Refining Inhalation Risk Assessment of a Contact Irritant using Computational Fluid Dynamics and the Mucilair™ Airway Assay

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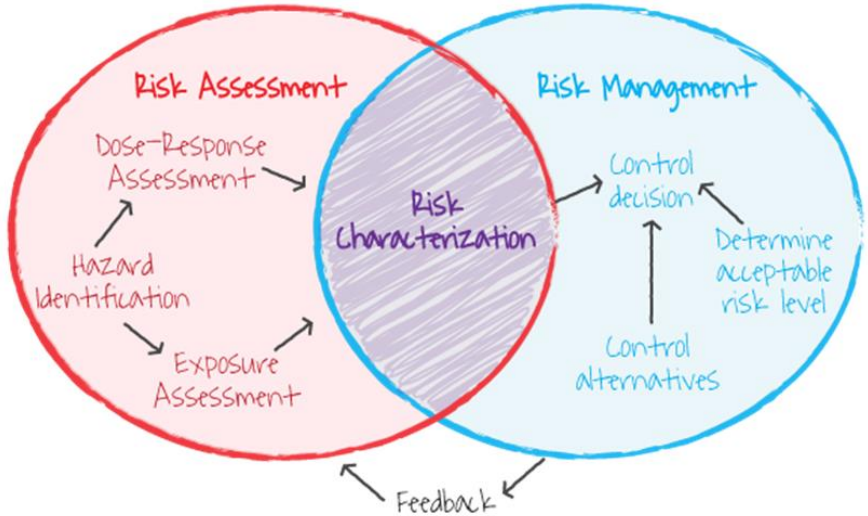
Overview

- Introduction
- Evaluation Framework for New Approach Methodologies for Human Health Safety Assessment
- Case Study: Chlorothalonil
  - Pesticide Exposure Data
  - Human CFD/Aerosol Simulation
  - MucilAir™ Assay
  - Benchmark Dose (BMD)
- Conclusion
- Acknowledgements

Introduction

- Numerous agrochemicals need to be assessed in the context of regulatory authorities for pulmonary toxicity.
- The toxicology of air pollutants and chemicals has relied on in vivo and in vitro testing for decades.
  - There is a high demand to find and implement new approaches that encompass the Three Rs principles (replacement, reduction and refinement).
- **Health effects due to inhalation of substances are therefore of considerable interest to many and form the basis for the use and development of new approach methodologies.**



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Environment International 85 (2015) 120–132

Contents lists available at ScienceDirect

Environment International

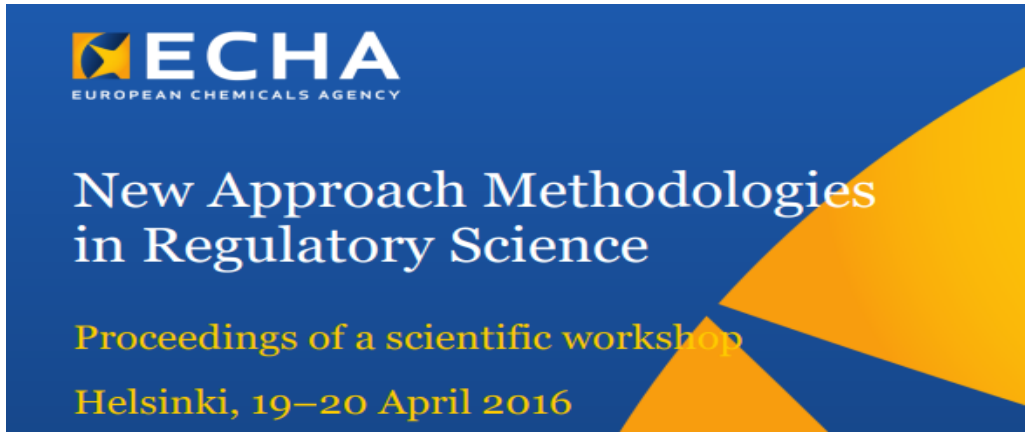
journal homepage: www.elsevier.com/locate/envint

Review article

Uncertainties in human health risk assessment of environmental contaminants: A review and perspective

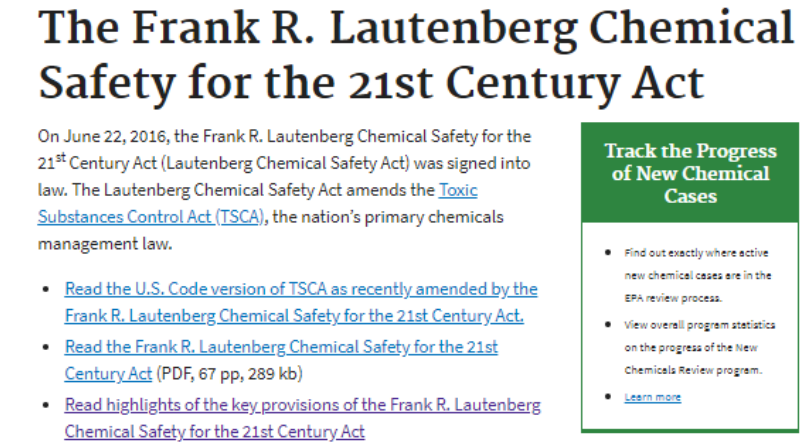
Zhaomin Dong, Yanju Liu, Luchun Duan, Dawit Bekele, Ravi Naidu *

The Faculty of Science and Information Technology, University of Newcastle, University Drive, Callaghan, NSW 2308, Australia
Cooperative Research Centre for Contamination Assessment and Remediation of the Environment, Mawson Lakes, SA 5095, Australia



- New approach methodologies (NAMs) - *in silico* and *in chemico* models and *in vitro* assays, as well as the inclusion of information from the exposure of chemicals in the context of hazard.

- NAMs for toxicity testing, including alternatives to animal testing approaches, may be able to provide a large amount of data to fill information gaps in both hazard and exposure.



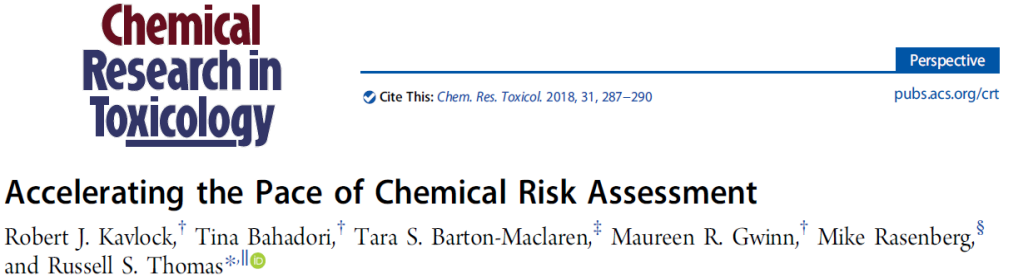
The Frank R. Lautenberg Chemical Safety for the 21st Century Act

On June 22, 2016, the Frank R. Lautenberg Chemical Safety for the 21st Century Act (Lautenberg Chemical Safety Act) was signed into law. The Lautenberg Chemical Safety Act amends the Toxic Substances Control Act (TSCA), the nation's primary chemicals management law.

