Development and application of in vitro methods for evaluating respiratory irritants.

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Describing the problem

Repeat dose 90-day inhalation study is a regulatory requirement.

- No new or additional information will be forthcoming from such a study that will improve human safety assessment.

No additional systemic risk from inhalation exposure.

- Contact irritation in respiratory tract.

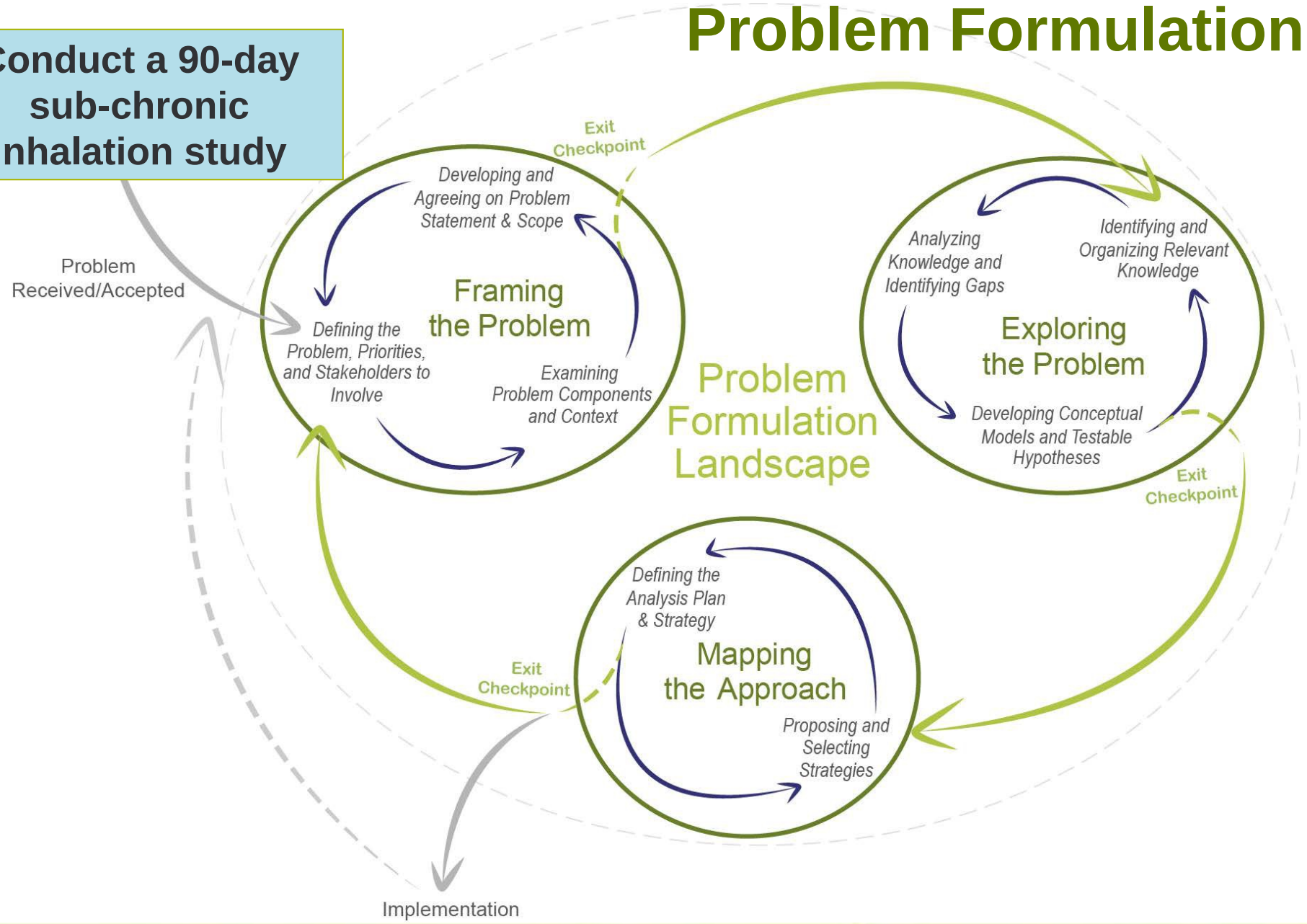
Long history of safe use, widely-used since 1966.

Question to Answer

What is the human health risk from intermediate- to long-term inhalation exposure to chlorothalonil?

Problem Formulation

Conduct a 90-day sub-chronic inhalation study



Sauve-Ciencewicki et al., Reg Tox Pharm 101:187-193, 2019

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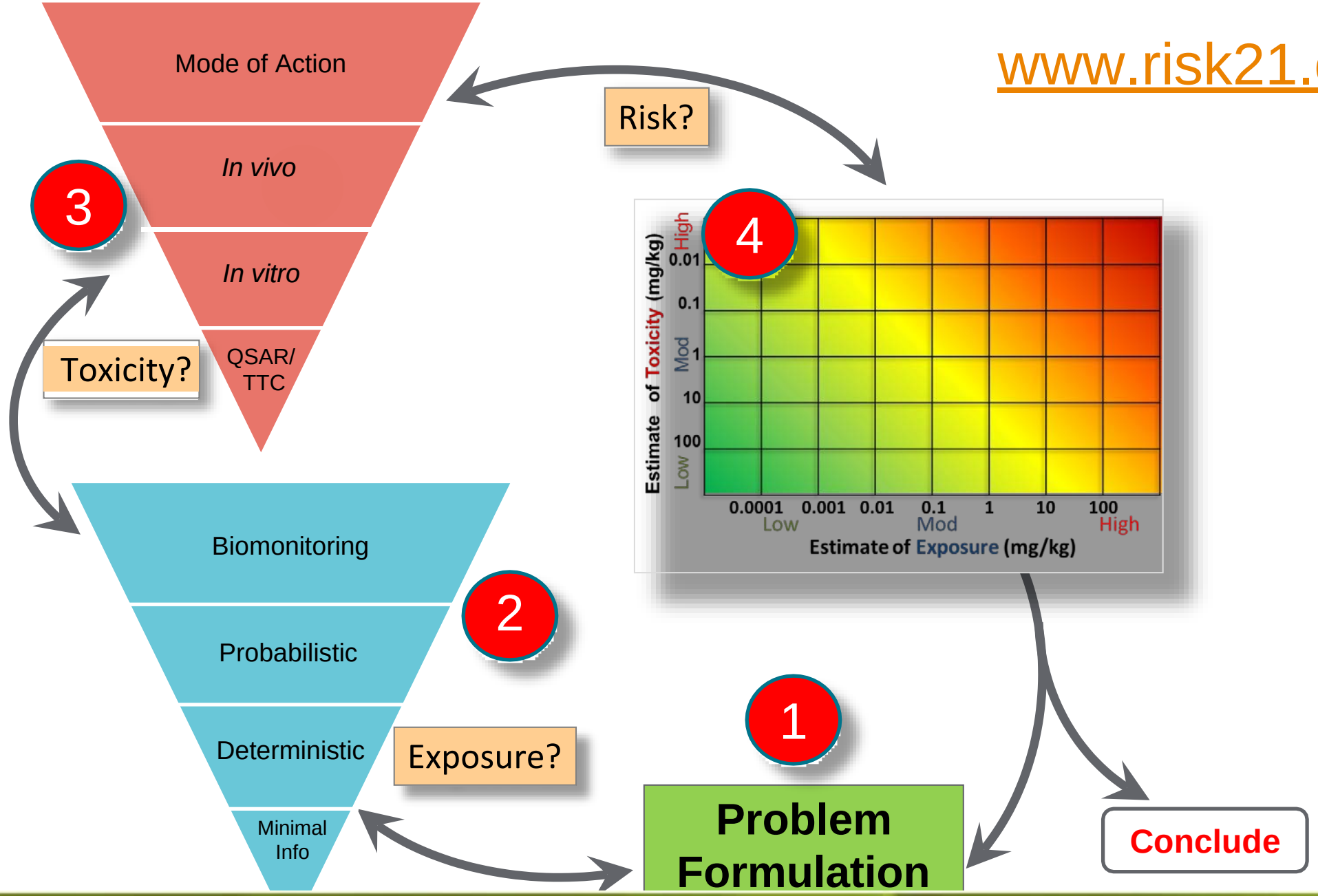

