What a Difference the Dose Makes:

Dosimetry Approaches to Aid Experimental Design, Evidence Integration, and Inferences for Risk Assessment

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Alternative Approaches for Acute Inhalation Toxicity to Address Global Regulatory and Non-regulatory Data Requirements
PETA International Science Consortium (PISC), Ltd.
NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)
Webinar Series
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Disclaimer:

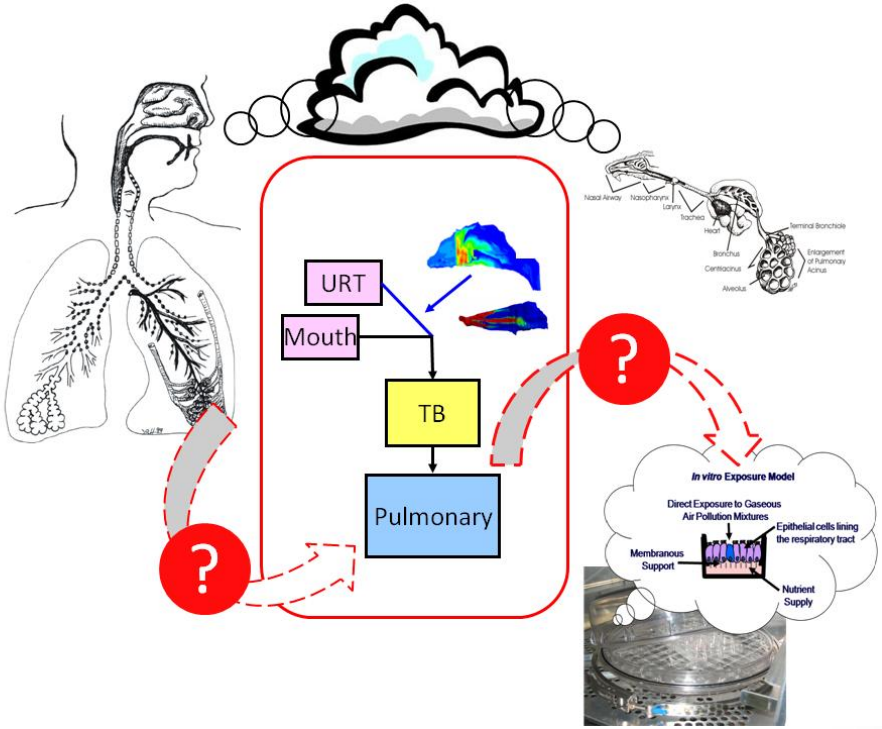
These views are those of the author and do not represent US EPA policy.

Topics

- **Conceptual basis: Critical determinants of comparative inhaled dose**
  - Anatomy
  - Physiology
  - Physicochemical properties
  - Exposure conditions
- **Contemporary toxicology: Motivation for mechanistic dosimetry modeling**
- **Context: Multi-scale characterization in risk assessment**
  - Hierarchy of adjustment factors and models
  - Dose metrics to describe key events of MOA or AOP
- **Challenges and considerations specific to *in vitro* test systems**
  - Experimental design
  - Dose and data translation

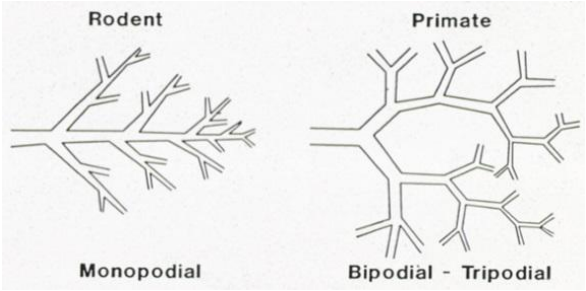
Conceptual Basis

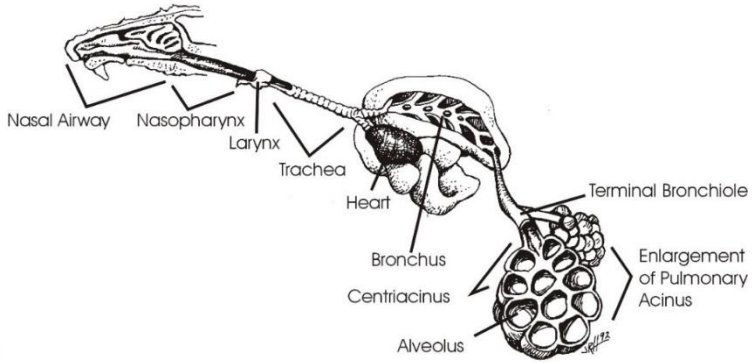
- **Methods and models used for interspecies extrapolation can be used to create context for characterizing in vitro to in vivo (IVIVE) extrapolation**
- **Inhalation dosimetry involves understanding critical biological features of the respiratory tract and how they interact with physicochemical properties of inhaled agents**
- **Exposure generation and characterization must similarly consider the dynamics of physicochemical properties, transport and transformation in the system**

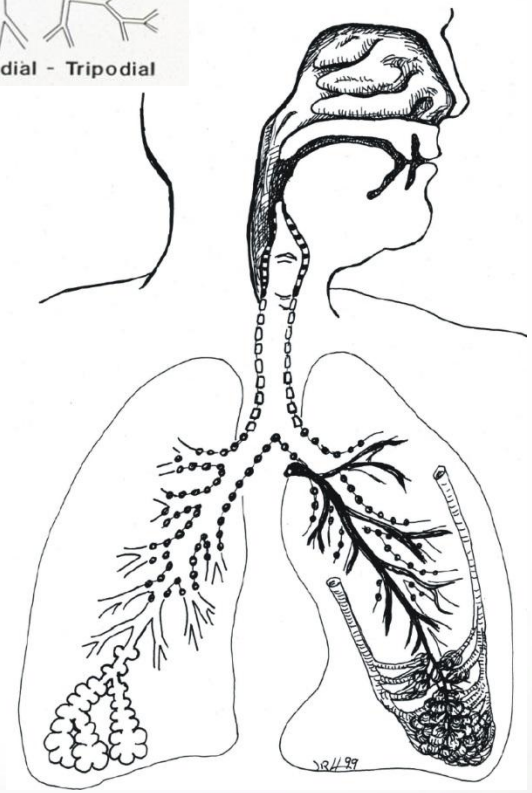


Comparative Airway Anatomy

- Airway dimensions
- Branching pattern
- Tissue dimensions
- Tissue volumes
- Cell types and distribution
- Mucus composition







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

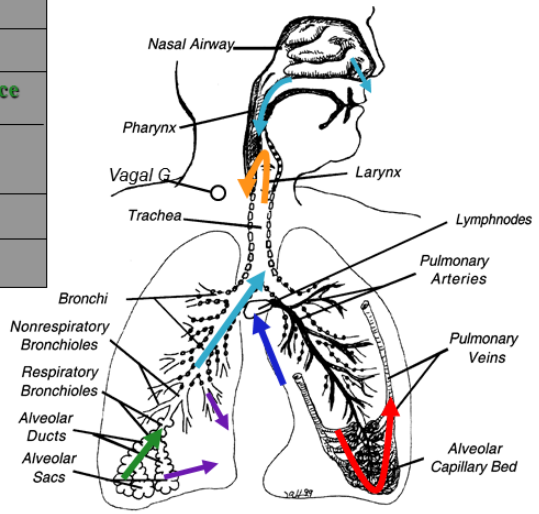
Comparative Airway Physiology

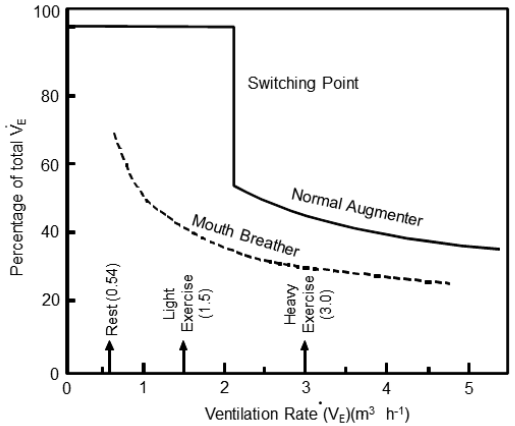
- Ventilation rate
- Breathing mode
- Mucociliary rates
- Metabolic capacities

