Inhalation Risk Assessment Using Computational and In Vitro Tools: A Brief Overview

Paul M. Hinderliter, PhD
Senior Research Scientist
Product Metabolism ADME
Syngenta Crop Protection
paul.hinderliter@syngenta.com

January 23, 2019

Acknowledgements

- Syngenta
  - Operator and Consumer Safety
    - Sheila Flack, Tharacad Ramanarayanan
  - Toxicology and Health Sciences
    - Doug Wolf, Alex Charlton, Bob Parr-Dobrzanski

- Pacific Northwest National Laboratory
  - Rick Corley, Andrew Kuprat, Sarah Suffield, Senthil Kabilan
- Epithelix
  - Samuel Constant
- Charles River – Edinburgh
  - Clive Roper

Defining the Problem

- Pesticide re-registrations examine the completeness of the toxicological database, including inhalation exposures
  - requirements often include a subchronic (90-day) inhalation study (Guideline 870.3465).

- “However, there may be “smarter testing”/alternative approaches that are suitable to inform …inhalation toxicity in lieu of a subchronic inhalation study”

- We have redefined the risk assessment paradigm using new tools of *in vitro* tools and dosimetry models

syngenta

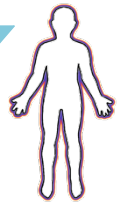
Source to Outcome Approach

- **Source**
  - Evaluate the particle size distribution of pesticide applications.
- **Exposure**
  - Investigate the impact of spray quality on inhalation exposure.
- **Dosimetry**
  - Compare deposition in human versus rat airways.
- **Outcome**
  - Predict human inhalation toxicity incorporating spray particle size.

Conceptual Model

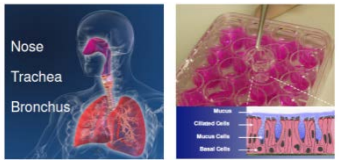
Inhalation Exposure

HEC



Risk Characterization

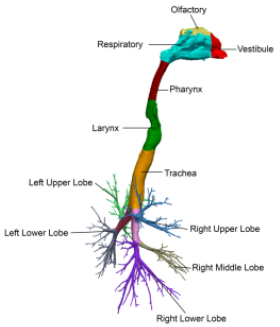
In vitro Testing



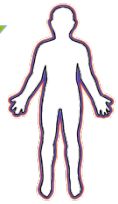
Particle Size Distribution of Inhalable Particles

