Adverse outcome pathways

as tools to assess chemical-induced toxicity

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1. Introduction

☑ Mode of action

- Initially described for human health risk assessment
  - ► Cancer: US Environmental Protection Agency (EPA)
  - ► Non-cancer endpoints: International Life Sciences Institute (ILSI)
- A series of key events along a biological pathway from the initial chemical interaction to the adverse outcome

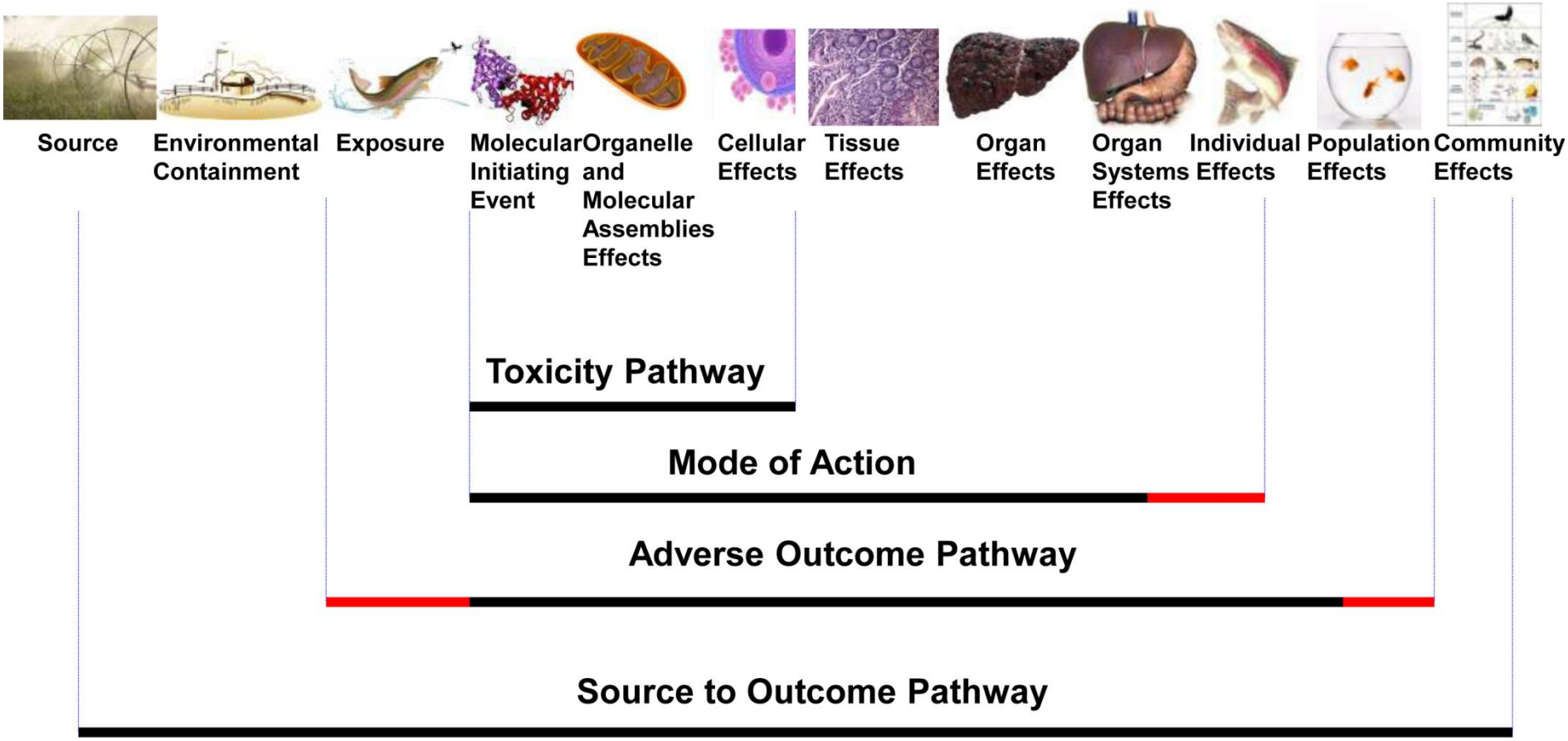
☑ Adverse outcome pathway (AOP)

- Initially described for ecological risk assessment
- Application in human health risk assessment
  - ► US National Academy of Science (2007): toxicity pathways
  - ► Organisation for Economic Co-operation and Development (OECD, 2012): AOP template
- A conceptual construct that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome at a biological level relevant to risk assessment

Ankley *et al.* (2010) Environ. Toxicol. Chem. 29: 730-741.

OECD (2012 and 2014) Proposal for a template and guidance on developing and assessing the completeness of adverse outcome pathways.