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Problem Statement

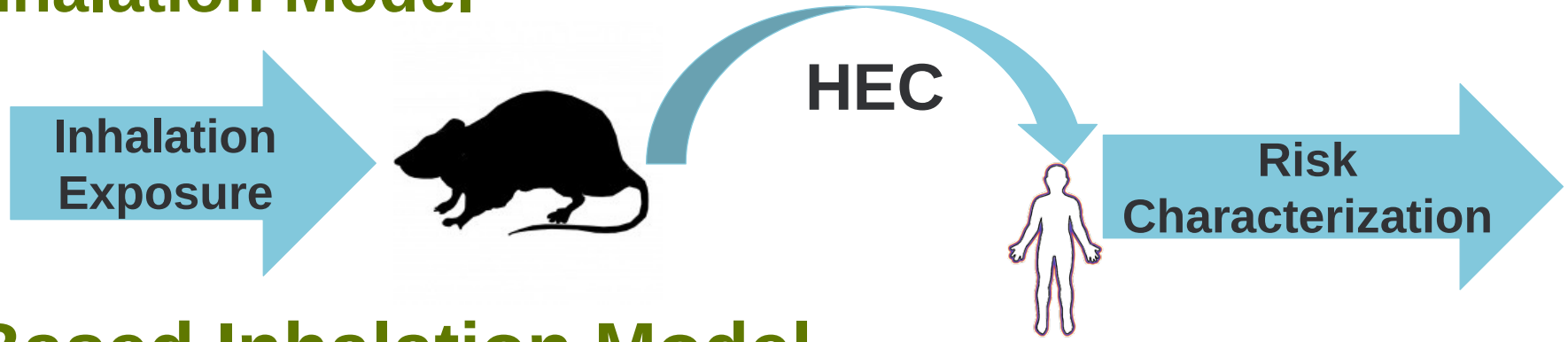
A new approach method can be developed and would be suitable to inform inhalation toxicity in lieu of a sub-chronic whole animal inhalation study.

Source to Outcome Approach

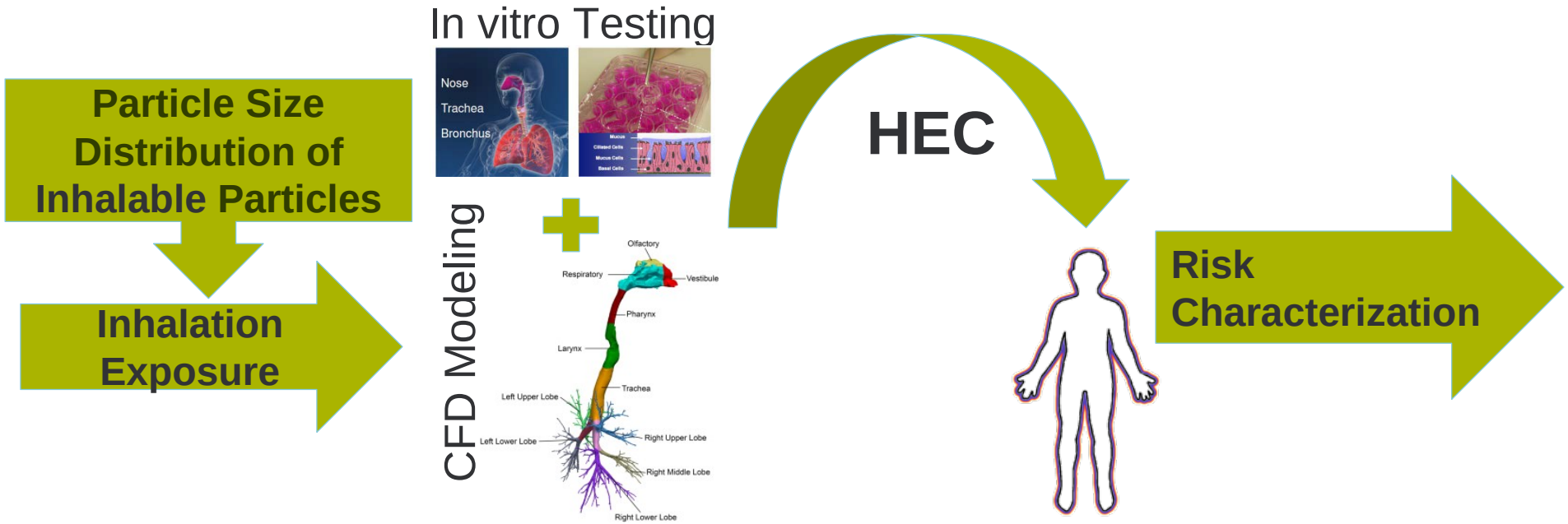
- **Source**
  - Evaluate the particle size distribution of pesticide applications.
- **Exposure**
  - Characterize aerosols captured on personal sampling devices.
- **Dosimetry**
  - Calculate deposition in human airways using Computational Fluid Dynamics (CFD) model.
- **Outcome**
  - Predict human inhalation toxicity incorporating spray particle size.

