

# Implementing alternative approaches for inhalation toxicity testing through multi-stakeholder collaborations

ICCVAM Communities of Practice Webinar  
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HECSB,  
PMRA

DOD; EPA OPP, OPPT;  
FDA CBER, CDER,  
CTP, CVM; OSHA



ANVISA



ECHA, EFSA,  
MHRA



CIBRC



ACICM, ICAMA,  
MEP, SAWS



MAFF, MOE



MAFF

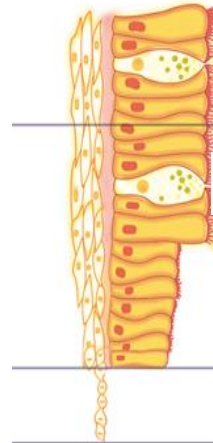
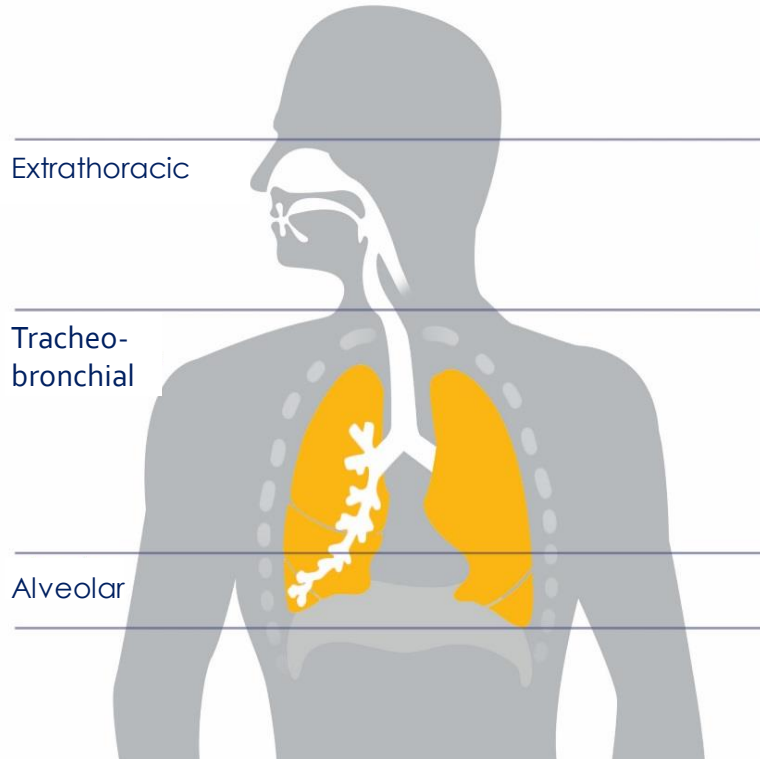


