Applying the Skin Sensitization AOP to Human Health Risk Assessment


Date: 22 March 2015

Abstract: Despite our understanding of the key events that drive skin sensitization (recently documented as an Adverse Outcome Pathway (AOP) – see figure below) our ability to combine non-animal data with exposure information to establish a safe level of human exposure for a sensitizing chemical remains a key gap. Our aim is to apply mechanistic understanding of skin sensitization to improve our ability to make risk assessment decisions. Central to our approach is mathematical modelling of the response and evaluation of model output against available clinical data on sensitization.

Our current model outputs naive CD8 T cell activation as a surrogate measure for sensitization induction in humans. Ordinary differential equations are used to model key events of the AOP: skin penetration (chemical diffusion and partitioning), haptenation of protein nucleophiles and antigen processing and presentation by skin dendritic cells. Biological parameters are taken from the immunological literature with human data used where possible. Bioavailability and chemical-specific parameters are derived from bespoke in vitro experiments and from sensitizer-specific literature.

The model has been used to simulate a study published previously by Friedmann et al. in which 132 healthy volunteers were exposed to one of five doses of the contact allergen 2,4-dinitrochlorobenzene (DNCB). As a significant proportion of each dose cohort were sensitised to DNCB within this study, comparison of model simulation results to these clinical data have provided an opportunity to explore the relationship between naive CD8 T cell activation and clinical sensitization. This analysis has enabled selection of an optimal model output parameter (T cell receptor trigger rate) for risk assessment decision-making and demonstrated the inherent difficulty in extrapolating from this cellular event to predict the extent of clinical sensitization. To address this finding, immune characterisation of allergic contact dermatitis patients is underway to enable mathematical modelling of the sensitizer-induced memory T cell response.

Application of model: 2,4-Dinitrochlorobenzene (DNCB) case study

Hypothesis:
- DNCB binds to protein via S$_x$Ar reaction with transient intermediate and first step being rate limiting

Reaction of DNCB with model peptides containing nucleophilic amino acids (cysteine peptide data shown) to estimate reaction kinetics with skin protein:
- DNCB incubated with 3 nucleophilic amino acids each over 3 concentrations
- Propose that in vivo reaction kinetics are also a single step 2$^{nd}$ order reaction

Skin Bioavailability and protein binding:
- ex vivo skin penetration experiment – modification of OECD TG 428 to include additional timepoints & measure fraction of chemical bound (Ref. Pickles et al. 2015 submitted)
- Skin PK model Davies et al. (2011). Toxicol. Sci. 119, 388-18
- Model fitting to experimental data provides parameters for DNCB

Acknowledgement: Skin bioavailability data generated in collaboration with In vitro Sciences, Charles River Laboratories, Edinburgh, UK

Model evaluation and next steps
- Currently performing uncertainty analysis on the model in partnership with J.P. Gosling (Univ. Leeds, UK; work funded by UK NCRM) and evaluating how the model can be applied to the human health risk assessment of cosmetic products
- Insights from these activities will guide future model development (e.g. explicit modelling of cellular immune response, formulation effects on skin bioavailability)

Class I MHC antigen processing and presentation

To predict the amount of haptenated peptide that will be presented to T cells from our skin bioavailability and reactivity model input data we have assumed that the CD8 T cell response will be induced through direct recognition of a haptenated ‘self’ peptide (i.e. ‘direct acting’ hypothesis).

We then estimate the average haptenated-pMHC surface density from considerations of:
1. the fraction of nucleophilic amino acids we expect to be haptenated by DNCB
2. probability that a pMHC contains a haptenated nucleophilic amino acid

Implementation of existing T cell activation model (Alekseev et al. 2010. Immunity, 32, 163–174) and review data from experimental antigens to identify sensitizer-relevant parameter sets (e.g. Stone et al. 2009. Immunology, 16, 165–174)

Simulation of Human immune response to DNCB

- Single exposure and site in humans to DNCB
- Subsequently, various exposure areas and sites

Acknowledgements: this research is informed by the wider skin allergy research programme that involves collaborations with the following organisations:

- University of Leeds
- University of Manchester
- Unilever
- NSF Foundation Trust
- Salford Royal NHS Foundation Trust