THE USE AND APPLICATIONS OF 3D TISSUE MODELS FOR RESPIRATORY TOXICOLOGY AND EFFICACY TESTING WITHIN THE CRO INDUSTRY

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DISCLAIMER

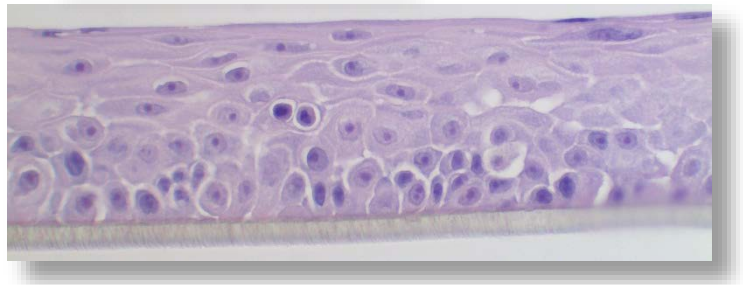
The opinions expressed in this presentation are those of the author, and do not represent the opinions, official policy or position of Charles River Laboratories, Inc.

Biosketch: Clive graduated with a toxicology PhD in *in vitro* dermal absorption and metabolism from Newcastle University. He joined Charles River in 1996 as a Research Officer to develop the *in vitro* skin penetration service. After serving in study director and scientific manager roles, he became Head, *In Vitro* Sciences in 2010 and Director, *In Vitro* Toxicology in 2020. His department currently performs *in vitro* skin absorption, *in vitro* safety pharmacology, investigational and mechanistic toxicology using advanced tissue models and *in vitro* respiratory toxicology. He has presented at many meetings and organized small meetings (Skin Metabolism) and large conferences (WC9). Clive has authored and co-authored many posters and abstracts as well as peer review papers. He is a peer reviewer for journals including TIV, Regulatory Pharmacology & Toxicology, Annals of Work Exposures, and Health and Skin Pharmacology and Physiology. He has been actively involved in advising regulatory agencies including NIH, SCCS, PMRA and EPA as well as industry bodies and has presented a webinar on *in vitro* toxicology with the FDA. He is also a member of the global Charles River 3Rs Working Group as well as being a founding member of the North American 3Rs Collaborative. In January 2021, Clive became a Member of the Board of the UK NC3Rs.

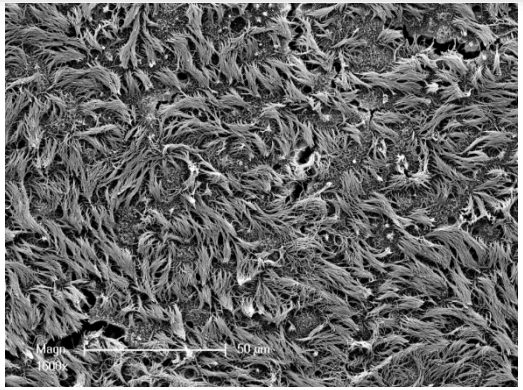
Abstract: 3D human tissue models have been used in regulatory toxicology for many years with test guidelines for many of the acute tests (e.g. skin irritation and corrosion and ocular irritation and severe damage). The availability of models has increased to include oral, gingival, vaginal and the lung. The airway is a particularly important route of administration of drugs as well as environmental pollutants, cosmetic (e.g. deodorant) ingredients, agrochemical sprays and pathogens. One of the fundamental questions is how relevant these models are in predicting toxicity or efficacy when our benchmark tests are using animals, rather than the patient/ consumer. Are the differences observed between the animal *in vivo* data and the human *in vitro* data to do with the species or the cellular model and if it is the former, is the latter a better model? How also can we protect animals, which are still required for regulatory testing? Therefore, a rat upper airway model has been created using the Charles River rat by MatTek. This presentation evaluates the different human upper and lower airway models and the rat upper airway model for predicting toxicity and also in evaluating efficacy in human disease derived tissue – the most important translation that we can assess is the human patient derived model. This presentation will also examine where the tests may be able to be further developed.

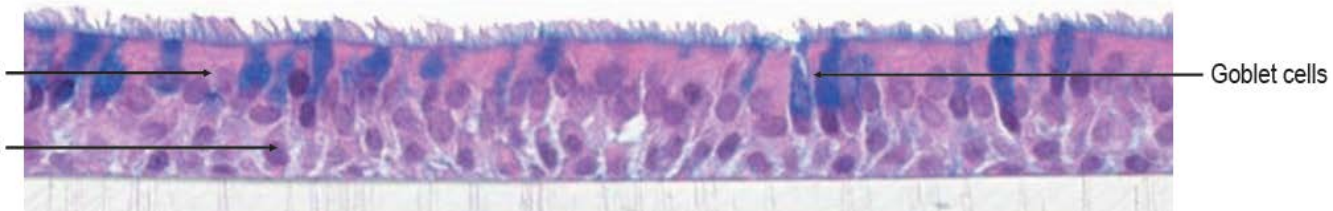
USES IN SAFETY ASSESSMENT

1. Regulatory: OECD Test Guideline Tests
    - Skin Irritation (OECD 439) – EpiDerm™ or EpiSkin ™
    - Skin Corrosion (OECD 431) – EpiDerm™ or EpiSkin™
    - Ocular Irritation (OECD 492) – EpiOcular™ or HCE ™
2. Regulatory: Non Guideline Tests
    - Respiratory Toxicology
        - Upper Airway – MucilAir™ or EpiAirway™ or SmallAir™
        - Lower Airway – EpiAlveolar™
    - Oral
        - Gingival – EpiGingival™
        - Buccal – EpiOral™
    - Vaginal – EpiVaginal™
3. Investigational



MatTek
LIFE SCIENCES





Epithelix

charles river

Toxicology in Vitro 31 (2016) 43–53

Contents lists available at ScienceDirect

Toxicology in Vitro

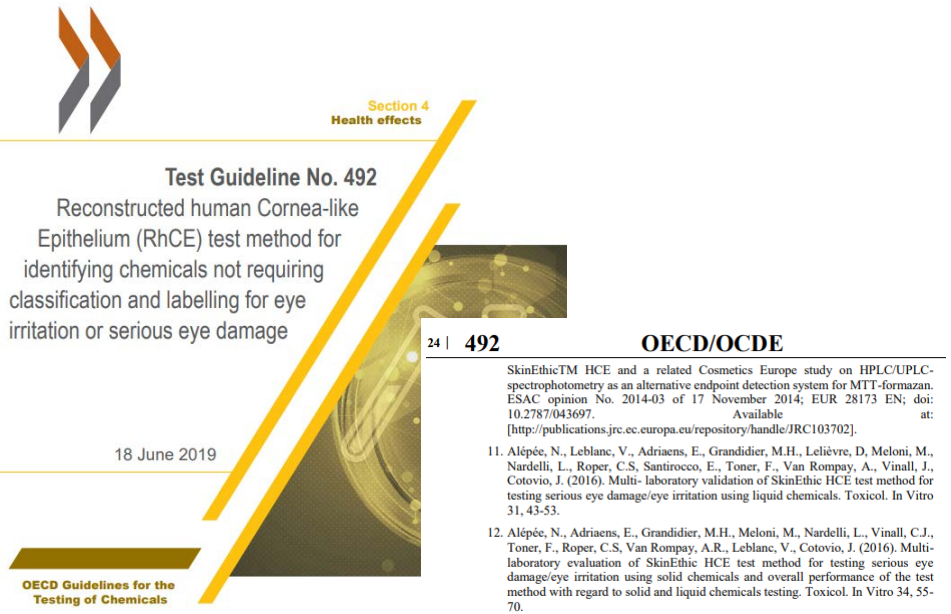
journal homepage: www.elsevier.com/locate/toxinvit

REGULATORY TOXICOLOGY

Multi-laboratory validation of SkinEthic HCE test method for testing serious eye damage/eye irritation using liquid chemicals

N. Alépée [a,*], V. Leblanc [a], E. Adriaens [b], M.H. Grandidier [a], D. Lelièvre [c], M. Meloni [d], L. Nardelli [a], C.S. Roper [e], E. Santirocco [d], F. Toner [e], A. Van Rompay [f], J. Vinall [e], J. Cotovio [a]

[a] L'Oréal Research & Innovation, Aulnay Sous Bois, France
[b] Adriaens Consulting, Aalter, Belgium
[c] Episkin SA, Lyon, France
[d] VitroScreen, Milano, Italy
[e] Charles River Laboratories, Edinburgh, United Kingdom
[f] VITO NV (Flemish Institute for Technological Research), Mol, Belgium



SkinEthicTM HCE and a related Cosmetics Europe study on HPLC/UPLC-spectrophotometry as an alternative endpoint detection system for MTT-formazan. ESAC opinion No. 2014-03 of 17 November 2014; EUR 28173 EN; doi: 10.2787/043697. Available at: [http://publications.jrc.ec.europa.eu/repository/handle/JRC103702].

11. Alépée, N., Leblanc, V., Adriaens, E., Grandidier, M.H., Lelièvre, D, Meloni, M., Nardelli, L., Roper, C.S, Santirocco, E., Toner, F., Van Rompay, A., Vinall, J., Cotovio, J. (2016). Multi- laboratory validation of SkinEthic HCE test method for testing serious eye damage/eye irritation using liquid chemicals. Toxicol. In Vitro 31, 43-53.

12. Alépée, N., Adriaens, E., Grandidier, M.H., Meloni, M., Nardelli, L., Vinall, C.J., Toner, F., Roper, C.S, Van Rompay, A.R., Leblanc, V., Cotovio, J. (2016). Multi-laboratory evaluation of SkinEthic HCE test method for testing serious eye damage/eye irritation using solid chemicals and overall performance of the test method with regard to solid and liquid chemicals testing. Toxicol. In Vitro 34, 55-70.

Toxicology in Vitro 34 (2016) 55–70

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N. Alépée [a,*], E. Adriaens [b], M.H. Grandidier [a], M. Meloni [c], L. Nardelli [a], C.J. Vinall [d], F. Toner [d], C.S. Roper [d], A.R. Van Rompay [e], V. Leblanc [a], J. Cotovio [a]

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[b] Adriaens Consulting, Aalter, Belgium
[c] VitroScreen, Milano, Italy
[d] Charles River Laboratories Edinburgh, EH33 2NE, United Kingdom
[e] VITO NV (Flemish Institute for Technological Research), Mol, Belgium

Toxicology in Vitro 52 (2018) 131–145

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

REGULATORY TOXICOLOGY

Review

Pathway-based predictive approaches for non-animal assessment of acute inhalation toxicity

Amy J. Clippinger[a,*], David Allen[b], Holger Behrsing[c], Kelly A. BéruBé[d], Michael B. Bolger[e], Warren Casey[f], Michael DeLorme[g], Marianna Gaça[h], Sean C. Gehen[i], Kyle Glover[j], Patrick Hayden[k], Paul Hinderliter[l], Jon A. Hotchkiss[m], Anita Iskandar[n], Brian Keyser[o], Karsta Luettich[n], Lan Ma-Hock[p], Anna G. Maione[k], Patrudu Makena[o], Jodie Melbourne[a], Lawrence Milchak[g], Sheung P. Ng[q], Alicia Paini[r], Kathryn Page[s], Grace Patlewicz[t], Pilar Prieto[r], Hans Raabe[c], Emily N. Reinke[u], Clive Roper[v], Jane Rose[w], Monita Sharma[a], Wayne Spoo[o], Peter S. Thorne[x], Daniel M. Wilson[m], Annie M. Jarabek[y]

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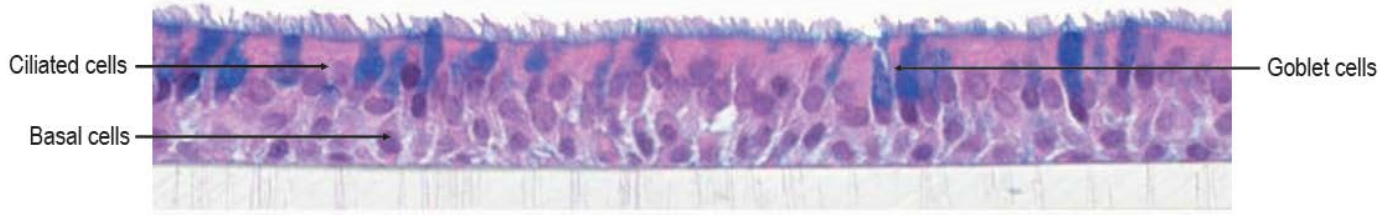
COMMERCIALLY AVAILABLE 3D MODELS: RESPIRATORY TRACT



Nasal/Laryngeal

- MucilAir (Epithelix)
- EpiAirway (MatTek)
- Human derived tissue
- Healthy/ smoker/ diseased

Epithelix

Small Airway / Bronchiole

- SmallAir (Epithelix)

Alveolar

- EpiAlveolar (MatTek)

MatTek
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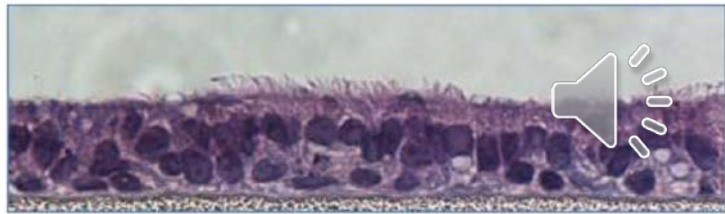
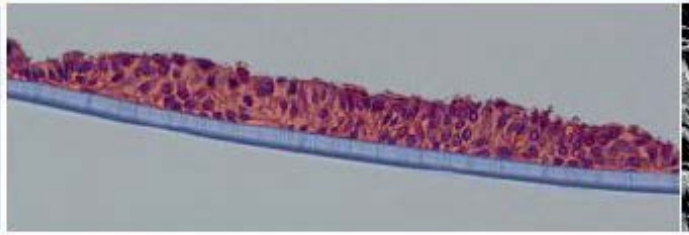
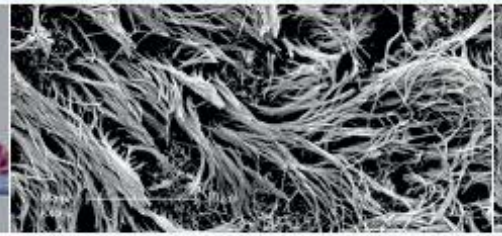
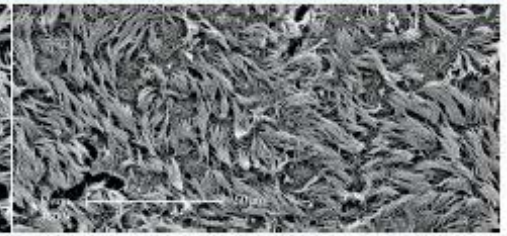
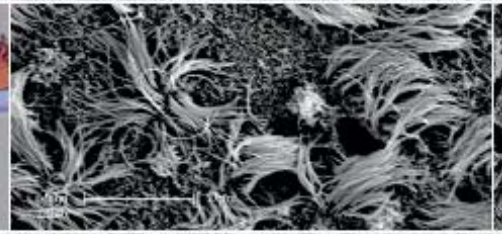
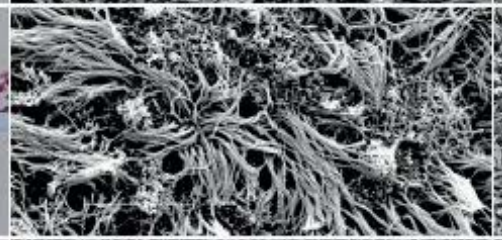
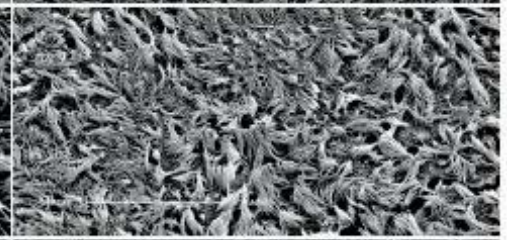
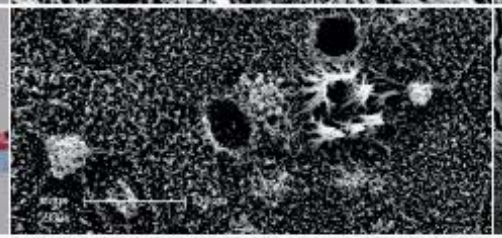
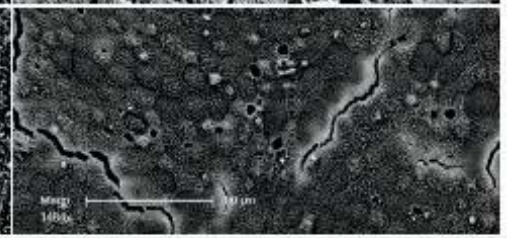
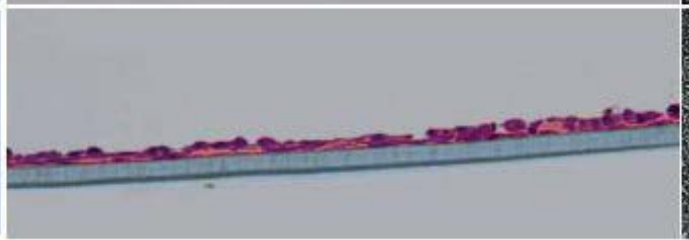
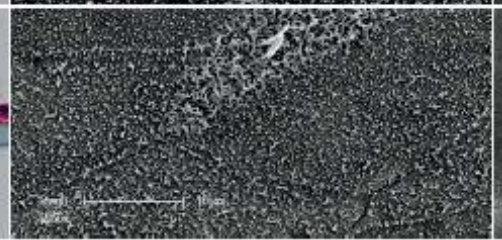
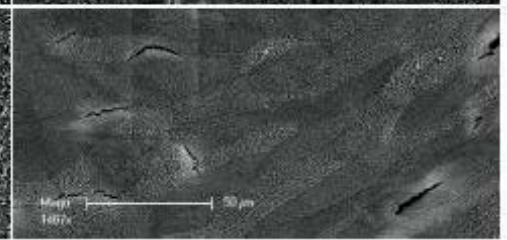
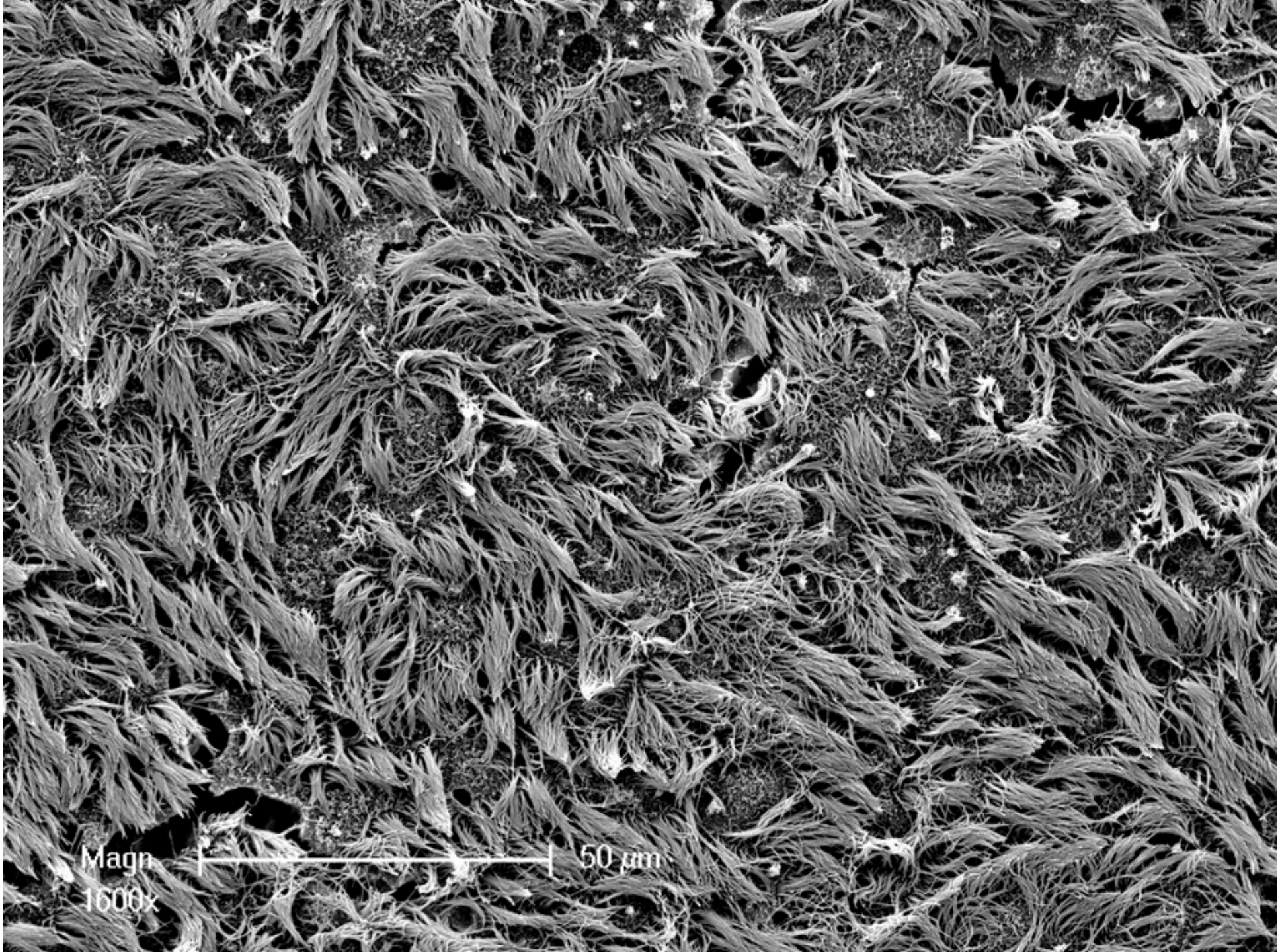


