

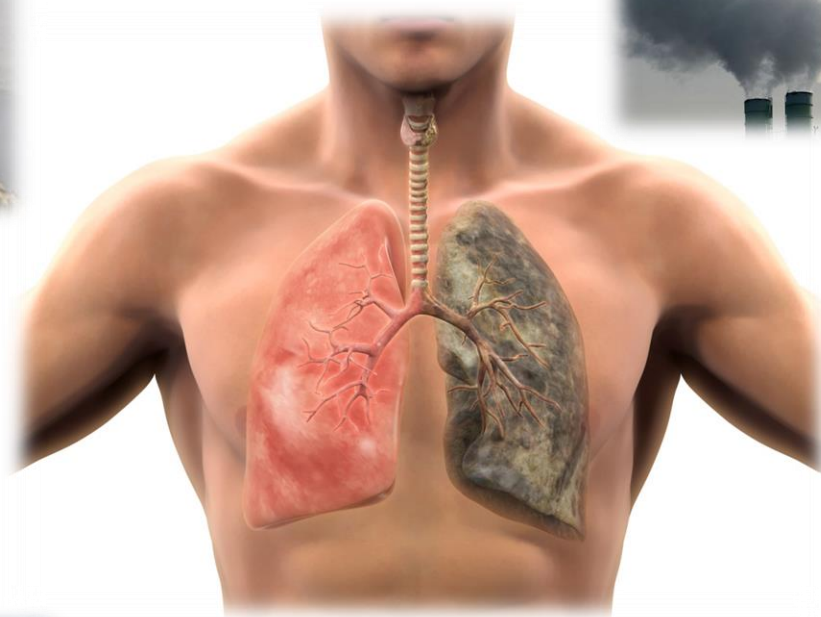
Pre-Validation of an Acute Inhalation Toxicity Assay Using the EpiAirway In Vitro Human Airway Model

George R. Jackson, Jr., Michelle Debatis, Anna G. Maione, Patrick J. Hayden




MatTek
CORPORATION


Exposure to potentially dangerous chemicals can occur through inhalation.



Regulatory systems for classifying the acute inhalation toxicity of chemicals


Figure 1B. Environmental Protection Agency (EPA) System

Category I	Category II	Category III	Category IV
	No Pictogram	No Pictogram	No Pictogram
Danger - Poison	Warning	Caution	Caution (Optional)
Fatal if inhaled	May be fatal if inhaled	Harmful if inhaled	
$\leq 0.05 \text{ mg/l}$	$> 0.05 \leq 0.5 \text{ mg/l}$	$> 0.5 \leq 2 \text{ mg/l}$	$> 2 \text{ mg/l}$





 Respirator Use Required

Regulatory systems for classifying the 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

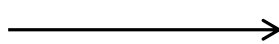
**Figure 2. Globally Harmonized System (GHS):
Specific Target Organ Toxicity - Single Exposure**

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

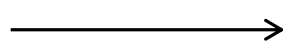
OECD 403/436 is the currently accepted test method for determining acute inhalation toxicity

OECD Test Guidelines 403/436: *In vivo* rat LD50 test (dose at which 50% of the animals die)

4 hour exposure



14 Days



Examination:

- Death
- Signs of toxicity
- Necropsy should be performed (not always reported)



Nose/Head only (preferred)