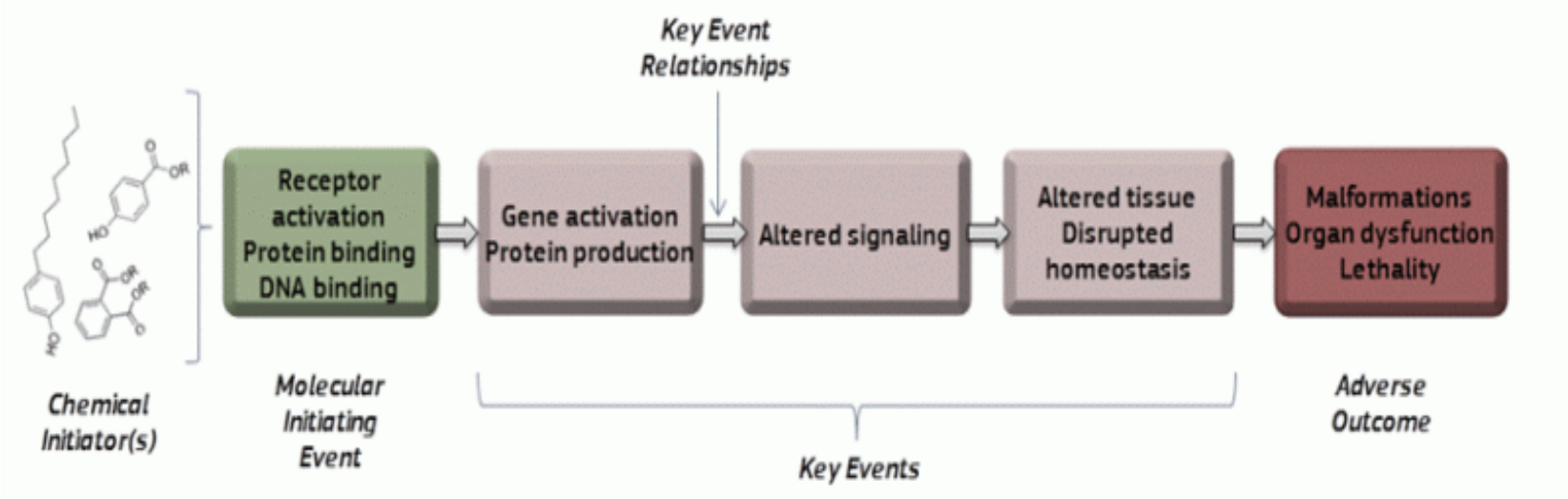
☑ Scope


Source
Environmental Containment
Exposure
Molecular Initiating Event
Organelle and Molecular Assemblies Effects
Cellular Effects
Tissue Effects
Organ Effects
Organ Systems Effects
Individual Effects
Population Effects
Community Effects
Toxicity Pathway
Mode of Action
Adverse Outcome Pathway
Source to Outcome Pathway