- Read the U.S. Code version of TSCA as recently amended by the Frank R. Lautenberg Chemical Safety for the 21st Century Act.
- Read the Frank R. Lautenberg Chemical Safety for the 21st Century Act (PDF, 67 pp, 289 kb)
- Read highlights of the key provisions of the Frank R. Lautenberg Chemical Safety for the 21st Century Act

Track the Progress of New Chemical Cases

- Find out exactly where active new chemical cases are in the EPA review process.
- View overall program statistics on the progress of the New Chemicals Review program.
- Learn more



Accelerating the Pace of Chemical Risk Assessment

Robert J. Kavlock,† Tina Bahadori,† Tara S. Barton-Maclaren,‡ Maureen R. Gwinn,† Mike Rasenberg,§ and Russell S. Thomas*,∥

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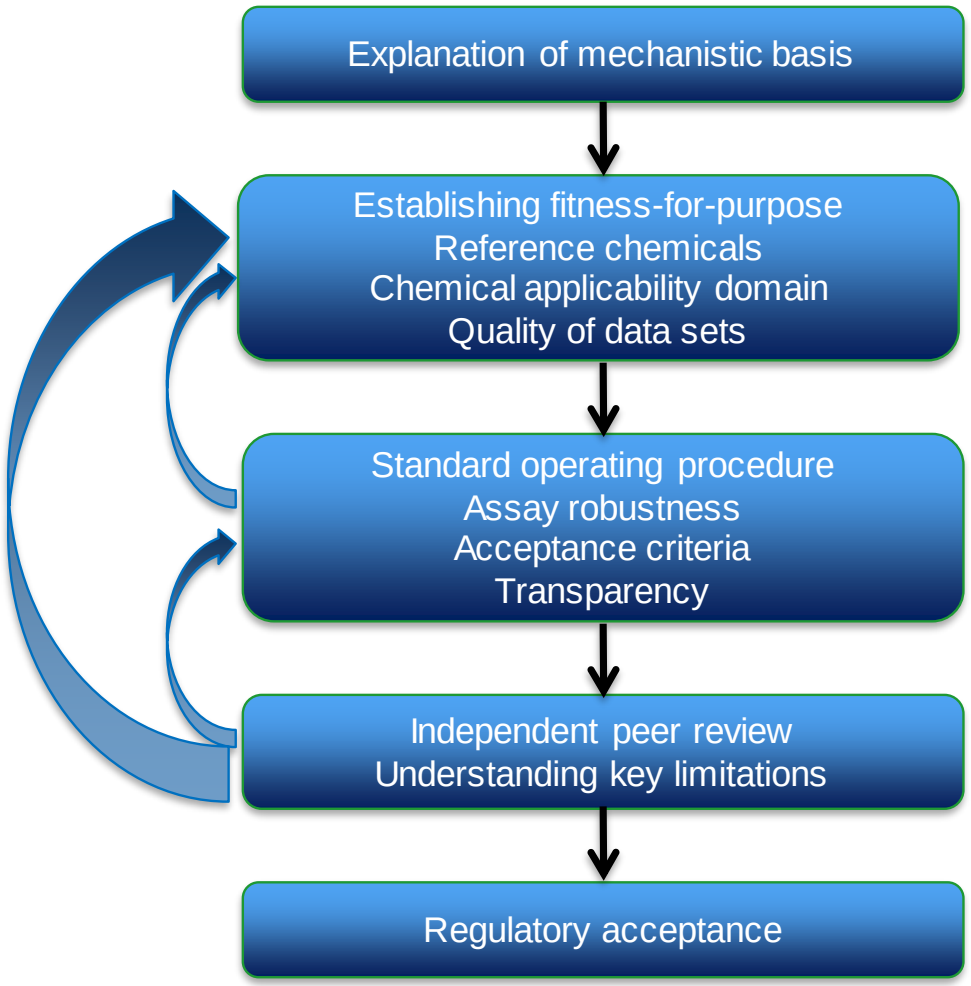
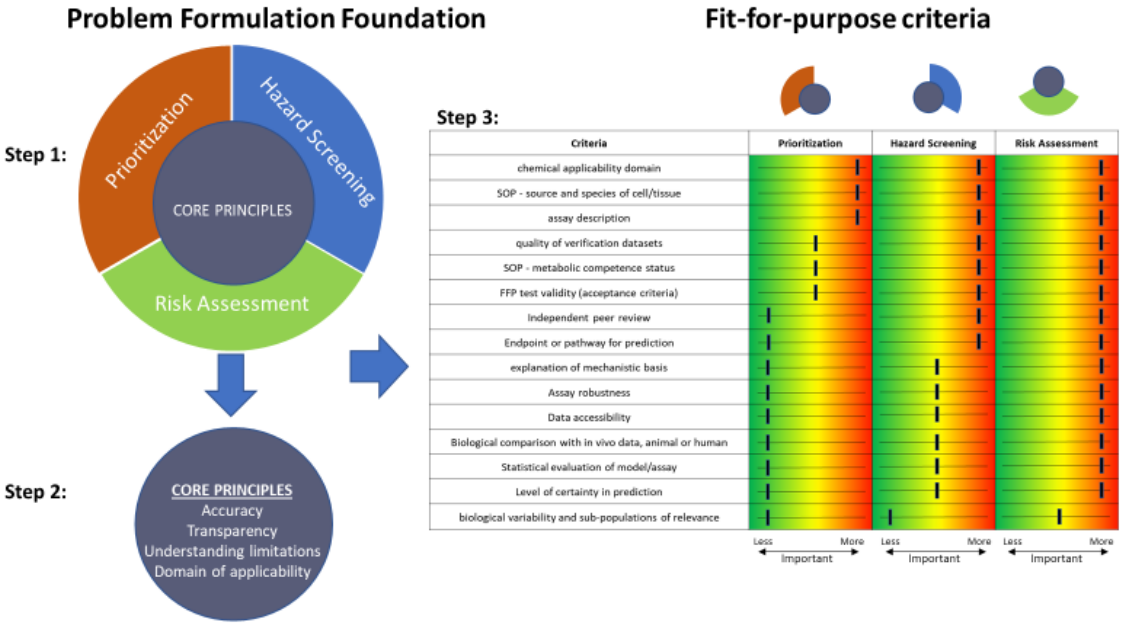
Establishing Fitness-for-Purpose of a New Approach Method





Parish et al. An Evaluation Framework for New Approach Methodologies (NAMs) for Human Health Safety Assessment. Reg Toxicol Pharmacol 112, doi: 10.1016/j.yrtph.2020.104592

