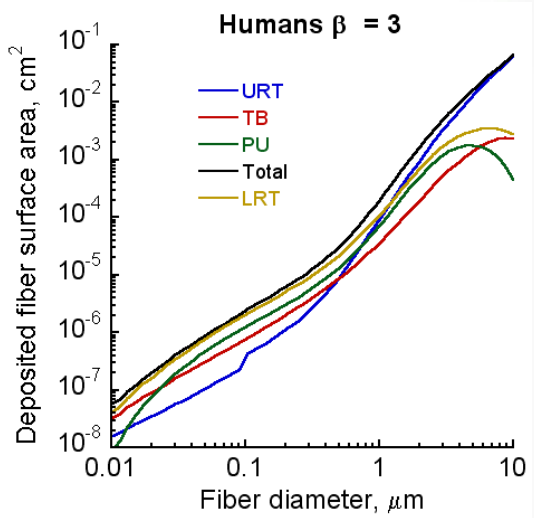
- **Difference in deposition due to shape is evident for a flat graphene ENM versus elongated nanotube of same dimension**

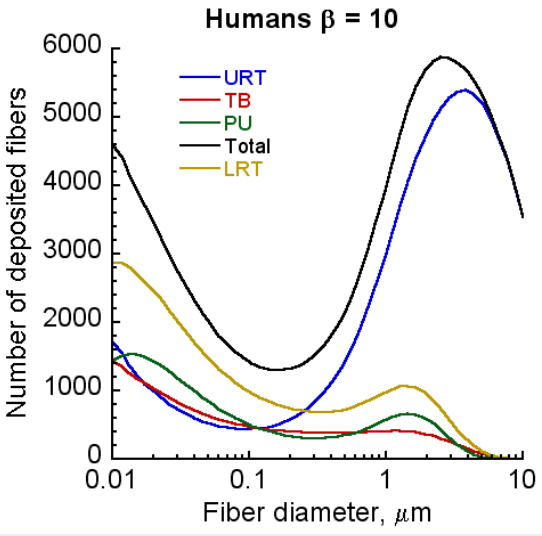


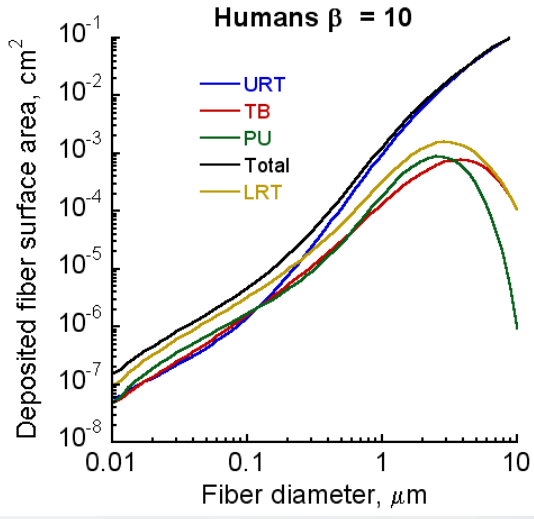
Deposition Differences due to Dose Metrics

- **Number (left) and Surface area (right)**
- **Aspect ratio = 3 (top) versus 10 (bottom)**
- **Metric and aspect ratio determine**
  - **Magnitude of deposition**
  - **Degree of regional differences**
  - **Species differences (*not shown*)**









Recommended ENM Measurements: Exposure

Metric	Measurement Recommendation
Mass – off-line	E (coupled with on-line)
Mass – on-line	E
Size distribution – off line	E
Size distribution – on line	E/D
Surface area – off line	O
Surface area – on line	O
Number – off line	N
Number – on line	E

E: These measurements are considered to be essential.
D: These measurements are considered to provide valuable information, but are not recommended as essential due to constraints associated with complexity, cost and availability.
O: These measurements are considered to provide valuable but non-essential exposure information.
N: These measurements are not considered to be of significant value to inhalation studies.

