Evaluation of *In Vitro* Models of the Rat and Human Airway Epithelium for Assessment of Acute Toxicity

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1 Introduction

Acute airway toxicity is an important consideration for the development of pharmaceuticals, chemicals, cosmetics and agrochemicals. Acute toxicity information is used to inform safe use of products, procedures for accidental exposure and dose range selection for *in vivo* studies. Currently validated methods require the use of animals yet it is widely acknowledged that these methods may not be appropriate for accurate prediction of human risk. To combat this, and as part of a 3R's strategy (refine, reduce, replace), *in vitro* models are being developed as an alternative strategy for assessment of inhalation toxicity.

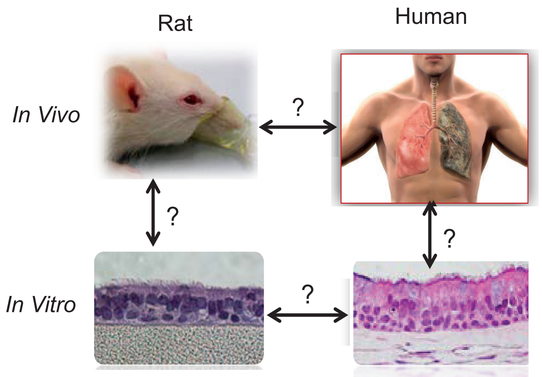

Figure 1: Together, Rat and Human EpiAirway cultures will provide additional information to aid in prediction of acute toxicity potential

One such commercially available airway model is EpiAirway™ (MatTek Corp., Ashland, MA, USA) constructed from primary human airway epithelial cells. At present there is no means to correlate the responses of these tissues to human outcomes as available *in vivo* data has been produced in rodents. To address this information gap, an *in vitro* airway model has been generated in a similar manner to the human EpiAirway™ model using cells from the airway epithelium of Charles River rats. In this study the rat and human models were compared on three independent occasions by two laboratories (Charles River and MatTek) using 14 test chemicals.

2 Methods

Human EpiAirway™: The human model was prepared by seeding normal human bronchial epithelial cells on a Millipore collagen coated membrane insert. Until a confluent monolayer was formed, the inserts were cultured submerged. When a confluent monolayer had developed, the inserts were further cultured at the air-liquid interface for up to 21 days. A fully differentiated airway model was formed (Figure 2).

Rat EpiAirway™: Airway epithelial cells isolated from the conducting airways of 8-week old male CD rats (Charles River Laboratories, MA, USA) and seeded onto Millipore membrane inserts. These cultures were cultivated at the air-liquid interface for up to 27 days (Figure 2).

Histological Evaluation: Human and rat cultures were fixed, embedded, sectioned and stained in H&E and examined.

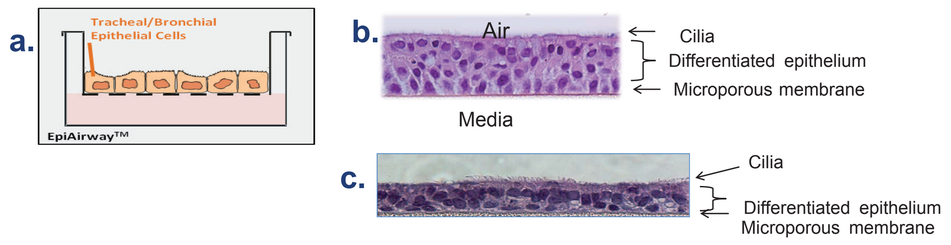

Figure 2: EpiAirway™ Tissue Models
(a) Schematic diagram of differentiated primary tracheal/bronchial epithelial cells grown at the air-liquid interface
(b) H&E-stained cross-section of human EpiAirway™ tissue shows ciliated cells and a pseudostratified morphology on a microporous membrane
(c) H&E-stained cross-section of rat EpiAirway™ tissue also shows ciliated cells and a pseudostratified morphology

Test Chemical Selection: A panel of 14 chemicals were selected to include a range of GHS classifications and chemical classes: acids, bases, oxidants, aldehydes, ketones, esters, alcohols, amines, halogenated aromatics, phenol and pyridine derivatives. A dose range finding experiment was conducted to determine appropriate test concentrations.

