NAM Based Prediction of Respiratory Toxicity using Human and Rat Airway Models

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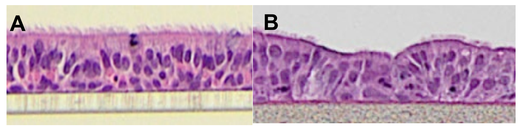
1 BACKGROUND & PURPOSE

Human lung organotypic cultures are currently used to assess direct toxicity of inhaled chemicals (OECD, 2022 and US EPA, 2023: reviewed in Wallace *et al.* 2025), however exact protocols are yet to be described in an OECD guideline. To work towards generating this guideline and work within a tiered risk assessment (Andersen *et al.* 2019), we evaluated the toxicity of a panel of chemicals with known point-of-contact inhalation toxicity in the lung.



Human EpiAirway (A) and rat EpiAirway (B). 200x magnification.

We developed a standard protocol for acute inhalation risk assessment and evaluated reproducibility and predictivity in two laboratories (Charles River, UK and MatTek, USA). We determined the EC25 values in 2D monolayer (Normal Human Bronchial Epithelial Cells: NHBEC), 3D human airway models (EpiAirway™:donor and passage matched to the 2D monolayer) and a 3D rat airway epithelial model (rat EpiAirway™), across both laboratories using this harmonized protocol and SOPs.

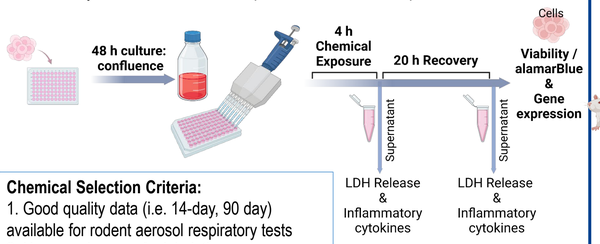
2 METHODS

10 chemicals were assessed in 2D monolayers and differentiated human (AIR-100-Day20) and rat (AIR-100-R) EpiAirway 3D tissue models. The assessed chemicals covered all human acute inhalation UN GHS hazard categories (right). Chemical exposure was performed in two labs (CRL & MTK) using a harmonized protocol. Each chemical/concentration was tested in each model at (n=4, 6 concentrations) by direct (liquid) application to the apical/epithelial surface of the tissue for 4 h, then 20h recovery. Membrane integrity and cell viability were assessed using transepithelial electrical resistance (TEER: 3D) and alamarBlue (all). The EC25 of each chemical was calculated by interpolation using the concentrations which straddled 25% reduction in viability compared to the vehicle control.

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

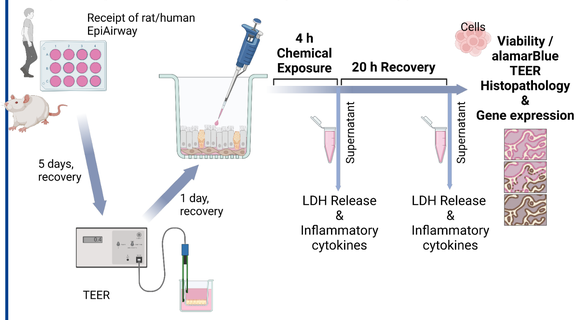
* Pubchem/ECHA/MSDS

2D Monolayer Human Bronchial Epithelial Cells - 4 h Exposure



Chemical Selection Criteria:
1. Good quality data (i.e. 14-day, 90 day) available for rodent aerosol respiratory tests
2. Human relevance for inhalation exposure
3. Mode of action for inhaled toxicity includes direct toxicity at the site of action (i.e. the upper airways/nasal)
4. Covering all GHS categories for respiratory toxicity
5. Covering a range of chemicals types and classes

3D EpiAirway Human and Rat - 4 h Exposure, direct application



3 RESULTS

2D-Human NHBEC EC25s (mg/mL)

Chemical (UN GHS acute inhalation classification)	2D-human alamarBlue	
	CRL	MTK
Isophorone diisocyanate (1)	0.03	0.15
Potassium dichromate (2)	0.01	0.01
Methylisothiazolinone (2)	0.03	0.02
Benzalkonium chloride (2)	0.01	0.01
2-Butyne-1,4-diol (3)	17.3	33.1
1,3-bis(aminomethyl) benzene (4)	0.22	0.21
Trimellitic anhydride (4)	1.51	1.52
Silica, fumed (5)	0.11	0.16
Lactose (5)	66.2	55.7
Propylene glycol (5)	37.6	29.0

3D-Human EpiAirway™ EC25s (mg/mL)

Chemical (UN GHS acute inhalation classification)	3D-human alamarBlue		3D-human TEER	
	CRL	MTK	CRL	MTK
Isophorone diisocyanate (1)	11.2	14.8	2.1	1.0
Potassium dichromate (2)	1.5	0.7	0.1	0.1
Methylisothiazolinone (2)	0.5	0.7	0.4	0.5
Benzalkonium chloride (2)	0.4	1.0	0.1	0.1
2-Butyne-1,4-diol (3)	2.0	1.5	1.9	0.6
1,3-bis(aminomethyl) benzene (4)	3.5	2.9	1.1	0.4
Trimellitic anhydride (4)	11.7	6.7	3.6	3.9
Silica, fumed (5)	60	2.2	11.7	5.2
Lactose (5)	450	450	450	450
Propylene glycol (5)	1036	1036	1036	501

Above top: 2D human EC25s, Above bottom: 3D human EC25s.
Right top: 3D rat EC25s, Right bottom: hazard class predictions based on EC25.

