# Joint Research Centre

## the European Commission's in-house science service

*Serving society*
*Stimulating innovation*
*Supporting legislation*


# From Bedside to Bench

adding human context
to *in vitro* models

## EUSAAT Annual Congress

26th August 2016

**Brigitte Landesmann**



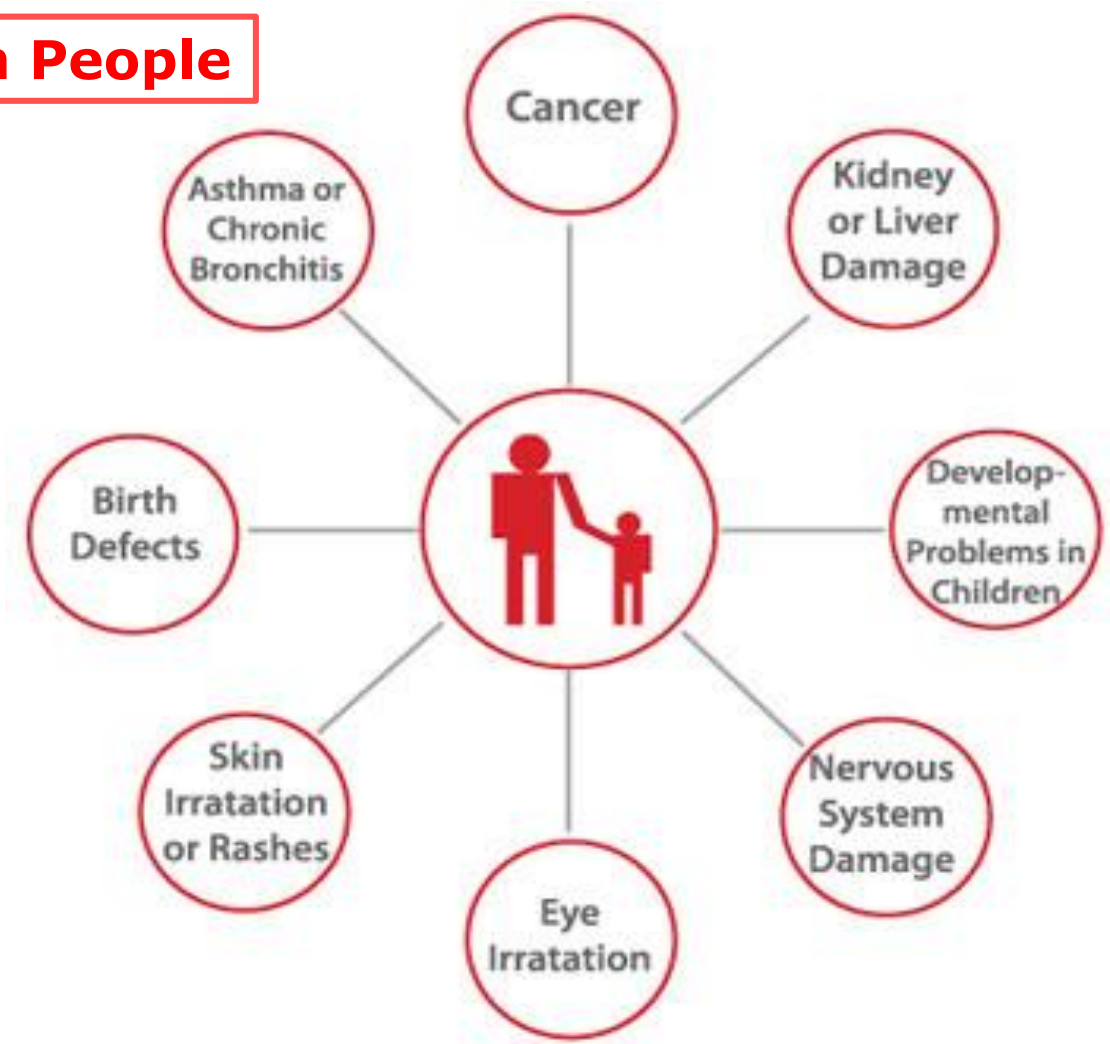
EURL ECVAM
European Union Reference Laboratory for Alternatives to Animal Testing

European Commission

# Human Health Risk Assessment

An estimation of the nature and probability of adverse health effects in humans who may be exposed to chemicals