Activity Pattern	Sleeping (.45 m³/h)		Sitting (.54 m³/h)		Activity Light (1.5 m³/h)		Activity Heavy (3.0 m³/h)		Total/Day	
	Hours	Total m³	Hours	Total m³	Hours	Total m³	Hours	Total m³	Hours	Total m³
Adult male, general population	8	3.6	8	4.32	8	12	0	0	24	19.9
Adult male, light work	8	3.6	6.5	3.5	8.5	1275	1	3	24	22.85
Adult male, heavy work	8	3.6	4	2.16	10	15	2	6	24	26.76

[a]International Commission on Radiological Protection (ICRP66, 1994).

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





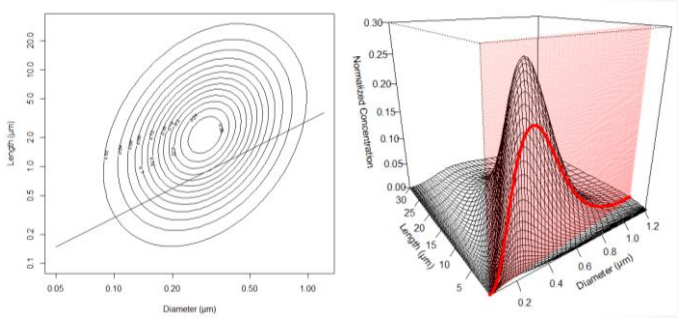
Source: International Commission on Radiological Protection (ICRP66, 1994).

Physicochemical Properties

- **Particles or fibers / engineered nanomaterials**
  - Density
  - Dimensions and distribution
  - Hygroscopicity
  - Solubility
  - Shape
  - Agglomeration state
  - 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
- **Gases**
  - **Molecular diffusivity**
  - **Reactive**
    - Hydrolysis
    - Protein binding
    - Metabolism / tissue reactions
  - **Soluble**
    - Blood:air and blood:tissue partitioning
    - Metabolism / tissue reactions

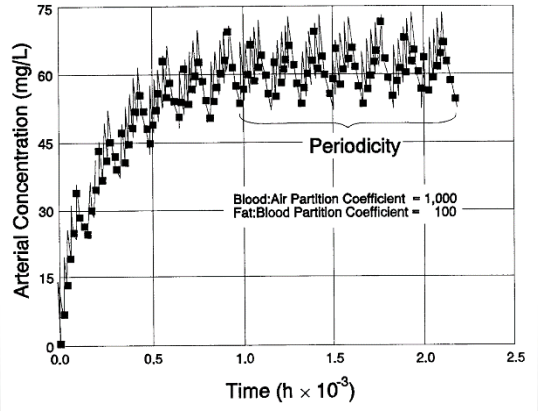
Exposure Conditions and Considerations

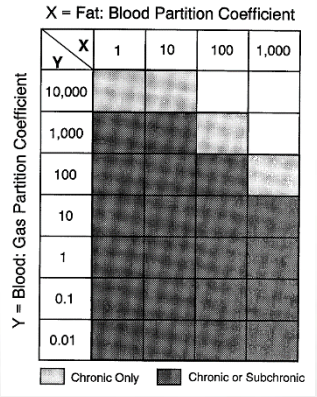
- **Generation and characterization**
  - Analytical methods and detection limits
  - Sizing conventions for particles
  - Dynamics of system to address temporal and spatial attributes of agent
- **Concentration**
- **Duration**
  - Hours / days / weeks / years
- **Experimental regimen (e.g., 6 hr/d and 5 d/wk)**
  - Definition of steady state or periodicity
  - Time course
    - ADME
    - Tissue reactions
- **Target human exposure scenario**
  - Acute emergent or episodic
  - Ambient
  - Occupational



Red line indicates truncation of exposure distribution by definition of fiber using aspect ratio (β) of 3:1

Exposure isopleth and bivariate distribution of 3.5 mg/m³ Libby asbestos (1-day with 0-hr recovery)





Contemporary Toxicology

- Bioassays and databases more comprehensive in scope
- Increased sophistication of measurements
- Growing understanding of mechanisms at molecular level
- Models of pathogenesis leading to disease states
- Emerging understanding of susceptibility factors
- Enhanced computational capacity to describe processes quantitatively

Mechanistic Modeling

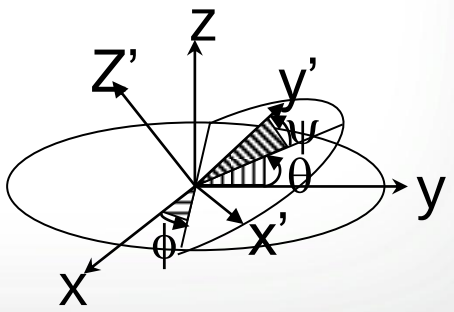
- **Qualitative agreement with biological understanding of a process**
- **Quantitative agreement with existing data describing the process**
- **Validation through prediction of experimental data not used in model construction and novel to the construction process**
- **Comparisons quantitatively characterized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

Defining Dose: Operational Dosimetry Modeling in Risk Assessment

- **"Dose"**
  - **Exposure versus internal amount**
    - Particle deposited or retained
    - Parent or metabolite
  - ***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:**
    - Particle mass, surface area (SA), number (#)
    - Blood or concentration or AUC, receptor binding…
  - **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**

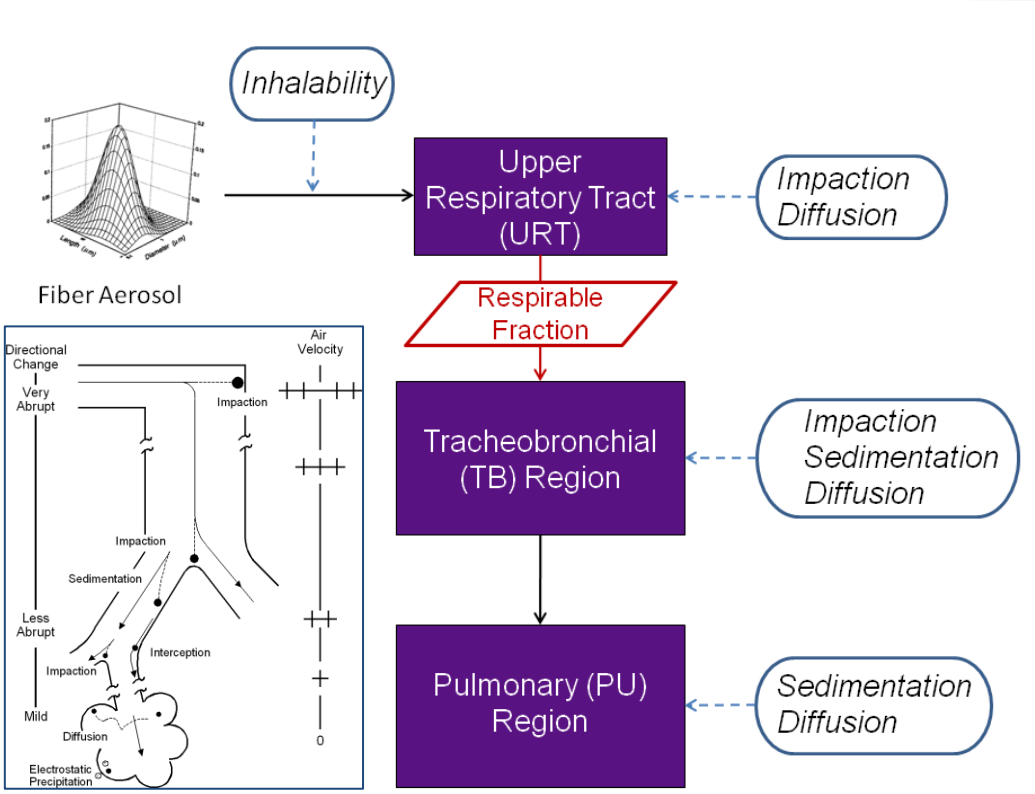
Anatomy, Airflow, Aerodynamics and Physics of Particle or Fiber Deposition

