

# 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***

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Vrije Universiteit Brussel



# 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**

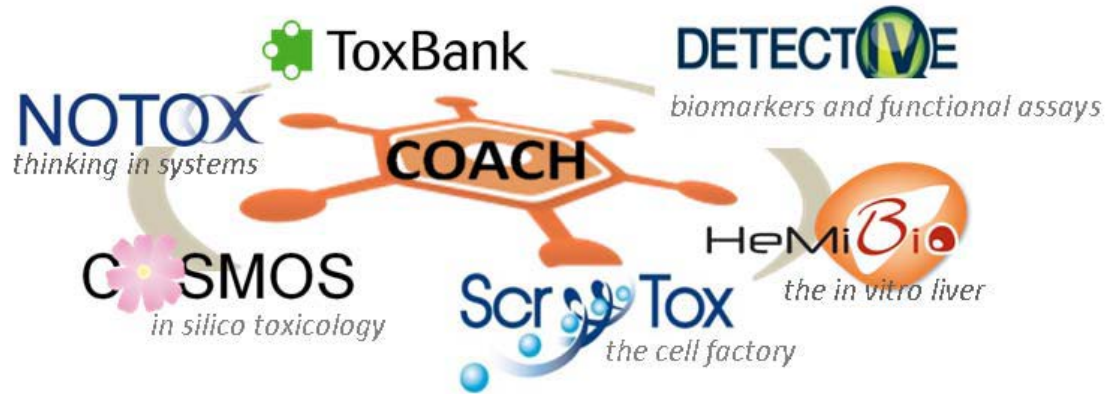
1 January 2011 - 31 December 2015

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**

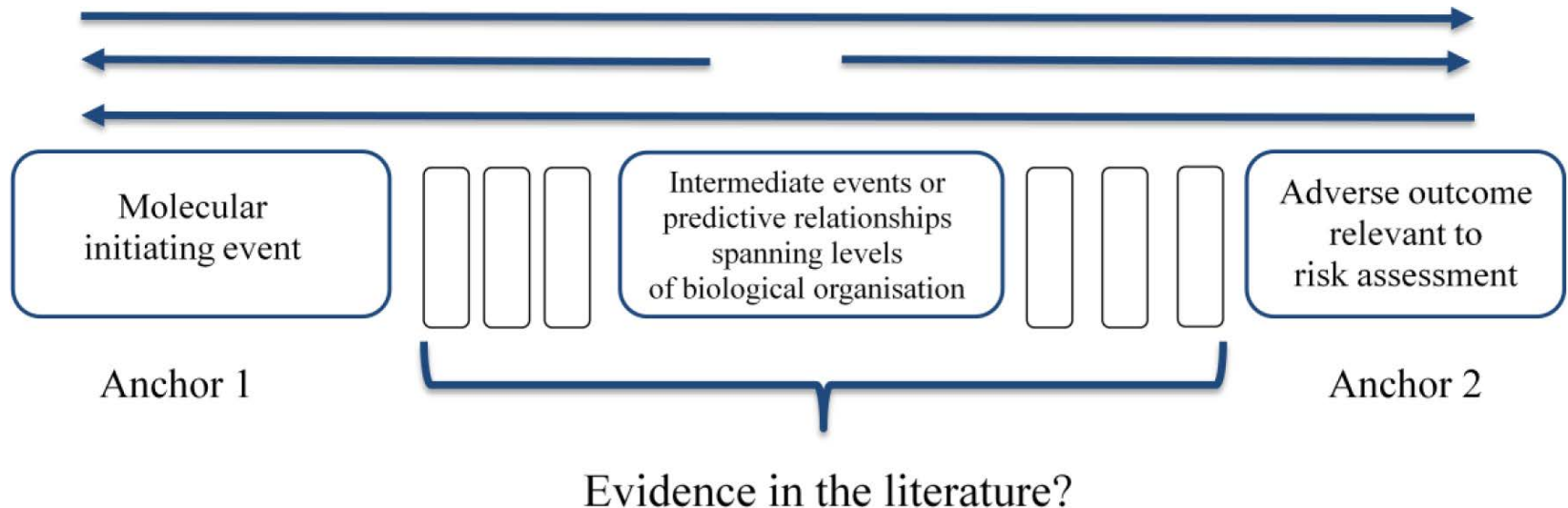
● **The liver is the most frequently targeted organ by cosmetics**

