



Evaluation of a Proposed Approach to Refine the Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology (NAM)

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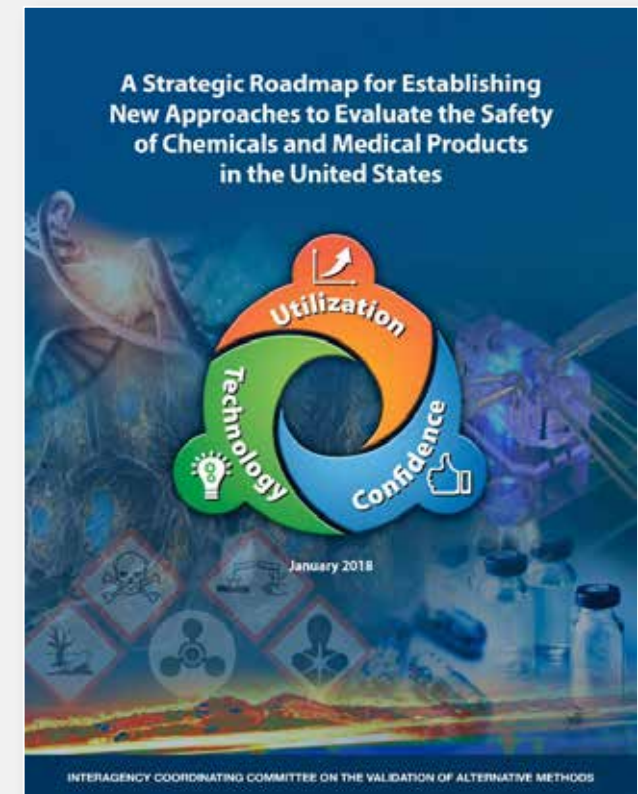
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ICCVAM Strategic Roadmap



- Connect end users with the developers of NAMs
- Foster use of efficient, flexible, and robust practices to establish confidence in new methods
- Encourage adoption and use of new methods and approaches by federal agencies and regulated industries

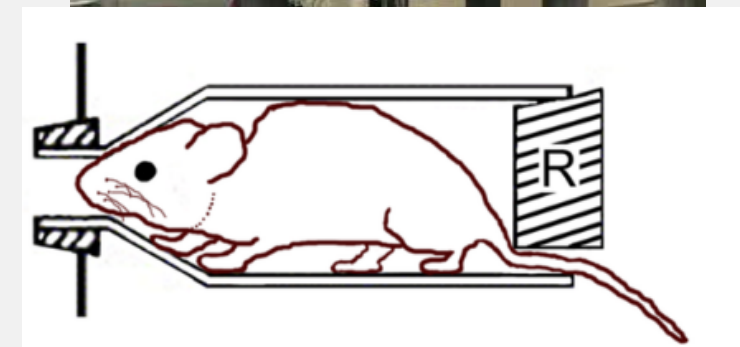


Inhalation Toxicity Studies

- Toxicological studies provide information on wide range of adverse health outcomes, routes of exposure, duration, species, and lifestages
 - Statutory requirements differ between FIFRA and TSCA
- EPA test guidelines specify Agency-recommended methods to generate data
 - Harmonized with Organisation for Economic Co-operation and Development (OECD)
- Inhalation test guideline requirements under OPPTS 870.3465, 40 CFR Part 798, OECD TG 412, and OECD TG 413

Inhalation Toxicity Studies

- Several groups of experimental animals (rat preferred) exposed to graduated concentrations of test substance
 - 10 animals/sex/test group
- Exposure as gas, vapor, or aerosol
- Observed daily for clinical signs
- Sacrificed and necropsied
- Histopathological examinations, including respiratory tract
- Satellite group may also be included to evaluate reversibility, persistence, or delayed occurrence of effects post-treatment
 - At least control and high concentration (10 animals/sex/test group)
- Determine lowest concentration where adverse effects are observed (LOAEC) and highest concentration at which no adverse effects observed (NOAEC)