Conceptual Models

Traditional Inhalation Model



Exposure Based Inhalation Model



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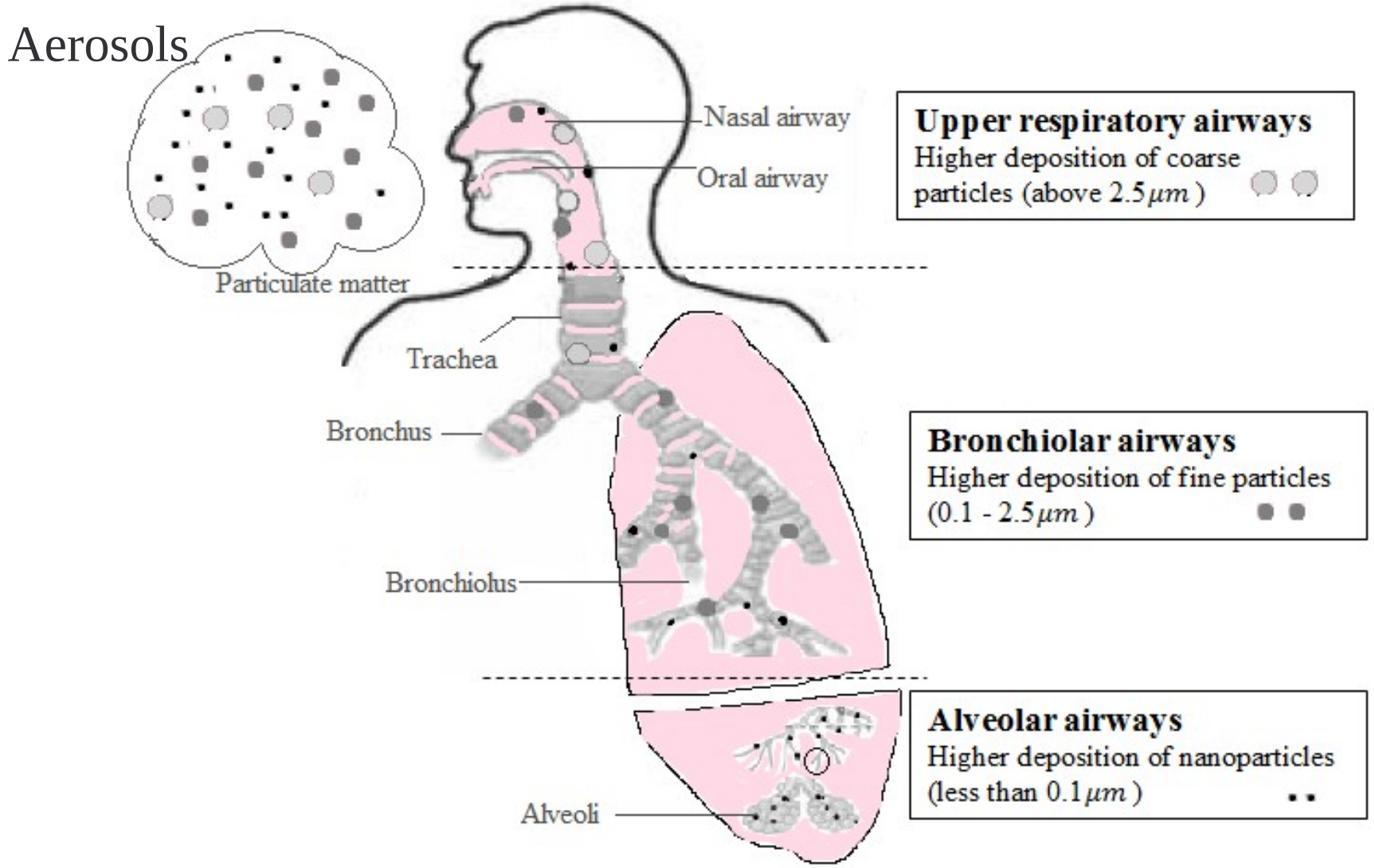
Pesticide Exposure

- Exposure data is commonly collected from agricultural workers using an OSHA Versatile Sampler (OVS) tube
- Typically, the OVS tube data is only reported as total concentration, without consideration of particle size
  - What is the particle size distribution captured by this device?
- Studies of spray particle size were undertaken at Syngenta to compare OVS tube with standard sizing methods

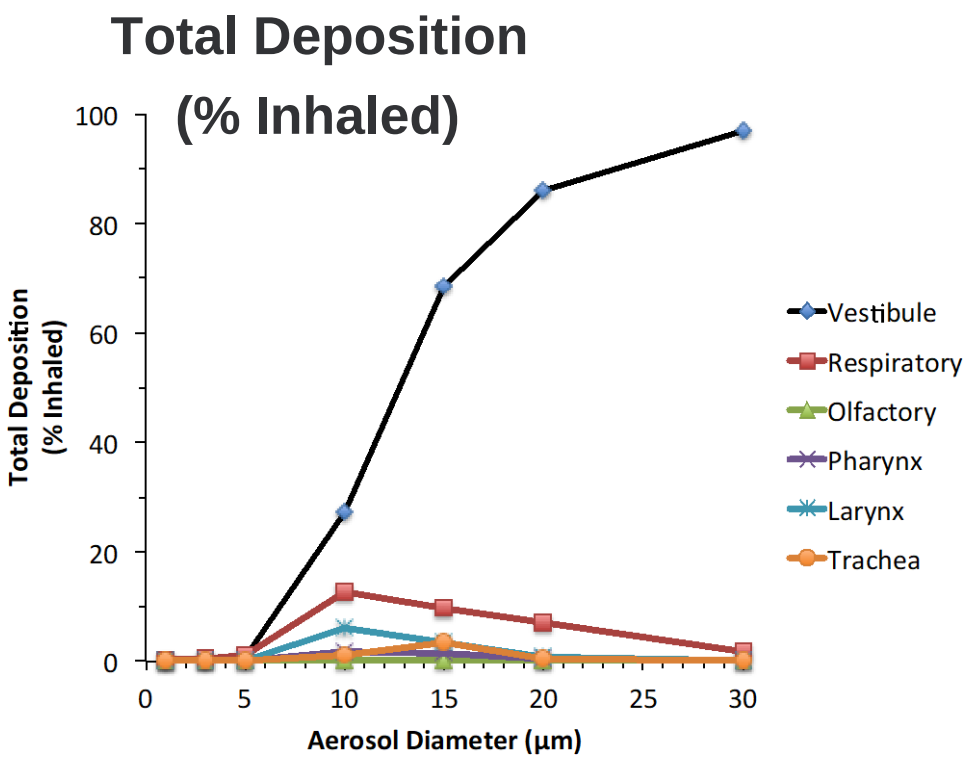
Flack et al., J Agri Safety Health 25:91-103, 2019

