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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?
Conduct a 90-day sub-chronic inhalation study
Mode of Action

In vivo

In vitro

QSAR/TTC

Toxicity?

Risk?

Biomonitoring

Probabilistic

Deterministic

Minimal Info

Exposure?

Problem Formulation

Conclude

www.risk21.org
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

Particle Size Distribution of Inhalable Particles

Inhalation Exposure

In vitro Testing

CFD Modeling

HEC

Risk Characterization
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
Human Respiratory Tract

- **Aerosols**
- **Nasal airway**
- **Oral airway**
- **Trachea**
- **Bronchus**
- **Bronchioles**
- **Alveoli**

**Upper respiratory airways**
Higher deposition of coarse particles (above $2.5 \mu m$)

**Bronchiolar airways**
Higher deposition of fine particles ($0.1 - 2.5 \mu m$)

**Alveolar airways**
Higher deposition of nanoparticles (less than $0.1 \mu m$)

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
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
Exposure to Chlorothalonil

Absorption into Respiratory Epithelial cell

Cellular Metabolism and Liberation of Reactive Oxygen Species

Overwhelming the Protective Anti-oxident System

Necrotic injury to luminal respiratory epithelial cells

Loss of surface epithelium
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 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

[Image of MucilAir™ 3D in vitro model](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

<table>
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<tr>
<th>Dose Level</th>
<th>Chlorothalonil Concentration (mg/L)</th>
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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.

<table>
<thead>
<tr>
<th>Donor</th>
<th>TEER</th>
<th>LDH</th>
<th>Resazurin</th>
<th>Mean</th>
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Calculating a Human Equivalent Concentration

CFD Deposition (monodisperse) mg CTN/cm²/breath

BMD_{1SD} mg CTN/cm²

Deposition (MMAD = 35 µm, GSD=1.5) mg CTN/cm²/breath

Total Daily Deposition (MMAD = 35 µm, GSD=1.5) mg CTN/cm²

HEC (mg/L) = \frac{BMD_{1SD}}{\text{Total daily deposition}} \times 1 \text{ mg/L}
Accurate and Health Protective Risk Assessments Based on human *in vitro* Assay and human *in silico* Model

**Operator Risk Assessment**

- **LOC = 10**
- **Applicator**
- **Mixer/Loader**

Exposure Based Inhalation Model (IATA)

Particle Size Distribution of Inhalable Particles

In vitro Testing

Inhalation Exposure

CFD Modeling

HEC

Risk Characterization
Outcome

This Source to Outcome Analysis Addresses:

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

Evaluation of a Proposed Approach to Refine the Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology (NAM)

Date and Time

Tuesday 12/04/2018 9:00AM EST to
Friday 12/07/2018 5:00PM EST

### Collaboration

#### Syngenta

<table>
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<th>Toxicology</th>
<th>Modeling</th>
<th>Risk Assessment</th>
<th>Application Technology</th>
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<tr>
<td>Alex Charlton</td>
<td>Paul Hinderliter</td>
<td>Sheila Flack</td>
<td>Mark Ledson</td>
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<td>Bob Parr-Dobrzanski</td>
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<td>Douglas Wolf</td>
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#### 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
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.
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

communication
• 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.