2. AOP structure

☑ Macrostructure

- **Overview**

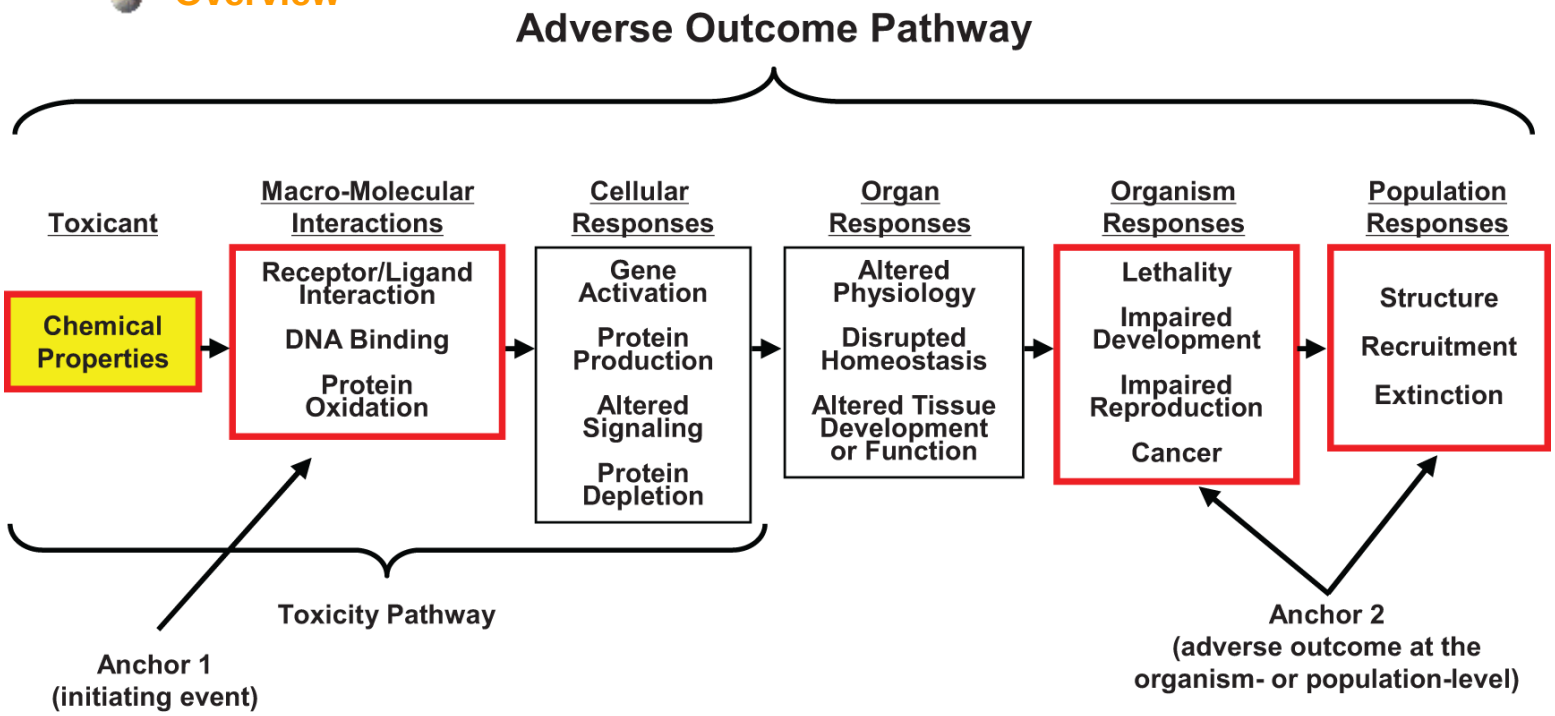


- **Main information blocks**
  - ► Anchor 1: molecular initiating event (MIE)
  - ► Key events (KE) and key event relationships (KER)
  - ► Anchor 2: adverse outcome (AO)

OECD (2012 and 2014) Proposal for a template and guidance on developing and assessing the completeness of adverse outcome pathways.

☑ Microstructure

- Overview



- Platforms
  - ► Biological levels
  - ► KEs
  - ► KERs

Ankley *et al.* (2010) Environ. Toxicol. Chem. 29: 730-741.

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3. AOP development

☑ Step 1 top-down/step 2 bottom-up: identification of the AO

- Change in morphology, physiology, … of an organism or system that results in impairment of the functional capacity or the capacity to compensate for stress
- Definition of the biological level
  - ► Cellular level
  - ► Organ level
  - ► Organism level
  - ► Population level
- Systemic or local effects

☑ Step 2 top-down/step 1 bottom-up: identification of the MIE

- Initial point of chemical-biological interaction within the organism
- Definition of the site of action
- Examples
  - ► Receptor-ligand interaction
  - ► Protein oxidation
  - ► DNA binding

OECD (2012 and 2014) Proposal for a template and guidance on developing and assessing the completeness of adverse outcome pathways.
Landesmann *et al.* (2012) JRC scientific and policy report.

☑ Step 3: identification of the KEs

- Change in biological state that is both measurable and essential to the progression of a defined biological perturbation leading to the AO
- Response matrix between the MIE and the AO
- Selected number of critical events
- Need for understanding normal physiological pathways
- Information resources
  - ► Literature data
  - ► *In vivo* data
  - ► *In vitro* data
  - ► *In chemico* data
  - ► *In silico* data
  - ► "-omics"-based data

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Landesmann *et al.* (2012) JRC scientific and policy report.

☑ Step 4: description of KERs

- **Scientifically-based relationship that connects KEs**
  - ► Defining a directed relationship between 2 KEs
  - ► Facilitating extrapolation of the state of the downstream KE from the upstream KE
- **May be direct or indirect**
  - ► Direct linkage between a pair of KEs that are adjacent in an AOP
  - ► Indirect linkages between a pair of KEs for which the relationship is thought to run through another KE or a gap in current understanding
- **May be qualitative or quantitative**
  - ► Dose-response relationships
  - ► Thresholds/points of departure
  - ► Mathematical equations
  - ► Biologically-based computational models
- **May be influenced by modulating factors**

OECD (2012 and 2014) Proposal for a template and guidance on developing and assessing the completeness of adverse outcome pathways.
Landesmann *et al.* (2012) JRC scientific and policy report.

4. AOP assessment

☑ Weight of evidence assessment: Bradford Hill criteria

- Concordance of dose-response relationships?
- Temporal concordance among the KEs and the AO?
- Strength, consistency and specificity of the MIE-AO association?
- Biological plausibility, coherence and consistency of the experimental evidence?
- Alternative mechanisms?
- Uncertainties, inconsistencies and data gaps?

