The European Commission's science and knowledge service

Joint Research Centre

Creating and Using an AOP

13th March 2018
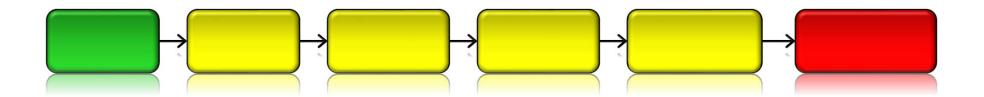**Brigitte Landesmann**

European Commission

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
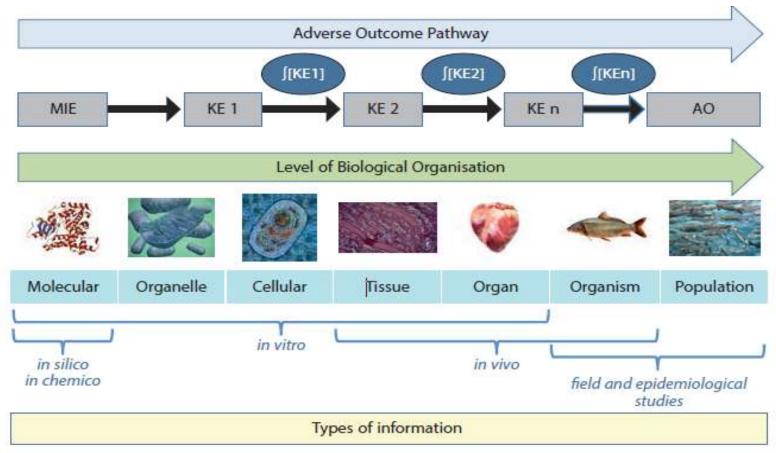
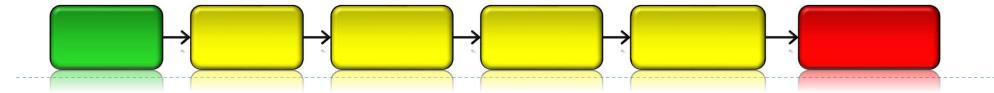# AOPs thrive because of the interactivity and multidisciplinarity of the crowd

Collection and organisation of various types of information
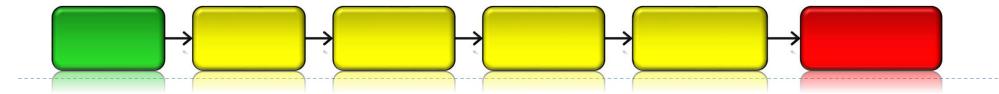
AOP and MOA

▸ **AOP:** chemical unspecific

▸ **MoA:** chemical specific

European Commission
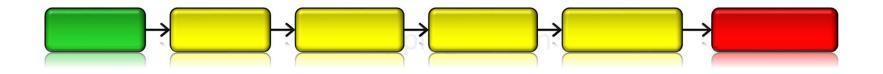
Building an AOP
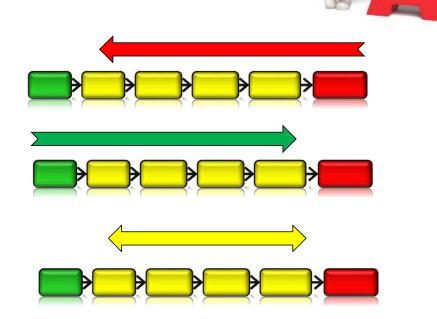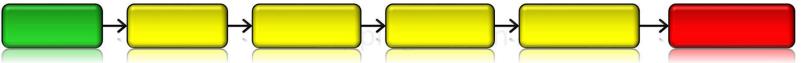
Q: **Where to start?**

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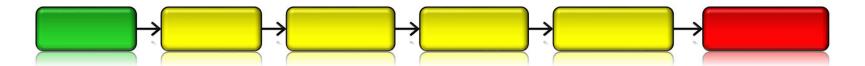
Q: **How many KEs should be included in an AOP?**
A: It depends

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

▶
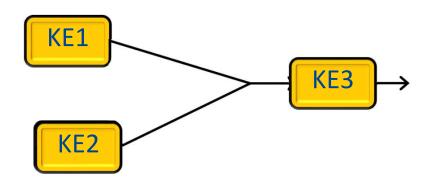
MIE:

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

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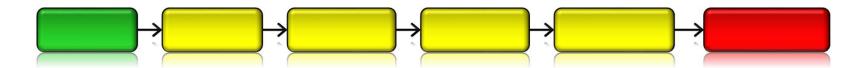
    Two events MUST occur to trigger the downstream KE.

