CASE STUDY PRESENTATION:
A QUANTITATIVE AOP FOR SKIN SENSITISATION RISK ASSESSMENT

GAVIN MAXWELL, CATHERINE CLAPP, RICHARD CUBBERLEY, SERAYA DHADRA, NIKKI GELLATLY, STEPHEN GLAVIN, SARAH HADFIELD, SANDRINE JACQUOILLEOT, AMELIA JARMAN, IAN JOWSEY, SUE LOVELL, JACK MAYNE, CRAIG MOORE, RUTH PENDLINGTON, JULIETTE PICKLES, JOE REYNOLDS, OUARDA SAIB, DAVID SHEFFIELD, RICHARD STARK, WENDY SIMPSON, VICKI SUMMERFIELD. DAWEI TANG, SAM WINDEBANK & CAMERON MACKAY

SAFETY & ENVIRONMENTAL ASSURANCE CENTRE (SEAC), UNILEVER R&D
TRADITIONAL SKIN SENSITISATION RISK ASSESSMENT

- Identify sensitisation potential
  - QSAR/read-across/analytical chemistry

- Identify sensitisation potency
  - LLNA (GPMT, Buehler)

- Define Human / HRIPT Threshold
  - No Expected Skin Sensitisation Induction Level (NESIL)

- Apply Sensitisation Assessment Factors (SAFs):
  - Inter-individual variability (x10)
  - Vehicle/product matrix effects (x1 - x10)
  - Use considerations (x1 – x10)
  - Acceptable Exposure Level (AEL)

- Compare AEL with Consumer Exposure Level (CEL)

- Decision on whether or not to market

- Benchmarking
  - Other Clinical data

- Consumer habits and practices data
NEW SKIN SENSITISATION RISK ASSESSMENT PARADIGM?

Modified from ‘Adverse Outcome Pathway (AOP) for Skin Sensitisation’, OECD
DEVELOP A MATHEMATICAL MODEL OF ALLERGIC CONTACT DERMATITIS TO ENABLE RISK ASSESSMENT DECISION-MAKING FOR NEW CHEMICALS

Model Inputs
- Concentration of ingredient due to exposure
- Molecular Initiating Event
- Cellular response
- Organ response

Model Outputs
- Vehicle Stratum Corneum
- Viable Skin
- Receptor Fluid
- Lymphatic Vessel
- Lymph Node

- Diffusion
- Partitioning

Model Inputs
- Exposure
- Skin Bioavailability
- Reactivity Kinetics

Model Outputs
- Hapten-specific T cell activation
- Dose of chemical applied to skin

Ex vivo human skin

Dendritic cell

Proliferating CD8+ T cell

Naïve CD8+ T cell

Chemical X

Donor Compound

1/8" OD x 1/32" Wall Tubing

Receptor Input

Receptor Chamber

Membrane

Compound and Receptor Output for Analysis

Chemical X

Dose of chemical applied to skin
DNCB CASE STUDY – BENCHMARK MODEL PREDICTION USING DATA FROM FRIEDMANN ET AL. 1983

Quantitative relationships between sensitizing dose of DNCB and reactivity in normal subjects

P. S. FRIEDMANN, CELIA MOSS, S. SHUSTER & JUDY M. SIMPSON* Department of Dermatology and *Department of Medical Statistics, University of Newcastle upon Tyne, Newcastle upon Tyne, UK.
MODELLING SKIN BIOAVAILABILITY OF EXPERIMENTAL SENSITISER 2,4-DI-NITRO CHLOROBENZENE (DNCB)

<table>
<thead>
<tr>
<th>Exposure Scenario</th>
<th>in vitro skin pen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>38.25 μg/cm²</td>
</tr>
<tr>
<td>Vehicle Volume</td>
<td>20 μL</td>
</tr>
<tr>
<td>Surface area</td>
<td>0.64 cm²</td>
</tr>
</tbody>
</table>

**Loss from skin**

**Loss from formulation**

**Partitioning**

**Diffusion**

**Estimation of Bioavailability in Skin** - based on OECD Test Guideline 428
- Penetration system modelled and partitioning and diffusion rates determined by fitting - Davies et al 2011 Toxicol. Sci. 119, 308-18
MODELLING OF SKIN PROTEIN HAPTENATION ➔ SKIN DENDRITIC CELL ANTIGEN PROCESSING, PRESENTATION & MIGRATION TO DRAINING LYMPH NODE

1. Concentration of ingredient due to exposure
2. Molecular Initiating Event
3. Cellular response
4. Organ response

- Vehicle
- Stratum Corneum
- Receptor Fluid
- Viable Skin
- Diffusion
- Partitioning
- Lymphatic Vessel
- Lymph Node
- Dendritic cell
- Naïve CD8+ T cell
- Proliferating CD8+ T cell