CAN THE 3D MODELS EFFECTIVELY IDENTIFY RESPIRATORY TOXICANTS?

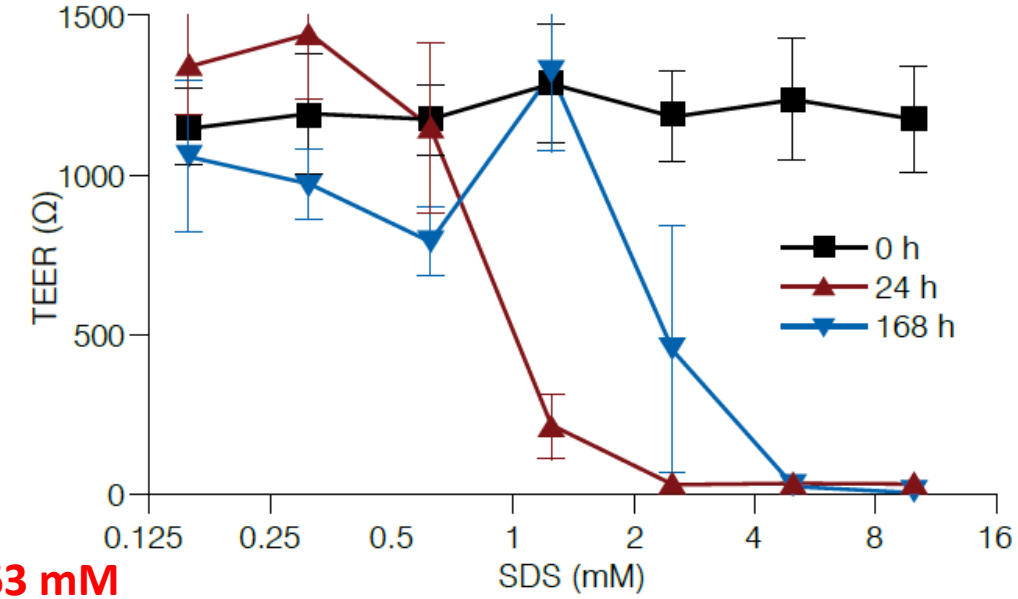
EFFECT OF SDS ON MUCILAIR™: HISTOLOGY AND MORPHOLOGY

SDS (mM)	Cross Sectional Histology (400x)	Surface Morphology	
0.00			
0.31			
0.63			
1.25			
2.50			

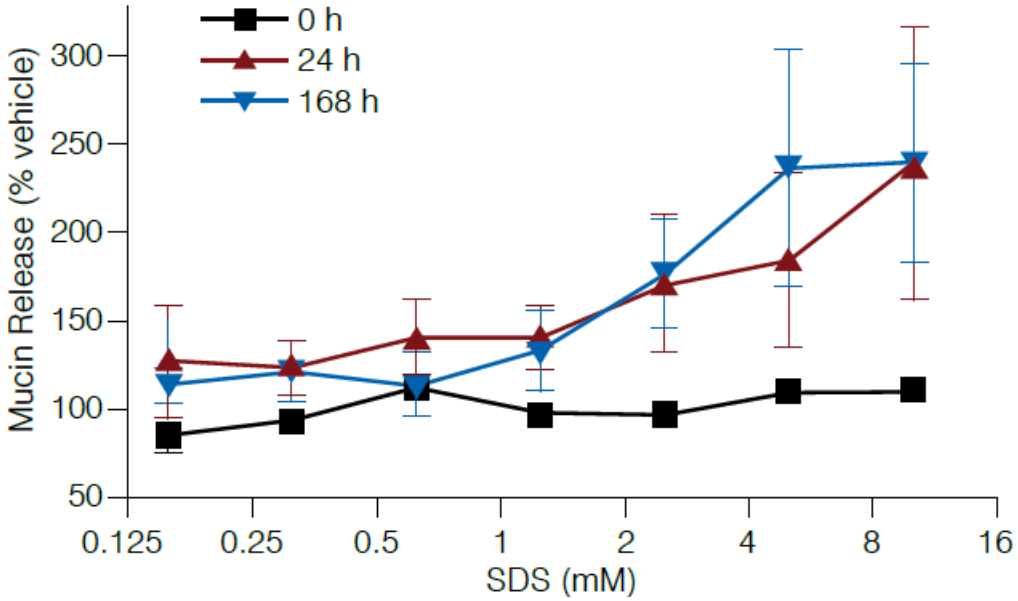


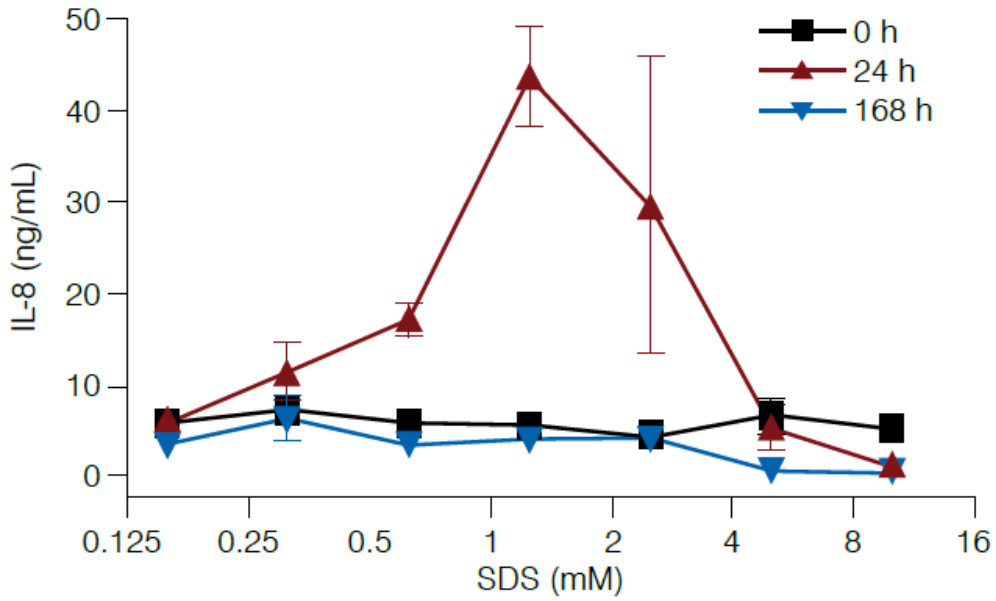
charles river

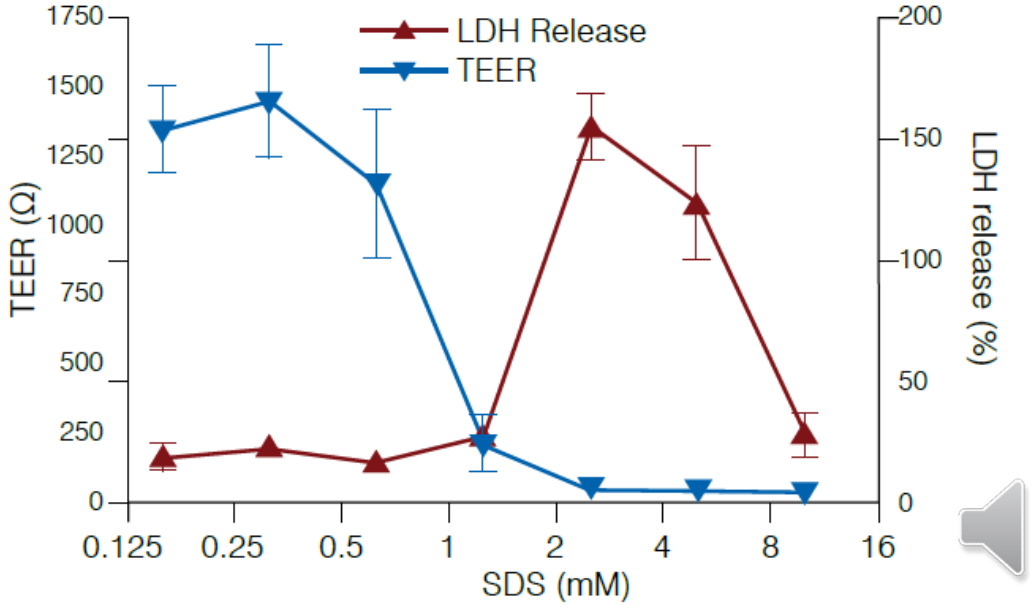
EFFECT OF SDS ON MUCILAIR™: BIOMARKERS



NOAEL = 0.63 mM







HOW CAN WE PROTECT ANIMALS WHICH ARE STILL REQUIRED FOR REGULATORY TESTING?

Determining a No Effect Level for Selection of a Point of Departure for Risk Assessment of an Irritant Aerosol Using a 3D *in vitro* Assay

A. J. Charlton[1], B. Parr-Dobranski[1], S. Flack[2], T. Ramanarayanan[2], P. Hinderliter[2], and D. C. Wolf[2]
Syngenta Crop Protection, [1] Bracknell, United Kingdom and [2] Greensboro, NC, USA

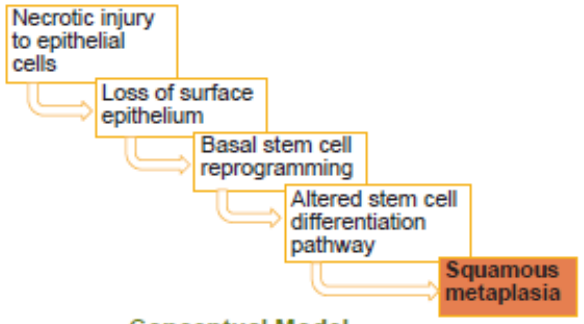
Abstract Number: 2377
Poster Board: 101

Background

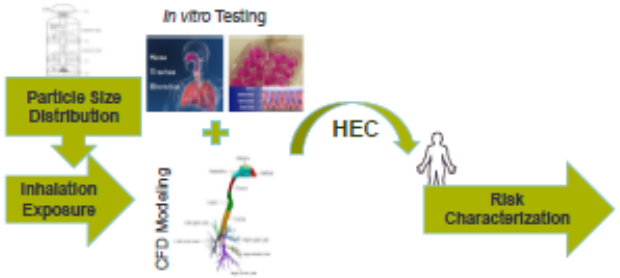
Chlorothalonil (CTN) is a broad spectrum fungicide that acts as a respiratory irritant when inhaled. A CTN containing formulation (Bravo 720®) was assessed for respiratory toxicity in a repeat dose inhalation toxicity study. This study showed a dose dependent increase in squamous metaplasia of the larynx at all doses tested. Additional animal studies to determine a no effect level would be unnecessary if a scientifically validated *in vitro* approach could be identified.

Syngenta developed such an alternative approach based upon an understanding of the mode of action that leads to squamous metaplasia of the larynx. A 3D *in vitro* model of respiratory epithelium was used to define the dose-response including a no effect level of the initial key event.

Adverse Outcome Pathway



Conceptual Model

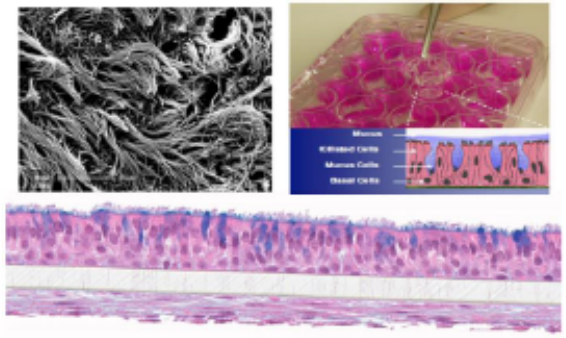


	Cell-cell interactions?	Representative tissue organisation?	Correlation with in vivo findings?
Immortalised cell monoculture	No	No	No
Primary cell monoculture	No	No	No
2D/3D cellular co-culture	Yes	Yes	No
Lung-on-a-Chip	Yes (Limited cell types)	No	No
MucilAir	Yes	Yes	Yes

MucilAir

MucilAir™ is a 3D model of the human airway epithelium formed from differentiated primary human cells. Syngenta has shown good concordance between MucilAir data and *in vivo* study outcomes. Measures of membrane and cell damage as markers of irritation selected were:

- Trans-epithelial electrical resistance (TEER) a decrease indicates a decrease in the integrity of tight junctions between cells in the membrane
- Lactate dehyrogenase (LDH) increased release indicates cellular cytotoxic membrane damage
- Resazurin metabolism Resazurin is metabolized to a fluorescent product in viable cells, decreased fluorescence indicates a decrease in metabolic competence

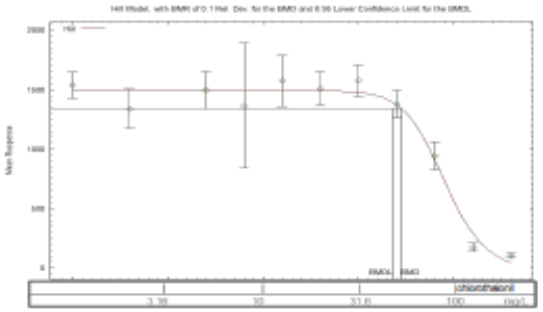


Study Design

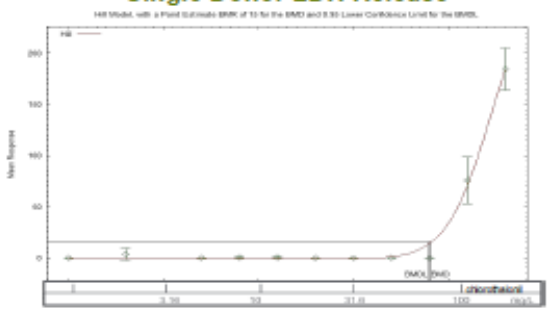
- Endpoints: TEER, LDH, and resazurin metabolism
- Tissues from 5 separate donors
- 24 hour topical exposure
- Chlorothalonil applied as Bravo 720 formulation
- 10 concentrations / donor
- 6 replicates / concentration / donor
- Concentration range 2 – 200 mg chlorothalonil/L

Data from each donor for each endpoint was fitted to a Hill model and benchmark dose analysis was conducted to derive benchmark dose lower 95% confidence interval (BMDL) values.