☑ Confidence assessment: OECD key questions

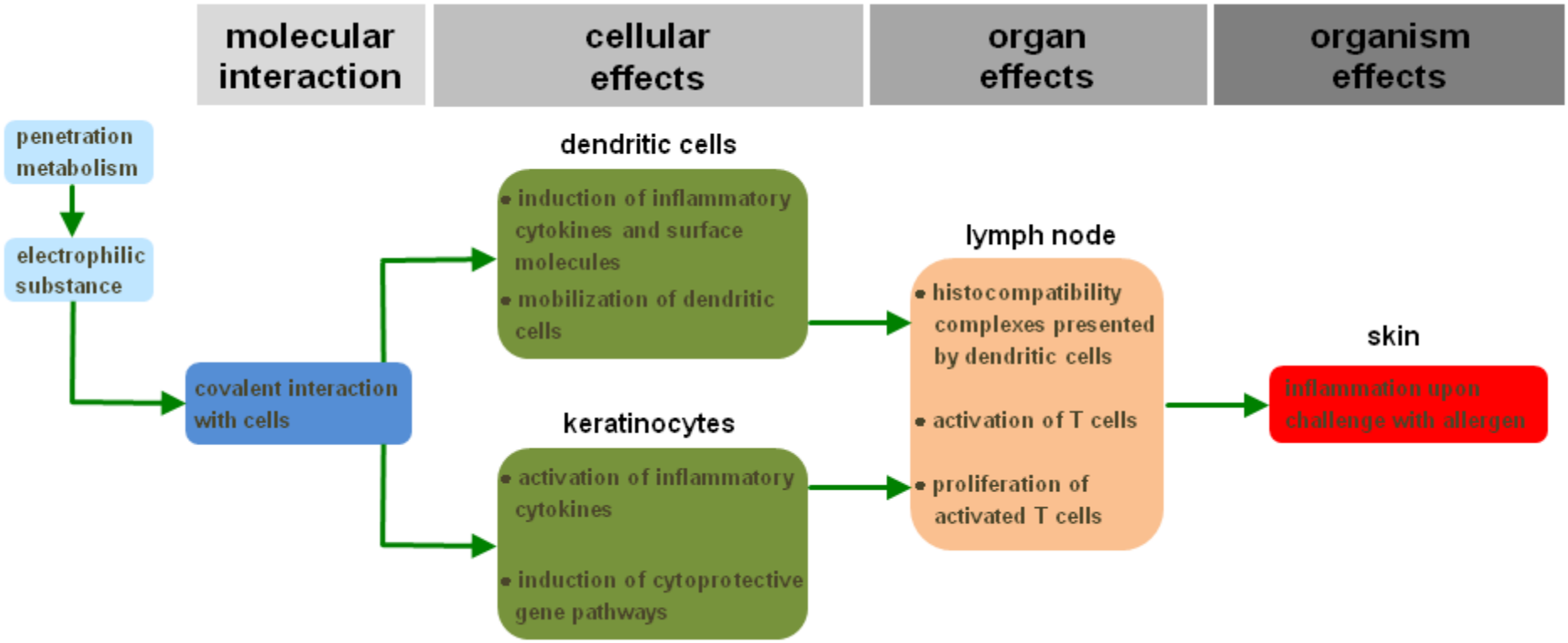
- How well characterised is the AOP?
- How well are the MIE and KEs causally linked to the AO?
- What are the limitations in the supporting evidence?
- Is the AOP specific to certain tissues, life stages or age classes?
- Are the MIE and KEs expected to be conserved across species?

Hill (1965) Proceed. Roy. Soc. Med. 58: 295-300.

OECD (2012) Proposal for a template and guidance on developing and assessing the completeness of adverse outcome pathways.