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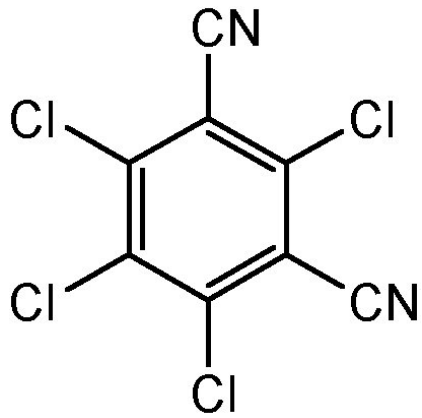
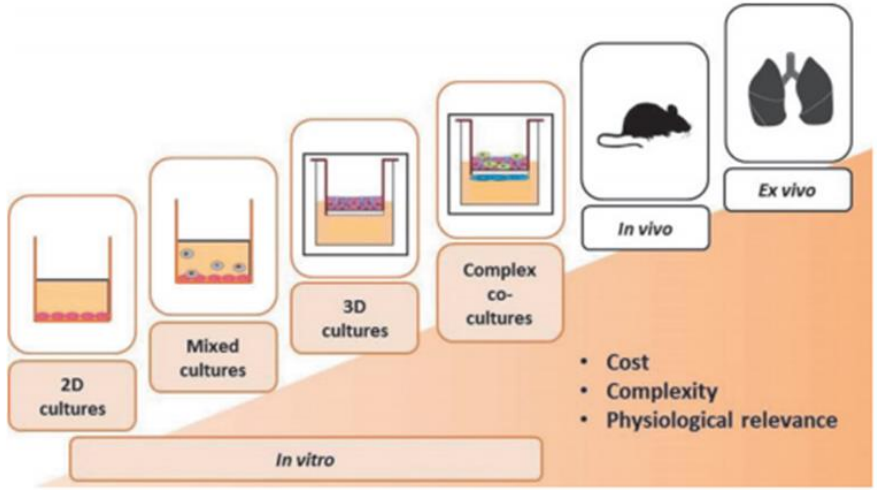
Problem Statement

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.

Exploring the Problem & Chlorothalonil

- During pesticide re-registration, the agency examines the completeness of the toxicological database to determine if it can still support risk assessments for safe use.
- Agency requested a 90-day inhalation study.
- Description of 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 safety assessment.
    - No additional systemic risk from inhalation exposure.
      - Contact irritation in respiratory tract.
- Broad spectrum, non-systemic fungicide
- Long history of safe use, widely-used since 1966.
- Contact irritant inducing respiratory tract effects in acute studies.
- The inhalation risk for non-volatile pesticides is very different from volatile chemicals
- Re-reg process started in 2010, docket 2012, DCI 2013, the NAM development started in 2014.





Conceptual Models

Traditional Inhalation Model

Inhalation Exposure

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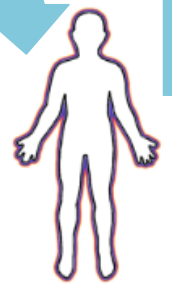


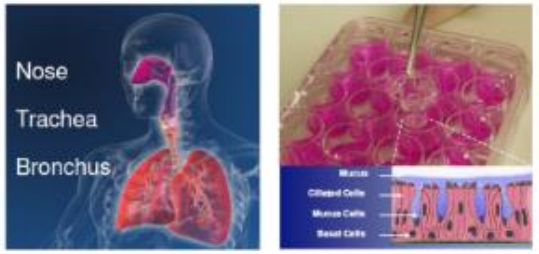
Risk Characterization

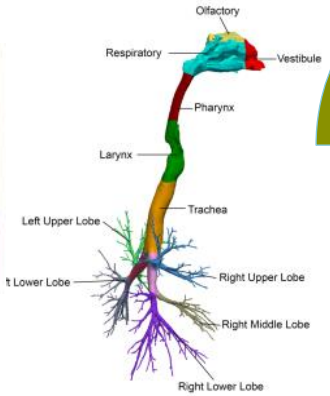
Human Relevant Exposure Based Inhalation Model

Particle Size Distribution of Inhalable Particles

Inhalation Exposure

In vitro Testing + CFD Modeling





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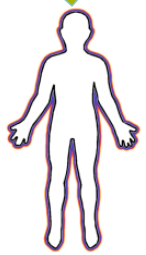

Risk Characterization

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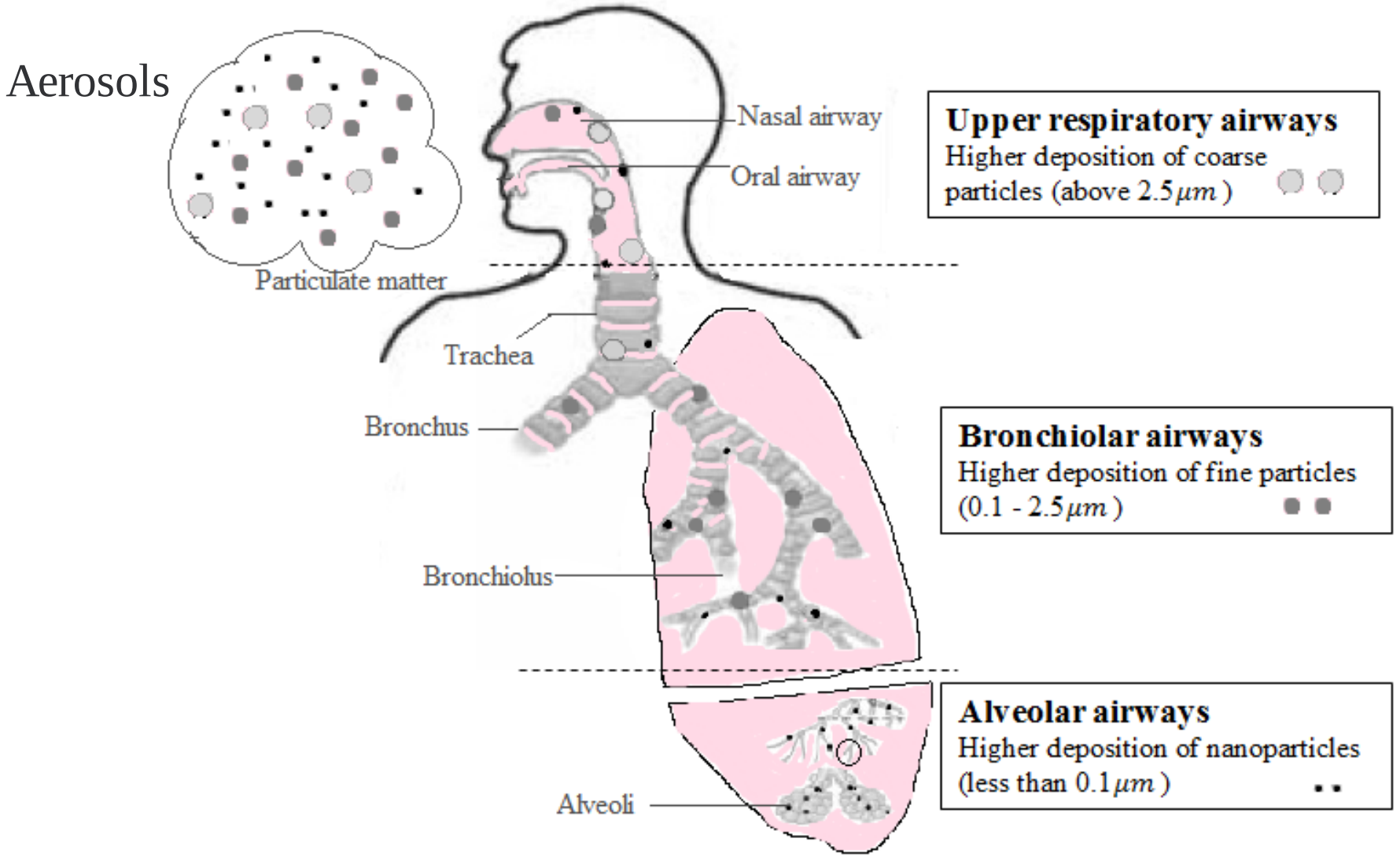
Pesticide Exposure Data

- 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 undertaken at Syngenta to compare OVS tube with standard sizing methods.