Inhalation Exposure

CFD Modeling



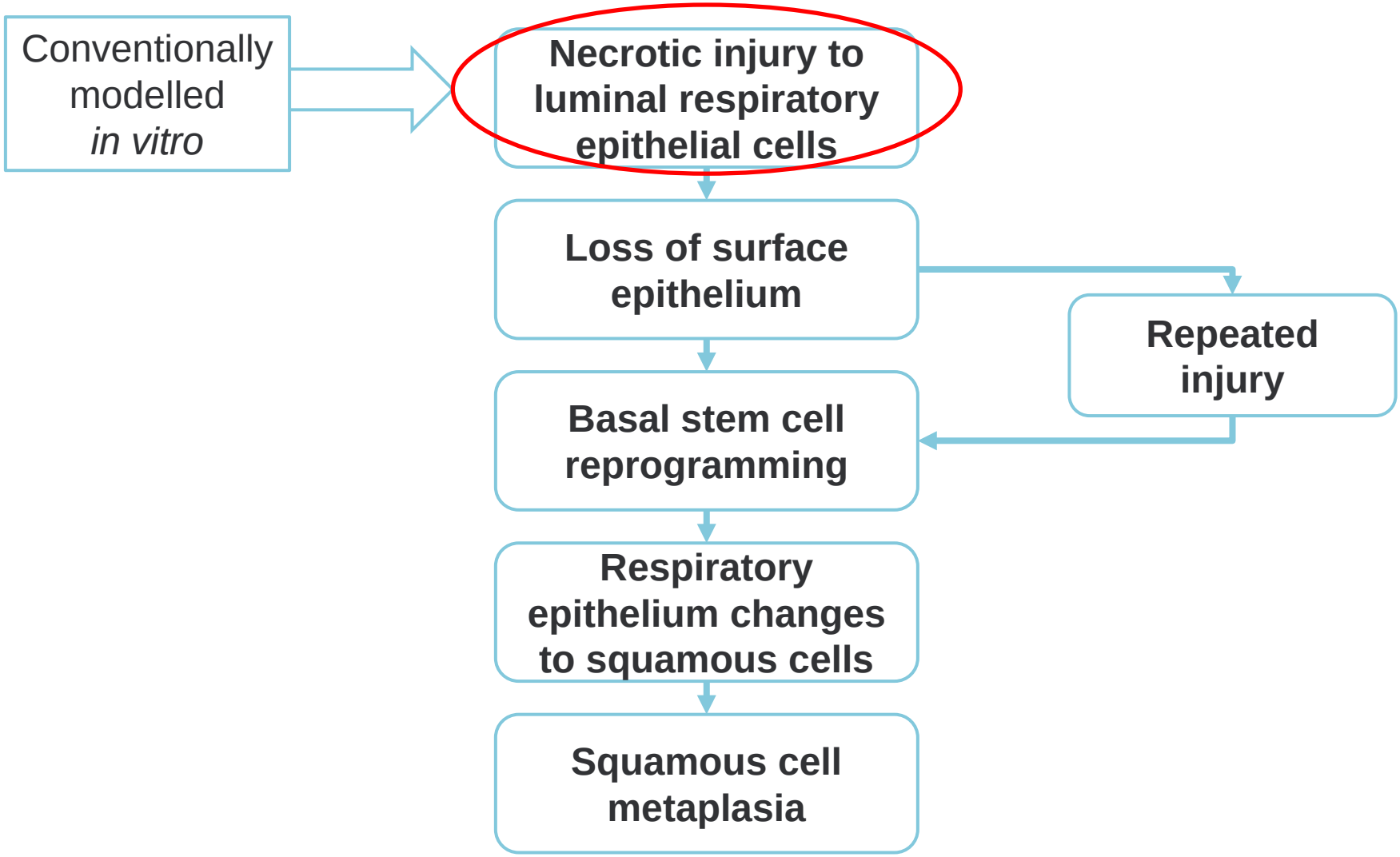
HEC



Risk Characterization

syngenta

Adverse Outcome Pathway for squamous cell metaplasia of the larynx

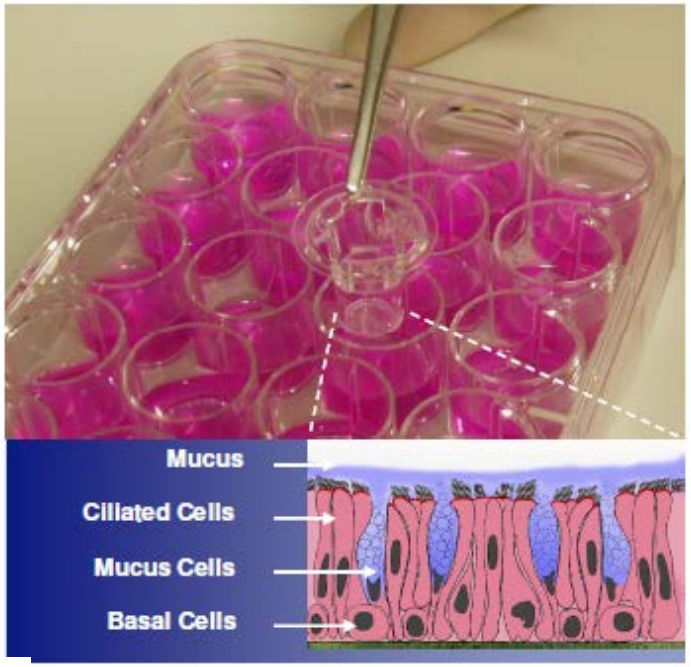


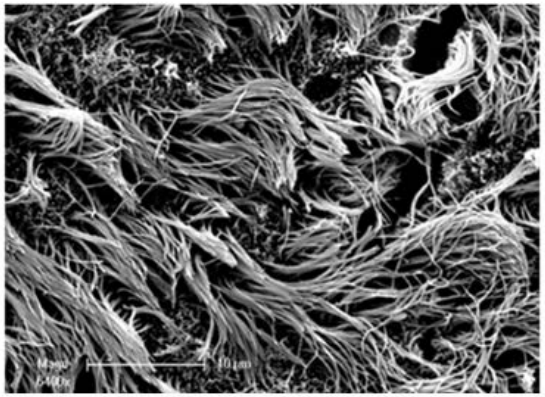
Adapted from: Renne R, Brix A, Harkema J, Herbert R, Kittel B, Lewis D, March T, Nagano K, Pino M, Rittinghausen S, Rosenbruch M, Tellier P, Wohrmann T (2009) Proliferative and Nonproliferative Lesions of the Rat and Mouse Respiratory Tract, Tox Path, 37:5D-73S

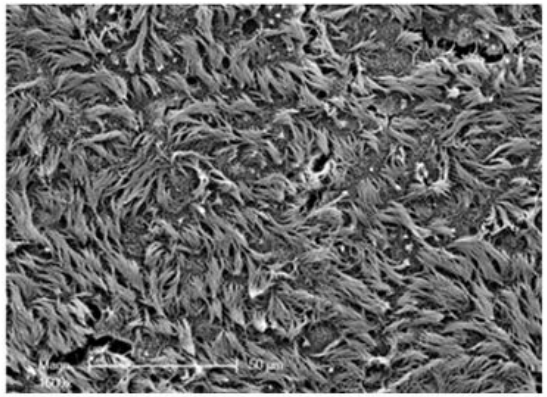
syngenta

In vitro toxicity assessment

MucilAir™ – 3D *in vitro* cell model of human upper airway epithelium prepared from differentiated primary human cells from a single healthy donor.

