Evaluation and Comparison of *In Vitro* Rat and Human Airway™ Epithelial Models for Inhalation Toxicity Testing

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1 INTRODUCTION

Acute airway toxicity is an important consideration during the development of pharmaceuticals, chemicals, cosmetics and agrochemicals. As part of our integrated toxicology and 3Rs testing programs, there is a requirement to assess *in vitro* 3D human and animal derived models to generate information on toxicity. This data can be used for screening, in support of dose range finding for *in vivo* testing or as an early prediction for translation from animal to human.

Human EpiAirway™ (MatTek Corporation) is a commercially available functional model of the human airway epithelium derived from cells collected from the airways of healthy donors and cultivated at the air-liquid interface. Rat EpiAirway™ (MatTek Corporation) uses airway epithelial cells collected from Charles River rats and is created similarly to the human model. The objective of this study was to evaluate and compare the responses of the two airway models.

2 METHODS

On arrival at Charles River, rat (AIR-100-R) and human (AIR-100 DAY20) EpiAirway™ were equilibrated in culture for 5 days prior to testing in a humidified incubator set to maintain 37°C, 5% CO_2. Media was changed at 2-3 day intervals. On the testing day, tissues were rinsed with phosphate buffered saline (PBS) to remove the mucus and transferred to fresh media. Transepithelial Electrical Resistance (TEER) was measured in the air liquid interface controls (ALI controls) prior to dosing. 14 test items were formulated in corn oil or ultrapure water at each of 4 different concentrations and tested in parallel to appropriate vehicle and positive (formaldehyde, 14.7 mg/mL) controls. ALI controls were treated exactly the same as test item treated tissues with the exception that they were not dosed.

The study protocol is summarised in Figure 1.

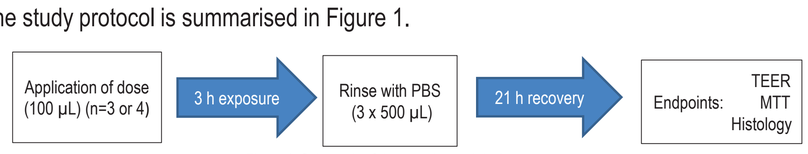

Figure 1: Schematic of the experimental design

All incubations were conducted in a humidified incubator set to maintain 37°C, 5% CO_2 with the exception of the formazan extraction which was conducted at room temperature. During the exposure period, Millicell caps were placed on top of each tissue to prevent evaporation of volatile test items (Figure 2). After a 3 h exposure, the tissues were rinsed with PBS to remove the test items and transferred to fresh pre-warmed media for a 21 h recovery incubation.

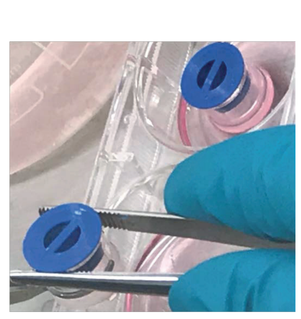

Figure 2: Capped EpiAirway ™ Tissues

Following recovery, post dose TEER was measured in all tissues. Prior to TEER measurements, the rat tissues were rinsed with PBS to remove mucus. After TEER was measured, tissues were transferred to MTT solution and incubated for 1.5 h then formazan extracted in extractant solution for 2 h on a shaking platform. The extract solution from the top and bottom of the tissue were combined and mixed prior to taking duplicate aliquots from each sample and analysed using a MultiSkan Go Spectrophotometer at 570 nm with correction at 650 nm. All test items were tested on at least 3 independent occasions. On one occasion, a fourth rat and human tissue were dosed with each test item and processed as described above with the exception that following TEER measurement they were fixed in 4% paraformaldehyde, embedded in paraffin then stained with H&E before cross sectioning. No MTT assay was conducted on these tissues. From the MTT and TEER data, an IC_{75} value (the concentration required to reduce the viability to 75% of the appropriate vehicle control) was calculated.

