In Vitro Respiratory Model for Acute Inhalation Toxicity Testing

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PETA INTERNATIONAL SCIENCE CONSORTIUM LTD.
Advancing 21st Century Toxicology

MatTek CORPORATION

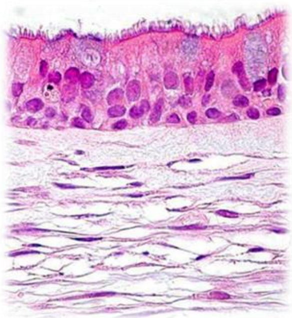
3D *in vitro* respiratory models available from MatTek

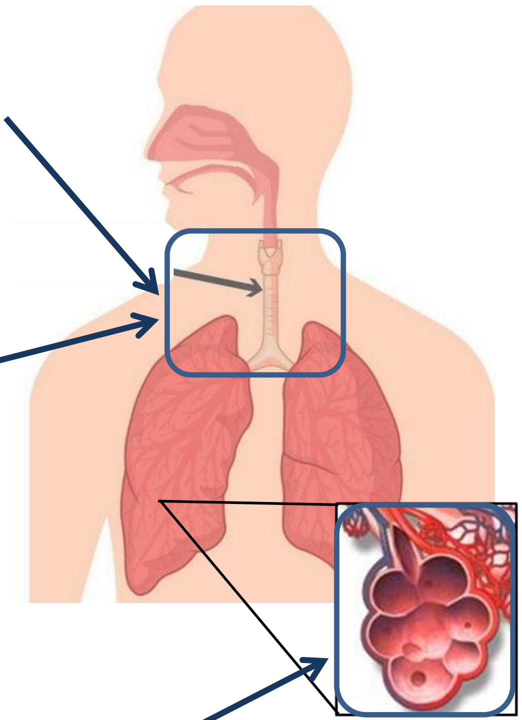
EpiAirway

-Primary tracheobronchial epithelial cells

EpiAirway Full Thickness

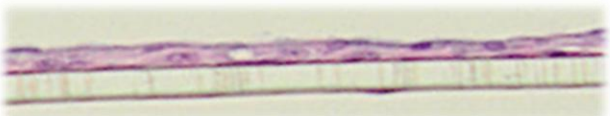
-Primary tracheobronchial epithelial cells & fibroblasts in an extracellular matrix