Office Of Pesticide Programs



- Guiding Principles for Data Needs for Pesticides
 - Purpose: provide consistency in the identification of data needs, promote and optimize full use of existing knowledge, and focus on the critical data needed for risk assessment.
 - <https://www.epa.gov/pesticide-registration/guiding-principles-data-requirements>
 - “...ensure there is sufficient information to reliably support registration decisions that are protective of public health and the environment while avoiding the generation and evaluation of data that does not materially influence the scientific certainty of a regulatory decision....”
 - “...avoid unnecessary use of time and resources, data generation costs, and animal testing.”
- Flexibility in implementing Part 158 data requirements (§158.30):
 - *Waivers* may be granted as permitted by 40 CFR Part 158.45;
 - Additional data beyond the 158 data requirements may be important to the risk management decision (§158.75), *alternative approaches* can be accepted, and other data can be used.

Office of Pollution Prevention and Toxics (OPPT)

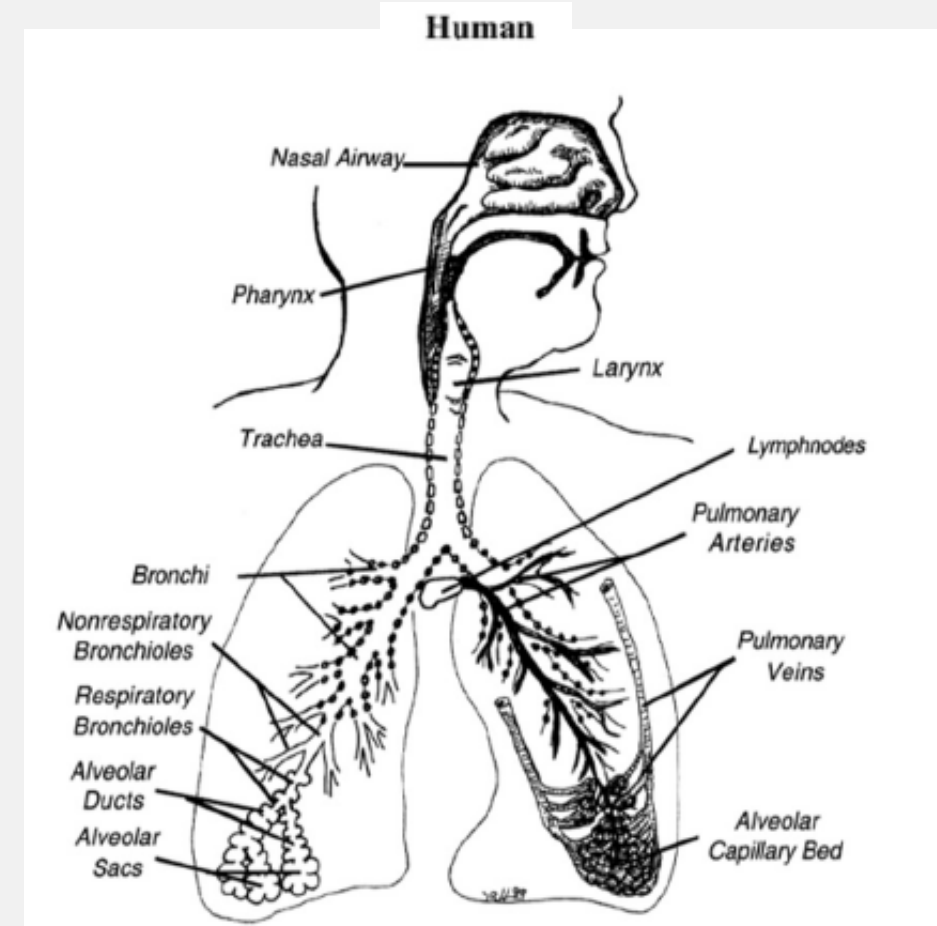
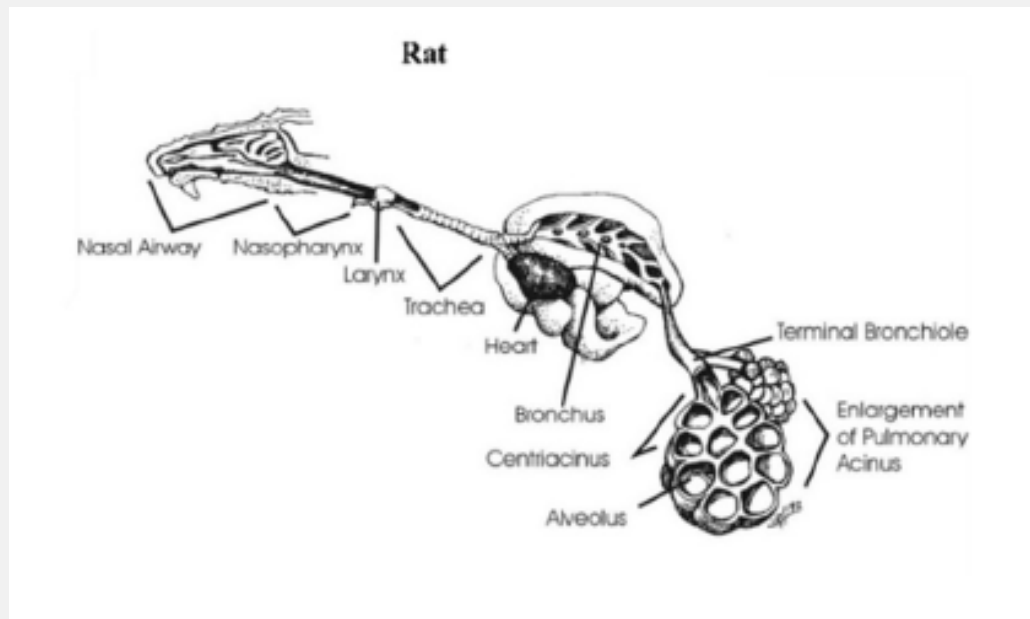