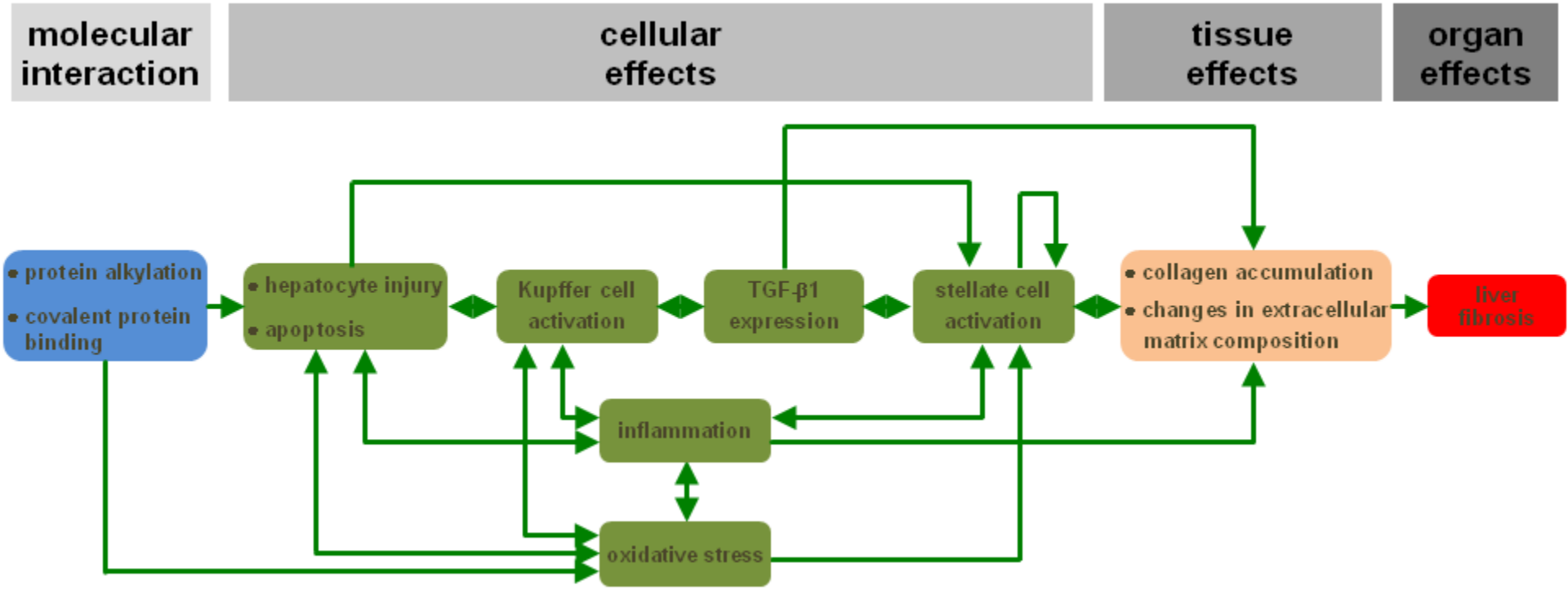
5. AOP examples

☑ Chemical-induced skin sensitisation



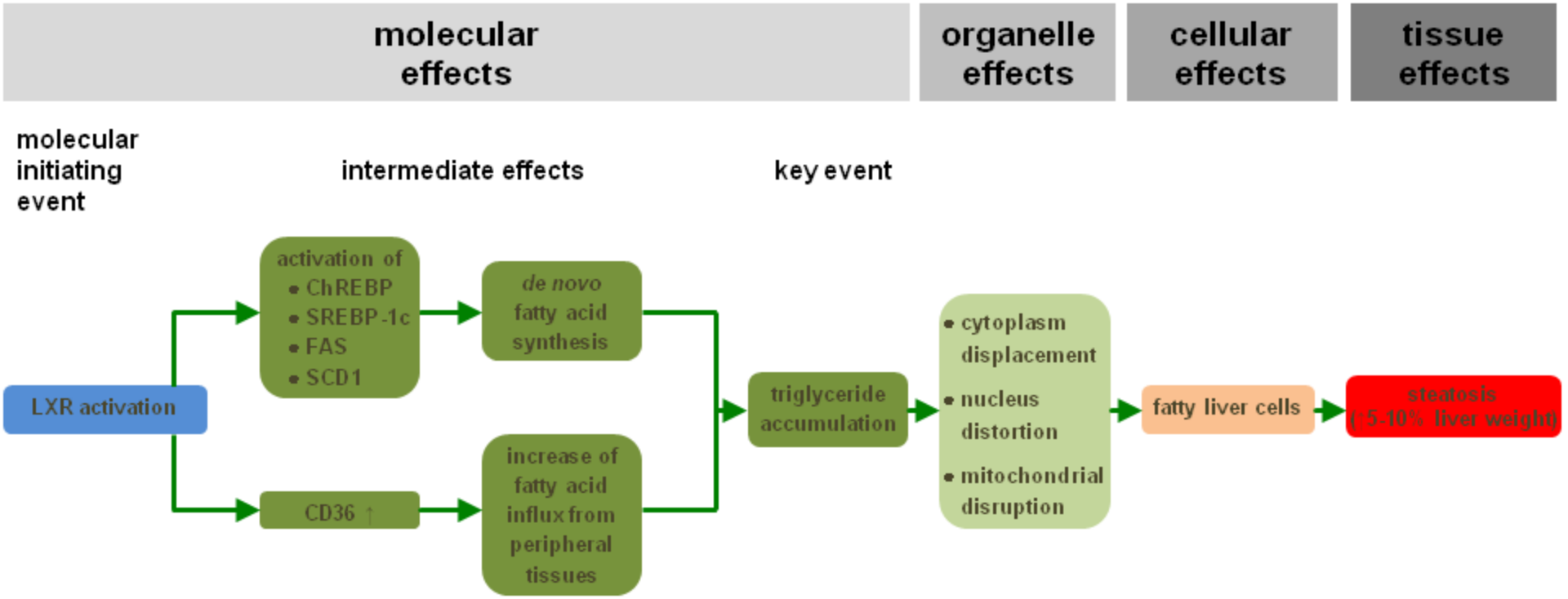
OECD (2012) The adverse outcome pathway for skin sensitisation induced by covalent binding of proteins part 1: scientific evidence.

☑ Drug-induced liver fibrosis



Landesmann *et al.* (2016) Toxicol. Sci.: submitted

☑ Drug-induced liver steatosis



Landesmann *et al.* (2012) JRC scientific and policy report.

