BRIDGE THE GAP
A Human Pathways Approach to Disease Research

BIOMED^{21}

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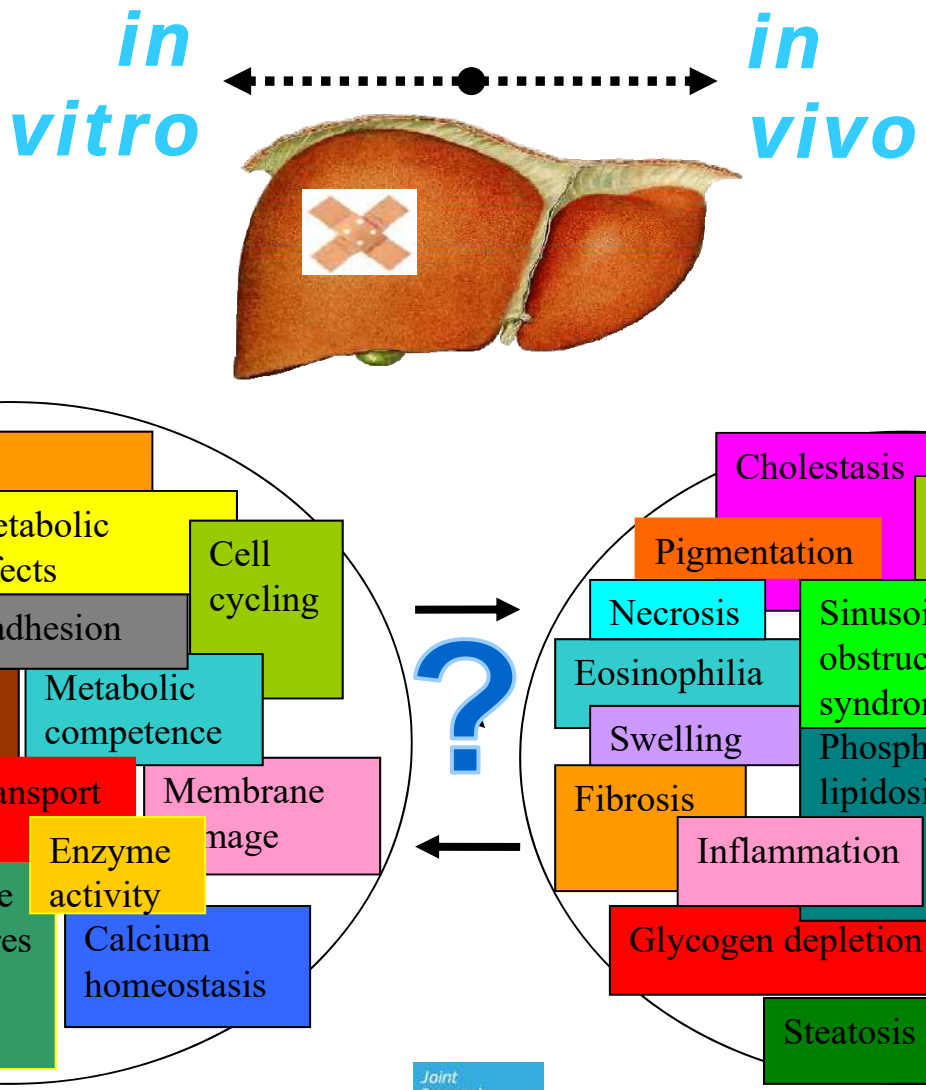
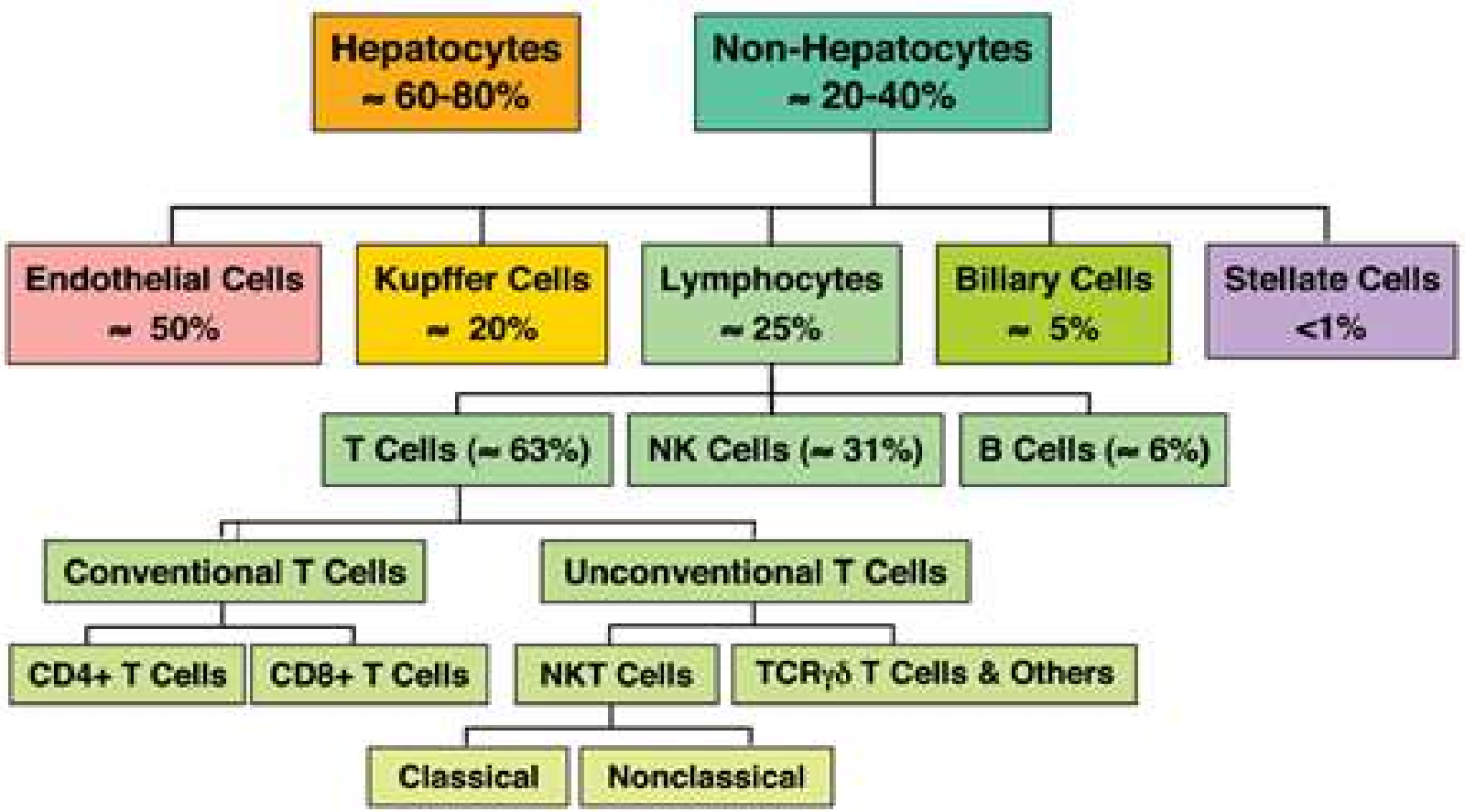