APVMA

## Respiratory tract region

## Cell types

## Deposition (particle size)



Ciliated cells – sweep debris

Goblet cells – secrete mucus

Club cells – divide and differentiate

Alveolar – AT1, AT2, macrophages

$>10\ \mu\text{m}$

$<10\ \mu\text{m}$   
 $>3\ \mu\text{m}$

PM10s penetrate the  
bronchiolar and alveolar  
regions of the lung

$<3\ \mu\text{m}$

PM2.5s can enter the bloodstream

Slide courtesy of Jason Adamson, British American Tobacco

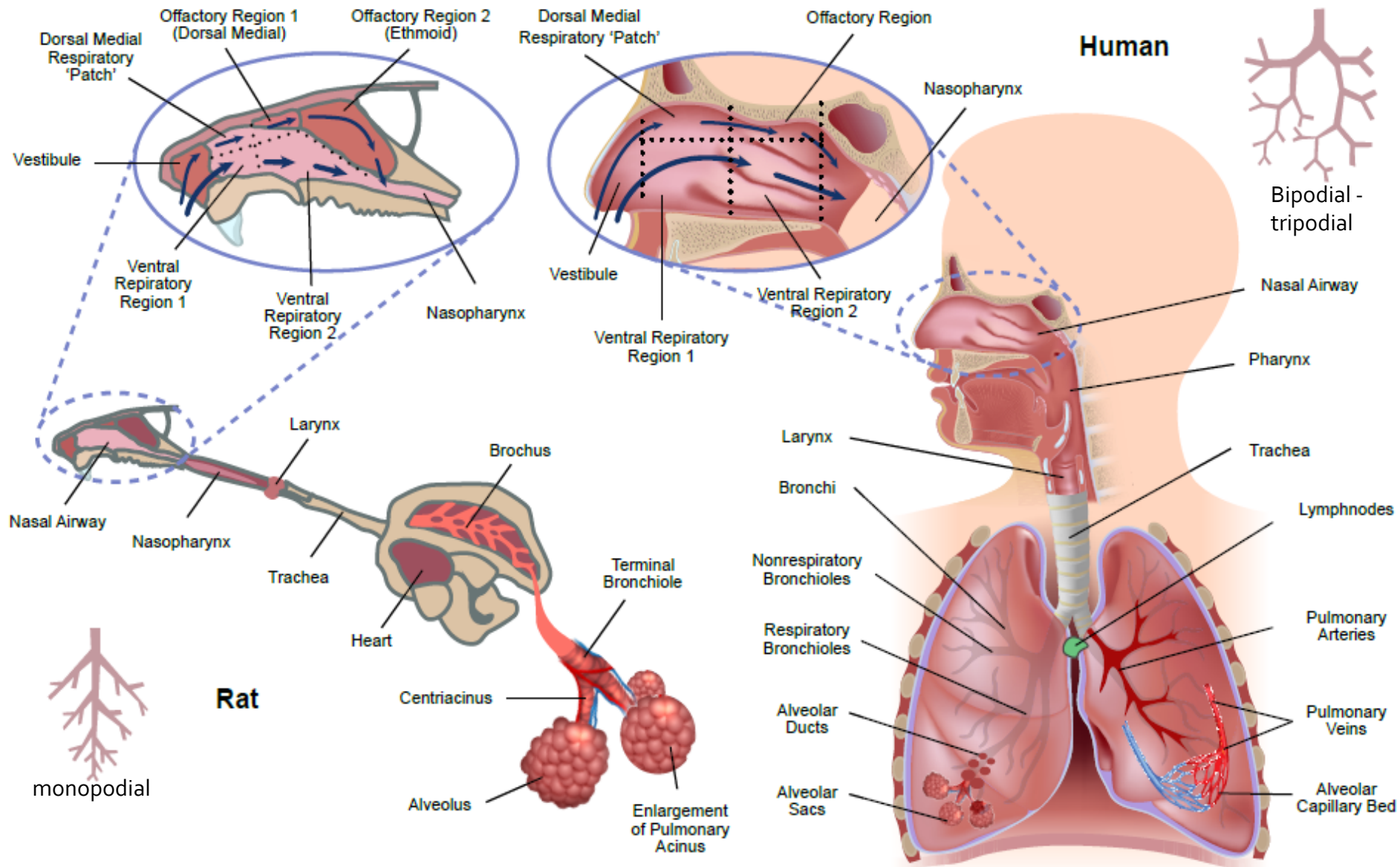


Illustration modified from Dr. Jack R. Harkema, Professor of Comparative Pathology, Michigan State University



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Contents lists available at [ScienceDirect](http://www.sciencedirect.com)

## Toxicology in Vitro

journal homepage: [www.elsevier.com/locate/toxinvit](http://www.elsevier.com/locate/toxinvit)



### Alternative approaches for acute inhalation toxicity testing to address global regulatory and non-regulatory data requirements: An international workshop report



Amy J. Clippinger<sup>a,\*</sup>, David Allen<sup>b</sup>, Annie M. Jarabek<sup>c</sup>, Marco Corvaro<sup>d</sup>, Marianna Gaça<sup>e</sup>, Sean Gehen<sup>f</sup>, Jon A. Hotchkiss<sup>g</sup>, Grace Patlewicz<sup>h</sup>, Jodie Melbourne<sup>a</sup>, Paul Hinderliter<sup>i</sup>, Miyoungh Yoon<sup>j</sup>, Dongeun Huh<sup>k</sup>, Anna Lowit<sup>l</sup>, Barbara Buckley<sup>c</sup>, Michael Bartels<sup>m</sup>, Kelly Bérubé<sup>n</sup>, Daniel M. Wilson<sup>g</sup>, Ian Indans<sup>o</sup>, Mathieu Vinken<sup>p</sup>