☑ Miscellaneous

- Voltage-gated sodium channel-mediated neurotoxicity
- Nephrotoxicity induced by 4-aminophenols
- Hemolytic anemia induced by anilines
- Acetylcholinesterase inhibition
- Embryonic vascular developmental toxicity
- Sustained activation of aryl hydrocarbon receptors
- Phototoxicity
- Acute aquatic toxicity initiated by weak acid respiratory uncoupling
- Fish cardiotoxicity induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

6. AOP uses

☑ Chemical categorisation/grouping

- **Focus on MIEs**
- **Establishment of (quantitative) structure-activity relationships**
- **Basis for read-across approaches**
- **Examples**
  - ► **Chemical-induced skin sensitisation**
    - ■ **Nucleophilic-electrophilic reaction**
    - ■ **Aldehydes, epoxides, imides and lactams**
  - ► **Drug-induced liver steatosis**
    - ■ **LXR binding**
    - ■ **Phenyl rings, chloro groups and methyl moieties**

OECD (2012) Proposal for a template and guidance on developing and assessing the completeness of adverse outcome pathways.
Vinken (2013) Toxicology 312: 158-165.

☑ Test development

- Focus on KEs
- *In vitro* and *in silico* methods
- Identification of *in vivo* relevant biomarkers
- Example: chemical-induced skin sensitisation
  - ► Dendritic cell activation as KE
  - ► Myeloid U937 skin sensitisation test (MUSST)
    - ■ Exposure of human histiocytic lymphoma cells to chemical
    - ■ Assessment of CD86 expression by flow cytometry
  - ► Human cell line activation test (hCLAT)
    - ■ Exposure of human monocytic leukemia cells to chemical
    - ■ Assessment of CD54 and CD86 expression by flow cytometry

OECD (2012) Proposal for a template and guidance on developing and assessing the completeness of adverse outcome pathways.
Vinken (2013) Toxicology 312: 158-165.

☑ Integrated testing strategies

- In European chemicals' legislation (REACH)
- Contribution to refinement, reduction or replacement of *in vivo* testing (3Rs)

☑ Prioritisation strategies

- Paraoxon for developmental toxicity
- Domoic acid for neurotoxicity

☑ Others

- OECD test guideline program
- Regulatory purposes

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Vinken (2013) Toxicology 312: 158-165.

7. AOP projects

- ☑ **OECD**
  - Pathway-targeted case studies
  - Guidance on AOP development and evaluation
- ☑ **US Hamner Institutes for Health Sciences**
  - Pathway-targeted case studies
  - Focus on estrogen-related and peroxisome proliferator-related signalling
- ☑ **US Center for Alternatives to Animal Testing**
  - Pathways of toxicity (PoT) and the human toxome
  - Implementation of “-omics”-based information in PoT development
- ☑ **EU Safety Evaluation Ultimately Replacing Animal Testing**
  - Focus on liver toxicity
  - Establishment of *in vitro* test methods and biomarkers

8. AOP optimization: cholestasis as an example

☑ Safety Evaluation Ultimately Replacing Animal Testing (SEURAT)

- **Raised in response to European Regulation (EC) No. 1223/2009**
  - ► Cosmetic products and their ingredients
  - ► Testing and marketing ban

- **Public - private research initiative**
  - ► European Commission/FP7 (25 million €)
  - ► Cosmetics Europe (25 million €)

European Commission

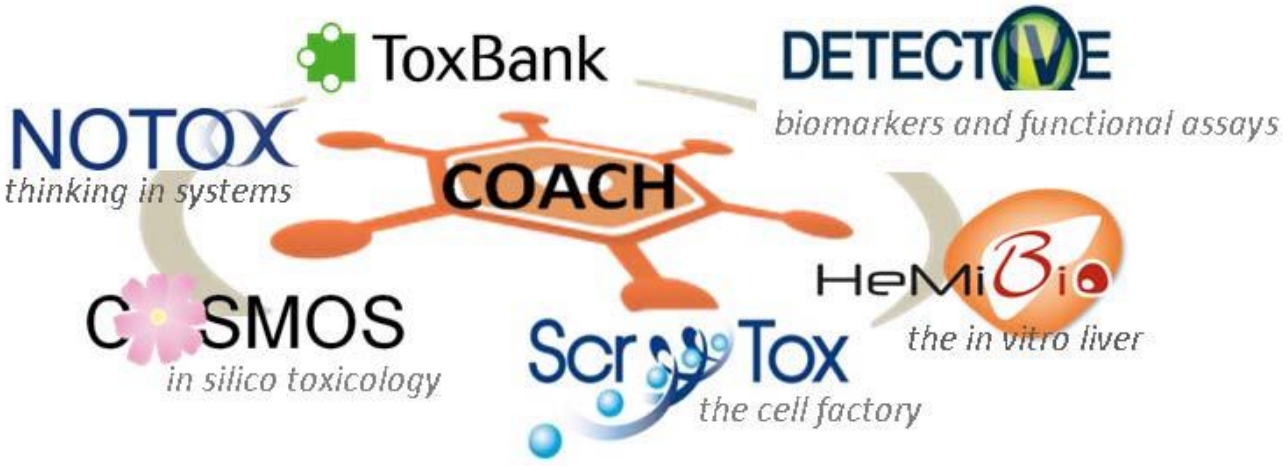
SEVENTH FRAMEWORK PROGRAMME

Cosmetics Europe
the personal care association

- **Organization**
  - ► 1 January 2011 - 31 December 2015
  - ► More than 70 research institutions
  - ► 6 projects and 1 coordinating action

