

# In Vitro Inhalation Toxicity: A Case Study in Building Confidence in New Methods

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## Funding



## Training, workshops, webinars



## Retrospective data reviews



## Standards making organizations



## Publications and presentations

Case study of developing and gaining confidence in a new method:  
*In vitro* approach for assessing portal-of-entry effects of chemicals  
exposed as liquids to a reconstructed human respiratory epithelial model



## Introduction



## Literature Review

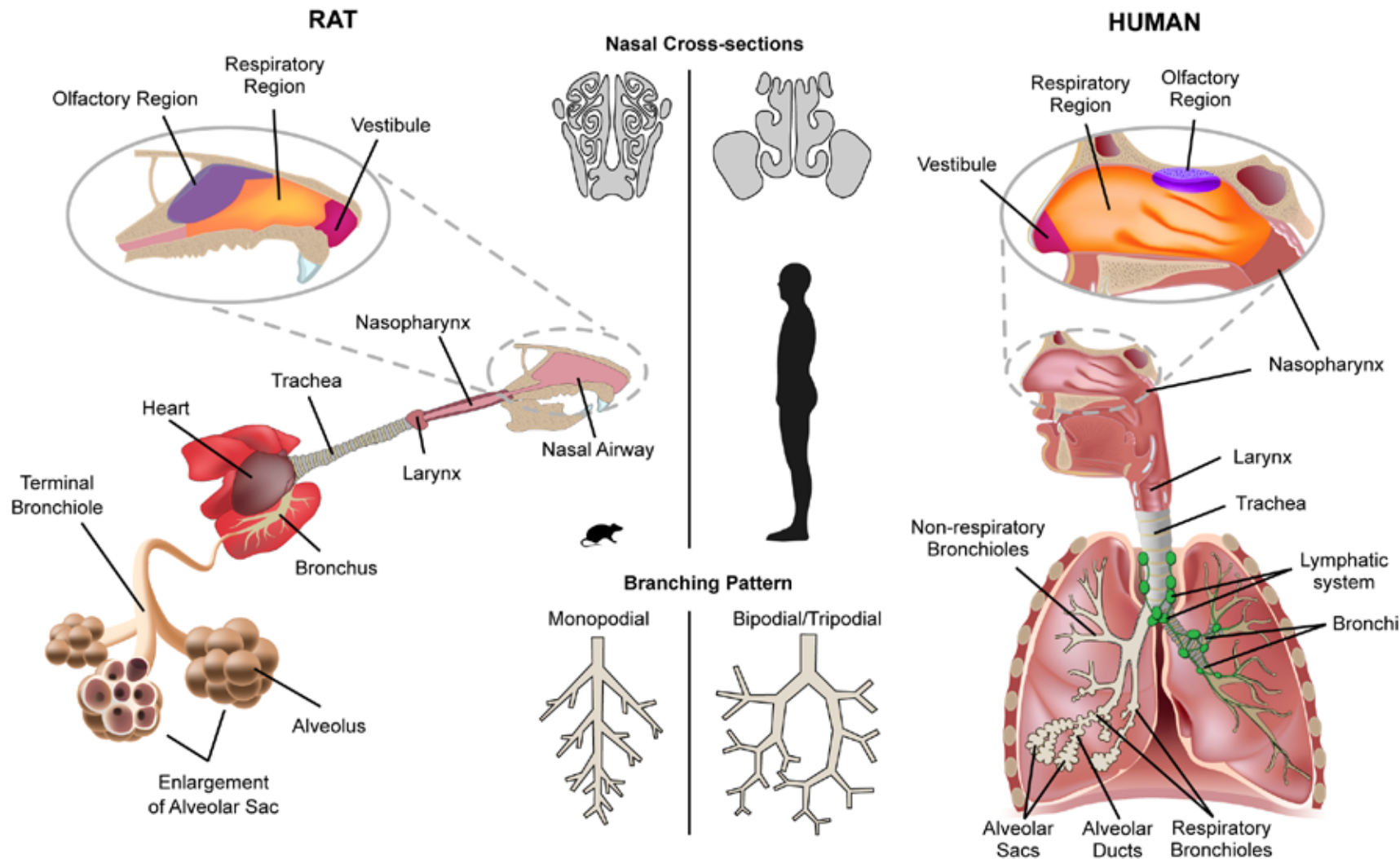
Reference Chemicals  
Testing Protocol



## Evaluating Scientific Confidence

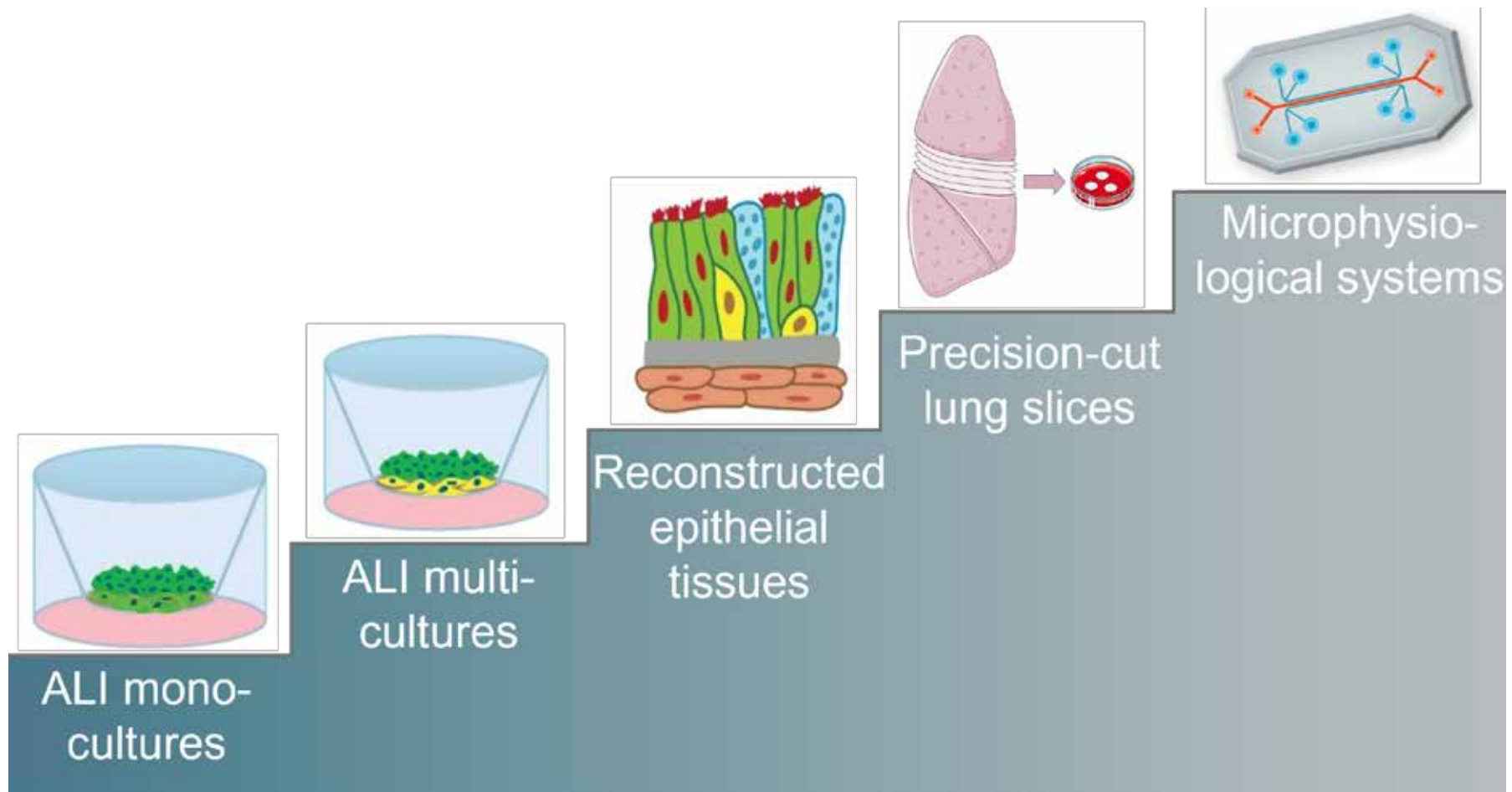
# Introduction

# Rat vs. human respiratory tract



Stucki AO, Sauer UG, Allen DG, Kleinstreuer NC, Perron MM, Yozzo KL, Lowit AB, Clippinger AJ. Differences in the anatomy and physiology of the human and rat respiratory tracts and impact on toxicological assessments. *Regul Toxicol Pharmacol.* 2024;150:105648.

