Aerosol Application for Prediction of Respiratory Toxicity using a Human Airway Model

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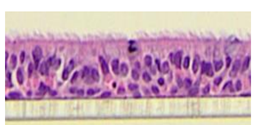
1 BACKGROUND & PURPOSE

Traditional in vivo inhalation toxicology studies are costly, require large numbers of animals, and often fail to translate effectively to human outcomes due to anatomical and physiological disparities between animal and human respiratory systems. Consequently, there is growing interest in human-relevant in vitro models for assessing inhalation toxicity. Human lung organotypic cultures are currently being used to assess direct toxicity of inhaled chemicals in a case-by-case basis. However, the absence of an OECD guideline limits their broader regulatory adoption. Establishing such a guideline would provide a standardized framework and promote regulatory acceptance of these models..



Differentiated 3D human airway tissue model Human EpiAirway at 200x magnification.

The objective of this project is to develop an in vitro inhalation toxicity screening protocol for risk-based decision making. We show that an EpiAirway™ protocol has the potential to allow prediction of human hazard classifications for acute inhalation risk by applying chemicals directly to the epithelial surface at the air liquid interface in solution or suspension. We also re-evaluate a subset of chemicals using aerosol exposure to determine whether the mode of application influences the resulting toxicity profiles.

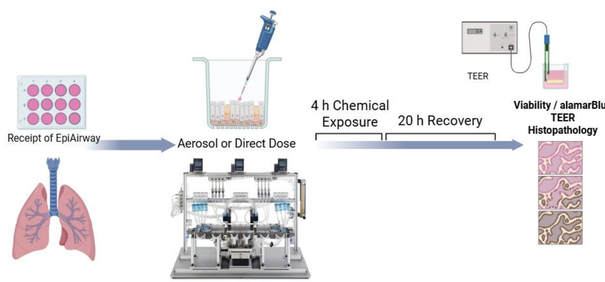
2 METHODS

A panel of ten chemicals with known inhalation point-of-contact toxicity, spanning a range of toxicities and mechanisms of action, were tested using the 3D human EpiAirway™ tissue model. Acute exposures were performed by applying the test materials directly to the epithelial surface for 4 h, followed by a 20 h recovery period, according to a harmonized protocol conducted in parallel at Charles River Laboratories (CRL) and MatTek. A subset of 4 chemicals were subsequently tested using aerosol conditions with a Vitrocell 12/12 Continuous Flow Exposure System (CRL only). Flow rates, temperatures and humidity of aerosols were closely regulated and continuously monitored. Barrier integrity (TEER), cell viability (alamarBlue) and morphology changes (H&E staining) were used as endpoint measurements. The drc package R was used to curve fit data, calculate EC10 values (the dose required to reduce a response by 10%), and compare results from the 2 laboratories.

Morphology: semi quantitative microscopic evaluation was performed for reduced numbers of cell layers, loss of surface cilia, erosion; intercellular separation, single cell degeneration, locally extensive necrosis, and squamous metaplasia. A composite median severity score was calculated for each test condition. Image analysis algorithms were developed in Visiopharm® to quantify decreased cell layer thickness, loss of cilia, intercellular separation and degenerating cells.

Chemical	UN GHS Acute Inhalation Hazard Classification*
Isophorone diisocyanate	1: H330
Potassium dichromate	2: H330
Methylisothiazolinone	2: H330
Benzalkonium chloride	2: (US EPA)
2-Butyne-1,4-diol	3: H331
1,3-bis(aminomethyl) benzene	4: H332
Trimellitic anhydride	4: H332
Silica, fumed	5: Not Hazardous
Lactose	5: Not Hazardous
Propylene glycol	5: Not Hazardous

* Pubchem/ECHA/MSDS



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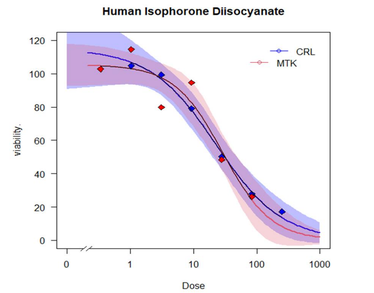
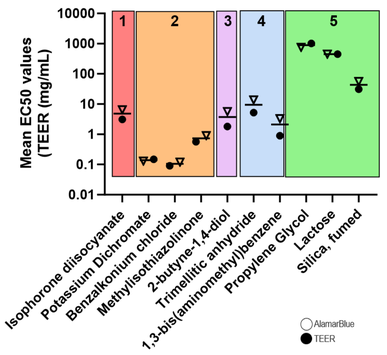
3 RESULTS

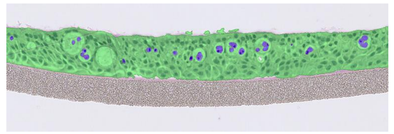




Comparable results were obtained between laboratories with p values of >0.05 and <2.5-fold difference denoting no biological relevance of differences. EC_{10} and EC_{50} generated from direct application to EpiAirway™ successfully ranked the chemicals in order of UN GHS inhalation hazard. Compounds producing EC_{10} < 5 mg/mL for TEER or viability endpoints (e.g., potassium dichromate) corresponded to the highest hazard categories, whereas chemicals with EC_{10} values exceeding the maximum testable concentration (e.g., lactose) were classified as non-hazardous.