**Focus is on People**

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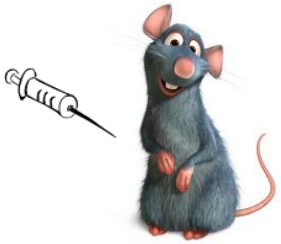
# The classical approach to chemical toxicity testing…

…is rather focused on animals

Animals are exposed to chemicals



and the outcome is observed

# PARADIGM SHIFT
# in regulatory toxicity testing and risk assessment


- to develop a more robust scientific basis for assessing adverse health effects of chemicals
- to replace, reduce, and refine the use of experimental animals (3Rs)
- to provide broad coverage of chemicals and chemical mixtures
- to reduce the cost and time of testing

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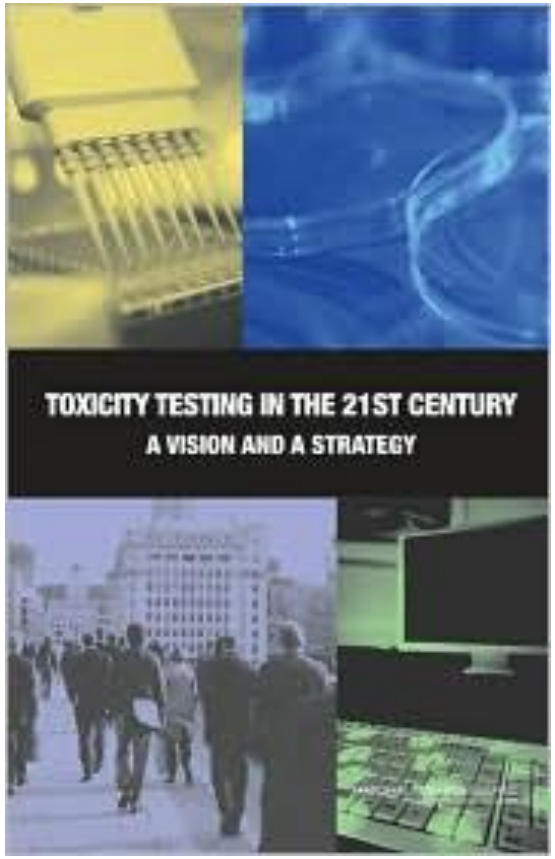
**Landmark Report**
NAS 2007

**Toxicity testing in the 21st century. A vision and a strategy**




"Transform toxicity testing from a system based on whole-animal testing to one founded primarily on *in vitro* methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin"

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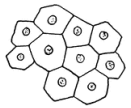
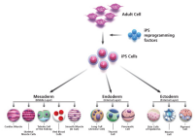
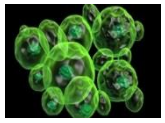
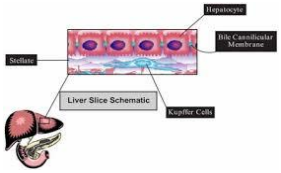
# 21st Century Toxicity Testing is here….

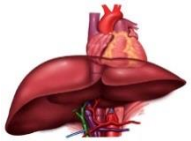
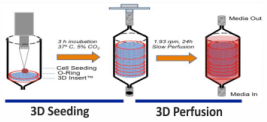
## We have a variety of cell models

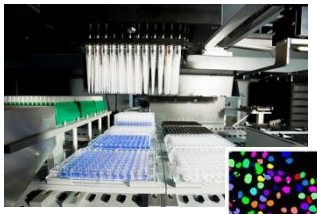
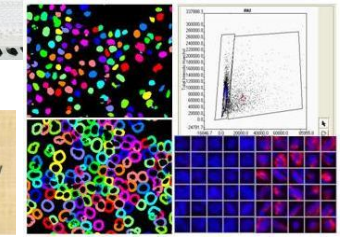
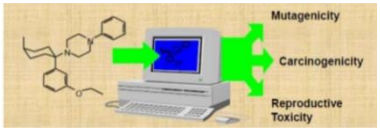



- Primary human cells
- Immortalized human cell lines
- Induced pluripotent stem cells human embryonic stem cells
- Co-cultures of various cell types
- 3D cultures
- Perfusion bio-reactors
- Precision-cut organ slices
- Isolated perfused organs




## and a variety of tools

- High throughput screening
- High content imaging
- Omics techniques
- Computer modelling

**Technology is rapidly further progressing…**

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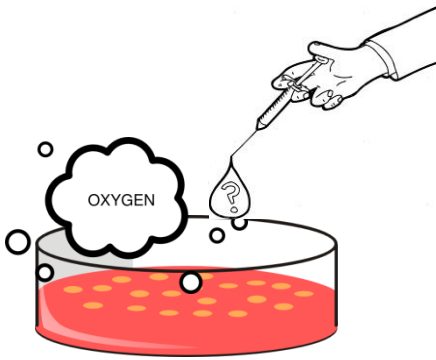
# 21st Century Toxicity Testing is here….