Arch Toxicol (2016) 90:1769–1783

DOI 10.1007/s00204-016-1717-8



CrossMark

#### MEETING REPORT

### Expert consensus on an in vitro approach to assess pulmonary fibrogenic potential of aerosolized nanomaterials

Amy J. Clippinger<sup>1</sup> · Arti Ahluwalia<sup>2</sup> · David Allen<sup>3</sup> · James C. Bonner<sup>4</sup> · Warren Casey<sup>5</sup> · Vincent Castranova<sup>6</sup> · Raymond M. David<sup>7</sup> · Sabina Halappanavar<sup>8</sup> · Jon A. Hotchkiss<sup>9</sup> · Annie M. Jarabek<sup>10</sup> · Monika Maier<sup>11</sup> · William Polk<sup>3</sup> · Barbara Rothen-Rutishauser<sup>12</sup> · Christie M. Sayes<sup>13</sup> · Phil Sayre<sup>14</sup> · Monita Sharma<sup>1</sup> · Vicki Stone<sup>15</sup>





Run Search Clear

Select Assays

- ☐ Acute Dermal Toxicity  
☐ Acute Oral Toxicity  
☒ Acute Inhalation Toxicity  
☐ Primary Skin Irritation  
☐ Primary Eye Irritation  
☐ Dermal Sensitization

Select Reference Lists.

Enter one CASRN per line.

Selected Assay Categories: Acute Inhalation Toxicity

Query Integrator Number of formulations = 469 (Number of active ingredients = 764 (unique: 249)). Showing 3 Endpoints.

Formulation ID	AI	CASRN	% AI	EPA Category	GHS Category	LC50 mg/L
▶ Bromuconazole(98.4%)		ICE_346464285		4	5	>5.05
▶ Cabrio EG Fungicide		ICE_4666705...		4	4	4.5
▶ Callisto Xtra		ICE_725870894		4	4	2.62
▶ Canguard BIT 20 DPG Preservative		ICE_4140063...		NA	NA	4.83
▶ Captan 4L		ICE_450334329		4	4	>2.03
▶ Boscalid		ICE_2112150...		4	5	>6.7
▼ Bravo Top		ICE_417649525		2	2	0.32 - 0.61
	Chlorothalonil	1897-45-6	40.0			
	Difenoconazole	110448-88-3	4.0			
▶ Britz Dryout Dust		ICE_4198918...		4	4	>2.02
▶ Bromuconazole(20%)		ICE_1180030...		NA	NA	>4.35
▶ Bioban 425 Antimicrobial		ICE_1813212...		2	2	0.38
▶ Bispyribac-sodium		ICE_4999578...		4	4	>4.48
▶ Bonide Cyper WSP Insecticide		ICE_1541848...		4	4	>2.04
▶ Bonide Spinosad Dust		ICE_1328035...		4	4	>2.03
▶ Bifenthrin 8% ME		ICE_4114833...		4	4	>2.32
▶ Bifenture 10WSB/10DF Insecticide/MI		ICE_361244420		4	5	>5.869
▶ Bifenture 17		ICE_4934514...		4	5	>5.06
▶ Biflex SFR		ICE_794588095		NA	NA	>7.25
▶ Chemsico Aerosol LEG		ICE_4384128...		4	4	>2.16
▶ Chemsico Pesticide Concentrate W-I		ICE_875544678		4	4	>2.09

Integrated Chemical Environment  
<https://ice.ntp.niehs.nih.gov/>  
Shannon Bell: [sbell@ils-inc.com](mailto:sbell@ils-inc.com)