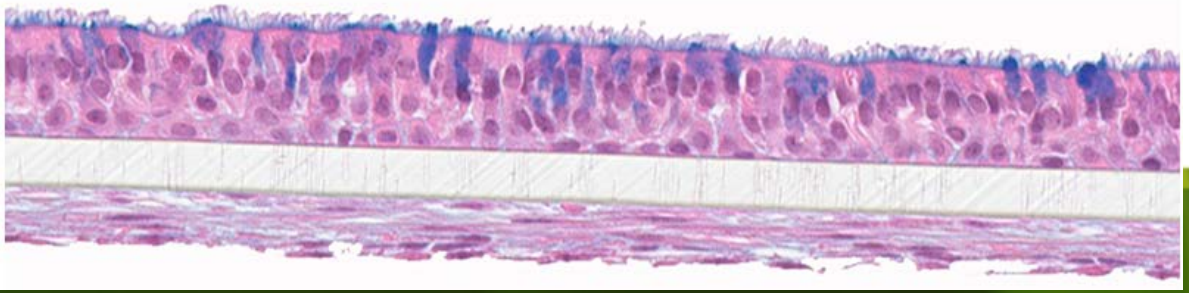






Electron microscopy at 45 days showing differentiated epithelia and cilia

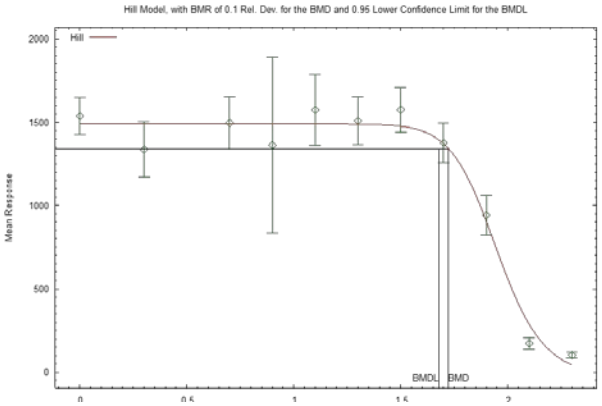
Graphics from
http://www.epithelix.com/



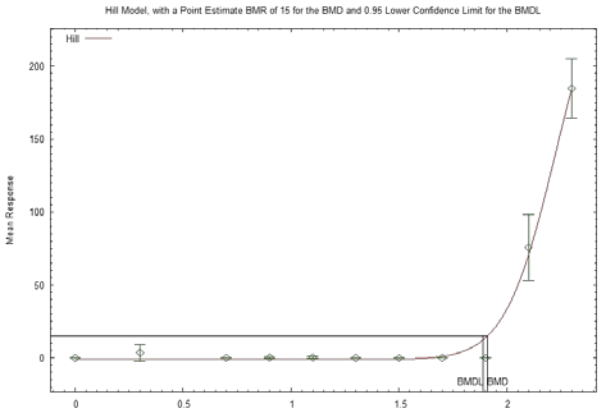
Cross section showing differentiated structure

MucilAir 3D *in vitro* model

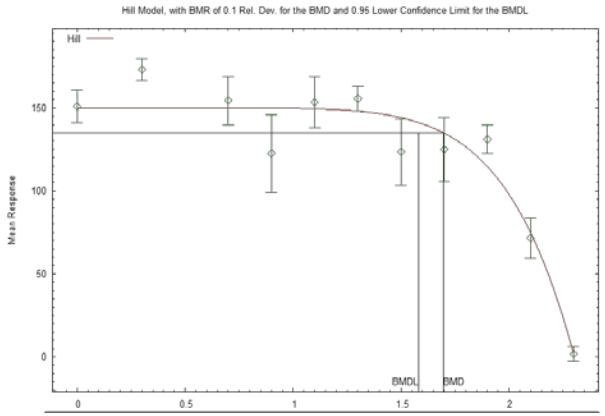
- Measures a variety of membrane and cell damage endpoints as markers of irritation
  - Trans-epithelial electrical resistance (TEER): measures the integrity of tight junctions between cells in the membrane
  - Lactate dehyrogenase (LDH): An enzyme present in most cells released when cells suffer cytotoxic membrane damage
  - Resazurin metabolism: reduced to a fluorescent product in viable cells used as a measure of metabolic competence



