Challenges and opportunities for using AOP-based *in silico* models in regulatory contexts

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Overview

• The “AOP-based in silico model” concept
• AOP-based in silico model – Altox’s Framework
• Examples: Balancing transparency, mechanistic interpretability and predictivity
• Challenges and opportunities for using these methods in regulatory contexts
Introduction

Adverse outcome pathways (AOP) framework

- An AOP is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organisation that lead to an adverse health or ecotoxicological effect (OECD, 2012)
  - **Regulatory challenge:** to define when there is sufficient confidence in predictivity to use one or more alternative models for regulatory purposes

Proposed “AOP-based in silico model” Concept

- A framework composed by individual *in silico* models used to identify chemicals that can activate the associated modular **AOP components** (MIE/KE) and based in these individual multilevel predictions, balanced by adjustments, relationships and weights, **to predict an adverse outcome.**

AOP-based *in silico* model – Altox’s Framework

Key Event Relationships (KERs)

Different weights for the predictions based in the AOP level, KERs and confidence levels along the AOP

- Molecular level
  - Chemical structures and physicochemical properties
    - Molecular weight
    - LogP
    - LogD
    - Topological and electronic descriptors
    - Fragments/fingerprints
    - Others

- Molecular Initiating Event (MIE)
  - Structural alerts
  - Mechanistic read-across
  - Statistical models for chemical interactions
  - Others

- Organ level
  - Key event 2
  - Key event 3
  - Key event 4

- Organism response
  - Global AOP-based in silico model balanced by KERs, adjustments, weight of evidence assessment in a logical framework
Example – AOP-Sens

- A logical framework balancing transparency, mechanistic interpretability and predictivity;
- Models to predict chemicals that **can activate** the AOP modular components (MIE/KEs);
- **A global model** integrating all multilevel predictions balancing predictivity, key events relationships and WoE adjustments, for predicting the outcome.
Molecular level

Structures and physicochemical properties

<table>
<thead>
<tr>
<th>Molecular Query</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMILES: CC=C=O=O(C)=OCC</td>
</tr>
<tr>
<td>Name: Molecule 2</td>
</tr>
<tr>
<td>logKow: 0.89</td>
</tr>
<tr>
<td>logD: 1.61</td>
</tr>
</tbody>
</table>

Metabolism prediction and potential for haptenation

To assess both direct and indirect haptenants, this module predicts the potential for metabolic activation (pro-hapten formation) by known Phase I reactions, i.e., it can be used to identify potential skin sensitizers which require some type of metabolism to an active metabolite (pro-haptenants) before initiation of the key event 1 (KBI) in a skin sensitization AOP (OECD Principle 5).

Molecular Initiating Event

Structural Alert Analysis

Result: (+) Positive

Alerts were found in the molecule. The results are in the table below and a description is provided at the end of the report.

<table>
<thead>
<tr>
<th>Category</th>
<th>Alert</th>
<th>Alert ID</th>
<th>References</th>
</tr>
</thead>
</table>

STR Contribution Map

Reactive / Active (+) Non-Reactive / Inactive (-)

Predicted endpoint/Method | Predicted class (Confidence) | STR Contribution Mapping |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Peptide Reactivity Assay (DPRA, OECD 442D)</td>
<td>Reactive (+) (82.8%)</td>
<td></td>
</tr>
</tbody>
</table>
Cellular and organ levels

Key Events

<table>
<thead>
<tr>
<th>Predicted endpoint/Method</th>
<th>Predicted class (Confidence)</th>
<th>STR Contribution Mapping</th>
</tr>
</thead>
<tbody>
<tr>
<td>KeratinoSens™ (OECD 442D)</td>
<td>Inactive (-) (89.7%)</td>
<td>![Chemical Structure]</td>
</tr>
<tr>
<td>Human Cell Line Activation Test (h-CLAT, OECD 442E)</td>
<td>Inactive (-) (76.5%)</td>
<td>![Chemical Structure]</td>
</tr>
<tr>
<td>Local Lymph Node Assay (LLNA, OECD 429)</td>
<td>Sensitizer (+) (76.4%)</td>
<td>![Chemical Structure]</td>
</tr>
</tbody>
</table>
Organism response

AOP-based algorithm = \[ \sum_{j=1}^{n} p_j c_j a_j w_j \]

Where \( p \) is a prediction result, \( c \) is the confidence level, \( a \) is the applicability domain and \( w \) is a weight (adjusted by KERs)

### Results

<table>
<thead>
<tr>
<th>Chemical Structure &amp; Predicted Properties</th>
<th>Predicted Physical-Chemical Properties:</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>( \log K_{ow} ): 0.49 ( \log D_{ow} ): 0.54</td>
</tr>
</tbody>
</table>