KE1 and KE2 must occur for KE3 to occur
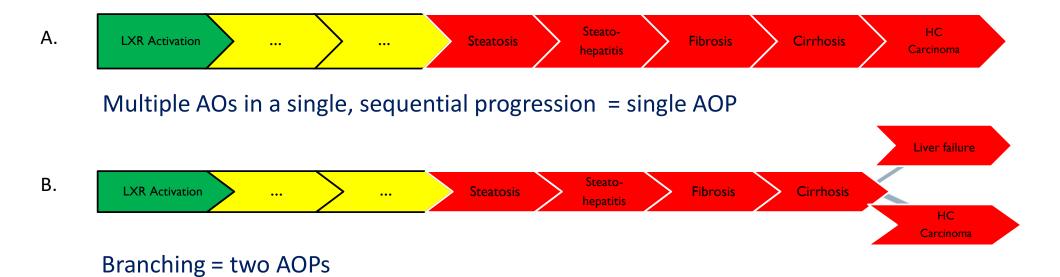
not
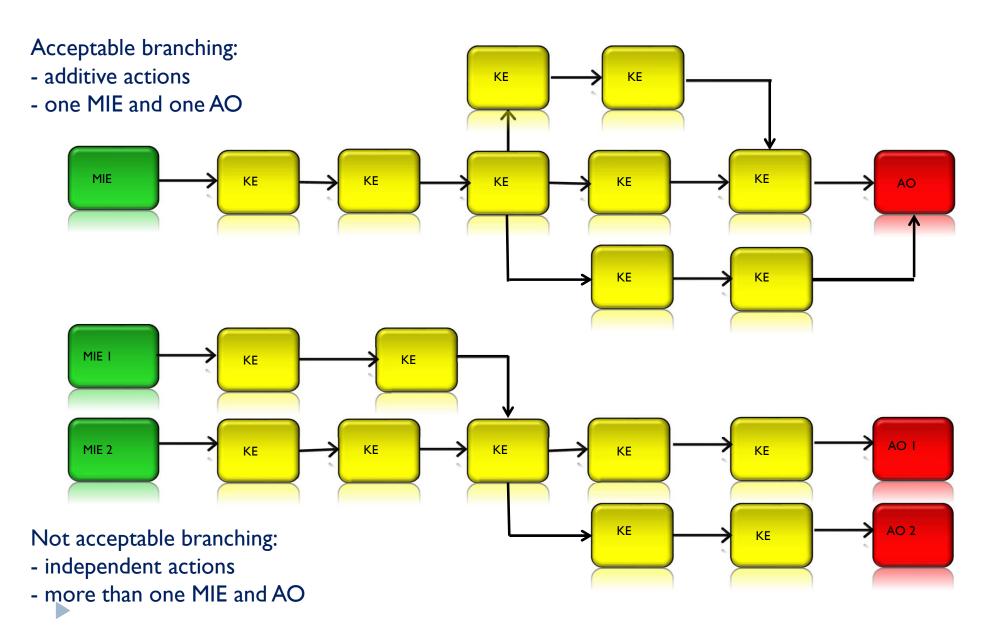
KE1 or KE2 must occur for KE3 to occur
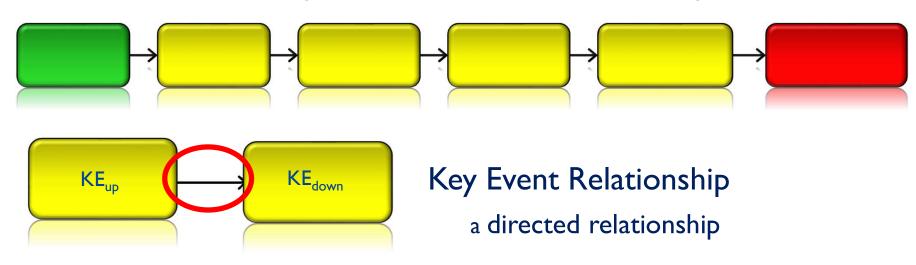
AO:

➤ 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 | Liver failure / 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

Key Event Relationship

a directed relationship

Functional unit of inference/extrapolation
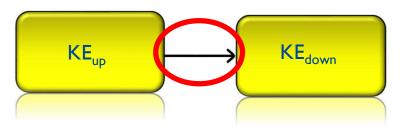
- Description
- Biological plausibility
- Empirical support
- Taxonomic applicability
- Quantitative understanding
  ▶

inconsistencies and uncertainties
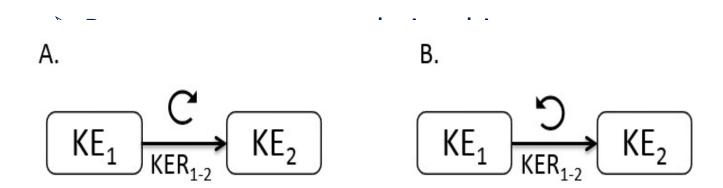
Adjacent/non -adjacent KERs

Quantitative Understanding of KERs

A.

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

B.

KE_1 $\xrightarrow{\ \circlearrowleft\ }$ KE_2
KER_{1-2}
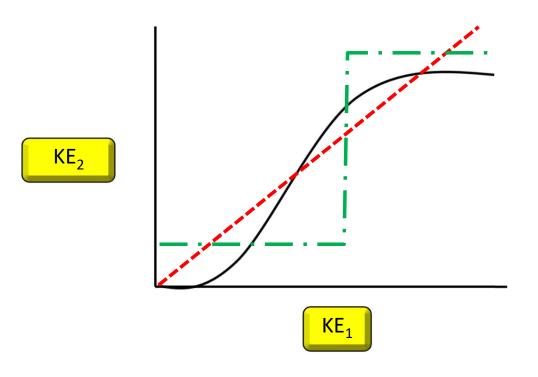
➢ Known feedback/feedforward loops influencing KER
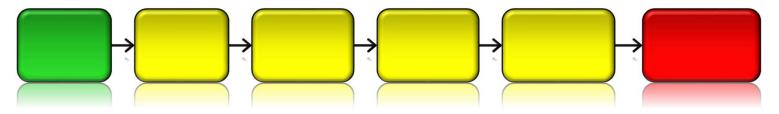
Quantitative Understanding of KERs

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

KE$_2$

KE$_1$
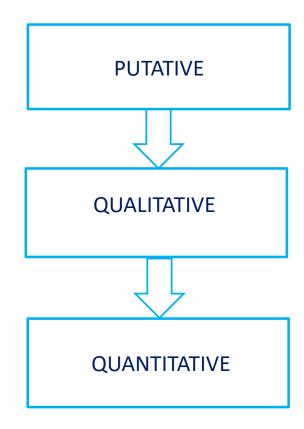
Nature of the
response-response
relationship

European
Commission

AOPs are living documents

PUTATIVE

QUALITATIVE

QUANTITATIVE

A quantitative AOP is <u>NOT EQUAL</u> 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
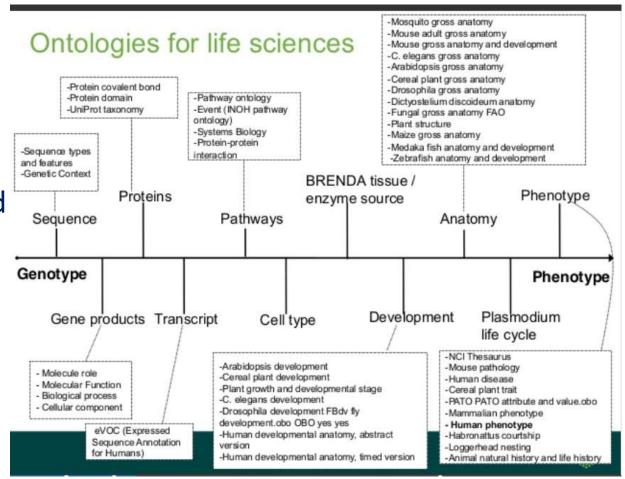
Ontologies