in vitro
in vivo
-omics
Steatosis
Metabolic effects
Cell viability
Cell cycling
Cell adhesion
Apoptosis
Cell funct ion
Chromosomal damage:
Metabolic competence
Ion transport
Membrane
DNA damage:
Enzyme activity
Gene expres sion
Mitochondrial damage
Calcium homeostasis
?
Cholestasis
Hypertrophy
Pigmentation
Bile duct inflamm ation
Necrosis
Sinusoidal obstruction syndrome
Eosinophilia
Swelling
Phospho lipidosis
Vacuolisation
Fibrosis
Inflammation
Granuloma
Glycogen depletion
Steatosis

The Liver



Cell composition of the healthy liver.

Numbers indicate the estimated frequency of each population relative to the total number of parenchymal and nonparenchymal cells in the liver. RACANELLI AND REHERMANN 2006

Cell models

Tools:
- high throughput screening
- high content imaging
- computer modelling
- omics techniques

Cell models:
- primary human hepatocytes
- immortalized human hepatocyte cell lines, such as HepG2 and HepaRG
- Liver cells derived from induced pluripotent stems or human embryonic stem cells
- co-cultures of hepatocytes and other cell types
- 3D liver models
- Perfusion bioreactors
- precision-cut liver slices
- isolated perfused liver

Shortcomings:
- immune system
- liver architecture
- limited life span, dedifferentiation
- Non-physiological conditions
- cell culture conditions (O_2, medium,….)
- efficacious doses, exposure,

- *In vitro* models cannot mimic whole human organs -"organ-on-a chip".
- But - the *in vivo* situation should always be the benchmark.
- A better understanding of the complex biological mechanisms in health and disease could enable the identification of endpoints and markers with translational relevance.

In vivo human data as benchmark

For this we need:

- Clinical studies to obtain *in vivo* human information, which may anchor *in vitro* research models to "real-world" illnesses in humans. E.g. the *in vitro* integration of signals that are released by injured hepatocytes in humans.

- Availability of clinical samples – from healthy persons and from patients with disease, like blood, serum, urine, omics data, histology (*ex vivo* biopsies or post-mortem).

Health and disease could be studied in synergy with toxicology using a combination of *in vitro* testing methods with human-based models and clinical data.

There is a disconnect between *in vitro* DILI endpoints and those used to assess hepatotoxicity in a clinical setting.

In vivo

- Serum enzymes:
  - AST, ALT
  - ALP, γGT
- Biosynthetic functions
  - Albumin
  - Immunglobulins
  - Coagulation factors
- Excretion and Detoxification
  - Bilirubin
  - Ammonia
- Others
  - Serology
  - Autoimmune markers
  - Imaging
  - Biopsy