# *In vitro/ex vivo* air-liquid interface systems



# Literature Review

# Literature review

Literature search was conducted to identify:

- chemicals that have been tested *in vitro* or *ex vivo*
- study designs used

The above was then used to inform:

- reference chemicals
- testing protocol

Overall, this information will be used to understand what's been done and what still needs to be done to gain confidence in the testing approach, including any additional testing needs.



# Literature review

**sysrev**

SysRev (Insilica) is the web platform used to conduct a reproducible, transparent, and thorough search of literature (PubMed).

# SysRev query

((("in vitro" OR "ex vivo") AND ("inhalation toxicity" OR "respiratory toxicity" OR "lung toxicity" OR "pulmonary toxicity" OR "airway toxicity" OR "respiratory irritation" OR "lung irritation" OR "lung effects" OR "respiratory effects")) OR ("EpiNasal" OR "EpiAirway" OR "EpiAlveolar" OR "AlveolAir" OR "MucilAir" OR "Epithelix" OR "ImmuOne"))

Search performed on July 15, 2024.

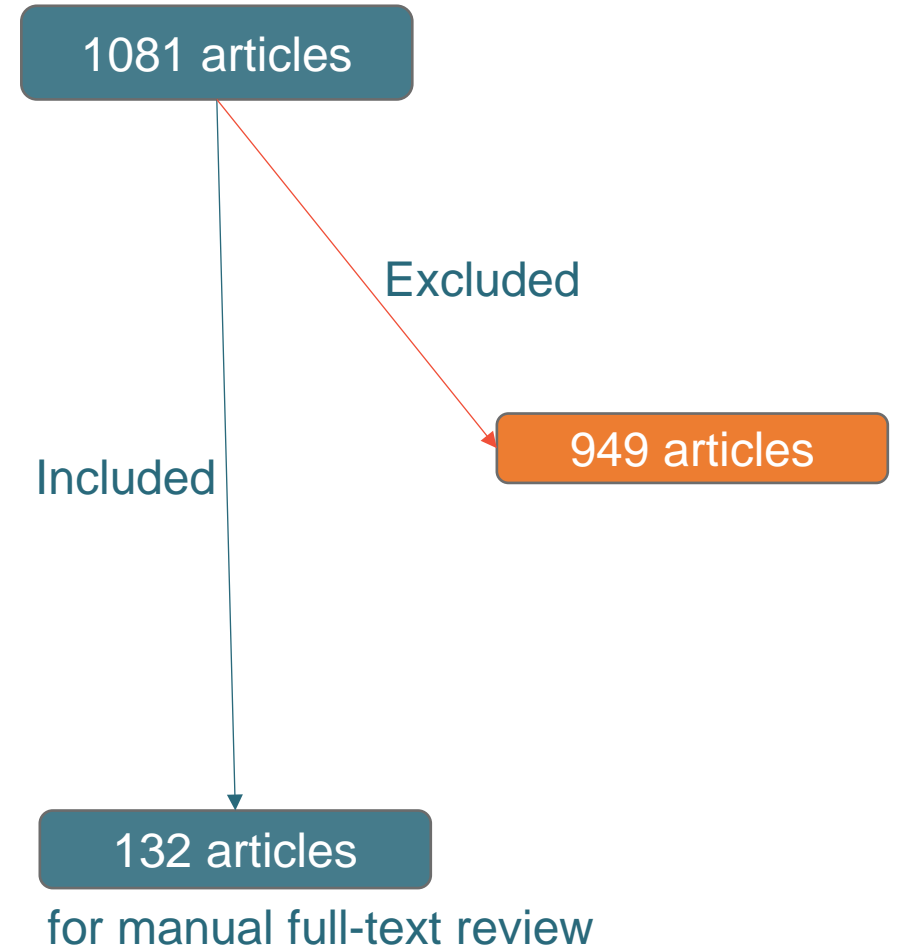
# Literature review

Step 1: Performed search using query. Returned 1081 articles

Step 2: SysRev autolabeler and humans reviewed the abstract/title of 1081 articles, with defined exclusion criteria applied.

