Use of Adverse Outcome Pathways (AOPs) to reduce uncertainty and animal use in chemical hazard and risk assessment

Catherine Willett
Director, Regulatory Toxicology, Risk Assessment and Alternatives
Humane Society of the United States
The need for a new approach to chemical assessment

Industrial chemicals:
- Growing concern over lack of information
- >10K – 100K chemicals worldwide
- Large-scale regulatory programs (i.e. REACH)

Pesticides:
- Registration requires the use of approximately 10,000 animals, millions of USD, and many years (decades)
- Need to identify “greener” chemistries

Pharmaceuticals:
- 92% of drug candidates fail in clinical studies
- More than $1 billion, a decade and innumerable animals
- Need to assess novel chemistries (i.e. nanomaterials)

Cosmetics:
- European Cosmetics Directives ban on animal testing
- Consumer concern over safety and animal testing worldwide
The time is right for a new approach

- Capitalize on advances in chemistry, biology, and engineering (since ~1970)
- Fully utilize all existing knowledge
- Increase assessment capacity (“throughput”)
- Increase efficiency (benefit/cost)
- Increase relevance to humans/species of concern
- Increase predictivity
One solution: a pathway-based approach

“envision a new toxicity-testing system that evaluates biologically significant perturbations in key toxicity pathways by using new methods in computational biology and a comprehensive array of in vitro tests based on human biology”

National Research Council in 2007 Report, Toxicity testing in the 21st century: A vision and a strategy
A biological map describing what happens when a chemical interacts with a living system, from the molecular initiating event through the resulting adverse outcome.

Building an AOP

- Start anywhere
- Gather all existing knowledge
- Evaluate and document the information
- Translate and capture information as a pathway


The OECD/EPA/JRC AOP project

- AOP Wiki
  Collaborative development of AOP descriptions and evidence

- Effectopedia
  Development of quantitative AOPs in a graphical environment

- Intermediate Effects DB
  Put chemical-related AOP components in a regulatory context

- AOP Xplorer
  Visualizes attribute networks to discover & explore AOPs in a broader context

- AOP-KB Hub
  Shared chemical, biological and toxicological ontologies

- Third party
  Applications, plugins

AOP Wiki: https://aopkb.org/aopwiki/index.php/Main_Page
- Publicly available to registered users
Using AOPs

Current use:

* Inform chemical categories and structure activity relationships
* Prioritization of chemicals for further assessment
* Hazard identification
* Increasing certainty of interpretation of both existing and new information
* Developing integrated testing strategies that maximize useful information gained from minimal testing

Future use:

* Identify key events for which non-animal tests can be developed, thereby facilitating mechanism-based, non-animal chemical assessment
* Create predictive toxicological assessments with low uncertainty and high human relevance
Using AOPs in hazard and risk assessment

<table>
<thead>
<tr>
<th>AOP Continuum</th>
<th>USE</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlative/qualitative</td>
<td>Chemical Categories/Read across</td>
<td>Narcosis due to respiratory failure</td>
</tr>
<tr>
<td>• mechanistic understanding of MIE/KE (Quantitative or not)</td>
<td>Prioritization</td>
<td>Mitochondrial Fatty Acid Beta-Oxidation Inhibition Leading to Steatosis</td>
</tr>
<tr>
<td>• simple statistical correlations with some biological plausibility between MIE/KEs and AOs</td>
<td>Hazard ID</td>
<td>Cancer caused by exposure to 1,4-dioxane</td>
</tr>
<tr>
<td>Qualitative</td>
<td>Integrated testing strategy, design</td>
<td>Skin Sensitization Initiated by Covalent Binding to Proteins</td>
</tr>
<tr>
<td>• some mechanistic understanding of linkages between MIE/KE and AO</td>
<td>Hazard characterization</td>
<td>Aromatase inhibition leading to reproductive dysfunction (in fish)</td>
</tr>
<tr>
<td>• some evidence for causal linkages</td>
<td>Quantitative Risk Assessments</td>
<td></td>
</tr>
<tr>
<td>Semi-quantitative</td>
<td>Predictive system</td>
<td></td>
</tr>
<tr>
<td>• some quantitative understanding</td>
<td>Predictive causally-linked quantitative models</td>
<td></td>
</tr>
<tr>
<td>• dose-response information, toxicokinetics, metabolism</td>
<td>Dose relationships</td>
<td></td>
</tr>
<tr>
<td>Quantitative</td>
<td>Some understanding of intersecting pathways</td>
<td></td>
</tr>
<tr>
<td>• Predictive causally-linked quantitative models</td>
<td>Increased certainty of likelihood of a particular AO vs some other outcome</td>
<td></td>
</tr>
</tbody>
</table>
Example: skin sensitization