● **Cholestasis is a prominent form of liver toxicity induced by cosmetics**

# 3. AOP development

## ☑ Step 1: identification of AOP anchors

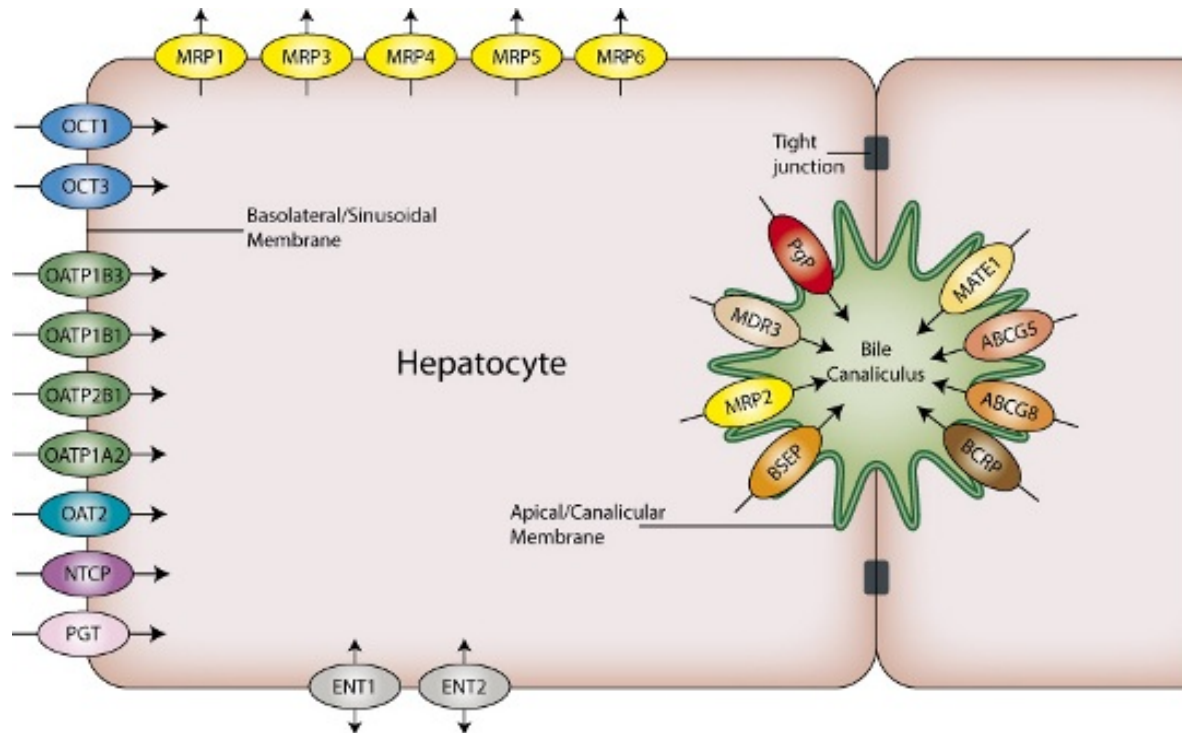
- Adverse outcome (AO): change in morphology or physiology of an organism or system that results in impairment of the functional capacity or the capacity to compensate for stress
- Molecular initiating event (MIE): initial point of chemical-biological interaction within the organism



● **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

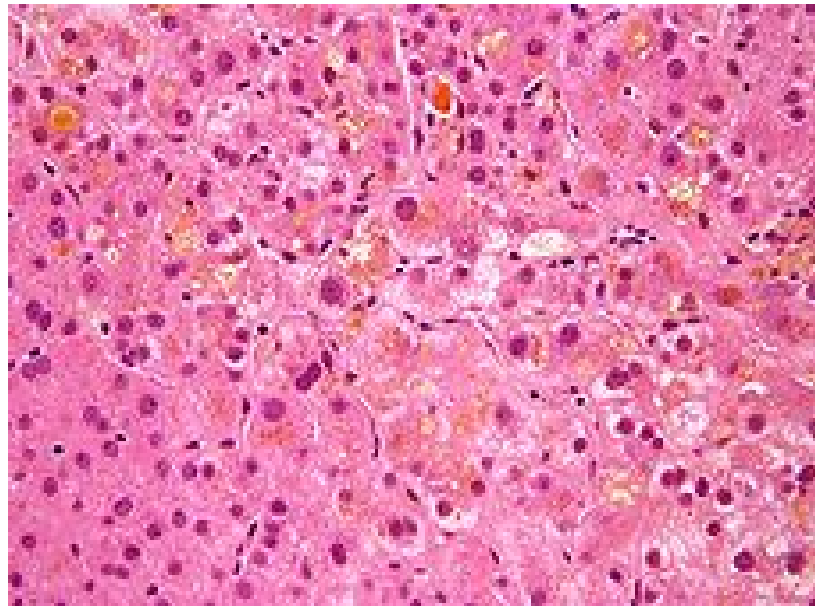
Impairment of bile flow from liver to duodenum