Flack SL, Ledson TM, Ramanarayanan TS. (2019). Particle Size Characterization of Agricultural Sprays Collected on Personal Air Monitoring Samplers. *Journal of Agricultural Safety and Health, 25*(2), 91-103.

Human Respiratory Tract

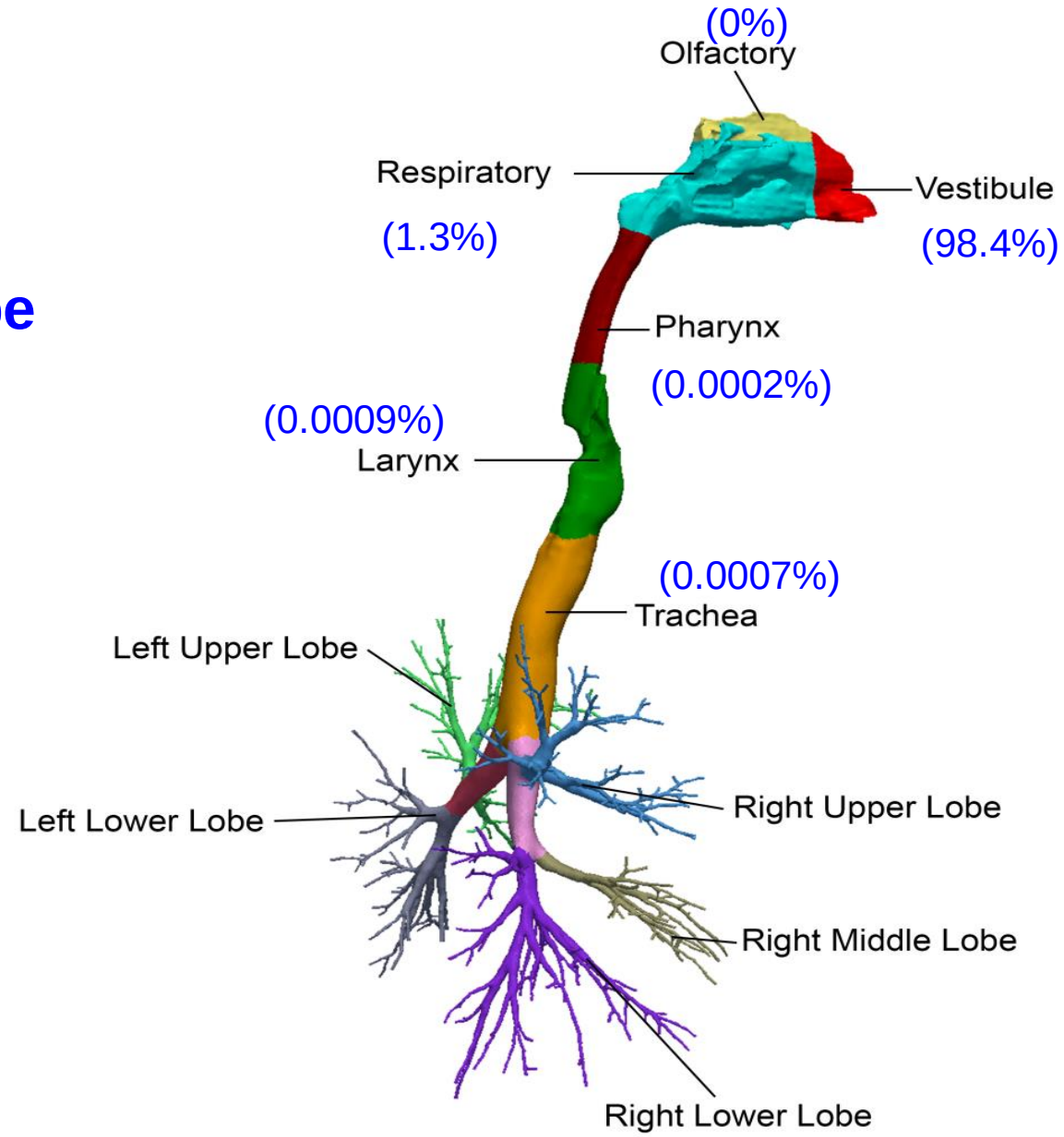


Adapted from: Kolanjiyil and Kleinstreuer J Biomech Eng. 2013 Dec;135(12):121003

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

% of Inhaled Mass Deposited by Tissue Type

- 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



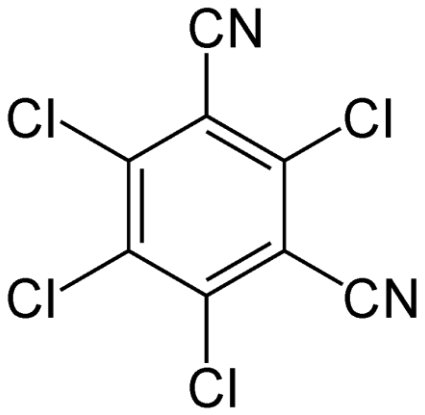
Corley R, Kuprat A, Suffield S, Kabilan S, **Hinderliter P**, Yugulis K, **Ramanarayanan, TS**. New Approach Methodology for Assessing Inhalation Risks of a Contact Respiratory Cytotoxicant: Computational Fluid Dynamics-Based Aerosol Dosimetry Modeling for Cross-Species and In Vitro Comparison. *Toxicological Sciences (in press).*

Acute Effects in Male and Female Rats

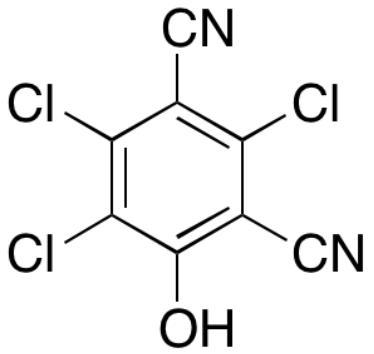
Nominal concentration → Tissue ↓	**Male 0.004 mg/L**	**Male 0.016 mg/L**	**Male 0.032 mg/L**	**Female 0.004 mg/L**	**Female 0.016 mg/L**	**Female 0.032 mg/L**
Larynx **2** hours Inflammation	*2 (0.4)	1 (0.2)	0	3 (0.6)	2 (0.8)	1 (0.4)
Larynx **2** hours Epithelial necrosis and ulceration	0	3 (1.8)	5 (3.8)	2 (1.0)	2 (2.2)	4 (2.8)
Larynx **4** hours Inflammation	4 (0.8)	1 (0.6)	0	0	0	0
Larynx **4** hours Epithelial necrosis and ulceration	1 (0.6)	3 (2.0)	5 (3.6)	5 (2.8)	5 (3.6)	5 (3.8)
Larynx **6** hours Inflammation	4 (1.4)	2 (0.8)	0	3 (1.4)	0	0
Larynx **6** hours Epithelial necrosis and ulceration	1 (0.4)	3 (2.0)	5 (3.4)	4 (1.6)	5 (4.0)	5 (4.0)

* Incidence (mean severity score); 5 animals/group

What Chlorothalonil (CTN) concentration in the aqueous/mucus layer covering the airway epithelium is necessary to damage the epithelium underneath?