**Nevertheless, there are important shortcomings**

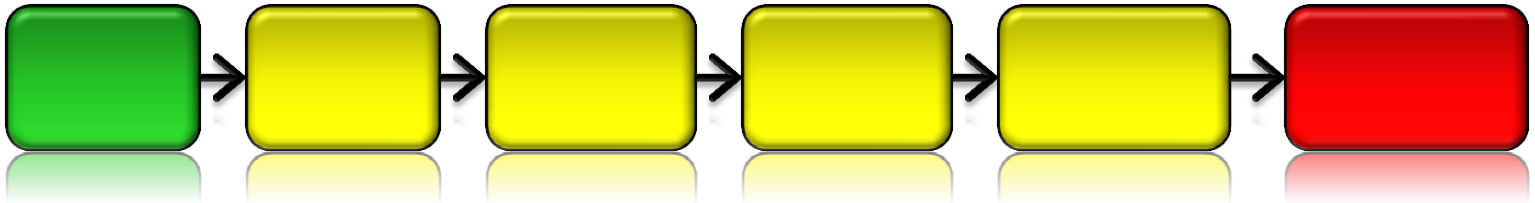
- Role of the immune system
- Specific organ architecture
- Limited life span of cells in culture, dedifferentiation
- Non-physiological conditions ($O_2$, medium,….)
- Efficacious doses, exposure




- *In vitro* models cannot mimic whole human organs
- But - the *in vivo* situation should be the benchmark
- A better understanding of **health**, **disease**, and **repair** mechanisms can support the identification of markers with translational relevance

# Scientific challenge:

How to make effective use of available mechanistic data and the wealth of existing scientific knowledge to support regulatory decision-making?



**Adverse Outcome Pathways are part of the solution.**

A tool for knowledge-based human health risk assessment

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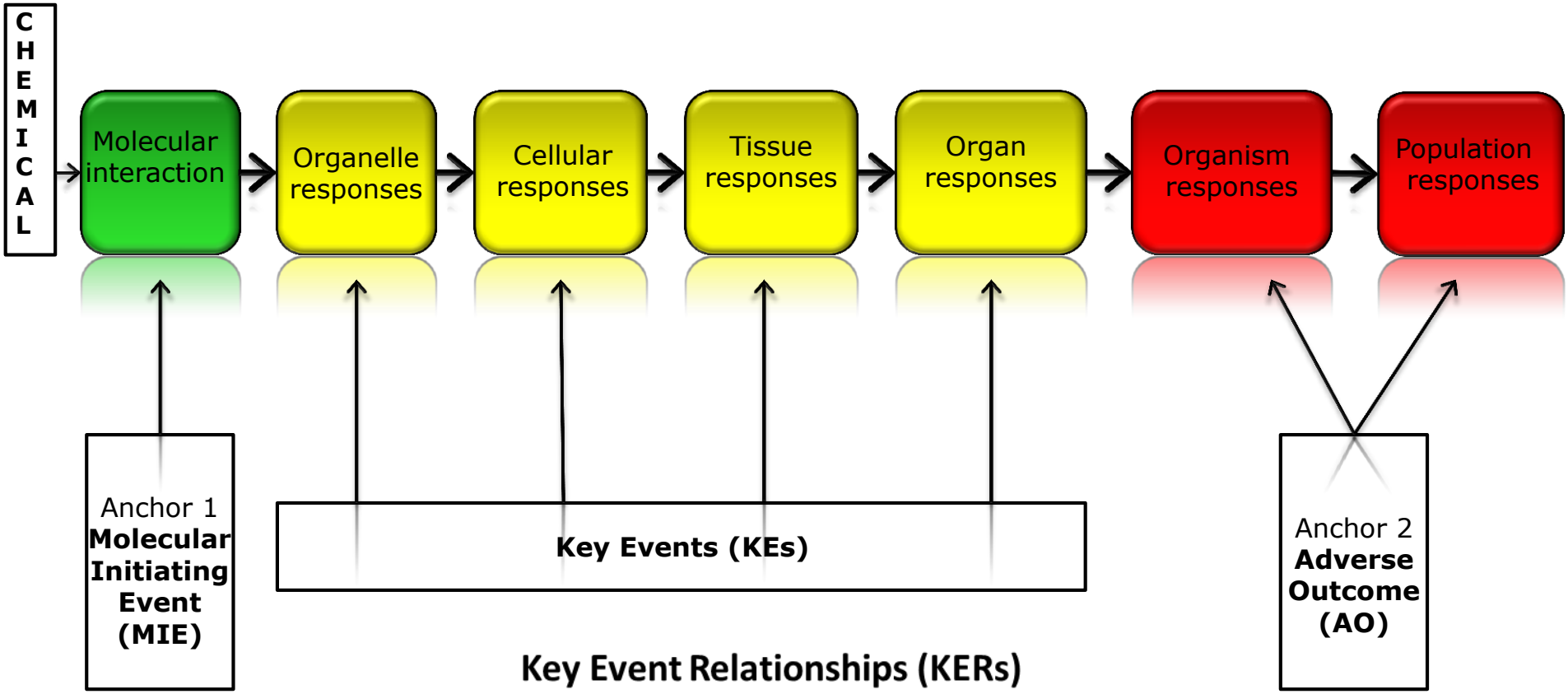
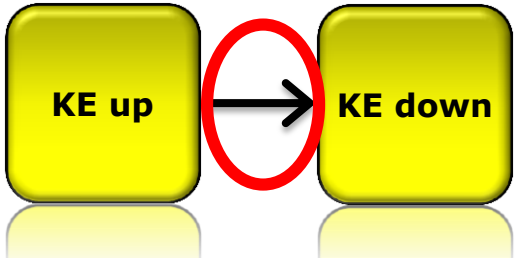
# Adverse Outcome Pathway