Phase I: Initiation

1. Chemical penetrates the skin
   a. Is either an electrophile or is metabolized to become one
2. Hapten reacts with proteins on the surface of keratinocytes and dendritic cells in the epidermis
3. Hapten-protein complex activates both cell types to produce cytokines
4. The dendritic cells also activated the immune system
Skin sensitization as an AOP

**Chemical Property**
- Electrophilic substance or precursor
- Covalent interaction with cell protein

**Molecular Initiating Event**
- QSARs
- In vitro skin absorption (OECD 428)
- Direct Peptide Reactivity Assay (DPRA; OECD 442C)

**Cellular Response**
- Dendritic cells
  - Induction of inflammatory cytokines and surface molecules
  - Mobilization of DCs
- Keratinocyte
  - Activation of inflammatory cytokines
  - Induction cyto-protective gene pathways

**Organ Response**
- Lymph node
  - Histocompatibility complexes presentation by DCs
  - Activation of T cells
  - Expansion of activated T-cells

**Organism Response**
- Skin
- Inflammation upon challenge with allergen

In vitro skin absorption (OECD 428)
- Direct Peptide Reactivity Assay (DPRA; OECD 442C)
- Human Cell Line Activation Test (h-CLAT; draft OECD 442E)
- KeratinoSens (OECD 442D)
## Integrated Approach to Testing and Assessment (IATA) informed by AOP

Compared to human test

<table>
<thead>
<tr>
<th>Compared to human</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard animal test</td>
<td>LLNA 89%</td>
</tr>
</tbody>
</table>

**Individual tests**

<table>
<thead>
<tr>
<th>Test</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPRA</td>
<td>87%</td>
</tr>
<tr>
<td>LuSens</td>
<td>82%</td>
</tr>
<tr>
<td>mMUSST</td>
<td>85%</td>
</tr>
<tr>
<td>h-CLAT</td>
<td>78%</td>
</tr>
</tbody>
</table>

**Combinations (1 out of 2 is positive)**

<table>
<thead>
<tr>
<th>Combination</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPRA + LuSENS</td>
<td>85%</td>
</tr>
<tr>
<td>DPRA + mMUSST</td>
<td>81%</td>
</tr>
<tr>
<td>DPRA + h-CLAT</td>
<td>83%</td>
</tr>
<tr>
<td>LuSens + mMUSST</td>
<td>80%</td>
</tr>
<tr>
<td>LuSens + h-CLAT</td>
<td>82%</td>
</tr>
</tbody>
</table>

**2 out of 3**

<table>
<thead>
<tr>
<th>Combination</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPRA + LuSens + mMUSST</td>
<td>94%</td>
</tr>
</tbody>
</table>

---

Risk assessors (regulatory or otherwise) need to:

- use many different kinds of information to make decisions
- weigh data in terms of relevance and reliability
- assess and document confidence in data and assumptions
- understand and acknowledge uncertainties surrounding the assessment
AOPs can decrease uncertainty by:

- Provides a framework for collecting, evaluating and quantifying data
  - confidence of underlying data is transparently catalogued
  - strengths of relationships within an AOP can be used to weigh data in a weight-of-evidence evaluation
- Provides a framework for IATA based on tests of key events
- Allows computational modeling of pathway elements
  - different types of modeling can be applied (probabilistic, deterministic)
  - safety can be predicted with known uncertainty/confidence
In summary

**AOPs can currently be used to:**

- reduce uncertainty by weighting or quantifying information
- indicate the most appropriate/valuable tests to use for assessment
- increase efficiency and effectiveness of chemical assessment
- reduce reliance on information from animal studies

**And in the future:**

- Predict outcomes of chemical and other perturbations by measuring a few, upstream events
Thank you!

Catherine Willett, PhD
Director, Regulatory Testing
Risk Assessment and Alternatives
Humane Society of the United States

Coordinator, Human Toxicology Project
Consortium

kwillett@humanesociety.org