- The Frank R. Lautenberg Chemical Safety for the 21st Century Act (signed June 2016)
 - Section 4(h)(1): *“The Administrator shall reduce and replace, to the extent practicable, scientifically justified, and consistent with the policies of this title, the use of vertebrate animals in the testing of chemical substances or mixtures...”*
 - Development of a strategic plan to promote development and implementation of alternative test methods and strategies within TSCA
 - Collaborated with other EPA programs, including OPP, and also sought and received input from other federal agencies (via ICCVAM) as well as other stakeholders
 - Section 4(h)(2)(A) of TSCA: *“...develop a strategic plan to promote the development and implementation of alternative test methods and strategies to reduce, refine, or replace vertebrate animal testing and provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment...”*
 - <https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/alternative-test-methods-and-strategies-reduce>

Rat vs Human Respiratory Tract

Differences lead to changes in airflow and deposition of inhaled substances

- Airway size and surface area
- Nasal turbinate systems
- Branching patterns
- Cell composition/distribution of surface epithelium
- Anatomy of larynx



Challenges associated with irritants



- Traditional *in vivo* inhalation studies are resource intensive in terms of animal use, expense, and time
- Respiratory irritants can elicit damage at very low doses
 - Clear no observed adverse effect concentration (NOAEC) may not be established
 - Animal welfare concerns
- Efforts to develop new approach methodologies (NAMs)

Case Study Using a NAM to Refine Inhalation Risk Assessment for Point of Contact Toxicity



- Submitted by Syngenta Crop Protection, one of the registrants for products containing the contact irritant chlorothalonil
- Proposal for refining inhalation risk assessment using a three-dimensional *in vitro* model to derive a point of departure
 - Initially presented to EPA in 2014
- Agency recognized the value of the proposal for chlorothalonil, as well as other respiratory contact irritants and encouraged further development