- **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**



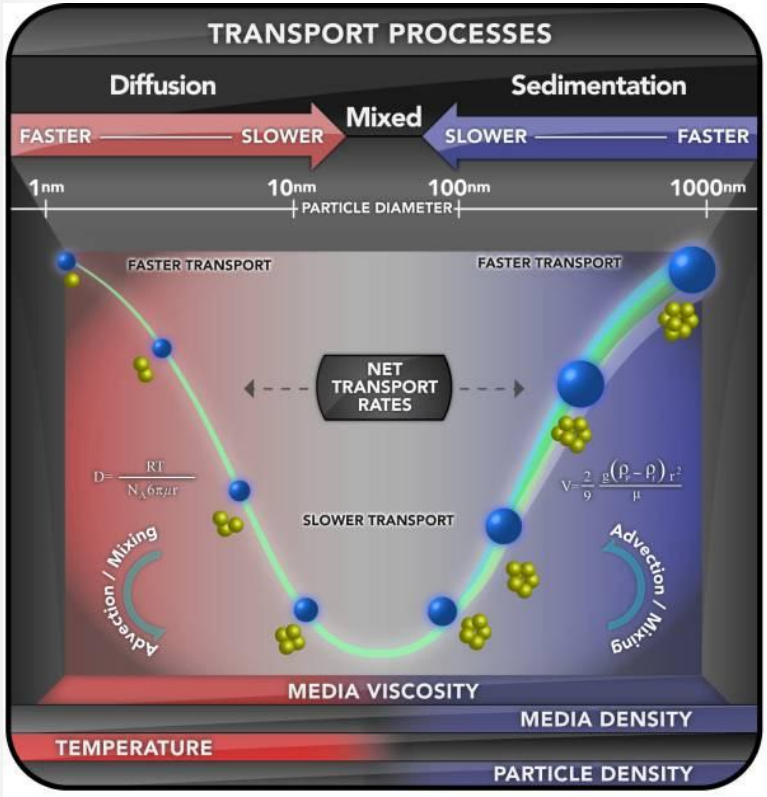
Retained burden = (Inhalability + Deposition) - Clearance

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

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 has extended coverage to nanoparticles: Version 3 soon to be released

Particle Deposition in the Dish

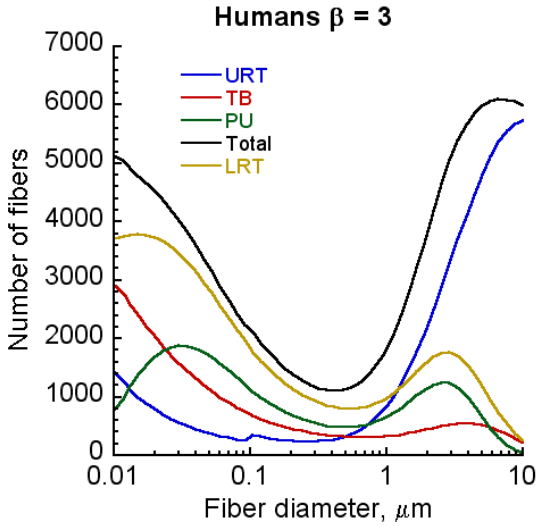


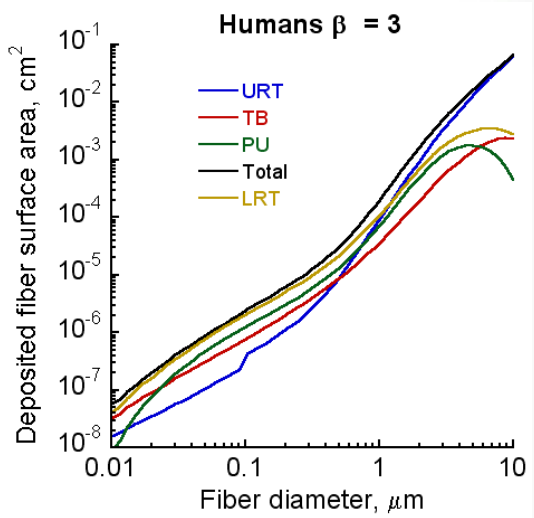
- **Considerations of transport mechanisms for particles in an *in vitro* system shown to be a major factor in delivered dose to cells.**
- ***In vitro* descriptions should be compared to predicted doses in the respiratory tract of test or target species in question to best estimate dose range for realistic testing**

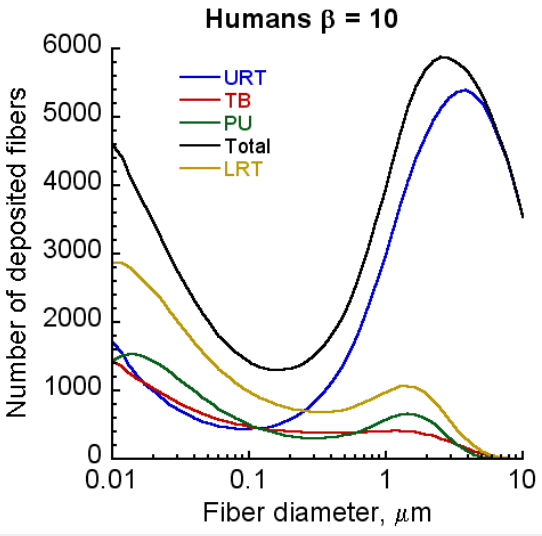
Hinderliter et al. (2010). ISDD: A computational model of particle sedimentation, diffusion, and target cell dosimetry for *in vitro* toxicity studies. Part Fibre Toxicol. Nov 30;7(1):36.

