Applying AOPs to the development of Integrated Approaches to Testing and Assessment (IATA)

Gavin Maxwell
Unilever
Safety & Environmental Assurance Centre (SEAC)
Bedford, UK
+44-1234-264-888
gavin.maxwell@unilever.com
Conflict of Interest Statement

Gavin Maxwell is an employee of Unilever, the company developing the ‘IATA for Skin Sensitisation Risk Assessment’ used as an example IATA in this presentation.
Course Objectives/Outline

- Introduction to Skin Sensitisation AOP
- Overview of non-animal test methods and IATA relevant to skin sensitisation hazard and risk assessment
- Case study: application of skin sensitisation AOP to develop an IATA for risk assessment
  - IATA scope, input data and output metrics
  - Application of IATA to risk assessment
  - Results of Uncertainty analysis
  - Ongoing research and next steps
- Learnings and insights for AOP-driven IATA development
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACD</td>
<td>Allergic Contact Dermatitis</td>
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<td>ADME</td>
<td>Absorption Distribution Metabolism Excretion</td>
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<td>DC</td>
<td>Dendritic Cell</td>
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<td>DNCB</td>
<td>2,4-Dinitrochlorobenzene</td>
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<td>IATA</td>
<td>Integrated Approaches to Testing and Assessment</td>
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<td>MHC</td>
<td>Major Histocompatibility Complex</td>
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<td>TK</td>
<td>Toxicokinetic</td>
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<td>TD</td>
<td>Toxicodynamic</td>
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<td>TcR</td>
<td>T cell receptor</td>
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<td>DPRA</td>
<td>Direct Peptide Reactivity Assay</td>
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<td>GARD</td>
<td>Genomic Allergen Rapid Detection</td>
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<td>GPMT</td>
<td>Guinea Pig Maximization Test</td>
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<td>hCLAT</td>
<td>human Cell Line Activation Test</td>
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<td>HRIPT</td>
<td>human repeat insult patch test</td>
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<td>hTCPA</td>
<td>human T Cell Proliferation Assay</td>
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<td>LLNA</td>
<td>mouse Local Lymph Node Assay</td>
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<td>Q(SAR)</td>
<td>Quantitative Structure Activity Relationship</td>
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<td>TIMES-SS</td>
<td>Times MEtabolism Simulator platform for predicting Skin Sensitization</td>
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Skin Sensitisation AOP

Adapted from OECD report, ‘The Adverse Outcome Pathway for Skin Sensitisation initiated by covalent binding to proteins’, 2012
Integrated Approaches to Testing and Assessment (IATA): working definition

‘A hypothesis-driven framework for hazard identification, hazard characterization and/or safety assessment of a chemical or group of chemicals, which strategically integrates and weights all relevant existing data and guides the targeted generation of new data where required to inform regulatory decision-making regarding potential hazard and/or risk.’

Modified from OECD STA No. 215
Information sources for Skin Sensitisation IATA

- Concentration of ingredient due to exposure
- Molecular initiating event
- Cellular response
- Organ response

**In vitro skin penetration** (e.g. OECD TG 428)
- Structure-activity relationship Q(SAR) models
- Toxicokinetic skin models
- Skin metabolism models (e.g. TIMES-SS)

- Nrf2 pathway activation assays (e.g. KeratinoSens - OECD TG 442D, LuSens)
- Peptide reactivity assays (e.g. DPRA – OECD TG 442C)
- Reconstructed human epidermis activation assays (e.g. SENS-IS, SenCeeTox)
- Dendritic cell activation assays (e.g. h-CLAT, U-Sens™, VITOSens, GARD, IL-8 Luc Assay, m-MUSST)

- Human T cell proliferation assays (e.g. hTCPA)
- Artificial lymph node tissue models
- Mouse local lymph node assay (LLNA – OECD TG 429)

- Human repeat insult patch test (HRIPT)
- Guinea pig elicitation test (Buehler, GPMT – OECD TG 406)

**In silico**
**In vitro**
**In vivo**
Development of AOP-based IATA

• Same information sources can be integrated/interpreted in many ways to enable different decisions to be made
  – context-specific (screening → risk assessment)
  – substance-tailored

• Multitude of possibilities precludes the prescription of one-size-fits-all IATA but creates a communication challenge
  – i.e. how to efficiently communicate my integration approach to a third party to enable scientific review?