SEURAT-1

- **SCR&Tox: stem cell differentiation for human organ-specific target cells**
- **HeMiBio: development of a hepatic microfluidic bioreactor**
- **DETECTIVE: identification and investigation of human biomarkers**
- **COSMOS: delivery of *in silico* tools to predict adverse effects of chemicals**
- **NOTOX: development of systems biology tools for organotypic cell cultures**
- **ToxBank: supporting integrated data analysis and servicing**
- **COACH: coordinating action**

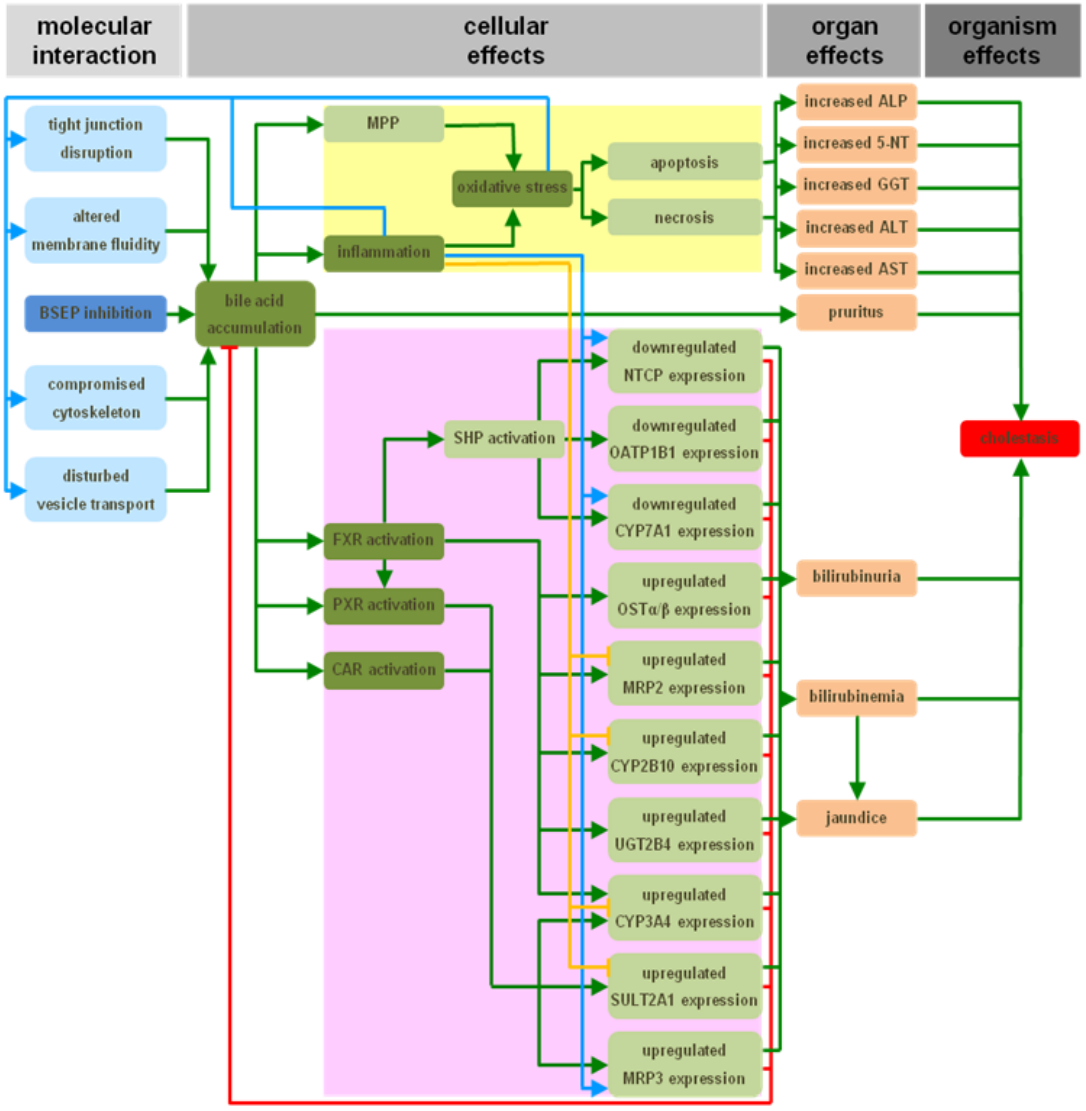


☑ AOP selection

- **Screening of cosmetic ingredient safety evaluation reports published by the Scientific Committee for Consumer Safety (SCCS) between 2000 and 2009**
  - ► SCCS safety evaluations of candidate cosmetic compounds to be included in the annexes of European Regulation (EC) No. 1223/2009
  - ► SCCS publishes the safety evaluation reports on open website
  - ► 253 safety evaluation reports covering 220 cosmetic substances
  - ► Focus on repeated dose toxicity testing
- **Outcome**
  - ► Liver, kidney and spleen are the most frequently targeted organs by cosmetic ingredients
  - ► Steatosis and cholestasis are the most prominent forms of liver toxicity induced by cosmetic ingredients

Vinken *et al.* (2012) Arch. Toxicol. 86: 405-412.

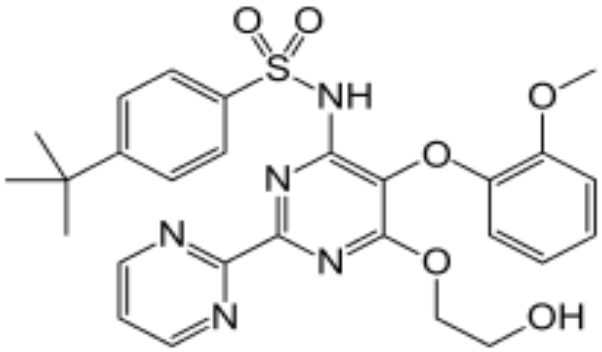
☑ AOP development



Vinken *et al.* (2013) Toxicol. Sci. 136: 97-106.

☑ Set-up

- **3 liver-based *in vitro* models**
  - ► Human primary hepatocytes
  - ► Human hepatoma HepaRG cells
  - ► Human skin-derived hepatic progenitor cells
- **AOP verification using a compound with a clear-cut toxicological profile**
  - ► Bosentan: drug for treatment of pulmonary arterial hypertension

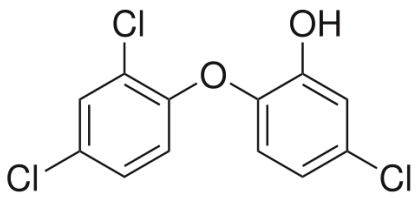


  - ► 3 concentrations (IC_{10}, $IC_{10}/4$, $IC_{10}/10$)
  - ► 3 exposure regimes (1 hour, 24 hours, 24 hours + 72 hours wash-out)

- **Detection of established biomarkers**
  - ► **BSEP inhibition functionality assay**
  - ► **Reporter gene assays for nuclear receptor activation**