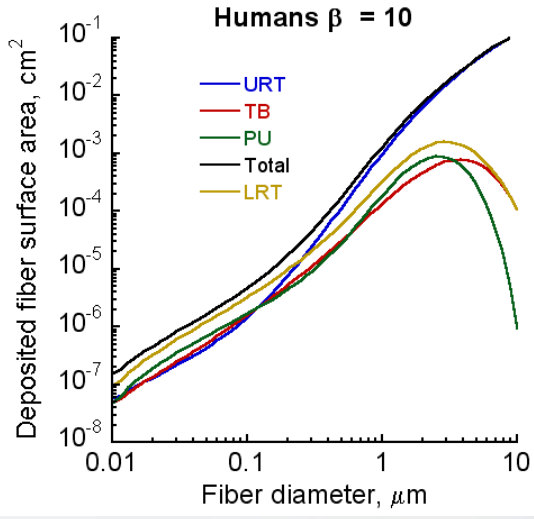
Deposition Differences due to Dose Metrics

- **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*)**

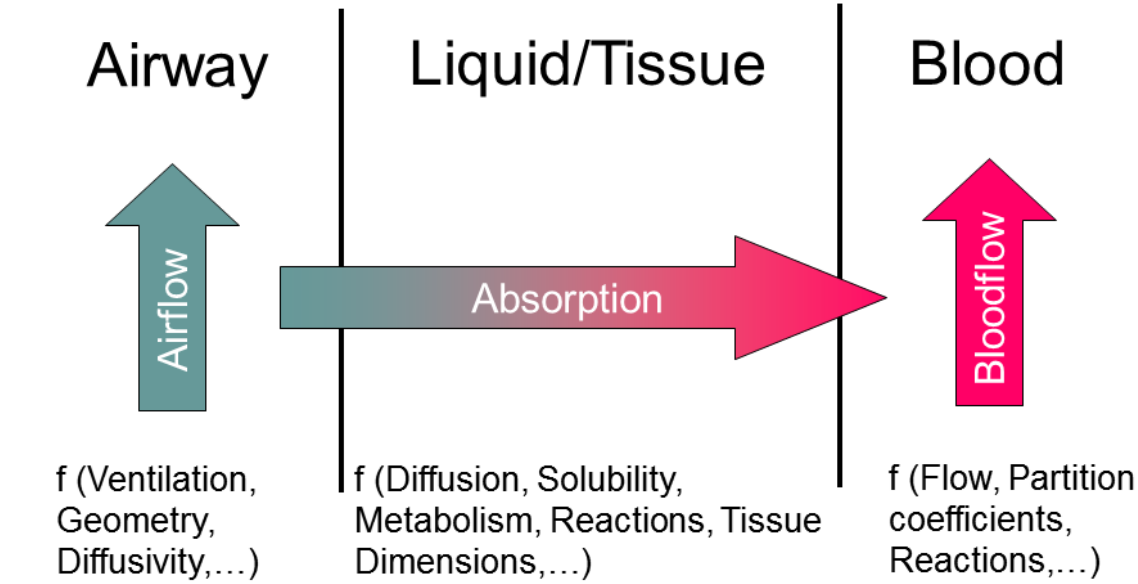


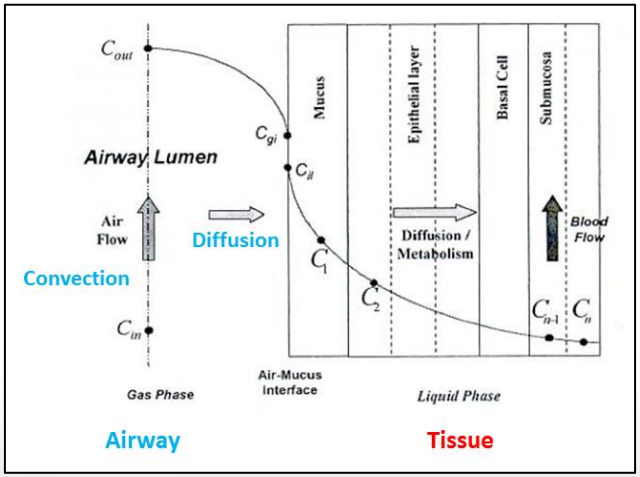




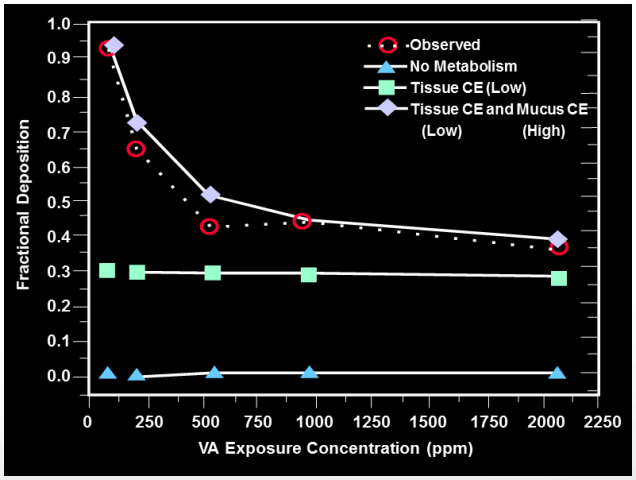


Gas Uptake: Conceptual Construct



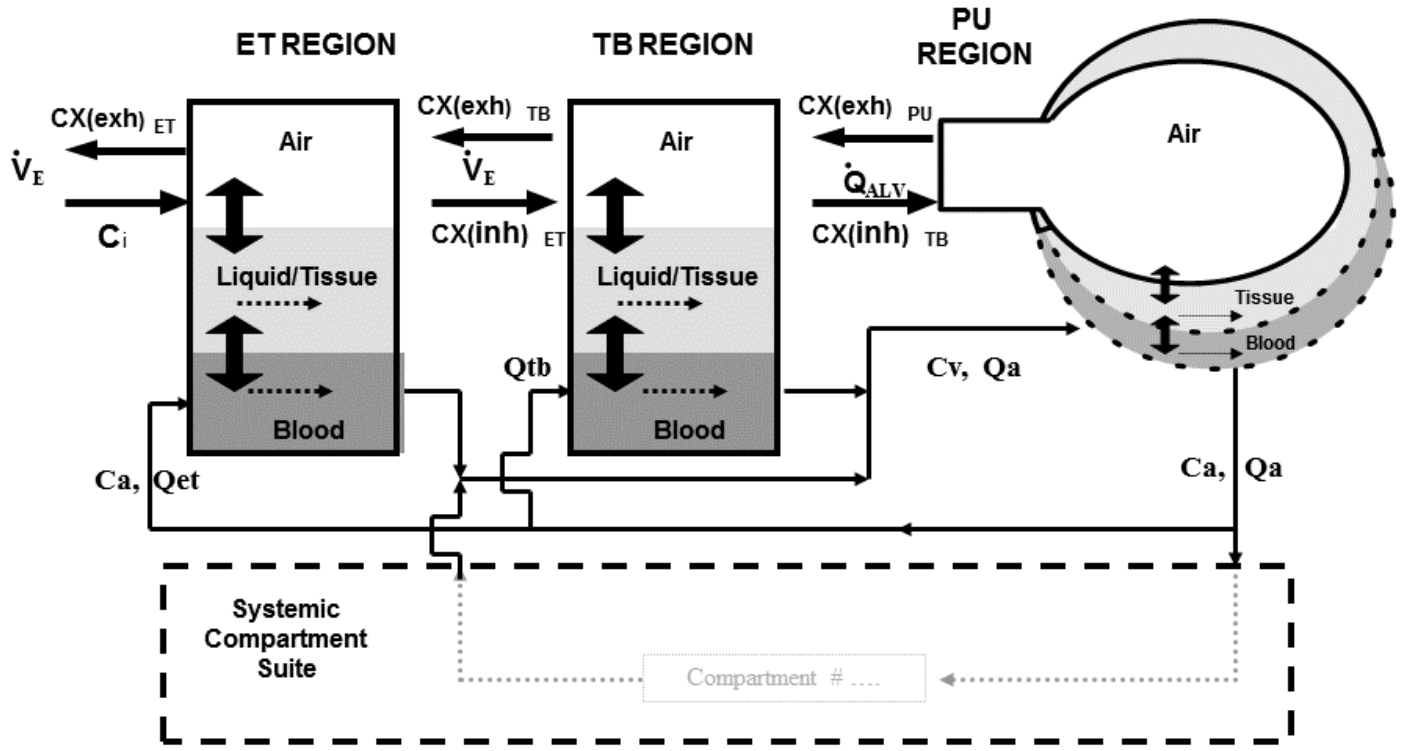


Bogdanffy and Jarabek (1995)