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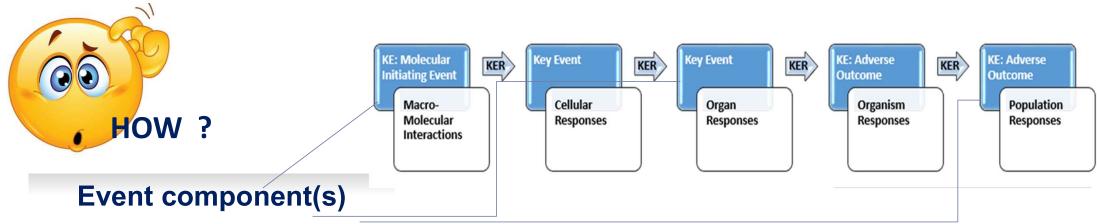
Provides more flexibility in creating new KEs.
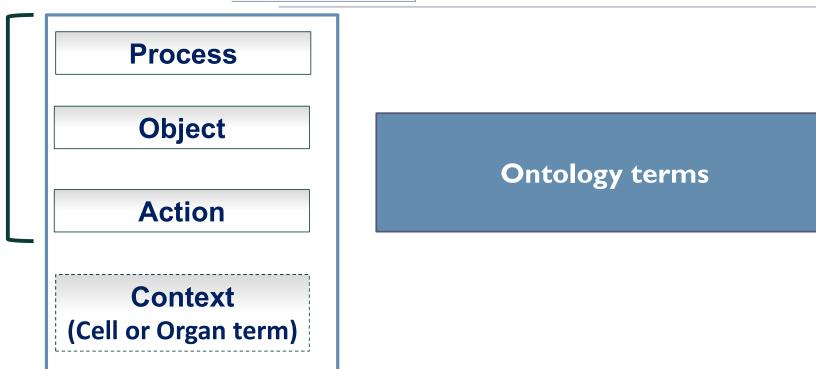
Facilitates reuse of KEs or KERs and reduces redundancy.
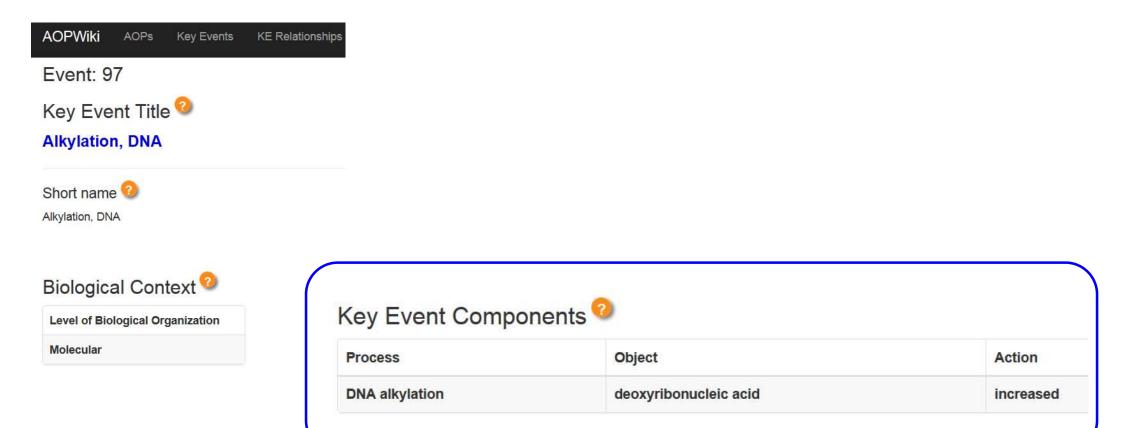
Supports building of AOP networks

HOW ?

Event component(s)

KE: Molecular Initiating Event	KER	Key Event	KER	Key Event	KER	KE: Adverse Outcome	KER	KE: Adverse Outcome
Macro-Molecular Interactions		Cellular Responses		Organ Responses		Organism Responses		Population Responses

