Advantages and implications of using Adverse Outcome Pathways (AOPs) for disease outcomes resulting from temporal exposures

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This talk does not necessarily reflect the views of the U.S. Environmental Protection Agency.
Outline

• Adverse Outcome Pathway Background
• AOPs and Temporal Exposure Issues
• Making It Happen
Goal: Bridge the Gap

• Tox21/SEURAT-1
  – High-throughput, *in vitro*
  – Capable of directly screening many chemicals

• Regulatory Decisions
  – Toxicity must relate to the impact on human health, endangered species, or wildlife populations
• Key Events (KEs) - nodes
  – Change in biological state
  – Measurable and essential for progression
  – MIE - initial point of chemical interaction
  – AO – adverse outcome of regulatory significance

• Key Event Relationships (KERs) - edges
  – Connections between two key events
  – Critical for assembling evidence in support of the AOP
Five Principles of AOP Development

1. AOPs Are Not Chemical Specific
2. AOPs Are Modular (consisting of KEs and KERs)
3. An Individual AOP Is a Pragmatic Unit of Development and Evaluation
4. For Most Real-World Applications, AOP Networks Are the Functional Unit of Prediction
5. AOPs Are Living Documents

OECD AOP Development Programme

What is an Adverse Outcome Pathway (AOP)

In 2012, the OECD launched a new programme on the development of Adverse Outcome Pathways. An Adverse Outcome Pathway (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 (see figure). AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning.

- Extended Advisory Group for Molecular Screening & Toxicogenomics (EAGMST)
- Guidance & Training
  - Guidance, User Handbook, many training options
- International Knowledgebase to capture information
  - >100 AOPs at various stages of development

Overlapping Phases of AOP Development

- Define AOP
- Evaluate the AOP
- Quantitatively describe the AOP
OECD Handbook
Step by step guide to AOP development


AOP-Wiki
Provides consistent structure based on the OECD handbook and facilitates collaborative AOP development

http://aopwiki.org/
AOP-KB Supports all Stages of Development

- **Putative AOPs**
- **Formal AOPs**
- **Quantitative AOPs**

- **AOP-Xplorer** – Helps assemble putative AOPs
- **AOP-Wiki** – Provides structured forms for evaluating AOPs
- **Effectopedia** – Provides tools for quantitatively describing AOPs
Current Status of AOP Development

- > 100 putative AOPs in the AOP-Wiki (most not under active development)
- 14 formal AOPs undergoing OECD review
- < 5 quantitative AOPs under development

Less Data Needs → Broader Coverage
Increasing Confidence → Broader Applicability
What do AOPs provide that can help when dealing with temporal exposures?
But we need to go further – AOPs are a step on the pathway to more formal representation of our knowledge on system composition and behaviour.

- Emphasise the need to understand, and not just measure - position modelling at the centre.
Match Measurements with Time Frame of Exposure

- Properties (QSAR)
- Disposition (Exposure Biomarkers)
- Toxicants
- Macro-Molecular Interactions
- Toxicity Pathways (HTS Assays)
- Key Events (Bioindicators)
- Cellular Responses
- Organ Responses
- Organism Responses
- Regulatory Endpoints (Adverse Outcomes)
- Population Responses

Time between exposure and effect increases

Predictivity of measurement for AO decreases
Alkylation of DNA in male pre-meiotic germ cells leading to heritable mutations

Short name: Alkylation of DNA leading to heritable mutations

Relationships Among Key Events and the Adverse Outcome

<table>
<thead>
<tr>
<th>Event</th>
<th>Description</th>
<th>Triggers</th>
<th>Weight of Evidence</th>
<th>Quantitative Understanding</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Alkylation</td>
<td>Directly Leads to</td>
<td>Insufficient or incorrect DNA repair, N/A</td>
<td>Strong</td>
<td>Moderate</td>
</tr>
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Factors Determining Predictivity of Early Key Events

- Evidence supporting the KERs between that KE and the AO
- Quantitative understanding of the downstream KERs
- Modifying factors that influence downstream KEs & KERs
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals

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<tr>
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<td>Directly Leads to</td>
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<td>Directly Leads to</td>
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<td>Weak</td>
</tr>
<tr>
<td>Thyroxin (T4) in neuronal tissue, Decreased</td>
<td>Indirectly Leads to</td>
<td>Cognitive Function, Decreased</td>
<td>Strong</td>
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<tr>
<td>Thyroxin (T4) in serum, Decreased</td>
<td>Directly Leads to</td>
<td>Hippocampal gene expression, Altered</td>
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AOP Title
VEGF Signaling and Vascular Disruption Leading to Adverse Developmental Outcomes
Short name: Developmental Vascular Toxicity

ToxCast: chemicals sorted by predicted vascular disruption (pVDCs)

This synthetic thalidomide analogue disrupts microtubule function in endothelial cells of immature blood vessels.

Slide courtesy of Tom Knudsen
5HPP-33 concentration response predicted in silico from ToxCast and demonstrated in vitro with a human endothelial cell assay.

Slide courtesy of Tom Knudsen

SOURCE: Kleinstreuer et al. (2013) PLoS Comp Biol
Factors Determining Predictivity of Early Key Events

- Evidence supporting the KERs between that KE and the AO
- Quantitative understanding of the downstream KERs
- Modifying factors that influence downstream KEs & KERs
Modifying Factors Emerge Naturally from AOP Networks


AOP:30
ER Antagonism

AOP:25
Aromatase Inhibition

AOP:23
AR Agonism

Key events shared by multiple AOPs

Linkages shared by multiple AOPs

Dan Villeneuve
AR Agonism

Hypothalamic Neurons (-) Feedback

Aromatase Inhibition

Granulosa Reduced E2 synthesis

Circulation Reduced E2 concentrations

Female Decreased spawning and cumulative Fecundity

Population Declining trajectory

↓ Estrogen Receptor Signaling

Hepatocyte Reduced VTG expression & production

Circulation Reduced VTG concentrations

Oocytes Reduced VTG uptake, impaired development

Molecular

Cell/Tissue

Organ

Individual

Population
Current Status of AOP Development

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Stages of AOP Development

- Computationally Predicted AOPs
- Putative AOPs
- Formal AOPs
- Quantitative AOPs

- Still have many assays without AOPs
  - EPA ToxCast: >400 targets
  - ~40 have AOPs
- Also need AOPs -> targets for new assays
- Automated data-mining can create hypothetical AOPs
Non-chemical Factors Also Emerge from AOP Networks
Acknowledgements

- AOP-Wiki Authors
- AOP-KB Development Team
- CSS AOP Discovery & Development Project Team
- OECD External Advisory Group on Molecular Screening & Toxicogenomics
- IPCS/WHO Mode of Action Steering Committee
Questions?

SYSTEMS TOXICOLOGY
Adverse Outcome Pathway

Molecular Initiating Event
- KER - Key Event
  - KER - Key Event
    - KER - Key Event
      - KER - Adverse Outcome

Chemical ADME

Target tissue
- Absorption, Distribution, Metabolism, Excretion

Tiered Approach to ADME Complements AOP Info

AOPs
- Computationally Predicted AOPs
- Putative AOPs
- Formal AOPs
- Quantitative AOPs

Exposure ADME
- Qualitative Screening
- Quantitative Ranking
- Quantitative Modeling

Exposure/ADME AOP Networks/Modifying Factors
Chemical-specific toxicity information
Integrated Approaches to Testing and Assessment (IATA)

- AOP Confidence
- ADME Confidence
- Chemical Data
  - QSAR
  - Read across
  - HTS
  - In vivo laboratory
  - Epidemiology/field studies
- Exposure Predictions
Source to Outcome Continuum
Tox21, MOA, AOP, IATA

Source
Environmental Contaminant

External Exposure

Internal Dose

Key Event

Cellular Effects

Individual

Population

Community

What is regulated

Exposure Predictions

Toxicity Pathway, NRC 2007

Mode of Action, IPCS/EPA/ILSI 2001-2008

Adverse Outcome Pathway, Ankley 2010, Villeneuve 2014

Criteria for regulation
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• IPCS/WHO Mode of Action Steering Committee

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