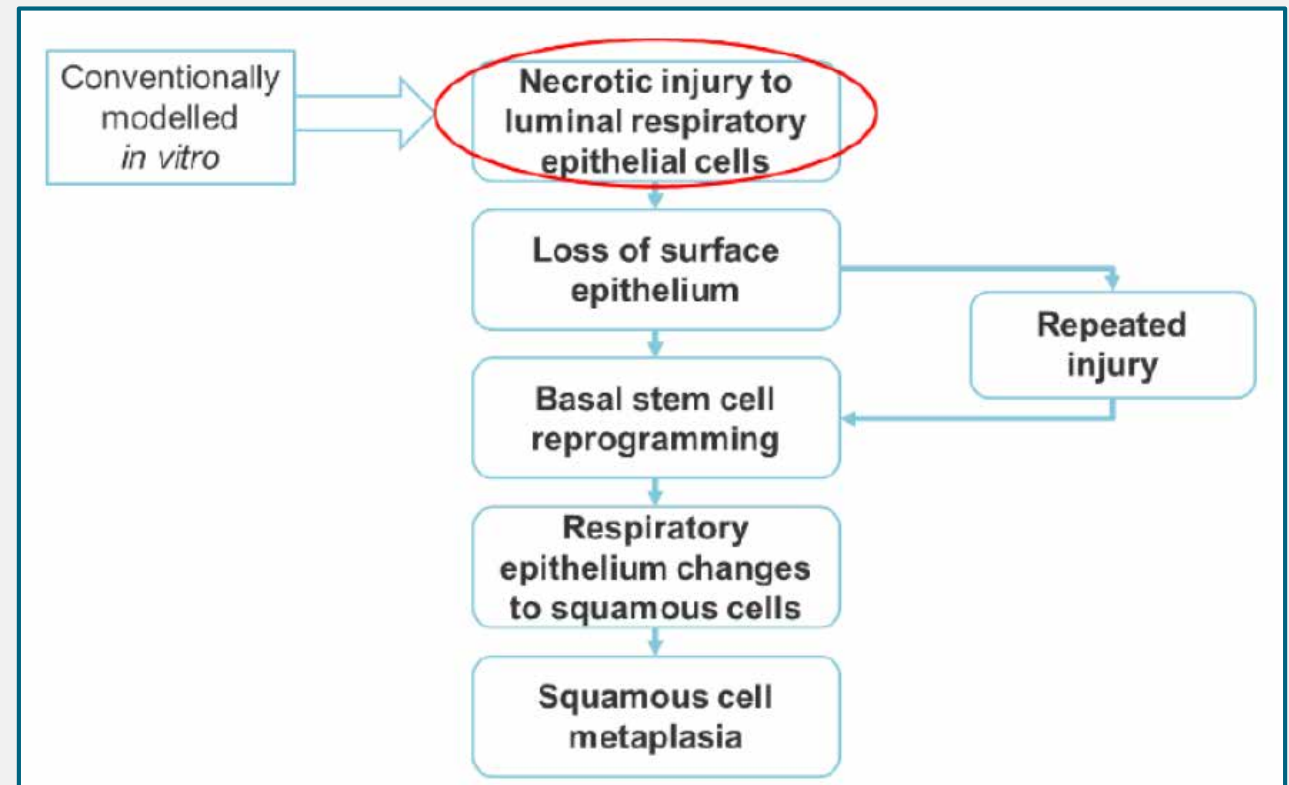
Case Study Using a NAM to Refine Inhalation Risk Assessment for Point of Contact Toxicity



- Continuous communication with Syngenta during development of the proposed approach
- Collaborated with NICEATM early in the process for review of the proposed approach
- Involvement from Office of Pollution Prevention and Toxics (OPPT)

