Testing of the predictive power and robustness of an adverse outcome pathway construct for bile salt export pump inhibition to cholestatic injury

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Adverse outcome pathways:

From research to regulation

September 3 - 5, 2014

Bethesda, Maryland, USA

DETECTIVE
Detection of Endpoints and Biomarkers for Repeated Dose Toxicity Using In Vitro Systems

Cosmetics Europe
the personal care association

SEVENTH FRAMEWORK PROGRAMME

Vrije Universiteit Brussel

FAFY

CePhaR

1. Context of AOP development and use

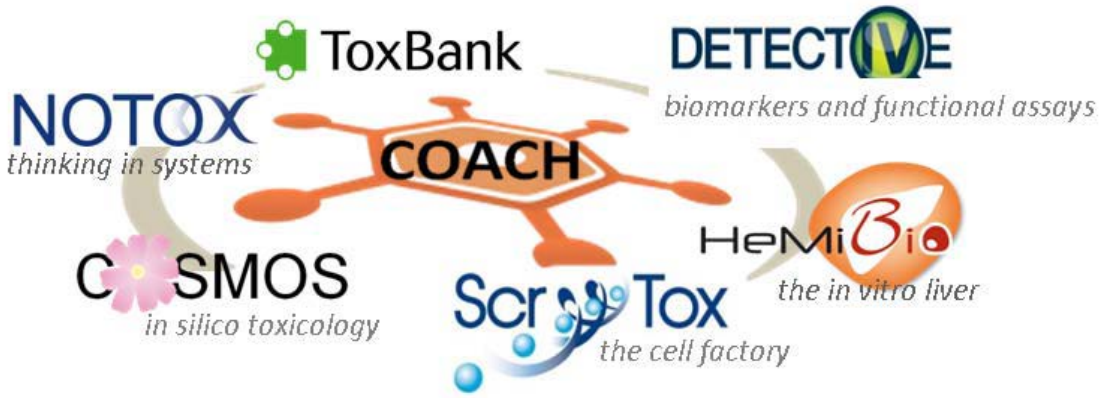
☑ Safety Evaluation Ultimately Replacing Animal Testing (SEURAT)

- **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 €)

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

☑ SEURAT-1 projects



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

2. AOP selection

☑ Resource and strategy

- **Scientific Committee of Consumer Safety (SCCS)**

  Performs safety evaluations of candidate cosmetic compounds to be included in the annexes of European Regulation (EC) No. 1223/2009

  Publication of safety evaluation reports on open website

- **Screening of SCCS safety evaluation reports published between 2000 and 2009**

  253 safety evaluation reports covering 220 cosmetic substances

  Focus on repeated dose toxicity testing

☑ Outcome

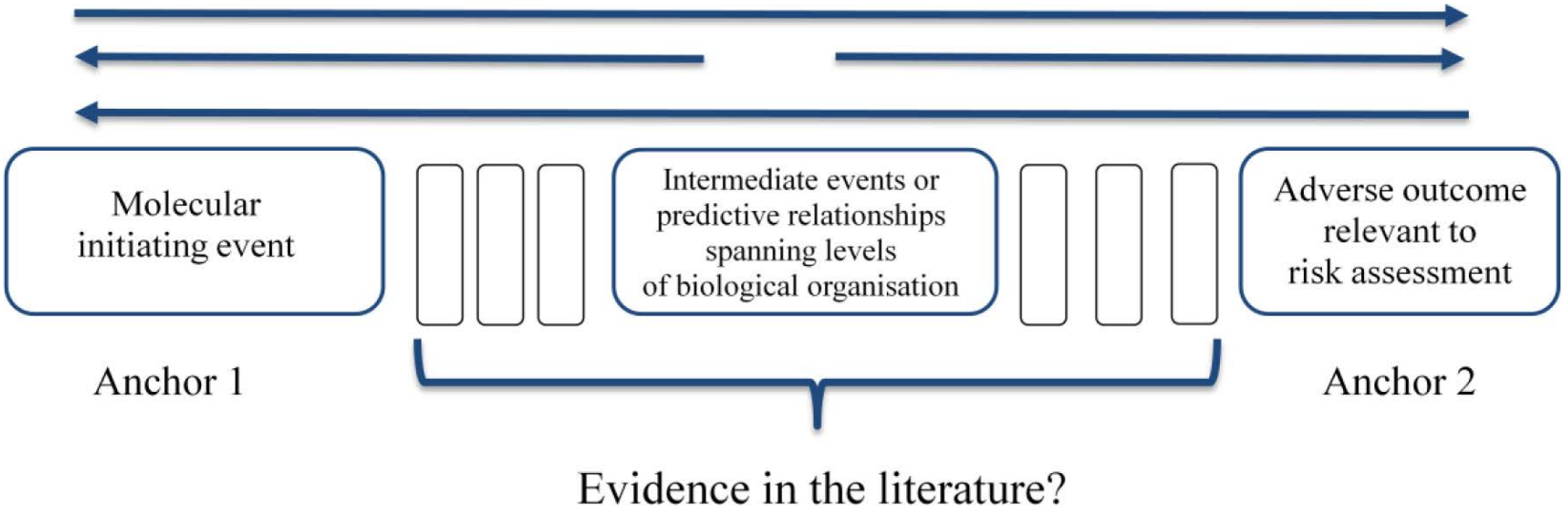
- **The liver is the most frequently targeted organ by cosmetics**
- **Cholestasis is a prominent form of liver toxicity induced by cosmetics**