Contents lists available at ScienceDirect

## Toxicology in Vitro

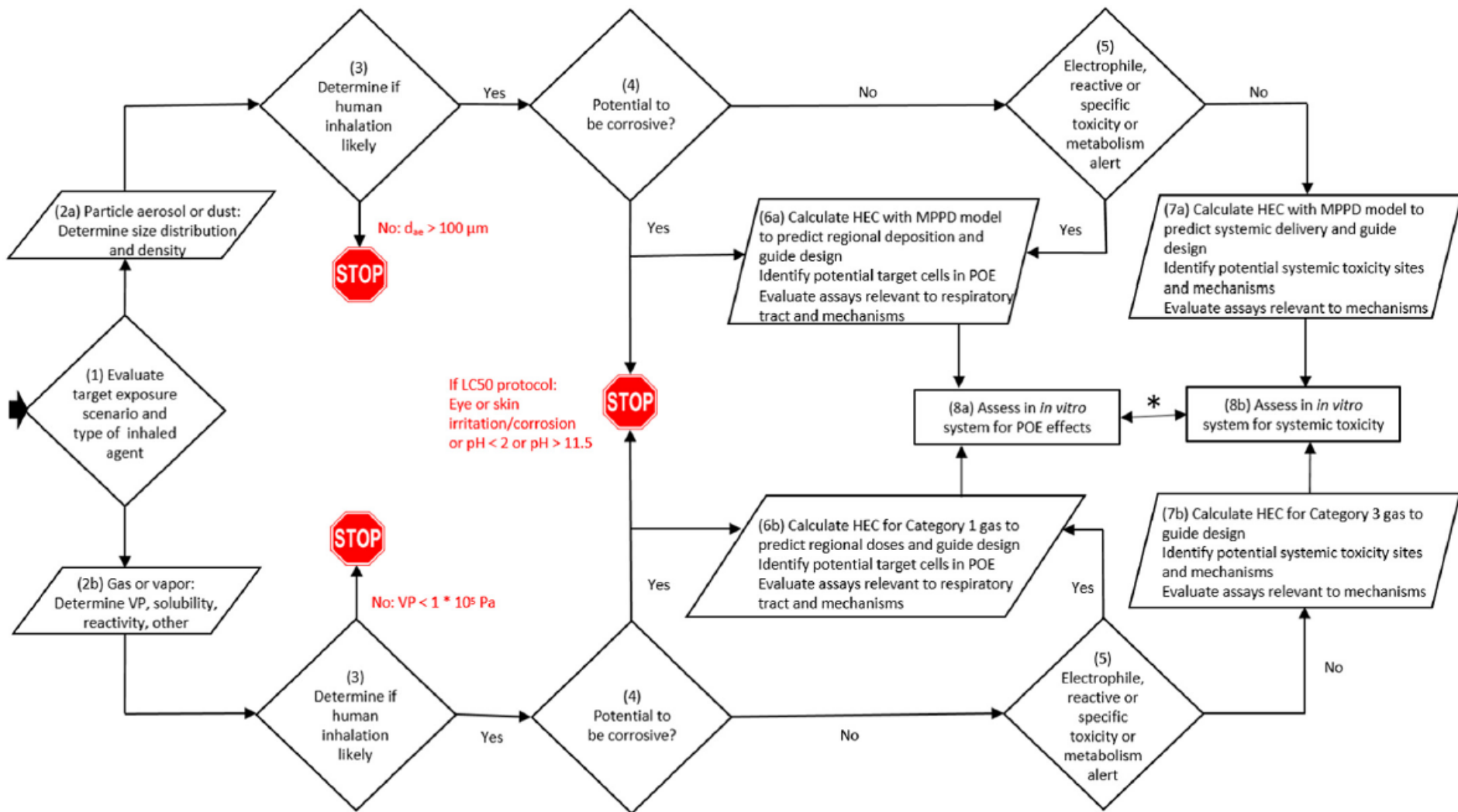
journal homepage: [www.elsevier.com/locate/toxinvit](http://www.elsevier.com/locate/toxinvit)



### Review

## Pathway-based predictive approaches for non-animal assessment of acute inhalation toxicity

Amy J. Clippinger<sup>a,\*</sup>, David Allen<sup>b</sup>, Holger Behrsing<sup>c</sup>, Kelly A. Bérubé<sup>d</sup>, Michael B. Bolger<sup>e</sup>, Warren Casey<sup>f</sup>, Michael DeLorme<sup>g</sup>, Marianna Gaça<sup>h</sup>, Sean C. Gehen<sup>i</sup>, Kyle Glover<sup>j</sup>, Patrick Hayden<sup>k</sup>, Paul Hinderliter<sup>l</sup>, Jon A. Hotchkiss<sup>m</sup>, Anita Iskandar<sup>n</sup>, Brian Keyser<sup>o</sup>, Karsta Luettich<sup>n</sup>, Lan Ma-Hock<sup>p</sup>, Anna G. Maione<sup>k</sup>, Patrudu Makena<sup>o</sup>, Jodie Melbourne<sup>a</sup>, Lawrence Milchak<sup>g</sup>, Sheung P. Ng<sup>q</sup>, Alicia Paini<sup>r</sup>, Kathryn Page<sup>s</sup>, Grace Patlewicz<sup>t</sup>, Pilar Prieto<sup>r</sup>, Hans Raabe<sup>c</sup>, Emily N. Reinke<sup>u</sup>, Clive Roper<sup>v</sup>, Jane Rose<sup>w</sup>, Monita Sharma<sup>a</sup>, Wayne Spoo<sup>o</sup>, Peter S. Thorne<sup>x</sup>, Daniel M. Wilson<sup>m</sup>, Annie M. Jarabek<sup>y</sup>