TEER



LDH



Resazurin

syngenta

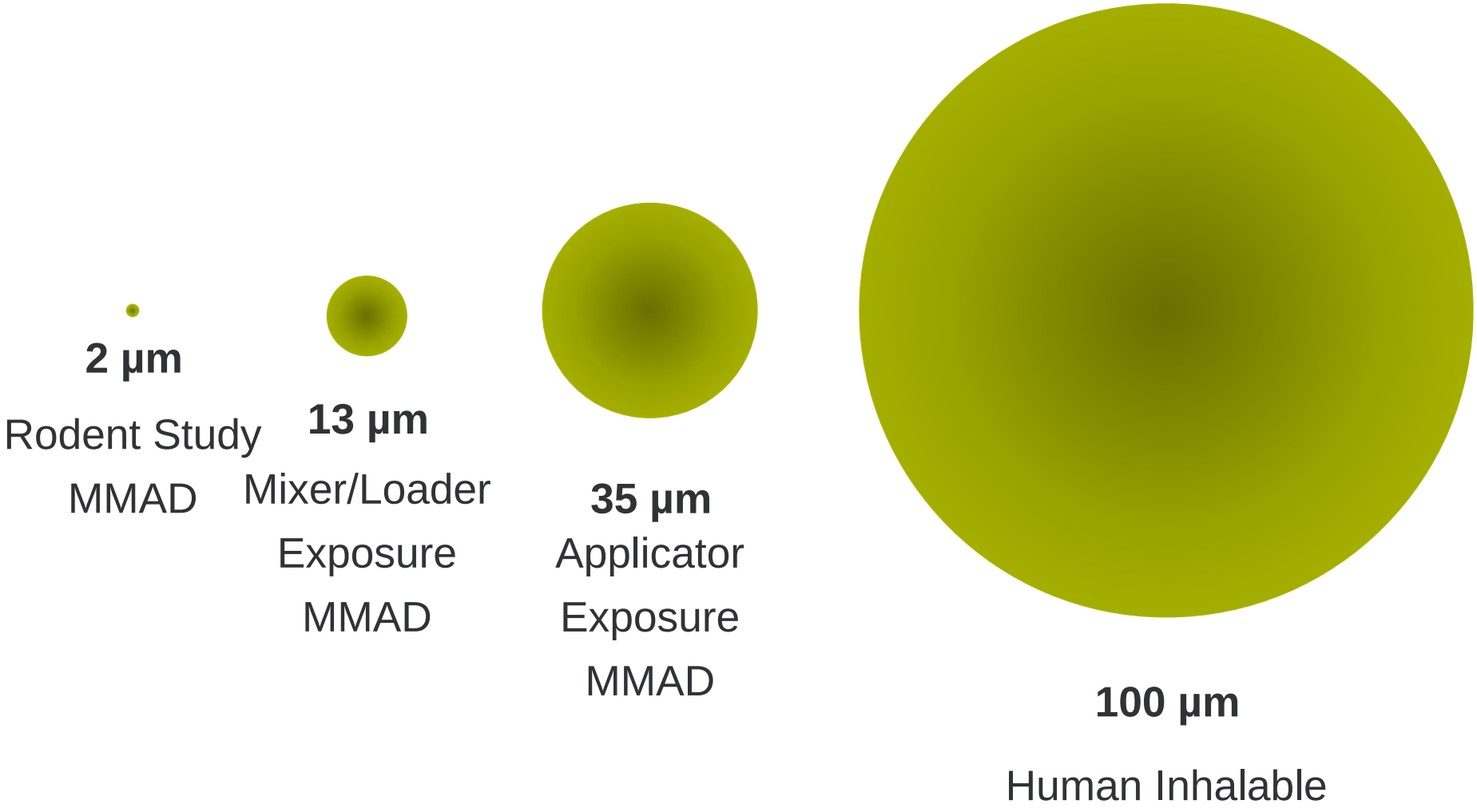
In vitro Point of Departure

- Low inter-donor variability across the 5 donors used
- All measured endpoints responded similarly and BMDL values were fit.
- Overall BMDL mean mass per unit surface area was determined (mg/cm^2).

	TEER BMDL (mg/cm^2)	LDH BMDL (mg/cm^2)	Resazurin BMDL (mg/cm^2)	**Mean**
Donor 1	0.00430	0.00703	0.00344	**0.00470**
Donor 2	0.00317	0.00963	0.00224	**0.00409**
Donor 3	0.00822	0.00878	0.00400	**0.00661**
Donor 4	0.00937	0.00998	0.00066	**0.00395**
Donor 5	0.00909	0.00875	0.00674	**0.00813**
Mean	**0.00625**	**0.00877**	**0.00267**	**0.00527**

- Necrotic injury to the luminal surface of the respiratory epithelium is a key event in squamous metaplasia of the larynx in treated rats.
- The initial irritant response can be assessed in MucilAir.
- Measured response is converted to a human BMDL which can then be used as the human Point of Departure (PoD) for risk assessment.

What is the Relevant Aerosol Droplet Size Distribution For Typical Agricultural Exposure?