Whole body



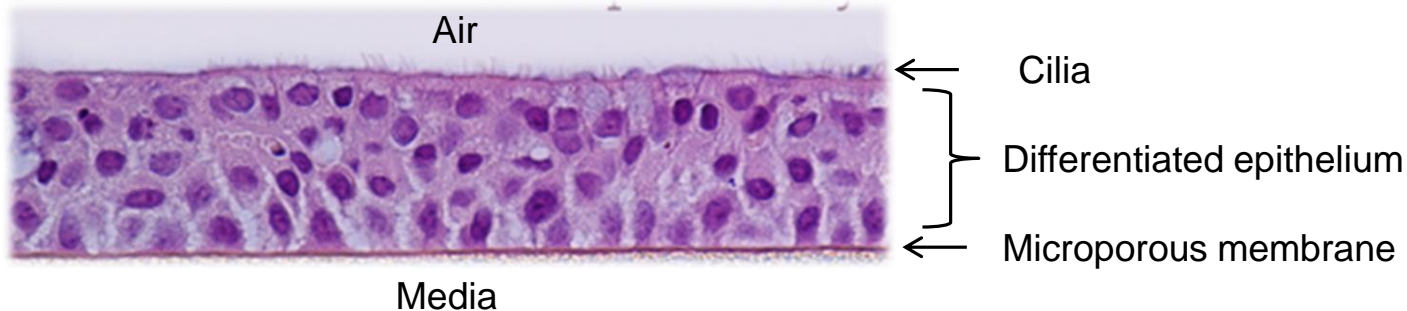
Repeat stepwise with additional concentrations as necessary

Our goal is to develop & validate an *in vitro* test for acute inhalation toxicity

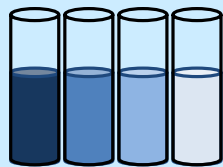
The EpiAirway Model

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

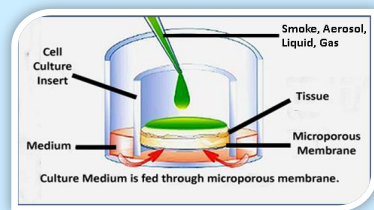
- Constructed from primary cells
- Highly reproducible
- Differentiated epithelium at the air-liquid interface
 - Beating cilia
 - Mucus secretion
 - Barrier function
- Physiologically relevant & predictive of the human outcome



EpiAirway™ acute inhalation toxicity test method



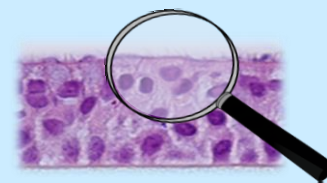
Prepare 4-point dose curve of chemical in dH₂O or corn oil



Apply chemical to the apical surface



Incubate for 3 hours

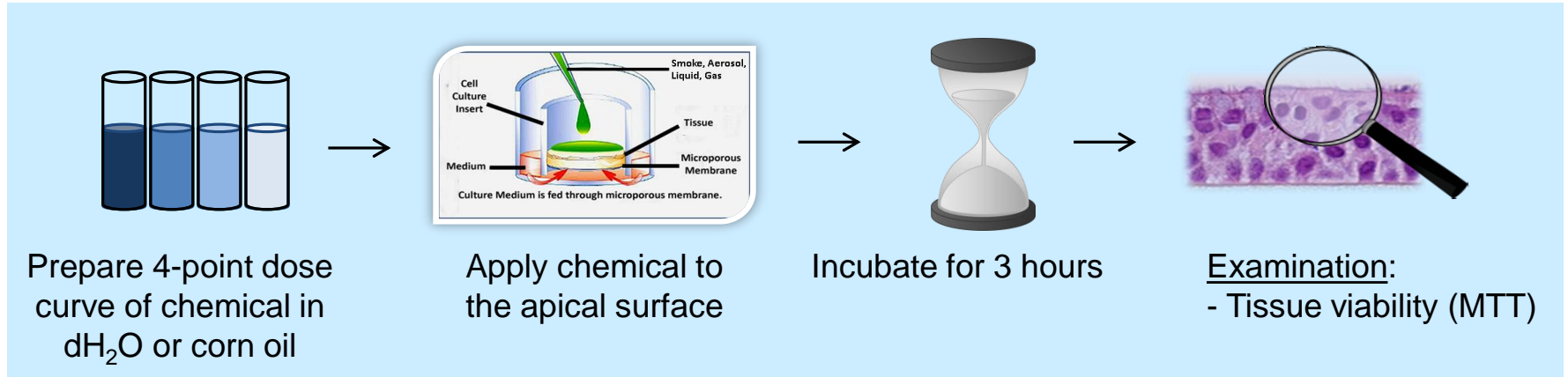


Examination:
- Tissue viability (MTT)

Advantages of using the *in vitro* EpiAirway test:

1. Exposure is straight-forward
 - Doesn't require extensive dosing characterization
 - No special training
2. Fast, high-throughput
3. No ethical concerns
4. Relatively low costs
5. Improved translation to human response

EpiAirway™ acute inhalation toxicity test: Preliminary results



-Tested 59 chemicals with a range of known inhalation toxicities (based on GHS, EPA and SDS)

-Determined IC₇₅ (dose at which tissues are 75% viable)

-Correlated *in vitro* data to *in vivo* data to develop a prediction model

In vivo data used for correlation were based on SDSs and the OECD eChemPortal database

SIGMA-ALDRICH

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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

1.2 Relevant identified uses of the substance or mixture and uses advised against
Identified uses : Laboratory chemicals, Synthesis of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich
3050 Spruce Street
SAINT LOUIS MO 63103
USA

Telephone : +1 800-325-5832
Fax : +1 800-325-5052

1.4 Emergency telephone number

Emergency Phone # : (314) 776-6555

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)
Flammable liquids (Category 2), H225
Acute toxicity, Oral (Category 4), H302
Acute toxicity, Inhalation (Category 4), H332
Acute toxicity, Dermal (Category 3), H311

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

Category 3
Respiratory System



Danger

335
May cause respiratory irritation

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

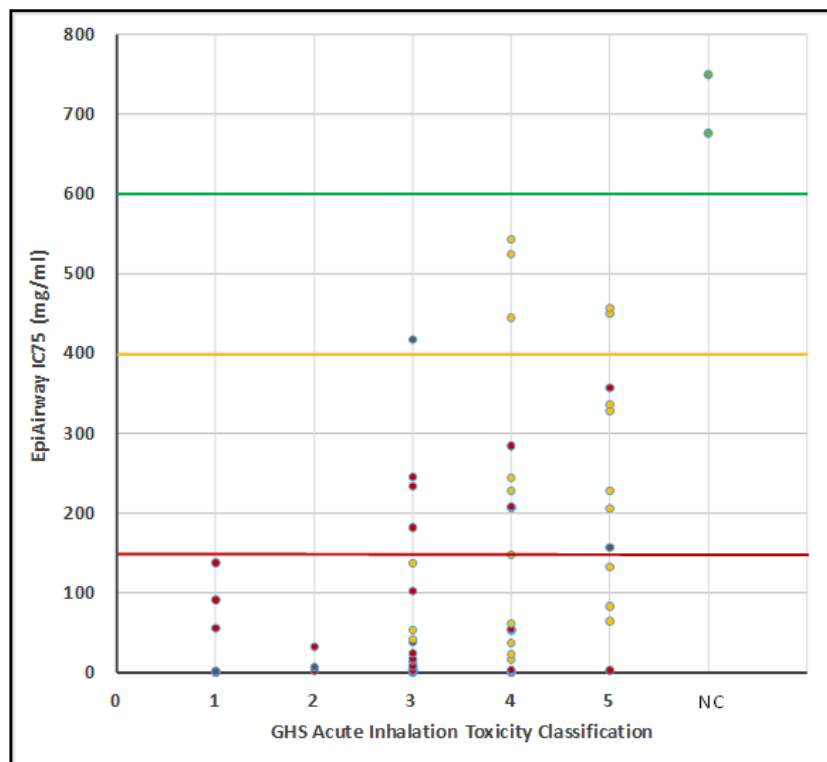
Components with workplace control parameters

