The European Commission’s science and knowledge service

Joint Research Centre
Creating and Using an AOP

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Increasing level of biological organization

Key Event Relationships (KERs)

KE up

KE down

_anchor 1 Molecular Initiating Event (MIE)

_anchor 2 Adverse Outcome (AO)
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 → Organellar → Cellular → Tissue → Organ → Organism → Population

Types of information

in silico in chemico → in vitro → in vivo → field and epidemiological studies
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 → HC Carcinoma

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

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

inconsistencies and uncertainties
Developing organism

MIE

KE₁

KE₂

Male

MIE

KE₁

KE₂

Female

In liver

KE₂

In lung

KE₁

KE₃

In brain

KE₄
Adjacent/non-adjacent KERs
Quantitative Understanding of KERs

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

A. 

$$KE_1 \xrightarrow{KER_{1-2}} KE_2 \xleftarrow{KER_{2-1}} KE_1$$

B. 

$$KE_1 \xrightarrow{KER_{1-2}} KE_2 \xleftarrow{KER_{2-1}} KE_1$$

- 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
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
Event component(s)

- Process
- Object
- Action
- Context (Cell or Organ term)

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**

- **Level of Biological Organization**
  - Molecular

**Cell term**

- Cell term
- eukaryotic cell

**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 in regulatory context

- **Mechanistic Support** for epidemiological studies

- **Defined Approaches**
  - IATA
  - In vivo test guidelines
  - In vitro test guidelines
  - Non-standard tests
  - QSAR models
  - Grouping and Read-across
  - Weight of Evidence

- **Expert Judgement**

- **ADME**

- **Exposure**

- **PBK models**
KEY

MESSAGES

Every AOP is useful

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

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

Virus
- HCV envelope glycoproteins E1 and E2 binding to cell membrane

Health
- Inflammation
- Oxidative stress

Disease
- 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

Protein Alkylation → Cell Injury/death → KC activation → TGF-β1 expression → HSC activation → ECM alteration → Liver Fibrosis

AOP 173: Resident cell activation leading to lung fibrosis

Induction of secretion of inflammatory cytokines → Induction of acute phase response, propagation of inflammatory response → Retention of or repeated exposure to foreign material leading to continuous inflammation → Cellular toxicity, cell death, reactive oxygen species synthesis → Tissue injury → TH2/M2 response, secretion and activation of interleukins, growth factors → Fibroblast proliferation, myofibroblast proliferation → Extracellular matrix deposition → 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 proinflammatory mediators
Increased leukocyte influx
HSC activation
Tissue Injury
Pro/anti proteolysis imbalance
Elastolysis
Lung Fibrosis

ECM Alteration/deposition
Fibroblast proliferation
Activation T-helper cells type 2
Activation T-helper cells type 2/M2

Impairment of learning and memory
Neurodegeneration in hippocampus and cortex

Cell Injury/death
Reduced, Release of BDNF
Decreased, Calcium influx
Inhibition, NMDARs
NMDARs, Binding of antagonist

Protein Alkylation

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
TPO inhibition, decreased

TH synthesis, decreased

Iodide in tissue, decreased

TH in neural tissues, decreased

T4 in serum, decreased

T4 in tissue, decreased

T3 in tissue, decreased

Metamorphosis, impaired

Survival, reduced

Anterior SB inflation, impaired

Hearing, reduced

Posterior SB inflation, impaired

Swimming performance, reduced

y.o.y survival, reduced

Population trajectory, decreased

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

Slide: courtesy of Dan Villeneuve, US EPA
Aggregate Exposure Pathway (AEP)

A flexible, data-driven framework to organize exposure data for supporting exposure based decision making, prediction, and risk assessment.
Aggregate Exposure Pathway

1 source

ENV Medium → External Exposure → Internal Exposure

Multiple source 1 chemical

ENV Medium → External Exposure → Internal Exposure

Multiple source multiple chemical

ENV Medium → External Exposure → Internal Exposure

Multiple source multiple stressors

ENV Medium → External Exposure → Internal Exposure

Adverse Outcome Pathway

1 Target site exposure (TSE)

1 TSE

Multiple TSE

KE1 → KE2 → KE3 → KE4 → AO

KE1 → KE2 → KE3 → KE4 → AO

KE1 → KE2 → KE3 → KE4 → AO

KE1 → KE2 → KE3 → KE4 → AO

AEP

PBK modeling

Adverse Outcome Pathway (AOP)

European Commission

Toxicodynamics
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
Two specialised KEs

- 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 networks

AOP 1: MIE 1 → KE 1 → KE 2 → KE 3 → AO 1 → AO 5 → ...
AOP 2: MIE 2 → KE 4 → KE 1 → KE 2 → KE 3 → AO 1 → AO 5 → ...
AOP 3: MIE 3 → KE 5 → KE 6 → KE 2 → KE 7 → AO 2 → AO 5 → ...
AOP 4: MIE 3 → KE 5 → KE 6 → KE 8 → KE 9 → AO 3 → AO 6 → ...
AOP 5: MIE 3 → KE 10 → KE 11 → KE 12 → KE 13 → AO 4 → AO 5 → ...

Key Events (KEs) shared by multiple AOPs

Key Events Relationships (KERs) shared by multiple AOPs
AOPs are a way of organizing existing knowledge

There is no objective “complete AOP”