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 ❓

Level of Biological Organization
Molecular

Cell term ❓
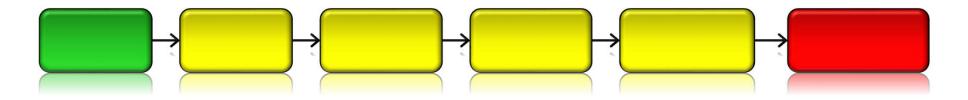
Cell term
eukaryotic cell

Key Event Components ❓

Process	Object	Action
DNA alkylation	deoxyribonucleic acid	increased
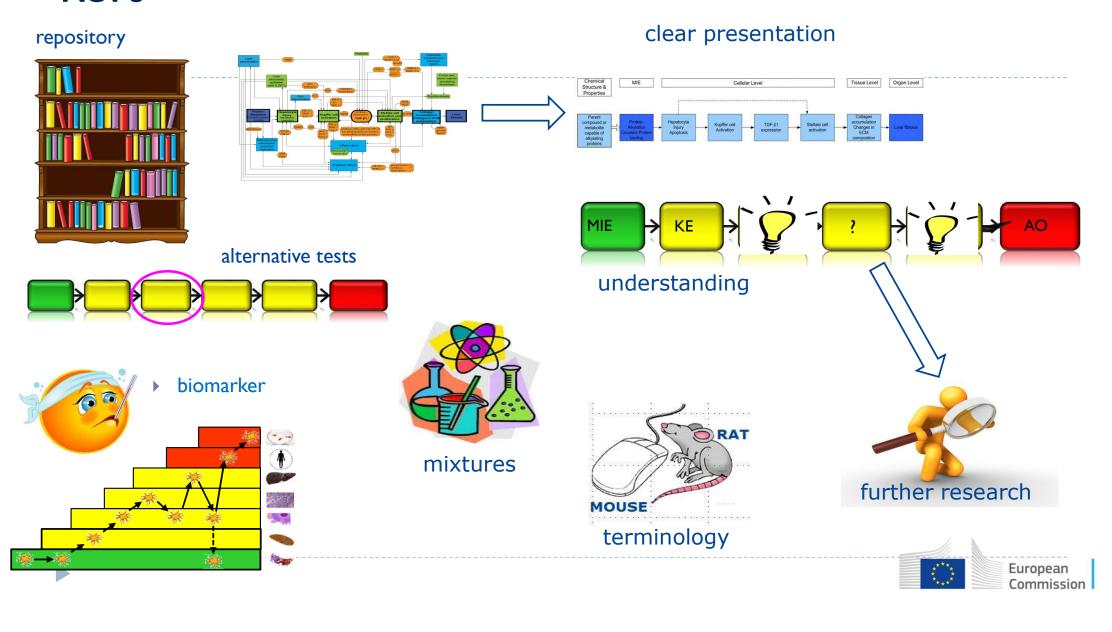
https://aopwiki.org/events/97
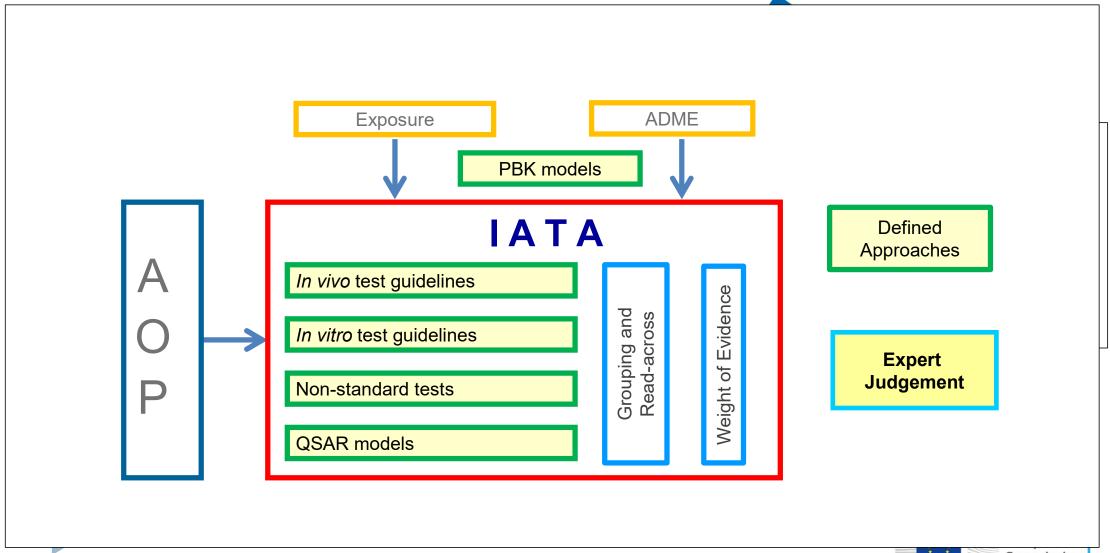
What are AOPs good for?

AOPs

repository

clear presentation

alternative tests

understanding

biomarker

mixtures

RAT

MOUSE

terminology

further research

MIE → KE → ? → AO

AOPs in regulatory context

Every AOP is useful

Integration of various types of information is necessary for risk assessment

KEY

MESSAGES

AOPs are living documents for collaboration and managing collective knowledge

European Commission

THANK YOU for your attention !

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

European Commission
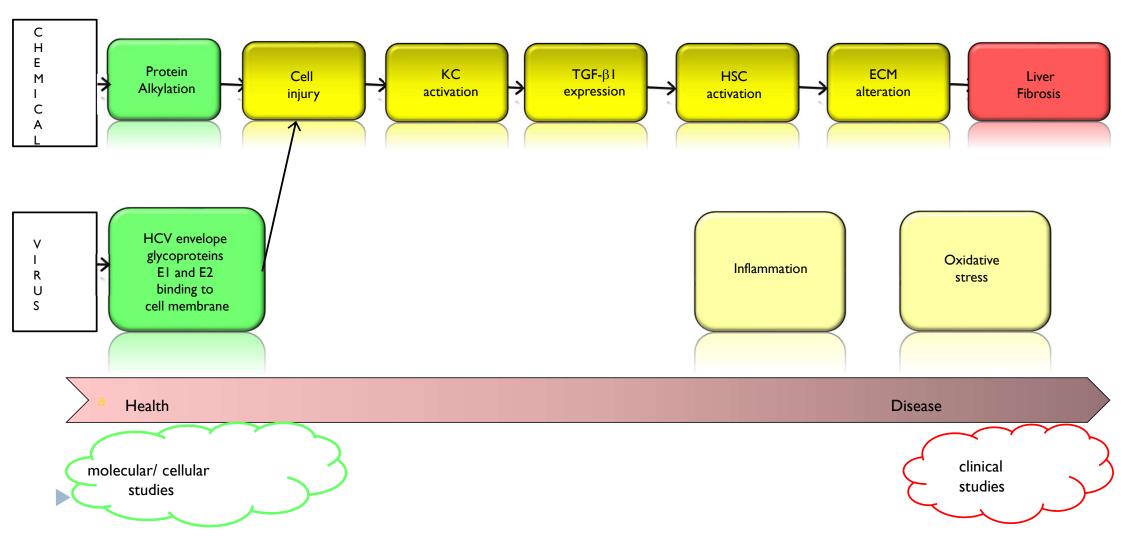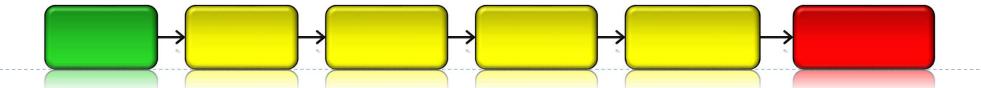
pathways to disease
AOP to Liver Fibrosis

