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

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PETA SCIENCE CONSORTIUM INTERNATIONAL e.V.

- Funding
- Training, workshops, webinars
- Retrospective data reviews
- Standards making organizations
- Publications and presentations

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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

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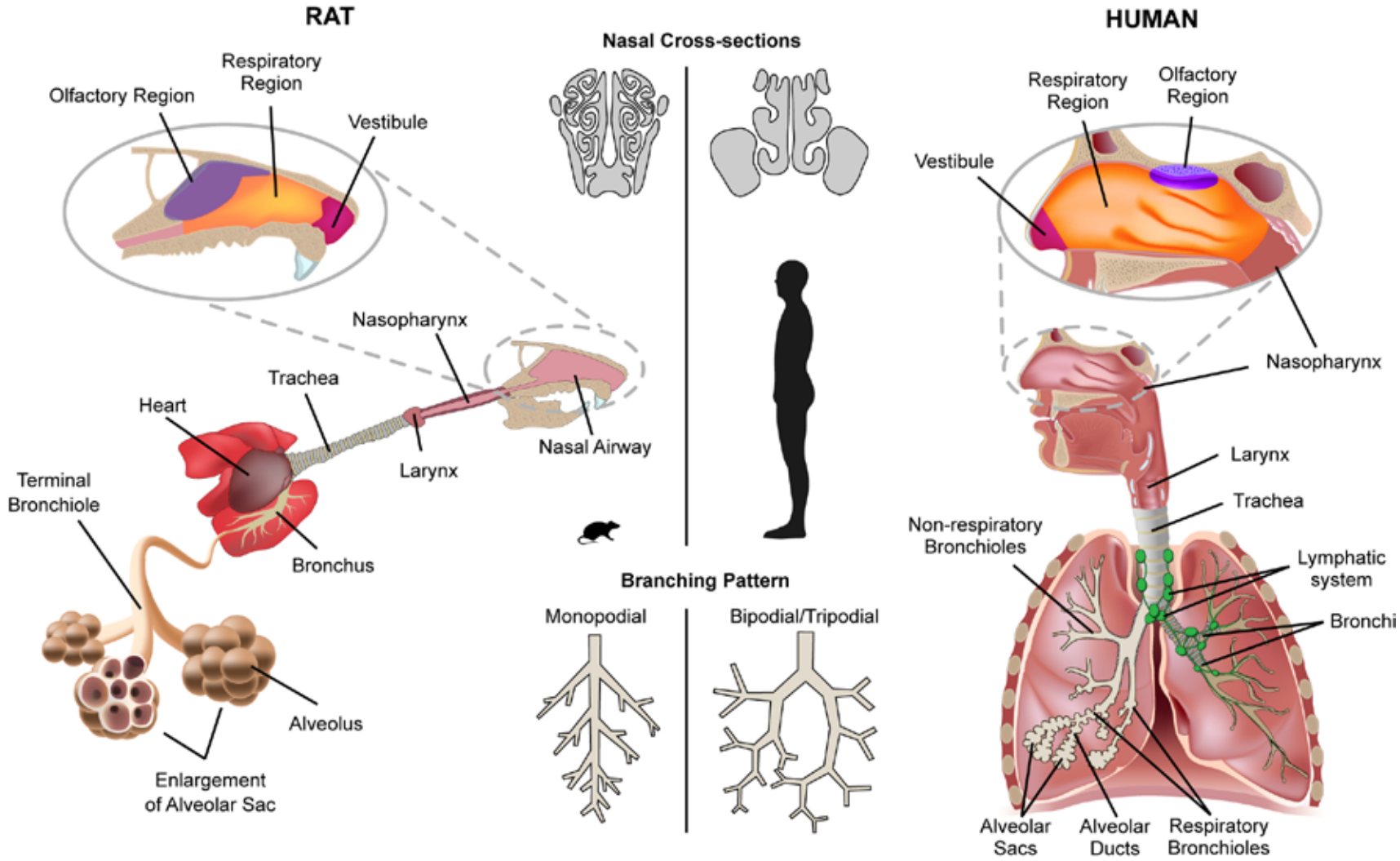
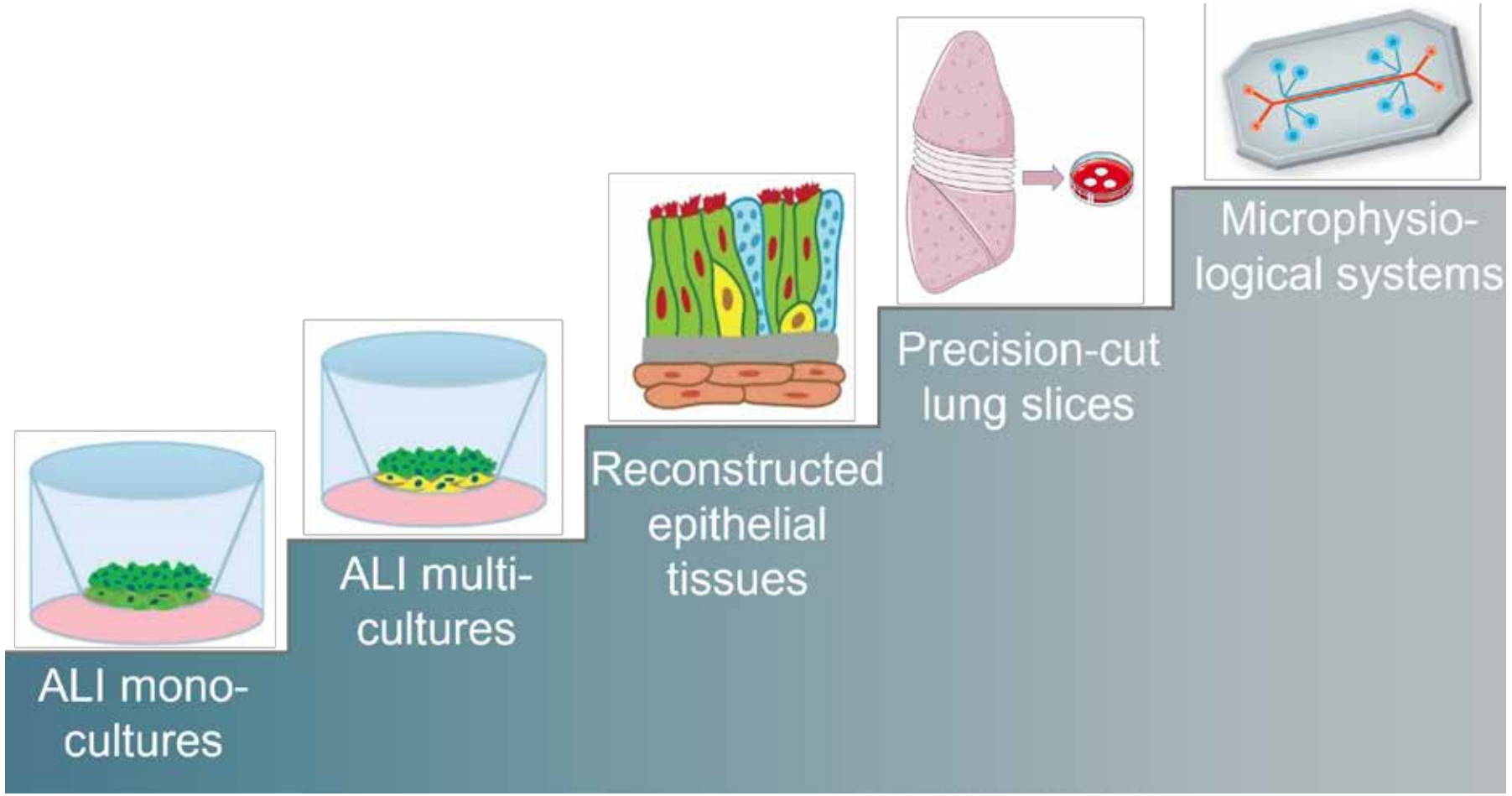
Introduction