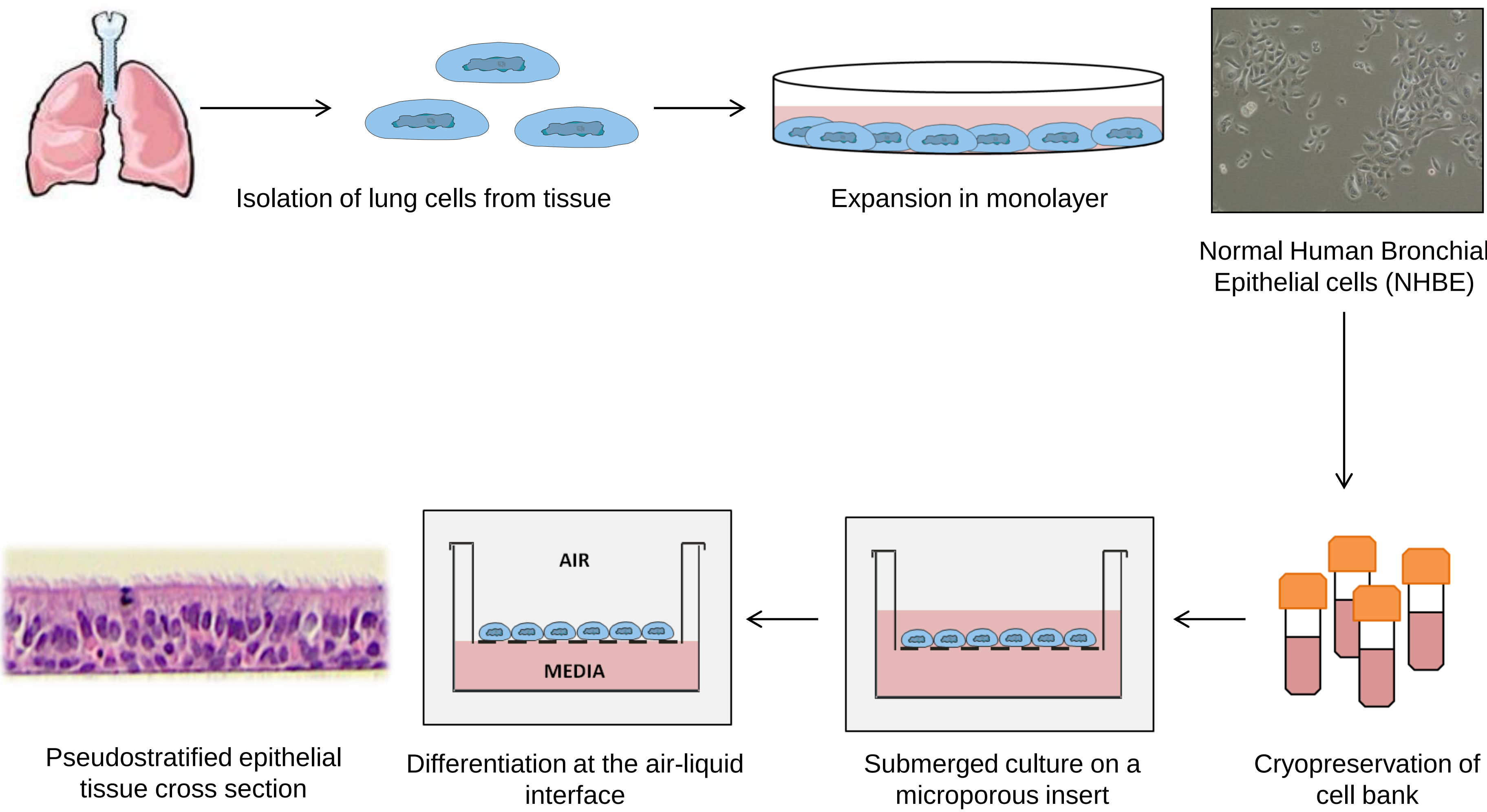


EpiAlveolar

-Primary alveolar epithelial cells, fibroblasts, endothelial cells and +/- macrophages



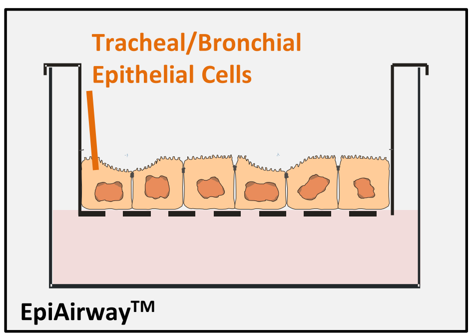
Production of human 3D *in vitro* respiratory tissue models

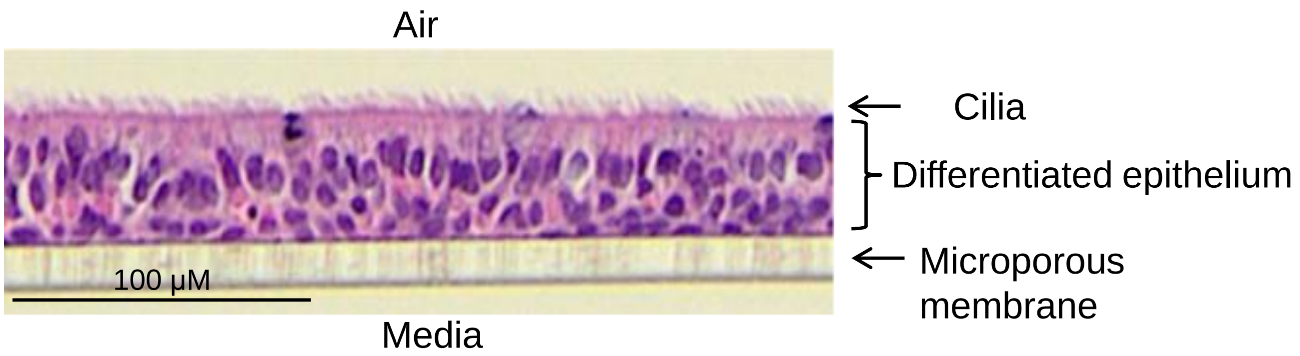


EpiAirway model

EpiAirway is an *in vitro* 3D organotypic model of human tracheal/bronchial epithelial tissue.

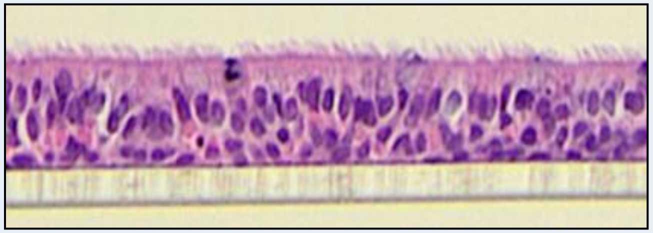
- Primary tracheobronchial epithelial cells grown at the air-liquid interface
- >35 donors available from various backgrounds
  - Healthy
  - COPD
  - Asthma
  - Smoker
- Variety of insert formats, including high throughput plates
- Highly reproducible, stable for long term culture, serum-free media
- Available worldwide
- >100 technical references