An 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.




**Key Event Relationships (KERs)**

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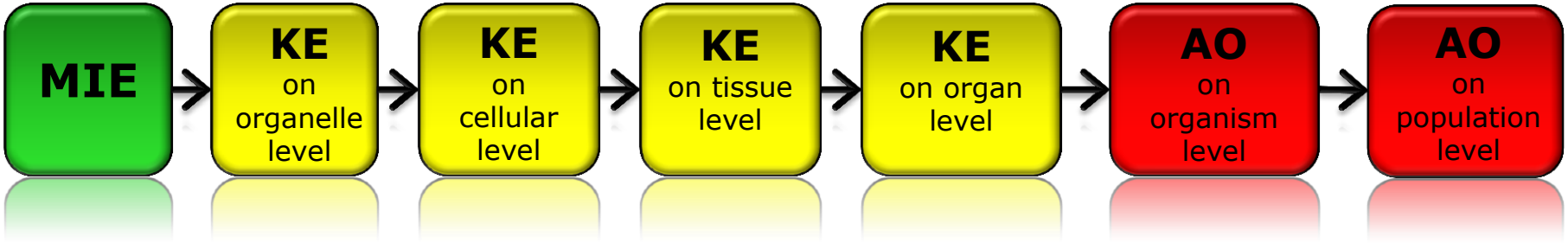
# Adverse Outcome Pathway (AOP)

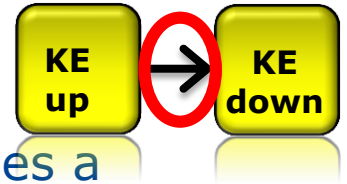



**Molecular initiating event (MIE)** – the initial point of chemical interaction on the molecular level within an organism

**Adverse Outcome (AO)** – correspondent to an established protection goal or equivalent to an apical endpoint in an accepted regulatory guideline toxicity test

**Key Event (KE)** - A change in biological state that is both measurable and essential to the progression to a specific AO

**Key event relationship (KER)** - A scientifically-based relationship that connects one KE to another; it defines a directed relationship between the two KEs, identifying one as upstream and the other as downstream

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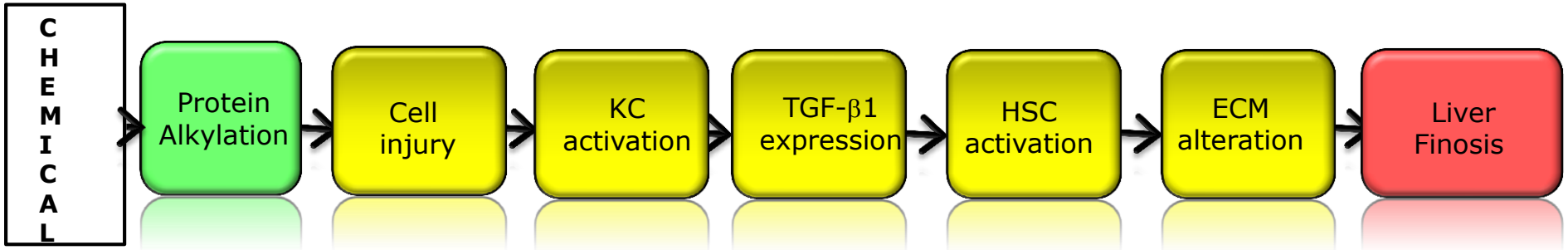
# AOP to Liver Fibrosis