#### Structural Alerts
- Positive (+)
  - Protein-binding alerts according to QSAR: 1A: 0, 1B: 1
- Metabolism: Number of metabolites 3, Major metabolite 29.9%
- DPRA: Reactive (+)
  - AD: Outside
  - Confidence: 81.6%

#### KeratinoSens
- Inactive (-)
  - AD: Within
  - Confidence: 79.1%

#### h-CLAT
- Active (+)
  - AD: Within
  - Confidence: 89.9%

#### LLNA
- Sensitizer (+)
  - AD: Within
  - Confidence: 59.7%

#### Human
- Non-Sensitizer (-)
  - AD: Within
  - Confidence: 61.9%

#### Combined Model
- Sensitizer (+)
  - AD: Within
  - Confidence: 95.6%

#### Metabolism
- Number of metabolites 4, Major metabolite 14.5%
- DPRA: Non-Reactive (-)
  - AD: Within
  - Confidence: 88.3%

#### Predicted KE4 and Adverse Outcome
- LLNA: Sensitizer (+)
  - AD: Within
  - Confidence: 78.4%
- Human: Sensitizer (+)
  - AD: Within
  - Confidence: 86.8%
- Combined Model: Sensitizer (+)
  - AD: Within
  - Confidence: 94.4%

#### AOP-based Prediction
- Concordance between KEs: 82.3%
- Confidence level (external validation): 87.5%
Challenges and opportunities

• Different results with variable confidence levels
• Identifying patterns of predictive combinations;
• Identifying patterns for pre and pro-haptens.
Measures of robustness and predictivity

**Benchmark - Combined dataset** (GMTP, LLNA and human data)

<table>
<thead>
<tr>
<th>(Q)SAR Models</th>
<th>Model Dataset</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>Accuracy</th>
<th><em>N (external dataset)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alert analysis</td>
<td>197</td>
<td>0.57</td>
<td>0.59</td>
<td>0.58</td>
<td>6422</td>
</tr>
<tr>
<td></td>
<td>128: 1A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>69: 1B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPRA</td>
<td>195</td>
<td>0.76</td>
<td>0.32</td>
<td>0.54</td>
<td>6422</td>
</tr>
<tr>
<td>KeratinoSens</td>
<td>190</td>
<td>0.78</td>
<td>0.27</td>
<td>0.52</td>
<td>4050</td>
</tr>
<tr>
<td>H-CLAT</td>
<td>161</td>
<td>0.65</td>
<td>0.41</td>
<td>0.53</td>
<td>4178</td>
</tr>
<tr>
<td>LLNA</td>
<td>997</td>
<td>0.46</td>
<td>0.68</td>
<td>0.57</td>
<td>4932</td>
</tr>
<tr>
<td>Human Skin</td>
<td>389</td>
<td>0.82</td>
<td>0.35</td>
<td>0.59</td>
<td>4177</td>
</tr>
<tr>
<td>Combined dataset</td>
<td>6971</td>
<td>0.75</td>
<td>0.92</td>
<td>0.84</td>
<td>1284</td>
</tr>
<tr>
<td>(GMTP, LLNA and human data)**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AOP-based prediction</td>
<td>-</td>
<td>0.76</td>
<td>0.71</td>
<td>0.74</td>
<td>6422</td>
</tr>
</tbody>
</table>

** Internal validation
Chemical and Toxicological Space for Skin Sensitization

Grouped by Similarity neighbors and Endpoint

Hybrid descriptor

Combined dataset = (GMTP, LLNA and human data)
Chemical and Toxicological Space for Skin Sensitization

Grouped by Similarity neighbors and all Endpoints Outcome: Positive (+) or Negative (-)

Hybrid descriptor
OECD Principles of (Q)SAR Validation for regulatory purposes

1. A defined endpoint;
2. An unambiguous algorithm;
3. A defined domain of applicability;
4. Appropriate measures of goodness-of-fit, robustness and predictivity;
5. A mechanistic interpretation, if possible.
Challenges and opportunities

- Balancing predictivity and mechanistic interpretation of the models at different levels (adverse outcome pathways - AOP)
- Looking for patterns with predictivity level similar or higher than biological assays
- Filling gaps in safety assessments with *in silico* predictions
- Making new regulatory criteria based in predictive integrated approaches containing *in silico* models
We are seeking partnerships and collaborations with our tools!

- iS-Ocular™
- Pred-CYP2D™
- Pred-Oral™
- DevTox-iS™
- Genotox-iS™
- AOP-Sens™
- IrriTest™
- iS-Liver™
- Acute-Tox™
- BCF-Test™
- Pred-Ecotox™
Thank you for your attention!

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