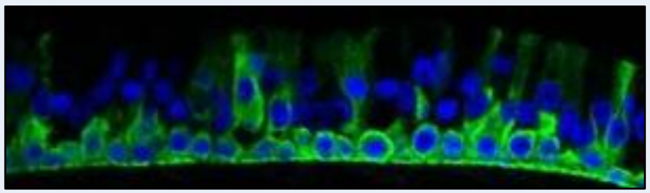


EpiAirway model recapitulates *in vivo* microenvironment

Pseudostratified epithelium



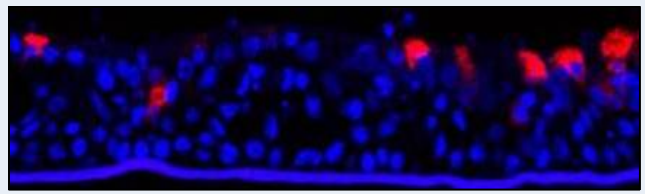
H&E



Keratin 5 DAPI

Goblet cells & mucus production

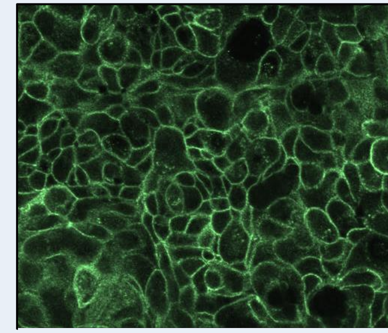
H&E Alcian Blue



MUC5AC DAPI

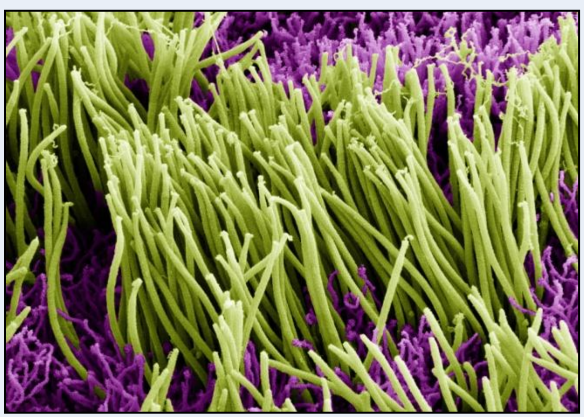
Barrier function

TEER ≥ 300 $\Omega*cm^2$

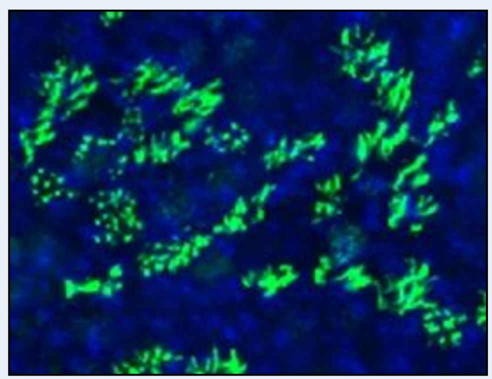


E-Cadherin
(Top view)

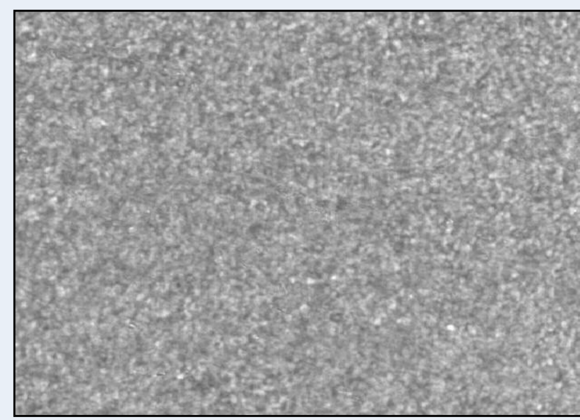
Beating cilia



SEM image (pseudocolored)



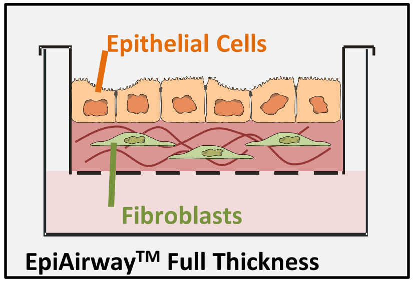
β-Tubulin DAPI (Top view)

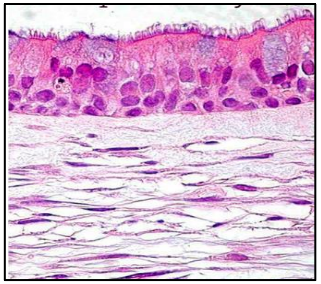


Beating cilia (Top view)

EpiAirway full thickness (EpiAirwayFT) model

- Primary tracheobronchial epithelial cells and fibroblasts within an extracellular matrix grown at the air-liquid interface
- >25 donors with matched fibroblasts available from various backgrounds