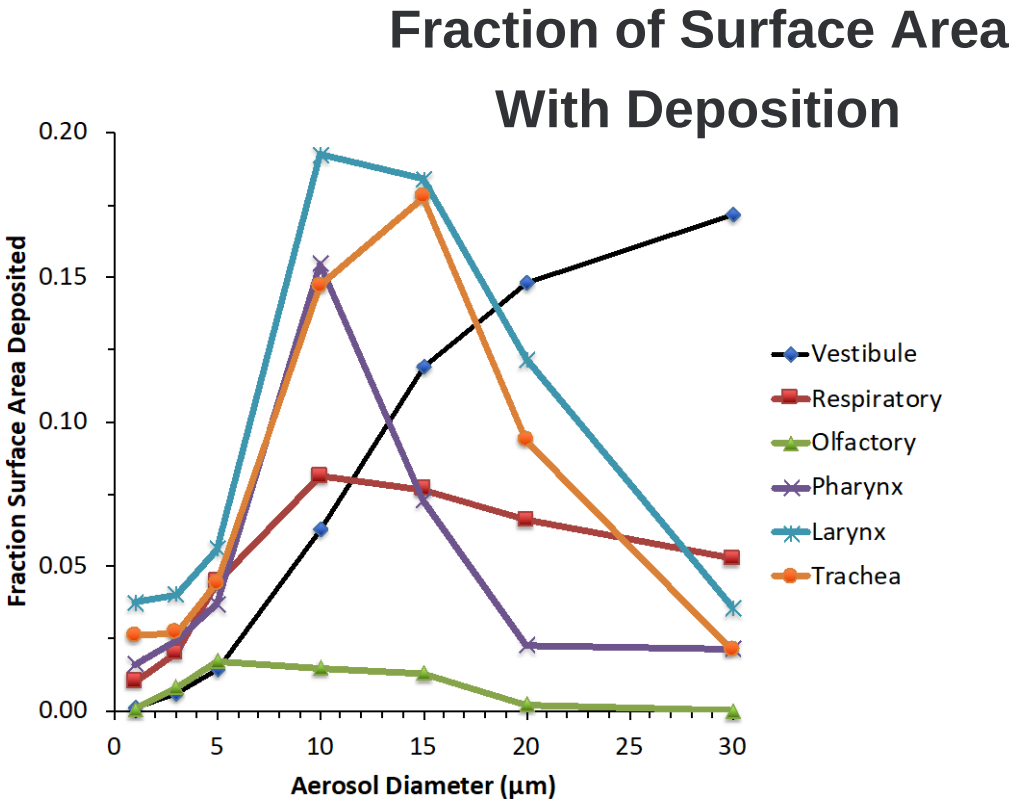
Human Respiratory Tract



Adapted from: Kolanjiyil and Kleinstreuer J Biomech Eng. 2013 Dec;135(12):121003

Regional Deposition in Humans





- Aerosols associated with worker exposures largely deposit in nasal vestibule
  - Peak regional airway exposures at 10-15 µm sized aerosols (excluding the vestibule)
- <20% of the regional airway surfaces receive any aerosol deposition at a high, 1 mg/L exposure

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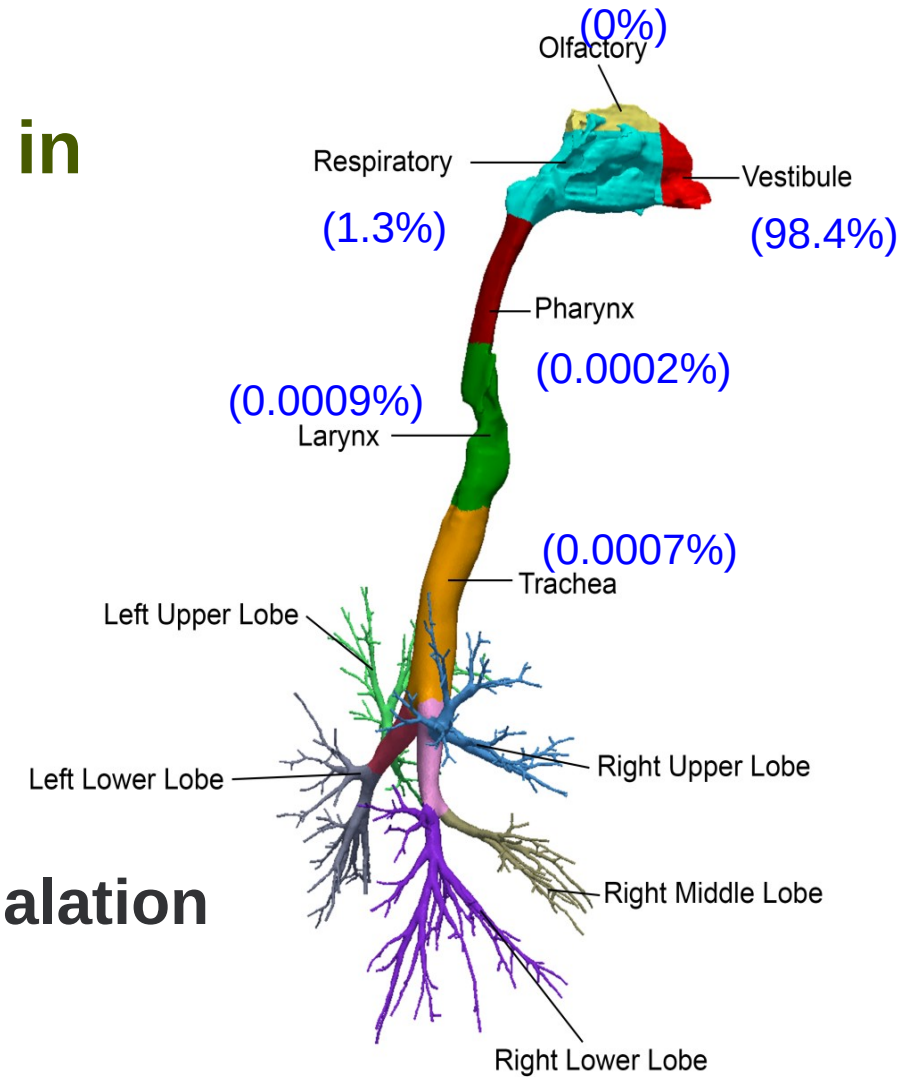
Human CFD/Aerosol Simulation (50 µm MMAD, 4 mg/L)