- **Characterisation of new biomarkers**
  - ► **Transcriptomics**
  - ► **Epigenomics**
  - ► **Metabonomics**
  - ► **Proteomics**

- **AOP application using a compound with a poorly documented toxicological profile**
  - ► **Triclosan: antimicrobial agent in consumer products**



  - ► **3 concentrations (IC_{10}, $IC_{10}/4$, $IC_{10}/10$)**
  - ► **3 exposure regimes (1 hour, 24 hours, 24 hours + 72 hours wash-out)**

9. AOP optimization: follow-up

- ☑ **Functional and *in silico* testing**
  - BSEP inhibition and nuclear receptor activation
  - Structural alerts and descriptors
- ☑ **Robustness and applicability testing**
  - Primary human hepatocytes
  - Drugs and cosmetic ingredients
- ☑ **Quantitative optimization**
  - Quantitative structure-activity relationships
  - Concentration-response relationships
- ☑ **Risk assessment optimization**
  - Kinetic data
  - Exposure data

10. Fit-for-purpose optimization: tiered testing strategy

☑ Qualitative testing: "yes/no" answer

- *In silico* testing
  - ► Molecular initiating event
  - ► Modelling and structural alerts
- *In vitro* testing
  - ► Intermediate steps and key events
  - ► Functional and '-omics' tests
- Weight-of-evidence approach
  - ► Decisive data: molecular initiating event and key event
  - ► Supporting data: intermediate steps

☑ Quantitative testing: no observed adverse effect level assessment

- Physiologically-based pharmacokinetic modelling
- Quantitative *in vitro-in vivo* extrapolation

11. Further reading

- ☑ Ankley *et al.* (2010) Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environmental Toxicology and Chemistry* 29: 730-741.

- ☑ Villeneuve *et al.* (2014) Adverse outcome pathway (AOP) development I: strategies and principles. *Toxicological Sciences* 142: 312-320.

- ☑ Villeneuve *et al.* (2014) Adverse outcome pathway (AOP) development II: best practices. *Toxicological Sciences* 142: 321-330.

- ☑ Vinken (2013) The adverse outcome pathway concept: a pragmatic tool in toxicology. *Toxicology* 312: 158-165.