H_2O



+ HCl → H^+ Cl^-

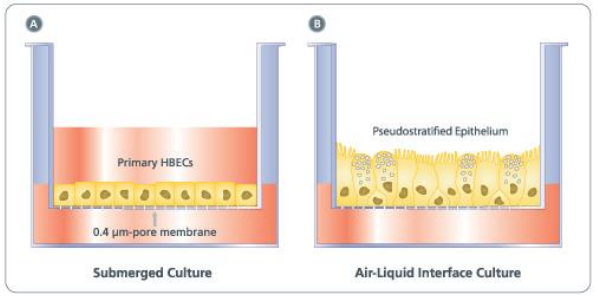
[CTN]

Aqueous and mucus layer

Hydrolysis and metabolism ROS

Aqueous and mucus layer

In vitro human airway epithelial cell culture



CTN dose-response
Cell degeneration
Cell death
Other endpoints?

Refine the model for dosimetry, deposition, dose-response

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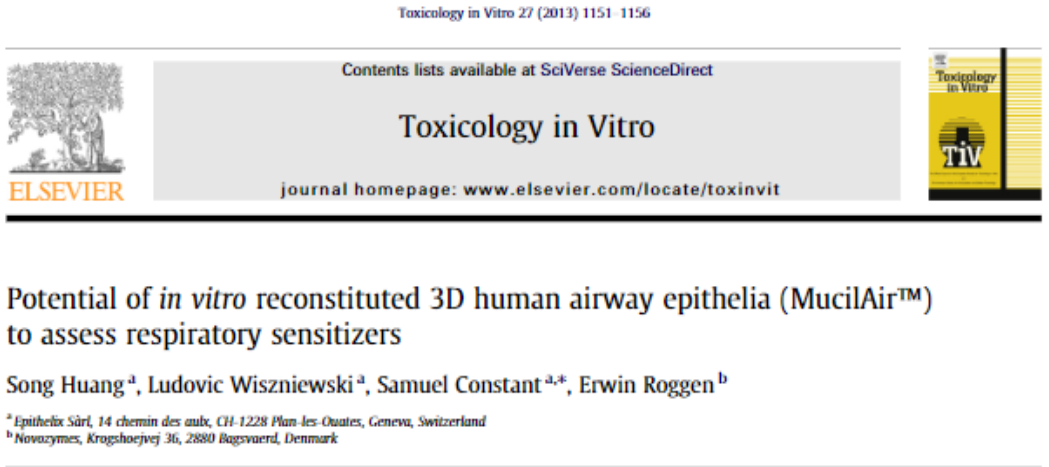
MucilAir™ Background

- 3D model of the human airway epithelium formed from differentiated primary human cells.
- MucilAir™ is derived from human airway cells collected from healthy donors. Cells are cultured at the air interface on Costar Transwell® polyester membrane units, using optimized chemically defined media.
- The culture process reconstructs a functional model of the human airway epithelium, exhibiting a pseudostratified, ciliated epithelium which secretes mucus.


Toxicology in Vitro 27 (2013) 1151–1156

Contents lists available at SciVerse ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Potential of *in vitro* reconstituted 3D human airway epithelia (MucilAir™) to assess respiratory sensitizers

Song Huang[a], Ludovic Wiszniewski[a], Samuel Constant[a,*], Erwin Roggen[b]

[a] Epithelix Sàrl, 14 chemin des aulx, CH-1228 Plan-les-Ouates, Geneva, Switzerland
[b] Novozymes, Krogshoejvej 36, 2880 Bagsvaerd, Denmark

	Cell-cell interactions?	Representative tissue organisation?	Correlation with in vivo findings?
Immortalised cell monoculture	No	No	No
Primary cell monoculture	No	No	No
2D/3D cellular co-culture	Yes	Yes	No
Lung-on-a-Chip	Yes (Limited cell types)	No	No
MucilAir	**Yes**	**Yes**	**Yes**

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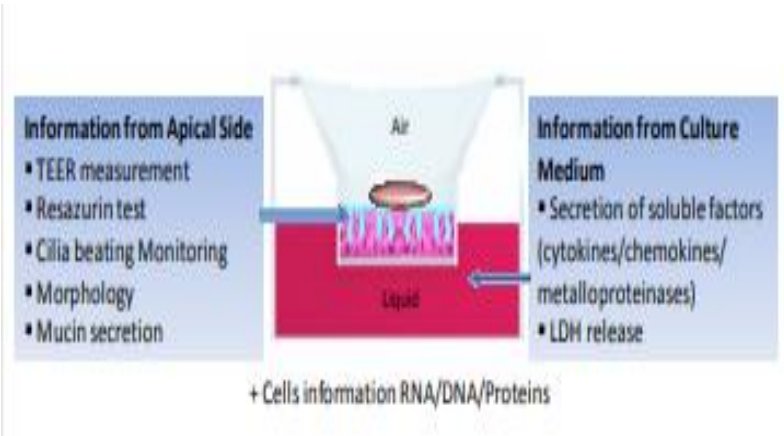
MucilAir™ Endpoint Parameters

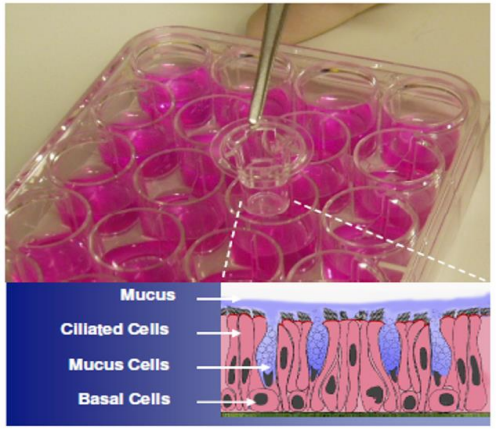
- Measures a variety of membrane and cell damage endpoints as markers of irritation.

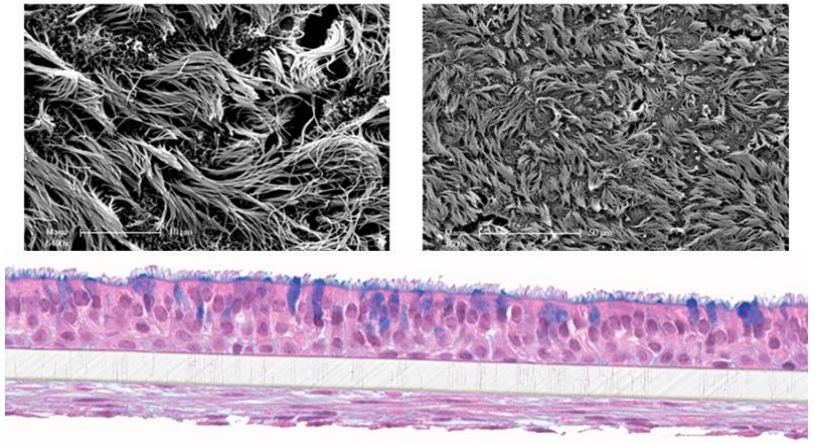
  Trans-epithelial electrical resistance (TEER): measures the integrity of tight junctions between cells in the membrane.