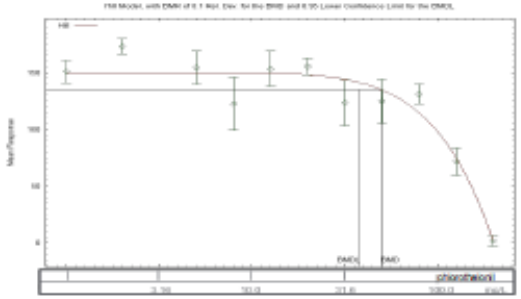
Single Donor TEER



Single Donor LDH Release



Single Donor Resazurin Metabolism



	BMDL (mg/cm²)			
	TEER	LDH	Resazurin	Mean
Donor 1	0.00430	0.00703	0.00344	0.00470
Donor 2	0.00317	0.00963	0.00224	0.00409
Donor 3	0.00822	0.00878	0.00400	0.00661
Donor 4	0.00937	0.00998	0.00066	0.00395
Donor 5	0.00909	0.00875	0.00674	0.00813
Mean	**0.00625**	**0.00877**	**0.00267**	**0.00527**

MucilAir assay data showed very low inter-donor variability with all 5 donors exhibiting similar BMD and BMDL values. Additionally, a good concordance between TEER, LDH release and resazurin metabolism endpoints was observed. Given the low inter-donor and inter-endpoint variability BMDL values were combined to derive an overall *in vitro* BMDL of 0.00527 mg chlorothalonil/cm² of the respiratory tract.

Conclusions

- Necrotic injury to the luminal surface of the respiratory epithelium is a key event in squamous metaplasia of the larynx in Chlorothalonil treated rats
- This initial irritant response can be assessed in MucilAir, a sophisticated 3D in vitro model of the human respiratory epithelium
- MucilAir tissues from 5 separate donors were exposed to 10 concentrations of Bravo 720 for a period of 24 hours
- TEER, LDH, and resazurin metabolism were measured following treatment and these results were interrogated to determine BMD and BMDL values
- BMDL values were in the 30 – 100 mg chlorothalonil /L range, translating to mass per unit surface area concentrations of 0.0027 - 0.0088 mg/cm2 (in vitro mean BMDL = 0.00527 mg/cm2)

Please see the application of this *in vitro* PoD on our companion poster #2379 (board 103).

For detailed information on study designs, please contact the authors: Alex.Charlton@syngenta.com

syngenta

https://www.epa.gov/sap/meeting-materials-december-4-7-2018-scientific-advisory-panel-0

charles river

An Alternative Approach For Evaluating The Human Health Risk From Exposure To An Irritant Aerosol

P. M. Hinderliter[1], R. Corley[2], S. Kabilan[2], A. Kuprat[2], S. Suffield[2], S. Flack[1], T. Ramanarayanan[1], A. J. Charlton[3], B. Parr-Dobrzanski[3], D. C. Wolf[1]
[1]Syngenta Crop Protection, Greensboro, NC, [2]Pacific Northwest National Laboratory, Richland, WA, [3]Syngenta Crop Protection, Bracknell, United Kingdom

Abstract Number: 2379
Poster Board: 103

Introduction

Modernizing strategies for evaluating human health risk from pesticide exposure requires new approaches that increase our ability to conduct an integrated evaluation of exposure and hazard. Accurate and relevant risk evaluation based on actual inhalation exposure scenarios and target site-specific respiratory surface concentrations is one such strategy to describe human health risks. Exposure data that includes measured aerosol characteristics of non-volatile pesticide formulations provides an improved input to exposure models. Coupling an airflow model with a 3D in vitro model of respiratory epithelium was used to determine a Human Equivalent Concentration. The in vitro assay was used to define the dose-response and a no effect level of the initial key event (see Abstract 2377, board 101 of this session for details of the *in vitro* assay).

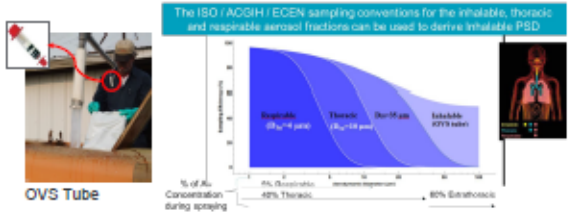
Computational Fluid Dynamic models can be used to describe target site-specific dosimetry for the rat and human and can calculate surface concentrations of deposited aerosol formulations in discrete regions of the respiratory tract. This approach can provide a more accurate reflection of the deposition necessary to initiate the cascade of events that result in an irritant mediated response in the upper respiratory tract.

Source to Outcome Approach

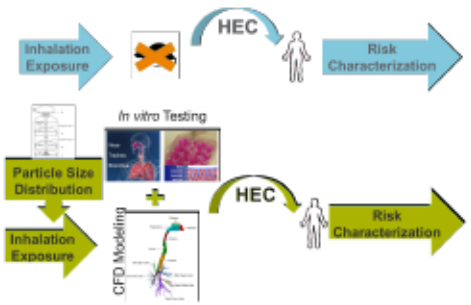
- ***Source*** Evaluate the particle size distribution of pesticide application and mixing/loading activities.
- ***Exposure*** Investigate the impact of spray quality and activity on inhalation exposure.
- ***Dosimetry*** Compare deposition in human versus rat airways.
- ***Outcome*** Predict human inhalation toxicity incorporating relevant particle size distributions.

Pesticide Exposure Data

- Exposure data is commonly collected from agricultural workers using an OSHA Versatile Sampler (OVS) tube. Typically, OVS tube data is only reported as total concentration, without consideration of particle size.
- Studies of spray particle size were undertaken at Syngenta to compare OVS tube with standard sizing methods. Side-by-side air sampling was conducted using OVS tube and Respicon for various agricultural nozzles/spray qualities.
- Air concentration of inhalable particles varies with spray quality, but particle size distributions are similar. Thus, can use same distribution for different spray applications.
- Identified particle size distribution of 35 µm (GSD=1.5) for applicator & residential bystander and 13 µm (GSD = 1.5) for mixer/loader

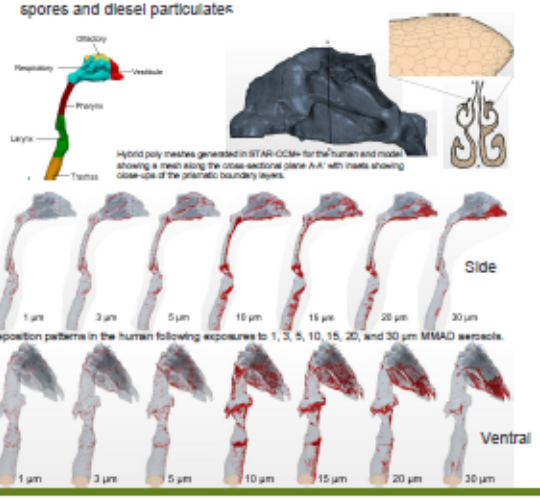


Conceptual Model



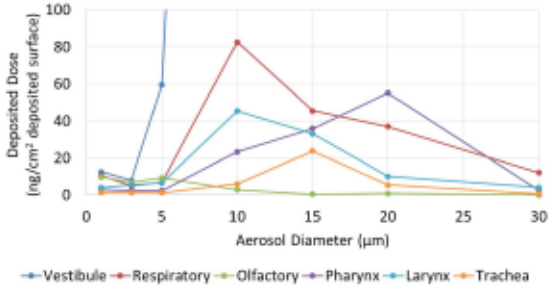
Computational Fluid Dynamics (CFD) Modeling

- Simulates the fluid dynamics of the airflow in the respiratory tract
- Surfaces are meshes of polygons, each of which can be monitored
- Commonly used in simulation of smaller particulates like bacteria spores and diesel particulates



Deposition from the CFD model – Human Simulation

Regional Dose: Top 25% deposited surface



Aerosol Diameter (µm)	Deposition (mg/cm2)					
	Vestibule	Respiratory	Olfactory	Pharynx	Larynx	Trachea
1	4.29E-06	3.04E-06	5.22E-06	1.71E-06	2.16E-06	7.34E-07
3	3.39E-06	2.43E-06	4.55E-06	1.26E-06	2.48E-06	7.69E-07
5	5.73E-06	2.86E-06	1.26E-05	1.48E-06	3.08E-06	6.36E-07
10	1.62E-04	4.50E-06	1.77E-06	5.41E-06	1.40E-05	1.30E-06
15	2.91E-04	2.90E-06	9.73E-07	3.49E-06	8.46E-06	1.39E-06
20	2.76E-04	2.27E-06	6.50E-07	1.85E-06	2.67E-06	5.57E-07
30	1.50E-04	1.89E-06	-	5.61E-07	1.03E-06	2.13E-07

Calculation of Human Equivalent Concentration - Applicators

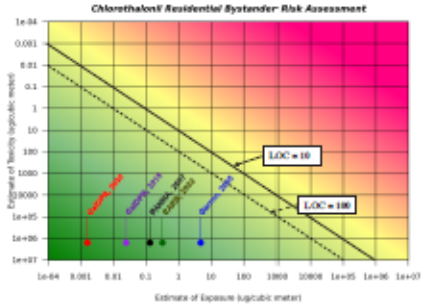
- Aerosol: 35 (1.5) µm polydisperse
- Breathing rate: 16.4 breaths/minute or 7872 breaths/8hr
- Dilution Factor 0.438 lb CTN/lb diluted formulation
- *In vitro* BMDL 5.27×10^{-3} mg CTN/cm²

	Respiratory	Olfactory	Pharynx	Larynx	Trachea
Deposition (mg/cm²/breath)	2.00E-06	1.41E-07	8.82E-07	1.61E-06	3.12E-07
Deposition (mg/cm²/8hr)	6.89E-04	4.85E-05	3.04E-04	5.54E-04	1.08E-04
HEC (mg/L)	**0.34**	**4.8**	**0.76**	**0.42**	**2.1**

- The exposure concentration is the air concentration necessary to match the *in vivo* 8 hour deposition with the *in vitro* BMDL

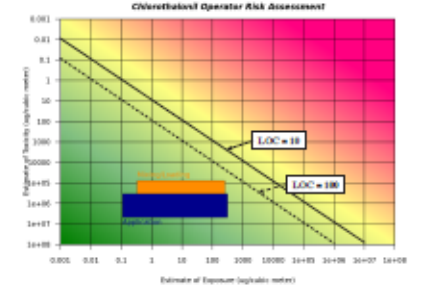
Residential Bystander Risk Assessment

- **Max Average Exposure Range** = 0.002 – 4.86 µg/m³
- **Toxicity (HEC) Range (mg/L converted to mg/m³)** = 112 – 1,590 mg/m³



Operator Risk Assessment

- **Exposure Ranges:**
  - Applicators = 0.11 – 313 µg/m³; Mixer/Loader = 0.33 – 259 µg/m³
- **Toxicity (HEC) Ranges (mg/L converted to mg/m³):**
  - Applicator = 335 – 4,760 mg/m³; Mixer/Loader = 77 – 616 mg/m³



For detailed information on study designs, please contact the authors: paul.hinderliter@syngenta.com
Current address for R. Corley: Greek Creek Toxicokinetics Consulting, LLC rcorley.gctc@gmail.com

OCCUPATIONAL TOXICOLOGY USING MUCILAIR™

Calculation of Human Equivalent Concentration - Applicators

- Aerosol: 35 (1.5) µm polydisperse
- Breathing rate: 16.4 breaths/minute or 7872 breaths/8hr
- Dilution Factor 0.438 lb CTN/lb diluted formulation
- *In vitro* BMDL 5.27×10^{-3} mg CTN/cm^2

	Respiratory	Olfactory	Pharynx	Larynx	Trachea
Deposition (mg/cm^2/breath)	2.00E-06	1.41E-07	8.82E-07	1.61E-06	3.12E-07
Deposition (mg/cm^2/8hr)	6.89E-04	4.85E-05	3.04E-04	5.54E-04	1.08E-04
HEC (mg/L)	**0.34**	**4.8**	**0.76**	**0.42**	**2.1**

- The exposure concentration is the air concentration necessary to match the *in vivo* 8 hour deposition with the *in vitro* BMDL

HOW DOES THE RAT 3D MODEL COMPARE WITH THE HUMAN 3D MODEL AND THE *IN VIVO* DATA?

TEST PANEL OF 14 CATEGORISED RESPIRATORY IRRITANT/ NON IRRITANTS WITH HUMAN AND RAT EPIAIRWAY™

Human and rat EpiAirway™ units were treated with 14 test chemicals formulated in either corn oil or ultrapure water at each of 4 different concentrations with vehicle and positive (formaldehyde) controls.

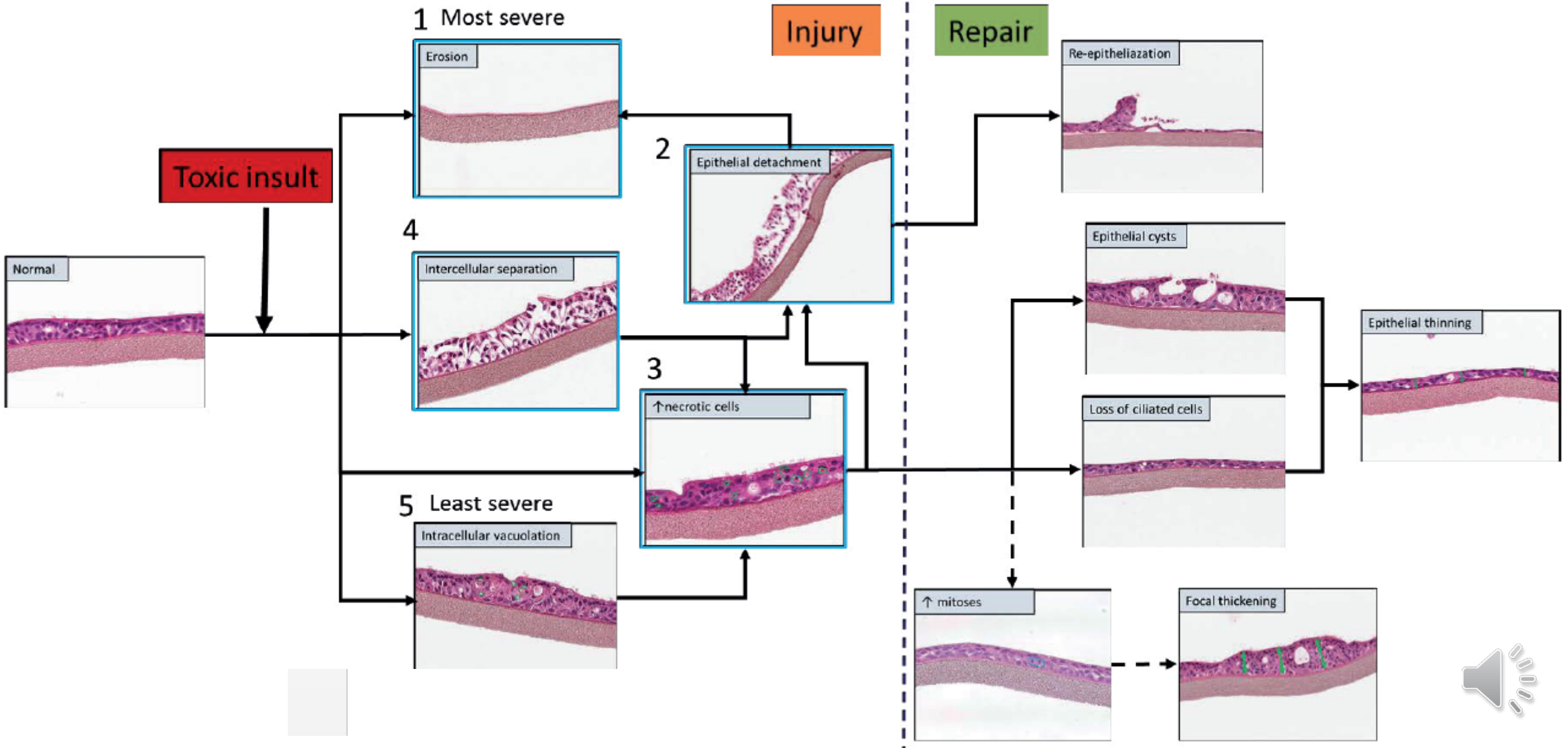
Experimental conditions and study design were similar except the tissues were rinsed with PBS at 3 h post dose and allowed to recover for 21 h.

TEER, MTT and histopathology were the endpoints.