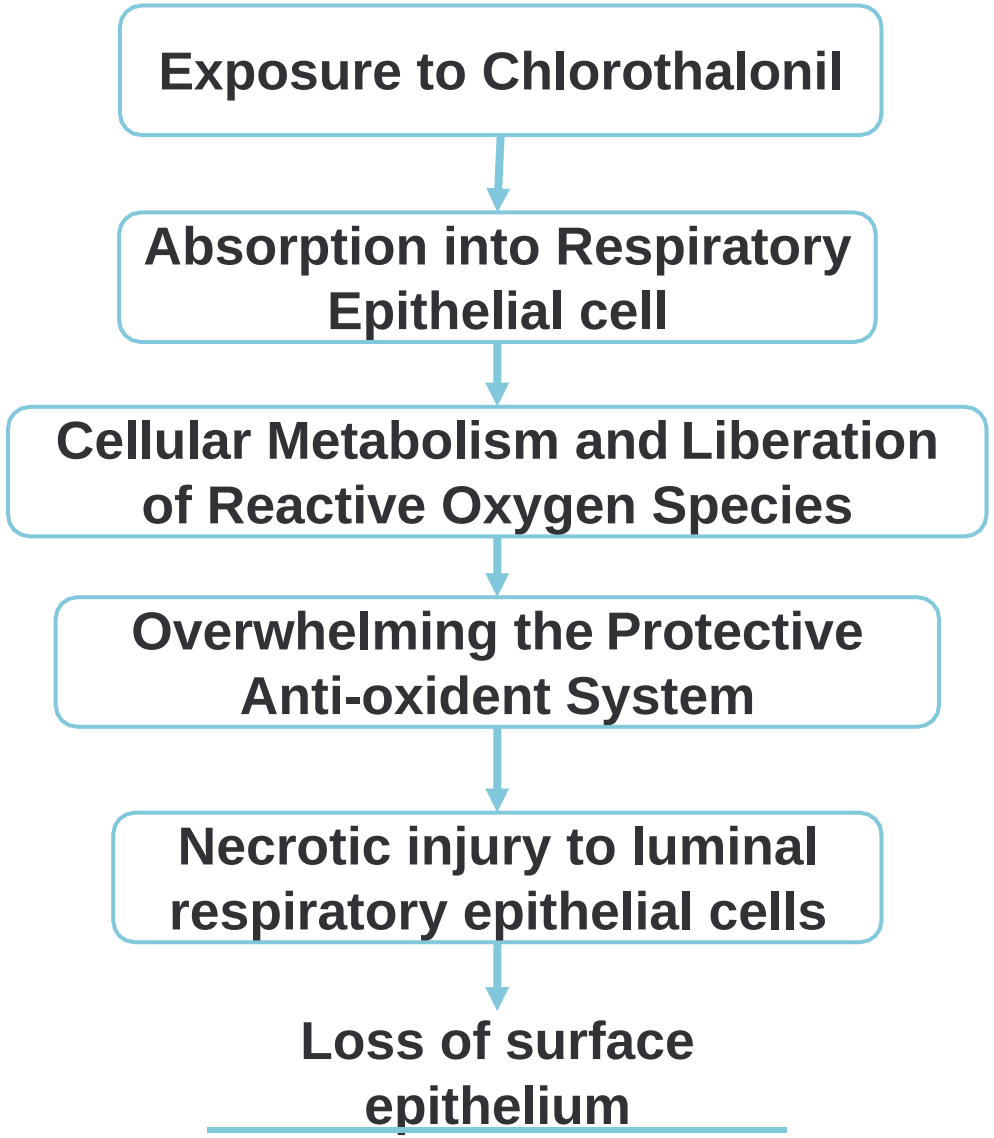
% of Inhaled Mass Deposited by Location in Respiratory Tract



99.7% Deposited in Nose, Pharynx, Larynx
0.0007% Deposited in Remaining Airways
0% Escape the 3D Lung
0.3% Remain suspended in airways at end of inhalation

Adverse Outcome Pathway leading to Cell Death

All can be Modelled *in vitro*



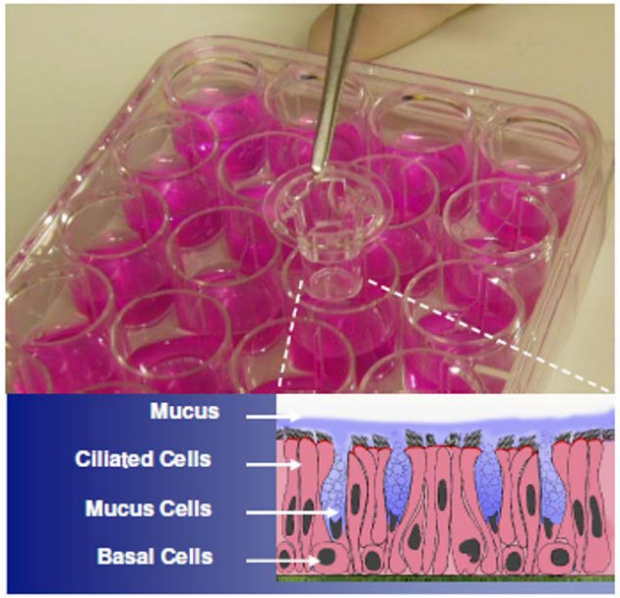
MucilAir™ 3D *in vitro* model

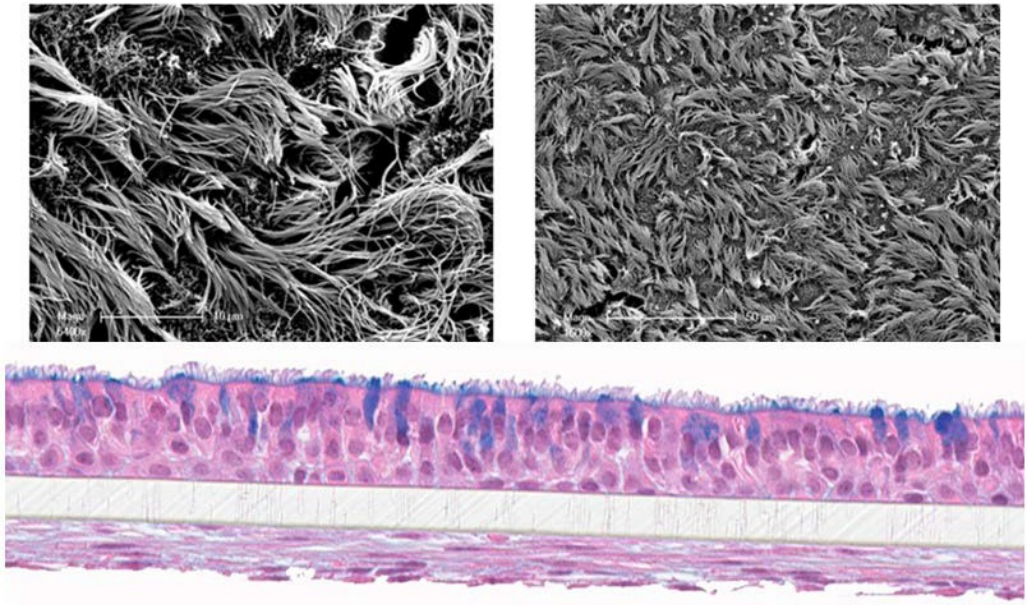
- 3D model of the human airway epithelium formed from differentiated primary human cells
- Measures a variety of membrane and cell damage endpoints as markers of irritation