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

3. AOP development

☑ Step 1: identification of AOP anchors

- **Adverse outcome (AO)**: change in morphology or physiology of an organism or system that results in impairment of the functional capacity or the capacity to compensate for stress
- **Molecular initiating event (MIE)**: initial point of chemical-biological interaction within the organism

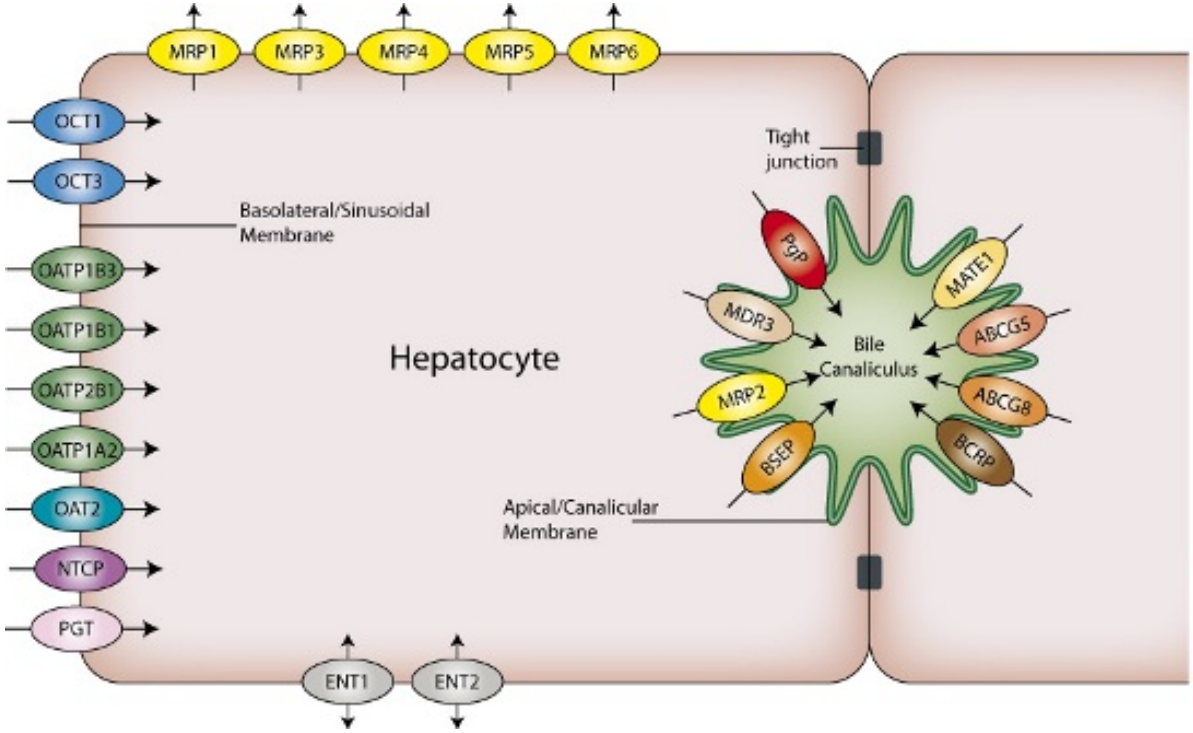


OECD (2012) Proposal for a template and guidance on developing and assessing the completeness of adverse outcome pathways.
Vinken (2013) Toxicology 312: 158-165.