  Lactate dehydrogenase (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.







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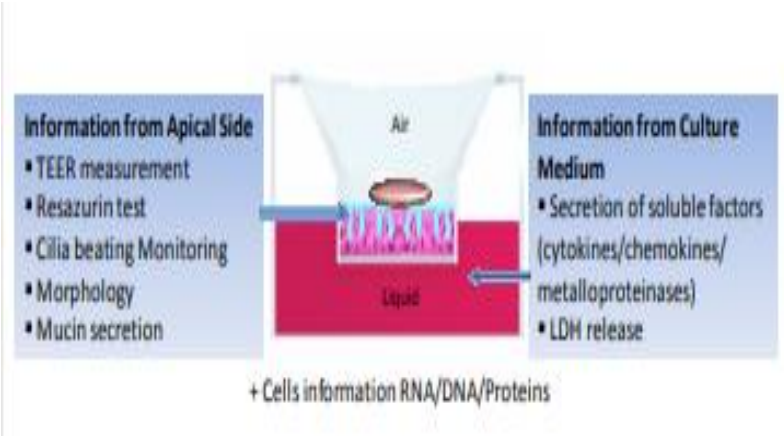
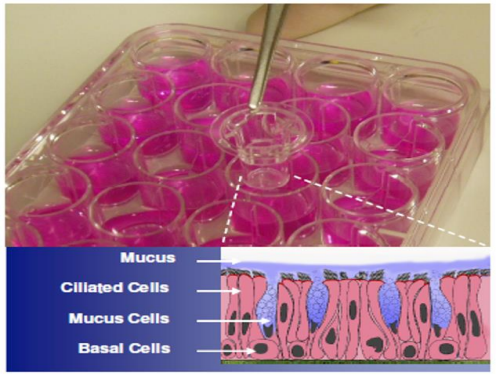
MucilAir™ Experimental Design

- Endpoints: TEER, LDH, and resazurin metabolism
  - **Trans-epithelial electrical resistance (TEER):** measures the integrity of tight junctions between cells in the membrane.
  - **Lactate dehydrogenase (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.
- Tissues from 5 individual healthy 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





Hargrove MM, Parr-Dobrzanski B, *Li L, Constant S, Wallace J*, **Hinderliter P, Wolf DC, Charlton A**. Use of the Mucilair™ Airway Assay, a New Approach Methodology, for Evaluating the Safety and Inhalation Risk of Agrochemicals. *Applied In Vitro Toxicology* (in press).

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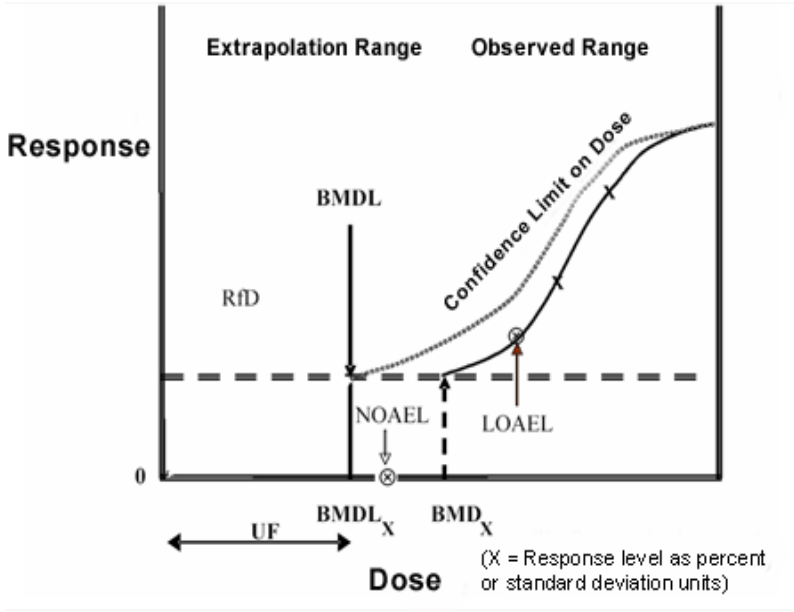
Benchmark Dose (BMD)

- **Benchmark dose (BMD) methods are used by the U.S. EPA and throughout the world for dose-response analyses to support chemical risk assessments and regulatory actions.**
- A benchmark dose (BMD) is a dose or concentration that produces a predetermined change in the response rate of an adverse effect.
- It is less dependent on dose selection and spacing and takes into account the shape of the dose-response curve.
- The estimation of a BMD 95% lower bound confidence limit (BMDL) results in a Point of Departure that accounts for study quality.
- The primary BMD tools developed by the U.S. EPA for this purpose are the Benchmark Dose software and Categorical Regression (CatReg) software.



https://www.epa.gov/bmds

Chlorothalonil 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, LDH, resazurin) responded similarly to Chlorothalonil in vivo.
- Multiple BMD approaches were examined, BMD_{1SD} was selected through consultation with the US EPA.

	BMDL (mg/L)			
Donor	TEER	LDH	Resazurin	**Mean**
1	51.21	67.60	66.72	**61.36**
2	53.09	92.85	80.47	**73.48**
3	88.24	90.15	91.85	**90.07**
4	110.20	91.00	42.45	**75.23**
5	124.40	101.60	113.2	**112.68**
Geometric Mean	**80.06**	**87.85**	**74.98**	**80.79**

	BMDL (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**

$$\text{BMDL (mg/cm}^2) = \text{BMDL (mg/L)} \times \frac{30\ \mu\text{L x } 1\text{x}10^{-6}\ \text{L}/\mu\text{L}}{33.18\ \text{mm}^2 \times 0.01\ \text{cm}^2/\text{mm}^2}$$