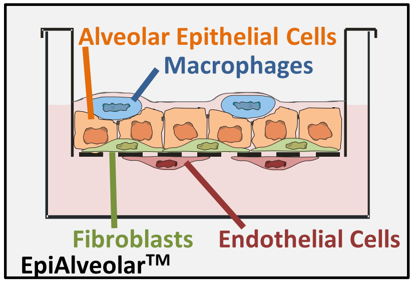


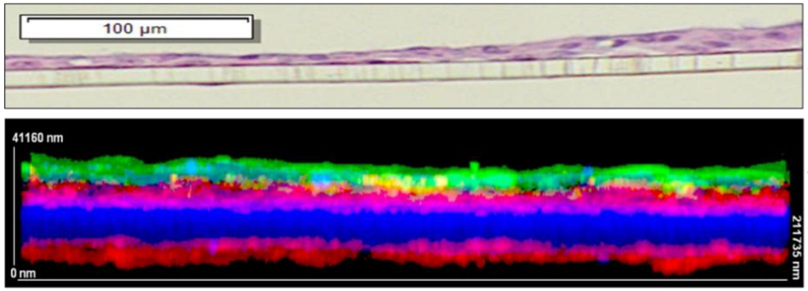
Pseudostratified epithelium

Fibroblasts in stromal extracellular matrix

EpiAlveolar model

- Primary alveolar epithelial cells, fibroblasts and endothelial cells grown at the air-liquid interface
- Able to include macrophages
- 3 donors available from various backgrounds





Epithelial & Fibroblast Layer
Microporous Membrane
Endothelial layer

Epithelial Layer
Fibroblast Layer
Microporous Membrane
Endothelial Layer

Cytokeratin 19 Vimentin DAPI

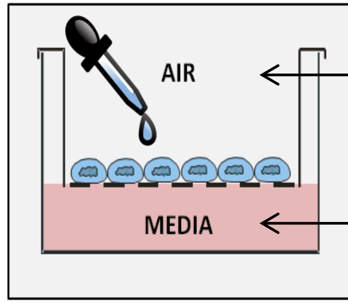
EpiAirway applications & assays

Applications

- Toxicity
- Viral or bacterial infection
- Drug delivery/permeation
- Goblet cell hyperplasia
- Inflammation
- Wound healing
- Fibrosis
- Disease modeling (asthma, COPD)

www.mattek.com/references/

Exposure methods



Apical exposure "Inhaled"/"Topical"

Submerged: Liquid, cream, powder

ALI: gas, vapor, aerosol

Basal exposure "Systemic"

VITROCELL® SYSTEMS

Assays

- Barrier function (TEER, permeation)
- Viability (MTT, LDH)
- Genotoxicity (Comet, micronucleus assays)
- Gene expression (qRT-PCR)
- Protein expression (staining, Western blot)
- Cytokine secretion (ELISA, multiplex)
- Cilia function (beat frequency, active area)
- Oxidative stress (NRF2 activity, glutathione assay)
- Mucus production (Alcian blue, PAS)
- Tissue architecture (histology)

Application:

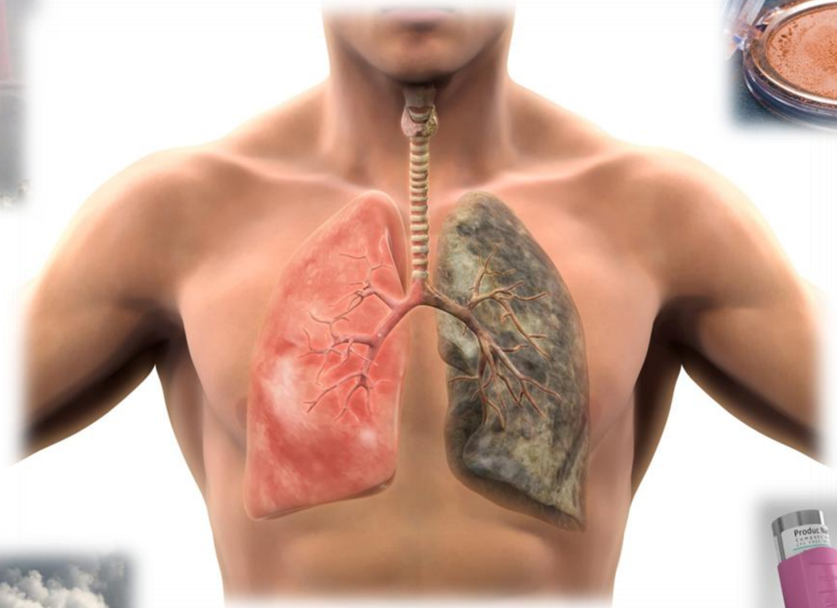
EpiAirway test for acute inhalation toxicity

APPLIED IN VITRO TOXICOLOGY
Volume 4, Number 2, 2018
Mary Ann Liebert, Inc.
DOI: 10.1089/aivt.2018.0004

Prevalidation of an Acute Inhalation Toxicity Test Using the EpiAirway *In Vitro* Human Airway Model

George R. Jackson, Jr., Anna G. Maione, Mitchell Klausner, and Patrick J. Hayden

Inhalation is a major route of exposure to potentially toxic chemicals

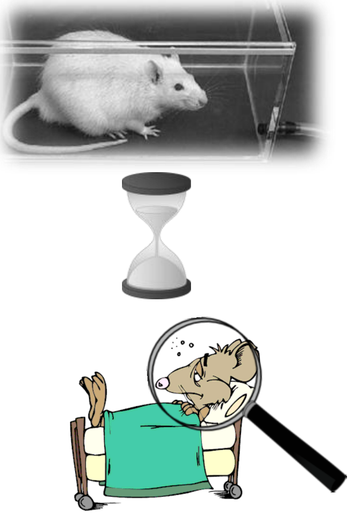


Regulatory agencies rely on animal tests to classify acute inhalation toxicity of chemicals