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

Inflammation

Oxidative stress

Health → Disease

molecular/ cellular studies

clinical studies
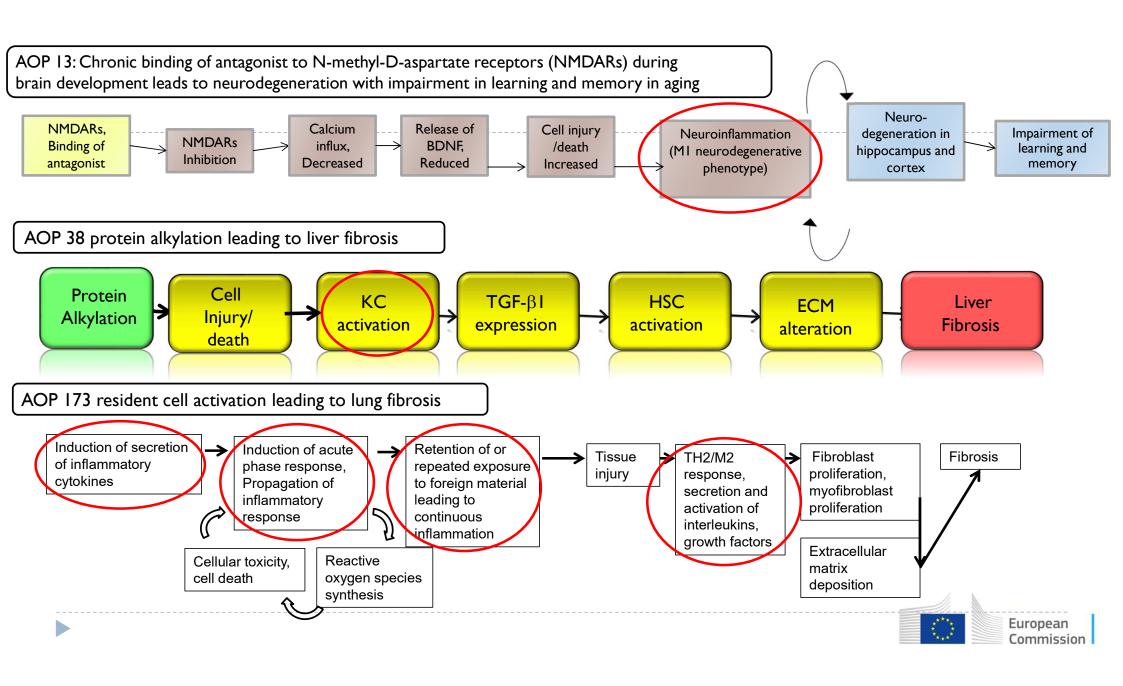
**How to represent
inflammation in AOPs
to facilitate network-building ?**

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

NMDARs, Binding of antagonist → NMDARs Inhibition → Calcium influx, Decreased → Release of BDNF, Reduced → Cell injury /death Increased → Neuroinflammation (M1 neurodegenerative phenotype) → Neuro-degeneration in hippocampus and cortex → Impairment of learning and memory

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 → Tissue injury → TH2/M2 response, secretion and activation of interleukins, growth factors → Fibroblast proliferation, myofibroblast proliferation → Fibrosis

