# Integrating Alternative Approaches to Replace Animals in Inhalation Toxicity Testing

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## Introduction

- Inhalation is a major route of chemical exposure for humans. The respiratory tract serves as the portal of entry from the external environment to the systemic circulation.

- Regulations require identification of hazards associated with inhaled substances such as particles, fibers, nanomaterials, reactive gases, and volatile organic chemicals.

- Acute inhalation toxicity tests are designed to identify chemicals that could cause illness or death after a short-term inhalation exposure.

- These tests historically use rats or mice in whole-body or nose-only test systems. However, differences in anatomy and physiology between the rodent and human respiratory tract (shown below) affect the precision with which the rodent test predicts the human response.

- Addressing this issue was a major goal of a workshop on September 22-23, 2016. An international group of experts discussed progress and challenges associated with the development, validation, and implementation of alternatives that could replace animal use for acute inhalation toxicity testing.

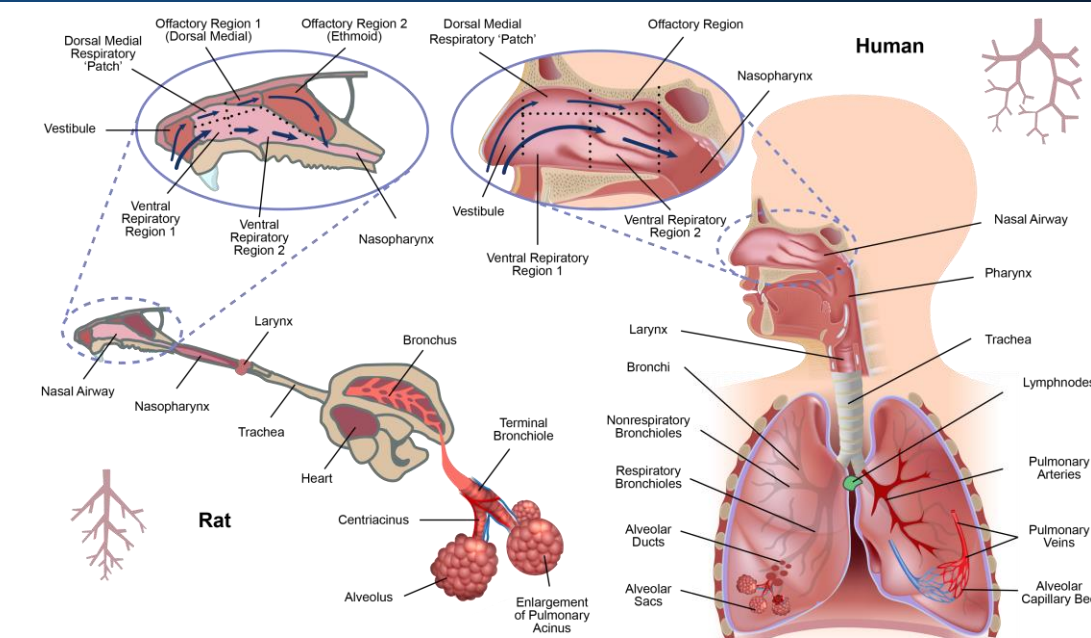## Rodent vs. Human Respiratory Tract



Figure compares anatomy and airflow in the human and rodent respiratory tracts. Illustration adapted from figure by Jack R. Harkema, D.V.M., Ph.D., Department of Pathobiology and Diagnostic Investigation, Michigan State University.

## Webinar Series

- A webinar series preceding the workshop reviewed:
  – Regulatory guidelines: when and how acute systemic toxicity data are used for assessing inhalation toxicity hazard potential
  – Existing alternatives: in vitro and in silico approaches
  – Mechanisms of acute toxicity that may constitute key events in an adverse outcome pathway for acute inhalation toxicity.

