Next Generation Risk Assessment approach for Inhalation: Polymer case studies

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Introduction

Next Generation Risk Assessment (NGRA) is an exposure-led, hypothesis driven approach that integrates one or more New Approach Methodologies (NAMs), which can be applied to ensure the safety of consumer products without the need for animal testing. Whilst there are different *in silico* and *in vitro* methods already available for testing lung toxicity, there is a need amongst both industry and regulatory risk assessors to create examples that can demonstrate the utility of these tools for decision-making on ingredient safety regarding pulmonary exposure.

We have identified 2 polymers as a case study to showcase a testing strategy in the area of inhalation risk assessment. The NGRA approach is shown as followed using one of the benchmark substances: Polyhexamethyleneguanidine phosphate (PHMG). PHMG causes acute interstitial pneumonia and pulmonary fibrosis in humans when it exposed to the lung (Song, et al. 2018).

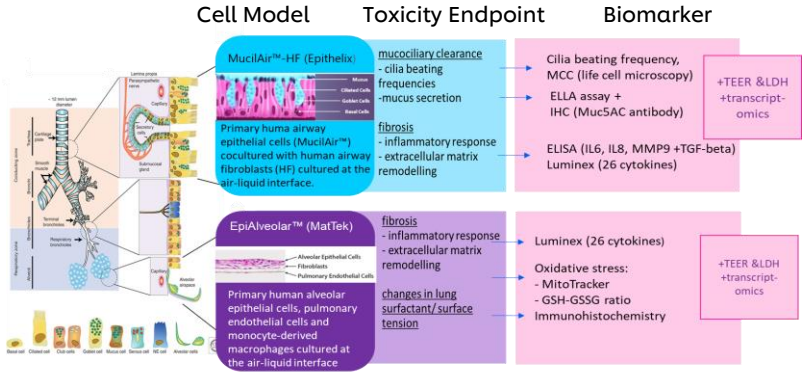
Exposure: prediction of realistic exposures and IVIVE

PHMG is an antimicrobial compound previously used in humidifiers. Exposure to PHMG caused an increase in lung-related deaths and permanent lung adverse effects (e.g. fibrosis). For this study the upper limit of the human exposure was estimated based on airborne concentration of 0.95 mg/m^3 and a worst case exposure duration of 11hrs per day. To estimate the equivalent deposited mass per surface area in the lung and compare to in vitro points of departure, MPPD was used to predict the daily, weekly and monthly human lung exposure (µg/cm^2) in the tracheobronchial and pulmonary region (no clearance) (Table 1). In vitro doses were selected based on these predictions.

Region of the lung	Daily exposure	Weekly exposure	Monthly exposure
Tracheobronchial deposition	0.07	0.49	2.1
Pulmonary deposition	0.0007	0.0049	0.021

Table 1: Prediction of human PHMG exposure (all values in µg/cm2).

Experimental design: chronic repeated exposure of compounds in 3D models



Modified after Bustamante-Marin, et al. 2017

Fig. 1: Experimental design. Cell models and biomarkers for toxicity measurements were selected based on the predicted toxicity endpoints that reflect the most prominent *in vivo* functionality of the upper and the lower airways. Cell were treated daily for up to 12 consecutive days with nebulised PHMG at stated dose levels on the air-liquid interphase using a cloud chamber from Vitrocell. To mimic in vivo clearance of particles in the lung, MucilAir-HF cells were washed after 6h of treatment while EpiAlveolar cells were left until next treatment 24h later. **Data Analysis:** Time and concentration-dependent effects were modelled using a nonlinear state space model. Here, the various endpoints measured in the data were represented in the model as different states that can dynamically evolve over time in response to treatment. Gaussian processes were used to the nonlinear dependencies between these states and the chemical treatment. The data were fitted to the model using Bayesian inference, which is a probabilistic approach that allows for quantification of uncertainties in the model predictions. For further information see (Svenson, et al. 2017).

Results: Upper airways

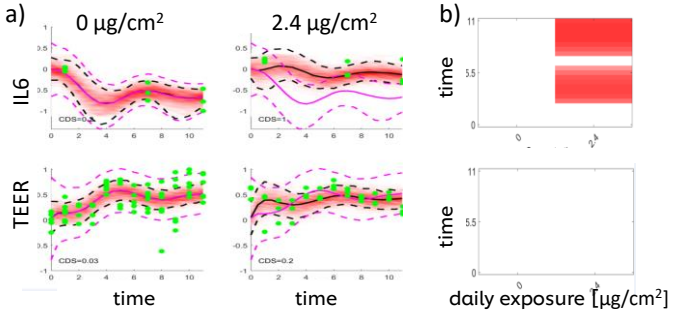


Fig. 2: Time and dose dependent response of PHMG treatment in MucilAir –HF. a) predictive distribution y plots showing 95% cred. range of mean response (black dashed lines) in comparison to the 95% cred. range of control (pink dashed lines). The green dots represent the actual data points. b) Concentration dependency score (CDS) plots summarising whether a particular biomarker is detected by the model as increasing (red) or decreasing (blue, see Fig. 3) at a given timepoint/dose. White implies no changes in comparison to the control.

Results: Lower airways

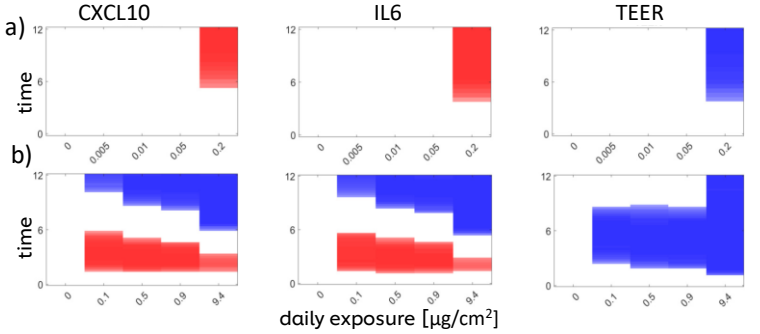


Fig. 3: Time and dose dependent response of PHMG treatment in EpiAlveolar cell model. CDS plots (as described in Fig 2) of PHMG treatment conducted in 2 independent laboratories (a and b). Different dose ranges were tested in the lab with the highest dose (0.2 µg/cm²) in a) overlapping with the lowest dose (0.1 µg/cm²) in b).

Conclusion and outlook

Treatment with PHMG leads to a time and dose dependent response in in both cell models. Daily exposure of 2.4 µg/cm² leads to only slightly elevated inflammatory cytokine release in MucilAir cell model (see Fig. 2 IL6) with no overall effect on tissue integrity (see Fig. 2 TEER). Daily exposure of 0.1-0.2 µg/cm² leads to loss of tissue integrity (TEER) in the EpiAlveolar cell system accompanied by increased release of cytokine markers (IL6/CXCL10) at early timepoints (see Fig.3) Decrease of cytokine release at later timepoints (blue) might be due cytotoxicity of the cells. These results might reflect the *in vivo* situation in humans where PHMG leads to acute interstitial pneumonia with diffuse alveolar damage.
Full analysis of the conducted study (ongoing) with the 2 case study and the 16 benchmark substances will help to investigate the suitability of the different cell models but also the gaps within the proposed NGRA framework.

References

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