Cellular toxicity, cell death

Reactive oxygen species synthesis

Extracellular matrix deposition

European Commission

Inflammation

Upstream Damage Signals

Stressor-dependent

Tissue Resident cell activation

Increased Pro-inflammatory Mediators

Leukocyte recruitment/ activation

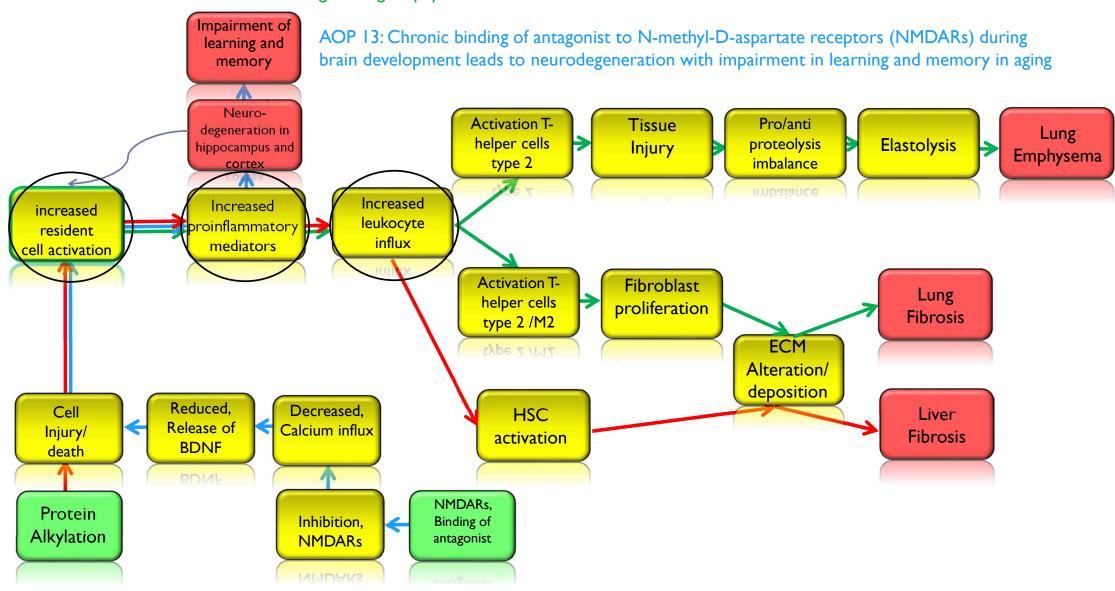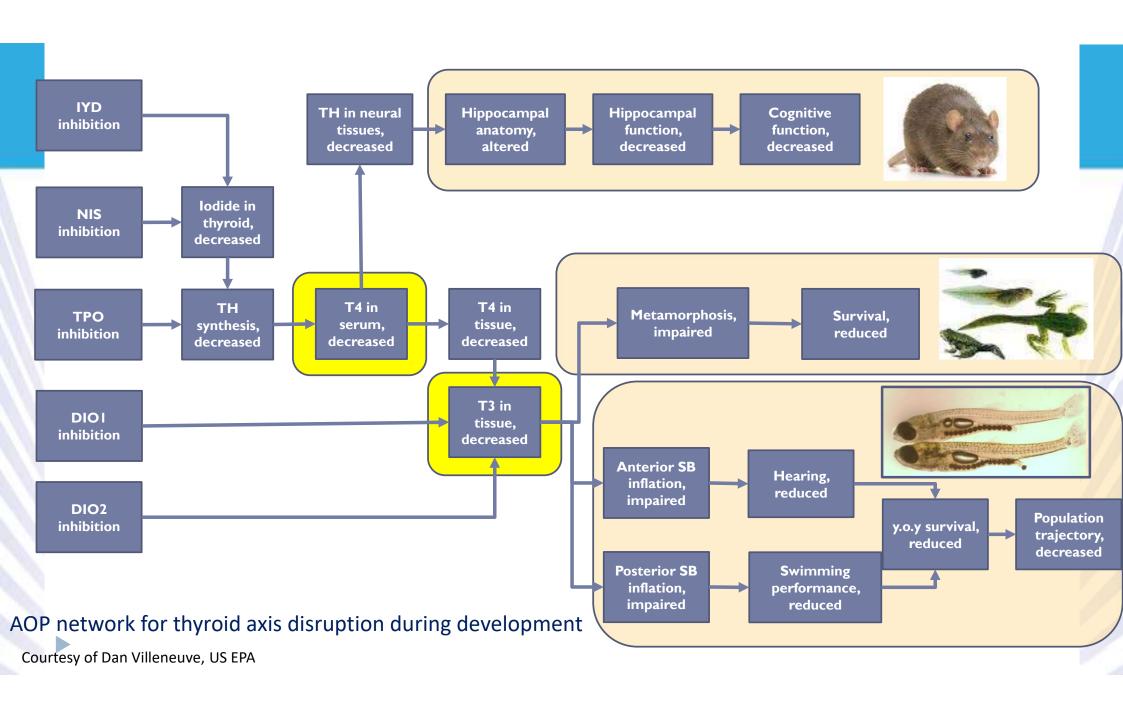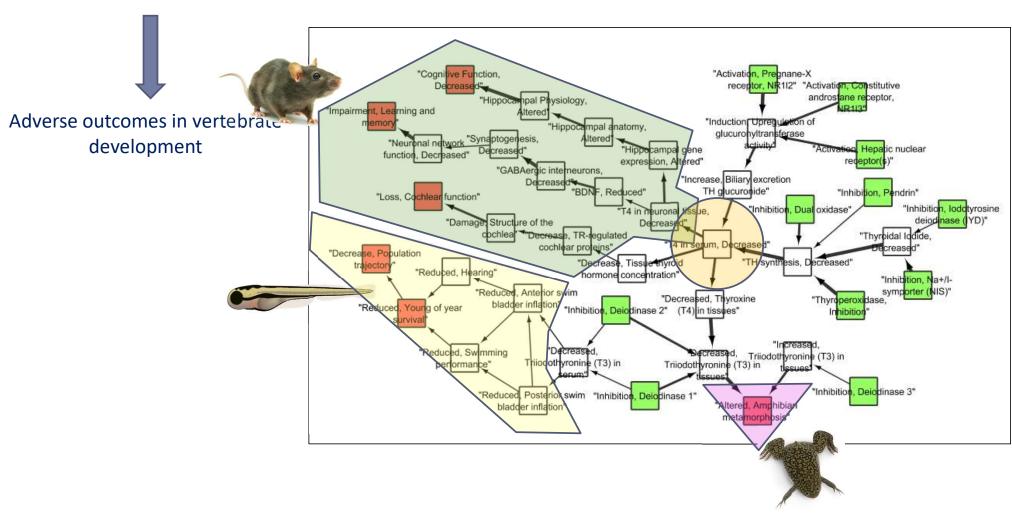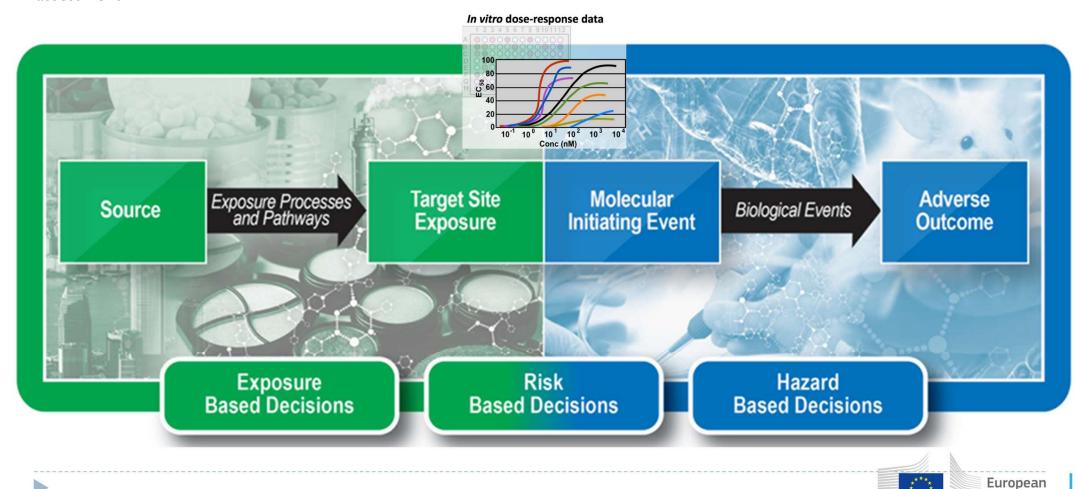Downstream Damage