## Webinar Series Presentations

| Topic | Speakers |
|---|---|
| Current Testing Practices: Regulatory Requirements and Non-regulatory Testing | Ian Indans, United Kingdom Health and Safety Executive<br><br>Jon Hotchkiss, Ph.D., The Dow Chemical Company |
| State-of-the-science, Practical Application, and Dosimetry Considerations for In Vitro and Ex Vivo Methods | Marianna Gaça, Ph.D., British American Tobacco<br><br>Annie Jarabek, Ph.D., National Center for Environmental Assessment, EPA ORD |
| State-of-the-science and Practical Application of In Silico Methods | Grace Patlewicz, Ph.D., National Center for Computational Toxicology, EPA ORD<br><br>Daniel Wilson, Ph.D., The Dow Chemical Company |
| GHS Additivity Approach to Classify Mixtures Based on Ingredient Toxicity | Marco Corvaro, Ph.D., Dow AgroSciences |
| Adverse Outcome Pathways | Mathieu Vinken, Ph.D., Free University of Brussels<br><br>Barbara Buckley, Ph.D., National Center for Environmental Assessment, EPA ORD |
| 21st Century Testing Approaches | Kelly BéruBé, Ph.D., Zoe Prytherch, Ph.D., School of Biosciences, Cardiff University<br><br>Dan Huh, Ph.D., University of Pennsylvania |

EPA ORD, U.S. Environmental Protection Agency Office of Research and Development

Slides and recordings of all webinars are available at http://www.piscltd.org.uk/acute-inhalation-toxicity/.

## Workshop Presentations

| Topic | Speakers |
|---|---|
| The Case for an Integrated Approach to Acute Inhalation Toxicity Testing and Assessment | Jon Hotchkiss, Ph.D., The Dow Chemical Company |
| An Alternative Framework for Acute Toxicity Using Mechanistic In Silico and In Vitro Approaches | Dan Wilson, Ph.D., The Dow Chemical Company |
| An Alternative Approach for Evaluating the Human Health Risk from Exposure to an Irritant Aerosol | Paul Hinderliter, Ph.D., Syngenta |
| Toxicokinetics in Risk Assessment: Evaluation of In Silico Approaches | Michael Bartels, Ph.D., ToxMetrics.com, LLC |
| Assessing Bioavailability and Systemic Delivery of Inhaled Compounds: Current Status and Future Directions | Miyoung Yoon, Ph.D., Scitovation, LLC |
| EPA OPP Regulatory Perspective on Acute Inhalation Toxicity Testing | Anna Lowit, Ph.D., EPA Office of Pesticide Programs |
| EPA OPPT Regulatory Perspective on Acute Inhalation Toxicity Testing | Iris Camacho, Ph.D., EPA Office of Pollution Prevention and Toxics |
| ICCVAM's Vision and Strategy for Acute Toxicity Testing | Grace Patlewicz, Ph.D., National Center for Computational Toxicology, EPA Office of Research and Development |

EPA, U.S. Environmental Protection Agency

Workshop slides are available at http://www.piscltd.org.uk/acute-inhalation-toxicity/.

## Alternative Approaches for Acute Inhalation Toxicity Testing to Address Global Regulatory and Non-regulatory Data Requirements

**September 22-23, 2016**
**Bethesda, MD**

September 22: Bldg. 35 Porter Neuroscience Center, NIH campus
September 23: Hilton Garden Inn Washington DC/Bethesda

More than 40 scientists from international regulatory agencies, academia, nongovernmental organizations, and industry attended the workshop. The workshop steering committee included representatives from the National Toxicology Program Interagency Center on the Validation of Alternative Toxicological Methods (NICEATM), the PETA International Science Consortium Ltd. (PISC), The Dow Chemical Company, Simulations Plus, Inc., the Netherlands Organisation for Applied Scientific Research, and the U.S. Environmental Protection Agency.



Breakout group discussions at the workshop produced four primary recommendations for advancing new approaches for acute inhalation toxicity testing. Working groups were established to address each recommendation.