syngenta

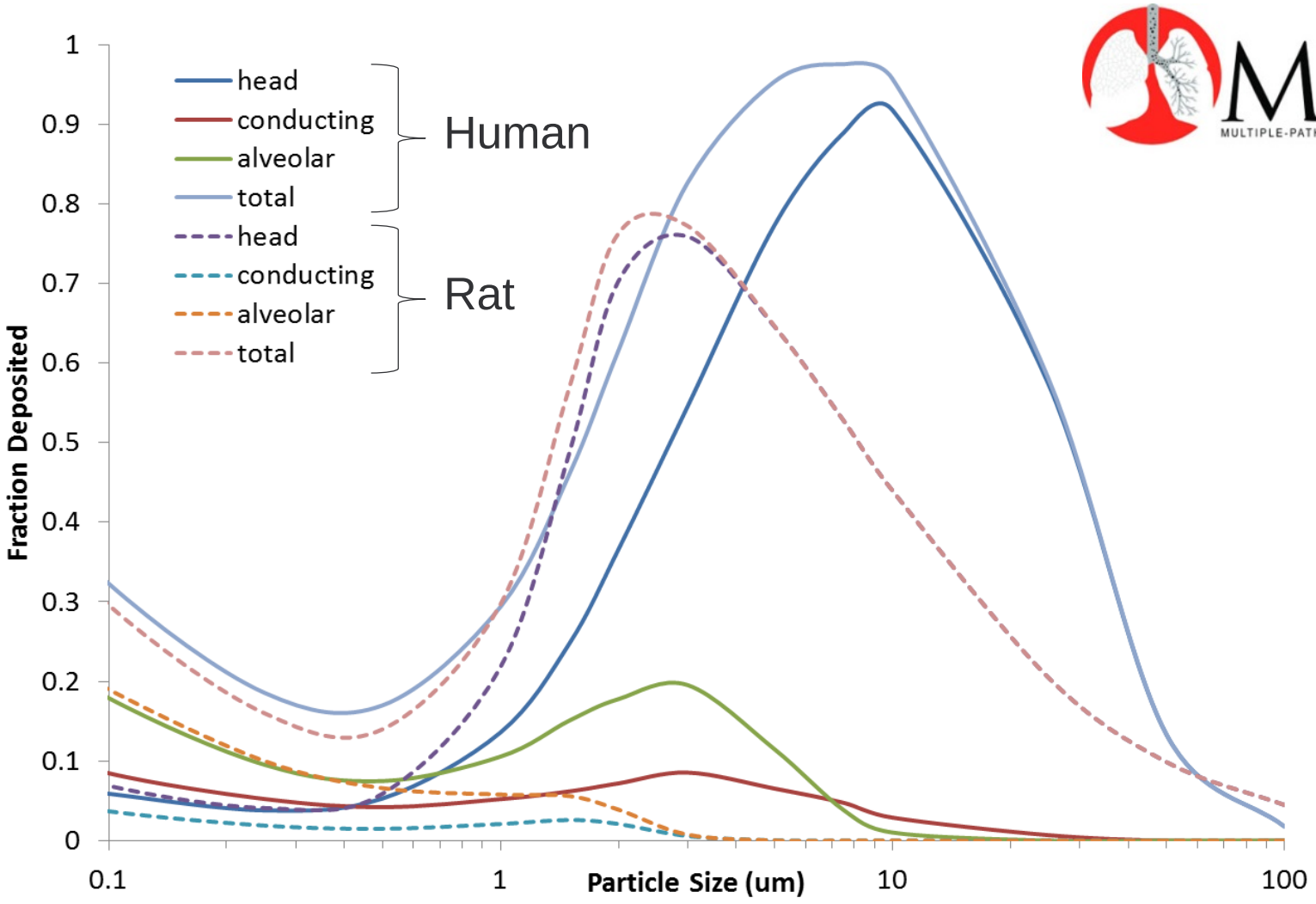
Inhalation Dosimetry Models

- Multiple-Path Particle Dosimetry Model (MPPD) calculates the deposition and clearance of aerosols in the respiratory tracts of rats and humans using theoretically derived efficiencies for deposition by diffusion, sedimentation, and impaction.
  - http://www.ara.com/products/mppd.htm

- In some cases, more precise models are needed to define exact locations in the respiratory tract (e.g. the U-shaped cartilage in the rat larynx).
- Computational fluid dynamics (CFD) simulate the deposition of a large number of individual particles.