Tissue and Context-dependent

European Commission

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

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

Neuro-degeneration in hippocampus and cortex

increased resident cell activation

Increased proinflammatory mediators

Increased leukocyte influx

Activation T-helper cells type 2

Tissue Injury

Pro/anti proteolysis imbalance

Elastolysis

Lung Emphysema

Activation T-helper cells type 2 /M2

Fibroblast proliferation

ECM Alteration/ deposition

Lung Fibrosis

Cell Injury/ death

Reduced, Release of BDNF

Decreased, Calcium influx

HSC activation

Liver Fibrosis

Protein Alkylation

Inhibition, NMDARs

NMDARs, Binding of antagonist

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
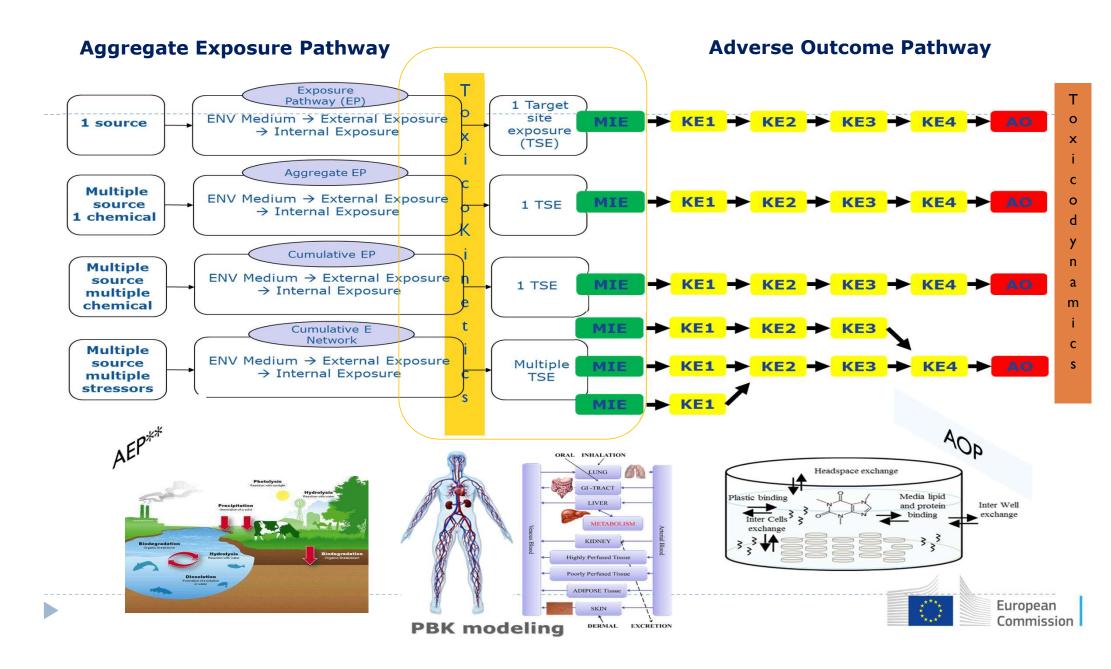
Slide: courtesy of Dan Villeneuve, US EPA

Aggregate Exposure Pathway (AEP)

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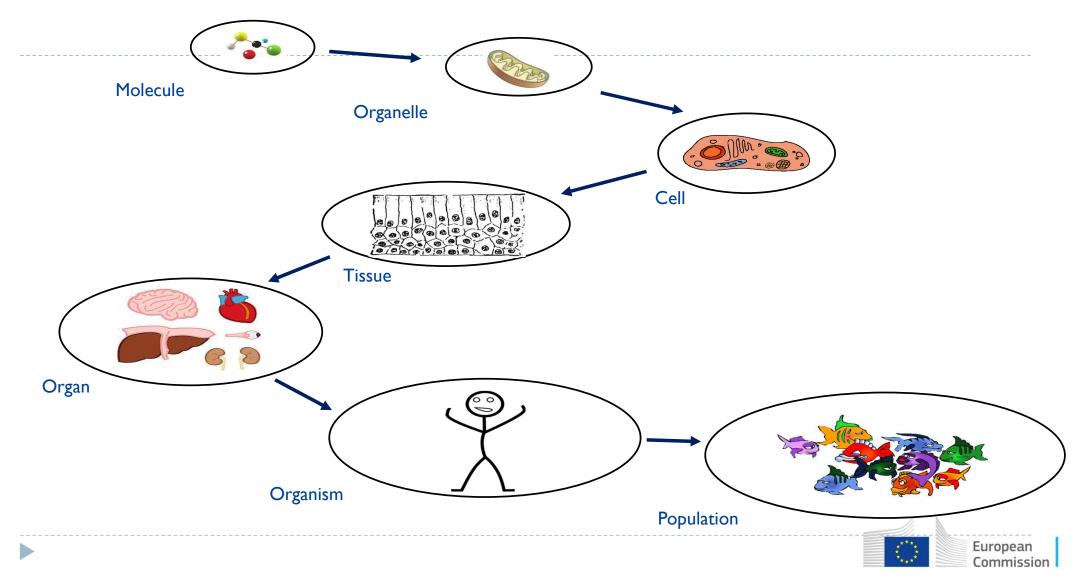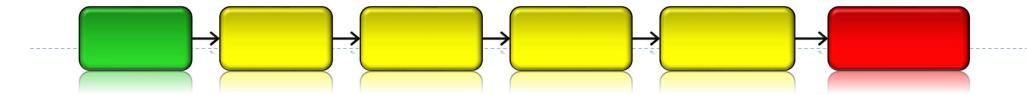
Aggregate Exposure Pathway

Adverse Outcome Pathway

Toxicodynamics

Toxicokinetics

| 1 source | Exposure Pathway (EP) | 1 Target site exposure (TSE) | MIE | KE1 | KE2 | KE3 | KE4 | AO |

ENV Medium → External Exposure → Internal Exposure

Multiple source 1 chemical — Aggregate EP — ENV Medium → External Exposure → Internal Exposure — 1 TSE — MIE — KE1 — KE2 — KE3 — KE4 — AO

Multiple source multiple chemical — Cumulative EP — ENV Medium → External Exposure → Internal Exposure — 1 TSE — MIE — KE1 — KE2 — KE3 — KE4 — AO

Multiple source multiple stressors — Cumulative E Network — ENV Medium → External Exposure → Internal Exposure — Multiple TSE — MIE — KE1 — KE2 — KE3 — KE4 — AO

MIE — KE1 — KE2 — KE3

MIE — KE1

AEP**

PBK modeling

ORAL   INHALATION
LUNG
GI -TRACT
LIVER
METABOLISM
KIDNEY
Highly Perfused Tissue
Poorly Perfused Tissue
ADIPOSE Tissue
SKIN
DERMAL   EXCRETION
Venous Blood   Arterial Blood

AOP

Headspace exchange
Plastic binding
Media lipid and protein binding
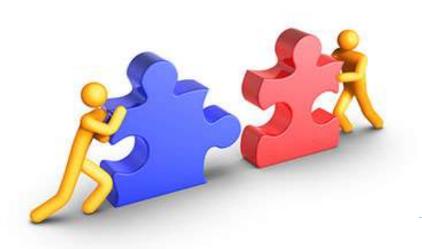Inter Cells exchange
Inter Well exchange