3 RESULTS AND DISCUSSION

The data are presented in Figures 3 and 4. For MTT, there were 3 or 4 acceptable runs of testing (*i.e.* where a dose response from the MTT assay was observed and an IC_{75} could be calculated) except for vinyl acetate in the human EpiAirway™. For TEER, acceptable data was achieved for all samples except for N,N-dimethyl formamide in human EpiAirway™. For rat TEER, stable measurements were not always possible or dose responses were not apparent resulting in a TEER IC_{75} for only 7 of the 14 test items on 3 or more occasions.

Overall, the data from the MTT and TEER assays were in good agreement predicting similar IC_{75} values for each test item. There was also good intra-run reproducibility in the predicted IC_{75} values showing consistency between batches for the two species.

For most of the test items, the IC_{75} values calculated from the human and rat tissues were very similar, although the rat tissues were particularly sensitive to methyl methacrylate and ethyl alcohol. Broadly, the 14 test items were ranked in the same order for each end point and for each species. The rank orders achieved are presented in Table 1.

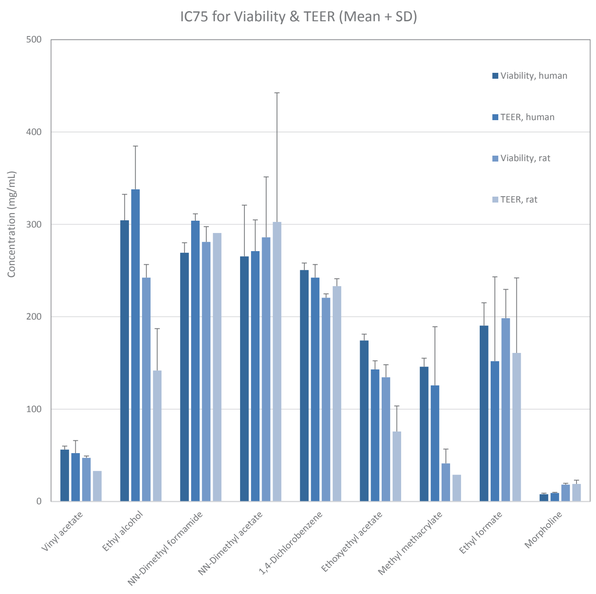

Figure 3: Comparison of Human and Rat IC_{75} Values (Mean ± SD) for 9 Test Items from MTT and TEER Data

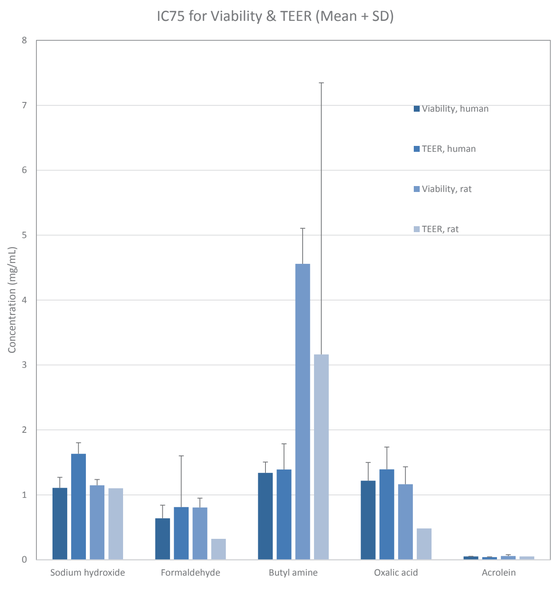

Figure 4: Comparison of Human and Rat IC_{75} Values (Mean ± SD) for 5 Test Items from MTT and TEER Data

Human and rat airway tissue morphology correlated well with *in-situ* primate and rat airway mucosae. Airway tissues exhibited lower numbers of ciliated and mucous cells and increased numbers of degenerate cells. A moderate increase in morphologic variability was seen in rat EpiAirway™ compared to the human. The spectrum of microscopic changes observed following exposure to known toxicants was similar as seen in animal model studies. The key injury related findings (Figure 5) were erosion, epithelial detachment, intercellular separation and an increase in necrotic cells. Key repair related findings were loss of ciliated cells, epithelial thinning, re-epithelialisation, cyst formation, increased mitoses, epithelial thinning and focal thickening. Using the key injury related findings, a composite scoring system was constructed to assign a single grade to each sample (Figure 6). Samples were scored in a blinded fashion and both human and rat airways were found to respond to toxicants in a similar manner with clear dose-relationships evident.