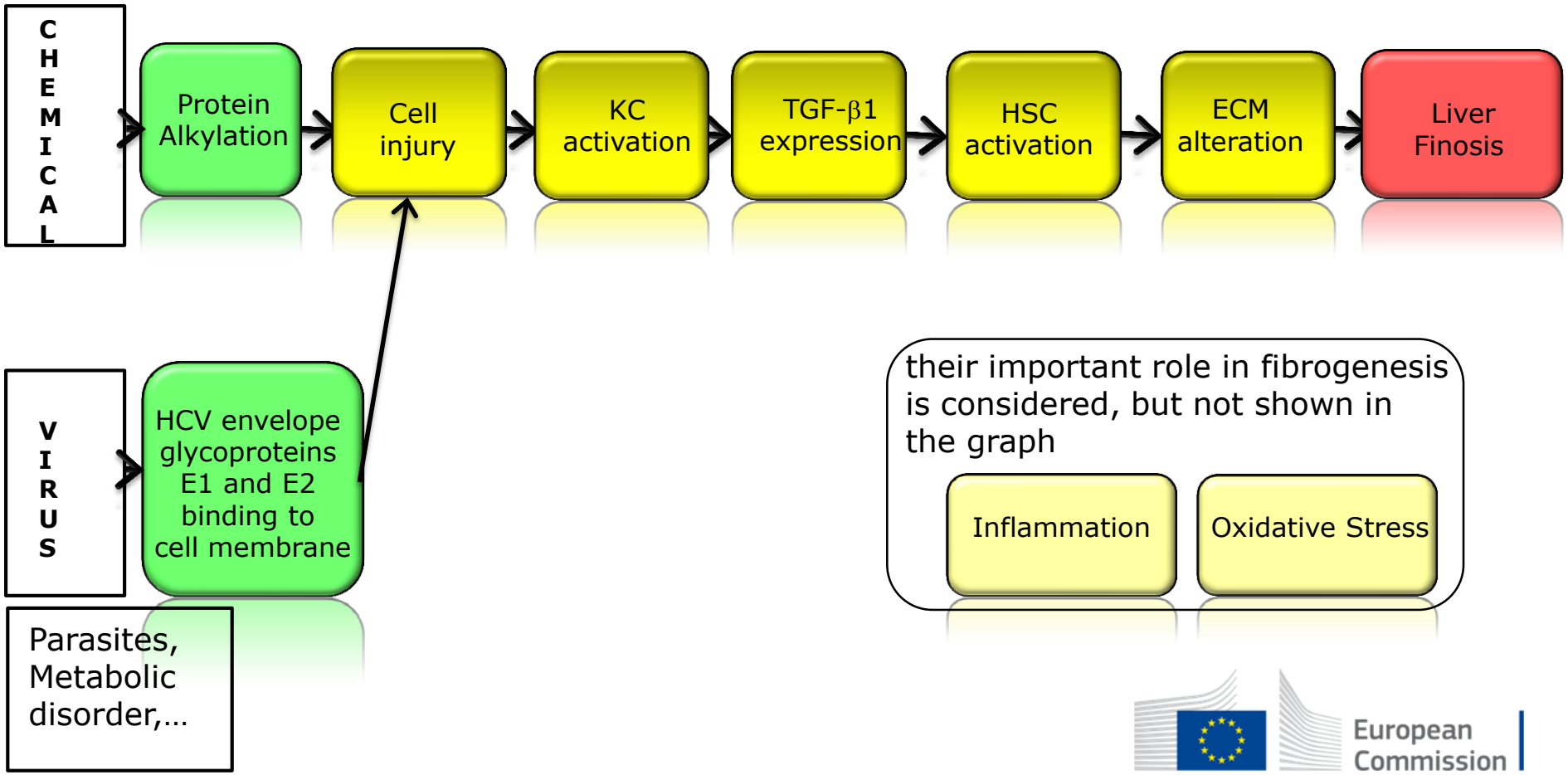

The MIE is protein alkylation, leading to structural and functional cell injury and cell death. Injured and apoptotic hepatocytes activate Kupffer cells, which are the main source of TGF-β1, the most potent pro-fibrogenic cytokine. TGF-β1 expression causes stellate cell activation, which leads to progressive collagen accumulation, changes in extracellular matrix composition and subsequently to liver fibrosis.