Component	CAS-No.	Value	Control parameters	Basis
Diethylamine	109-89-7	TWA	5.000000 ppm	USA, ACGIH Threshold Limit Values (TLV)
	Remarks	Upper Respiratory Tract irritation Eye irritation Not classifiable as a human carcinogen Danger of cutaneous absorption		
		STEL	15.000000 ppm	USA, ACGIH Threshold Limit Values (TLV)
		Upper Respiratory Tract irritation Eye irritation Not classifiable as a human carcinogen Danger of cutaneous absorption		
		TWA	10.000000 ppm 30.000000 mg/m3	USA, NIOSH Recommended Exposure Limits
		ST	25.000000 ppm 75.000000 mg/m3	USA, NIOSH Recommended Exposure Limits
		TWA	25.000000 ppm 75.000000 mg/m3	USA, Occupational Exposure Limits (OSHA) - Table Z-1 Limits for Air Contaminants
		The value in mg/m3 is approximate.		
		TWA	5 ppm	USA, ACGIH Threshold Limit Values (TLV)
		Upper Respiratory Tract irritation Eye irritation Skin irritation Not classifiable as a human carcinogen Danger of cutaneous absorption		
		STEL	15 ppm	USA, ACGIH Threshold Limit Values (TLV)
		Upper Respiratory Tract irritation Eye irritation Skin irritation		

Reproducibility of the In Vivo Rat Test

Test Article	Rat LC50 Cat
Acrolein	II
Acrolein	II
Acrolein	I
Acrolein	I
Acrolein	I
ACROLEIN	I
ACROLEIN	II
Calcium Oxide	III
Formic acid	IV
FORMIC ACID	I
FORMIC ACID	IV
BENZOYL CHLORIDE	III
BENZOYL CHLORIDE	III
Crotonaldehyde	III
Crotonaldehyde	IV
Crotonaldehyde	IV
Crotonaldehyde	II
Crotonaldehyde	IV
CROTONALDEHYDE	II
CROTONALDEHYDE	IV
Acrylonitrile	III
Acrylonitrile	IV
ACRYLONITRILE	III
Camphor	II
Camphor	IV

Classification of GHS categories: *In vivo* rat vs. the EpiAirway test



EpiAirway Prediction model based on combined GHS Acute Inhalation plus STOT Classifications

IC75 > 600 mg/ml: No Classification

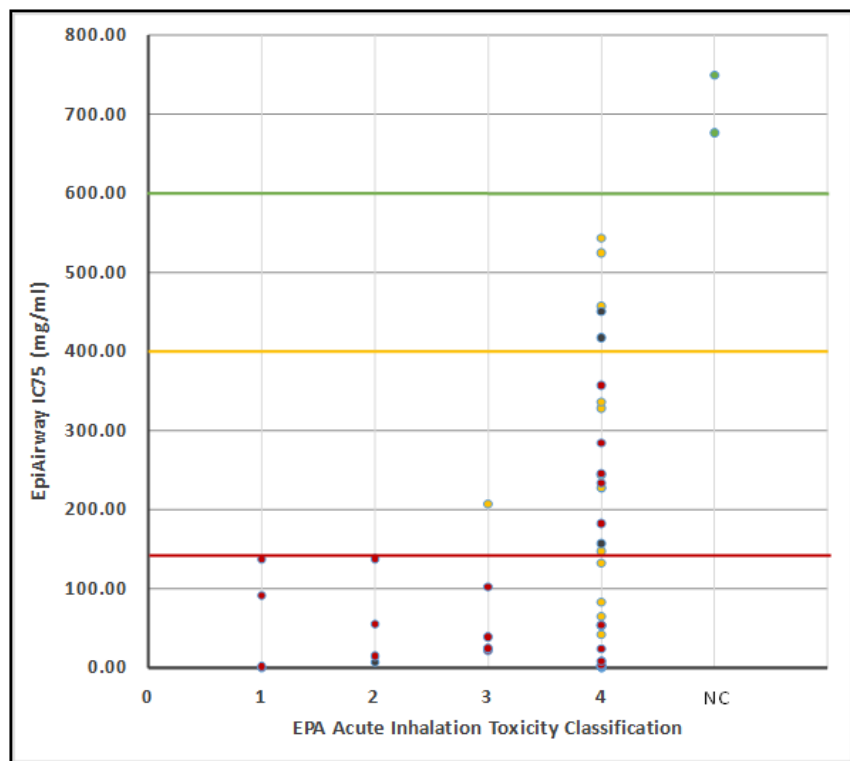
400 < IC75 ≤ 600 mg/ml: GHS 4-5

150 < IC75 ≤ 400 mg/ml: GHS 3 and/or irritating to the respiratory tract or narcotic effects