Trans-epithelial electrical resistance (TEER): measures the integrity of tight junctions between cells in the membrane

Lactate dehyrogenase (LDH): An enzyme present in most cells released when cells suffer cytotoxic membrane damage

Resazurin metabolism: reduced to a fluorescent product in viable cells used as a measure of metabolic competence





https://www.epithelix.com/

Design of MultiDonor MucilAir™ study

- Endpoints: TEER, LDH, and resazurin metabolism
- Tissues from 5 separate donors
- 24 hour topical exposure
- Chlorothalonil applied as Bravo 720 formulation
- 10 concentrations / donor
- 6 replicates / concentration / donor

Dose Level	Chlorothalonil Concentration (mg/L)
1	2
2	5
3	8
4	13
5	20
6	32
7	50
8	79
9	126
10	200

MultiDonor MucilAir™ study results

- Individual donor responses were very similar across the 5 donors used in this study, suggesting low inter-donor variability in sensitivity
- All endpoints (TEER, LHD, resazurin) responded similarly to chlorothalonil challenge
- Multiple BMD approaches were examined, BMD_{1SD} was selected through consultation with the US EPA

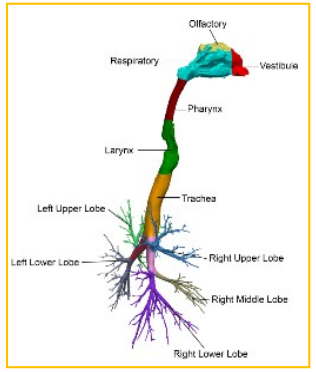
	BMD_{1SD} (mg/cm^2)			
Donor	TEER	LDH	Resazurin	**Mean**
1	0.00463	0.00611	0.00603	**0.00555**
2	0.00480	0.00840	0.00728	**0.00664**
3	0.00798	0.00815	0.00830	**0.00814**
4	0.00996	0.00823	0.00384	**0.00680**
5	0.01125	0.00919	0.01024	**0.0102**
Mean	**0.00724**	**0.00794**	**0.00678**	**0.00730**

Calculating a Human Equivalent Concentration

CFD Deposition

(monodisperse)

mg CTN/cm^2/breath



BMD_{1SD}

mg CTN/cm^2

Deposition

(MMAD = 35 µm, GSD=1.5)

mg CTN/cm^2/breath

Total Daily Deposition

(MMAD = 35 µm, GSD=1.5)

mg CTN/cm^2

HEC

(mg/L)

$$\text{HEC (mg/L)} = \frac{BMD_{1SD}}{\text{Total daily deposition}} \times 1 \text{ mg/L}$$

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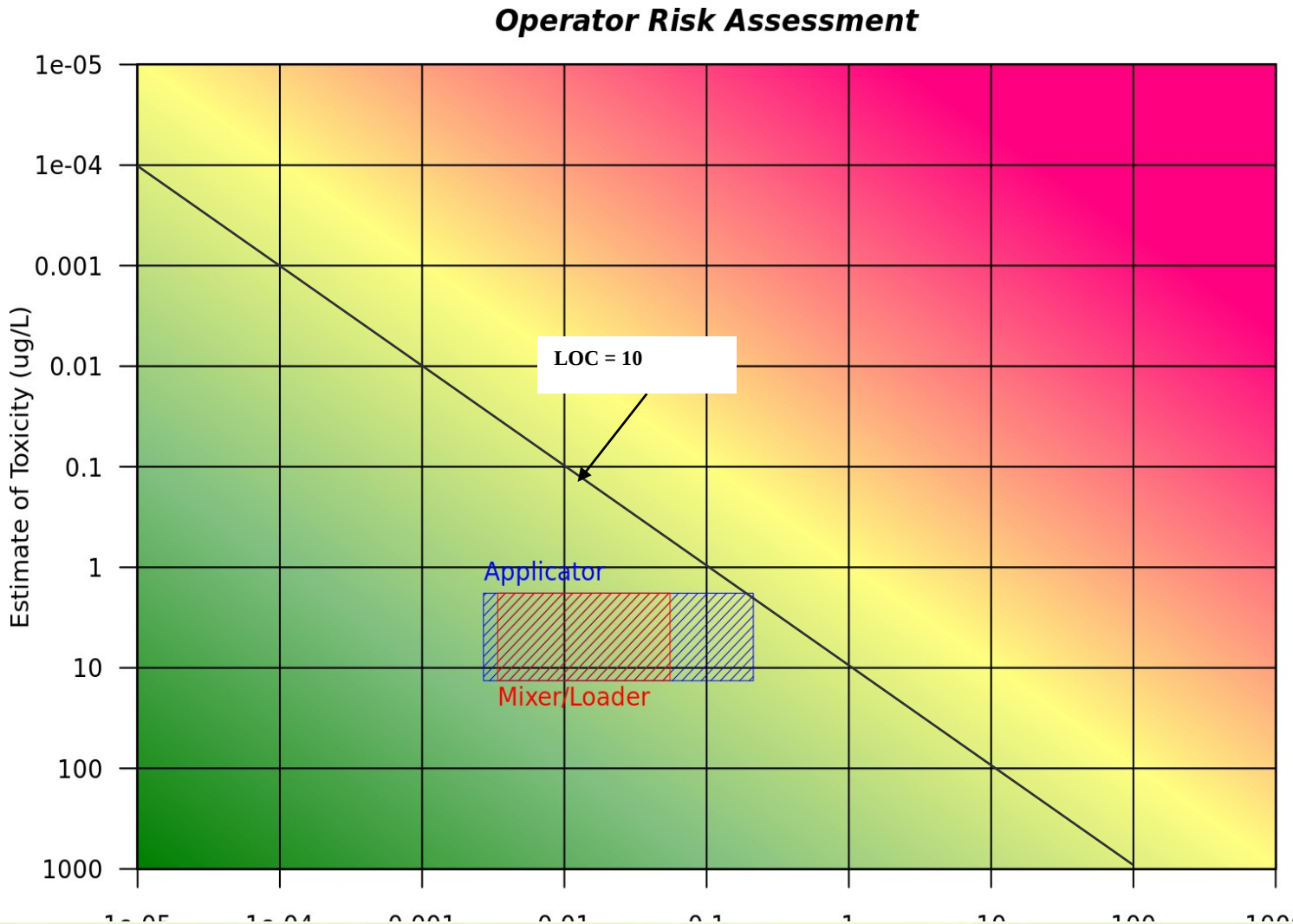
Accurate and Health Protective Risk Assessments Based on human *in vitro* Assay and human *in silico* Model