Figure 1A. Globally Harmonized System (GHS): Acute Toxicity				
Category 1	**Category 2**	**Category 3**	**Category 4**	**Category 5**
				No pictogram
Danger	Danger	Danger	Warning	Warning
330 Fatal if inhaled	**330** Fatal if inhaled	**331** Toxic if inhaled	**332** Harmful if inhaled	**333** May be harmful if inhaled

Figure 2. Globally Harmonized System (GHS): Specific Target Organ Toxicity - Single Exposure		
Category 1	**Category 2**	**Category 3**
Danger	Warning	Warning
370 Causes damage to organs (or state all organs affected, if known)	**371** May Cause damage to organs (or state all organs affected, if known)	**335** May cause respiratory irritation

OECD Test Guideline 403/436:
Acute Inhalation Toxicity Test: Rat LD_{50} test



4 hour exposure (whole body or head/nose only)

↓

14 day wait

↓

Assess lethality & any signs of toxicity

Repeat with additional concentrations as necessary

Drawbacks to current animal inhalation toxicity testing

Significant drawbacks to animal testing method:

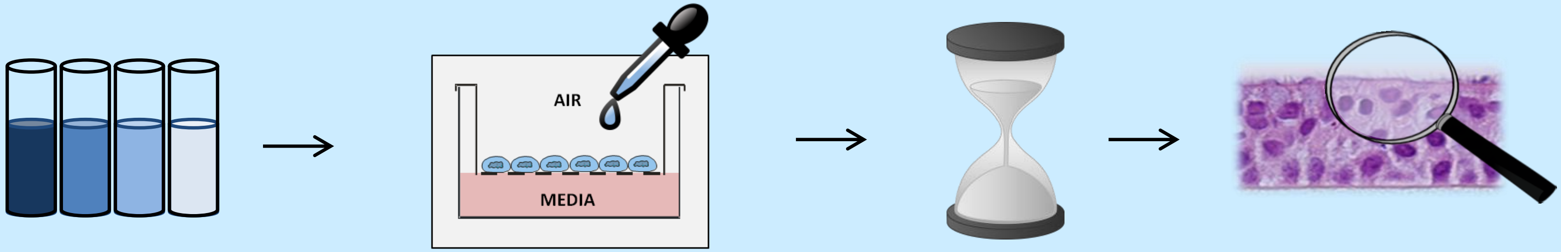
1. Not always predictive of human response
   - Primarily endpoint is lethality; miss non-lethal effects – not ideal for safety assessment
   - No mechanistic information
   - Species-specific differences in response, breathing, deposition

2. Ethical concerns
   - Increasing regulatory pressures to avoid animal use (Tox21, 3Rs, EU Cosmetics Directive)

3. Inefficient
   - Costly, time consuming, low throughput
   - Variability between test
   - Poor control over delivery & ability to measure effective dosing

Alternative, non-animal testing strategies are greatly needed.

EpiAirway™ acute inhalation toxicity test method.



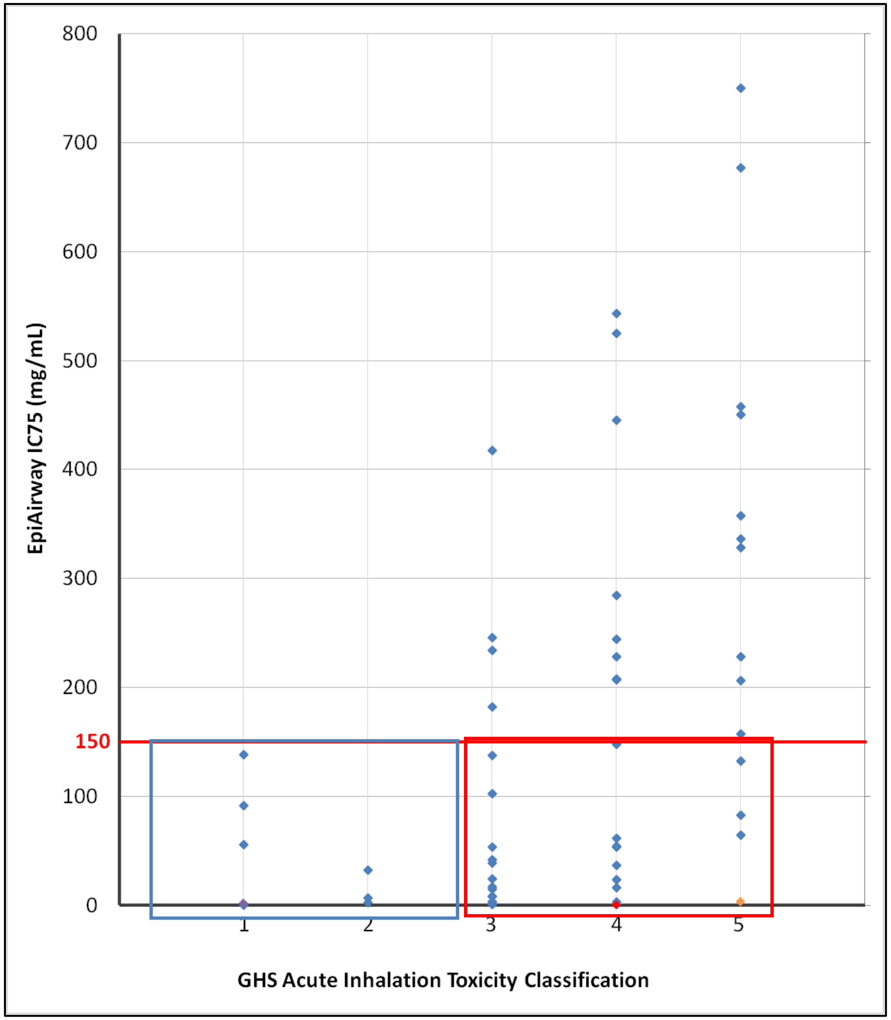
Prepare 4-point dose curve of chemical in dH_2O or corn oil → Apply chemical to the apical surface → Incubate for 3 hours → Examination:
- Tissue viability (MTT)