2 Laboratories

- MatTek Corp., Ashland, MA, USA
- Charles River, Edinburgh, UK

ADVERSE OUTCOME PATHWAY
EFFECT OF TOXIC INSULT ON EPIAIRWAY™



TEST PANEL OF 14 CATEGORISED RESPIRATORY IRRITANT/ NON IRRITANTS WITH HUMAN AND RAT EPIAIRWAY™ (IC_{75})

	Charles River Edinburgh				MatTek						
	MTT		TEER		MTT		TEER		Known Toxicity Information**		
Test Chemical	Human	Rat	Human	Rat	Human	Rat	Human	Rat	GHS Category	Known RI*	Skin/Eye Irritant
Acrolein	0.05	0.06	0.04	0.05	0.04	0.03	0.03	0.03	1	Y	Y
Formaldehyde	0.64	0.81	0.81	0.32	0.17	0.13	0.20	0.18	3	Y	Y
NaOH	1.11	1.15	1.63	1.10	0.73	0.73	0.88	0.31	No data	Y	Y
Butyl Amine	1.34	4.56	1.39	3.16	0.92	1.07	0.89	0.51	3	Y	Y
Oxalic Acid	1.22	1.16	1.39	0.48	1.16	0.67	0.88	0.27	No data	Y	Y
Morpholine	7.90	18.3	9.13	19.0	10.6	16.2	11.8	16.2	3	Y	Y
Vinyl Acetate	56.2	47.2	52.4	33.0	21.3	18.8	16.1	10.1	4	Y	N
Ethyl Formate	190	199	152	161	109	115	63.2	62.4	4	Y	N
2-Ethoxyethyl Acetate	174	134	143	75.7	117	72.6	57.5	57.3	4	N	N
Methyl Methacrylate	146	41.3	126	28.9	132	29.9	130	14.9	5	Y	N
Dimethyl acetamide	265	286	271	303	266	221	263	147	4	N	N
N,N-Dimethylformamide	269	281	304	291	311	285	309	229	4	N	N
Ethyl Alcohol	304	242	338	142	320	217	306	140	5	Y	N
p-Dichlorobenzene	251	221	242	233	346	240	358	235	5	N	N

TEST PANEL OF 14 CATEGORISED RESPIRATORY IRRITANT/ NON IRRITANTS WITH HUMAN AND RAT EPIAIRWAY™ (IC_{75})

	Charles River Edinburgh				MatTek						
	MTT		TEER		MTT		TEER		Known Toxicity Information**		
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Vinyl Acetate	56.2	47.2	52.4	33.0	21.3	18.8	16.1	10.1	4	Y	N
Ethyl Formate	199	132	161	109	119	63.4	4	Y	N		
2-Ethoxyethyl Acetate	174	134	143	75.7	117	72.6	57.5	57.3		N	N
Methyl Methacrylate	146	41.3	126	28.9	132	29.9	130	14.9	5	Y	N
Dimethyl acetamide	265	286	271	303	266	221	263	147	4	N	N
N,N-Dimethylformamide	269	281	304	291	311	285	309	229	4	N	N
Ethyl Alcohol	304	242	338	142	320	217	306	140		Y	N
p-Dichlorobenzene	231	242	233	346	340	255	5	N	N		

TEST PANEL OF 14 CATEGORISED RESPIRATORY IRRITANT/ NON IRRITANTS WITH HUMAN AND RAT EPIAIRWAY™ (IC_{75})

	Charles River Edinburgh				MatTek						
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Oxalic Acid	1.22	1.16	1.39	0.48	1.16	0.67	0.88	0.27	No data	Y	Y
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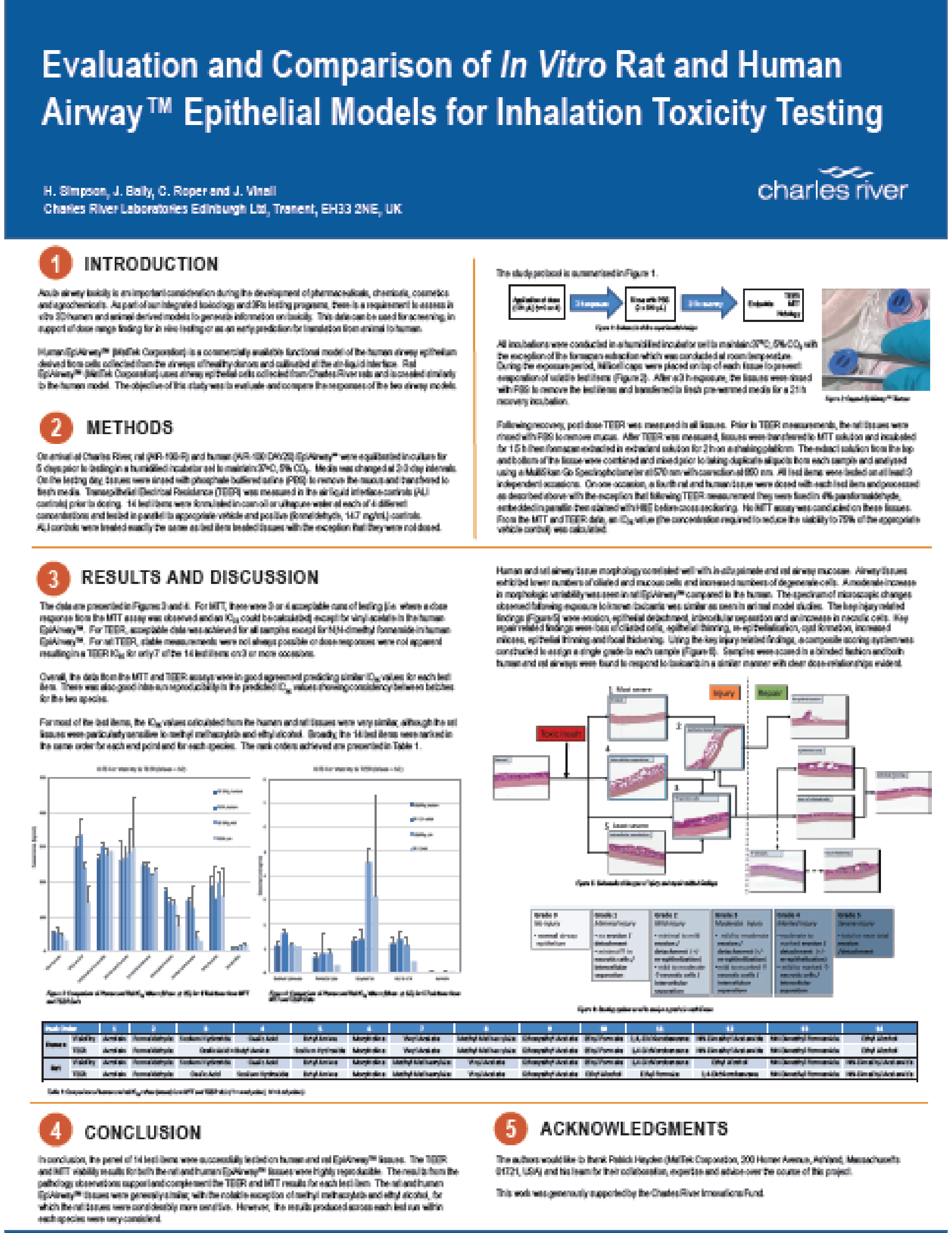
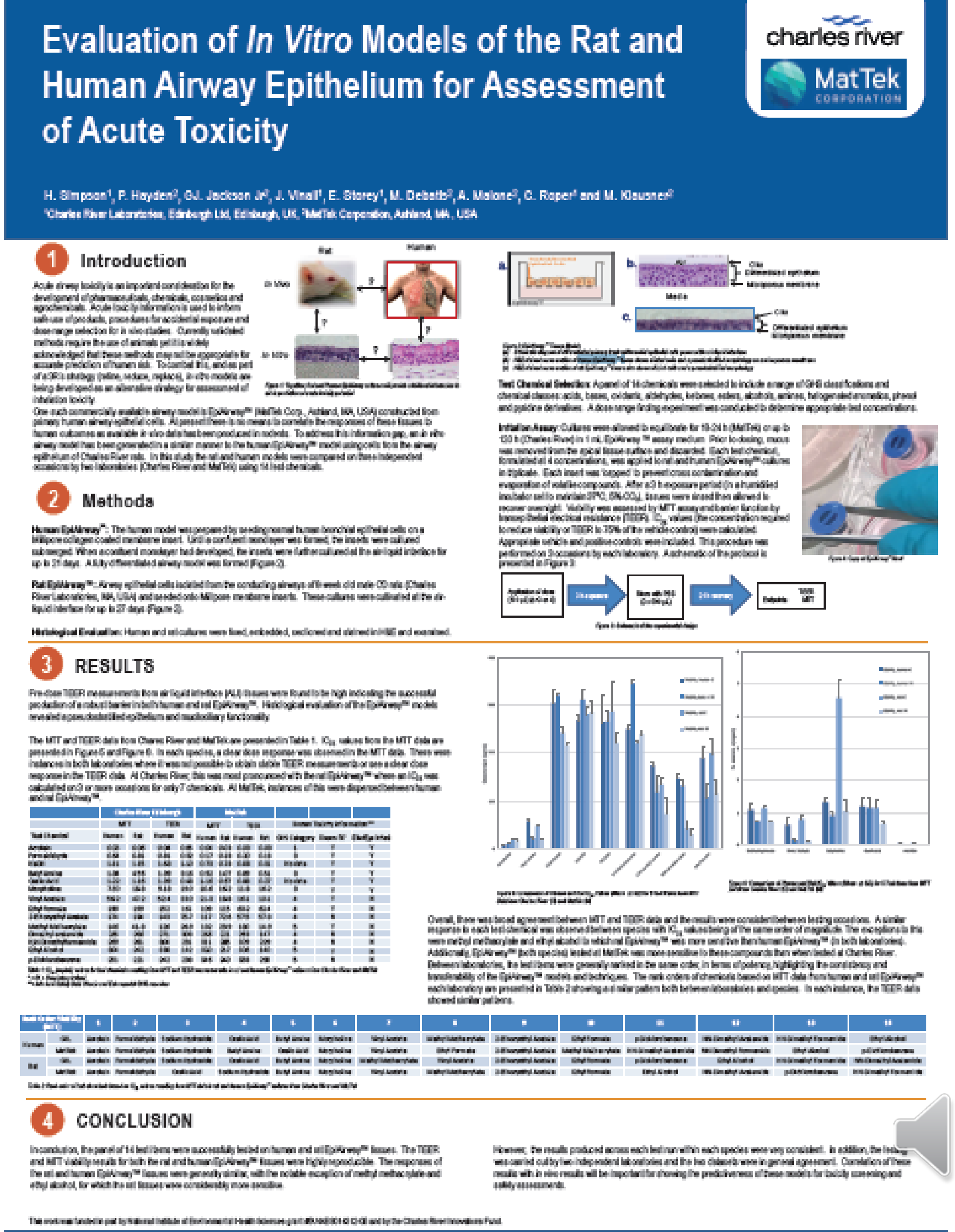
Dimethyl acetamide	265	286	271	303	266	221	263	147	4	N	N
N,N-Dimethylformamide	269	281	304	291	311	285	309	229	4	N	N
Ethyl Alcohol	304	242	338	142	320	217	306	140	5	Y	N
p-Dichlorobenzene	251	221	242	233	346	240	358	235	5	N	N


Evaluation and Comparison of In Vitro Rat and Human Airway™ Epithelial Models for Inhalation Toxicity Testing
H. Simpson, J. Baily, C. Roper and J. Vinall
Charles River Laboratories Edinburgh Ltd, Tranent, EH33 2NE, UK
charles river
1 INTRODUCTION
2 METHODS
3 RESULTS AND DISCUSSION
4 CONCLUSION
5 ACKNOWLEDGMENTS

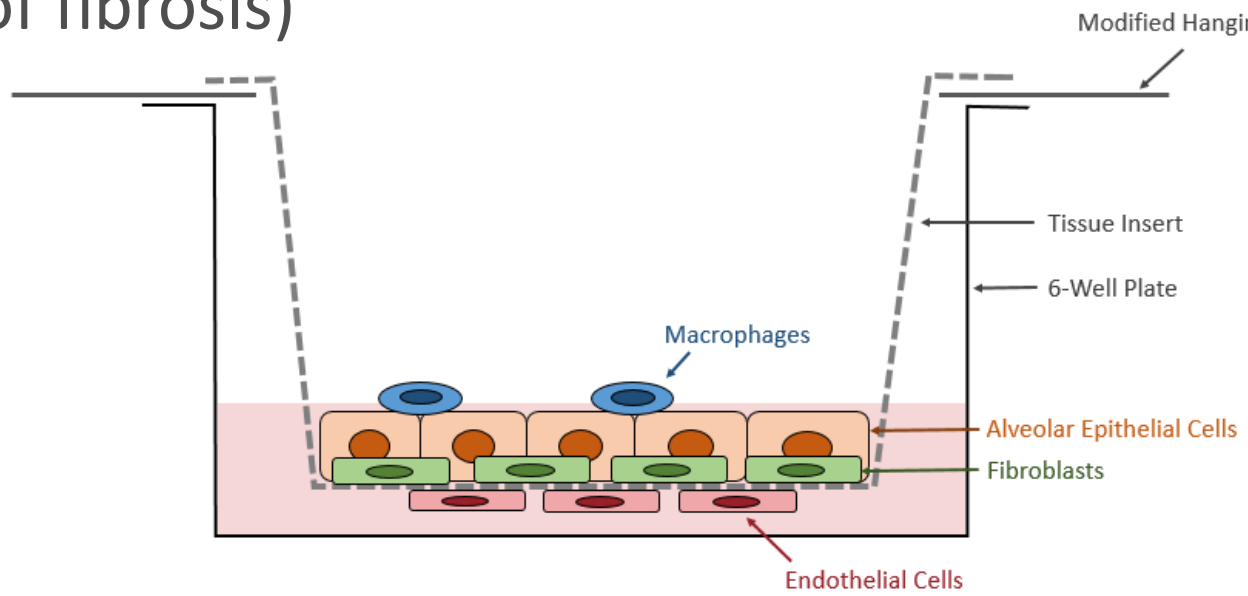

Evaluation of In Vitro Models of the Rat and Human Airway Epithelium for Assessment of Acute Toxicity
charles river
MatTek CORPORATION
H. Simpson1, P. Hayden2, G.J. Jackson Jr2, J. Vinall1, E. Storey1, M. Debatt2, A. Malone2, C. Roper1 and M. Klausner2
1Charles River Laboratories, Edinburgh Ltd, Edinburgh, UK, 2MatTek Corporation, Ashland, MA, USA
1 Introduction
2 Methods
3 RESULTS
4 CONCLUSION

HOW CAN WE USE THE ALVEOLAR MODELS?

ALVEOLAR TOXICITY AND FIBROTIC POTENTIAL

MODEL RESPIRATORY FIBROTIC AGENT: TGF-β1

- TGF-β1 dosed at the liquid interface at 1, 5 and 10 ng/mL
- Media containing TGF-β1 was changed every 2 days, exposure to 14 days
- TEER measured every 2 days prior to media change
- On Days 2, 6, 10 and 14, tissues were processed for histopathology
- The spent media analyzed by ELISA for fibronectin, IL-6 and Pro-Collagen I α1 (biomarkers of fibrosis)



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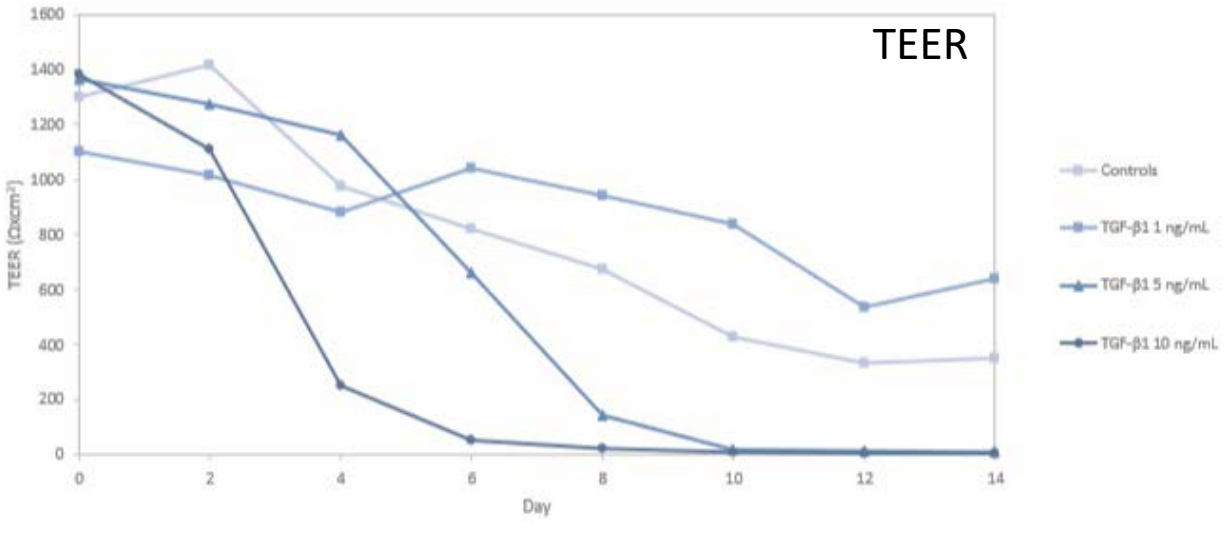
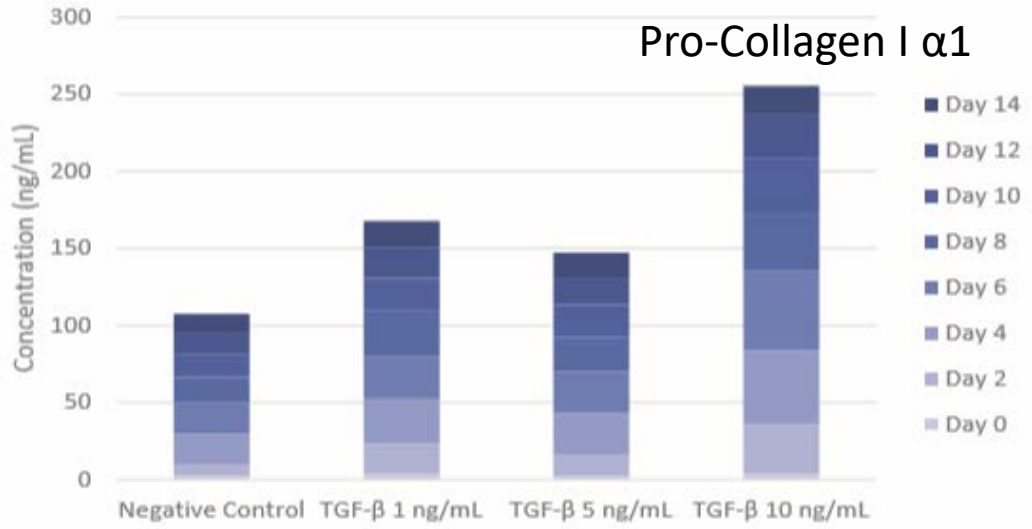
ALVEOLAR TOXICITY AND FIBROTIC POTENTIAL

