Increasing level of biological organization

**Key Event Relationships (KERs)**

- KE up
- KE down

**Anchor 1**
Molecular Initiating Event (MIE)

**Anchor 2**
Adverse Outcome (AO)

**Key Events (KEs)**
The Five Principles of AOP Development

- AOPs are NOT chemical-specific
- AOPs are MODULAR
- AOPs are a pragmatic functional unit of development and evaluation
- AOP networks are the functional unit of prediction
- AOPs are living documents
AOPs thrive because of the interactivity and multidisciplinarity of the crowd
Collection and organisation of various types of information

Adverse Outcome Pathway

MIE → KE 1 → KE 2 → KE n → AO

Level of Biological Organisation

Molecular → Organelle → Cellular → Tissue → Organ → Organism → Population

in silico, in chemico → in vitro → in vivo → field and epidemiological studies

Types of information

OECD (2017), Guidance document for the use of AOPs in developing IATA, Series on Testing & Assessment No. 260,
AOP and MOA

- **AOP**: chemical unspecific
- **MoA**: chemical specific
Building an AOP
Q: *Where to start?*

- Top-down AOP development
- Bottom-up AOP development
- Middle-out AOP development
Q: What is the minimum number of elements that can constitute an AOP?
A: Three.

Q: What is the maximum number of KEs that can be included in an AOP?
A: In theory, there is no maximum number of KEs.

Q: How many KEs should be included in an AOP?
A: It depends

Convention:
• One MIE
• Desirably, one KE at each level of biological organization
• One AO (AOPs can have more than one AO)
MIE:

- Typically one per AOP
- Can link to any number of separate AOPs

(rare) exception:
Two events MUST occur to trigger the downstream KE.

KE1 and KE2 must occur for KE3 to occur

not

KE1 or KE2 must occur for KE3 to occur
AO:

- Potentially more than one per AOP - if they represent a single progression of injury

A. LXR Activation → ... → ... → Steatosis → Steato-hepatitis → Fibrosis → Cirrhosis → HC Carcinoma

Multiple AOs in a single, sequential progression = single AOP

B. LXR Activation → ... → ... → Steatosis → Steato-hepatitis → Fibrosis → Cirrhosis

Branching = two AOPs
Acceptable branching:
- additive actions
- one MIE and one AO

Not acceptable branching:
- independent actions
- more than one MIE and AO
Key Event Relationships

Functional unit of inference/extrapolation

Key Event Relationship

- Description
- Biological plausibility
- Empirical support
- Taxonomic applicability
- Quantitative understanding

inconsistencies and uncertainties
Developing organism

Adult organism

Male
Female

In liver
In lung
In brain
Adjacent/non-adjacent KERs
Quantitative Understanding of KERs

- Response-response relationships
- Time-scale
- Known modulating factors
- Known feedback/feedforward loops influencing KER


A.  

\[ \text{KE}_1 \xrightarrow{\text{KER}_{1-2}} \text{KE}_2 \]

B.  

\[ \text{KE}_1 \xleftarrow{\text{KER}_{1-2}} \text{KE}_2 \]

- Known feedback/feedforward loops influencing KER
Quantitative Understanding of KERs

How much change in $KE_{up}$ and/or for how long is needed to evoke some unit of change in $KE_{down}$?

Nature of the response-response relationship
AOPs are living documents

PUTATIVE

QUALITATIVE

QUANTITATIVE
A quantitative AOP is NOT EQUAL to a computer model

Quantitative KER descriptions support the development of computational models aligned with an AOP.

A qAOP model can be described as a statistical or mathematical construct that models one or more of the KERs.

The choice of the modeling method is dependent on the addressed question and the available data.
Ontologies

Ontology – a kind of controlled vocabulary of well-defined terms with specified relationships between those terms, capable of interpretation by both humans and computers.

National Center for Biomedical Ontology (NCBO)

Courtot M, EMBL-EBI,, from https://www.slideshare.net/mcourtot/ontologies-for-life-sciences-examples-from-the-gene-ontology
Why add ontology terms in the AOP Wiki?

- Provides more flexibility in creating new KEs.
- Facilitates reuse of KEs or KERs and reduces redundancy.
- Supports building of AOP networks.
HOW?