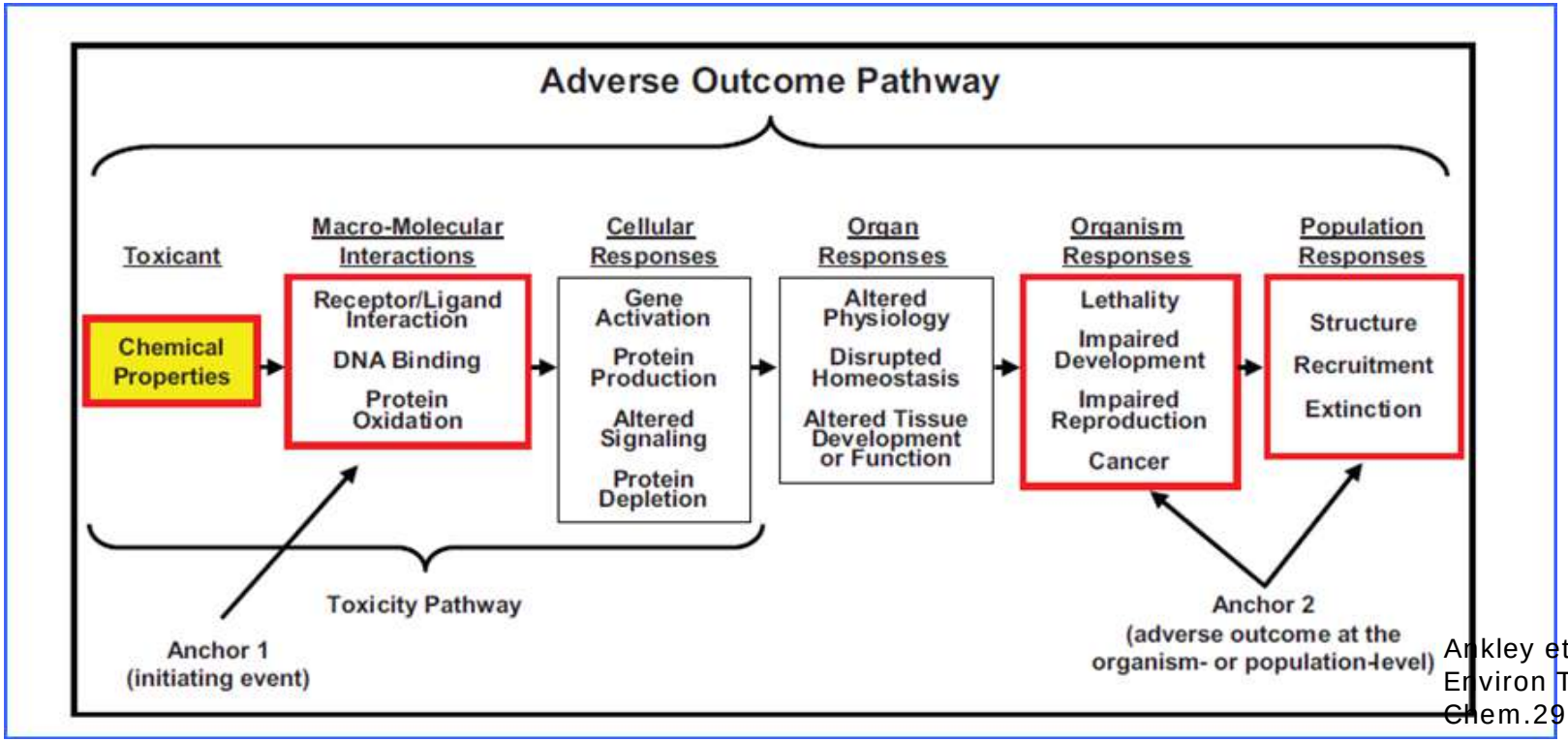
In vitro

- Protein adducts
- Apoptosis, Necrosis
  - LDH leakage, Caspases
- Mitochondrial dysfunction
  - MMP
- Oxidative stress
  - ROS, GSH
- Kupffer cell activation
  - Cytokines
- Stellate Cell activation
  - Morphology, α-SMA, collagen
- Steatosis
- Cholestasis
- Gene expression levels

Adverse Outcome Pathway (AOP)

