

A Primer on Acute Inhalation Toxicity Testing

Where do Alternative Methods Fit?

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Outline

- Why do we test?
- Current test guidelines
- How do we test?
- Potential alternatives
- Opportunities and challenges



Why test?

- Major route of human exposure
- Unique interface between environment and systemic circulation
 - Upper (URT) and lower (LRT) respiratory tract important
- Exposure-response data for hazard identification
 - Integration of material properties, deposition, absorption, transport, metabolism and elimination
- Identify critical responses to inhaled materials
 - Portal of entry effects
 - Cells and tissues of URT and LRT
 - Systemic effects
 - Internal organs and tissues



How is the Data Used?

- Hazard Identification
 - Guideline testing for registration
 - Classification and Labeling
 - Handling and Shipping
 - Safety Data Sheets
- Risk Assessment
 - Occupational Exposure Levels
 - Emergency Response
- Product Stewardship
 - Data Gaps
 - Read Across
 - Reformulations
 - New Product Selection



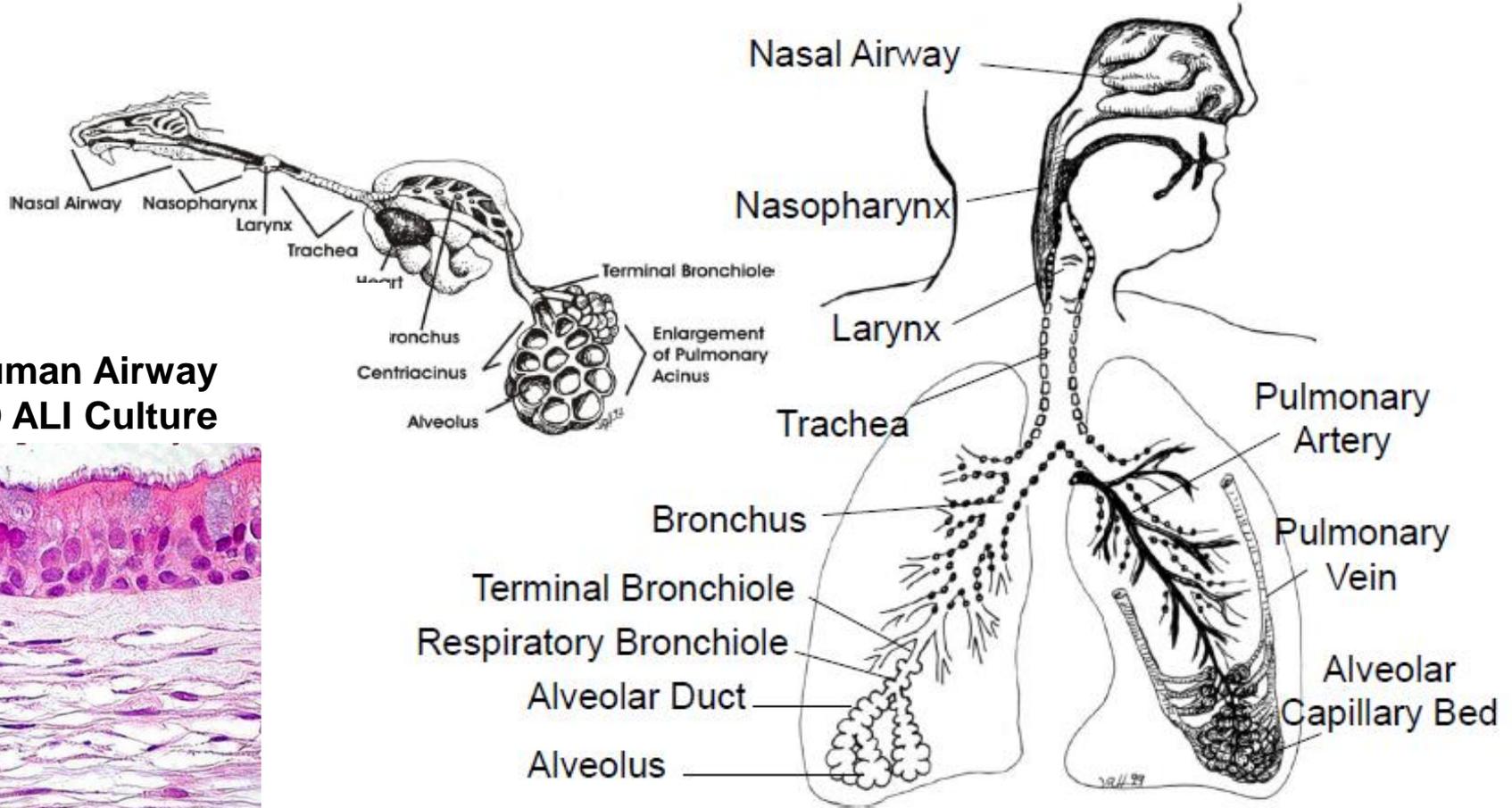
What is the goal?