MODEL RESPIRATORY FIBROTIC AGENT: TGF-β1

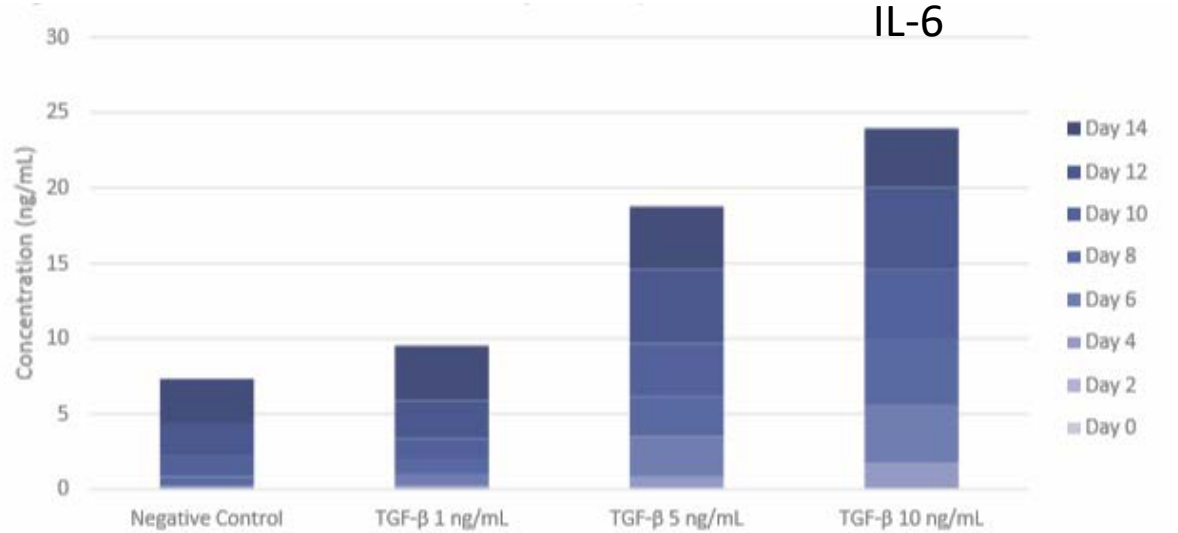
TEER



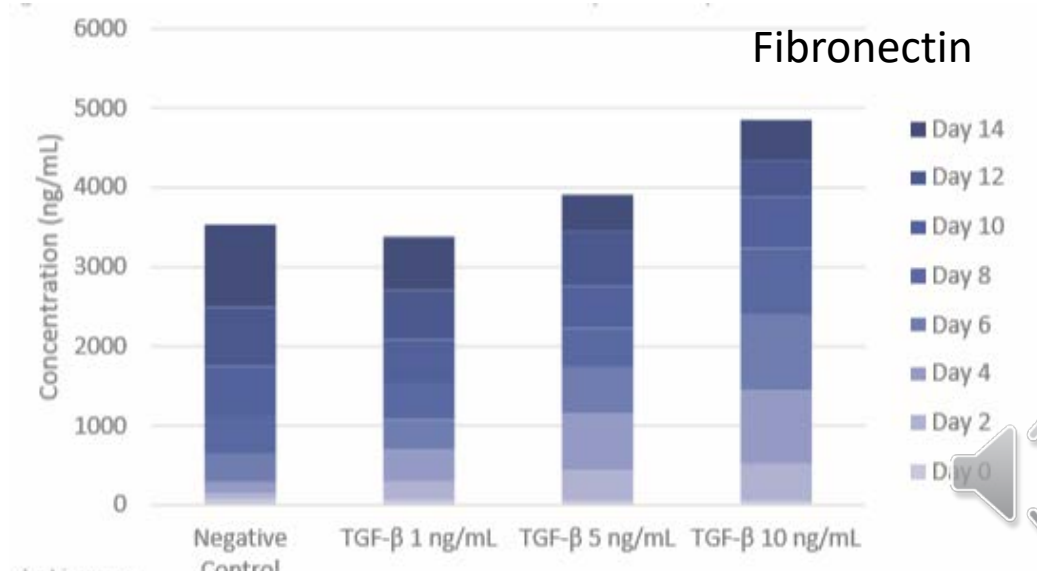
Pro-Collagen I α1



IL-6



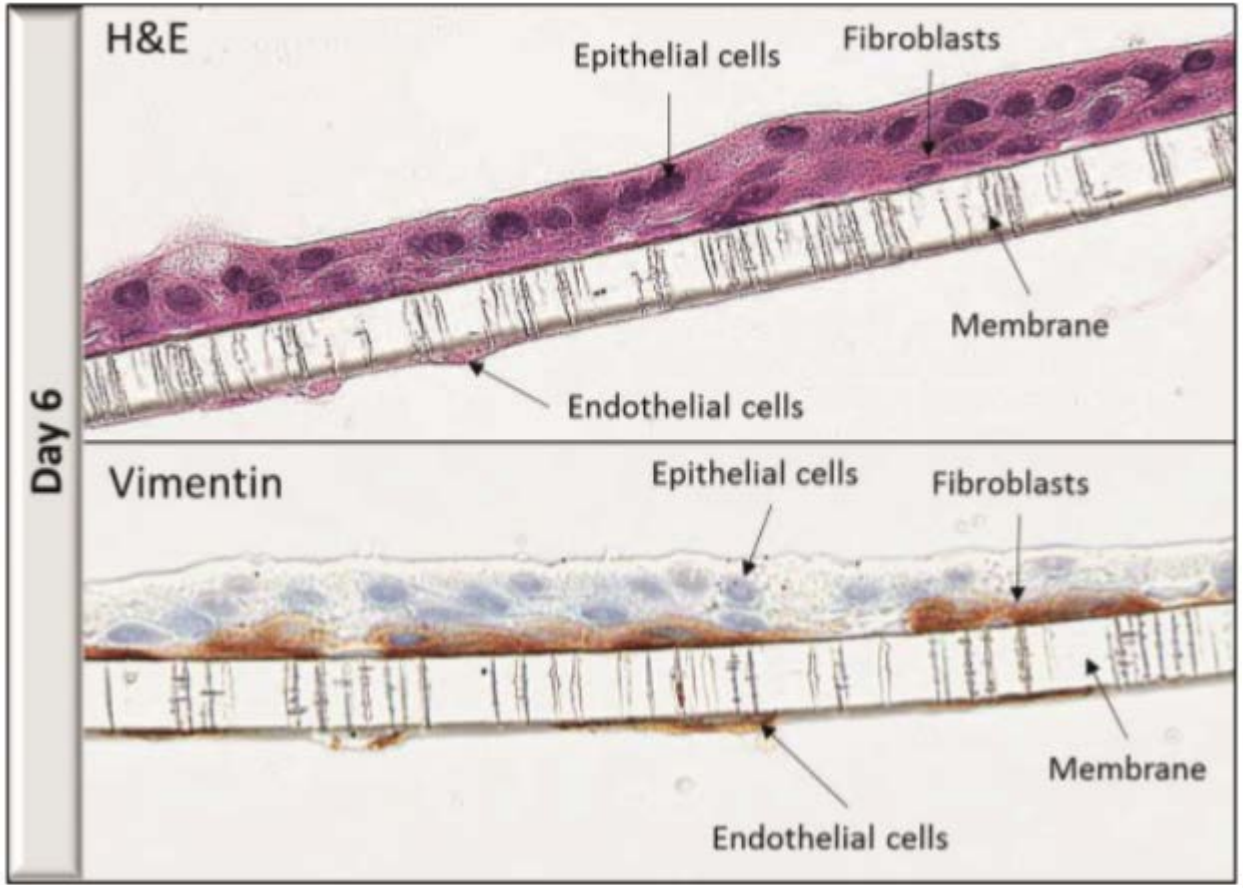
Fibronectin

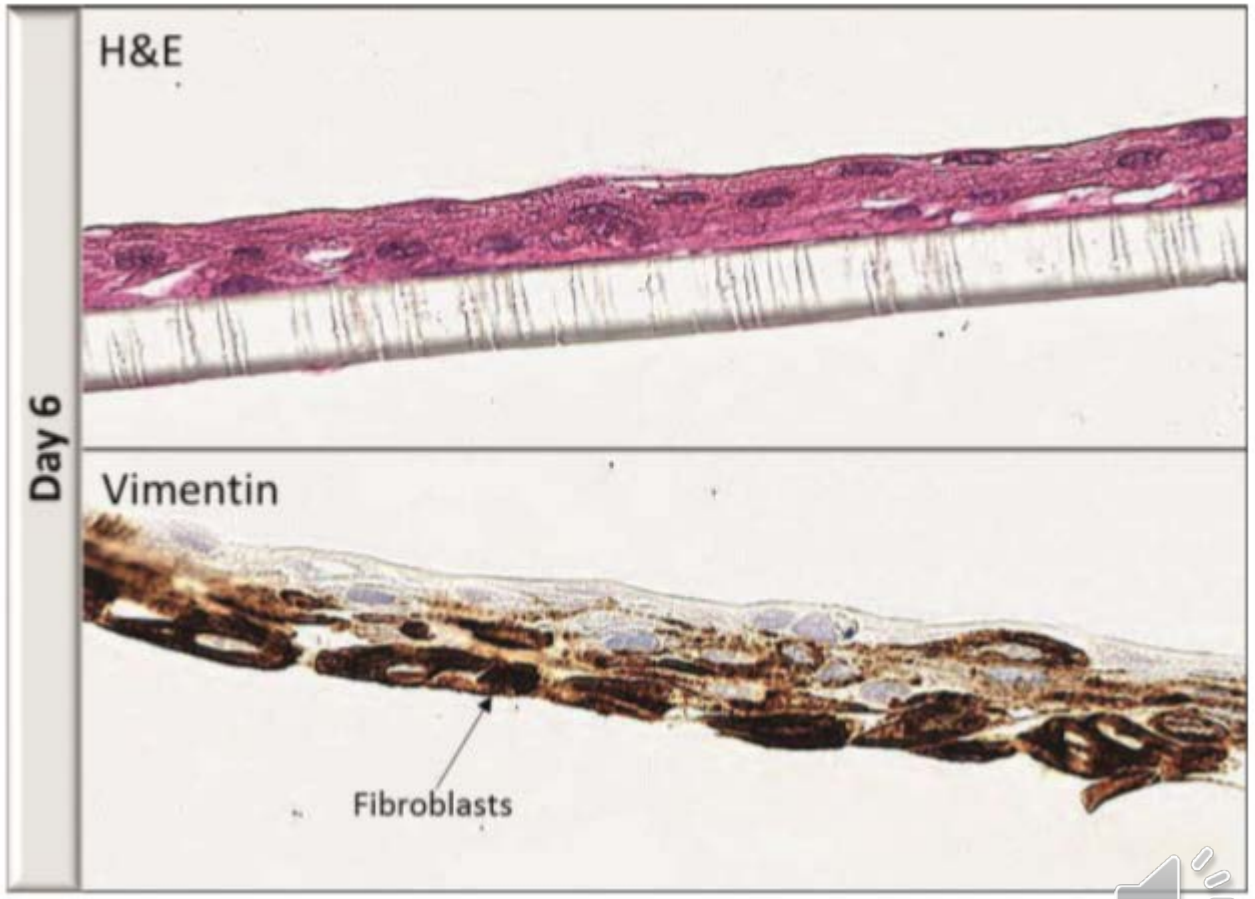


ALVEOLAR TOXICITY AND FIBROTIC POTENTIAL

MODEL RESPIRATORY FIBROTIC AGENT: TGF-β1

Representative Images of Untreated and TGF-β1 Treated Samples on Day 6





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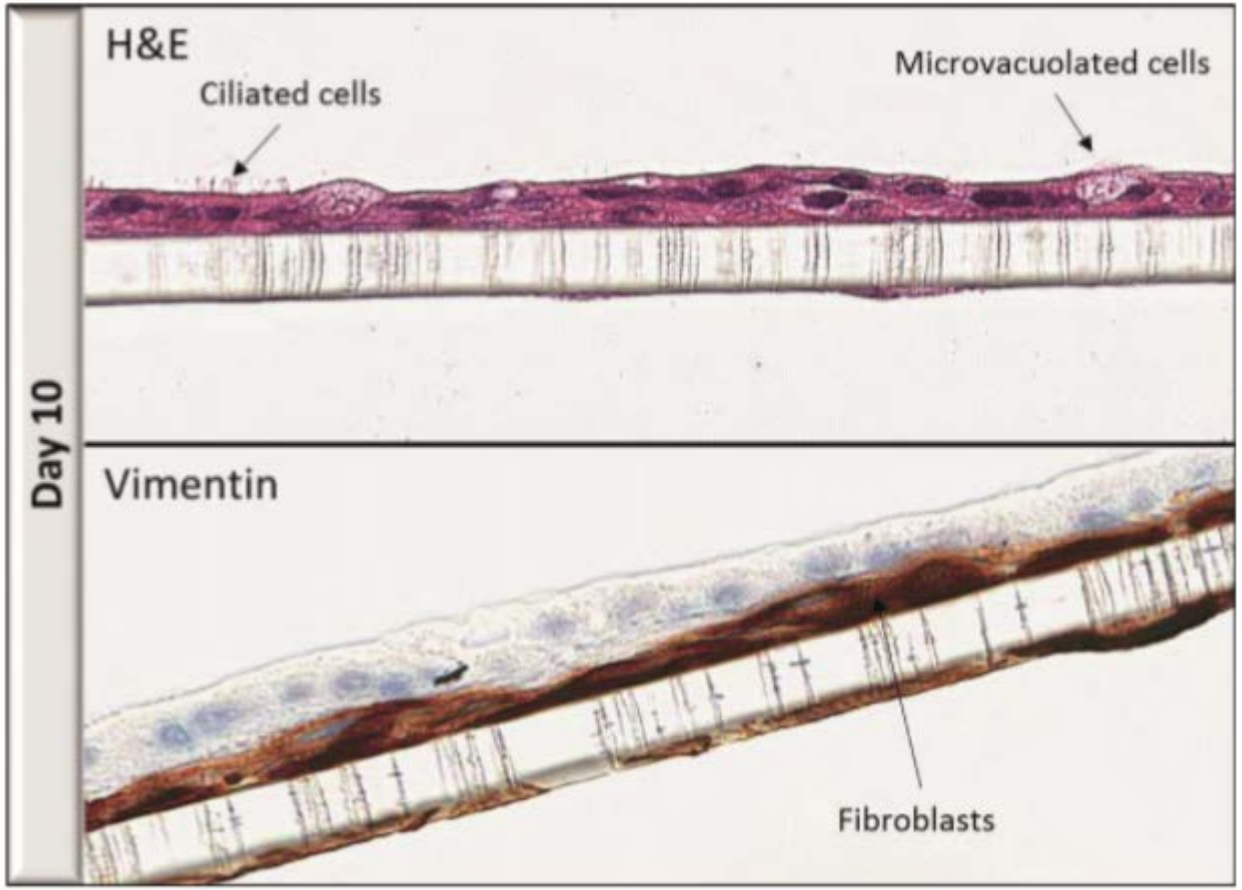
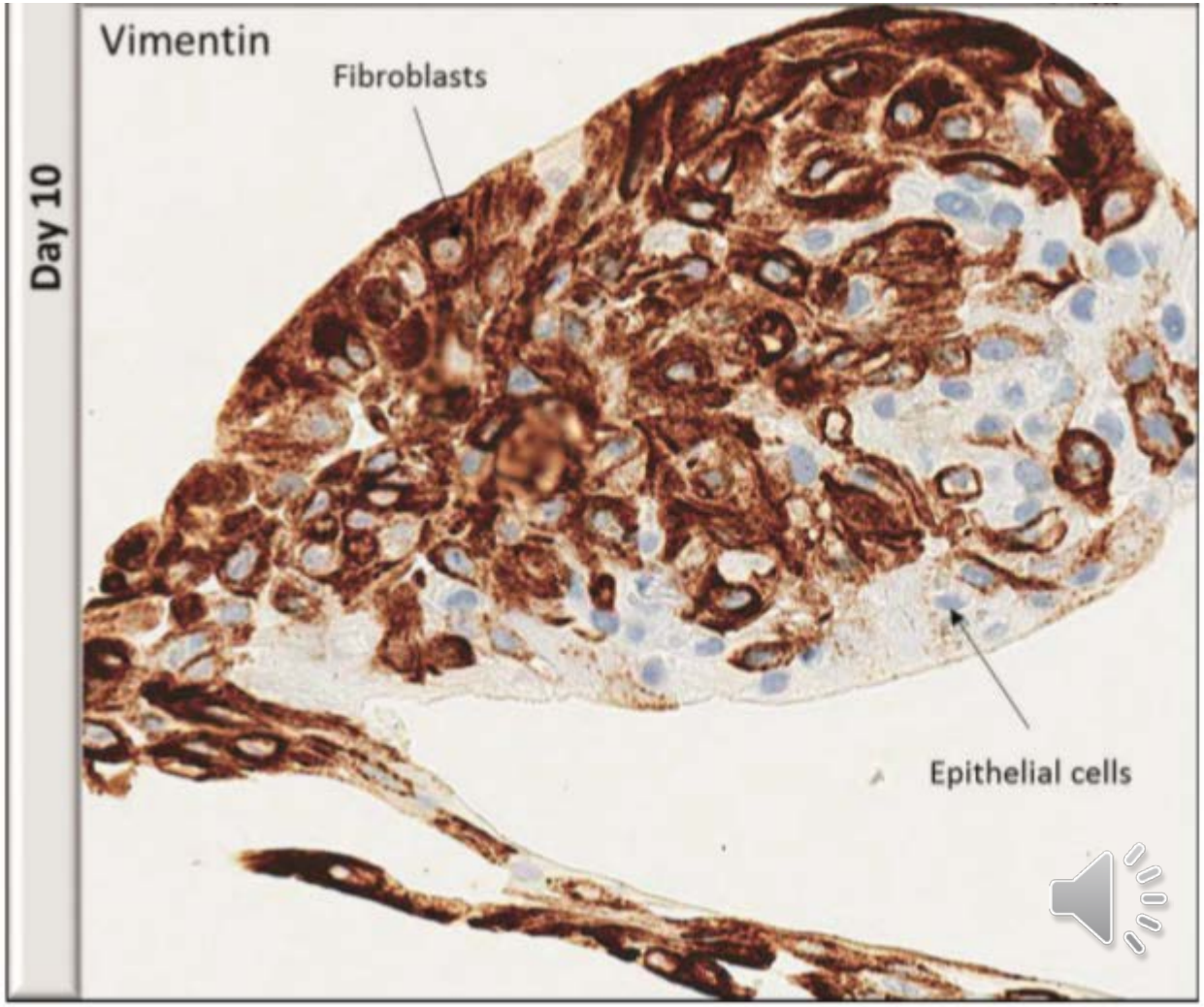
ALVEOLAR TOXICITY AND FIBROTIC POTENTIAL