Event component(s)

- Process
- Object
- Action

Context (Cell or Organ term)

Ontology terms

Ives et al, Creating a Structured AOP Knowledgebase via Ontology-Based Annotations, Applied In Vitro Toxicology (under Press)
Event: 97

Key Event Title
**Alkylation, DNA**

Short name
Alkylation, DNA

Biological Context

<table>
<thead>
<tr>
<th>Level of Biological Organization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular</td>
</tr>
</tbody>
</table>

**Key Event Components**

<table>
<thead>
<tr>
<th>Process</th>
<th>Object</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA alkylation</td>
<td>deoxyribonucleic acid</td>
<td>increased</td>
</tr>
</tbody>
</table>

https://aopwiki.org/events/97
What are AOPs good for?
AOPs

- Repository
- Alternative tests
- Biomarker
- Mixtures
- Understanding
- Terminology
- Further research

The diagram illustrates a flow from AOPs repository to clear presentation, highlighting alternative tests and the need for further research on biomarkers, mixtures, and understanding terminology.
AOPs in regulatory context

Defined Approaches

In vitro test guidelines
In vivo test guidelines
Non-standard tests
QSAR models

Grouping and Read-across

Weight of Evidence

Expert Judgement

Exposure
ADME
PBK models
KEY

Every AOP is useful

MESSAGES

Integration of various types of information is necessary for risk assessment

AOPs are living documents for collaboration and managing collective knowledge
Stay in touch

JRC Science Hub: www.ec.europa.eu/jrc

Twitter: @EU_ScienceHub

LinkedIn: european-commission-joint-research-centre

YouTube: JRC Audiovisuals

Vimeo: Science@EC

THANK you for your attention!
pathways to disease
AOP to Liver Fibrosis

Protein Alkylation → Cell injury → KC activation → TGF-β1 expression → HSC activation → ECM alteration → Liver Fibrosis

HCV envelope glycoproteins E1 and E2 binding to cell membrane

Inflammation → Oxidative stress

molecular/cellular studies

clinical studies
How to represent inflammation in AOPs to facilitate network-building?
AOP 13: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development leads to neurodegeneration with impairment in learning and memory in aging.

AOP 38 protein alkylation leading to liver fibrosis

AOP 173 resident cell activation leading to lung fibrosis
Inflammation

Upstream Damage Signals
Stressor-dependent

Tissue Resident cell activation

Increased Pro-inflammatory Mediators

Leukocyte recruitment/activation

Downstream Damage
Tissue and Context-dependent
AOP 173: Resident cell activation leading to lung fibrosis
AOP 1.25: Resident cell activation leading to lung emphysema

AOP 38: Protein alkylation leading to liver fibrosis

- Increased resident cell activation
- Increased proinflammatory mediators
- Increased leukocyte influx
- Activation T-helper cells type 2
- Tissue Injury
- Pro/anti-proteolysis imbalance
- ECM Alteration/deposition
- Elasticity
- Lung Emphysema

- Cell Injury/death
- Reduced, Release of BDNF
- Decreased, Calcium influx
- HSC activation
- ECM Alteration/deposition
- Fibroblast proliferation
- Tissue Injury
- Elastolysis

- Protein Alkylation
- Inhibition, NMDARs

AOP 13: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development leads to neurodegeneration with impairment in learning and memory in aging

- Impairment of learning and memory
- Neurodegeneration in hippocampus and cortex
- Decreased, Release of BDNF
- Decreased, Calcium influx
- Binding of antagonist to NMDARs
- Neurodegeneration in hippocampus and cortex

- Lung Fibrosis
- Liver Fibrosis
AOP network for thyroid axis disruption during development

Courtesy of Dan Villeneuve, US EPA
Network of 14 AOPs
Decreased serum T4

Adverse outcomes in vertebrate development
Aggregate Exposure Pathway (AEP)

A flexible, data-driven framework to organize exposure data for supporting exposure based decision making, prediction, and risk assessment.
Levels of biological organisation

- Molecule
- Organelle
- Cell
- Tissue
- Organ
- Organism
- Population
Principles of AOP development
AOPs are NOT chemical-specific

Biological motifs of failure
AOPs are MODULAR KEs

- measurable
- essential

Functional unit of observation/verification

- Description
- Methods for observing/measuring
- Taxonomic applicability
Molecular initiating event (MIE)

Adverse Outcome (AO)
AOPs are a pragmatic functional unit of development and evaluation.

Linear, no branches.
AOP networks are the functional unit of prediction

Key events shared by multiple AOPs

KERs shared by multiple AOPs

AOP 1

MIE 1 → KE → KE → KE* → KE' → AO

AOP 2

MIE 2 → KE* → KE' → AO

AOP 1

MIE1 → KE → KE 2 → KE* → KE' → AO

AOP 2

MIE2
AOPs are a way of organizing existing knowledge

There is no objective “complete AOP”