- **AOP from bile salt export pump inhibition (MIE) to cholestasis (AO)**

Bile salt export pump (BSEP/ABCB11) inhibition

ATP-binding cassette transporter



Located at canalicular membrane surface of hepatocytes

Active transport of bile and drugs from hepatocytes to the bile duct

Cholestasis

Definition

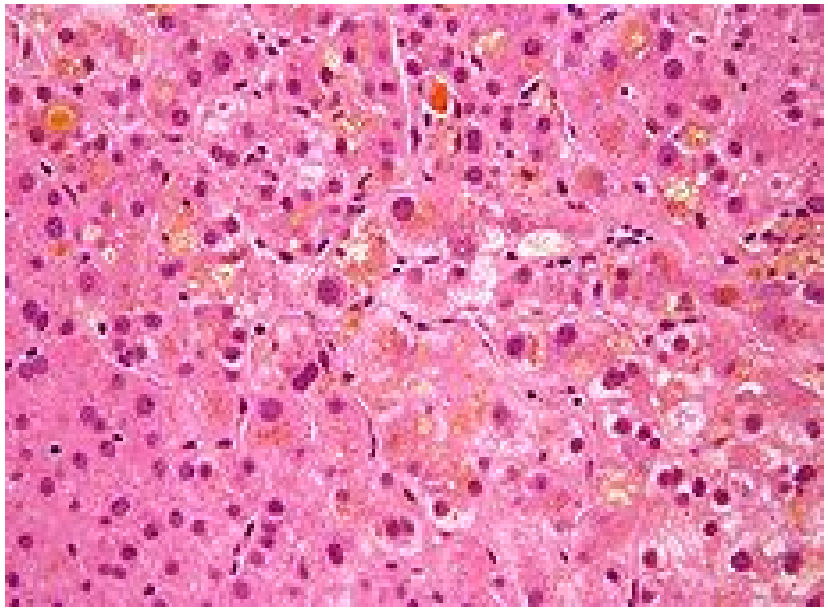
Impairment of bile flow from liver to duodenum

Accumulation of bile plugs in canalicular areas or bile ducts

Types

Intrahepatic: blockage inside the liver

Extrahepatic: blockage outside the liver



☑ Step 2: identification of intermediate steps and key events

- **Response matrix between the MIE and the AO**
- **Key events are intermediate steps that are**
  - **Toxicologically relevant**
  - **Experimentally measurable/quantifiable**
- **Located at different levels of biological organization**
- **Need for understanding normal physiological pathways**
- **Resources**
  - **Literature data**
  - ***In vivo* data**
  - ***In vitro* data**

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AOP from BSEP inhibition to cholestasis

Intermediate steps

Cellular level

Altered expression of drug metabolizing enzymes
Altered expression of drug transporter proteins
Mitochondrial disruption
Apoptotic cell death
Necrotic cell death

Organ level

Extracellular leakage from cytosolic enzymes
Pruritus
Bilirubinuria
Bilirubinemia
Jaundice

Key events (cellular level)

Bile acid accumulation

Inflammation

Oxidative stress

Activation of nuclear receptors

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

☑ Step 3: data linkage and representation

- **Linear graphical representation**

- **Linkage of events**

  - **Established mechanistic links (quantitative data)**
  - **Plausible links (limited data*)***
  - **Empirical links (quantitative exposure - response data)**
  - **Predictive model links (quantitative exposure - response data/chemical structure data)**
  - **Hypothetical links**