In Silico
In Vitro

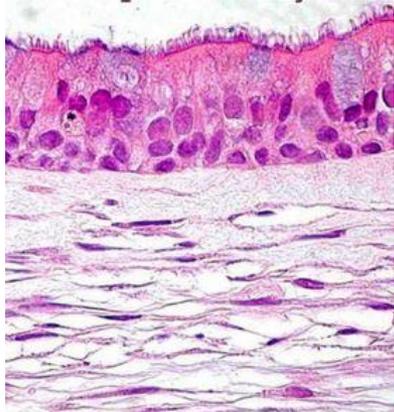
In Vivo



Human



**Human Airway
3D ALI Culture**



MatTek EpiAirwayFT



The **Rt** Formula
RIGHT

Acute *In Vivo* Inhalation Guidelines

	EPA OPPTS 870.1300	OECD TG 403	OECD TG 436	Draft OECD TG433
Limit test Concentration Duration = 4h	2 mg/L	20,000 ppm (gas) 20 mg/L (vapor) 5 mg/L (aerosol)	20,000 ppm (gas) 20 mg/L (vapor) 5 mg/L (dust/mist)	5000 ppm (gas) 20 mg/L (vapor) 5 mg/L (dust/mist)
# Exposure Groups (Main Study)	3/n=5 per sex	Traditional: 3/n=5 per sex C x t: 4 or 5 at multiple durations/n=1 per sex (or 2 of more susceptible sex)	≥1/n=3 per sex (or n=6 of more susceptible sex)	≥1/n=5 (most susceptible sex based on sighting study)
Recommended exposure	Nose only	Nose only	Nose only	Nose only
Observation period	14 days	14 days	14 days	14 days
Observations	Daily clinical obs; weekly body weight; TOD; gross necropsy (optional histo)	Daily clinical obs; body weight on d 0, 1, 3, 7, weekly; TOD; gross necropsy	Daily clinical obs; body weight on d0, 1, 3, 7, weekly; TOD; gross necropsy	Daily clinical obs; body weight on d0, 1, 3, 7, weekly; TOD; gross necropsy (optional histo); Evident toxicity
MMAD range	1-4 μM	1-4 μM	1-4 μM	1-4 μM
Notes	Covers entire range of the concentration- mortality relationship – LC ₅₀ point estimate	Covers entire range of the concentration-mortality relationship – LC ₅₀ point estimate; C x t can derive AEGs; Better estimates of toxicity at lower exposure concentration boundaries	Refinement and reduction – serial steps/fixed concentrations; LC ₅₀ range estimate	Refinement alternative; evident toxicity or death TG not adopted yet



Acute Inhalation Hazard Categories

GHS & OSHA	Category 1 DANGER  <i>Fatal if Inhaled</i>	Category 2 DANGER  <i>Fatal if Inhaled</i>	Category 3 DANGER  <i>Toxic if Inhaled</i>	Category 4 WARNING  <i>Harmful if Inhaled</i>	Category 5 WARNING <i>May be Harmful if Inhaled</i>
Gases (ppm/V)	≤ 100	>100 ≤ 500	>500 ≤ 2500	>2500 ≤ 5000	> 5000
Vapors (mg/L)	≤ 0.5	>0.5 ≤ 2.0	>2.0 ≤ 10	>10 ≤ 20	> 20
Dusts/Mists (mg/L)	≤ 0.05	>0.05 ≤ 0.5	>0.5 ≤ 1.0	>1.0 ≤ 5	> 5

EPA	Category I	Category II	Category III	Category IV
Acute Inhalation <i>(No Distinction Between TM States)</i>	≤ 0.05 mg/L	>0.05 thru 0.5 mg/L	>0.5 thru 2 mg/L	> 2 mg/L