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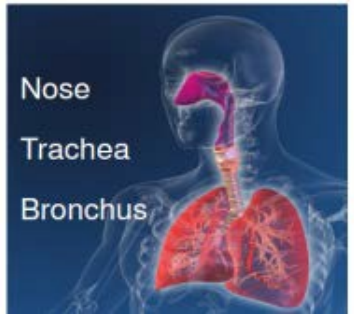
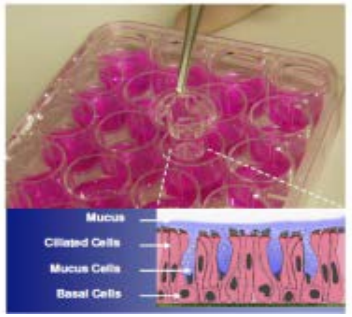
Exposure Based Inhalation Model (IATA)

Particle Size Distribution of Inhalable Particles

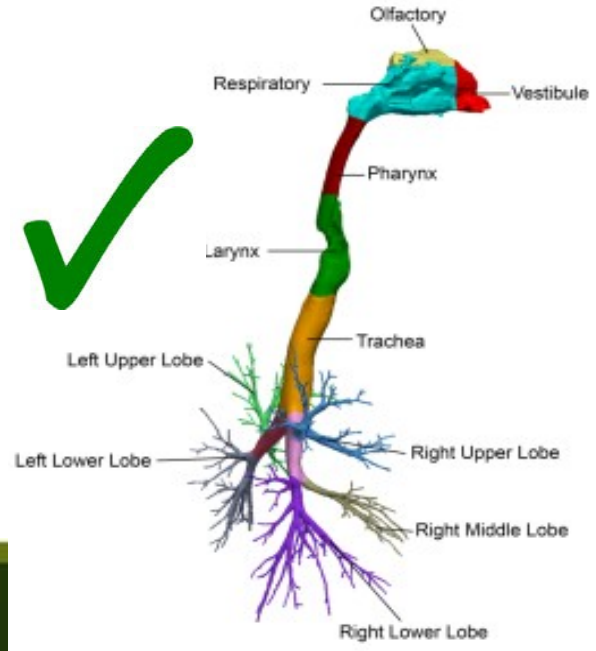
Inhalation Exposure

In vitro Testing

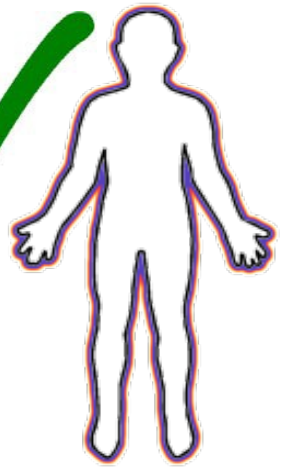




CFD Modeling



HEC



Risk Characterization

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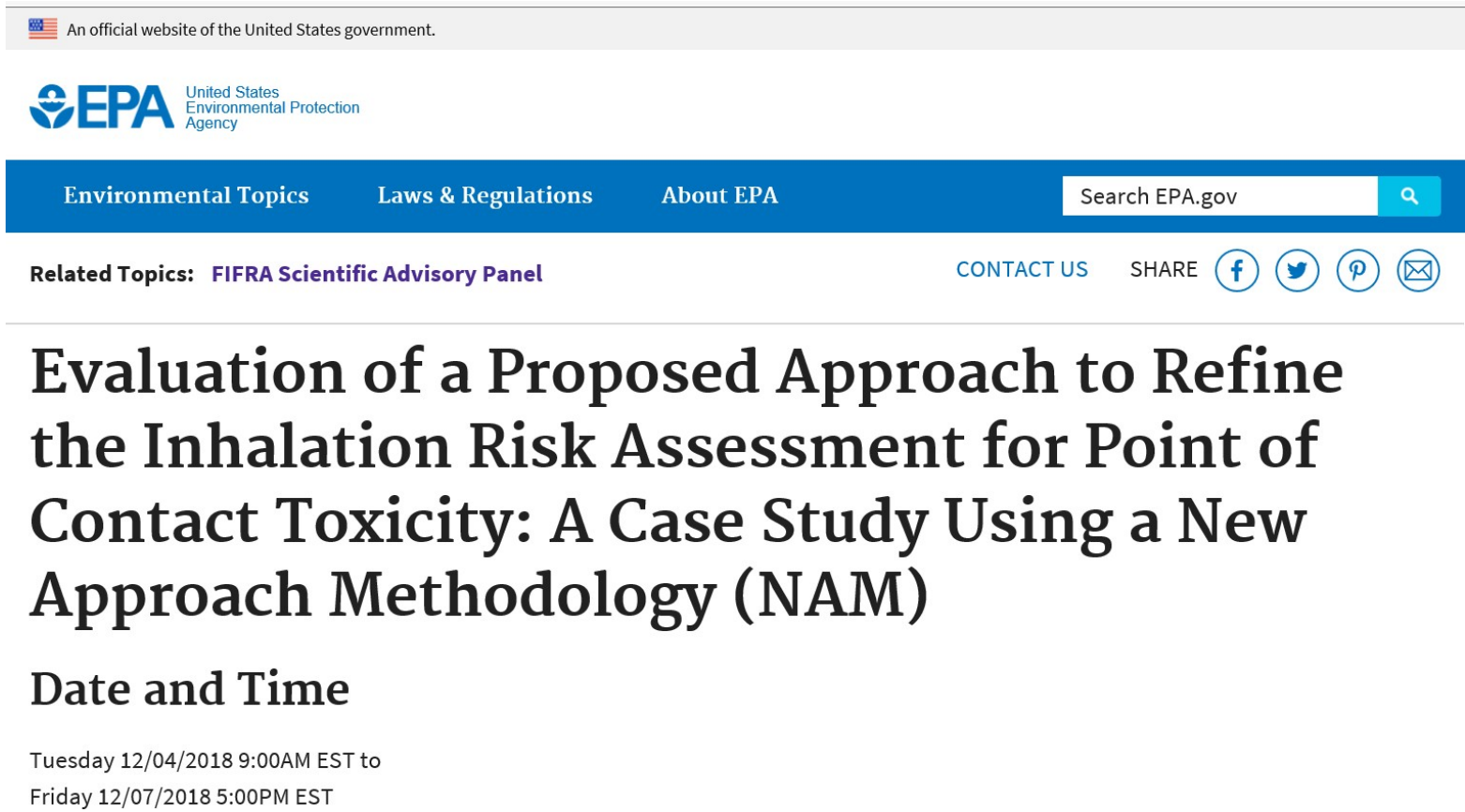
Outcome