## Opportunities for Reduction or Replacement Identified at the Workshop

### Waivers

- Mixtures or formulated products that are substantially similar to well-characterized mixtures or products
- Substances that cause severe local irritation and corrosivity
- Substances with low volatility (non-volatile actives not aerosolized or otherwise made inhalable as a gas or vapor)
- Formulations with large particle size

### Additivity

- Classify mixtures based on the toxicity and concentration of their individual components, without conducting new tests
- Base classification on an acute toxicity estimate (ATE) of the mixture, calculated using ATE values for relevant ingredients:

$$\frac{100}{ATE_{mix}} = \sum_{i=1}^{n} \frac{C_i}{ATE_i}$$

$C_i$ = concentration of ingredient i
$ATE_i$ = acute toxicity estimate of ingredient i

### Multiple Path Particle Dosimetry

- Estimate airway particle dosimetry, deposition and clearance

### In Vitro Systems

- Monolayer cultures
- 3D airway epithelium
- Lung-on-a-chip

## Workshop Recommendations and Subsequent Progress



**Working Group 1: Develop a database of existing acute systemic toxicity data**

- Consolidate existing databases and obtain all available data (published and unpublished): currently in progress
- Prioritize an efficient and user-friendly format



**Working Group 2: Prepare a state-of-the-science review on mechanisms and assays for acute inhalation toxicity**

- Identify methods that may be furthest along in the development/validation process, and consider how to integrate these methods into defined approaches
- Manuscript in preparation for submission this fall
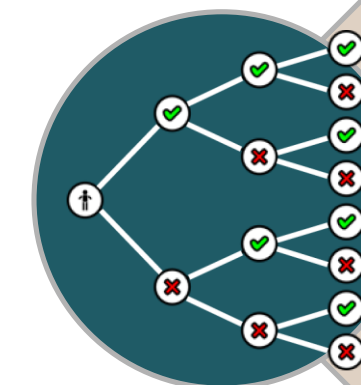


**Working Group 3: Develop an in silico decision tree for determining when acute inhalation testing should be performed**

- The decision tree will inform both the need to conduct testing and what testing approach to follow.
- Multiple tiered models predictive of variables critical to acute inhalation toxicity may be required to inform exposure and dosimetry models to establish whether exposure via the inhalation route is feasible.
- Both continuous variables and classification models will be considered.
- The utility of using chemical reactivity to inform next steps will be evaluated.
- Repeat dose toxicity will also be considered in the context of its usefulness to inform acute toxicity.
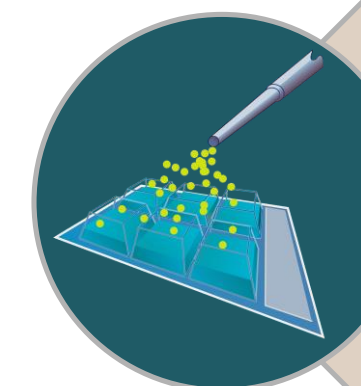


**Working Group 4: Optimize in vitro assays and standardized protocols that can be used across laboratories**

- Study will optimize in vitro assays and standardized protocols.
- The research of other working groups will inform selection of relevant assays and reference chemicals based on availability of curated in vivo data.
- Initial testing will focus on a specific chemical domain (e.g., agrochemical testing for EPA OPP or smoke testing for the U.S. Food and Drug Administration Center for Tobacco Products) or may be based on a specific chemical space, such as oxidant irritants or volatile organic compounds.
- Awaiting completion of the review article to further define study goals and details.

## Summary

- Currently accepted guidelines for inhalation toxicity tests share core principles. These principles inform essential testing needs to be addressed by alternative approaches and opportunities for existing information to enable waivers of required testing.

- A variety of available alternative test methods can reliably identify potential cytotoxicants, but none can single-handedly assess the multiple mechanisms of acute systemic toxicity following inhalation exposure.

- Integrated approaches to testing and assessment will be needed to address the breadth of different mechanisms, ensure good coverage of the relevant chemical landscape, and leverage the collective strengths of the most promising testing methods and non-testing approaches.

- To realize success, input will be needed from industrial sectors, academic disciplines, federal agencies, stakeholder organizations, and international partners.

## Acknowledgements

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