Bogdanffy et al. (1995)

Generalized Structure for Gases

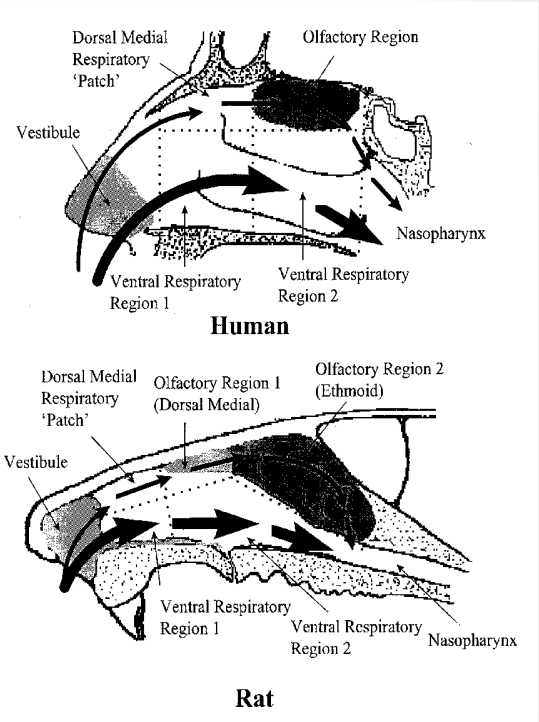


- Sequential regions account for fractional penetration
- Most models do not address exhalation
- Tissue compartments can be regional or more localized
- Blood flow links to remote compartments to describe systemic tissues

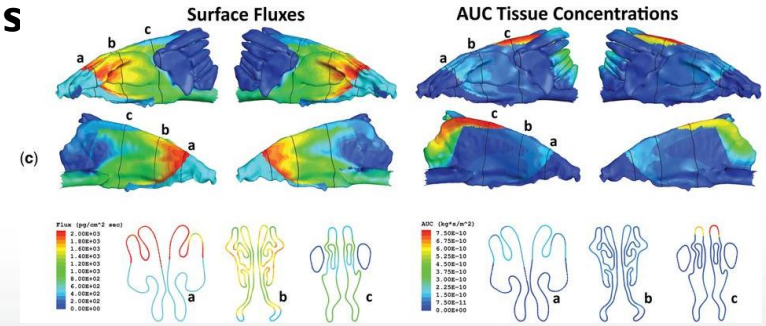
Continuing Challenges to Describe Pathogenesis

- **Interspecies extrapolation must address differences in dosimetry due to**
  - Anatomy
  - Physiology
  - Physicochemical properties
  - Exposure conditions

Note: Noses *NOT* to scale!



- **Models have matured and rapidly becoming more mechanistic to address these differences and at localized levels**
- **Pathogenesis is not restricted to specific durations in testing paradigms**
- **Different dose metrics**



Corley et al. *Toxicol. Sci.* 2015;146:65-88

Creating Context: Human Equivalent Concentrations

$$POD_{HEC}\ (mg/m^3) = POD_{ADJ} \times DAF$$

Replace with Response Model

Replace with Dosimetry Model

Where:

$POD(ADJ) = POD(mg/m^3) \times \#\ hr / 24\ hr \times \#\ d / 7\ d$

DAF = Dosimetric Adjustment Factor, either

$RDDR_r$ = Regional Deposited Dose Ratio for particles

or

$RGDR_r$ = Regional Gas Dose Ratio for gases

r is surface area for respiratory tract region of observed effect or other **normalizing factor** (e.g., BW for systemic effects; # of alveolar macrophages)

Human Equivalent Concentration (HEC) Calculation

- **RDDR illustrated for regional deposited dose (RDD) of particles in animals (A) and humans (B) but can be calculated for any other particle 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)**

$$(RDDR)_r = \frac{(RDD)_A}{(RDD)_H} = \frac{(C_1)_A}{(C_1)_H} \times \frac{(\text{Normalizing Factor})_A}{(\text{Normalizing Factor})^*_H} \times \frac{(\dot{V}E)_A}{(\dot{V}E)_H} \times \frac{(F_r)_A}{(F_r)_H}$$