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

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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

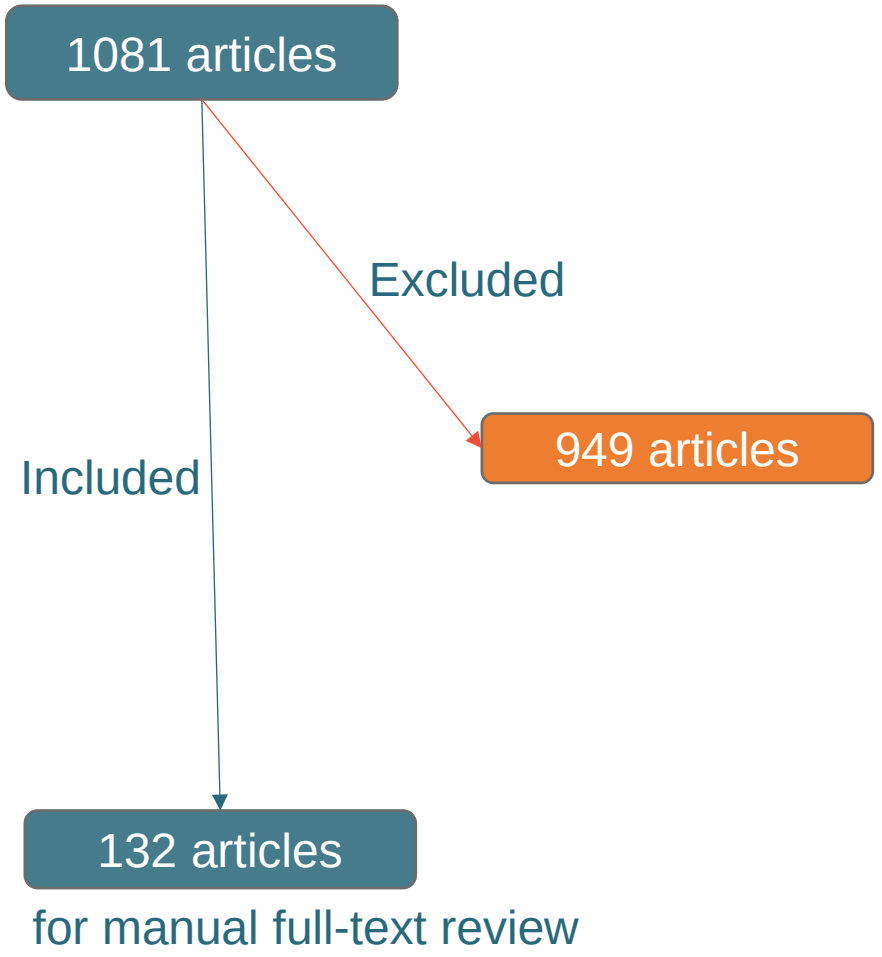
Step 1: Performed search using query. Returned 1081 articles

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

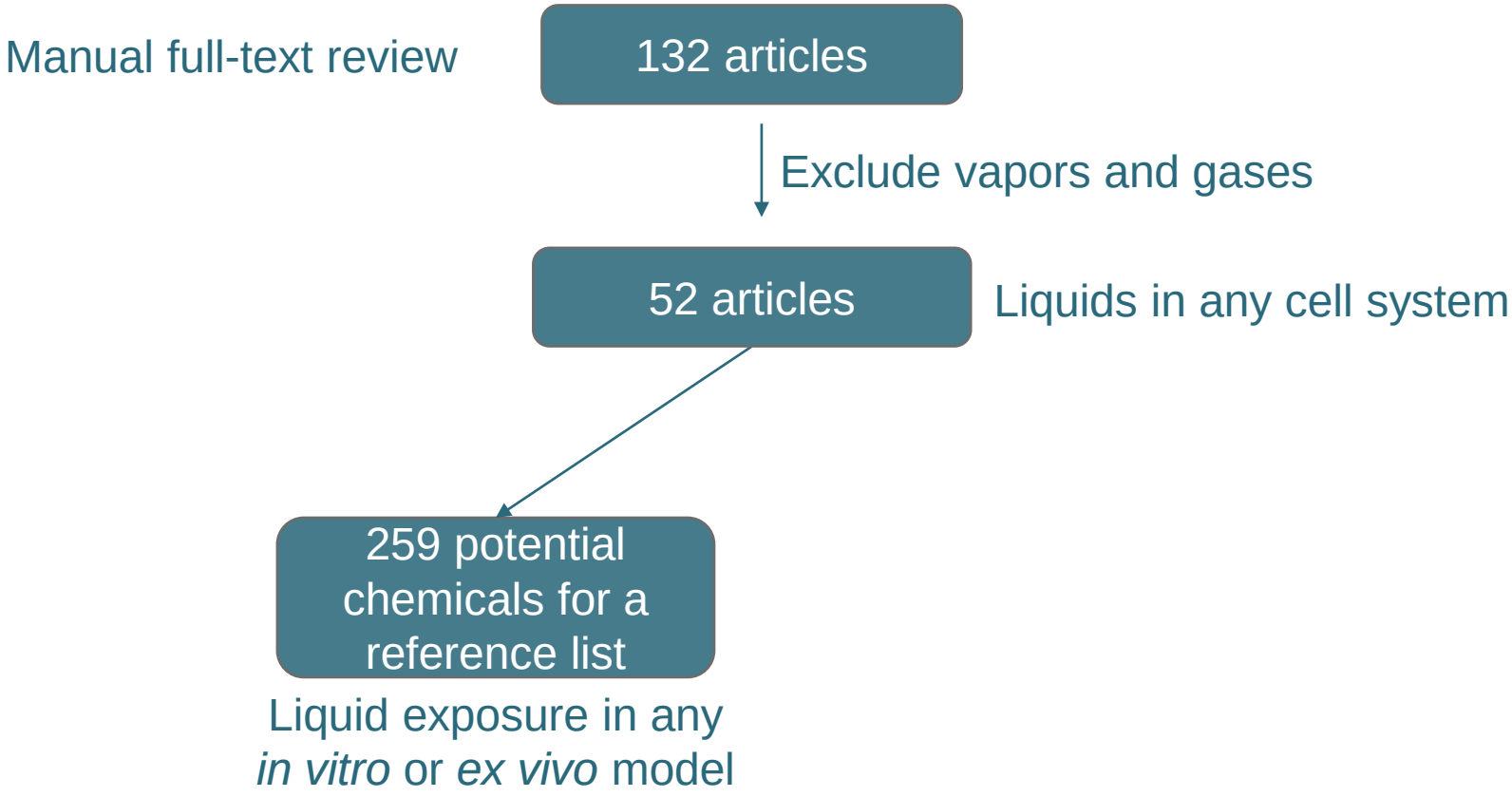
- Review articles with no underlying data
- Papers without any *in vitro* or *ex vivo* data
- Papers only testing solid aerosols/particles
- Articles without cellular toxicity information