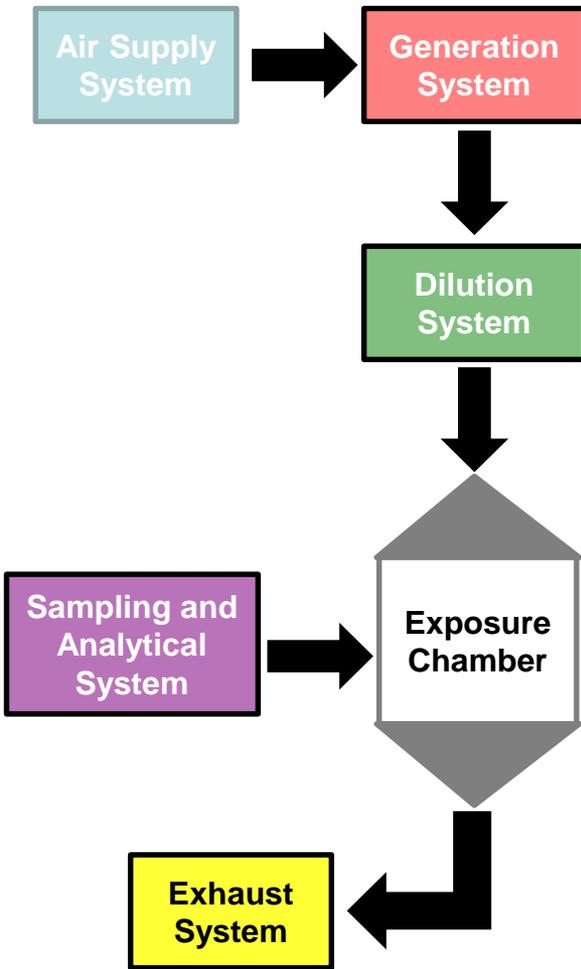


Acute Studies Do Not Provide...

- Histopathology of the respiratory tract or other systemic organ systems
- The types/severity of lesions
- Persistence or reversibility of lesions
- Information on target organ toxicity
- No data related to mechanism of toxicity
- ***Guideline studies provide none of the data essential to develop/validate in silico and in vitro alternative methods!***



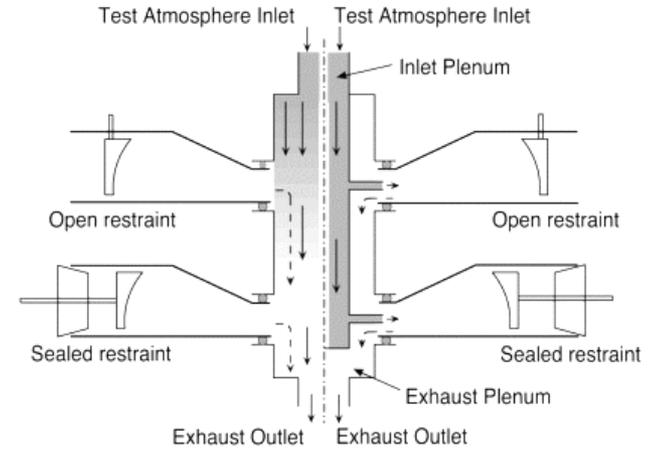
Acute Inhalation Testing



Whole-Body



Nose-Only



Types of Test Atmospheres

- Gases

- Exist in gaseous state under normal conditions

- Vapors

- Gas phase fraction of volatile solids/liquids

- Saturated vapor (ppm) = $V_p \text{ (mm Hg)} \times 10^6 / \text{AtmP (mm Hg)}$
- $\text{mg/L (vapor or gas)} = \text{ppm} \times (\text{molecular wt}/24450)$

- Aerosols

- Liquid

- Suspension of liquid droplets

- Solid

- Suspension of solid particles

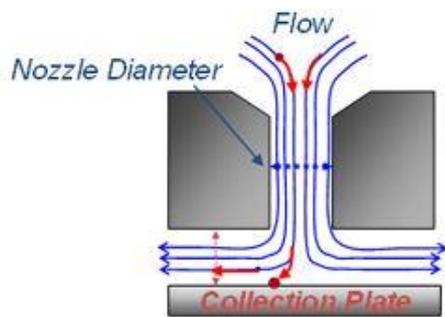
- Formed from mechanical size reduction, molten TM, or dried from liquid aerosol