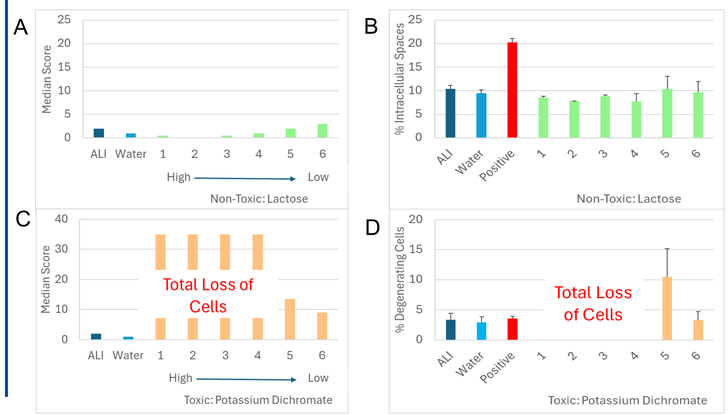
Left Above: Curve fits for CRL (blue) and MTK (red) showing inter-lab comparison for an example data set (human EpiAirway alamarBlue viability following Isophorone diisocyanate treatment)
Left Below: Mean EC_{50} for alamarBlue viability and TEER predict UN GHS hazard categories (1-5) for inhalation risk

4 MORPHOLOGY CHANGE

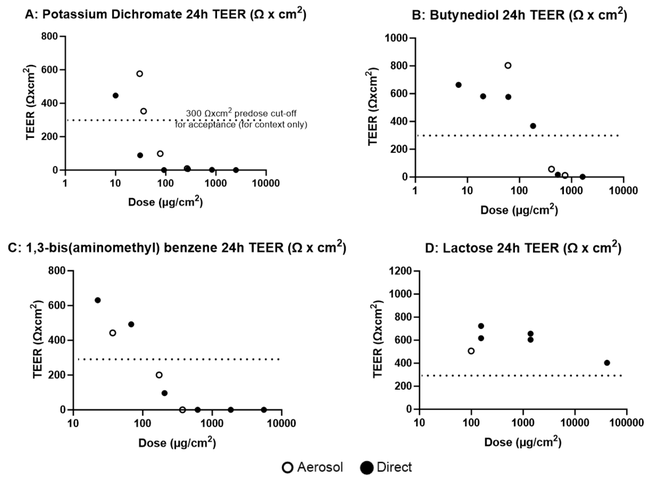


Above: Image analysis algorithms were developed to quantify morphology change. Green = epithelial layer, blue = degenerating cells, grey/granular = plastic membrane.
Below: Morphology change assessed by pathologist evaluation (A+C) and Visiopharm quantitative analysis (B+D) for example non-toxic (Lactose, A+B) and toxic (potassium dichromate, C+D) chemicals.

Samples exhibiting TEER < 50 Ωxcm² generally lacked sufficient structural integrity for histological analysis. For analyzable tissues, concentration-dependent morphological alterations were evident for both labs with both assessment methods. Note that severe injury resulting in loss of cells resulting a high composite severity score in the path eval but no score on the image analysis side as there were few remaining cells to analyse.



5 COMPARISON OF AEROSOL VS DIRECT APPLICATION



Aerosol application of vehicle (humidified air) did not significantly alter TEER or viability compared to incubator controls (P>0.05: one way ANOVA, not shown). Furthermore, when normalized to surface area dose (µg/cm²), the proof-of-concept dose–response relationships obtained from aerosol exposures closely aligned with those from direct applications for TEER (shown above) and alamarBlue viability (not shown) for three of the 4 chemicals (B to D above). The exception is potassium dichromate for which very similar estimated aerosol delivery of 36 µg/cm² and liquid delivery of 31 µg/cm² resulted in viable and non-viable TEER results, respectively. We are currently investigating this further.

6 CONCLUSIONS & NEXT STEPS

These findings demonstrate that this tiered in vitro testing strategy shows strong potential for predicting human acute inhalation hazard classifications. The approach represents a key step toward developing a predictive, risk-based testing framework for aerosolized chemicals.

Work is ongoing to further investigate dose responses following both liquid and aerosol application. This will allow better understanding of the effect of dose application route at dose levels that will be used to help inform safe exposure levels and suggest, if any, safety factors that may need to be included for liquid application.

The broader research program aims to establish an integrated in vitro protocol capable of supporting regulatory decision-making for inhalation risk assessment. Ongoing and future work include in silico modelling for in vitro to in vivo extrapolation, comparison of different donors, further work to clarify the relationship between aerosol and direct application dosimetry, and testing of additional chemicals to verify the prediction model.

This initiative directly supports the global regulatory objective of replacing animal testing with scientifically robust, translatable, and cost-efficient alternative methods.

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C.O.I.: SA, KG and MK are employees of MatTek Corporation. JW, BS, AM, JB and MM are employees of Charles River Laboratories.