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**



## ● 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**

## ☑ **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

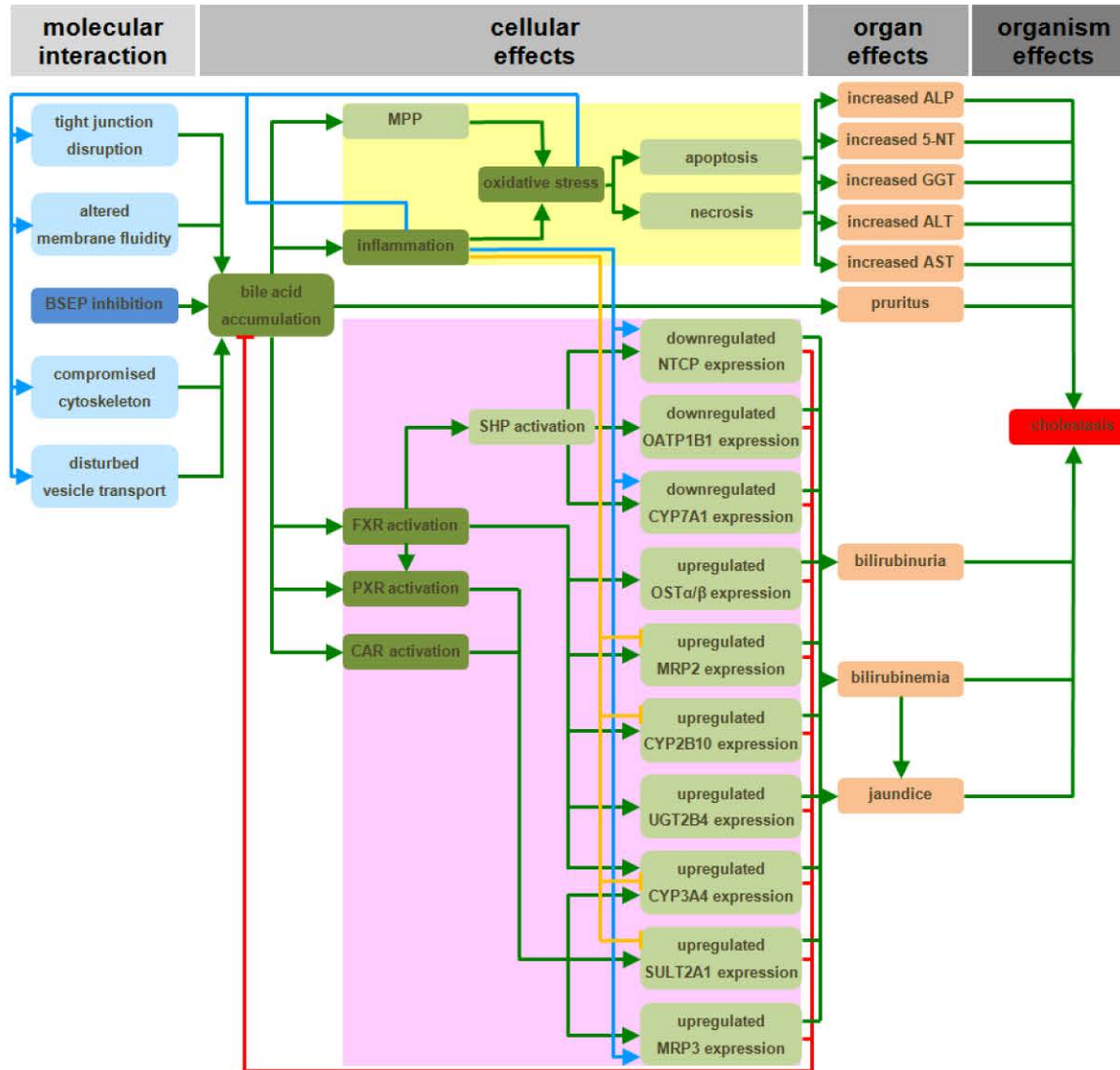
- **Subjective evaluation of the strength of linkage of the AOP components**