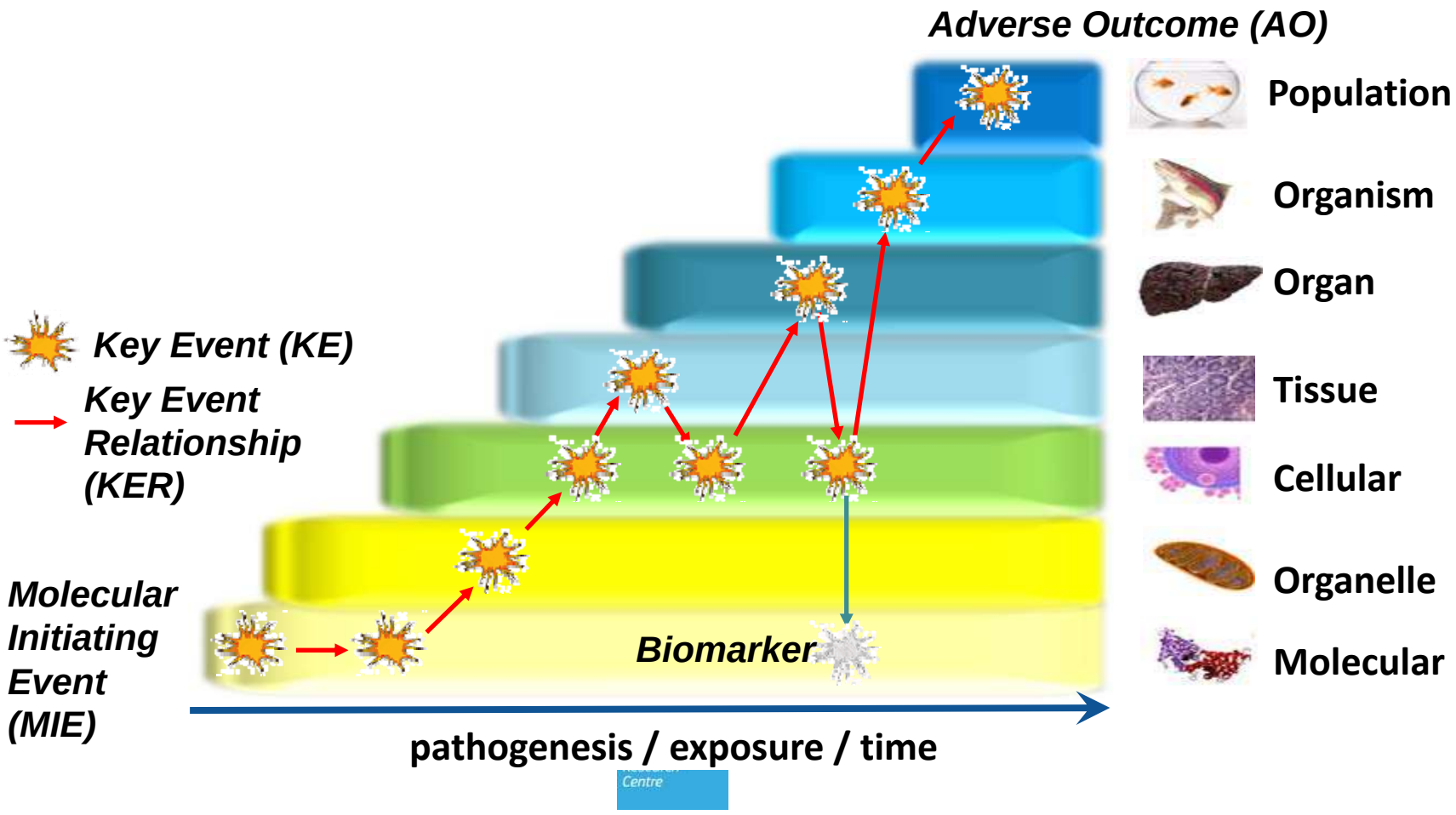
European Commission

An **Adverse Outcome Pathway** (AOP) is a conceptual construct that describes a **sequential chain of causally linked events** starting on molecular level and leading through different levels of biological organisation to an adverse health or eco-toxicological outcome.
AOPs are the central element of a **toxicological knowledge framework** being built to support chemical risk assessment **based on mechanistic reasoning**.