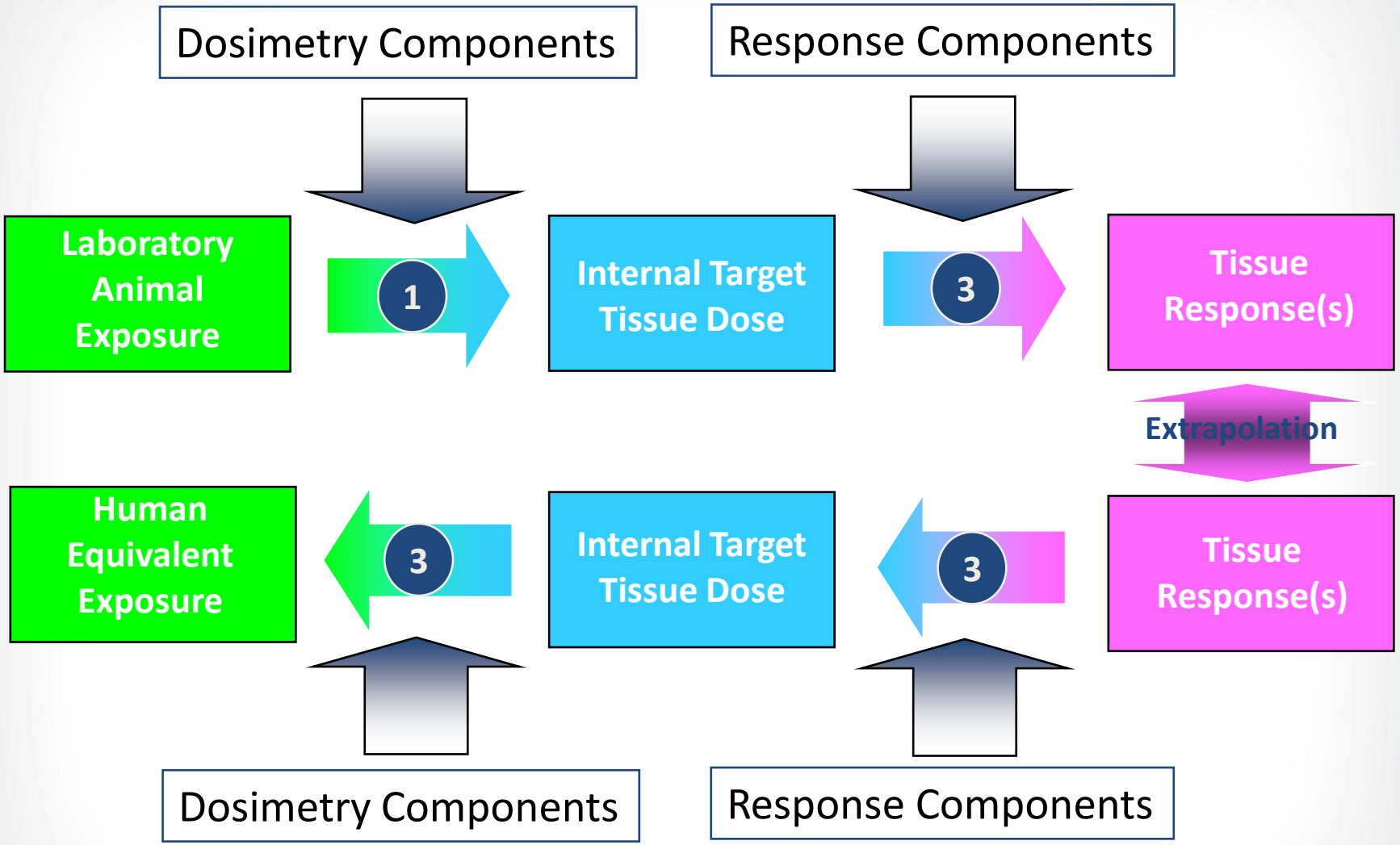
$(\dot{V}E)$ = Ventilation rate

F_r = fraction of mass deposited in region predicted with model

r = Region of observed toxicity for extrapolation

* = SA for respiratory effects and BW for remote effects

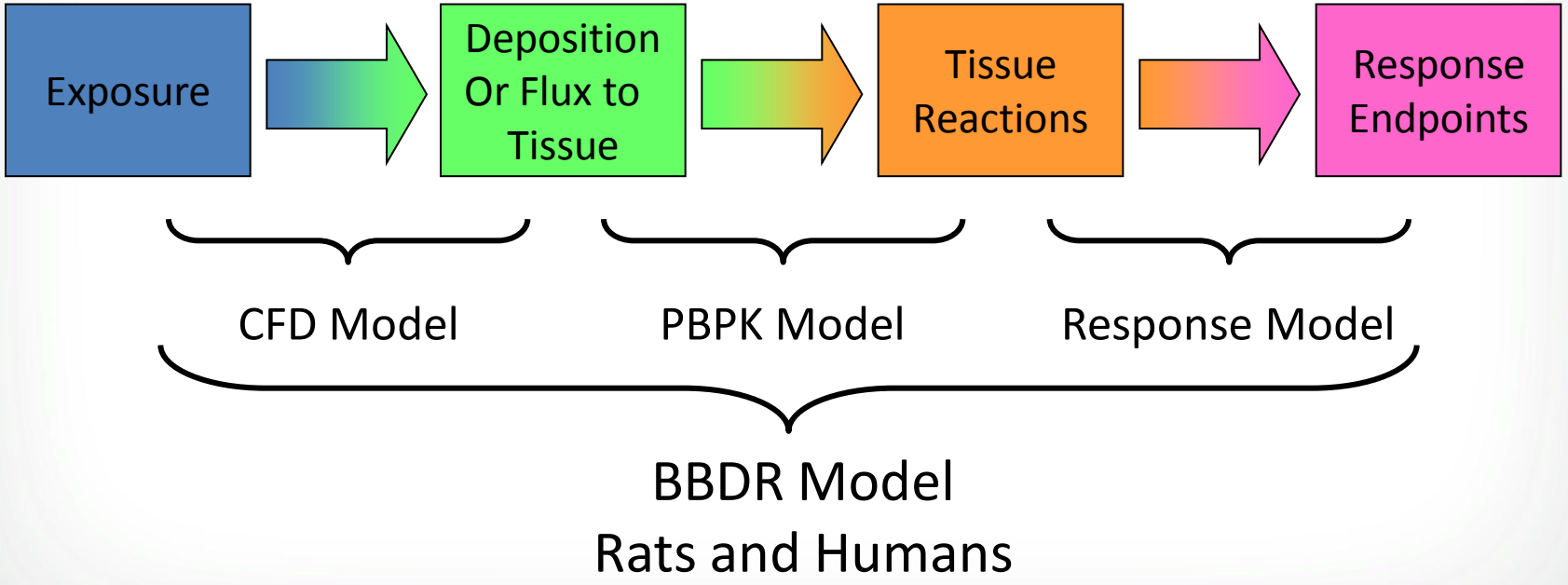
Laboratory Animal Data



Human Data

Bridging to Systems Biology: Conceptual Computational Approach

- **Construct conceptual model**
- **Define parameters and implement computational model**
- **Evaluate in comparative context**
  - **Clarify terminology (example: "clearance")**

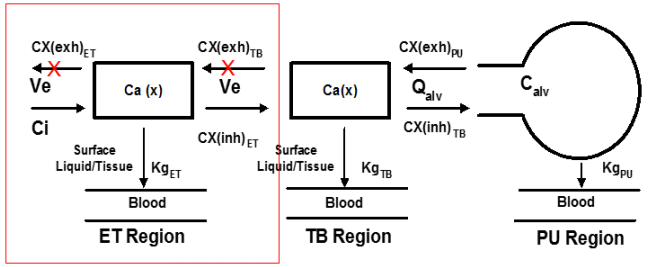


Model Selection Criteria

- Data availability
- Physicochemical characteristics: Particle / Gas
- Location of toxicity (Portal-of-entry or systemic)
- Level of observation (cellular to population)
- Toxicity time frame versus exposure duration
- Mode of action
- Dose metric description

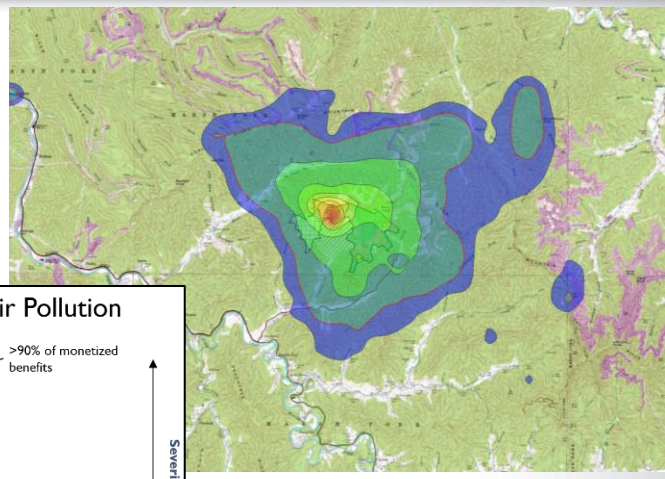
Model Structure Hierarchy

- **Exposure adjustment**
  - Default uncertainty factors for toxicokinetic differences in animals and humans
- **Categorical defaults**
  - Allometric scaling
  - Reduced-form analytical solutions
  - Data-derived uncertainty factors
- **Chemical-specific**
  - Computational fluid dyanamics (CFD)
  - Single-path mass transfer
  - Physiologically-based pharmacokinetic model (PBPK)
  - Data-derived uncertainty factors
- **Comprehensive**
  - Biologically-based dose-response (BBDR) descriptions

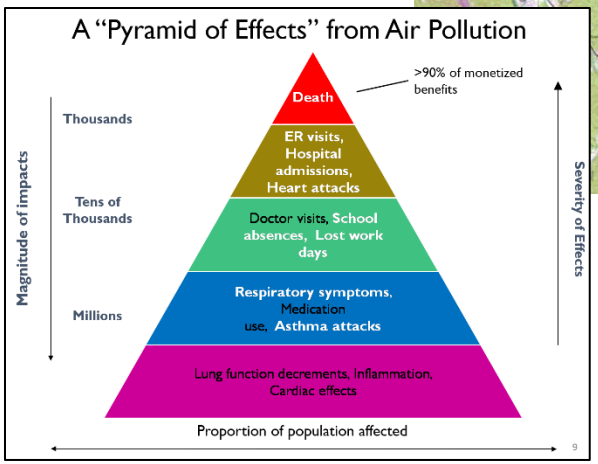


Accuracy

Assessment Applications: Multi-scale Modeling



- **Flexibility required to**
  - **Characterize different exposure scenarios**
    - Acute
    - Episodic
    - Chronic
  - **Address different scales**
    - Geographical
    - Temporal
    - Biological



  - **Describe <u>disease dimensions</u> (e.g., early or late event) and <u>key events</u>**
  - **Develop probabilistic approaches based on prognostic significance for key events and evolve benefits:cost analyses**