-Tested 59 chemicals with a range of inhalation toxicities

-Determined IC_{75} (dose at which tissues are 75% viable)

-Correlated *in vitro* data to *in vivo* rat LD_{50} data (GHS category) to develop a prediction model (also compared against EPA category, not shown)

EpiAirway test is highly sensitive for identifying potential toxicants.



Compared to GHS Rat Data	
Sensitivity	8/8 = 100%
Specificity	22/51 = 43%
Overall Accuracy	30/59 = 51%

What chemicals is the rat LD_{50} test missing?

Rat LD_{50} test fails to identify many respiratory corrosives based on GHS STOT-SE classification.

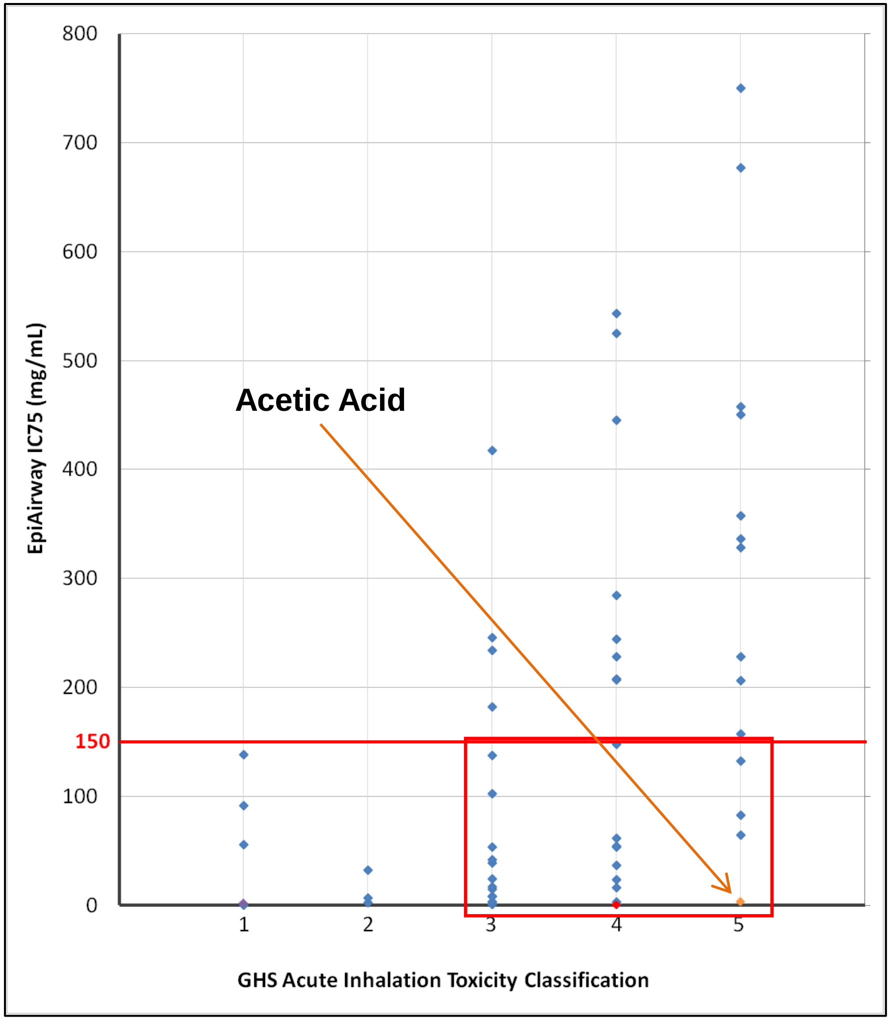
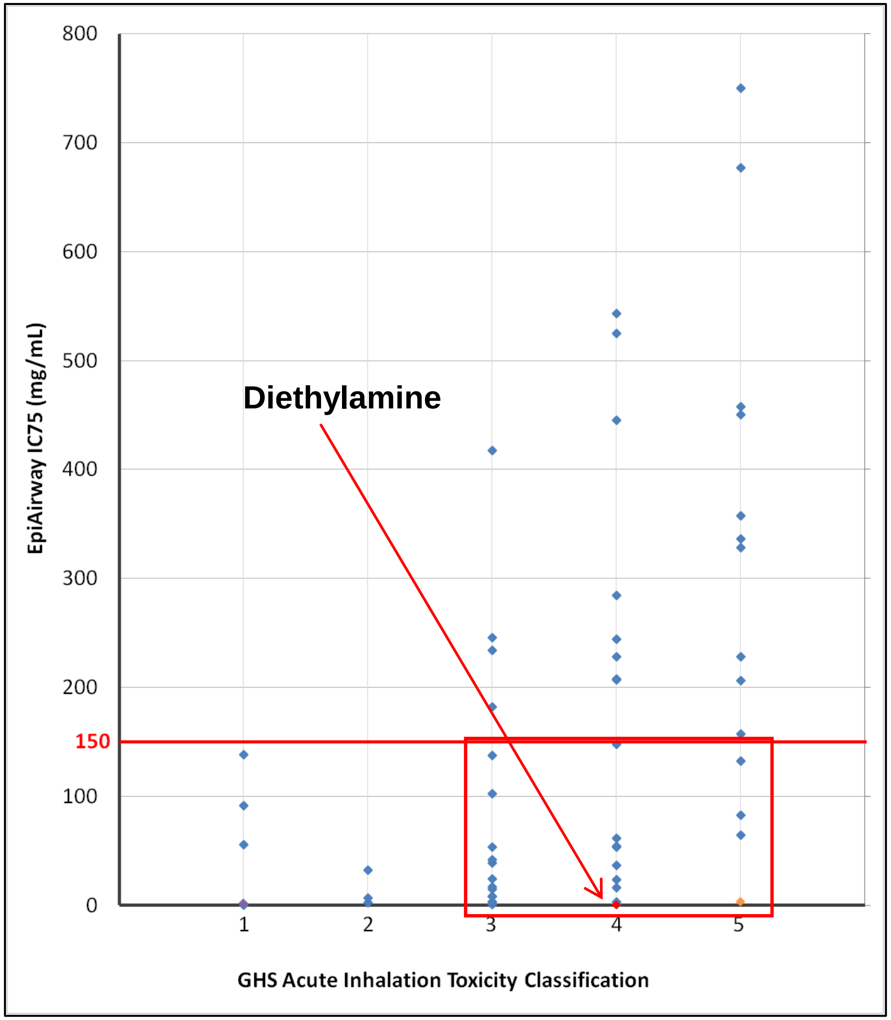