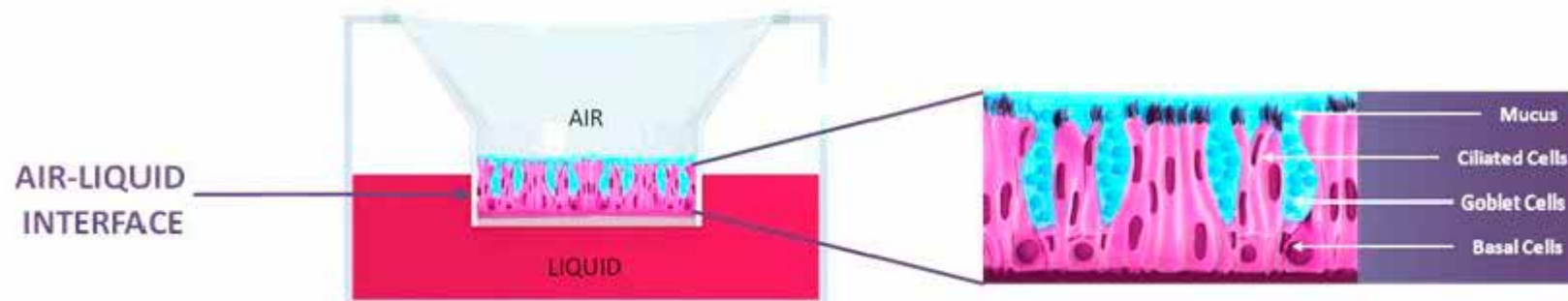
Outcome

- Epithelial cell damage occurs from initial inhalation exposure to chlorothalonil and causes cell death
- Following repeated exposure, cell death results in metaplastic response and transformation of respiratory epithelium into stratified squamous epithelium
- Sufficient amount of chlorothalonil is needed at the cell surface to result in cell death in pathway



Outcome

- Syngenta considered available *in vitro* models for assessing damage to respiratory epithelial cells and identified MucilAir™ as optimal model
- MucilAir™ is a three-dimensional *in vitro* test system derived from human epithelial cells from nasal, tracheal, or bronchial tissues
 - Proposed approach used nasal tissue since it was the only model available at the time and cellular composition is similar to tracheal and bronchial epithelia (i.e., similar responses expected across tissue types for evaluating cell damage from irritation)
- Cell damage evaluated using measurements of transepithelial electrical resistance (TEER), resazurin metabolism, and lactate dehydrogenase (LDH).