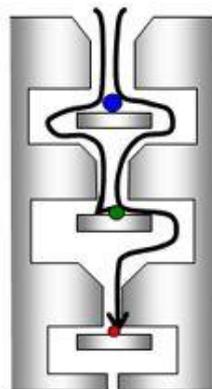
Aerodynamic Particle Size

Gravimetric Impactors

Principle of Operation



Single Stage



Cascade Impactor

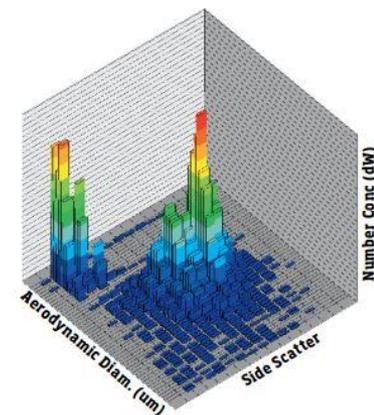
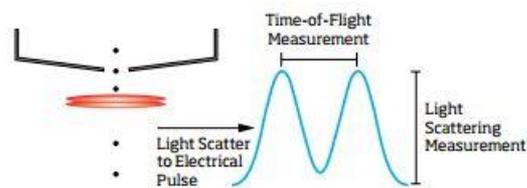
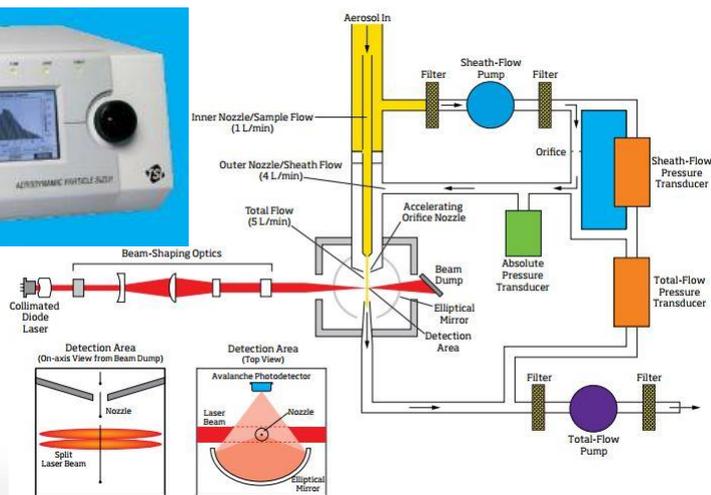
Stage 3: > 10 μ m