IC75 ≤ 150 mg/ml: GHS 1-2

Prediction Model Categories	
Category 1-2	Category 3
Acrolein	Benzene
Butylamine	Chloroform
Dimethylamine	Dimethylphthalate
Diethylamine	Benzoyl chloride
Formic acid	p-Xylene
Crotonaldehyde	Cyclohexane
Chloroacetaldehyde	Trichloroethylene
Formaldehyde	Allyl chloride
Acetic acid	Tetrachloroethylene
Ethanolamine	Carbon Tetrachloride
Benzyl chloride	Acetonitrile
Diethyl(amino)ethanol	n-Heptane
Diisopropylamine	Hexane
Methyl acrylate	p-Dichlorobenzene
Morpholine	
Allyl alcohol	Category 4-5
Vinyl Acetate	2-Ethoxyethanol
H2O2	p-tert-Butyltoluene
4-Ethylmorpholine	Acetone
Phenol	Ethyl alcohol
p-Anisidine	Dimethylacetamide
Cyclohexanol	N,N-Dimethylformamide
Aniline	
Allyl glycidyl ether	
Ethyl acrylate	
2-Butoxyethanol	No Classification
Cyclohexanone	Triethylene glycol
Dichloroethyl ether	Propylene Glycol
Ethyl formate	
Isophorone	
1-Butanol	
Paraquat	
Glycidol	
Methyl methacrylate	
Hexone	
Ethylene chlorohydrin	
2-Ethoxyethyl acetate	

Classification of EPA categories: *In vivo* rat vs. the EpiAirway test



EpiAirway Prediction model based on combined EPA Acute Inhalation plus STOT Classifications

IC75 > 600 mg/ml: No Classification

400 < IC75 ≤ 600 mg/ml: EPA IV

150 < IC75 ≤ 400 mg/ml: EPA III and/or irritating to the respiratory tract or narcotic effects

IC75 ≤ 150 mg/ml: EPA I-II

Prediction Model Categories	
Category I-II	Category III
Acrolein	Benzene
Butylamine	Chloroform
Dimethylamine	Benzoyl chloride
Diethylamine	Cyclohexane
Formic acid	Trichloroethylene
Crotonaldehyde	Allyl chloride
Chloroacetaldehyde	Tetrachloroethylene
Acetic acid	Carbon Tetrachloride
Diethyl(amino)ethanol	Acetonitrile
Diisopropylamine	n-Heptane
Methyl acrylate	Hexane
Morpholine	p-Dichlorobenzene
Allyl alcohol	
4-Ethylmorpholine	
Phenol	Category IV
Aniline	2-Ethoxyethanol
Allyl glycidyl ether	p-tert-Butyltoluene
Ethyl acrylate	Acetone
2-Butoxyethanol	Ethyl alcohol
Cyclohexanone	Dimethylacetamide
Dichloroethyl ether	N,N-Dimethylformamide
Isophorone	
1-Butanol	
Paraquat	No Category
Methyl methacrylate	Triethylene glycol
Hexone	Propylene Glycol
Ethylene chlorhydrin	
2-Ethoxyethyl acetate	