MODEL RESPIRATORY FIBROTIC AGENT: TGF-β1

Representative Images of Untreated and TGF-β1 Treated Samples on Day 10





Assessment of Alveolar Toxicity and Fibrotic Potential *In Vitro* using the MatTek EpiAlveolar™ Model

CS Roper, JE Baily, HJ Simpson and JL Vinall
Charles River Laboratories, Edinburgh, UK

charles river
MatTek CORPORATION
Unilever

1 INTRODUCTION

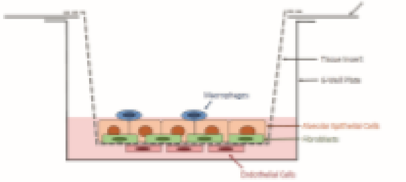
Pulmonary fibrosis (PF) is a debilitating, typically fatal, condition caused by a variety of factors including environmental or occupational exposures, drugs, radiation and genetic predisposition. No current *in vitro* (immortalized cell lines), *ex vivo*, *in silico* or *in vivo* models of PF fully recapitulate all salient features of the human disease.

In this study, a novel *in vitro* organotypic 3D airway model from primary human cells (EpiAlveolar™ from MatTek Corporation) with macrophages was examined to evaluate alveolar toxicity and the capacity of the model as a method to assess fibrotic potential *in vitro*. Previously, this model has been shown to behave in a manner reflecting the parent tissue and under some experimental conditions can develop aspects of PF seen in the lower respiratory tract[1].

The study design was based on the protocol provided by MatTek[2]. EpiAlveolar™ tissues were challenged to develop aspects of PF, by exposure to known PF causing agents; bleomycin (12, 0.12 or 0.0012 µg/mL) or TGF-β1 (10, 5 or 1 µg/mL) in the culture media, or to repeated aerosol applications of silica (50 nm particle size, *ca* 10 or 1 µg/cm²), for up to a 14 day period. Tissue viability was assessed by transepithelial electrical resistance (TEER) and lactate dehydrogenase (LDH) release every second day. Tissue samples and spent culture media were collected throughout the time course for analysis by pathology, immunohistochemistry (IHC) and ELISA to further characterise the healthy model and to identify aspects of PF developing over time.

2 MATERIALS AND METHODS

Figure 1: Schematic of EpiAlveolar™ Tissues in Culture



EpiAlveolar™ is a novel commercially available functional model of human alveoli, derived from primary human alveolar epithelial cells, pulmonary endothelial cells, fibroblasts and monocyte-derived macrophages (THP-1s), cultured at the air-liquid interface (Figure 1).

Tissue viability was assessed by TEER and LDH release assays on Day 0. Following this, a group of untreated tissues were processed for histology/ pathology. In addition, 11 tissues per treatment group were treated on Day 0 (either aerosol exposure then incubation in blank media for 2 days or incubation in media containing doses for 2 days). The following groups were tested:

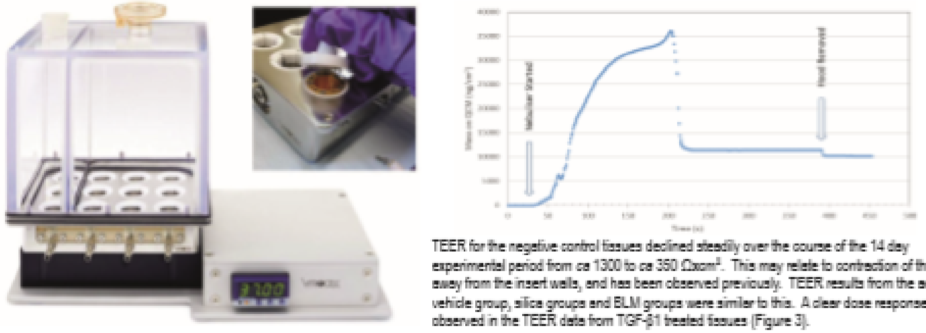
- Silica nanospheres (50 nm) aerosol application at *ca* 1 µg/cm² and *ca* 10 µg/cm² doses
- Bleomycin sulfate (BLM) at 12 (0.1 Unit), 0.12 (1 mU) and 0.0012 µg/mL (0.01 mU)
- TGF-β1 at 10, 5 and 1 ng/mL
- Controls: vehicle control for aerosol and negative control (control for BLM /TGF-β1).

Aerosol exposure was conducted using a saline vehicle in the Vitrocell Cloud 12 exposure chamber with associated quartz crystal microbalance (QCM) for assessment of applied dose (Figure 2).

Dosing was repeated every 2 days. The TEER and LDH measurements were repeated after every 2 day incubation. On Day 2, Day 6 and Day 10, 2 tissues per group were processed for histology/ pathology. Five tissues per group underwent the full 14 day regime, at which point all remaining tissues were processed for histology/ pathology. Spent culture media at each time point was retained for ELISA analysis (2 tissues that underwent 14 day treatment per group) for the following markers: fibronectin, IL-6 (kits obtained from R&D Systems) and Pro-Collagen I α1 (Abcam).

3 RESULTS

Figure 2: Vitrocell Cloud12 Chamber and QCM Balance and Representative QCM Trace for Aerosol Silica (10 µg/cm² Dose)



TEER for the negative control tissues declined steadily over the course of the 14 day experimental period from *ca* 1300 to *ca* 350 Ω.cm². This may relate to contraction of the tissues away from the insert walls, and has been observed previously. TEER results from the aerosol vehicle group, silica groups and BLM groups were similar to this. A clear dose response was observed in the TEER data from TGF-β1 treated tissues (Figure 3).

Compared to the untreated control, the 5 and 10 ng/mL TGF-β1 treated groups TEER dropped to close to zero by Day 10 and Day 6, respectively. LDH release each day was compared to an independent set of Triton X-100 lysed tissues prepared daily and calculated as a percentage of that value. Generally, compared to the lysed controls, the levels of LDH released from all samples were negligible. However, on Day 6 and Day 8, a small increase in released LDH was measured in all TGF-β1 groups, silica (1 µg/cm²) and the top BLM dose group (data not shown). These data indicate that the TGF-β1 treated tissues, in particular, are being put under stress, manifesting as a reduction in tight junction integrity (TEER) and leaking of some cell contents, peaking between 6 and 8 days into the treatment regime. However, the LDH released did not approach lysed cell values, indicating that the majority of the cells remained alive. A clear dose response was measured for secreted extracellular matrix proteins, Pro-Collagen I α1 and Fibronectin and the inflammatory marker, IL-6, from the TGF-β1 treated groups over time (Figures 4-6). Pro-Collagen I α1 secretion was also analysed for the other treatment groups with no clear patterns or dose responses apparent.

Figure 3: TEER Following TGF-β1 Treatment for up to 14 Days

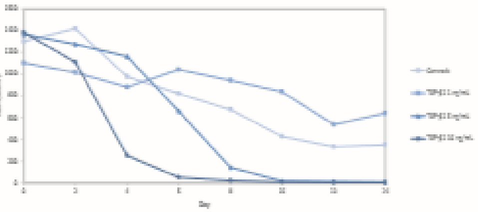


Figure 4: Cumulative Pro-Collagen I α1 Secretion Over 14 Days of TGF-β1 Treatment *

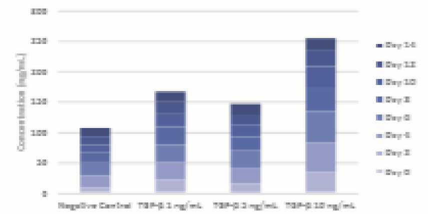


Figure 5: Cumulative IL-6 Secretion Over 14 Days of TGF-β1 Treatment *

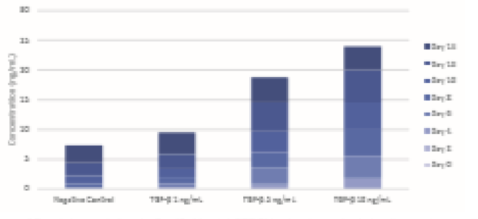
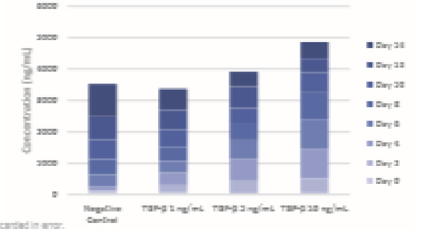


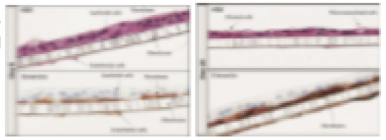
Figure 6: Cumulative Fibronectin Secretion Over 14 Days of TGF-β1 Treatment *



* Secreted concentrations for Day 12 of 1 ng/mL TGF-β1 treatment are estimated, as the samples were discarded in error.

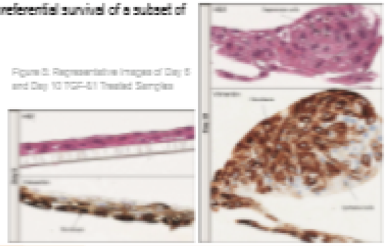
4 PATHOLOGY AND IMMUNOHISTOCHEMISTRY

Figure 7: Representative Images of Day 6 & Day 10 Untreated Samples



<u>Model Characterisation</u> (Figure 7)
Up to Day 6, untreated controls displayed consistent morphology with a single deep layer of vimentin (VM) positive spindle shaped fibroblasts covered by a layer of 2 to 3 cells thick of polygonal VM negative epithelial cells. Rare ciliated cells and microvacuolated cells were observed in the surface layer. Degenerate cells were also rare and occasional VM positive endothelial cells were observed on the underside of the membrane. From Day 10, there was overall thinning of the epithelial cell layer with increased numbers of VM positive cells and surface layer ciliated and microvacuolated cells. This suggests a change in phenotype between Day 6 and Day 10 characterised by proliferation of the fibroblast subpopulation and proliferation/ preferential survival of a subset of airway epithelial cells included in the initial population.

Figure 8: Representative Images of Day 6 and Day 10 TGF-β1 Treated Samples



<u>TGF- β1 Treatment</u> (Figure 8)
By Day 6 of TGF- β1 (10 ng/mL) treatment, the overall thickness of the cell layer was similar to untreated controls, however, the majority of the cell population expressed a spindle shaped VM positive fibroblast phenotype. From Day 10, samples exhibited multifocal thickening and, in places, protuberant foci composed of multiple layers of VM positive fibroblasts mixed with lesser VM negative epithelial cells. Increased numbers of degenerate cells were observed in these samples.

5 DISCUSSION AND CONCLUSION

Silica and BLM treatments at the selected dose levels did not show clear patterns in their responses and were generally similar to their respective controls, indicating that the 14 day challenge with these materials did not result in toxicity or induce markers of PF in the EpiAlveolar™ tissue model. However, LDH release (cytotoxicity) increased slightly after approximately 6 days of treatment for all TGF-β1 dose levels. TGF-β1 treated tissues also displayed a dose response reduction in TEER (tight junction integrity) around the same time point and increased secreted Pro-Collagen I α1 over time (precursor of collagen type 1, for which production and accumulation is the hallmark of PF). This data correlates well with the histopathology observations. Lower TEER values and increased Pro-Collagen I α1 are likely due to the change from a predominantly epithelial to fibroblast phenotype whilst increased LDH is likely a result of increased numbers of degenerate cells observed from Day 6 of TGF-ß treatment.

These data suggests that repeat dosing of EpiAlveolar™ via both apical (aerosol) and basal routes can be successfully performed over a 14 day period and that this would be a valuable model to identify alveolar toxicological end-points. Furthermore, these data indicate that while TGF-β1 treatment for 14 days has not caused large scale toxicity, it has induced a number of markers of PF in the still viable cells. Therefore, the model has the potential be used to identify PF causing agents and new PF treatments whilst reducing the need for *in vivo* testing and for improving translation to human outcomes.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude for provision of the tissue model and advice regarding study design to Patrick Hayden, Anna Malone and the team at MatTek Corporation, 200 Homer Avenue, Ashland, Massachusetts 01721, USA.

The authors would like to thank Iris Muller, Maria Baltazar and Anthony Bowden (SEAC, Unilever Research, Sharnbrook, Bedfordshire, MK44 1LQ, UK) for their innovative ideas on study design.

The authors would like to thank Eleanor Storey, Joy Millican, Kamil Czuchnowski, Nici Smith and Maria Aitchison (Charles River) for their technical assistance in running the experiment.

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NEXT STEPS
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Early De-risking Approaches for Identification of Anti-Fibrotics for Idiopathic Pulmonary Fibrosis (IPF) Treatment

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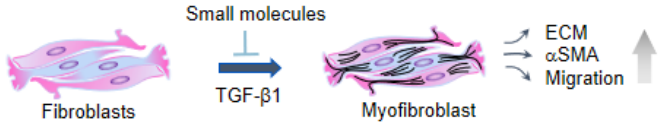
1 INTRODUCTION

Idiopathic Pulmonary Fibrosis (IPF) is a chronic lung disease leading to a progressive and irreversible decline in lung function caused by repetitive micro-injuries to the alveolar epithelium. To date, only nintedanib and pirfenidone have been licensed to treat IPF, therefore, there is an increased effort to discover new therapies. We have created a testing strategy for identifying new and improved therapies for IPF using *in vitro* and *in vivo* models. The strategy starts with the *in vitro* high throughput screening model, *in vitro* FMT Assay, followed by the low throughput 3D, EpiAlveolar model (which needs further optimization as it was developed for assessment of lung toxicity), and finally testing in the well established *in vivo* bleomycin model. This testing strategy provides an integrated (human *in vitro* and animal *in vivo*) testing program with 3Rs benefits.

2 *IN VITRO* FMT ASSAY

METHOD: Lung-derived primary human bronchial fibroblasts were seeded and subsequently refreshed in preparation for addition of small molecule compounds and the fibrotic trigger TGF-β1 (Figure 1). After 3 days, cells were fixed and stained for αSMA and DAPI and imaged by high-content analysis (Figure 2).

Figure 1. Schematic Overview of TGF-β1-Mediated Trans-differentiation of Fibroblasts to Myofibroblasts (FMT) and Modulation by Small Molecules



RESULTS: Exposure to TGFβ-1 demonstrated a clear, concentration dependant increase in αSMA levels (Figure 3). This was fully inhibited by treatment with the ALK-5 inhibitor (SB525334) and nintedanib. IC_{50} values for nintedanib are consistent across donors and strong Spearman rank correlation denotes consistency between biological replicates.