Stage 2: > 2.5 μ m

Stage 1: > 1 μ m

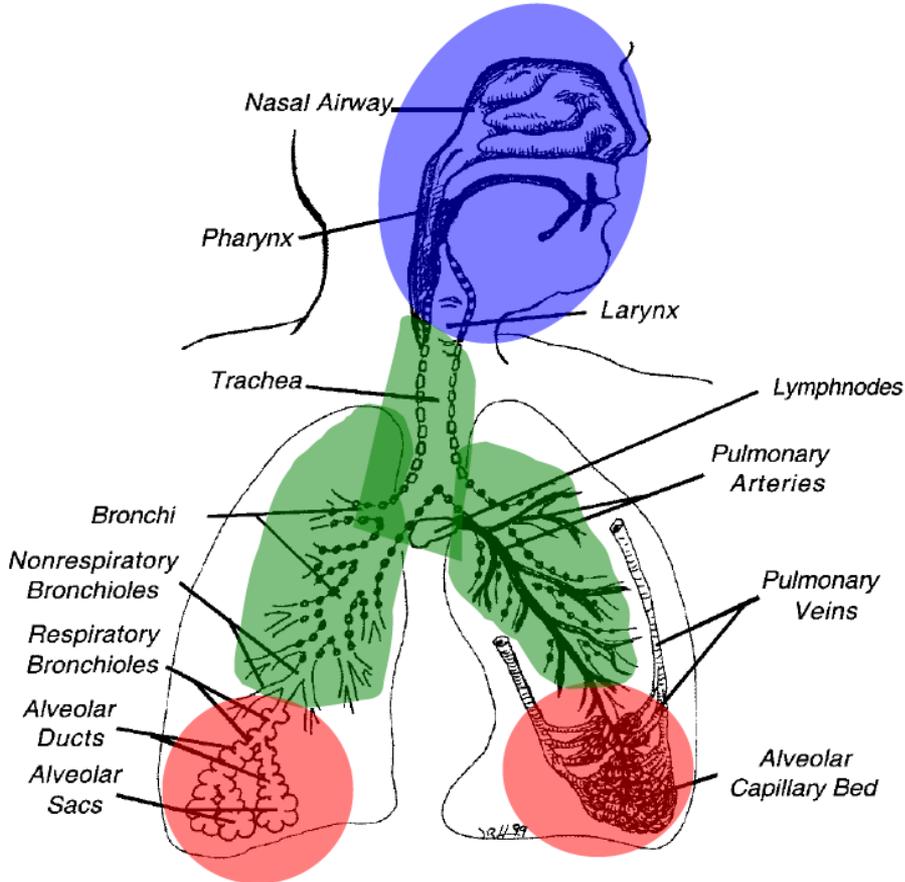


Aerosol Particle Size Spectrometer

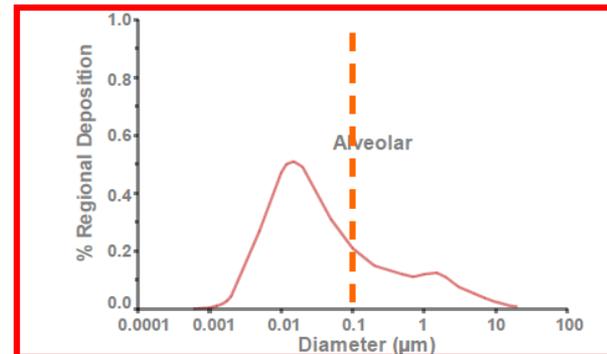
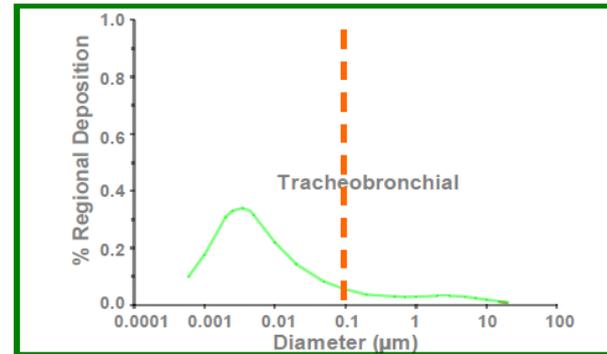
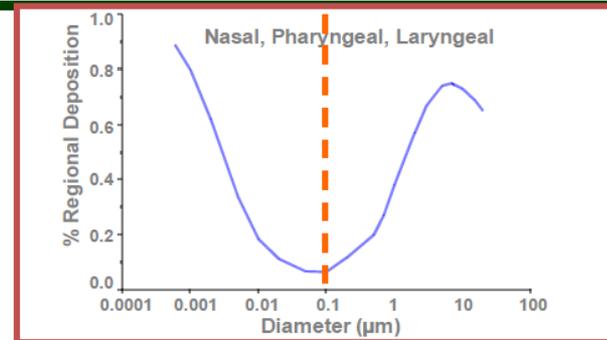


Aerosol Particle Deposition

(ICRP Model, 1994; Nose-breathing)



Slide courtesy of G. Oberdörster
Figure courtesy of J.Harkema



Aerosol Dosimetry

■ Inhalable Fraction

- The fraction of particles that enter the body through the nose and/or mouth during breathing ($d_{ae} \leq 100 \mu\text{m}$)
 - Relevant to health effects anywhere in the respiratory tract and systemic effects

■ Thoracic Fraction

- Subfraction of inhalable particles that can penetrate into the tracheo-alveolar region ($d_{ae} < 30 \mu\text{m}$)
 - Important for asthma, bronchitis, and lung cancer

■ Respirable Fraction

- Subfraction of inhalable particles that penetrate into the alveolar region ($d_{ae} \leq 10 \mu\text{m}$)
 - Chronic respiratory diseases: pneumoconiosis, emphysema



“Dose” Estimates

■ Inhaled dose

- Concentration x minute ventilation x duration
 - Rat: $(\text{mg/L}) \times (0.78 \text{ L/min} \cdot \text{kg}) \times \text{min} = \text{mg/kg}$
 - Mouse: $(\text{mg/L}) \times (1.533 \text{ L/min} \cdot \text{kg}) \times \text{min} = \text{mg/kg}$
 - Human: $(\text{mg/L}) \times (0.089 \text{ L/min} \cdot \text{kg}) \times \text{min} = \text{mg/kg}$
- Assumes 100% deposition and absorption

■ Deposited dose

- Fractional Deposition x Inhaled Dose
 - Better – often quite good for particles (use MPPD model)

■ Absorbed dose

- Mass transport (flux) x Deposited Dose
 - Even better – requires knowledge of regional deposition, mass transport
 - Response modified by local metabolism and or sensitivity of cell populations