Dose Translation and Data Integration

- **Data from diverse sources and approaches require dose translation to facilitate interpretation**
  - **Community and ecosystem sensors**
  - **Human studies (clinical, epidemiological)**
  - **Laboratory animal (*in vitro, ex vivo, in vivo*) → IVIVE**
  - **Biomonitoring**
  - **Clinical chemistry**
  - **Virtual tissues**
  - **HTS / HC**

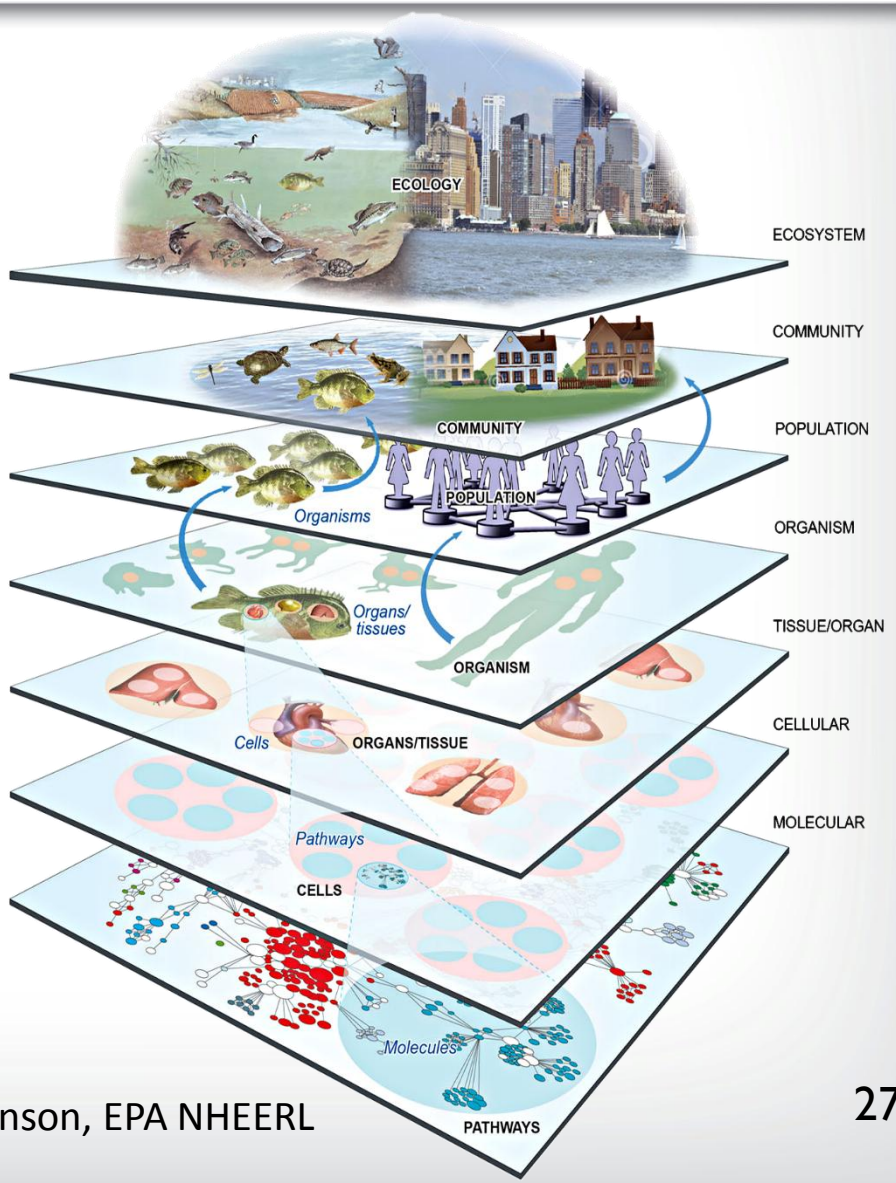
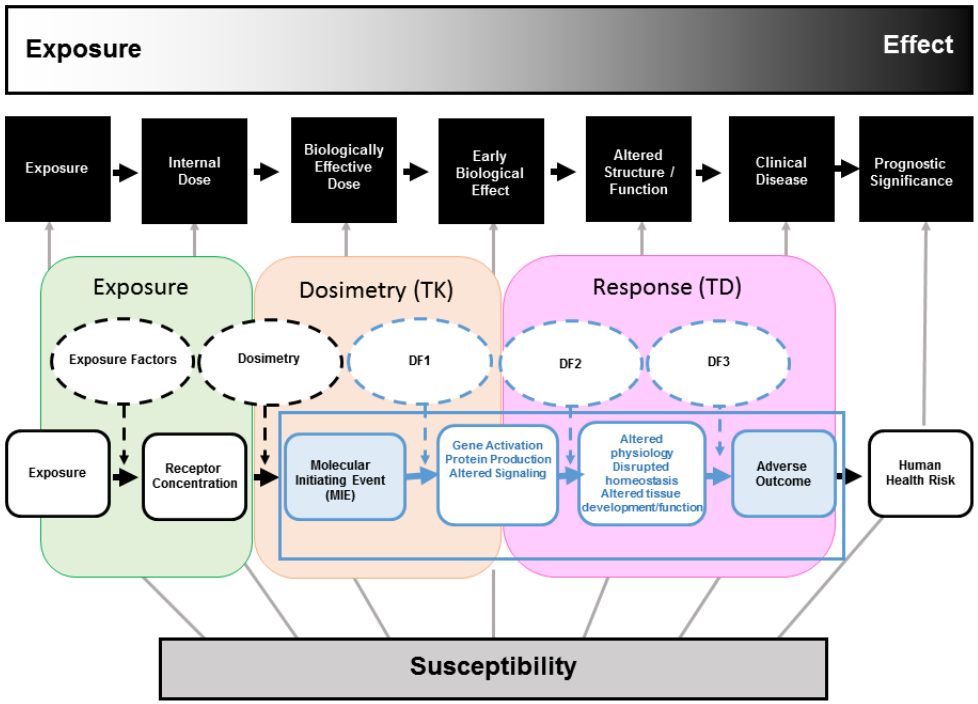