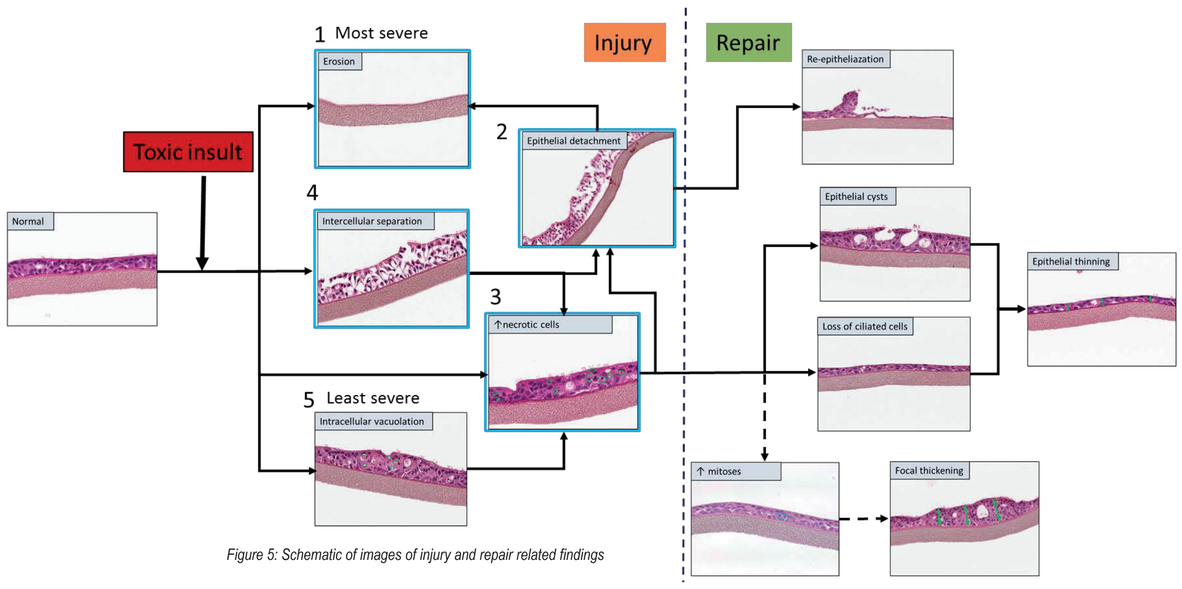

Figure 5: Schematic of images of injury and repair related findings

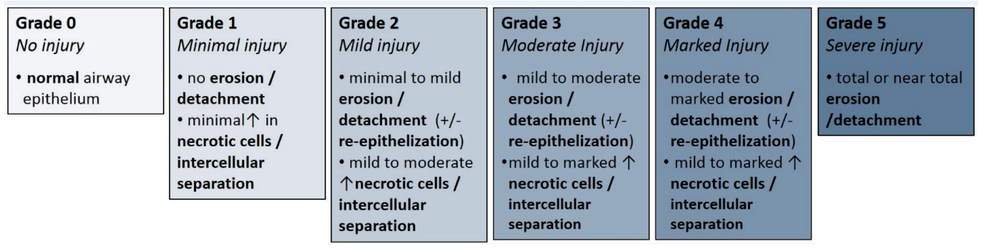

Figure 6: Scoring system used to assign a grade to each tissue

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

Table 1: Comparison of human and rat IC_{75} values (mean) from MTT and TEER data (1 = most potent, 14= least potent).

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 results from the pathology observations support and complement the TEER and MTT results for each test item. 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.

5 ACKNOWLEDGMENTS

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