European Commission

Levels of biological organisation

Principles
of AOP development

AOPs are NOT chemical-specific

Chlorpyrifos
Diazinon
Fenthion
Parathion
Malthion
Aldicarb
Carbaryl
Propoxur
Methomyl
…………

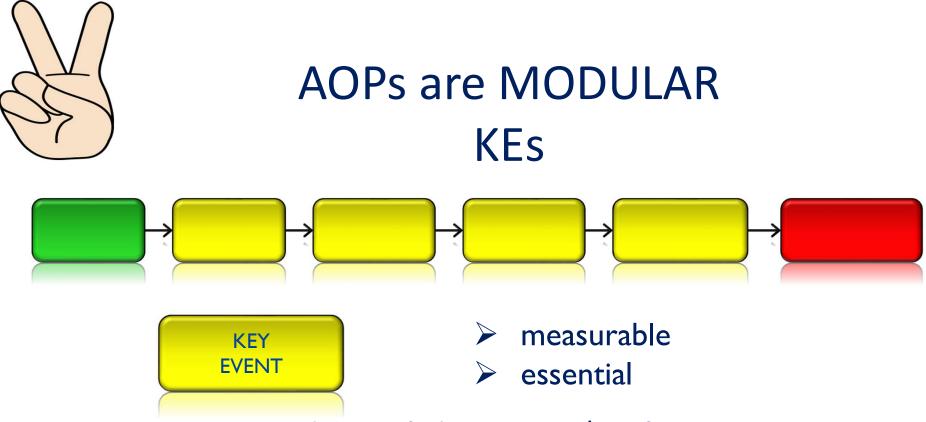Biological motifs of failure

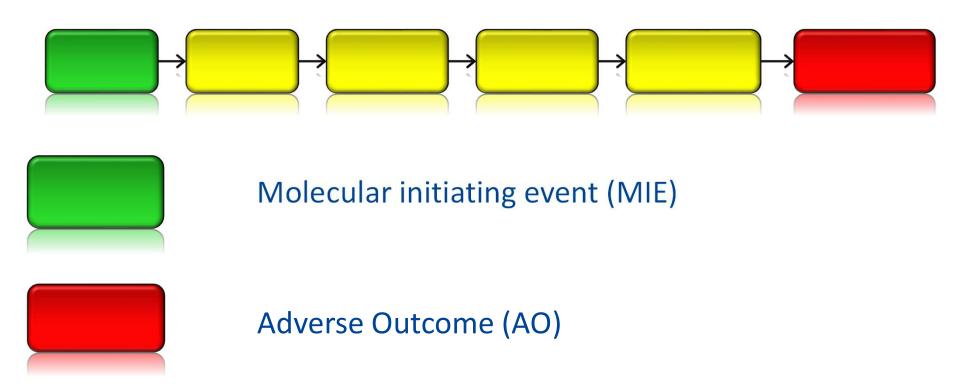# AOPs are MODULAR
KEs

KEY
EVENT

➤ measurable
➤ essential

Functional unit of observation/verification

- Description
- Methods for observing/measuring
- Taxonomic applicability

Two specialised KEs
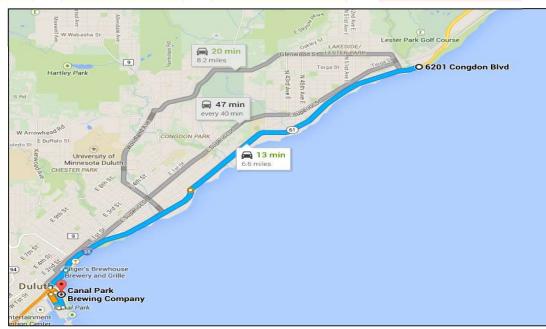

Molecular initiating event (MIE)

Adverse Outcome (AO)

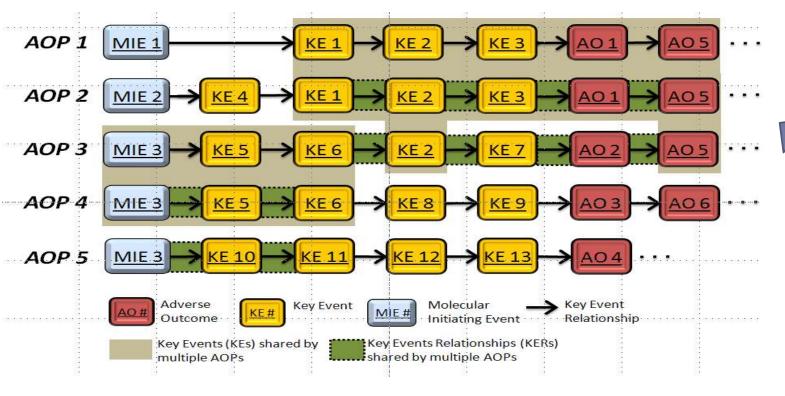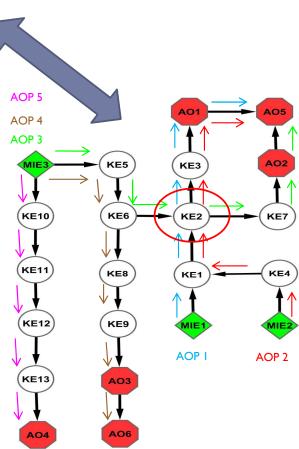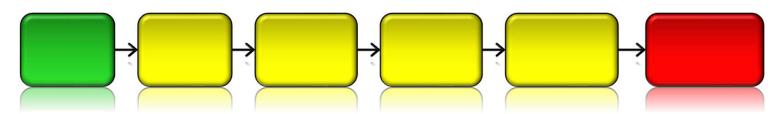# AOPs are a pragmatic functional unit of development and evaluation

Linear, no branches

AOP networks are the functional unit of prediction

Key events shared by multiple AOPs

KERs shared by multiple AOPs

AOP 1

MIE 1 → KE → KE → KE* → KE' → AO

AOP 2

MIE 2 → KE* → KE' → AO

KE → AO 1

AOP 1

MIE1 → KE → KE 2 → KE* → KE' → AO

AOP 2

MIE2 → KE*

AOP networks

AOPs are living documents

AOPs are a way of organizing existing knowledge

There is no objective "complete AOP"