Modelling Local and Systemic Toxicity: Incorporation of *In Silico* Predictions in the Development of Adverse Outcome Pathways

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**In Silico Prediction**

- Activity (e.g. toxicity) of a chemical $\propto$ Molecular (structural) properties

- Certain endpoints easier to predict than others

- Methods need to be transparent

- Newer methods such as category formation (*grouping*) & read-across can be applied to complex endpoints & are transparent

| Toxicity | ⚫ | ← | O | ← | ⚫ |
Adverse Outcome Pathway for Skin Sensitisation

- The AOP provides a method to represent the **Key Events** involved
- **Key Events** are measurable and toxicologically relevant
- NB pathway may be non-linear; adaptive & regulatory responses may be involved
- Realistic exposure scenario need to be considered
Key Events in Skin Sensitisation AOP

**Key Event 1**
- Molecular Initiating Event
- Covalent binding interaction

**Electrophile**

**Key Event 2**
- Cellular response
- Keratinocyte inflammatory response

**Key Event 3**
- Cellular response
- Dendritic cell activation

**Key Event 4**
- Organ Response
- Lymph node - activation of T-cells, proliferation of activated T-cells

**Adverse Outcome**
- Skin: Inflammation on challenge with antigen
Key Events in Skin Sensitisation

Key Event 1: Molecular Initiating Event
- Covalent binding interaction
- Electrophile: Nucleophile on skin protein (cysteine/lysine)

Key Event 2: Cellular response
- Keratinocyte inflammatory response

Key Event 3: Cellular response
- Dendritic cell activation

Key Event 4: Organ Response
- Lymph node - activation of T-cells, proliferation of activated T-cells

Adverse Outcome
- Skin: Inflammation on challenge with antigen
Accumulating Information to Develop AOPs

- The Molecular Initiating Event (MIE) (initial interaction between chemical and biological system) is a **Key Event**
  - Hypothesis or evidence required for Key Events
  - Evidence can be accumulated from a range of sources

- Capturing the chemistry underlying an MIE can be used to develop Structural Alerts / Profilers for **grouping** chemicals into categories

**In Silico**
- Creating Structural Alerts / Profilers
- Identifying MIEs

**In Chemico**
- Direct Peptide Reactivity Assay
- Glutathione depletion

**In Vitro**
- MUSST/h-CLAT
- Ames Test
- Mitochondrial Damage
- Oxidative Stress

**In Vivo**
- GPMT
- LLNA
- Human Patch Test
- Toxicity Assays
Identifying Groups in Chemical Space
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Structural Analogues
Identifying Groups in Chemical Space
Identifying Groups in Chemical Space

Mode of Action Analogues
(Binding to oestrogen receptor)
Identifying Groups in Chemical Space
Identifying Groups in Chemical Space

Mechanistic analogues (Michael addition reaction)
Mechanistic Structural Alerts and Profilers Developed:

Protein binding relating to skin & respiratory sensitisation

Use knowledge of mechanistic chemistry domain e.g. Acylation, Schiff Base Formation

Michael Addition $^{\text{\textgreater}r}$ → Mechanistic Alert: (e.g. polarised alkenes)

Structural Alerts: e.g. $\alpha,\beta$ unsaturated aldehydes, ketones, amides

104 structural alerts developed for protein binding (46 associated with skin sensitisation); 52 structural alerts for respiratory sensitisation

Also 57 structural alerts developed for DNA binding

Derive associated SMARTS$^1$ patterns (e.g. CC=OC; C=NCCR)

Encode into profilers

$^1$ freely available – also encoded in KNIME workflows

$^2$ available in OECD QSAR Toolbox;
Identifying Groups in Chemical Space
Identifying Groups in Chemical Space

Structural Similarity
Grouping Chemicals for Read-Across

Analogue search → Chemical Dataset → Apply mechanistic “profilers”

(unknown mechanism)

Group according to similarity

Confirm acceptability of group
- adequate numbers
- visual assessment of similarity

Identify key fragment responsible for activity
- literature search for mechanism
- propose Molecular Initiating Event (MIE)

Code fragment as structural alert (chemotype)
- incorporate in “profilers”

Proceed to grouping and read-across

Automation e.g. KNIME workflows; OECD QSAR Toolbox
951 compound dataset* (650 +ve)

Grouped by structural similarity - using Toxmatch software

Group (category) acceptable if:
- contained \( \geq 6 \) compounds
- similarity index \( \geq 0.6 \)
- visually appeared similar

Alerts used to re-screen dataset

Literature search for putative mechanisms of toxicity

Mechanisms proposed for \( \sim 25\% \) of hepatotoxicants in dataset

Example SMARTS Patterns
1. C=CC=CC=CC=O
2. ClCCNCCl
3. O=C1CCC2C3CCC4CCCCC4C3CCC2=C1

16 structural alerts# (key fragments) identified

1

2

3


Using the Information to Predict Toxicity

- Profilers can be used to form groups of compounds (categories)
- Structural or mechanistic knowledge of category members can be used to infer information concerning an (unknown) compound of interest i.e. a read-across prediction

Enables transparent, justifiable predictions to be made

Cefotaxime predicted to be in toxicity class ‘B’

Formation of Structural Categories to Allow for Read-Across for Teratogenicity
Tools for Category Formation & Read-Across: OECD QSAR Toolbox

- Input query chemical
- Profile (identify key features)
- Gather endpoint information
- Develop and refine category
- Use compounds of known activity to fill data gap
- Produce report

Profilers: Mechanistic, Empiric, Toxicological
Endpoint specific or by affiliation

Under further development...
The COSMOS Project

Integrated *in silico* models for the prediction of repeated dose toxicity of **COSMetics to Optimise Safety**

- Developing tools for predicting repeat dose toxicity
  - database of relevant toxicity & ADME data
  - building (Q)SARs
  - identifying structural alerts – creating profilers for category formation
- Freely available as KNIME workflows

[www.cosmostox.eu](http://www.cosmostox.eu)
Conclusions and Outlook

- AOPs provide a framework for organising information
  - The MIE is a key event (⚓) in an AOP
- Understanding mechanistic chemistry enables structural alerts / profilers to be built associated with an MIE
- Categories (groups) can be based on structural similarity & potential mechanisms investigated
- Literature or experimental evidence provides support for AOPs
  - Link *in silico* investigation to directed *in vitro* analysis
    - Confirmation of effects; defining chemical space of alert
- Improvements needed in tools to capture and use information
  - Toolbox; Effectopedia
  - Mitigating factors need to be considered
  - More quantitative predictions in future

Category formation (grouping) & read-across provide more transparent, acceptable methods of predicting toxicity
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Thank you for your attention!