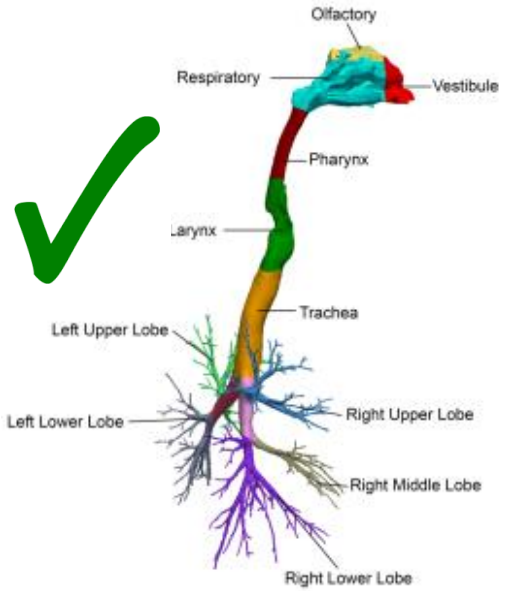
Exposure Based Inhalation Model

In vitro Testing

Particle Size Distribution of Inhalable Particles

Inhalation Exposure

CFD Modeling



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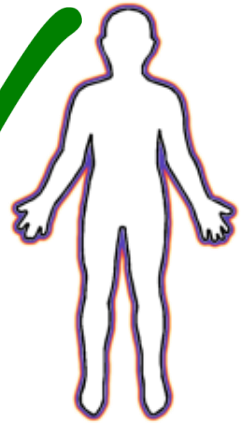
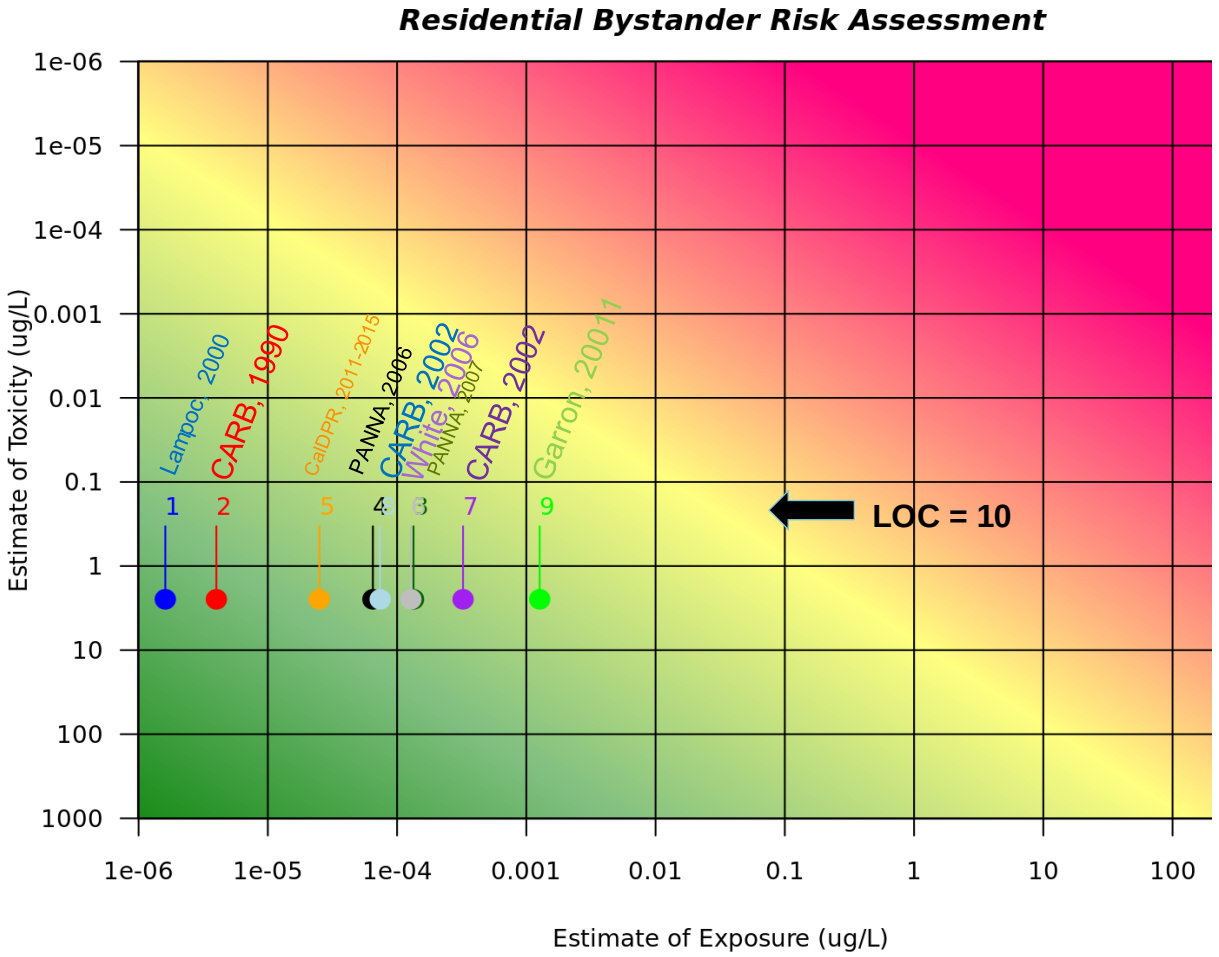
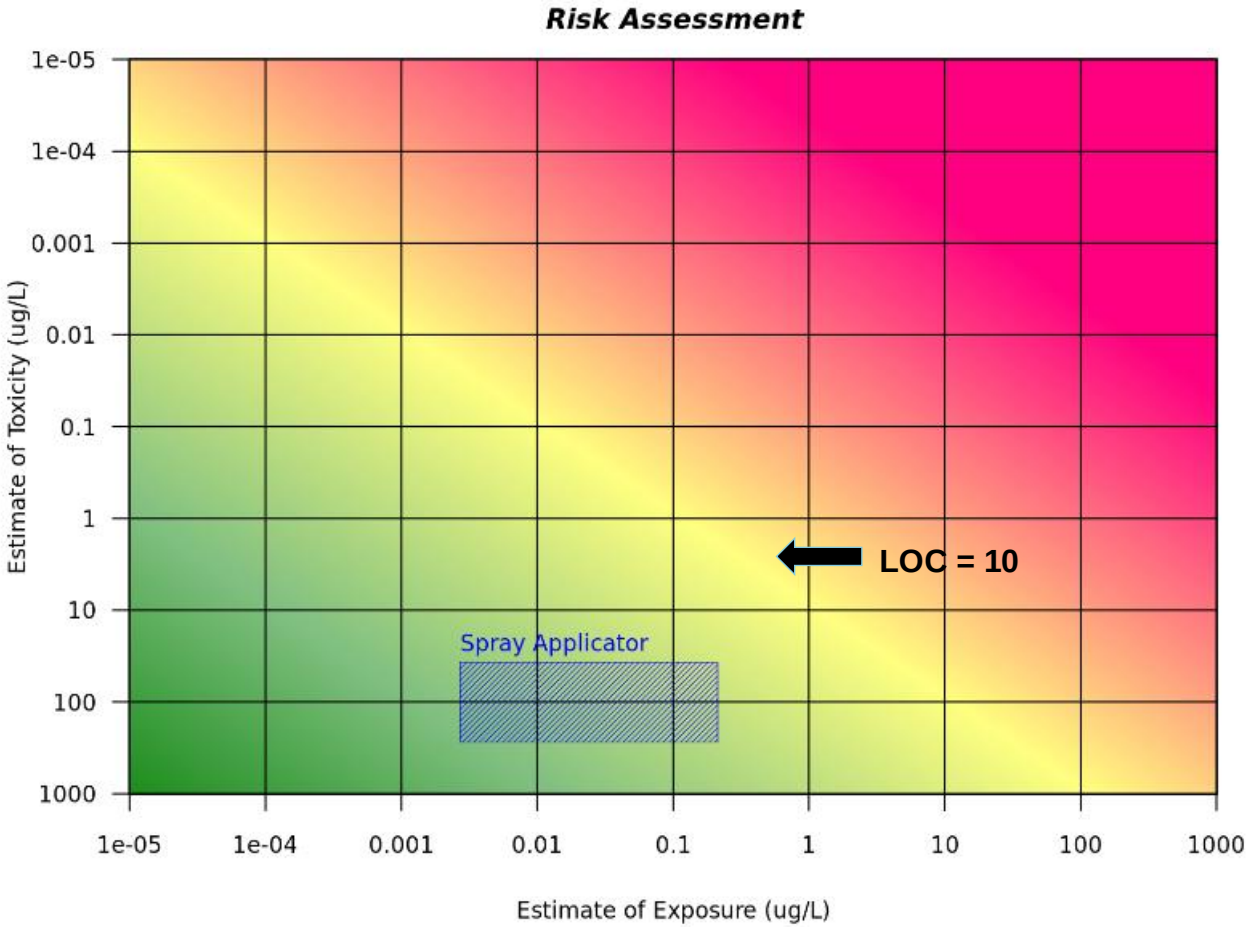