https://aopwiki.org/wiki/index.php/Aop:38

*An AOP does not provide a comprehensive molecular description of every aspect of the biology involved (the mechanism of action), but focuses on the critical steps in the pathway.*

The AOP concept systematically describes the links between causes and outcomes – potentially also describing **Pathways of Disease**.

Any chronic liver disease may result in fibrosis.
Though the causes/initiators are different, the further downstream pathway to the AO/disease remains.

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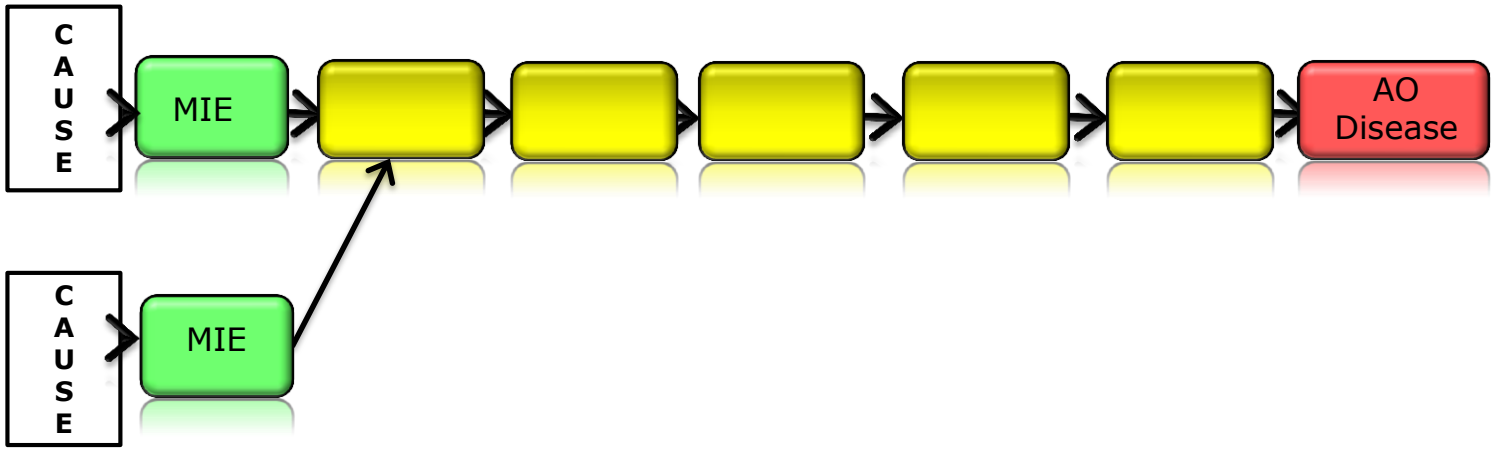



There is a disconnect between the concepts and data associated with molecular/cellular studies and those associated with clinical studies.

Sharing information between these research communities, thus closing data gaps between *in vitro* findings and clinical knowledge, would be beneficial for all stakeholders.

# E.g. there is a disconnect between *in vitro* DILI endpoints and the data 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


# How to interrelate these findings?

# *In vivo* human data as benchmark!

**Clinical studies** to obtain *in vivo* human information, to anchor *in vitro* research models to "real-world" illnesses in humans.

**Clinical samples** from healthy persons and from patients (*ex vivo* or post-mortem) like blood, serum, urine, omics data, histology,….
Data that are regularly taken in the course of the diagnostic and curative process could support – consent provided - better understanding and improved relevance of *in vitro* testing.

Aiming at a combination of *in vitro* testing methods with human-based models and clinical data.