Dosimetry

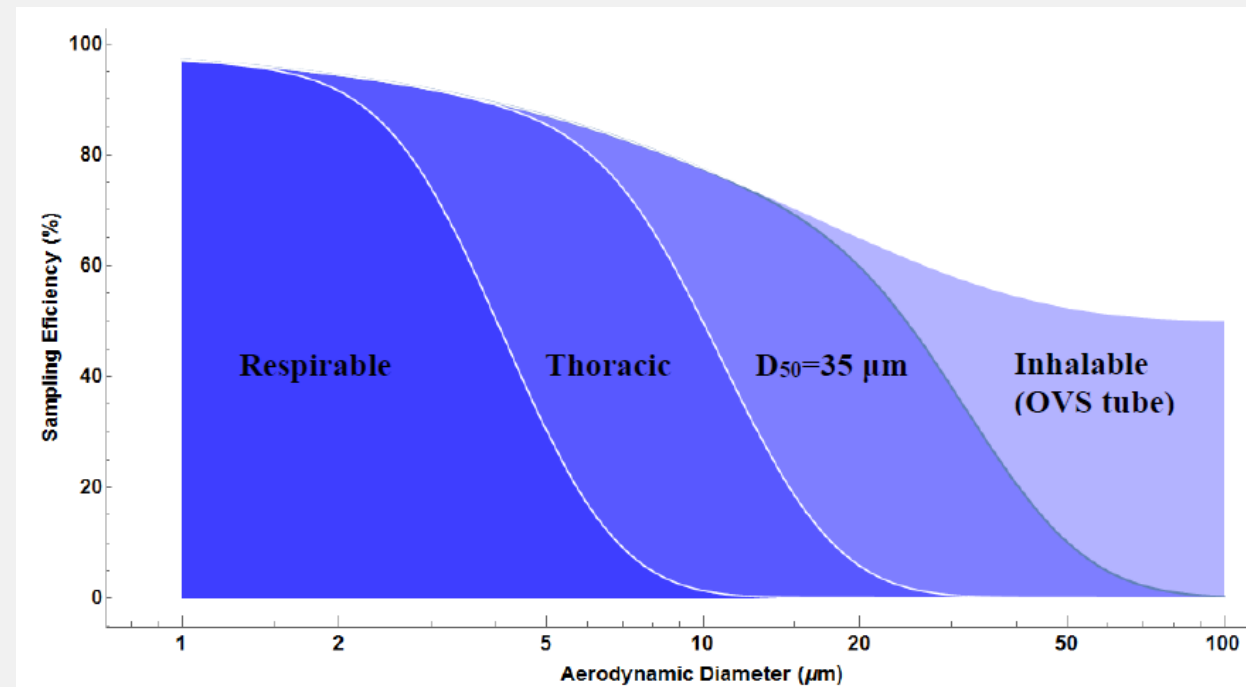


- Site-specific deposition in human upper respiratory tract predicted by computational fluid dynamic (CFD) modeling
- CFD models for upper respiratory tract developed for several species including rats, monkeys, and humans
- Utilize computational mesh based on species-specific anatomical data to develop airflow patterns that are used with boundary conditions, chemical-specific diffusivity, and mass transfer coefficients to predict localized deposition of inhaled material
- Incorporate human relevant particle size distributions (PSDs)

Dosimetry



- For case study, Syngenta mathematically derived human relevant PSD for inhalable particles for spray applicators
- Mathematically defined distributions of the inhalable, thoracic, and respirable size fractions internationally recognized
- Maximum cut-off of 100 μm for particles that are inhalable incorporated
- PSD of adjusted inhalable fraction results in median geometric diameter of 35 μm and geometric standard deviation (GSD) of 1.5



Proposed Inhalation Risk Assessment Utilizing Refined Approach



- Site-specific HECs calculated by integrating dosimetry and outcome results
 - Benchmark dose modeling of *in vitro* data
 - Total Daily deposition calculated from CFD model results
 - Includes calculations to generate polydisperse particle distributions and incorporate relevant exposure duration

Peer Review: FIFRA Scientific Advisory Panel

- December 4-7, 2018
- Charge questions regarding:
 - How the biological understanding informs the applicability of the *in vitro* testing
 - Use of *in vitro* system (study design, methods, selected measurements, robustness of data, data reporting)
 - Assumptions and calculations using CFD model to calculate cumulative deposition
 - Calculation of human equivalent concentrations
 - Strengths and limitations of using approach for other contact irritants, as well as potential for use with other chemicals that cause portal of entry respiratory tract effects

Peer Review: FIFRA Scientific Advisory Panel

- SAP Report released in April 2019
 - <https://www.epa.gov/sap/fifra-scientific-advisory-panel-meetings>
- No panelists supported using laboratory animal study
- In general, panel recommended:
 - Use of most sensitive endpoint
 - Repeat dosing with in vitro assay
 - Consideration of several parameter assumptions for CFD model
 - Particle size distributions based on empirical data
 - Additional clarifications and/or request for supporting information

Continued Communication Post-SAP



- Following SAP, EPA has continued to work with Syngenta to address panel recommendations
- Hold regular calls with Syngenta to obtain status updates and further discuss HEC calculations
- Meeting held in June 2019 to discuss potential path forward for particle size distributions with EPA staff, registrants, consultants, and other experts