Irritation Assay: Cultures were allowed to equilibrate for 18-24 h (MatTek) or up to 120 h (Charles River) in 1 mL EpiAirway ™ assay medium. Prior to dosing, mucus was removed from the apical tissue surface and discarded. Each test chemical, formulated at 4 concentrations, was applied to rat and human EpiAirway™ cultures in triplicate. Each insert was 'capped' to prevent cross contamination and evaporation of volatile compounds. After a 3 h exposure period (in a humidified incubator set to maintain 37°C, 5% CO_2), tissues were rinsed then allowed to recover overnight. Viability was assessed by MTT assay and barrier function by transepithelial electrical resistance (TEER). IC_{75} values (the concentration required to reduce viability or TEER to 75% of the vehicle control) were calculated. Appropriate vehicle and positive controls were included. This procedure was performed on 3 occasions by each laboratory. A schematic of the protocol is presented in Figure 3.

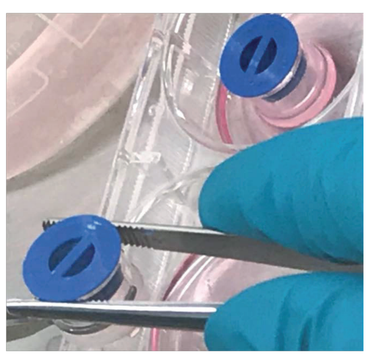

Figure 4: Capped EpiAirway™ Insert

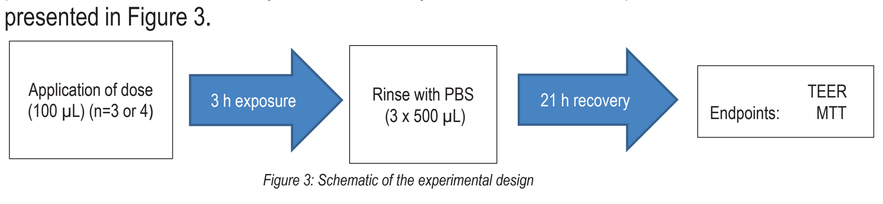

Figure 3: Schematic of the experimental design

3 RESULTS

Pre-dose TEER measurements from air liquid interface (ALI) tissues were found to be high indicating the successful production of a robust barrier in both human and rat EpiAirway™. Histological evaluation of the EpiAirway™ models revealed a pseudostratified epithelium and mucilociliary functionality.

The MTT and TEER data from Chares River and MatTek are presented in Table 1. IC_{75} values from the MTT data are presented in Figure 5 and Figure 6. In each species, a clear dose response was observed in the MTT data. There were instances in both laboratories where it was not possible to obtain stable TEER measurements or see a clear dose response in the TEER data. At Charles River, this was most pronounced with the rat EpiAirway™ where an IC_{75} was calculated on 3 or more occasions for only 7 chemicals. At MatTek, instances of this were dispersed between human and rat EpiAirway™.

	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

Table 1: IC_{75} (mg/mL) values for test chemicals resulting from MTT and TEER measurements in rat and human EpiAirway™ cultures from Charles River and MatTek
** = RI = Respiratory Irritant*
***= Info from Safety Data Sheets and Echemportal GHS searches*

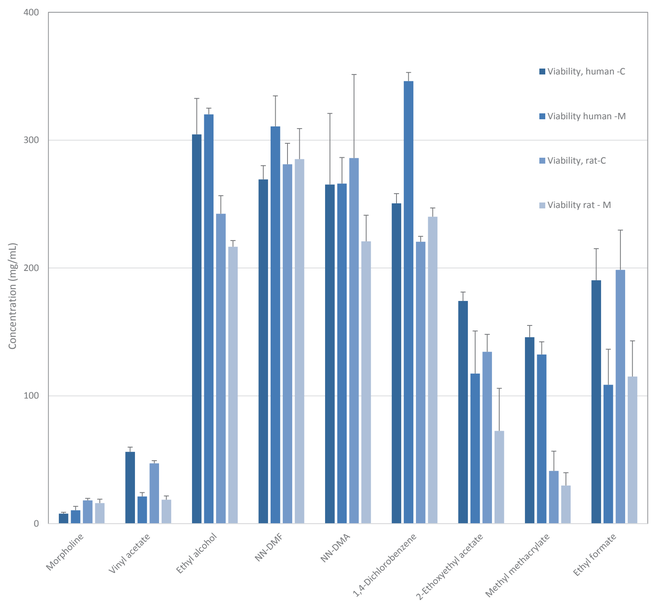

Figure 5: Comparison of Human and Rat IC_{75} Values (Mean ± SD) for 9 Test Items from MTT Data from Charles River (C) and MatTek (M)