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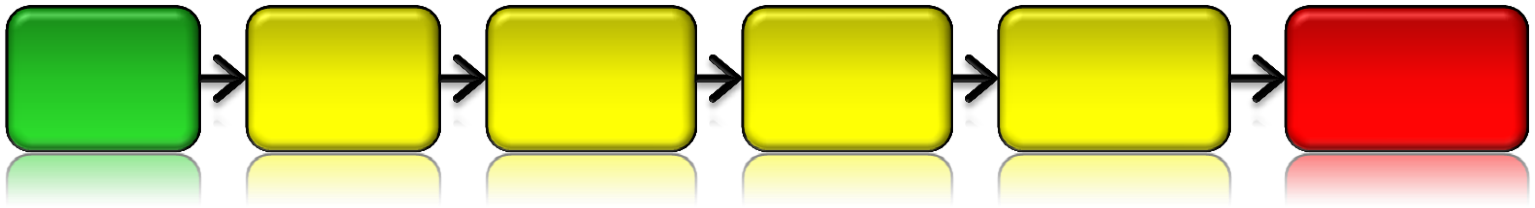


# The AOP framework – a tool to support

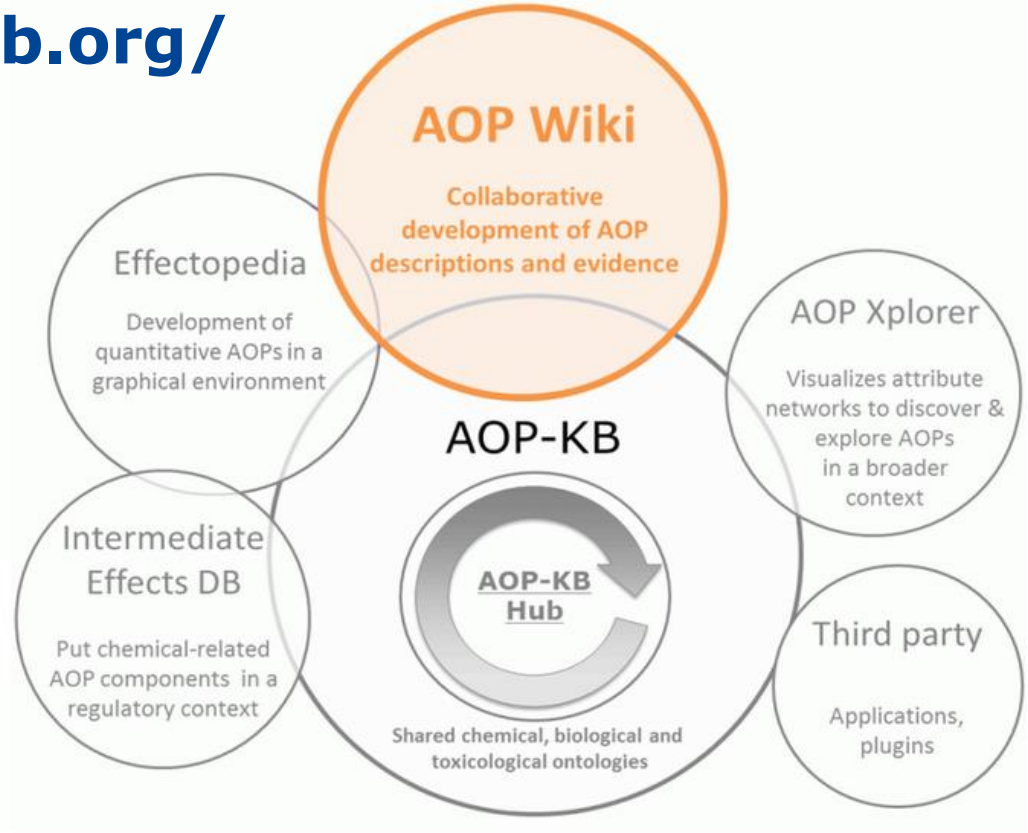
- the integration of available knowledge
- mechanistic understanding
- the identification of biomarkers
- the identification of gaps and uncertainties
- the direction of further research for closing these gaps
- the collaboration between scientists from various disciplines

http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm

# Adverse Outcome Pathway Knowledge Base (AOP-KB)

|| AOP-KB || Background || How to contribute ||

**https://aopkb.org/**




Please click on any of the AOP-KB elements you want to use.
Please note that the AOP-KB is work in progress and more elements will become available over time.

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# Adverse Outcome Pathway Knowledge Base AOP KB

The AOP-KB is a combination of four individually developed platforms - AOP-Wiki, Effectopedia, AOP Xplorer and Intermediate Effects DB – with different emphasis on the type of the captured Information. All four modules (and potentially compatible third party systems) share, exchange and synchronise information via the AOP-KB Hub.