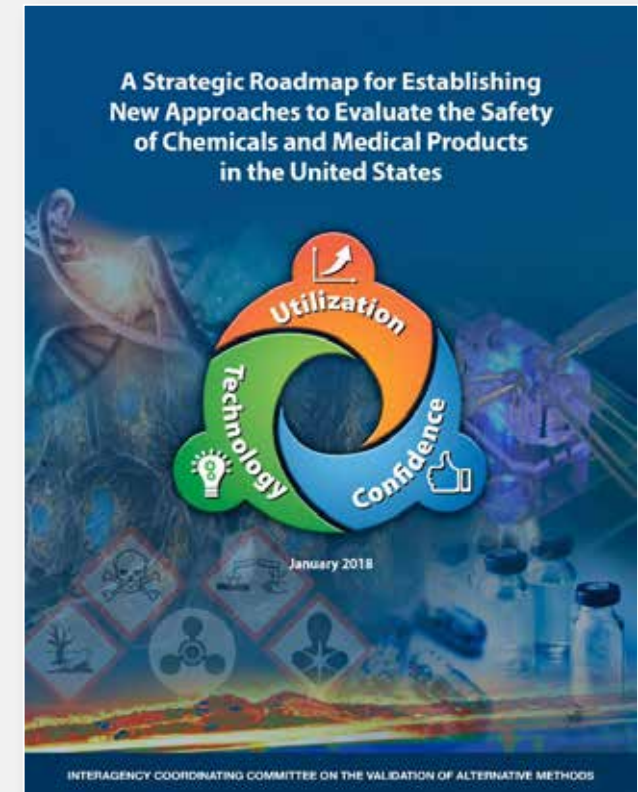
Continued Progress for Inhalation Alternatives

- ORD Research Project
 - Proof-of-concept study exposing commercially available 3D human lung culture models and 2D human primary bronchial epithelial cells at air-liquid interface
 - Chemicals nominated by OCSPP, including known irritants
 - Evaluation of transcriptomic data and functional biomarkers (cell viability, barrier integrity)
- NIEHS Phase 2B grant:
 - Validation of a human airway epithelial model for identifying acute toxicity
 - Steering committee comprised of ICCVAM agencies (NIEHS, DoD, EPA, CPSC)
 - Lead laboratory (Mattek) and 2 additional laboratories to assess relevance and reliability
- PETA-ISC: Case study with a phased approach using increasingly more complex *in vitro* systems using silanes
- SOT Ancillary Meeting: Discussion of the state of the science and potential for refinement/replacement of *in vivo* inhalation study for other chemicals

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EPA Administrator Memo Prioritizing Efforts to Reduce Animal Testing, September 10, 2019



- EPA will reduce its requests for, and our funding of, mammal studies by 30 percent by 2025
- EPA will eliminate all mammal study requests and funding by 2035. Any mammal studies requested or funded by the EPA after 2035 will require Administrator approval on a case-by-case basis.
- Form a working group of agency experts in this field who will provide a work plan within six months.
- This plan will include:
 - Validation to ensure that NAMs are equivalent to or better than the animal tests replaced;
 - Demonstration that NAMs are applicable for use in risk assessment and that new decision-making approaches are as protective of human health and the environment as existing approaches;
 - Recognition that statutory and regulatory requirements for animal testing currently exist and that a plan to adopt more flexible requirements should be developed;
 - Outreach to all stakeholders to incorporate their knowledge and address concerns; and
 - Establishment of baselines, measurements and reporting mechanisms to track the agency's progress.
- OCSP and ORD hold a joint annual conference on NAMs for presentations by leading scientists in the NAMs field, with the first conference to be held in 2019.
- <https://www.epa.gov/environmental-topics/administrator-memo-prioritizing-efforts-reduce-animal-testing-september-10-2019>

Thank you!