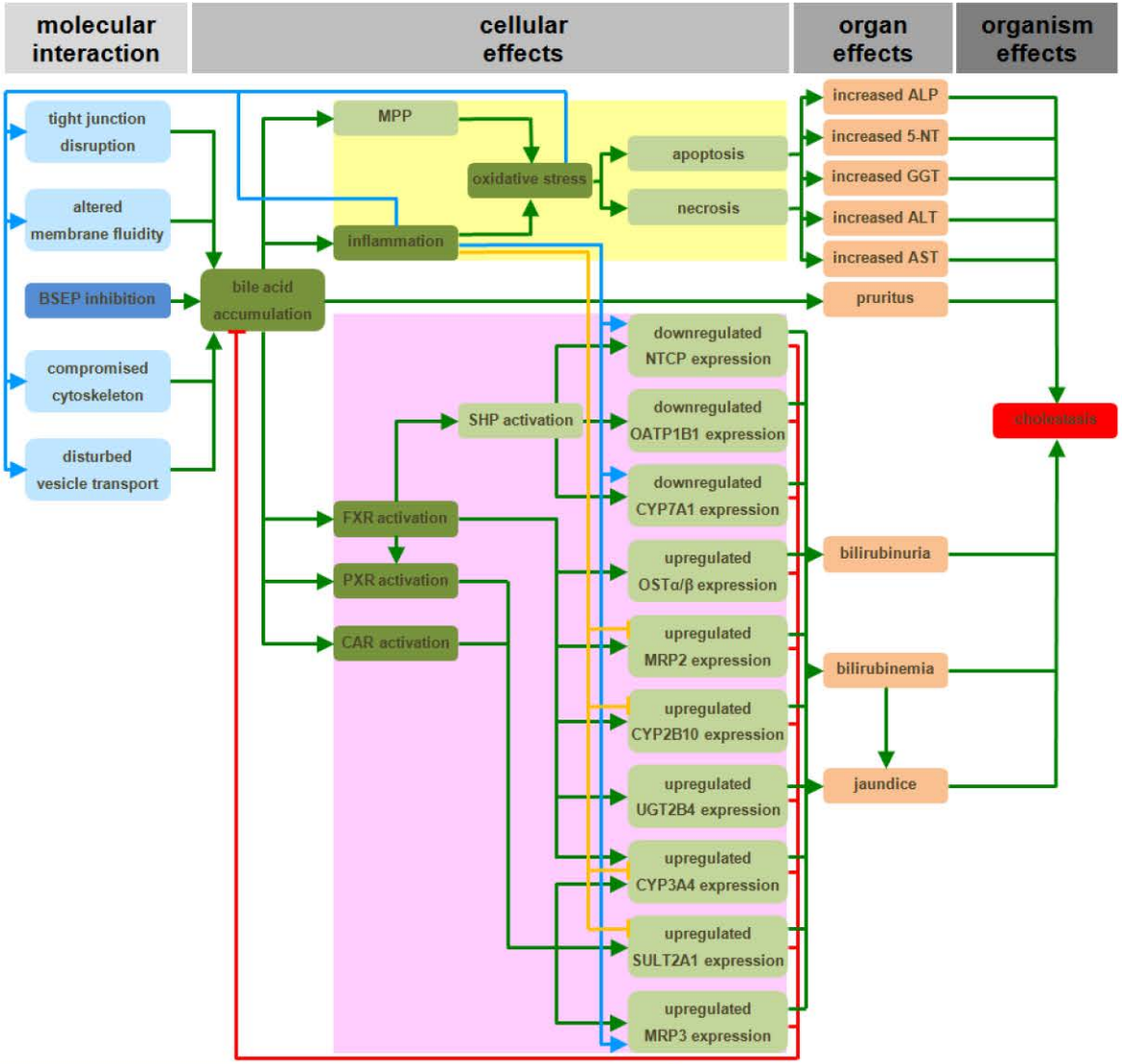
- **Subjective evaluation of the strength of linkage of the AOP components**

  - **(Very) strong**
  - **Moderate**
  - **Weak**

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

Vinken (2013) Toxicology 312: 158-165.

AOP from BSEP inhibition to cholestasis



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

4. AOP evaluation

☑ Weight of evidence assessment: Bradford-Hill criteria

- Concordance of dose-response relationships?
- Temporal concordance among the key events 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 key events 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 key events expected to be conserved across species?