- Review articles with no underlying data
- Papers without any *in vitro* or *ex vivo* data
- Papers only testing solid aerosols/particles
- Articles without cellular toxicity information (e.g., effects on viral load or drug transport studies)

Step 3: Returned 132 articles for full text review



# Extracting relevant information

Manual full-text review

132 articles

↓ Exclude vapors and gases

52 articles

Liquids in any cell system

259 potential  
chemicals for a  
reference list

Liquid exposure in any  
*in vitro* or *ex vivo* model

# Reference Chemicals

*used to establish the validity of a method and give known, consistent results*

# Selecting reference chemicals

## Considerations:

- Coverage of the range of responses to be predicted
- Availability of high-quality data
- Ease of procurement (commercially available, not prohibitively expensive to acquire/dispose)
- Coverage of the applicability domain (representation across the types of substances for which the method will be used)
- Reproducible response
- Avoiding reference chemicals with borderline results near “cut-off” values
- Incorporation of expert feedback

The number of reference chemicals can vary depending on the method (e.g., the range of responses or the availability of data)

# Selecting reference chemicals

Based on the criteria outlined on the previous slide and from the list of 259 chemicals identified in the literature review, we've curated a draft reference chemical list, to be shared for expert input.

- Chemical name
- CASRN
- Concentration
- Chemical class
- GHS classification (acute inhalation toxicity)

# Testing Protocol



# Extracting relevant information

Manual full-text review

132 articles

↓ Exclude vapors and gases

52 articles

Liquids in any cell system

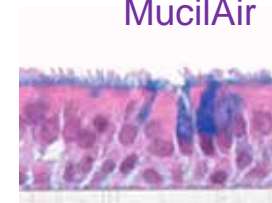
259 potential  
chemicals for a  
reference list

Liquid exposure in any  
*in vitro* or *ex vivo* model

20 articles with  
study designs

Liquid exposure in  
RHRE tissue models

MucilAir



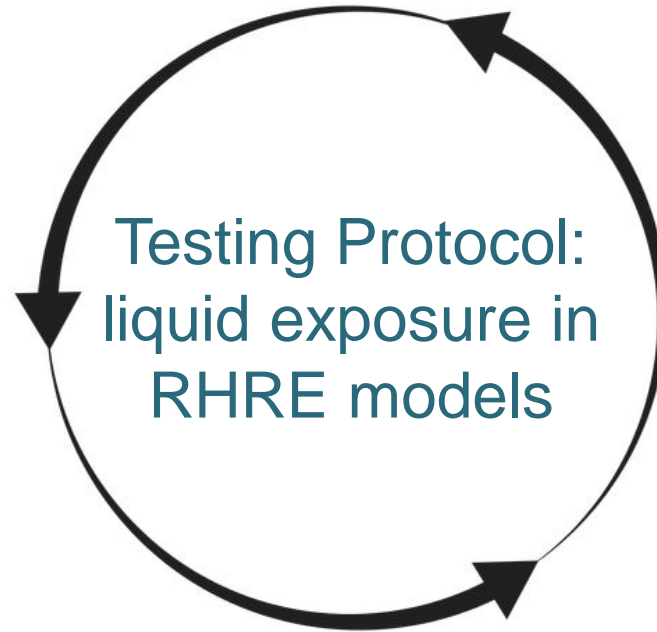
 EPITHELIX

EpiAirway



 MATTEK  
A BICO COMPANY

PETA SCIENCE CONSORTIUM  
INTERNATIONAL e.V. 