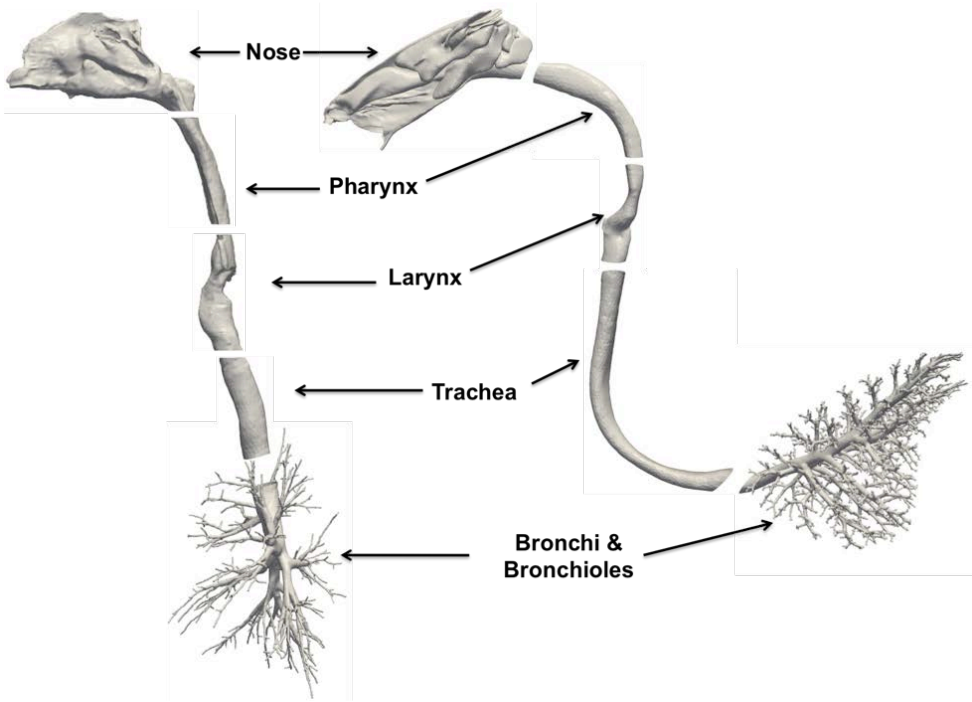
syngenta

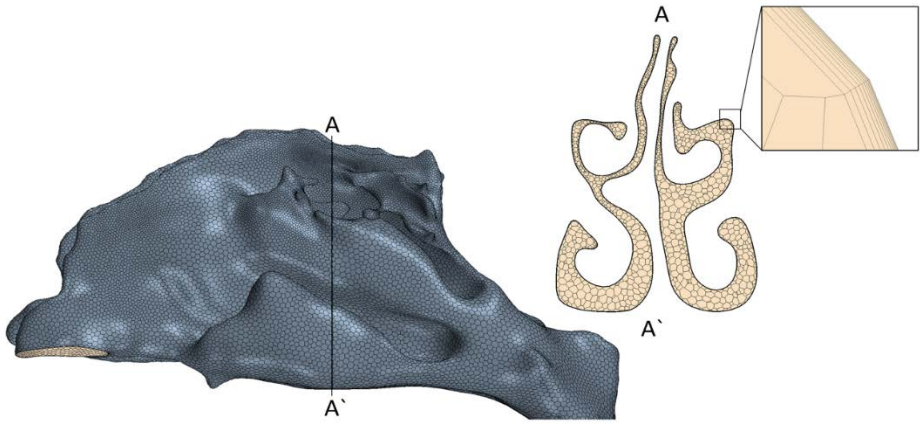
Comparative Deposition (Human vs. Rat)