Figure courtesy of Bill Benson, EPA NHEERL

Advancing AOP and MOA

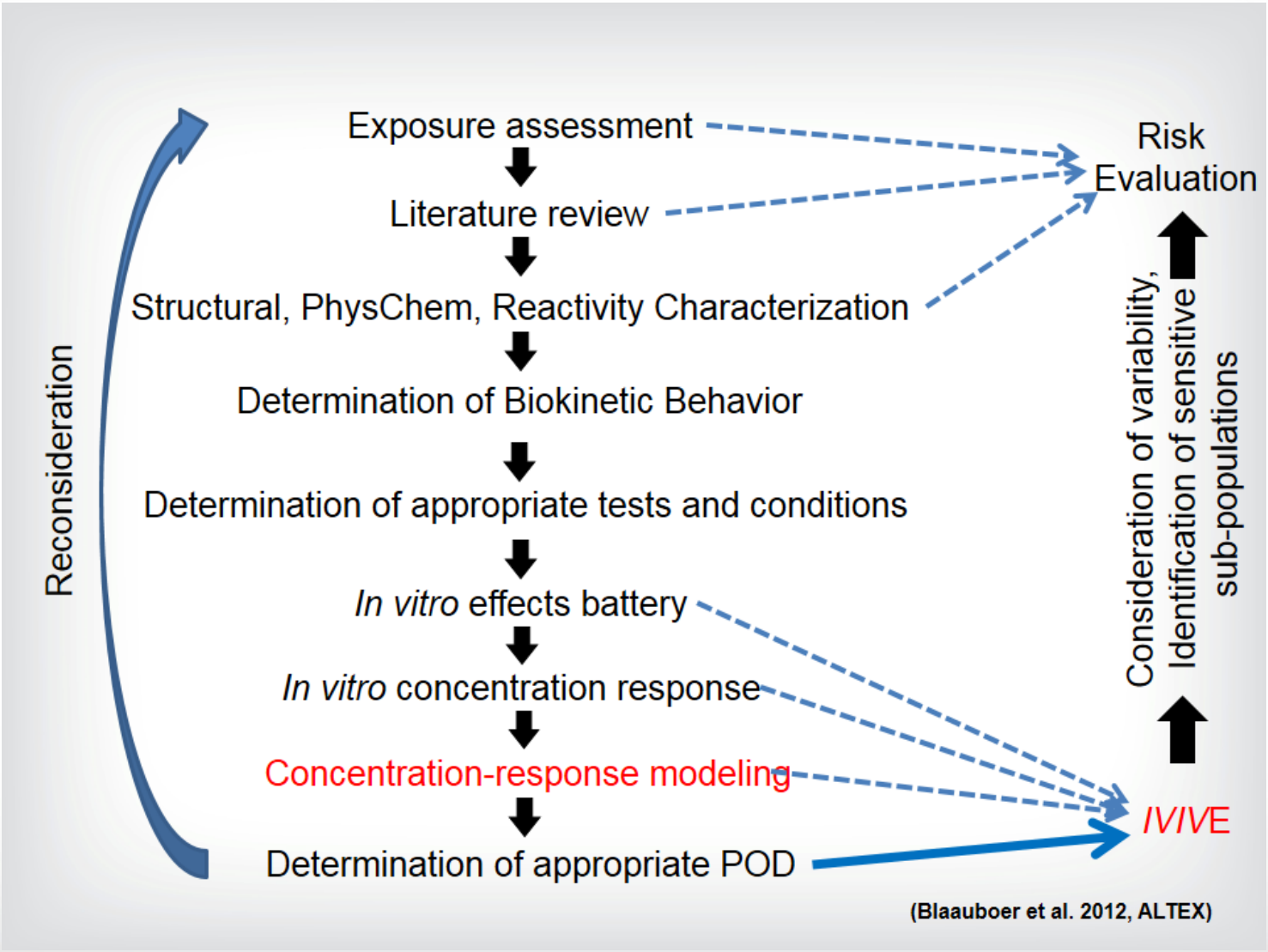
- **Need to define different dose metrics in order to apply key events of adverse outcome pathways (AOP) and mode of action (MOA) in risk assessment**
  - Screening dosimetry insufficient for quantitative response analysis
  - Portal-of-entry descriptions
  - Broad context re: both endpoints and chemical classes
- **Support transparency, causal linkage and interoperability along continuum: exposure to dose-response analysis**



Source: US EPA Human Health Risk Assessment (HHRA) FY16-19 Strategic Research Action Plan

https://www.epa.gov/research/strategic-research-action-plans-2016-2019

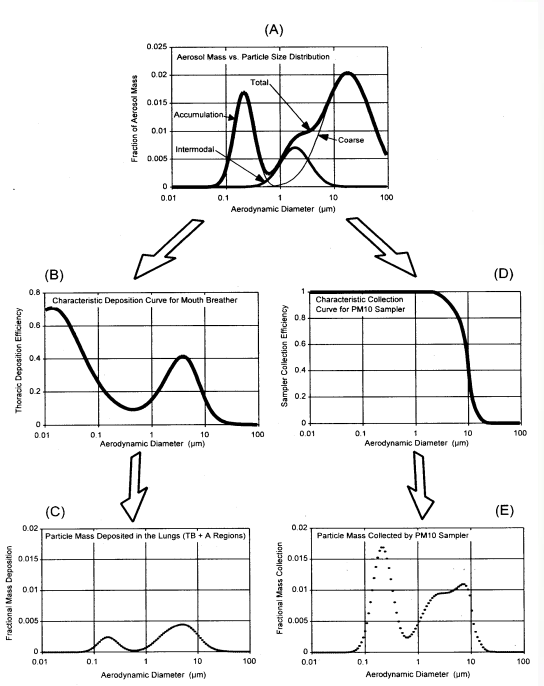
Creating Context: In vitro to In Vivo (IVIVE) Extrapolation



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 via air at cell level
- **Impact on inferences**
  - **Biases introduced based on**
    - Exposure system
    - 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

IVIVE: 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**
    - Operating specifications of generation / characterization and *in vitro* system
    - Concentration, duration
    - Periodic, ambient constant, workplace
  - **Individual anatomical and physiological parameters**
    - Age-specific anatomy and ventilation rate; disease status
    - Cell type(s) or tissue(s) relevant to pathogenesis
    - Activity pattern (e.g., rest, exertion)
    - Breathing mode (nasal, oronasal or mouth)
  - **Physicochemical properties – related to both exposure and biological system**
    - **Particles:** Diameter, distribution, density, durability
    - **Gases:** Diffusivity, reactivity, solubility

Advantages to Mechanistic Modeling of Inhaled Agents for IVIVE

- **Builds on current understanding of biological and physicochemical mechanisms in mode of action (MOA)**
- **Aids comparisons and translation of results**
  - Quantifies and explores differences systematically
  - Different agents (particles/fibers/ENM or gases)
  - *in vitro* to *in vivo (IVIVE)* context
    - Across exposure conditions
    - Between species
    - Target scenarios
- **Facilitates comparisons of regional to local estimates of different doses metrics with disease endpoints and measurements**
  - Provides insights on MOA inferences and integration
  - Refines risk assessment predictions

Thank you

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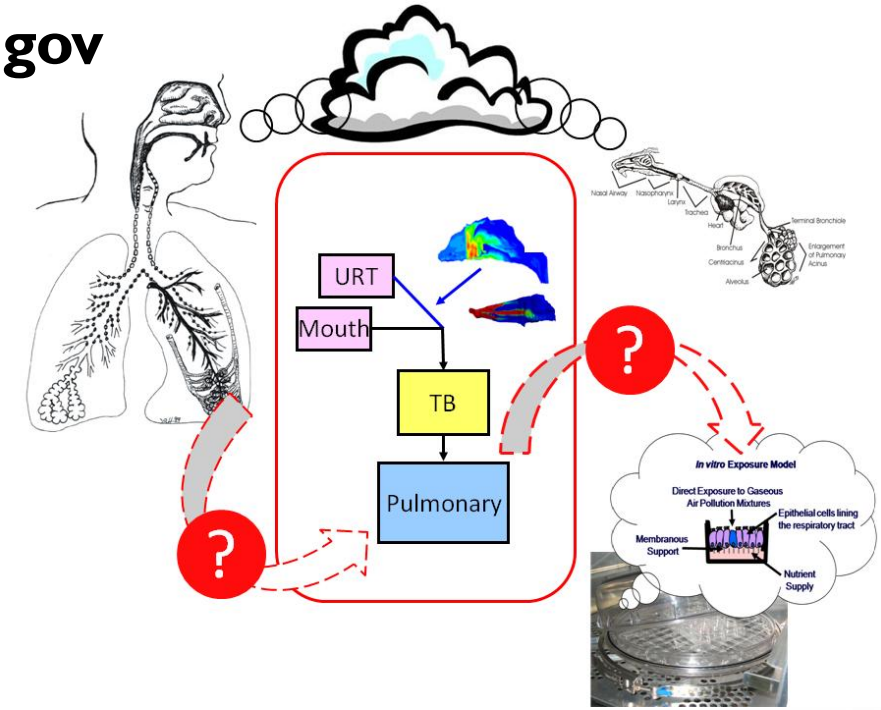

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