Source: Oberdorster et al. (2005). Principles for characterizing the potential human health effects from exposure to nanomaterials: Elements of a screening strategy. Part Fibre Toxicol. 2005 Oct 6;2:8.

Recommended ENM Characterization in Studies

Characterization (Off-line)	Human exposure	Toxicity Screening Studies		
		Supplied material	Administered material	Material in vivo/in vitro
Size distribution (primary particles)	E (Combine with agglomeration state)	E	D	D
Shape	E	E	O	O
Surface area	D	E	D	O
Composition	E	E	O	O
Surface chemistry	D	E	D	D/O
Surface contamination	D	N	D	N
Surface charge – suspension/solution	O	E	E	O
Surface charge – powder (use bio fluid surrogate)	O	E	N	O
Crystal structure	O	E	O	O
Particle physicochemical structure	E	E	D	D
Agglomeration state	E	N	E	D
Porosity	D	D	N	N
Method of production	E	E	–	–
Preparation process	–	–	E	–
Heterogeneity	D	E	E	D
Prior storage of material	E	E	E	–
Concentration	E	–	E	D

E: These characterizations are considered to be essential.
D: These characterizations are considered to provide valuable information, but are not recommended as essential due to constraints associated with complexity, cost and availability.
O: These characterizations are considered to provide valuable but non-essential information.
N: These characterizations are not considered to be of significant value to screening studies.

Source: Oberdorster et al. (2005). Principles for characterizing the potential human health effects from exposure to nanomaterials: Elements of a screening strategy. Part Fibre Toxicol. 2005 Oct 6;2:8.

Advantages to Mechanistic Modeling of Nanomaterials

- Builds on current understanding of biological and physicochemical mechanisms in mode of action (MOA)
- Aids comparisons and translation of results
  - *in vitro* to *in vivo* context
  - Across fiber types
  - Between species
- Facilitates comparisons of regional to local estimates of different fiber doses metrics with disease endpoints and measurements
  - Provides insights on MOA inferences and integration
  - Refines risk assessment predictions

Selected References

Gangwal S, Brown JS, Wang A, Houck KA, Dix DJ, Kavlock RJ, Cohen Hubal EA. (2011). Informing selection of nanomaterial concentrations for ToxCast in vitro tesing based on occupational exposure potential. Environ Health Perspect 119, 1539-1546.

Hinderliter PM, Minard KR, Orr G, Chrisler, WB, Thrall BD, Pounds JG, Teeguarden JG. (2010). ISDD: A computational model of particle sedimentation, diffusion, and target cell dosimetry for *in vitro* toxicity studies. Part Fibre Toxicol. Nov 30;7(1):36.

Holsapple MP, Farland WH, Landry TD, Monteiro-Riviere NA, Carter JM, Walker NJ, Thomas KV. Research strategies for safety evaluation of nanomaterials, Part II: Toxicological and safety evaluation of nanomaterials, current challenges and data needs. Toxicol Sci Nov;88(1):12-7

Jarabek AM, Asgharian B, Miller FJ. (2005). Dosimetric adjustments for interspecies extrapolation of inhaled poorly soluble particles (PSP). Inhal Toxicol Jun-Jul; 17(7-8), 317-334.

Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D, Olin S, Monteiro-Riviere N, Warheit D, Yang H; ILSI Research Foundation/Risk Science Institute Nanomaterial Toxicity Screening Working Group. (2005). Principles for characterizing the potential human health effects from exposure to nanomaterials: Elements of a screening strategy. Part Fibre Toxicol. 2005 Oct 6;2:8.

Teeguarden J, Hinderliter PM, Orr G, Thrall BD, Pounds JG. (2007). Particokinetics In Vitro: Dosimetry considerations for in vitro nanoparticle toxicity assessments. Toxicol Sci 95(2), 300-312.

Thank you

Annie M. Jarabek

(919) 541-4847

Jarabek.Annie@epa.gov