Figure 2. Globally Harmonized System (GHS): Specific Target Organ Toxicity - Single Exposure

Category 1	Category 2	Category 3
Danger	Warning	Warning
370 Causes damage to organs (or state all organs affected, if known)	**371** May Cause damage to organs (or state all organs affected, if known)	**335** May cause respiratory irritation

Test Chemical	Mean IC75 (mg/ml)	GHS	GHS STOT-SE	Skin/Eye Corrosive
Acrolein	0.17	1	1	skin, eye
Butylamine	0.71	3	1	skin, eye
Dimethylamine	0.73	4	1	skin, eye
Diethylamine	0.75	4	1	skin, eye
Formic acid	1.04	3	1	skin, eye
Chloroacetaldehyde	2.22	2	1	skin, eye
Formaldehyde	2.98	3	1	skin, eye
Acetic acid	**3.14**	**5**	**1**	**skin, eye**
Ethanolamine	3.27	4	1	skin, eye
Benzyl chloride	3.35	3	1	eye
Diethyl(amino)ethanol	3.52	3	1	skin, eye
Methyl acrylate	8.13	3	1	

Rat LD_{50} test fails to identify many respiratory irritants based on SDS data.



SIGMA-ALDRICH sigma-aldrich.com

SAFETY DATA SHEET

Version 5.5
Revision Date 06/02/2016
Print Date 07/27/2016

1. PRODUCT AND COMPANY IDENTIFICATION

1.1 **Product identifiers**

Product name : Diethylamine

Product Number : 471216
Brand : Sigma-Aldrich
Index-No. : 612-003-00-X

CAS-No. : 109-89-7

11. TOXICOLOGICAL INFORMATION

11.1 **Information on toxicological effects**

Acute toxicity
LD50 Oral - Rat - male - 540 mg/kg
(OECD Test Guideline 401)

LC50 Inhalation - Rat - female - 4 h - 17.3 mg/l
(OECD Test Guideline 403)