3D-Rat EpiAirway™ EC25s (mg/mL)

Chemical (UN GHS acute inhalation classification)	3D-rat alamarBlue		3D-rat TEER	
	CRL	MTK	CRL	MTK
Isophorone diisocyanate (1)	83.3	2.5	4.5	3.9
Potassium dichromate (2)	0.7	0.5	0.1	0.1
Methylisothiazolinone (2)	1.5	1.3	0.7	0.6
Benzalkonium chloride (2)	0.5	0.3	0.1	0.1
2-Butyne-1,4-diol (3)	19.6	0.6	5.0	4.0
1,3-bis(aminomethyl) benzene (4)	5.0	0.5	2.1	2.8
Trimellitic anhydride (4)	19.7	11.4	12.8	4.7
Silica, fumed (5)	60	3.1	60	36.3
Lactose (5)	450	450	450	450
Propylene glycol (5)	1036	1036	752.9	289.1

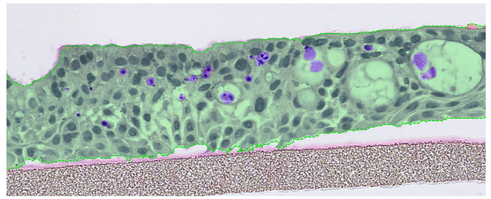
2D-human alamarBlue EC25s were highly reproducible between labs (within 2-fold) for 9/10 chemicals. The exception was isophorone diisocyanate, for which the difference was 5-fold. As expected, 2D-human monolayer cultures were more sensitive than 3D-human tissues for 9/10 chemicals (5 to 300-fold). 2-Butyne-1,4-diol was the only chemical which showed greater sensitivity in 3D tissues than the 2D monolayers (9-fold and 22-fold, respectively at CRL and MTK).

For the 3D-human tissue model, the EC25s for 8-9/10 chemicals were within 2.5-fold of each other when comparing CRL and MTK, indicating reproducibility between labs. 3D-human EC25's generally ranked chemicals in the order of UN GHS acute respiratory hazards (scale of 1 to 5 with 1 being life threatening and 5 being non-toxic, table below). Data from the 3D-rat model was similarly reproducible between labs, with 7 (alamarBlue) and 8 (TEER) EC25s being within 2.5-fold of each other when comparing CRL and MTK. EC25s for the rat model were slightly higher than from the human tissue. See table below for EC25 hazard predictions. For specific chemicals, rat alamarBlue EC25 values were 19x to 30x fold different between labs while TEER values were similar. We will review these data in the context of histology assessment (pending); we suspect the alamarBlue maybe sensitive to interference and will investigate further as required as part of the protocol development.

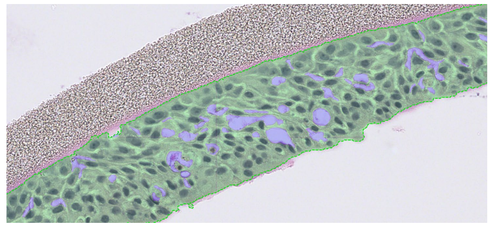
Model	EC25 concentration	Hazard Class Prediction
3D human EC25	≤3 mg/mL	Toxic, Cat 1-3
	>3 mg/mL	Non-toxic, Cat 4
	> highest concentration tested	Non-toxic, Cat 5
3D rat EC25	≤5 mg/mL	Toxic, Cat 1-3
	>5 mg/mL	Non-toxic, Cat 4-5

4 HISTOLOGY NEXT STEPS

Visiopharm machine deep learning algorithms are being developed for quantitative assessment of morphology changes (degenerating cells, intercellular spaces, ciliated surface and tissue thickness) in 3D human and rat EpiAirway.



Above: Overlay for algorithm detecting degenerating cells. Green=tissue, blue/purple=degenerating cells.



Below: Overlay for algorithm detecting intercellular spaces. Green=tissue, blue/purple=intercellular spaces

5 CONCLUSIONS & NEXT STEPS

Initial results demonstrate the general usefulness of the 2D model for setting dose levels for further testing using 3D models. These results demonstrate that reproducibility between labs can be obtained with harmonized protocols and SOPs. The rat model is a useful tool to aid translation between in vivo rodent data and in vitro human outcomes, and will be used to model predictions of in vivo outcomes. Finally, the data show that this EpiAirway protocol has the potential to allow prediction of human hazard classifications for acute inhalation risk.

Ongoing analysis of the current data set includes histopathology review, analysis of a panel of secreted inflammatory cytokines, and changes in gene expression for samples from both labs. This work is also part of a larger program which includes repeat dose responses (14 day), aerosol vs liquid application, modelling in vitro-in vivo extrapolations, and donor differences, with the eventual goal of generating a prediction model for inhalation risk of aerosol chemicals.

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

5 REFERNECES

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