Risk Characterization

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





Ramanarayanan TS, Szarka A, Flack SL, *Corley R*, **Wolf DC**. Inhalation risk assessment using endpoints derived from new assessments methods (NAM) and inhalation dosimetry models (under review).

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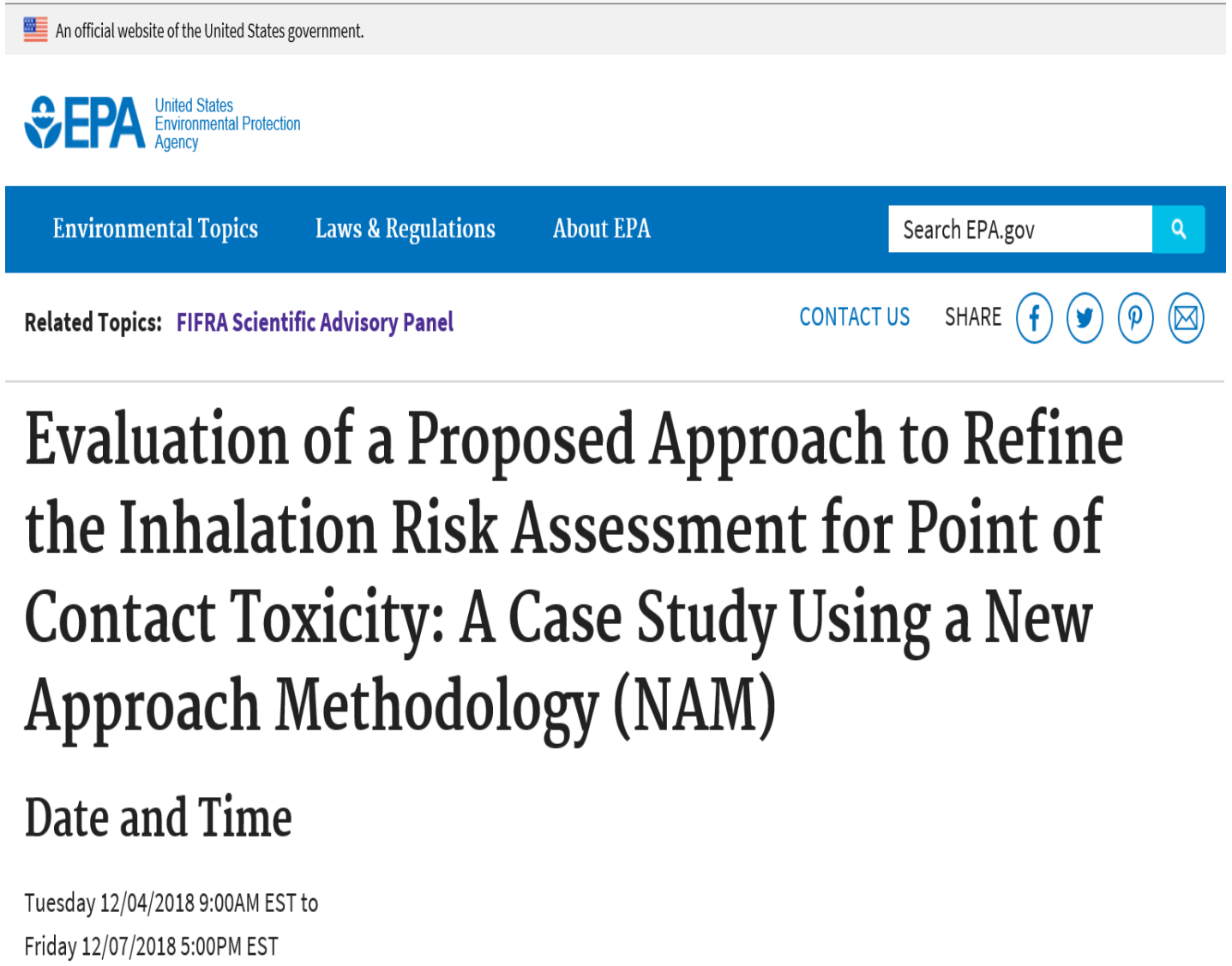
Outcome

This Source to Outcome Analysis Addresses:

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



Conclusions/Key Learnings

- Chlorothalonil is an important fungicide under registration review by the US EPA which requested a 90-day inhalation study as a requirement to re-registration.
- The goal was to develop and demonstrate that an *in vitro* assay combined with an *in silico* model was fit for purpose as a new approach method, using chlorothalonil as a case study, that would answer the Agency's risk question for inhalation and for any respiratory irritant.
- For respiratory irritants, such as chlorothalonil, an alternative *in vitro* approach may be taken when *in vivo* data are not adequate or not available to establish a toxicity endpoint for inhalation risk assessment.
- The characterization of the PSD of aerosols (in pesticide applications) collected with OSHA Versatile Sampler (OVS) tubes can be used to refine inhalation risk assessments for agricultural workers and bystanders.
- The MucilAir™ system was identified as the optimal *in vitro* model to assess damage to respiratory epithelial cells caused by exposure to Chlorothalonil.
- Using three different endpoints that measured the integrity of tight junctions between cells in the membrane, cytotoxicity, and cell metabolic competence, a Benchmark Dose Level of 0.00730 mg/cm^2 was derived for Chlorothalonil.
- This information proved useful in calculating the Human Equivalent Concentration to inform inhalation risk assessment.
- **The US EPA is using the MucilAir™ assay to inform chronic inhalation risk.**

Acknowledgments

- Douglas C. Wolf, D.V.M., Ph.D.
- Robert Parr-Dobrzanski, Ph.D.
- Alexander Charlton, Ph.D.
- Sheila Flack, Ph.D.
- Tina Stevens, Ph.D.
- Paul Hinderliter, Ph.D.
- Tharacad Ramanarayanan, Ph.D.
- Arpad Szarka, Ph.D.
- Richard Corley, Ph.D.
- Clive Roper, Ph.D.
- Joanne Wallace, Ph.D.
- Hazel Paulo, M.S.