• OECD (in partnership with EU Commission JRC) currently developing a guidance document for the reporting of IATA using twelve Skin Sensitisation IATA as case studies
### Skin Sensitisation IATA: matrix of input datasets

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<tr>
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<th>Phys-Chem Info.</th>
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## IATA: sensitiser potential, potency, risk assessment

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<td>Kasting Skin PBPK model</td>
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Skin Sensitisation IATA case study references


6. & 7. Takanouchi et al. Test battery with the human cell line activation test, direct peptide reactivity assay and DEREK based on a 139 chemical data set for predicting skin sensitizing potential and potency of chemicals, J Appl Toxicol. 2015. 35. 1318-32


Objective: TKTD model scope should be simplest representation of the chemistry and biology capable of reproducing the induction of contact allergy to DNCB to enable prediction of a safe level of skin exposure.
**PANEL A = TOXICOKINETIC MODEL:**

1. Diffusion and partitioning into the stratum corneum and skin;
2. Sensitiser clearance by dermal capillaries;
3. Covalent modification of protein nucleophiles by hapten.

**PANEL B = TOXICODYNAMIC MODEL:**

4. Proteasome processing of protein nucleophiles to form small peptides and transport to the endoplasmic reticulum (ER);
5. Binding of peptides and hapten-peptide complexes to Class I MHC and transport to plasma membrane;
6. Binding of pMHC and hapten-pMHC to CD8+ T cell receptors and
7. Activation of naïve specific CD8+ T cells.
TKTD model: founding assumptions

1. Extent of naïve CD8+ T-cell receptor (TcR) triggering is the key determinant of human allergic status
2. Existence of at least one T-cell specific to the ‘antigen’
3. Required T-cell co-stimulatory signals are sufficient
4. Accompanying CD4+ T-cell response is optimal
5. DC migration from exposure site is sufficient
DNCB case study: human benchmark data


Quantitative relationships between sensitizing dose of DNCB and reactivity in normal subjects
P. S. Friedmann, C. Moss, S. Schuster & J. M. Simpson

- 165 healthy human volunteers divided into five dose groups
- Single exposure to between 62.5 - 1000 µg DNCB applied to 7.1cm² volar forearm in 100µL acetone vehicle
- Sensitisation assessed four weeks later by DNCB challenge
- Sensitisation varied between 8 - 100% across dose groups
TK model of Skin Penetration and Protein Binding

- **S**: sensitiser
- **Nu**: protein nucleophiles
- **\( \hat{\text{Nu}} \)**: protein hapten-nucleophiles

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**A**

1. Sensitiser enters stratum corneum
2. Sensitiser binds to protein nucleophiles
3. Combination of sensitiser and protein nucleophiles enters skin

**B**

1. Protein nucleophiles bind to DC surface
2. DCs internalise protein nucleophiles
3. Protein nucleophiles enter DC cytosol
4. Peptide and MHC bind
5. MHC and peptide presented
6. Peptide MHCI binds TcR
7. TcR activates CD8+ T cell

- **p**: peptide
- **\( \hat{\text{p}} \)**: hapten-peptide
- **MHC**: MHCI
- **\( \hat{\text{MHC}} \)**: peptide MHCI
- **\( \hat{\text{MHC}} \)**: hapten-peptide MHCI
- **TcR**: CD8+ T cell Receptor
Characterising dose of DNCB in viable skin

- Ex vivo human skin penetration experiment – OECD TG 428 to include multiple time points and chemical free/bound measurements
- Fitting to experimental data provides parameters for DNCB TK Model
Characterising rate of DNCB protein binding

Bespoke method:
- Radiolabelled DNCB in solution incubated with tape-stripped ex vivo human skin for up-to 24hrs
- Reaction diffusion system modelled and reaction rate determined by fitting to experimental data
TD model of protein degradation, hapten-peptide-MHC binding & TcR signaling