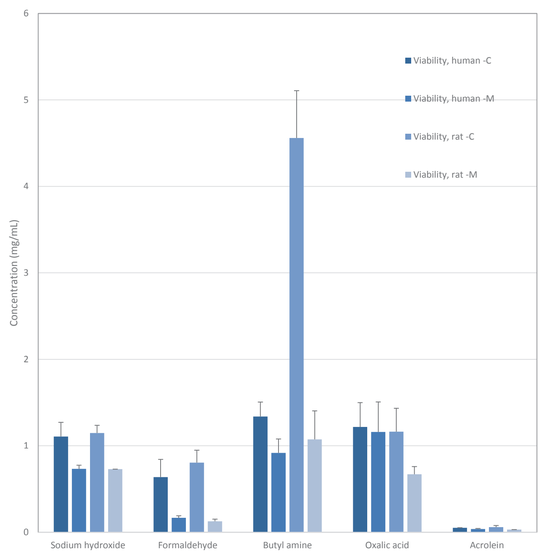

Figure 6: Comparison of Human and Rat IC_{75} Values (Mean ± SD) for 5 Test Items from MTT Data from Charles River (C) and MatTek (M)

Overall, there was broad agreement between MTT and TEER data and the results were consistent between testing occasions. A similar response to each test chemical was observed between species with IC_{75} values being of the same order of magnitude. The exceptions to this were methyl methacrylate and ethyl alcohol to which rat EpiAirway™ was more sensitive than human EpiAirway™ (in both laboratories). Additionally, EpiAirway™ (both species) tested at MatTek was more sensitive to these compounds than when tested at Charles River. Between laboratories, the test items were generally ranked in the same order, in terms of potency, highlighting the consistency and transferability of the EpiAirway™ models and techniques. The rank orders of chemicals based on MTT data from human and rat EpiAirway™ each laboratory are presented in Table 2 showing a similar pattern both between laboratories and species. In each instance, the TEER data showed similar patterns.

Rank Order: Viability (MTT)		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Human	CRL	Acrolein	Formaldehyde	Sodium Hydroxide	Oxalic Acid	Butyl Amine	Morpholine	Vinyl Acetate	Methyl Methacrylate	2-Ethoxyethyl Acetate	Ethyl Formate	p-Dichlorobenzene	NN-Dimethyl Acetamide	NN-Dimethyl Formamide	Ethyl Alcohol
	MatTek	Acrolein	Formaldehyde	Sodium Hydroxide	Butyl Amine	Oxalic Acid	Morpholine	Vinyl Acetate	Ethyl Formate	2-Ethoxyethyl Acetate	Methyl Methacrylate	NN-Dimethyl Acetamide	NN-Dimethyl Formamide	Ethyl Alcohol	p-Dichlorobenzene
Rat	CRL	Acrolein	Formaldehyde	Sodium Hydroxide	Oxalic Acid	Butyl Amine	Morpholine	Methyl Methacrylate	Vinyl Acetate	2-Ethoxyethyl Acetate	Ethyl Formate	p-Dichlorobenzene	Ethyl Alcohol	NN-Dimethyl Formamide	NN-Dimethyl Acetamide
	MatTek	Acrolein	Formaldehyde	Oxalic Acid	Sodium Hydroxide	Butyl Amine	Morpholine	Vinyl Acetate	Methyl Methacrylate	2-Ethoxyethyl Acetate	Ethyl Formate	Ethyl Alcohol	NN-Dimethyl Acetamide	p-Dichlorobenzene	NN-Dimethyl Formamide

Table 2: Rank order of test chemicals based on IC_{75} values resulting from MTT data in rat and human EpiAirway™ cultures from Charles River and MatTek

4 CONCLUSION

In conclusion, the panel of 14 test items were successfully tested on human and rat EpiAirway™ tissues. The TEER and MTT viability results for both the rat and human EpiAirway™ tissues were highly reproducible. The responses of the rat and human EpiAirway™ tissues were generally similar, with the notable exception of methyl methacrylate and ethyl alcohol, for which the rat tissues were considerably more sensitive.

However, the results produced across each test run within each species were very consistent. In addition, the testing was carried out by two independent laboratories and the two datasets were in general agreement. Correlation of these results with *in vivo* results will be important for showing the predictiveness of these models for toxicity screening and safety assessments.

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