

INTEGRATED TESTING STRATEGIES FOR ACUTE INHALATION TOXICITY ASSESSMENT

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ABSTRACT

Progress has been made to reduce the numbers of animals used in acute inhalation toxicity tests, particularly for classification and labeling of respiratory hazards. In addition, there exist a plethora of respiratory tissue-specific cell and tissue models, encompassing different cell types, model architectures, and exposure conditions, as well as double and triple co-culture models. However, the development of non-animal models that could assess acute inhalation toxicity has not been pursued as comprehensively as models or strategies for acute oral toxicity, despite the scientific and animal welfare implications of *in vivo* acute inhalation testing. This presentation proposes an Intelligent Testing Strategy, organized according to pulmonary system architecture and using existing models, with the aim of providing coverage of the full potential toxicological sequelae of acute airborne agent exposure. This would include the breadth of different regions of the respiratory system, the diversity of potential toxicities (such as tissue damage or respiratory sensitization,) and the consequences of systemic migration of the molecule or particle. Because sufficient data were not available from the literature to complete such a simulation, we present two hypothetical "test case" agents, and use theoretical agent-specific characteristics to illustrate the flexibility of this strategy. Moving ahead to further develop this testing strategy and the models it uses will require a coordinated effort from all stakeholders.

METHODS

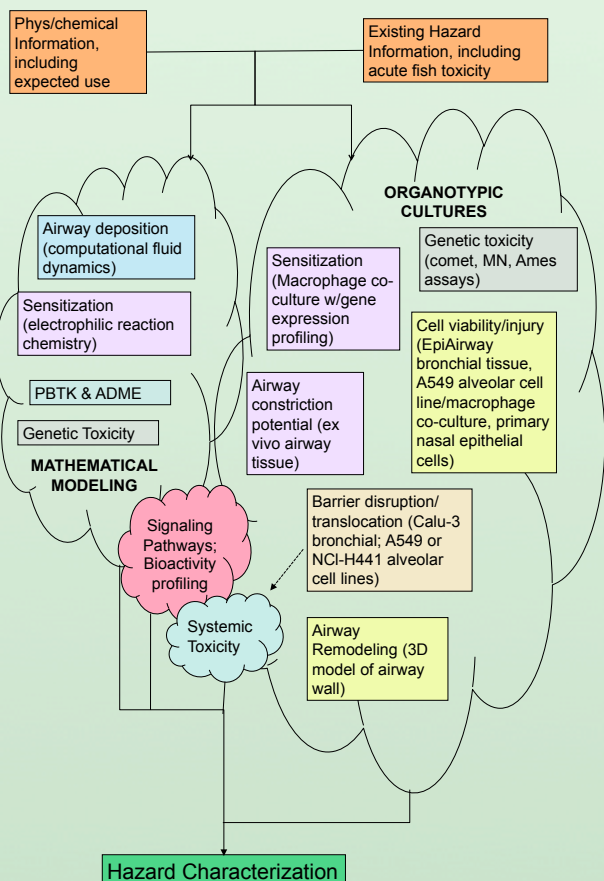
Tissue Damage <ul style="list-style-type: none"> •Irritation or corrosion •Cell death •Mucus/surfactant alterations •Olfactory dysfunctions •Decreased elasticity/scar formation 	Barrier properties <ul style="list-style-type: none"> •Functional alterations •Translocation 	System-wide Considerations <ul style="list-style-type: none"> •Chemical properties •Airway deposition •ADME
Genetic Toxicity <ul style="list-style-type: none"> •Mutagenicity & Chromosomal Damage 	Reactivity <ul style="list-style-type: none"> •Bronchoconstriction •Sensitization •Immune function 	

To successfully model potential acute pulmonary toxicity without animals, Integrated Testing Strategies should encompass several key toxicity endpoints (above, adapted from previous work at ECVAM and ICCVAM). Each of the endpoints can be explored using non-animal models currently in various stages of maturity; chemical-specific information completes a weight-of-evidence assessment approach. Models or test systems specified were chosen from review of the literature and with an attempt to balance maturity, scientific adequacy, ease of use and familiarity; other comparable systems could be substituted if appropriate.