(e.g., effects on viral load or drug transport studies)

Step 3: Returned 132 articles for full text review



Extracting relevant information



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

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Extracting relevant information

Manual full-text review

132 articles

Exclude vapors and gases

52 articles

Liquids in any cell system

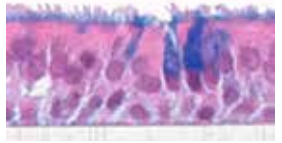
259 potential chemicals for a reference list

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

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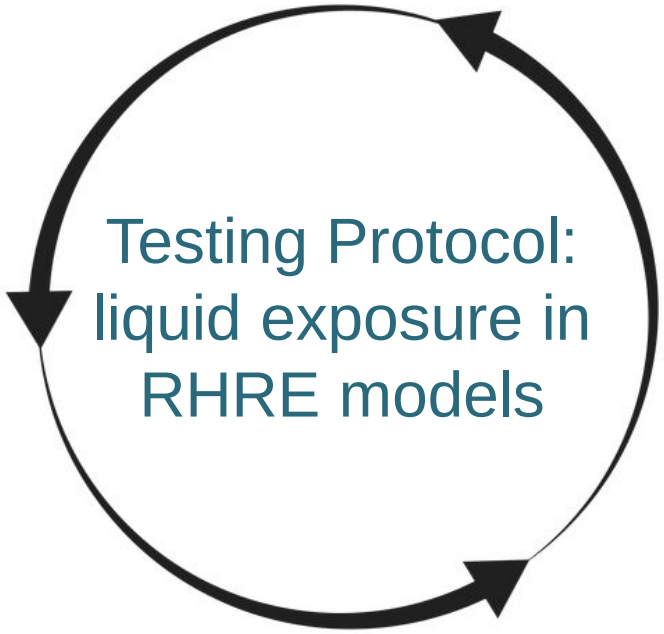

- Starting point: Protocol developed under “The INSPIRE Initiative”
- 20 papers with relevant study designs found in the literature review
- Unpublished test protocols, best practices, and lessons learned e.g., RespTox
- Now sharing the draft protocol with additional experts for input