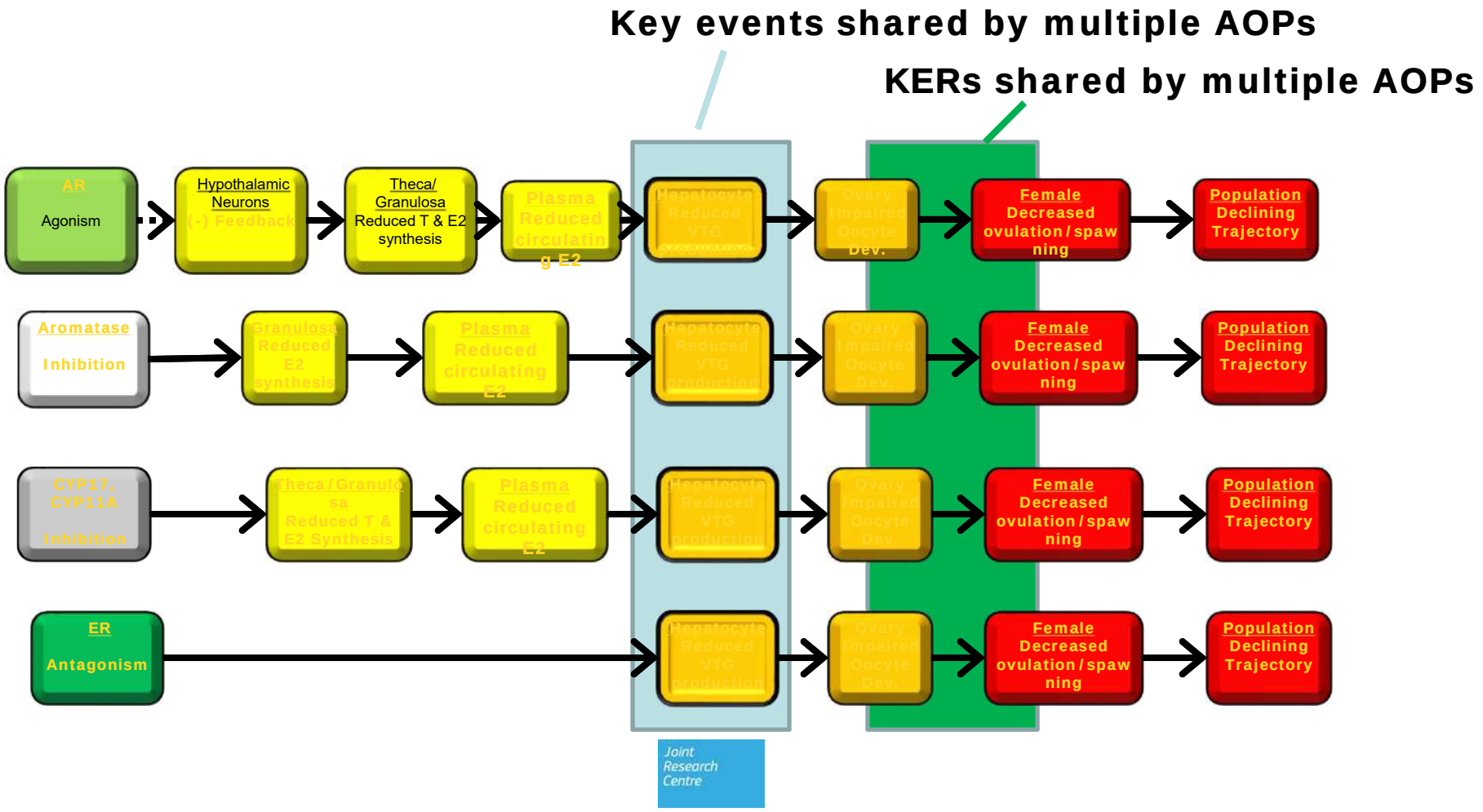


Ankley et al (2010) Environ Toxicol Chem.29(3):730-41

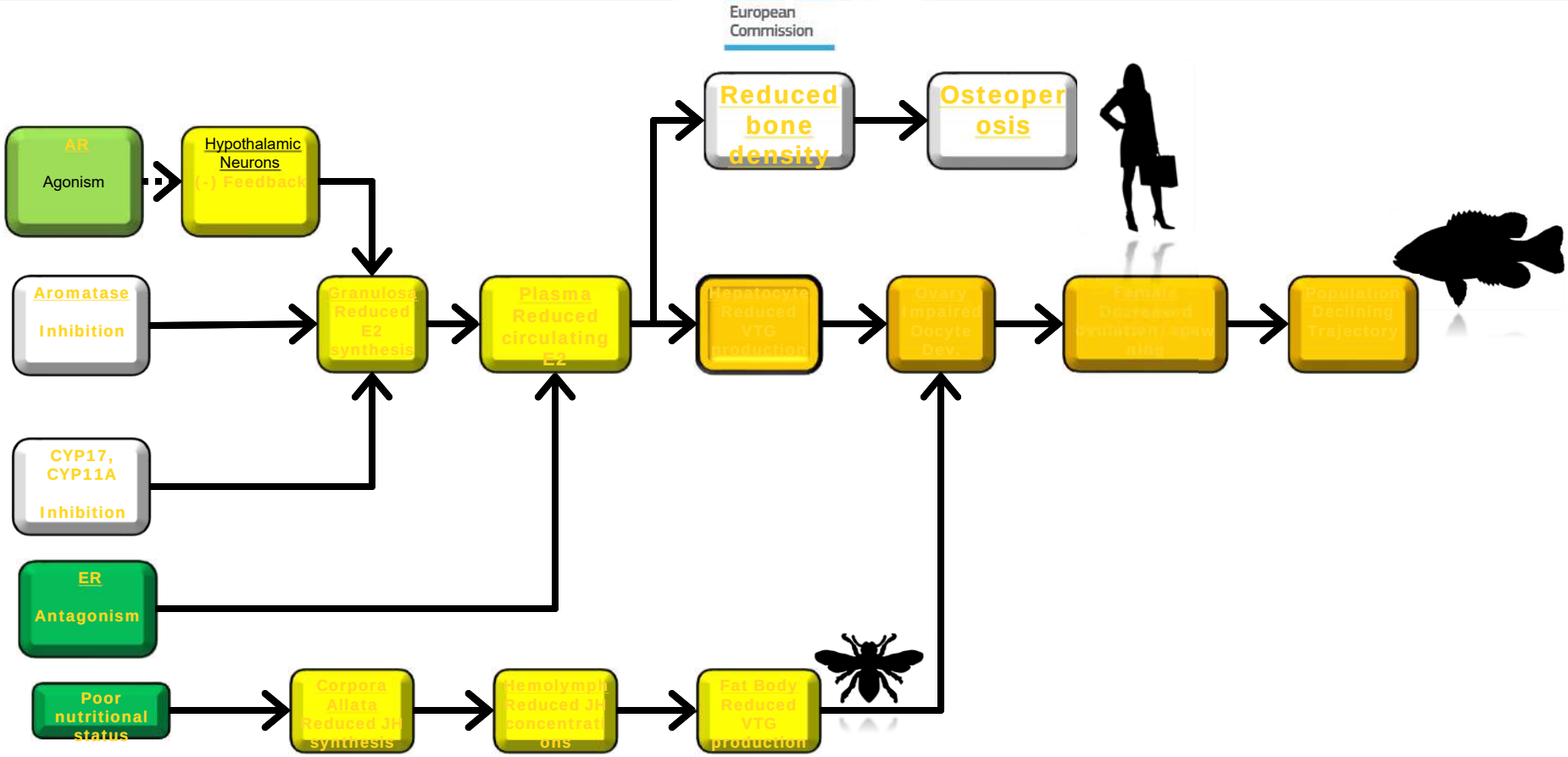
Adverse Outcome Pathway (AOP)



Principles of AOP Development



Principles of AOP Development



AOP networks also a way to represent conservation and divergence of toxicological responses across taxa, life stages, etc.