# Webinar Series on the Use of NAMs in Risk Assessment

MPPD and CFD Modeling to Predict Dosimetry of Inhaled Substances

Bahman Asgharian, Applied Research Associates

Richard Corley, Greek Creek Toxicokinetics Consulting

January webinar is being rescheduled due to  
the partial US government shutdown

[www.piscltd.org.uk/nam-webinars](http://www.piscltd.org.uk/nam-webinars)

**PhysiciansCommittee**  
for Responsible Medicine



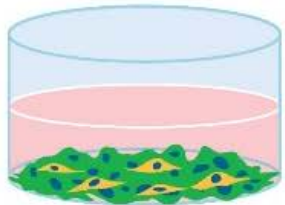
PETA INTERNATIONAL   
SCIENCE CONSORTIUM LTD.

## QSAR models:

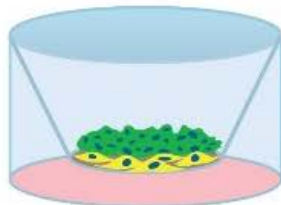
- TOPKAT (Biovia)
- CASE Ultra (MultiCASE)
- REACHAcross (UL)

## Modeling challenge?

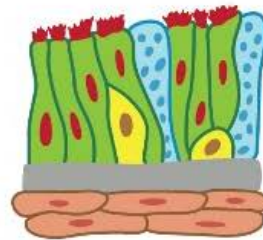
- E.g., CATMoS: Collaborative Acute Toxicity Modeling Suite (Kleinstreuer et al. *Comp Toxicol.* 2018; 8:21-24 and Mansouri et al, *in preparation*)



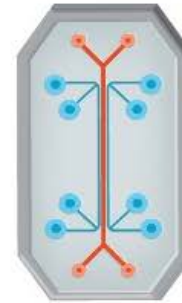
**Submerged  
co-cultures**



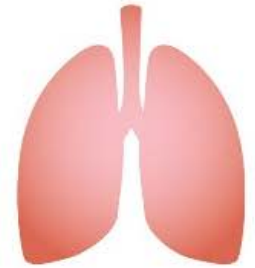
**Co-cultures  
grown at  
the ALI**



**3D reconstructed  
human tissues  
grown at the ALI**



**Microfluidic  
human lung-on-  
a-chip**



**Human PCLS**

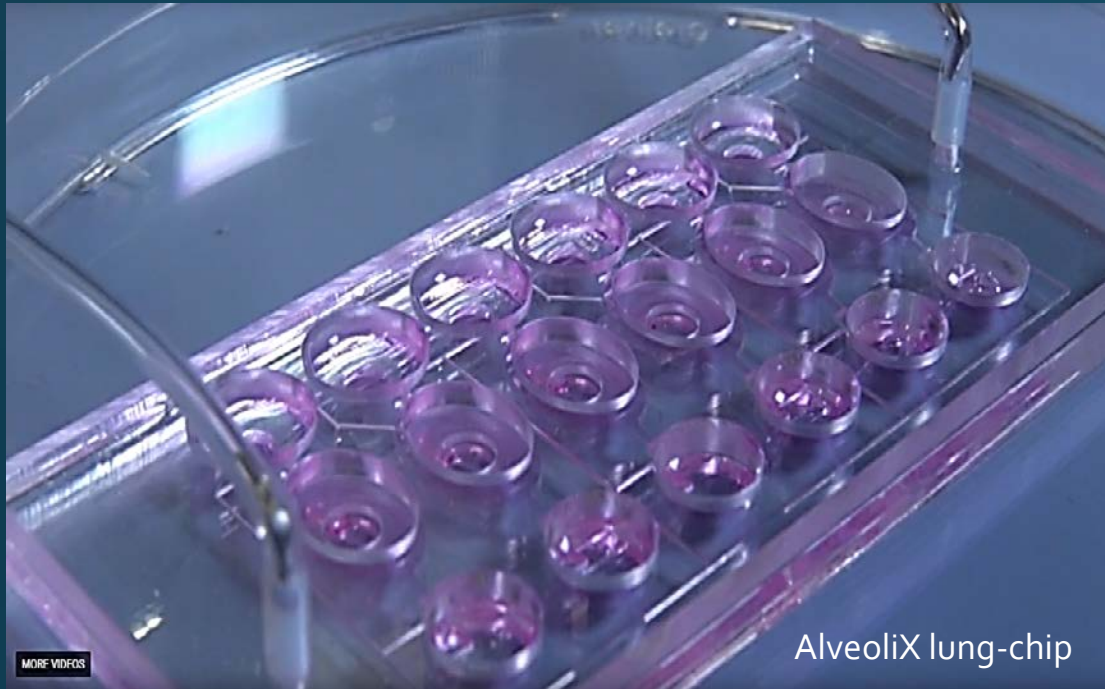
3D reconstructed human tissue models e.g.,