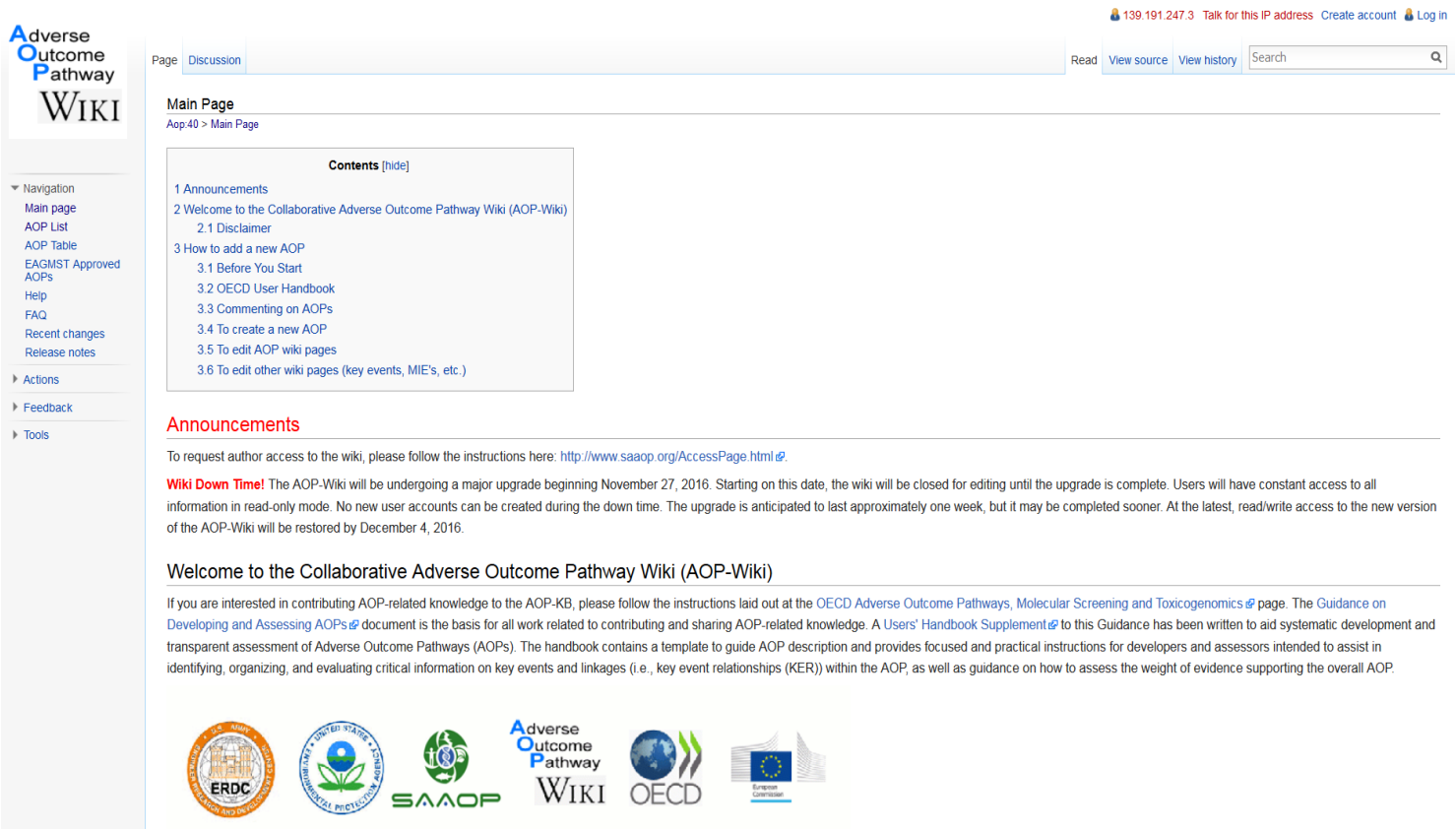
The AOP-KB project is an OECD initiative, executed in collaboration between the European Commission's Joint Research Centre (JRC), the United States Environmental Protection Agency (US EPA), and the US Army Engineer Research & Development Center (ERDC).

**https://aopkb.org/**




# AOP Wiki

The AOP-Wiki is one component of the AOP Knowledge Base that enables the scientific community to share, develop, and discuss AOP-related knowledge.

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# Exchange between toxicology and clinical medicine

## A **bedside**-to-**bench**-to-**bedside program**

⇒ to inform relevant *in vitro* testing

⇒ to allow extrapolation of *in vitro* information to disease and regeneration mechanisms *in vivo*.

**collaboration**

health care professionals, academia, and industry

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