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5. AOP project/SEURAT-1 case study

☑ Set - up

- **3 Liver-based *in vitro* models**

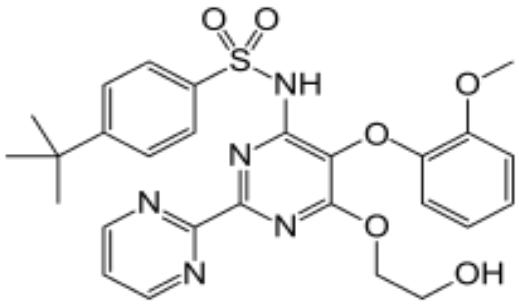
  Human primary hepatocytes

  Human hepatoma HepaRG cells

  Human skin-derived hepatic progenitor cells (hSKP-HPCs)

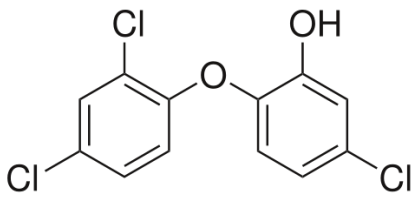
- **AOP verification using a compound with a clear-cut toxicological profile**

  Bosentan: drug for treatment of pulmonary hypertension



24 hours exposure to 3 concentrations

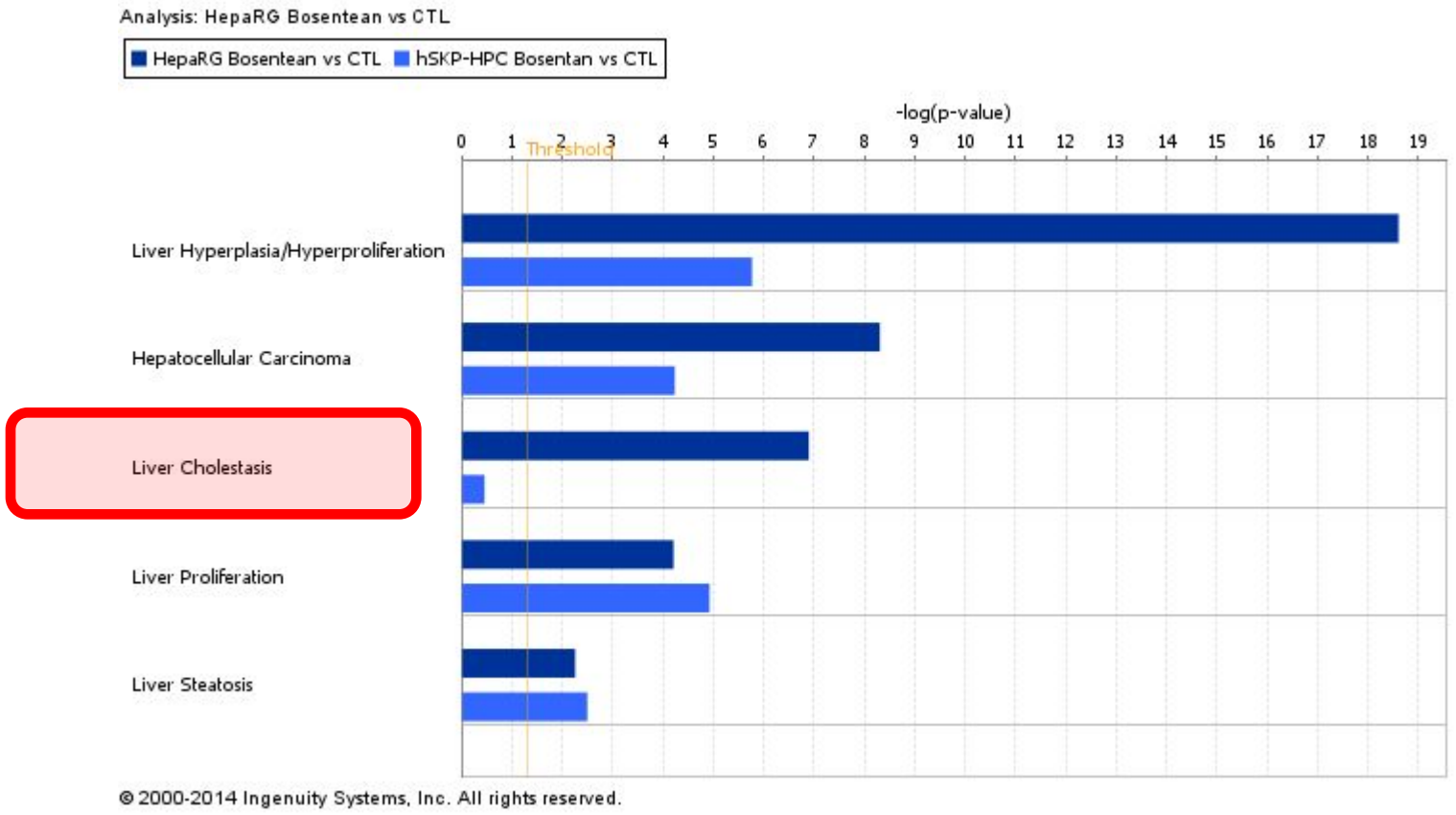
- **Detection of established biomarkers**
  - BSEP inhibition functionality assays
  - Reporter gene assay for nuclear receptor activation
  - Expression of key events: microarray analysis/qRT-PCR analysis
- **Characterization of new biomarkers**
  - Transcriptomics
  - Epigenomics
  - Metabonomics
  - Proteomics
- **AOP application using a compound with a poorly documented toxicological profile**
  - Triclosan: antimicrobial agent in consumer products