Figure 2. High Content Imaging of αSMA and Nuclei Numbers (DAPI Stained) with an INCell Analyzer 2200

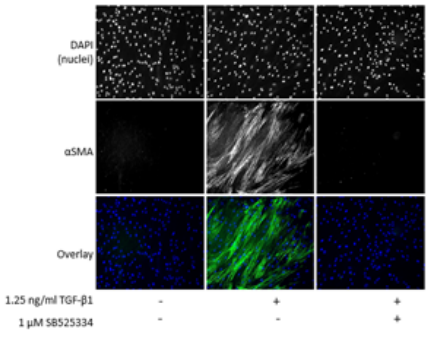
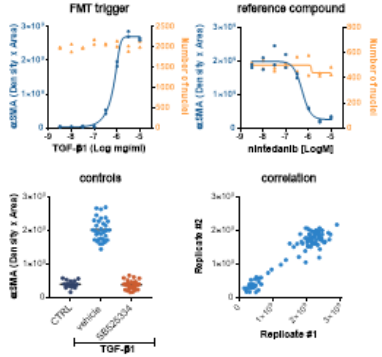


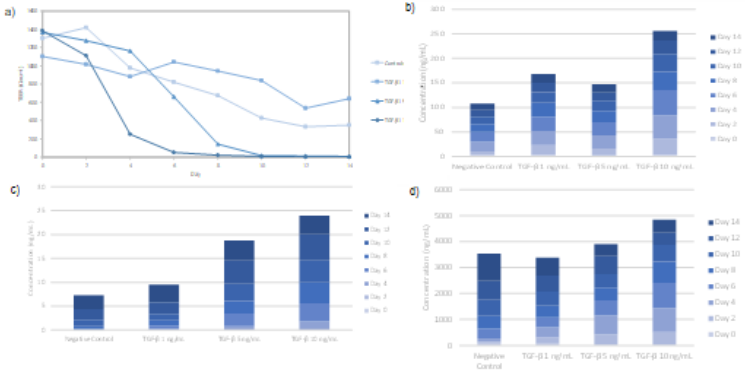
Figure 3. NEEDS A TITLE



3 *IN VITRO* EPIALVEOLAR™ MODEL

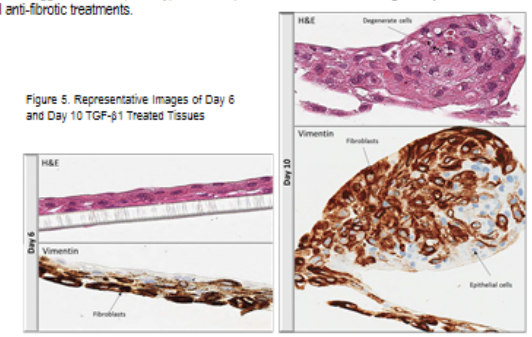
METHOD: A novel *in vitro* organotypic 3D airway model from primary human cells (EpiAlveolar™ from MatTek Corporation) with macrophages was examined to evaluate alveolar toxicity and the capacity of the model as a method to assess fibrotic potential *in vitro*. EpiAlveolar™ tissues were challenged to develop aspects of pulmonary fibrosis, by exposure to known TGF-β1 (1, 5 and 10 µg/mL). Doses were replenished every second day over a 14 day period. Tissue viability was examined by TEER and LDH every second day and tissues were terminated at various intervals throughout the 14 day period to allow examination by histopathology throughout the period. Additionally, spent media was assayed by ELISA for fibronectin, IL-6 and Procollagen I α1.

Figure 4. a) TEER b) Cumulative Pro-Collagen I α1 Secretion* c) Cumulative IL-6 Secretion* and d) Cumulative Fibronectin Secretion*



RESULTS: A dose response reduction in TEER was observed (Figure 4a) but no cell death measured in LDH release (data not shown). There were dose response increases in the fibrotic makers of Pro-Collagen, IL-6 and Fibronectin (Figures 4a, b and c). By Day 6 of TGF- β1 (10 ng/mL) treatment, the overall thickness of the cell layer was similar to untreated controls, however, the majority of the cell population expressed a spindle shaped VM positive fibroblast phenotype (Figure 5). From Day 10, samples exhibited multifocal thickening and, in places, protuberant foci composed of multiple layers of VM positive fibroblasts mixed with lesser VM negative epithelial cells. Increased numbers of degenerate cells were observed in these samples. These data show that it is possible to trigger a fibrotic phenotype in the EpiAlveolar™ model indicating it may be a useful tool to investigate the efficacy of novel anti-fibrotic treatments.

Figure 5. Representative Images of Day 6 and Day 10 TGF-β1 Treated Tissues

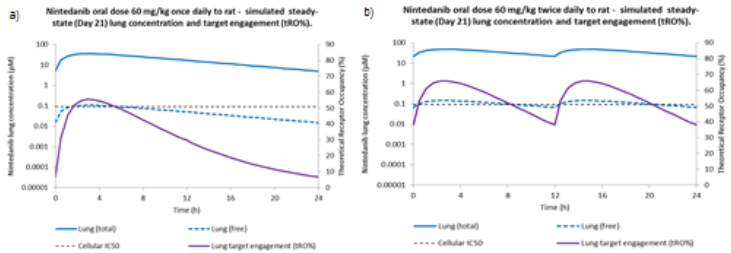


* Secreted concentrations for Day 12 of 1 ng/mL TGF-β1 treatment are estimated, as the samples were discarded in error

4 *IN VIVO* BLEOMYCIN MODEL

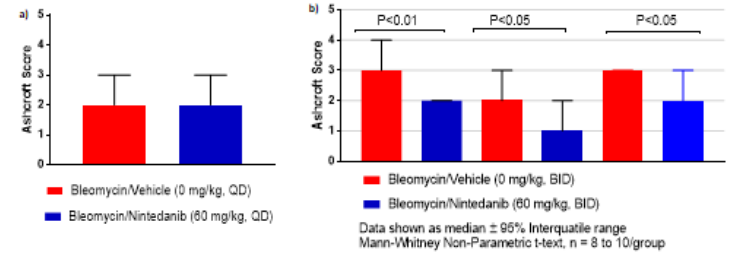
METHOD: Studies were performed in male SD rats (250-300 g). Lung exposure was modelled using integrated pharmacokinetic data, estimates of free nintedanib lung concentrations and receptor affinity data. Pharmacokinetic (PK) parameters were determined following administration of nintedanib at 60 mg/kg (QD or BID) and free drug concentrations were determined using rat lung tissue and plasma protein binding data. The repetitive fibrosis model was established by direct administration of bleomycin (1.66 units/kg, Days 1, 2, 3, 6 and 7); nintedanib or vehicle were dosed orally from Day -1 to Day 27. On Day 28, the right lung was inflation-fixed and scored for fibrosis using the modified Ashcroft scale (blind assessment on 6 lung sections per right lung). The left lung was retained for hydroxyproline determination using an LCMS assay (n = 8 to 10 rats/group).

Figure 6. Nintedanib Pharmacokinetic Modelling Following a) Once or b) Twice Daily Dosing in the Rat



RESULTS: PK modelling of unbound exposure, relative to cellular potency, demonstrated that 60 mg/kg nintedanib (BID) gave 40-70% target coverage between dose occasions (Figure 7b) compared to 60 mg/kg (QD) which gave only 10% target coverage at C_{trough} (Figure 7a). These data suggest twice daily dosing will be more efficacious compared to once daily dosing at 60 mg/kg. Nintedanib (60 mg/kg, BID) significantly reduced fibrosis in three independent studies (Figure 7B). Nintedanib (60 mg/kg, QD) was not consistently antifibrotic in three independent studies (data from one study presented) (Figure 7A).

Figure 7. Effect of a) Once or b) Twice Daily Nintenanib Dosing on Fibrosis Score (a + b)

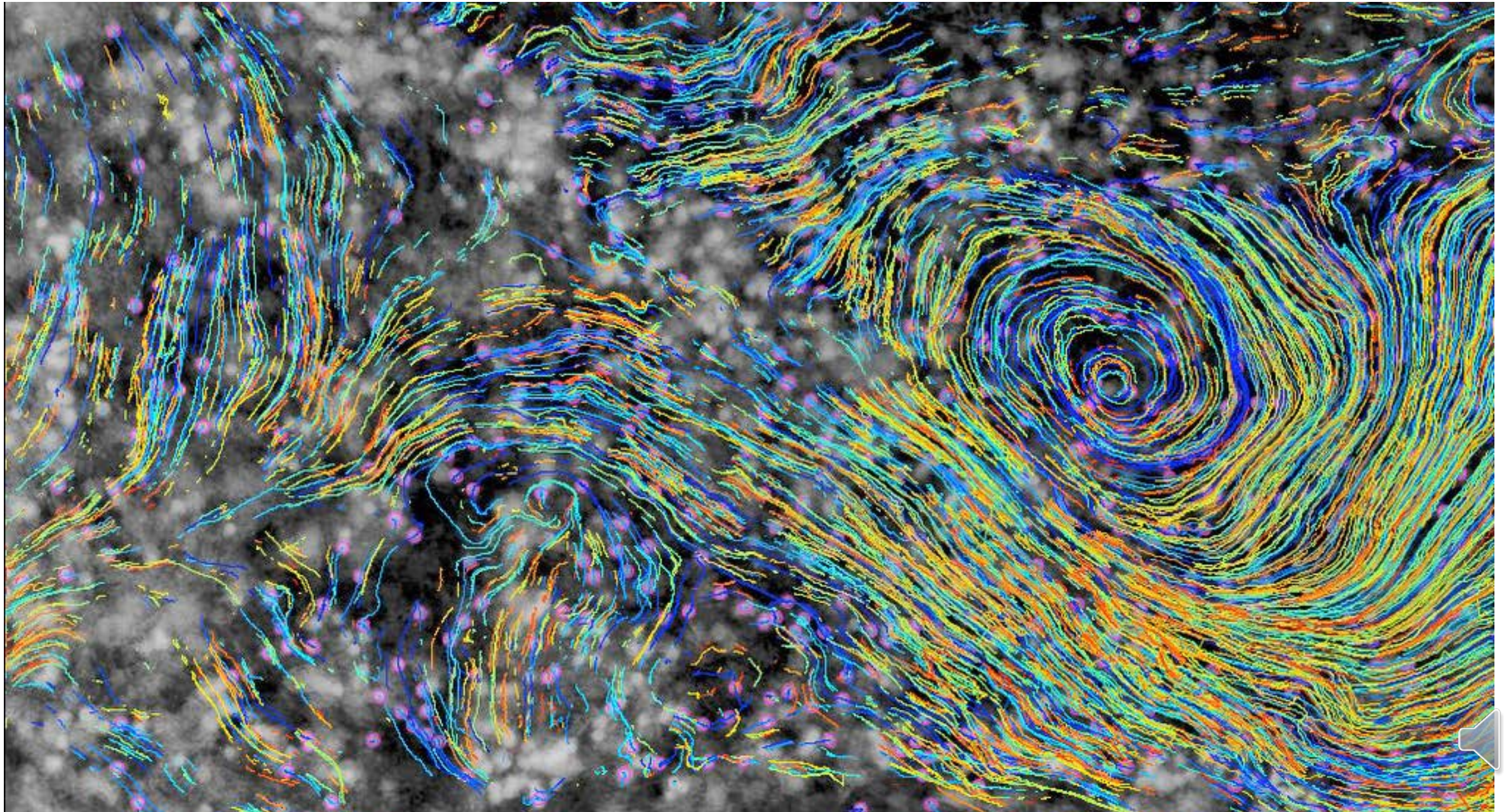


5 CONCLUSION

In conclusion, human-derived *in vitro* models can be used to assess potency and toxicity of novel fibrotic compounds in disease relevant assays (e.g. FMT). Only compounds with the optimum potency and low toxicity will move forward into *in vivo* assays to assess exposure and efficacy. Integration of *in vitro* and *in vivo* data will improve with screening and selection for novel therapeutic anti-fibrotic drugs.

TRANSLATION AND EFFICACY

MUCOCILIARY CLEARANCE FROM CYSTIC FIBROSIS DERIVED MUCILAIR™



Assessment of Mucociliary Clearance and Cilia Beating Frequency in MucilAir™-Cystic Fibrosis *In Vitro*

C. S. Roper, E. Storey, H. Paulo, and J. Wallace
Charles River Laboratories, Edinburgh, UK

charles river

1 INTRODUCTION

The lungs are cleared of particles and kept sterile by cilia beating the mucus resulting in mucociliary clearance (MCC) of these materials. In cystic fibrosis patients, the mucus produced is thicker and, therefore, the MCC system does not function effectively. MucilAir™-Cystic Fibrosis (MucilAir™-CF) tissues are a functional model of the human airway epithelium derived from cystic fibrosis patients' cells (homozygote ΔF503 mutation) cultured at the air-liquid interface resulting in a similar morphology to the patient, with functioning cilia, but defective MCC. Efficacy testing for novel cystic fibrosis treatments requires assessment of cilia beating frequency (CBF) and MCC. Therefore, this proof of concept study was performed to asses the effects of a known surfactant, Tyloxapol, in MucilAir™-CF on % active cilia, CBF and MCC, with cell viability assessed by Transepithelial Electrical Resistance (TEER). MucilAir™-CF was chosen as the test system for this study as this is an *in vitro* model for the respiratory airways known to reflect the disease conditions of cystic fibrosis patients.

The aim of the study was to measure the effects of the surfactant drug, Tyloxapol, β-Lactose (negative control) both in physiological saline, and physiological saline on the critical end-points for cystic fibrosis drug efficacy testing; % active cilia, CBF and MCC.

2 MATERIALS AND METHODS

A dose range finding test (data not presented) was performed to select the concentrations of Tyloxapol in saline for the main test. For the main experiment, triplicate MucilAir™-CF were dosed (30 µL) with Tyloxapol (Low Dose; 0.0005%, v/v and High Dose; 0.1%, v/v) in physiological saline. The tissues were exposed for 1, 10 and 30 min and 1, 2 and 24 h. Triplicate MucilAir™-CF tissues were also dosed with the vehicle control (30 µL; physiological saline) or the negative control (30 µL; ß-Lactose; 75 µM in physiological saline) for 24 h only. Figure 1 shows a schematic of the cells in culture.

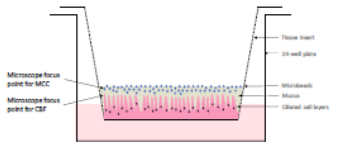


Figure 1: Schematic of MucilAir™-CF Tissue in Culture

At each exposure timepoint, CBF and % active cilia (active cilia were considered to have a CBF >3.5 Hz) were assessed immediately using CiliaX software (Epithelix, Switzerland). Microbeads (10 µm, dark blue) were then applied to the surface of each tissue and *ca* 30 s videos were captured. The videos were analysed using the TrackMate plugin on ImageJ (NIH). This software tracks the beads as they move and determines the rate at which they move (Figure 2). TEER is a sensitive and reliable method to confirm the integrity of the monolayer and was assessed in all tissues following MCC analysis. Six untreated tissues were analysed as for the treated tissues. Individual tracked images are presented in Figure 3.

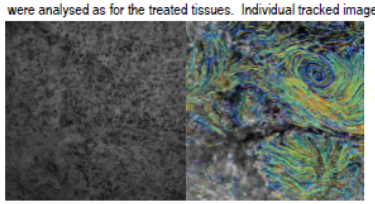
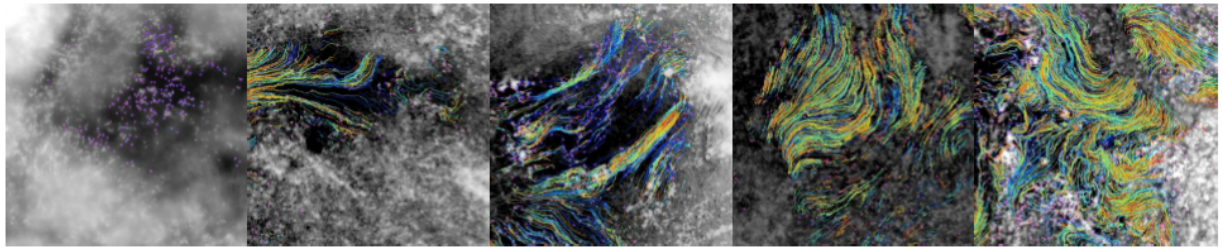


Figure 2: Representative Images of Microbeads on Tissue With & Without Tracks

3 RESULTS: MONOLAYER INTEGRITY, % ACTIVE CILIA, CBF & MCC

Figure 3: Single Image from MCC Analysis with Overlaying Microbead Tracks at 1, 10, 60, 120 min and 24 h Post Application of Low Dose Tyloxapol



All values are given as mean ± SD. Undosed results demonstrated that MucilAir™-CF recapitulates the disease (% active cilia, CBF and MCC (displacement) were 82 ± 16%, 4.3 ± 0.4 Hz and 3.5 ± 7.0 µm).

Cell viability was unaffected by the Low Dose Tyloxapol treatment (Figure 4a). The % active cilia (Figure 5a), CBF (Figure 6a) and MCC (Figure 7a) all increased immediately (1 min) to 93 ± 8%, 6.5 ± 1.4 Hz and 20.8 ± 26.3 µm, respectively. % active cilia and CBF were sustained to 24 h. MCC was sustained to 60 min, increased to 61.9 ± 34.0 µm at 2 h and remained at this high level to 24 h (62.1 ± 31.9 µm). These results are summarised in Table 1.

Conversely, following the High Dose treatment of Tyloxapol, cell viability was reduced from 30 min onwards (Figure 4b). However % active cilia (Figure 5b) and CBF (Figure 6b) were not reduced until 24 h. MCC increased up to 2 h (68.3 ± 54.1 µm), but fell by 24 h (7.3 ± 7.4 µm), presumably due to Tyloxapol toxicity.

At 24 h, viability (Figure 8), % active cilia (Figure 9) and CBF (Figure 10) were unaffected by physiological saline, B-lactose or Low Dose Tyloxapol treatment. MCC (Figure 11) was improved for physiological saline (36.2 ± 20.8 µm), C (52.9 ± 40.7 µm) and Low Dose Tyloxapol (62.1 ± 32.0 µm) treatment compared to High Dose Tyloxapol that was reduced (7.3 ± 7.4 µm). The results are summarised in Table 2.