- Sensitiser: $S$
- Protein nucleophiles: $Nu$
- Protein hapten-nucleophiles: $\tilde{Nu}$

Diagram:

- **A**
  - Sensitiser $S$ in stratum corneum
  - Protein nucleophiles $Nu$ react with $S$ to form $\tilde{Nu}$
- **B**
  - CD8$^+$ surface
  - DC surface
  - TcR
  - Peptide $p$ and MHC
  - Hapten-peptide MHCI
  - CD8$^+$ T cell Receptor

Symbols:

- $p$: Peptide
- $\tilde{p}$: Hapten-peptide
- MHC: Major Histocompatibility Complex
- $pMHC$: Peptide-MHC complex
- $\tilde{p}MHC$: Hapten-peptide-MHC complex
- $CD8^+$: CD8$^+$ T cell
Parameterising TK model using literature data: e.g. proteasomal protein degradation

- Proteasome
- Damaged proteins
- Peptides

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<table>
<thead>
<tr>
<th>Reference</th>
<th>System/Species</th>
<th>Experimental Method</th>
<th>Interpretation (refer to the actual data considered)</th>
<th>Source of Uncertainty (start a new row for each)</th>
<th>Direction &amp; Magnitude</th>
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<tr>
<td>Boisvert et al., 2012</td>
<td>HeLa cells were cultured in vitro</td>
<td>Study of HeLa cells in cultured in SILAC media. Analysis by Gel electrophoresis followed by LC-MS/MS and bioinformatics analysis for determination of peptide turnover.</td>
<td>8,041 endogenous HeLa proteins were identified. FIG. 5. Shows the distribution of protein 50% turnover time in various subcellular compartments including cytoplasm. Fig 5C, shows the distribution of protein 50% turnover time in cytoplasm.</td>
<td>System extrapolation (from HeLa cells in vitro to skin DC in vivo). Expectation that cancer cells and in vitro cells may display a higher turnover and protein degradation rate than skin DC under DNAC exposure</td>
<td>-/+44</td>
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- Sensitisation assessed four weeks later by DNCB challenge
- Rates of sensitisation vary between 8 - 100%
TKTD model simulation of single 16.5\(\mu\text{gcm}^{-2}\) exposure

Median, 5\textsuperscript{th} & 95\textsuperscript{th} %tile over 10,000 simulations

Fraction of pMHC bound

Fraction of peptides bound
Thresholding model output to predict adverse response

- Threshold defined (using literature data) that predicts the rate of human naïve CD8+ TcR signaling that would be sufficient to confer allergic status
Thresholding model output to predict adverse response

- Threshold defined (using literature data) that predicts the rate of human naïve CD8⁺ TcR signaling that would be sufficient to confer allergic status.
- Reverse Dosimetry used to back-calculate the doses of DNCB that would cross the TcR signal rate threshold/cause contact allergy.
Learnings and insights for AOP-driven IATA development

- AOP provides a framework/scaffold for IATA development by summarizing our mechanistic understanding and thereby providing a ‘working hypothesis’ for data weighting and integration
Learnings and insights for AOP-driven IATA development

- IATA scope, input data and output data should all follow the purpose (i.e. what question are you trying to answer?)

‘A hypothesis-driven framework for hazard identification, hazard characterization and/or safety assessment of a chemical or group of chemicals, which strategically integrates and weights all relevant existing data and guides the targeted generation of new data where required to inform regulatory decision-making regarding potential hazard and/or risk.’
Learnings and insights for AOP-driven IATA development

- Quantitative modelling of AOPs can help refine our mechanistic understanding by checking the value of each key event for informing a regulatory/safety decision.

**Objective:** model scope should be simplest representation of the chemistry and biology capable of reproducing the induction of contact allergy to DNCB to enable prediction of a safe level of skin exposure.
Learnings and insights for AOP-driven IATA development

- Uncertainty analysis is a powerful tool for providing decision-makers with a quantitative understanding of how model assumptions and imperfect parameter data translate into overall prediction uncertainty.
References

**AOP → IATA background literature**

**Case study: IATA for Risk Assessment background literature**