  - 24 hours exposure to 3 concentrations

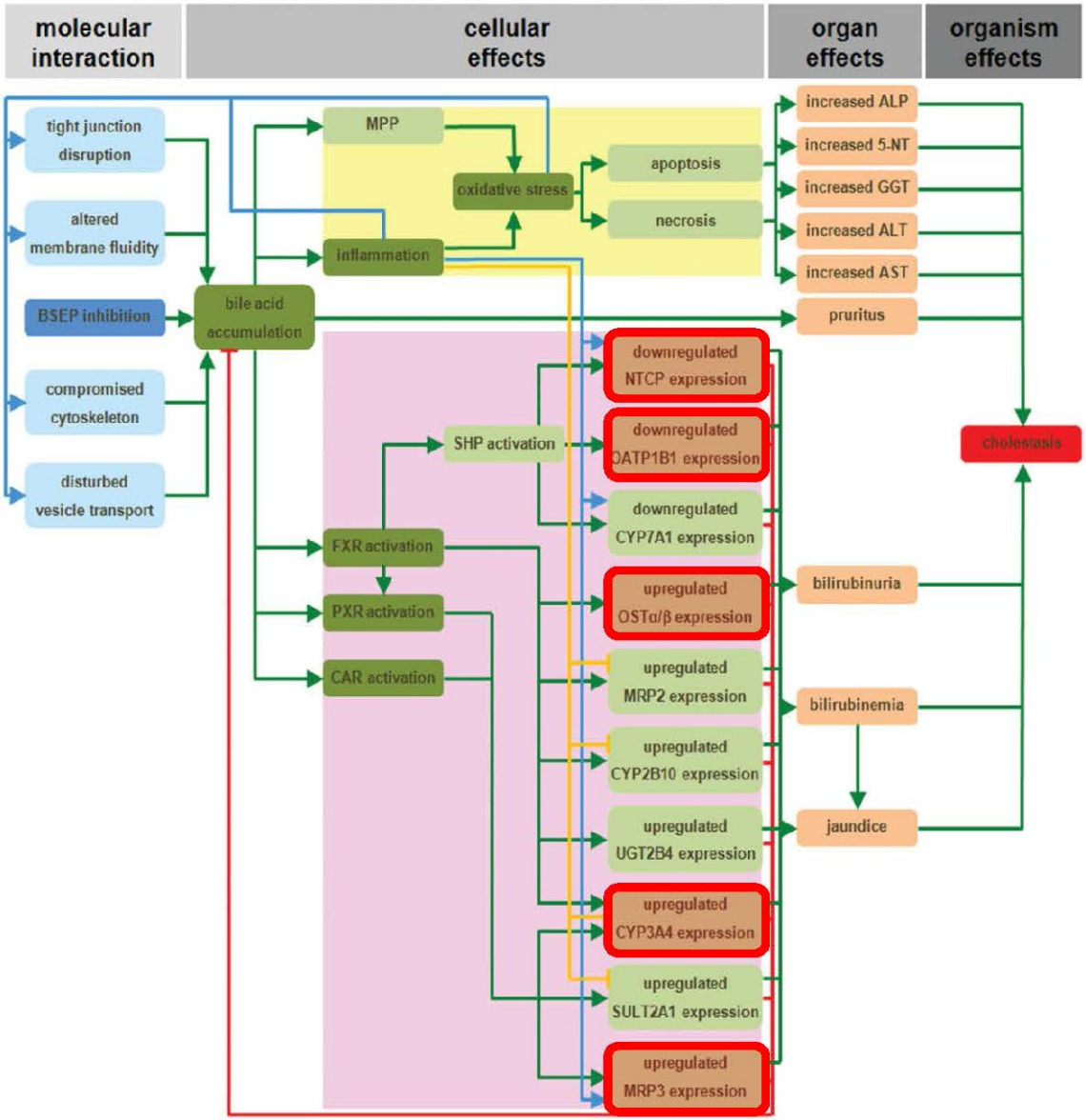
☑ Preliminary results

- **HepaRG cells: 135 µM bosentan**
- **Cholestasis is the third most significantly enriched toxicity function in HepaRG cells**



- **5 genes correctly modulated**

Gene	HepaRG vs. control	Prediction
SLCO1B1 (OATP1B1)	-2.59	Correct
OSTα	2.97	Correct
ABCC2 (MRP2)	1.27	Correct
UGT2B4	-5.15	Wrong
CYP3A4	5.05	Correct
ABCC3 (MRP3)	1.78	Correct


molecular interaction
cellular effects
organ effects
organism effects
tight junction disruption
altered membrane fluidity
BSEP inhibition
compromised cytoskeleton
disturbed vesicle transport
bile acid accumulation
MPP
oxidative stress
inflammation
apoptosis
necrosis
SHP activation
FXR activation
PXR activation
CAR activation
downregulated NTCP expression
downregulated OATP1B1 expression
downregulated CYP7A1 expression
upregulated OSTα/β expression
upregulated MRP2 expression
upregulated CYP2B10 expression
upregulated UGT2B4 expression
upregulated CYP3A4 expression
upregulated SULT2A1 expression
upregulated MRP3 expression
increased ALP
increased 5-NT
increased GGT
increased ALT
increased AST
pruritus
bilirubinuria
bilirubinemia
jaundice
cholestasis

- **Identification of new (transcriptomics) biomarkers of cholestasis**

  ABCB4: multidrug resistance protein 3

  ADH1C: alcohol dehydrogenase 1C

  AKR1D1: aldo-keto reductase 1D1

  GPX2: glutathione peroxidase 2