Figure 4: Viability for (a) Low Dose and (b) High Dose Tyloxapol

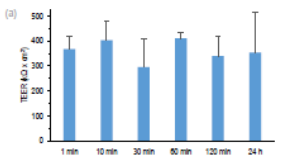


Figure 5: Active Cilia for (a) Low Dose and (b) High Dose Tyloxapol

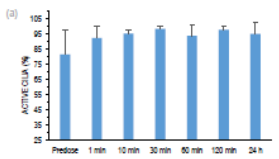


Figure 6: CBF for (a) Low Dose and (b) High Dose Tyloxapol

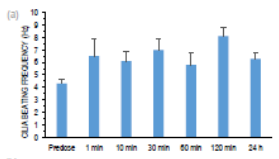


Figure 7: Displacement for (a) Low Dose and (b) High Dose Tyloxapol

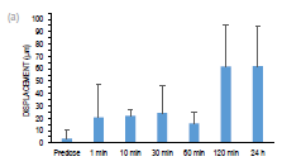


Figure 8: Viability at 24 h Post Dose

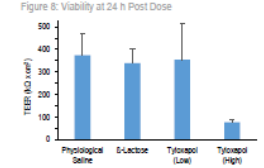


Figure 9: Active Cilia at 24 h Post Dose

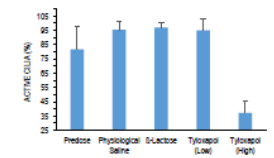


Figure 10: CBF at 24 h Post Dose

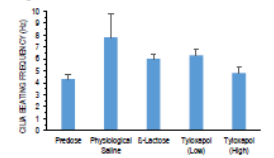
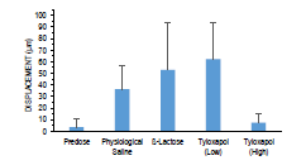


Figure 11: Displacement at 24 h Post Dose



4 DISCUSSION

This study demonstrated that efficacy can be measured and changes observed in MucilAir™-CF using % active cilia, CBF and MCC. Toxicity can also be observed in parallel using TEER.

The study design clearly demonstrated efficacy and toxic effects of the treatments. The Low Dose Tyloxapol was both efficacious and non toxic. Conversely, the High Dose Tyloxapol was efficacious only over a short period but resulted in reduced MCC at 24 h.

Both the vehicle control (physiological saline) and negative control (ß-Lactose) resulted in efficacy without causing toxicity to the MucilAir™-CF cells. Efficacy was, therefore, at least partly due to the mucus thinning effect of physiological saline used for all treatments. The surfactant properties of Tyloxapol increased MCC further at 24 h than physiological saline or ß-Lactose.

Table 1: Summary of CBF (Hz), % Active Cilia, MCC and TEER for Low Dose Tyloxapol Treatment

	CBF (Hz)		% Active Cilia		MCC (µm)		TEER	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Undosed	4.3	0.4	81.6	15.7	3.5	7.0	-	-
1 min	6.5	1.4	92.3	7.6	20.8	26.3	368	50
10 min	6.1	0.8	95.3	2.2	22.1	5.0	403	77
30 min	7.0	0.9	98.4	1.4	24.2	22.6	295	116
60 min	5.8	1.0	93.9	7.4	15.9	8.9	411	24
120 min	8.1	0.7	97.8	2.6	61.9	34.0	339	82
24 h	6.3	0.5	95.0	7.9	62.1	32.0	354	162

Table 2: Summary of CBF (Hz), % Active Cilia, MCC and TEER at 24 h Post Treatment

	CBF (Hz)		% Active Cilia		MCC (µm)		TEER	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Predose	4.3	0.4	81.6	15.7	3.5	7.0	-	-
Physiological Saline	7.8	2.0	95.4	6.0	36.2	20.8	374	97
ß-Lactose	6.0	0.4	97.0	3.4	52.9	40.7	338	66
Tyloxapol (Low)	6.3	0.5	95.0	7.9	62.1	32.0	354	162
Tyloxapol (High)	4.8	0.5	37.2	8.5	7.3	7.4	76	13

5 CONCLUSION

In conclusion, this study demonstrated that % active cilia, CBF and MCC can be measured and changes observed in MucilAir™-CF. Therefore, it is proposed to use these endpoints in this test system to identify improved and novel treatments for patients with cystic fibrosis.

6 REFERENCES

- Huang S., Wiszniewski L., & Constant S. (2011). The Use of *In Vitro* Cell Models in Drug Development for Respiratory Diseases. The Use of *In Vitro* 3D Cell Models in Drug Development for Respiratory Diseases, Drug Discovery and Development - Present and Future. Kapetanović I. (Ed.).
- Beubler E., Fischer R., Untersteiner G. and Strohmaier W. (2016). Influence of the Surfactant Tyloxapol on Mucociliary Clearance in Human Respiratory Cystic Fibrosis Cells. Pharmacology: 98; 1-3.

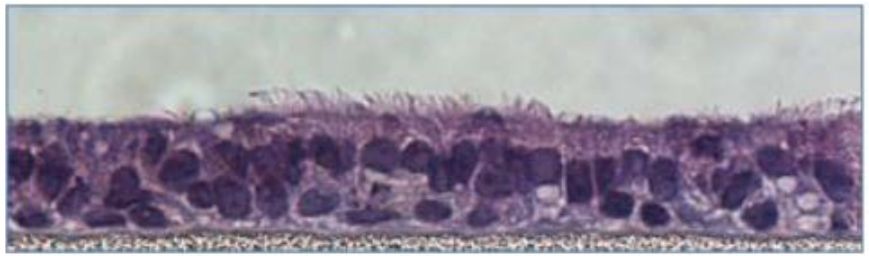
TRANSLATION – STUDY DESIGN

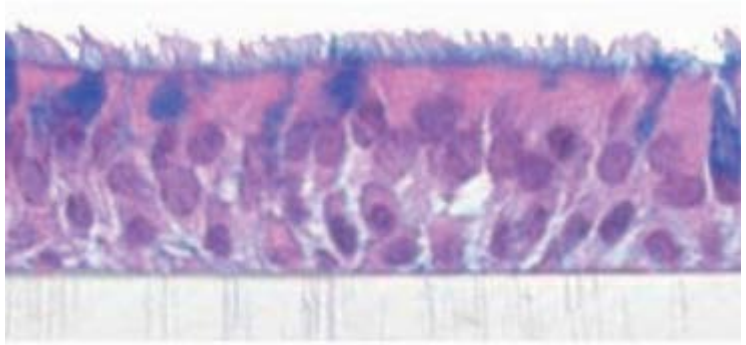
RAT *VERSUS* HEALTHY HUMAN *VERSUS* DISEASED HUMAN

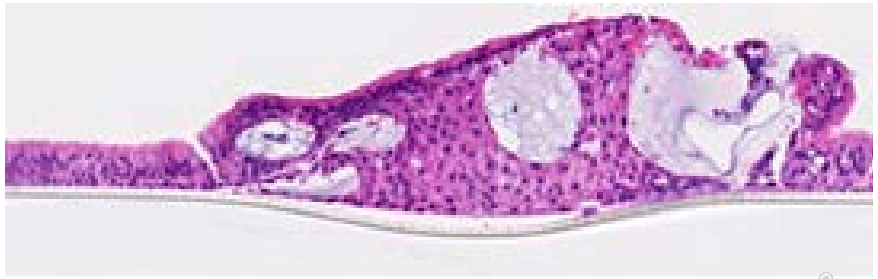
Rat EpiAirway™

Healthy human MucilAir™

Diseased human OncocilAir™







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IS THIS EASY TO DESIGN AND RUN?

KEY POINTS FOR STUDY DESIGN

WHAT SHOULD AN *IN VITRO* RESPIRATORY STUDY LOOK LIKE?

- Tissues arrive
- Returned to culture
- Recovery overnight or longer, model dependant

- Dose response curve
- Typically 4-6 replicates at each of 4-6 concentrations
- Half log dilutions?

Vehicle Controls?

- Numerous possible measures
- Cell viability (LDH, MTT, resazurin)
- Pathology, ELISA, PCR……
- Replicate virtually anything possible *in vivo*
- Others: TEER, MCC, CBF

Tissue Receipt & Recovery → Dose Applied Apically Liquid or Aerosol → Exposure Duration Rinse → Single or Repeat Dosing → Recovery → Measurements?

- Rinse with saline or PBS
- Very gently to avoid perturbing system

Measurements?

ALI and/or Untreated Control?

Positive Control? What Effect are we Assessing?

CONCLUSIONS

CONCLUSIONS

- *In vitro* models can be used to effectively screen out toxicants prior to *in vivo* toxicology assessment or identify potential candidates for drug development following efficacy testing
- *In vitro* models can be used to generate human equivalent concentrations in occupational toxicology as well as identify no effect levels in toxicology. From these data, formulations or active ingredients can be screened out. For test articles chosen for *in vivo* testing, this data can be used in support of identifying DRF starting doses
- Using the rat, healthy human and diseased human models in parallel provides translational information that often could not be considered until after first in man or later. Therefore, these data can be used in support of clinical decision making

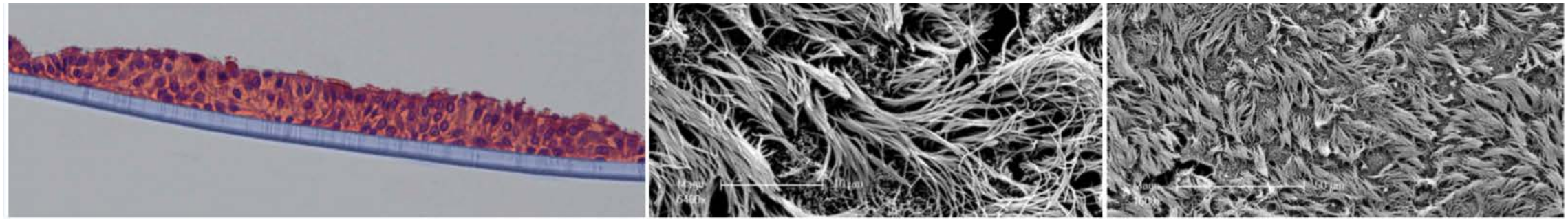
CONCLUSIONS

- As new microfluidic/ on-a-chip technologies and tissue models become available, so this science will rapidly expand to answer more complex questions such as chronic diseases as the current research has focussed on the acute effects
- These tests will have tangible 3Rs and animal welfare benefits
- Integration of these tests into testing strategies should reduce the costs of bringing products to market

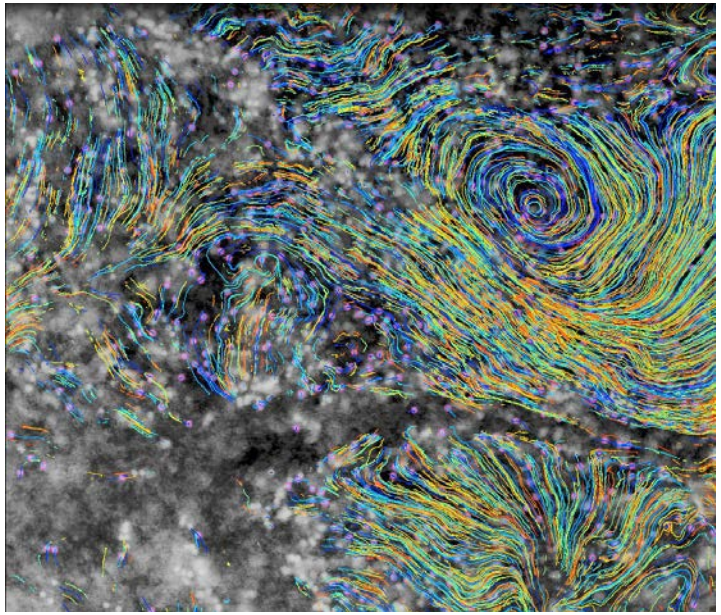
- And we didn't even discuss applications of these technologies for COVID-19 research!!!

ORGANOTYPIC 3D TISSUE MODELS FOR RESPIRATORY SAFETY AND EFFICACY TESTING

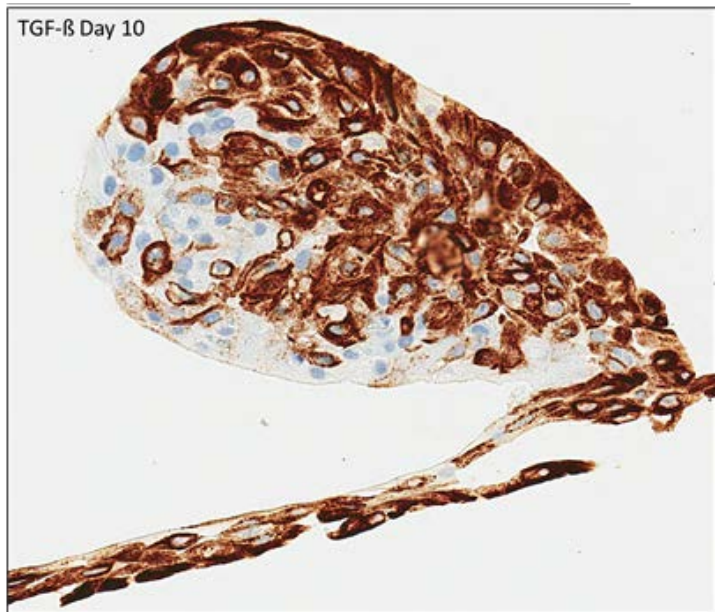
MucilAir™ Upper Airway Cross Section and SEM.



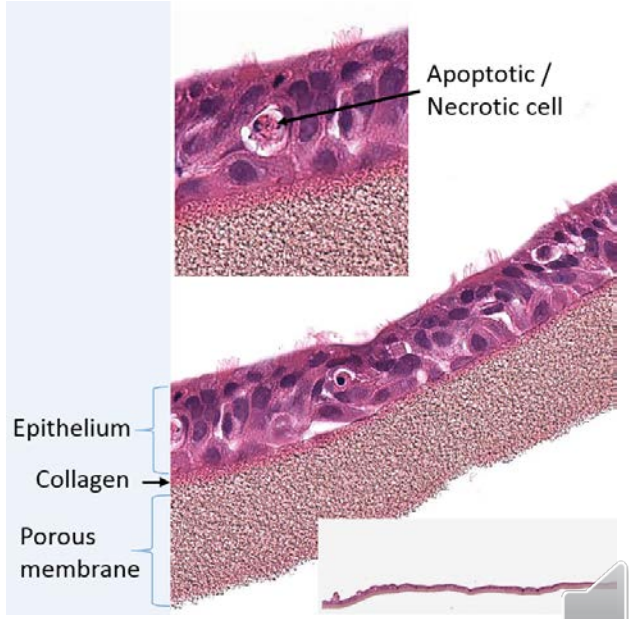
Mucociliary Clearance from CF MucilAir™



Fibrotic Protuberant Foci from EpiAlveolar™



Histopathology of EpiAirway™



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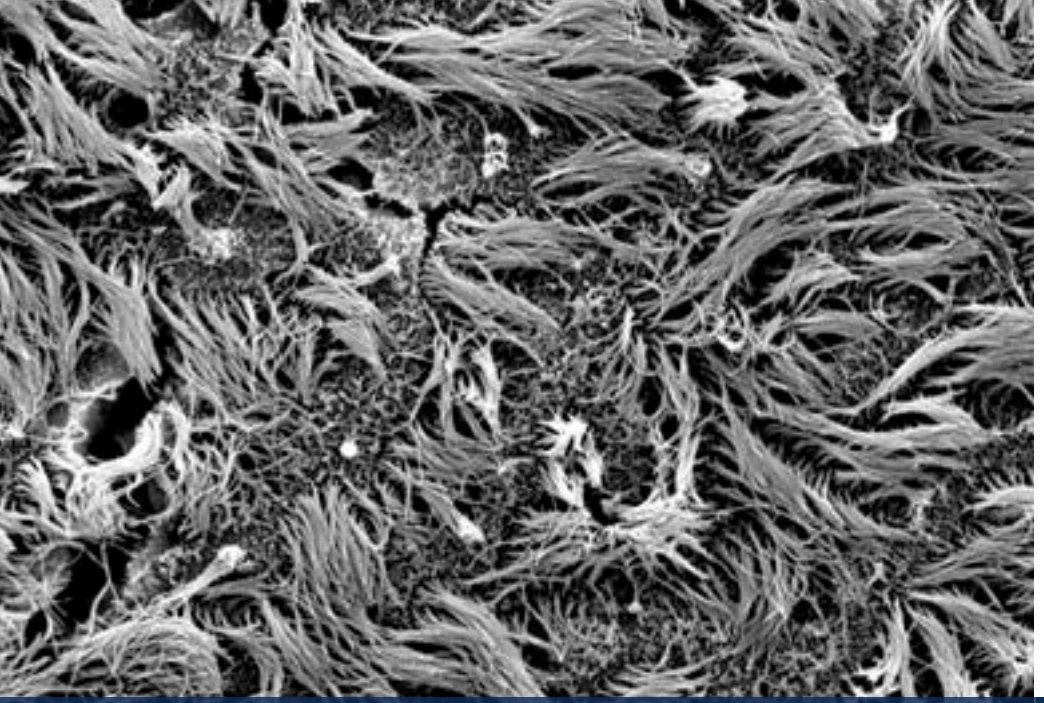

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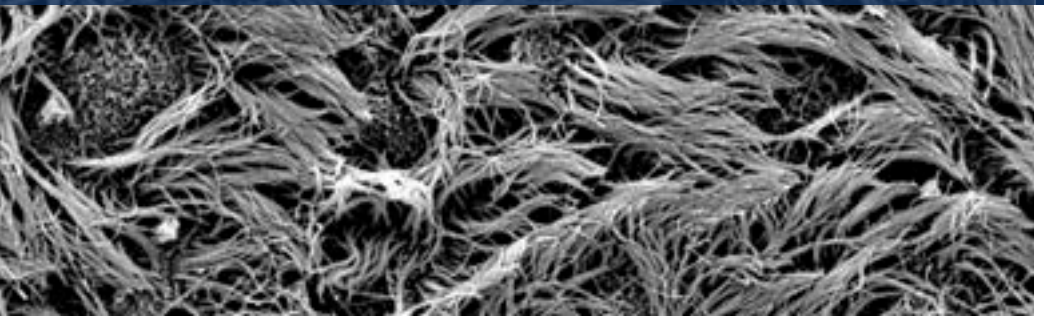

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