syngenta

Computational Fluid Dynamics Modeling

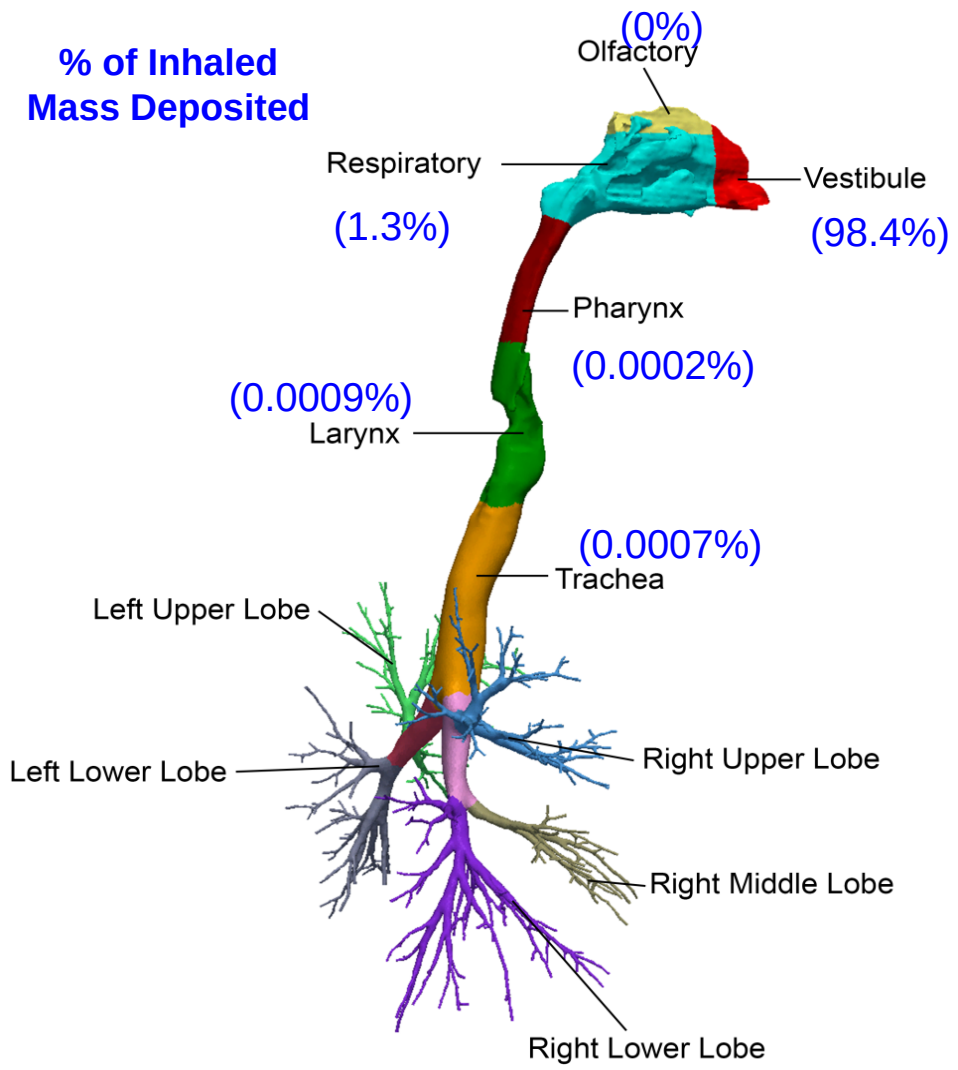


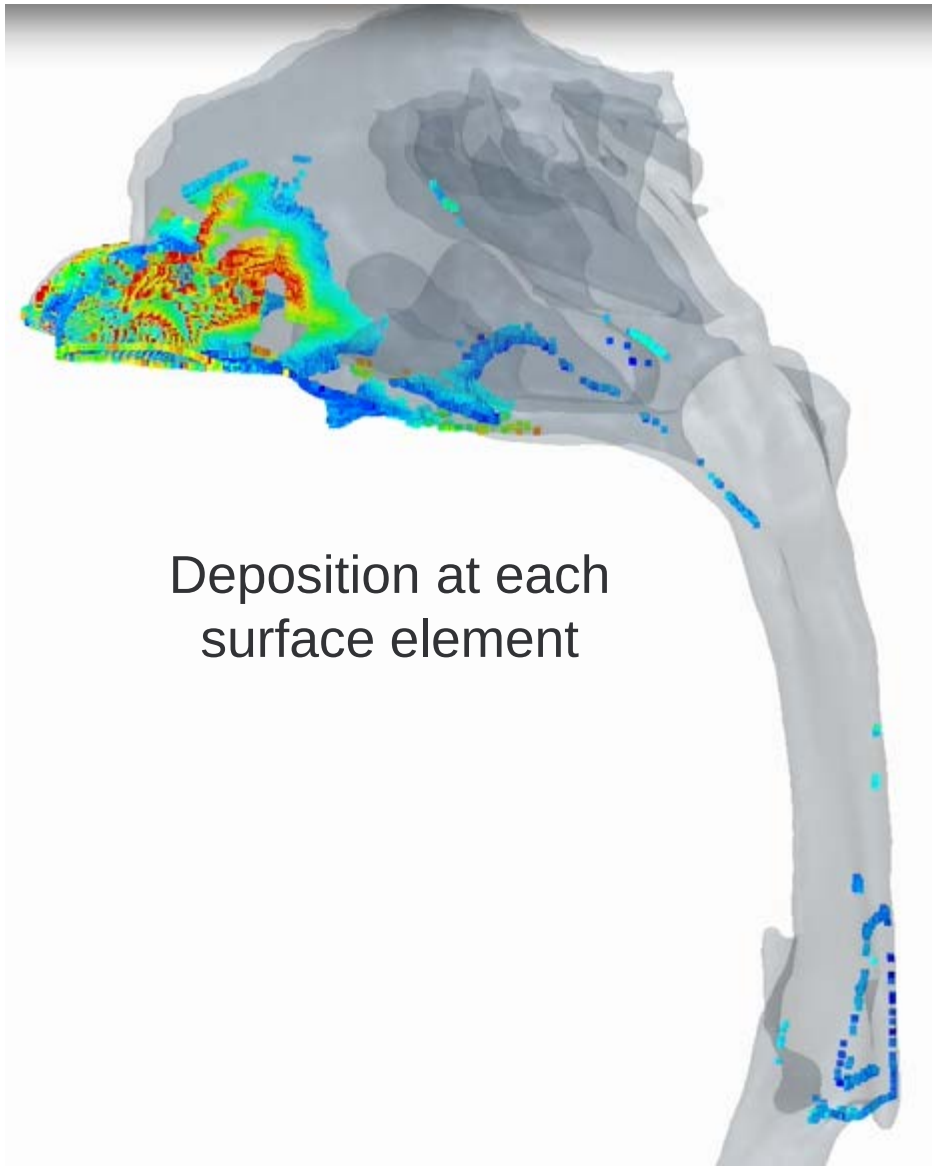


- Simulates the fluid dynamics of the airflow in the respiratory tract.
- Surfaces are meshes of polygons, each of which can be monitored.
- Commonly used in simulation of smaller particulates like bacteria spores and diesel particulates.

syngenta

Human CFD/Aerosol Simulation (50 µm MMAD)