- Starting point: Protocol developed under “The INSPIRE Initiative”
- 20 papers with relevant study designs found in the literature review
- Unpublished test protocols, best practices, and lessons learned e.g., RespTox
- Now sharing the draft protocol with additional experts for input

# Scientific Confidence



## A framework for establishing scientific confidence in new approach methodologies

Anna J. van der Zalm<sup>1</sup> · João Barroso<sup>2</sup> · Patience Browne<sup>3</sup> · Warren Casey<sup>4</sup> · John Gordon<sup>5</sup> · Tala R. Henry<sup>6</sup> · Nicole C. Kleinstreuer<sup>7</sup> · Anna B. Lowit<sup>6</sup> · Monique Perron<sup>8</sup> · Amy J. Clippinger<sup>1</sup>

<sup>1</sup> PETA Science Consortium International e.V.

<sup>2</sup> European Commission, Joint Research Center

<sup>3</sup> Organisation for Economic Co-Operation and Development, Hazard Assessment and Pesticides Programmes

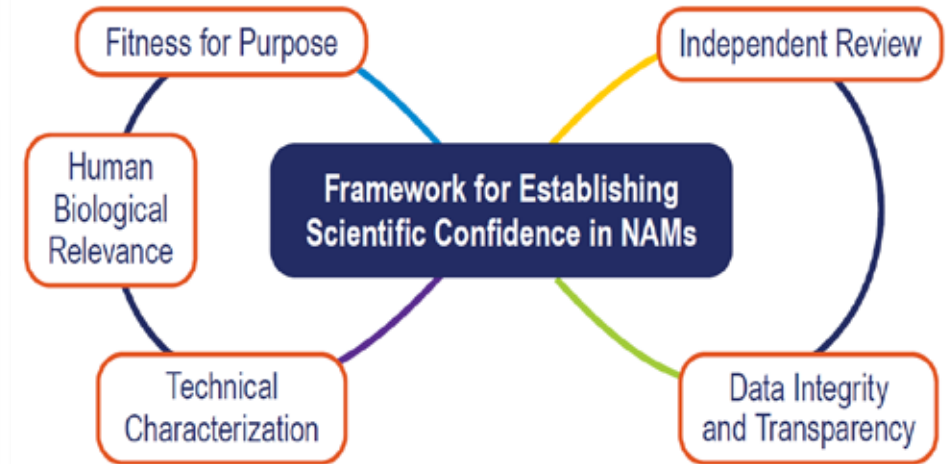
<sup>4</sup> National Institutes of Health, DNTP, National Institutes of Environmental Health Sciences

<sup>5</sup> US Consumer Product Safety Commission

<sup>6</sup> US Environmental Protection Agency, Office of Pollution Prevention and Toxics

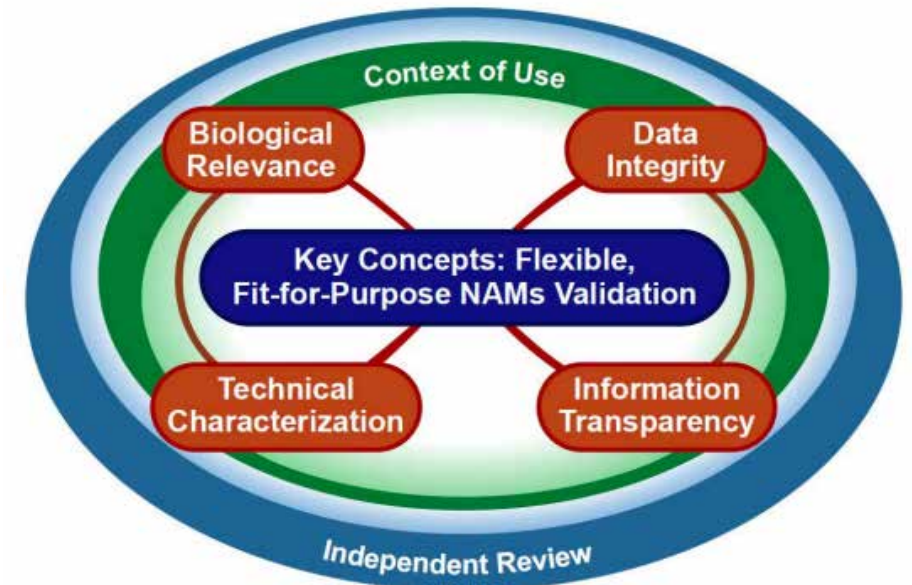
<sup>7</sup> National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