- MucilAir (Epithelix)
- SmallAir (Epithelix)
- EpiAirway (MatTek)
- Alveolar models from Epithelix and MatTek to be launched in 2019

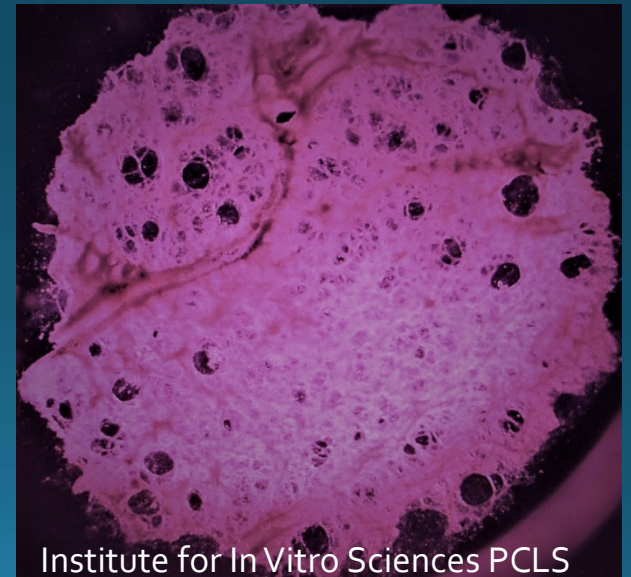








AlveoliX lung-chip



Institute for In Vitro Sciences PCLS

# Adverse Outcome Pathways

**Molecular  
Initiating Event**



**Cellular  
Response**



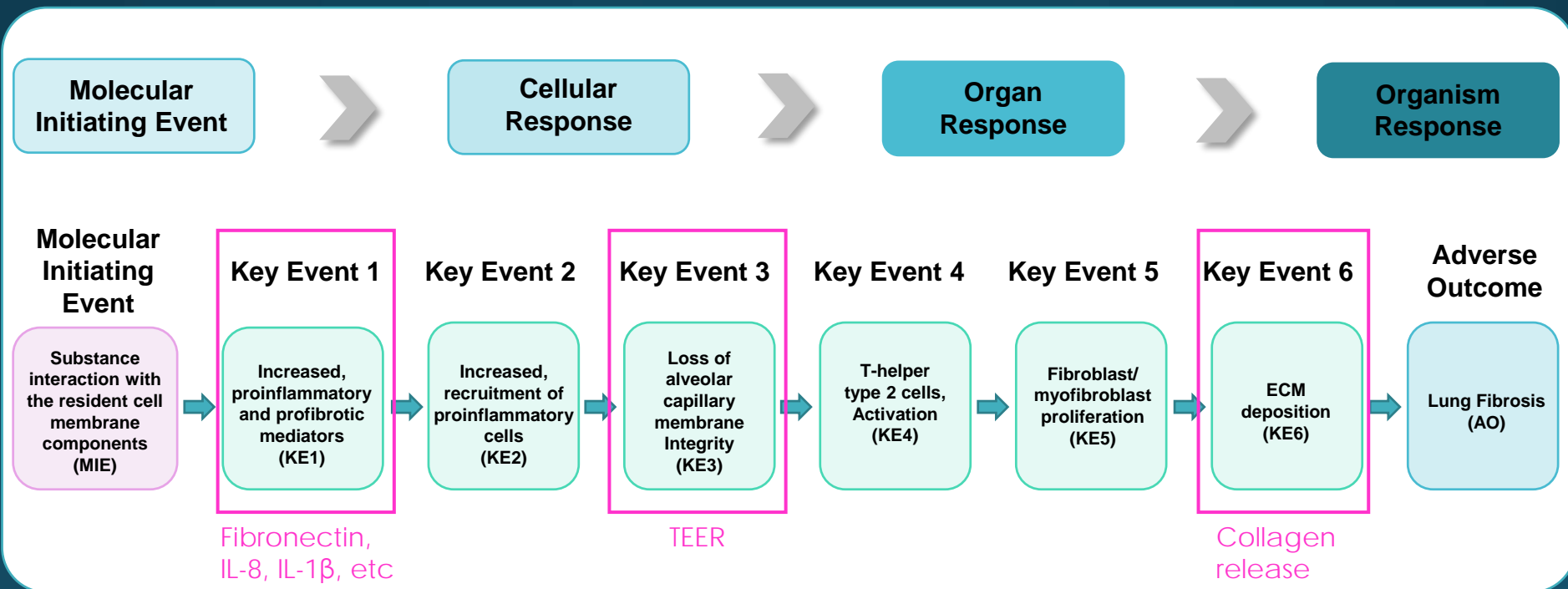
**Organ  
Response**

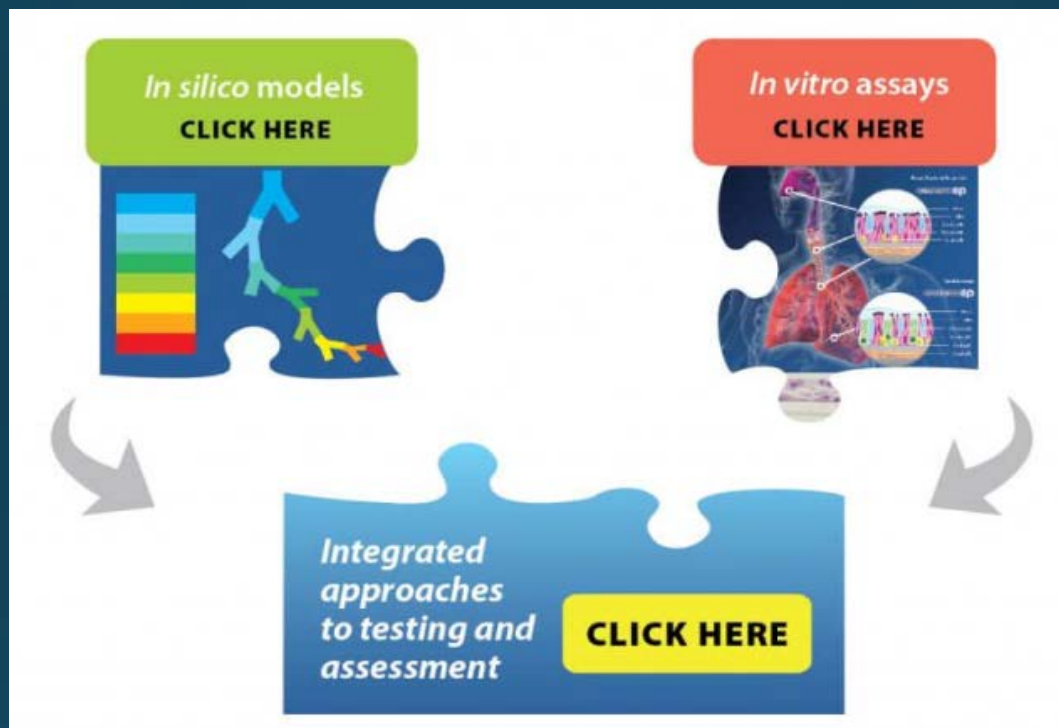


**Organism  
Response**



# AOP 173: Increased Substance Interaction with the Resident Cell Membrane Components Leading to Lung Fibrosis





<https://www.pisc ltd.org.uk/inhalation-webinars/>



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FOR THE WORKING ENVIRONMENT



# Conclusions

- Multiple *in silico* and *in vitro* approaches will be needed to assess the various mechanisms of toxicity following inhalation exposure
- Multi-stakeholder collaborations on data sharing and validation efforts foster the development of *in vitro* and *in silico* approaches that can be used to protect human health without using animals





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