LD50 Dermal - Rabbit - male - 582 mg/kg

No data available

Skin corrosion/irritation
Skin - Rabbit
Result: Causes severe burns. - 1 min

Category 4

Warning

332
Harmful if inhaled

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 **Control parameters**

Components with workplace control parameters

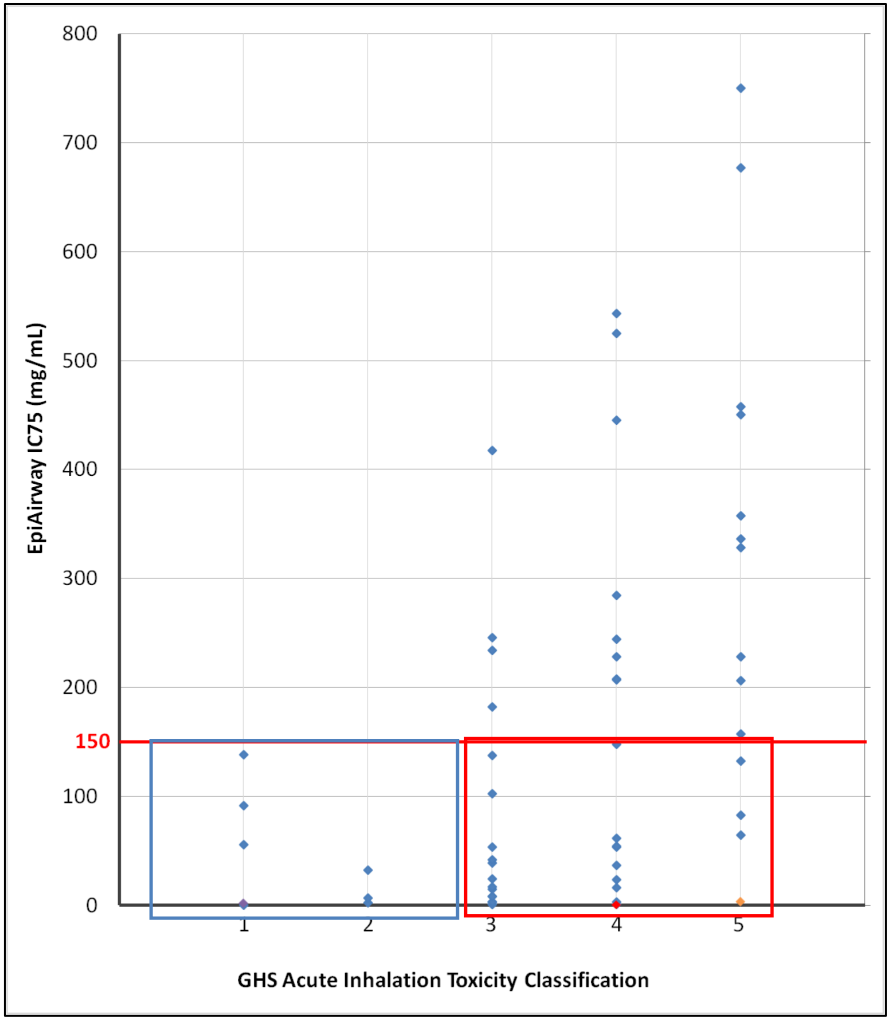
Component	CAS-No.	Value	Control parameters	Basis
Diethylamine	109-89-7	TWA	5.000000 ppm	USA. ACGIH (TLV)
	Remarks	Upper Respiratory Tract irritation Eye irritation Not classifiable as a human carcinogen Danger of cutaneous absorption		
		STEL	15.000000 ppm	USA. ACGIH (TLV)
		Upper Respiratory Tract irritation Eye irritation Not classifiable as a human carcinogen Danger of cutaneous absorption		
		TWA	10.000000 ppm	USA. NIOSH

Category 1

Danger

370
Causes damage to organs (or state all organs affected, if known)

Advantages of EpiAirway test over rat LD_{50} test



1. More predictive than rat LD_{50} test
   - Correctly identified 100% of the highly toxic chemicals (Categories 1 & 2)
   - Successfully identified respiratory corrosives & irritants based on GHS STOT-SE and SDS data that were missed by the rat test
   - Eliminates interspecies differences; potential for better human translation

2. Simple test & classification scheme
   - Fast & easy dosing strategy
   - Cost-effective
   - Higher throughput
   - No animal-associated ethical concerns

Ongoing Work

1. How does the EpiAirway test distinguish between systemic toxicity (Category 1/2 chemicals) and respiratory corrosives & irritants?

   - Tiered testing with skin corrosion test (OECD TG 431)
   - Read across, chemical properties
   - Additional endpoints, including mechanistic experiments

2. Refine and finalize prediction model with additional chemicals

   - Inter-laboratory testing of chemical subset
   - Submission to OECD

3. Extended to sub-chronic and chronic inhalation toxicity tests?

   - Repeat dosing strategy

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Olivia O'Connell
Zach Sellman

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References

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www.mattek.com
Anna Maione: amaione@mattek.com
Patrick Hayden: phayden@mattek.com

<u>Regulatory considerations</u>:
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-STOT-SE https://www.schc.org/assets/docs/ghs_info_sheets/specific_target_organ_toxicity-single_exposure.pdf
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