Scientific Confidence

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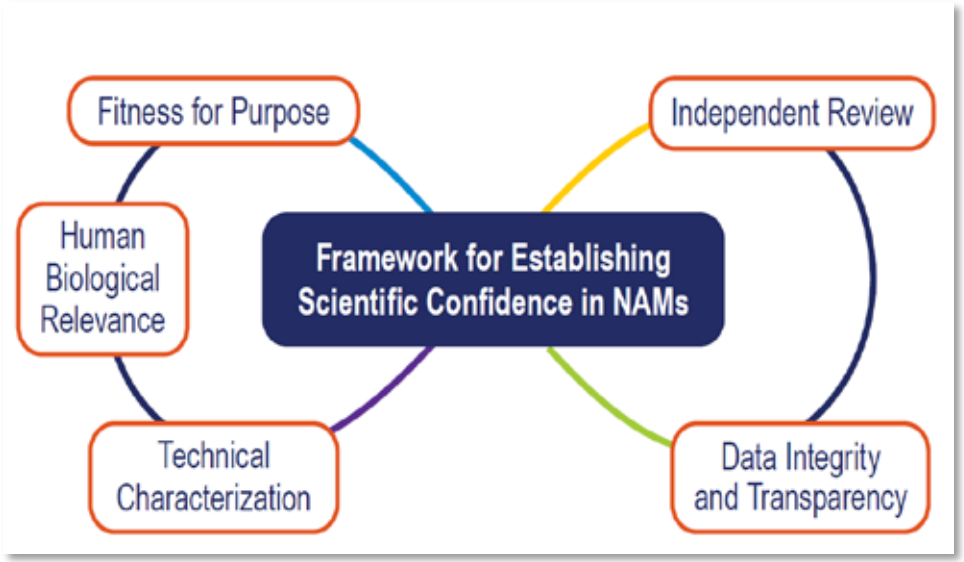
Archives of Toxicology (2022) 96:2865–2879
https://doi.org/10.1007/s00204-022-03365-4

REVIEW ARTICLE

A framework for establishing scientific confidence in new approach methodologies

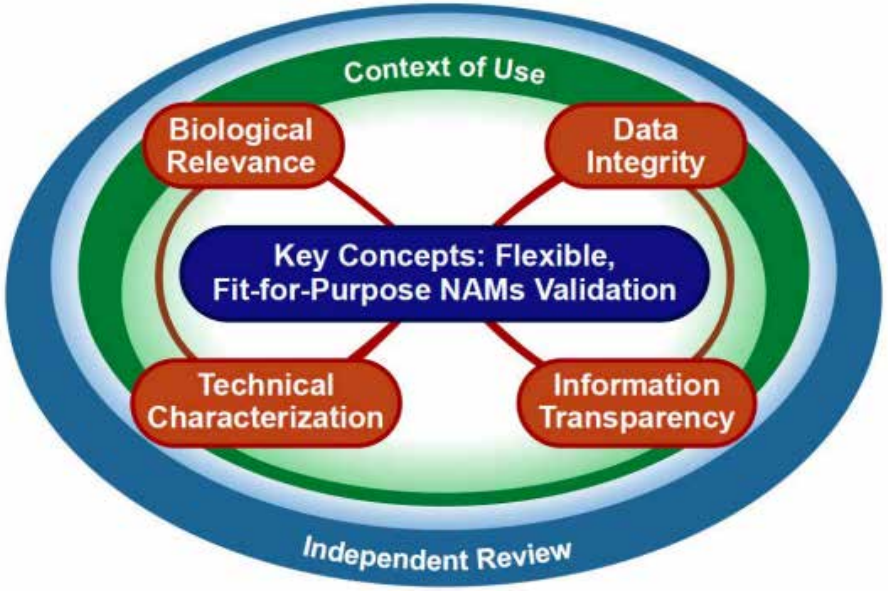
Anna J. van der Zalm[1] · João Barroso[2] · Patience Browne[3] · Warren Casey[4] · John Gordon[5] · Tala R. Henry[6] · Nicole C. Kleinstreuer[7] · Anna B. Lowit[6] · Monique Perron[8] · Amy J. Clippinger[1]

[1] PETA Science Consortium International e.V.
[2] European Commission, Joint Research Center
[3] Organisation for Economic Co-Operation and Development, Hazard Assessment and Pesticides Programmes
[4] National Institutes of Health, DNTP, National Institutes of Environmental Health Sciences
[5] US Consumer Product Safety Commission
[6] US Environmental Protection Agency, Office of Pollution Prevention and Toxics
[7] National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
[8] 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



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Literature Review

- Reference chemicals
- Testing protocol

Metabolic capabilities of respiratory models

Minimizing variability

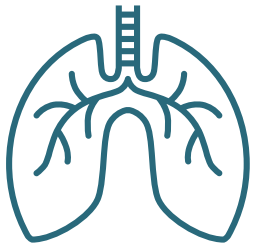
FBS: Chary et al. *Toxicol in Vitro*. 2022.
rAbs for IL-6 & 8 under development.

Reporting standards

Sharma et al. *Arch Toxicol. In Press.*

Gaining scientific confidence

Van der Zalm et al. *Arch Toxicol*. 2022.



OECD TG for assessing portal-of-entry effects of chemicals exposed as liquids to a reconstructed human respiratory epithelial model

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

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