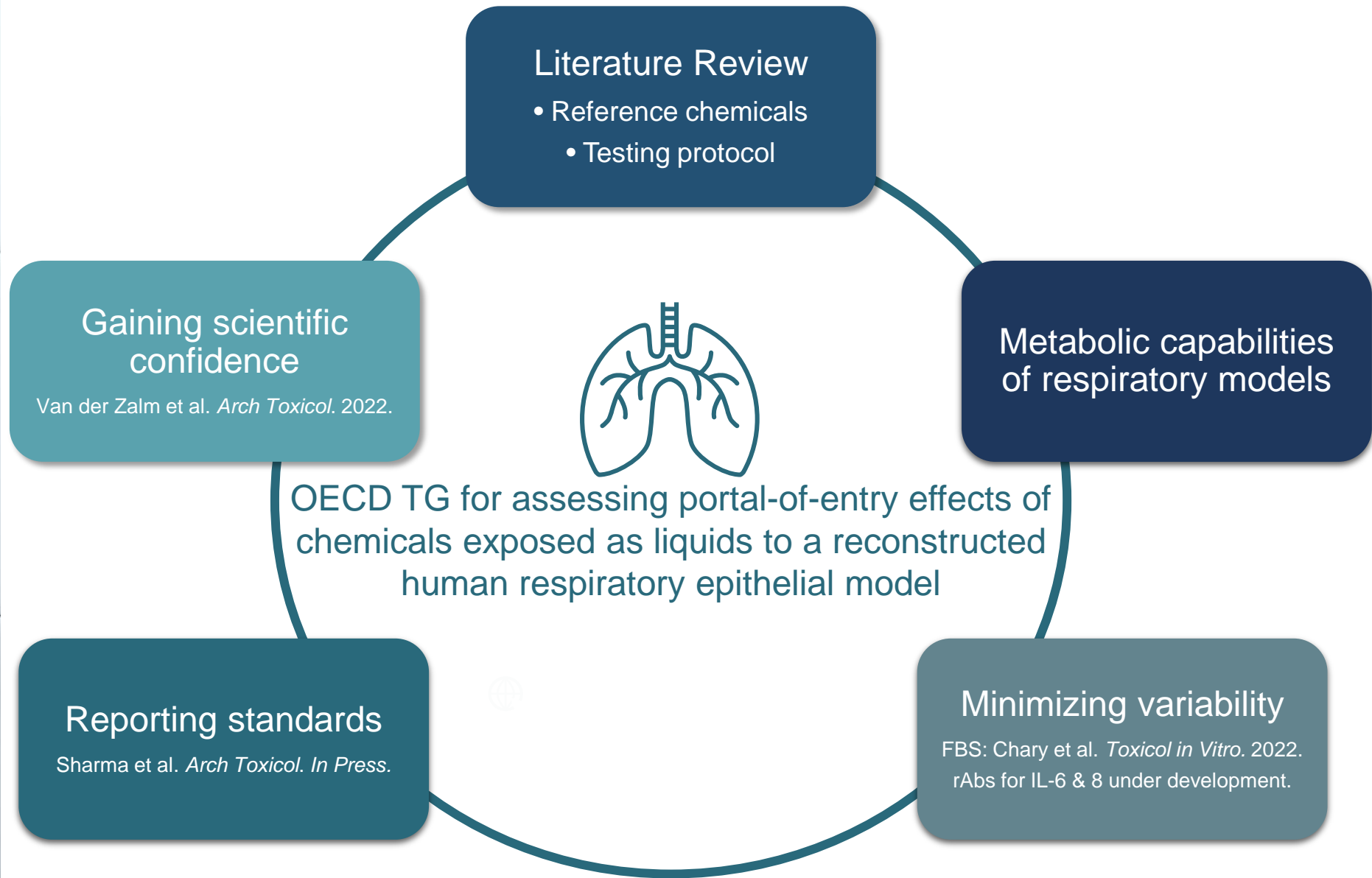
<sup>8</sup> US Environmental Protection Agency, Office of Pesticide Programs



## Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies

A Report of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Validation Workgroup





Ø Collaboration and iterative incorporation of expert feedback facilitate the advancement of new approaches that effectively and efficiently protect human health.

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