AOPs

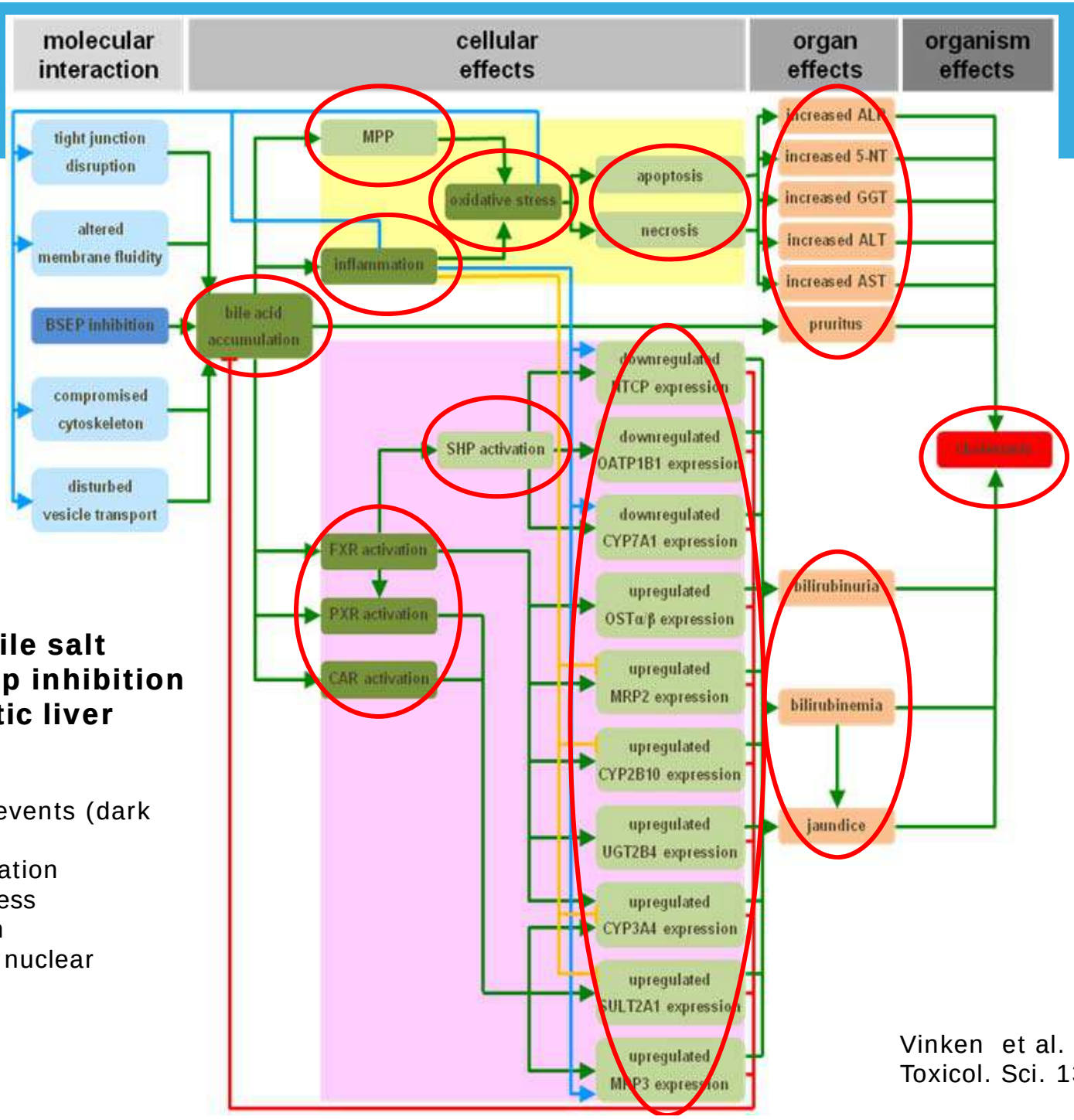
A concept that systematically describes the links between causes and outcomes – potentially also including causes of disease.

Incorporation of the AOP construct into human health research and drug discovery could provide mechanistic rationales for diagnostic, preventative, and therapeutic interventions.

A better understanding of disease and repair mechanisms in humans can enable an earlier diagnosis, a better prediction of the clinical outcome and the development of targeted therapies.

A reduction in bile flow (cholestasis) can result from:

- gall stones
- impingement from a local tumor
- intrahepatic cholestasis of pregnancy
- genetic deficiency in bile export proteins
- autoimmune disorders (primary biliary cirrhosis
- primary sclerosing cholangitis
- associated with infections
- associated with total parenteral nutrition
- neonatal disorders (as progressive familial intrahepatic cholestasis and biliary atresia)
- ...
- Drug induced (toxic)



AOP from bile salt export pump inhibition to cholestatic liver injury

Identified key events (dark green)

- bile accumulation
- oxidative stress
- inflammation
- activation of nuclear receptors.

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

AOPs are a powerful tool to support

- the integration of available knowledge, information and data from various sources (**clinical data**!)
- the mechanistic understanding of toxicological processes - **and diseases**
- the identification of biomarkers
- the identification of gaps and uncertainties
- the direction of further research
- **the collaboration between scientists from various disciplines**

www.aopwiki.org

Exchange between toxicology and clinical medicine

A **bedside-to-bench-to-bedside** program is needed that will not only inform relevant *in vitro* testing, but also allow the extrapolation of *in vitro* information to disease and regeneration mechanisms *in vivo*.

This can only be achieved through a better collaboration between
health care professionals, academia, and industry.

European Commission

Joint Research Centre