Classification of GHS categories: *In vivo* rat vs. the EpiAirway test

	Test Chemical	Mean IC75 (mg/ml)	GHS Acute Classification ¹	GHS STOT-SE Classification GHS-J3	EPA Acute Classification ⁴	U/LRTI (SDS) ⁵	Skin/Eye Corrosive ⁶
1	Acrolein	0.17	1	1	1	y	skin, eye
2	Butylamine	0.71	3	1	4	y	skin, eye
3	Dimethylamine	0.73	4	1	4	y	skin, eye
4	Diethylamine	0.75	4	1	4	y	skin, eye
5	Formic acid	1.04	3	1	4	y	skin, eye
7	Chloroacetaldehyde	2.22	2	1	4	y	skin, eye
8	Formaldehyde	2.98	3	1		y	skin, eye
9	Acetic acid	3.14	5	1	4	y	skin, eye
10	Ethanolamine	3.27	4	1		y	skin, eye
11	Benzyl chloride	3.35	3	1		y	eye
12	Diethyl(amino)ethane	3.52	3	1	4	y	skin, eye
14	Methyl acrylate	8.13	3	1	4	y	
15	Morpholine	8.53	3	1	4	y	skin, eye
16	Allyl alcohol	14.98	3	1	2	y	
18	H2O2	17.30	3	1		y	skin, eye
20	Phenol	24.74	3	1	3	y	skin, eye
21	p-Anisidine	32.62	2	1			
23	Aniline	38.98	3	1	3		eye
24	Allyl glycidyl ether	41.70	3	1	4	y	eye
25	Ethyl acrylate	53.48	3	1	4	y	
26	2-Butoxyethanol	53.61	4	1	4	y	
27	Cyclohexanone	54.41	4	1	4	y	eye
28	Dichloroethyl ether	55.69	1	1	2	y	
29	Ethyl formate	61.94	4	1		y	
32	Paraquat	91.51	1	1	1		
33	Glycidol	102.52	3	1		y	eye
36	Ethylene chlorohydrin	138.06	1	1	2		eye
38	Benzene	157.17	5	1	4		
39	Chloroform	182.36	3	1	4		
42	p-Xylene	208.06	4	1		y	
45	Allyl chloride	233.83	3	1	4	y	

EpiAirway test Sensitivity, Specificity and Overall Accuracy compared to rat LD50 test or human STOT data.

Compared to EPA	
Sensitivity	8/8 = 100%
Specificity	20/40 = 50%
Overall Accuracy	28/48 = 58.3%

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

Compared to GHS STOT Data	
Sensitivity	27/36 = 75%
Specificity	13/23 = 56%
Overall Accuracy	40/59 = 68%

Problems to Address:

- How do we distinguish between a respiratory corrosive and a systemic CAT1/2 chemical?
 - Further mechanistic assays
 - Electrophilic reactivity (Nrf2, GHS depletion)
 - Oxidation
 - Corrosion
 - Membrane disruption
 - ER stress
 - Inflammation (NFkB, AP1, etc.)
 - Read across with other known chemicals
 - Related tests with other in vitro models
 - OECD skin corrosion assay
 - Liver, cardiac, kidney, immunotox models

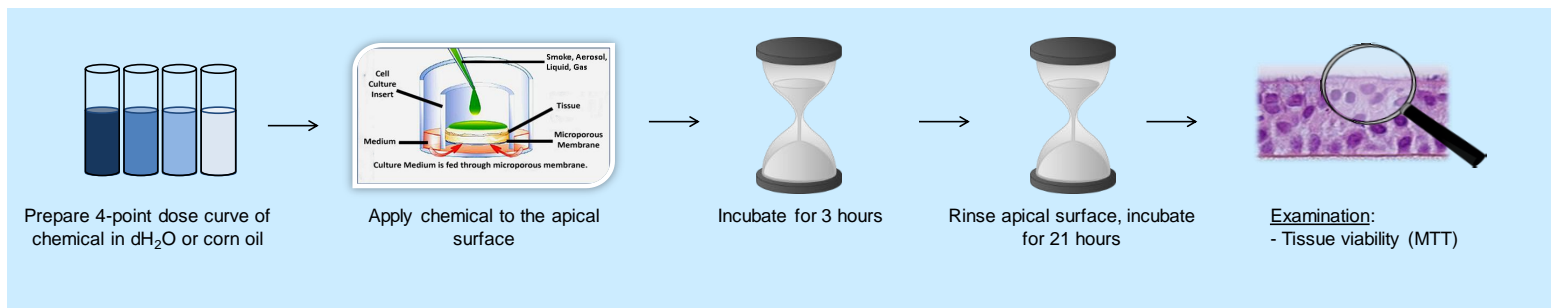
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Ongoing Work

- Testing additional 75 chemicals to refine and finalize a prediction model
- Test the prediction model 25 chemicals in multi-laboratory ring trials
- Submission to OECD



MatTek Project Team

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