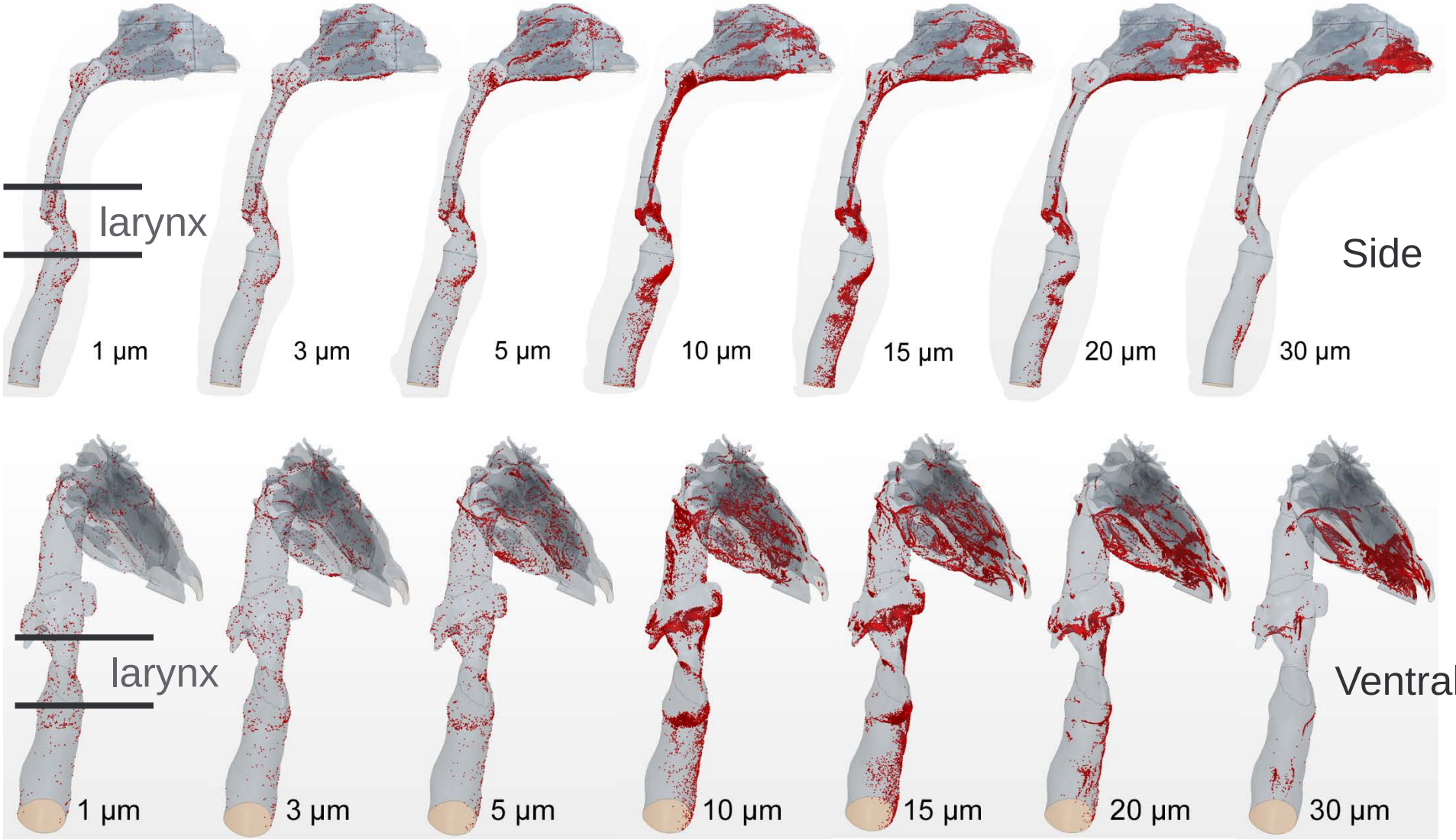




Deposition at each surface element

syngenta

Deposition Patterns in Humans



syngenta

Calculation of Human Exposure Concentration - Applicators

- Aerosol: 35 (1.5) µm polydisperse
- Breathing rate: 16.4 breaths/minute or 7872 breaths/8hr
- Dilution Factor 0.438 lb/lb diluted formulation
- *In vitro* BMDL 5.27×10^{-3} mg/cm^2

	Respiratory	Olfactory	Pharynx	Larynx	Trachea
Deposition (mg/cm^2/breath)	2.00E-06	1.41E-07	8.82E-07	1.61E-06	3.12E-07
Deposition (mg/cm^2/8hr)	6.89E-04	4.85E-05	3.04E-04	5.54E-04	1.08E-04
Exposure Concentration (mg/L)	**0.34**	**4.8**	**0.76**	**0.42**	**2.1**

- The exposure concentration is the air concentration necessary to match the *in vivo* 8 hour deposition with the *in vitro* BMDL

syngenta

Human Equivalent Concentration (HEC)

- HEC calculated using BMDL (*in vitro* model) and total daily deposition (CFD model).

Exposure Scenario	Human Equivalent Concentration (mg/L)				
	Respiratory	Olfactory	Pharynx	Larynx	Trachea
Spray Applicator	0.0013	0.0075	0.0019	0.0010	0.0055
Mixing/Loading Liquids	0.00079	0.0014	0.00071	0.00029	0.0024

$$\text{HEC} = \frac{\text{BMDL}}{\text{Total daily deposition}} \times 1 \text{ mg/L}$$

syngenta

Conclusions

- This Source to Outcome analysis addresses:
  - Inhalation study requirement
  - Point of Departure
  - Uncertainty Factors
    - Database, LOAEL to NOAEL, Interspecies

- Most importantly:
  - A human derived endpoint with dosimetry provides a more accurate estimate of risk.
  - Animal use is reduced.

- EPA Science Advisory Panel review of this work was held in December 2018.