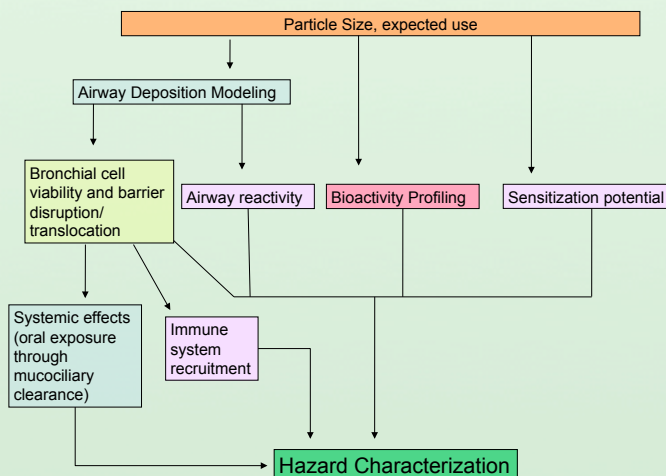
A literature review reveals a data-driven preference for *in vitro* models that are derived from human cells and tissues, are grown and used at the air/liquid interface, and provide greater physiological relevance through the use of 3-D architecture or co-cultures. Optimal exposure devices differ; the selected device should realistically mimic particle deposition and/or vapor flow.

The Testing Strategy is flexible depending on the chemical and the data obtained. Two hypothetical agents, a particle and a vapor, were chosen to show how such a strategy might be used.

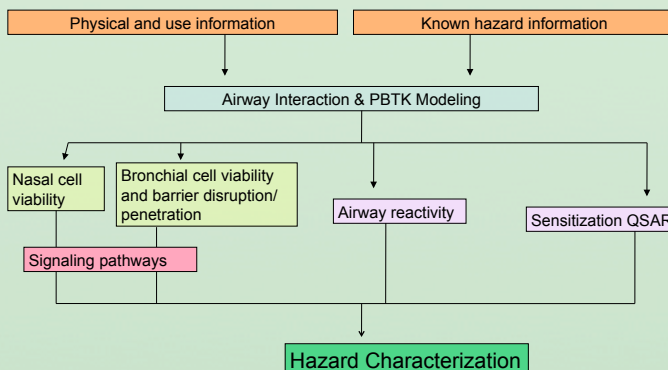
GENERAL STRATEGY



1-5 µm PARTICLE



SOLUBLE VAPOR



DISCUSSION/CONCLUSION

- + A flexible, chemical-specific testing strategy has been proposed from *in vitro* and *in silico* models currently under laboratory investigation.
- + From a regulatory standpoint, components of this strategy could be used for screening and/or initial hazard characterization, depending on the chemical in question. Bioactivity profiling and *in vitro* screens for tissue damage can be used now to prioritize classes or categories of chemicals, such as inhaled nanoparticles or low production industrial chemicals, or to characterize the toxicity of some chemicals.
- + It may be possible to assess a combination of endpoints in one model; here, we combined cell viability and barrier disruption and translocation in one assay.
- + A validation project is ongoing for A549 cells, testing for cell damage endpoints, at ZEBET. Other models are also very promising, but lack specificity testing. To date most are challenged with known entities such as cigarette smoke, diesel exhaust, or common vapors.
- + Baseline toxicity (or non-specific toxicity) should be a primary consideration for industrial chemicals; under this theory, acute fish toxicity and solubility could inform inhalation toxicity. A recently-published QSAR model uses electrophilic activity to screen for respiratory sensitization.
- + Emerging models include A549 microscale cultures, such as Microlung (Cardiff University) and another perfused model (Johns Hopkins University).
- + Techniques to ensure optimum exposure conditions (air flow, particle deposition, etc.) should be standardized.
- + For efficient progress, a coordinating committee should be convened, composed of industry, academic, and regulatory stakeholders with traditional *in vitro* and *in vivo* disciplines, modeling (both SAR and virtual anatomy), database, and high-throughput expertise.