Graphs:
- Haptenated nucleophiles in the skin
- Skin DC density (cells cm⁻²)
- Fraction of hapten-pMHC on skin DCs
- LN DC density (cells mm⁻³)
- Fraction of hapten-pMHC on LN DCs

Time t (days): 0, 5, 10, 15, 20, 25
## DNCB CASE STUDY – MODEL OUTPUT & CLINICAL DATA

Predicted synapse formation rate

Percentages indicate the observed sensitisation rate at the corresponding dose (Friedmann et al. 1983)

### Sensitisation assessed by challenge with DNCB 4 weeks after application

- Single exposure to DNCB
- 7.1cm² volar forearm
- 100μL acetone vehicle

---

Friedmann et al. 1983

Quantitative relationships between sensitizing dose of DNCB and reactivity in normal subjects

P. S. FRIEDMANN, CELIA MOSS, S. SHUSTER & JUDY M. SIMPSON* Department of Dermatology and *Department of Medical Statistics, University of Newcastle upon Tyne, Newcastle upon Tyne, UK.

---

Prediction of maximum immune synapse rate in the 4 weeks following application

---

### Predicted synapse formation rate

Percentages indicate the observed sensitisation rate at the corresponding dose (Friedmann et al. 1983)
Sensitisation assessed by challenge with DNCB 4 weeks after application

Friedmann 2007
- Single exposure to DNCB
- Various exposure areas, sites, vehicles & volumes

Prediction of maximum immune synapse rate in the 4 weeks following application

<table>
<thead>
<tr>
<th>Diameter (cm)</th>
<th>Area (cm²)</th>
<th>Total (µg)</th>
<th>Concentration (µg cm⁻²)</th>
<th>Number of subjects</th>
<th>Percentage sensitised</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>7.1</td>
<td>1000</td>
<td>142</td>
<td>24</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>7.1</td>
<td>500</td>
<td>71</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>7.1</td>
<td>250</td>
<td>35.4</td>
<td>30</td>
<td>83</td>
</tr>
<tr>
<td>3</td>
<td>7.1</td>
<td>125</td>
<td>17.7</td>
<td>30</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>7.1</td>
<td>62.5</td>
<td>8.8</td>
<td>24</td>
<td>8</td>
</tr>
<tr>
<td>1.5</td>
<td>18.2</td>
<td>62.5</td>
<td>35.4</td>
<td>7</td>
<td>86</td>
</tr>
<tr>
<td>2.1</td>
<td>3.5</td>
<td>50</td>
<td>16.4</td>
<td>22</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>7.1</td>
<td>116</td>
<td>16.4</td>
<td>34</td>
<td>50</td>
</tr>
<tr>
<td>4.25</td>
<td>14.2</td>
<td>232</td>
<td>16.4</td>
<td>15</td>
<td>66</td>
</tr>
<tr>
<td>1 cm paper</td>
<td>0.8</td>
<td>30</td>
<td>38</td>
<td>28</td>
<td>93</td>
</tr>
<tr>
<td>3 mm paper</td>
<td>0.08</td>
<td>3</td>
<td>38</td>
<td>15</td>
<td>26</td>
</tr>
</tbody>
</table>

The relationships between exposure dose and response in induction and elicitation of contact hypersensitivity in humans

P.S. Friedmann

DOI: 10.1111/j.1365-2133.2007.08162.x

Predicted synapse formation rate per hour

Percentages indicate the observed sensitisation rate at the corresponding dose (Friedmann et al 2007)
OVERALL SUMMARY

• We have developed a mechanistic, mathematical model of DNCB-induced CD8⁺ T cell activation in humans with the purpose of informing skin sensitisation risk assessment
• The model predicts various measures of sensitiser-induced CD8⁺ T cell activation for different DNCB exposure scenarios using a combination of chemical- & exposure-specific non-animal data on skin bioavailability and reactivity

NEXT STEPS

1. Progress human CD8⁺ T cell response model development to allow mechanistic prediction of allergic contact dermatitis
2. Determine the uncertainty in our model predictions through characterising parameter & model uncertainty
ACKNOWLEDGEMENTS

Unilever

University of Leeds
Sheeja Krishnan, Grant Lythe & Carmen Molina-París
John-Paul Gosling – sponsored by:

University of Manchester
Rebecca Dearman, Chris Griffiths, Matt Harries, Amy Popple & Ian Kimber

Salford Royal NHS Foundation Trust
Jason Williams

University College London
Benny Chain & Theres Oakes