This Source to Outcome Analysis Addresses:

- Inhalation Study Requirement
- Point of Departure
- Uncertainty Factors
  - Database
  - LOAEL to NOAEL
  - Interspecies



https://www.regulations.gov/docket?D=EPA-HQ-OPP-2018-0517

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Collaboration

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Toxicology

- Alex Charlton
- Bob Parr-Dobrzanski
- Douglas Wolf

Modeling

- Paul Hinderliter

Risk Assessment

- Sheila Flack
- Tharacad Ramanarayanan

Application Technology

- Mark Ledson

External Partners

Charles River Labs

Epithelix

NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

Pacific Northwest National Lab

Research Triangle Institute

USEPA

Thank You

Bringing plant potential to life

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Roadmap

HESI RISK ASSESSMENT SUMMIT 2020

The HESI RISK21 Committee is pleased to invite you to the first **Risk Assessment Summit,** which will be held on **February 18-19,2020, in Washington, DC**!

The Summit will convene a broad group of stakeholders to discuss risk assessment approaches

Transparent
- Visual

The Summit will be structured around specific case examples presented by government, industry, academic, and NGO stakeholders, to illustrate various real-world applications of these principles in the context of human health and ecological risk assessment (e.g., prioritization, categorization, study design, new product development, risk management and communication, etc.). There will also be a poster session on the evening of February 18th to allow a broader group of attendees to share their case studies.

We would like this meeting to provide a forum to discuss risk assessment challenges, foster collaboration, and leverage expertise to address gaps and needs, and we hope you will join us to learn about lessons and insights in the risk assessment space, and also share your experience with this community of practice.

□ Click Here to Register!

□ About RISK21®

Mission

Harmonize the approach to

Principles of RISK21®

• Problem-formulation based
• Risk-based at each step of

Impact

• Developed a risk assessment approach that embraces advance in scientific knowledge and

Next Steps

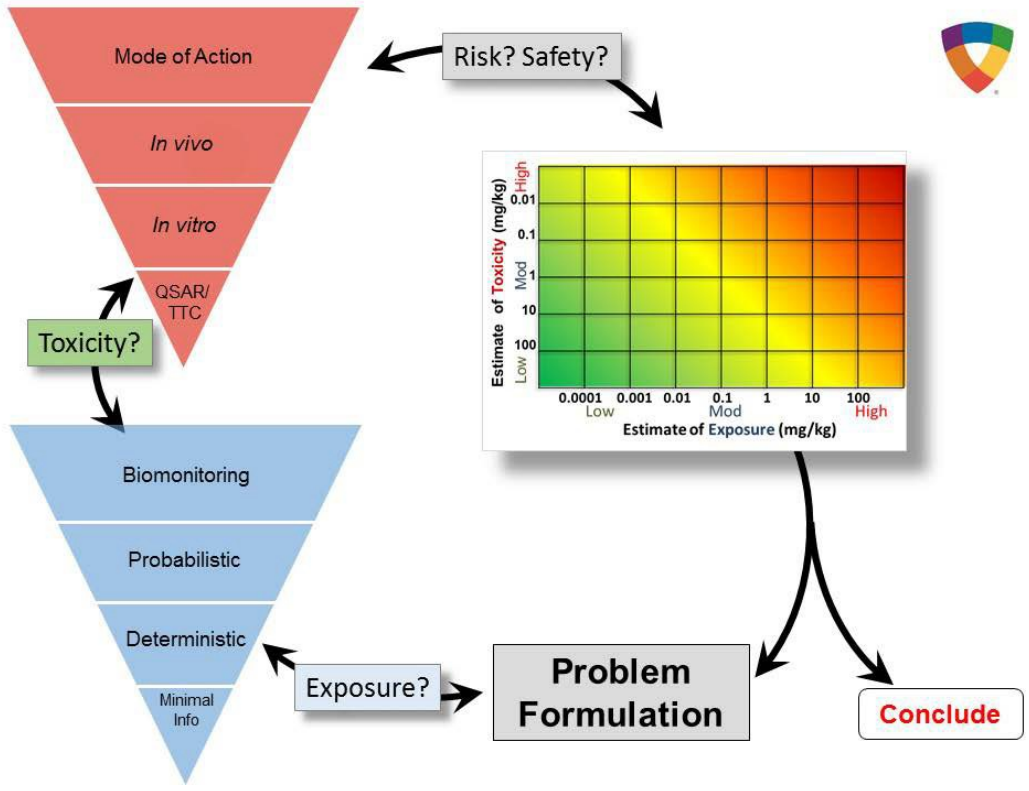
• Continued outreach and

how the world evaluates chemicals using a risk-based approach.

the process
• Use of prior knowledge
• Fit for purpose
• Value of information
• “Enough precision to make the decision”
• Provide a framework that is flexible, transparent, visual

methods
• Actively involved over 120 participants (government, industry, academia, NGOs) through 6 years of work
• Published 8 manuscripts in Critical Reviews in Toxicology
• Reached over 500 participants in 5 countries on three continents via 7 hands-on case-study workshops
• Revised current thinking about how to approach the science and art of risk assessment

communicatio n
• Additional illustration and application via case studies
• Ongoing dialog on advances and needs



Version 2.0 of the RISK21® Webtool is

Now Available!

21st Century Framework

The webtool supports a problem formulation-based, exposure-driven, tiered acquisition approach that leads to an informed decision.

Transparent and Consistent

The webtool approach allows users to optimize the use of resources and information available for chemical risk assessment.

Create Custom Plots

Users can interact with the RISK21 webtool application to visualize their own risk data, creating a custom plot which will be displayed on the screen.

RISK21® Roadmap and Matrix

The RISK21 webtool application allows users to input estimated exposure and toxicity data for each chemical, and will plot the intersection area, overlaying a risk matrix represented as a heat map.