  LBP: lipopolysaccharide-binding protein

  SLC22A1: organic cation transporter 1

6. AOP use

☑ Chemical categorization/grouping

- Focus on MIE
- Establishment of (quantitative) structure - activity relationships
- Basis for read - across approaches and *in silico* modeling
- Example: BSEP inhibition

  Contributing structural determinants: esters bound to a carbon atom of heterocyclic groups

  Counteracting structural determinants: hydroxyl groups bound to aliphatic carbon atoms

☑ Identification of biomarkers and test development

- **Focus on MIE and key events**
- **Establishment of mechanistic *in vitro* methods**
- **Characterization of *in vivo* - relevant biomarkers**
- **Examples**

  BSEP inhibition

  Cholyl - lysyl - fluorescein assay

  Vesicular transport assay

  BSEP ATPase assay

  Nuclear receptor activation: gene reporter constructs

Vinken (2013) Toxicology 312: 158-165.

7. Take-home message

- ☑ **Generation of hepatic AOPs in SEURAT-1**
  - Cholestasis
  - Fibrosis
  - Steatosis
- ☑ **Fine-tuning of hepatic AOPs**
  - More time points (reversibility of effects)
  - More chemicals (structure-activity effects)
  - More analyses (functional and -omics read-outs)
- ☑ **Follow-up**
  - Testing in SEURAT- 1 case study program
  - Testing in OECD AOP program
  - Included in AOP wiki

8. Acknowledgements

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