Alternative Approaches: Waivers

- Inability to generate a toxic concentration
 - Gas, vapor or aerosol
 - If formulation inerts interfere then use toxicity profile of active
- Low volatility
 - $< 7.5 \times 10^{-5}$ mm Hg (indoor uses); $< 7.5 \times 10^{-4}$ mm Hg (outdoor uses)
 - If not aerosolized, heated, evaporated or made inhalable during use, storage, handling or transport – or contained in nonvolatile matrix
- Non-inhalable aerosol particle size
 - If $> 99\%$ of particles are $> 100 \mu\text{m}$ aerodynamic diameter
 - Large aerosol diameter during application not relevant
 - Resistant to mechanical size deduction by attrition

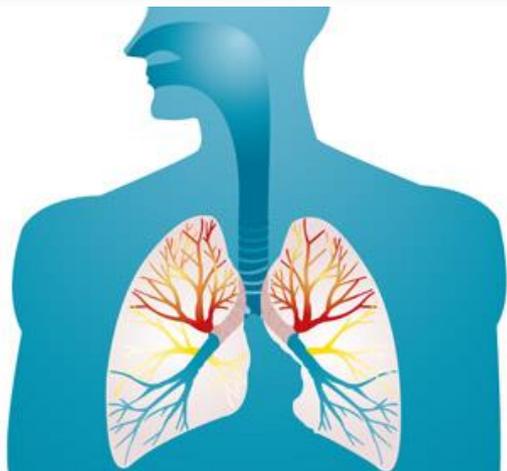


Predictive *In Silico* Approaches

- Predictive *in silico* models of acute inhalation toxicity are rudimentary
 - There is a need for more curated inhalation data
- Require significant expert judgment to yield reliable results
- A commercial QSAR model for acute rat inhalation toxicity showed poor sensitivity
- Read across of acute inhalation toxicity to intravenous or oral data was better than global QSAR model results



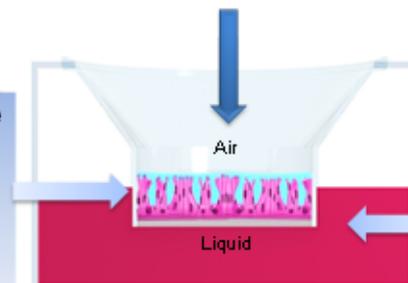
In Vitro Alternative Methods



Exposing Cells at the Air-Liquid Interface (ALI)

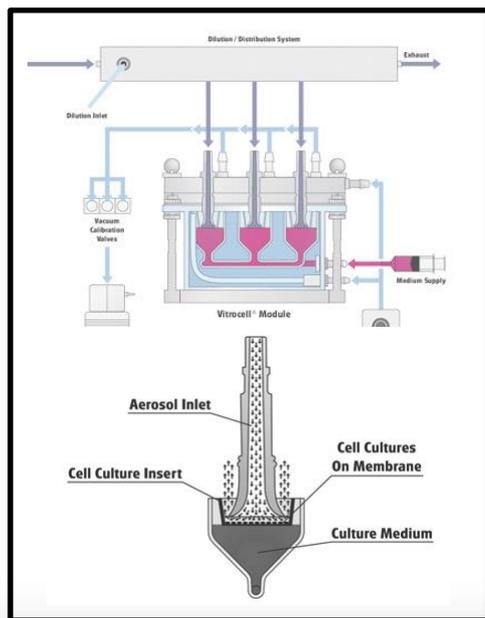
Information from Apical Side

- TEER measurement
- Resazurin test
- Morphology
- Cilia beating frequency
- Mucociliary Clearance



Information from Culture Medium

- LDH release
- IL-8, IL-6 release



- Alternative model systems
 - Submerged cultures
 - “Any cell will do” or Site-specific (transformed) cells
 - Air-liquid interface (ALI) cultures
 - Transformed or primary human cells
 - 3D organotypic models
 - Lung on a chip
 - +/- other organs-on-chips
- Equivalent dosimetry *in vivo* and *in vitro*



Opportunities and Challenges.....

- Growing acceptance of predictive *in silico* and *in vitro* models for hazard assessment
 - The models must be robust, transparent, reproducible & accurate
 - Greater impact and acceptance early in development cycle
- Incorporate data from alternative methods into read-across arguments
 - Anchor predictive methods to existing data sets
- Be smart when implementing *in vivo* TGs
 - Regulatory need; existing data (other routes; similar structures)
- Simplification/standardization of alternative model systems will facilitate regulatory acceptance
- ***Ultimately, it's up to us to advocate for change***