  - (Very) strong

  - Moderate

  - Weak

## AOP from BSEP inhibition to cholestasis



## 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?

## 5. AOP project/SEURAT-1 case study

### ☑ Set - up

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

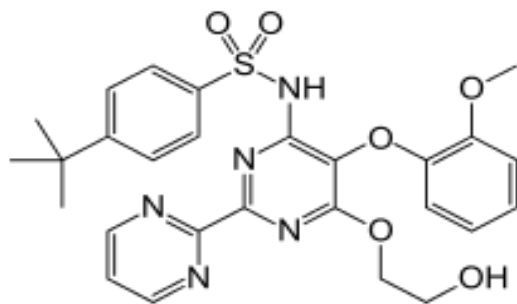
Human primary hepatocytes

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

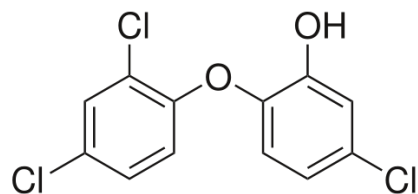
  - Epigenomics

  - Metabonomics

  - Proteomics

- **AOP application using a compound with a poorly documented toxicological profile**

  - Triclosan: antimicrobial agent in consumer products





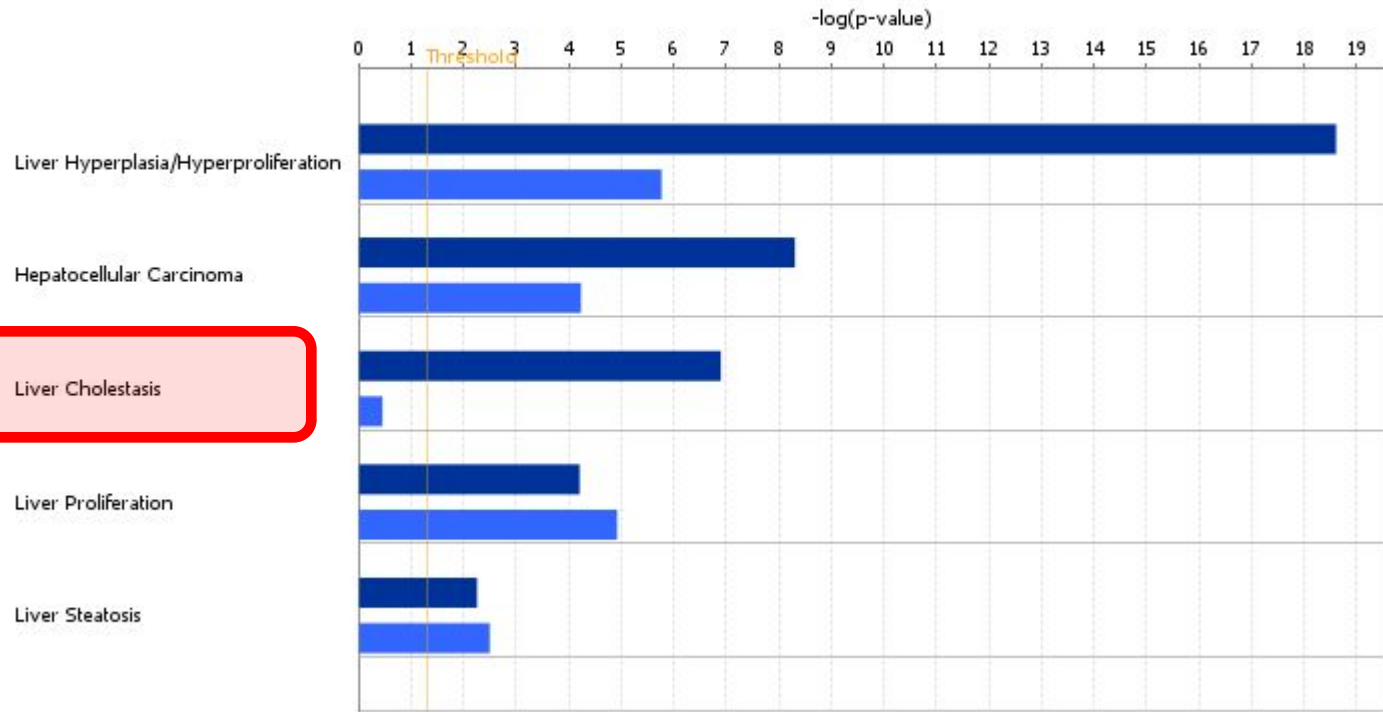
  - 24 hours exposure to 3 concentrations

## ✓ Preliminary results

- HepaRG cells: 135  $\mu$ M bosentan
- Cholestasis is the third most significantly enriched toxicity function in HepaRG cells

Analysis: HepaRG Bosentan vs CTL

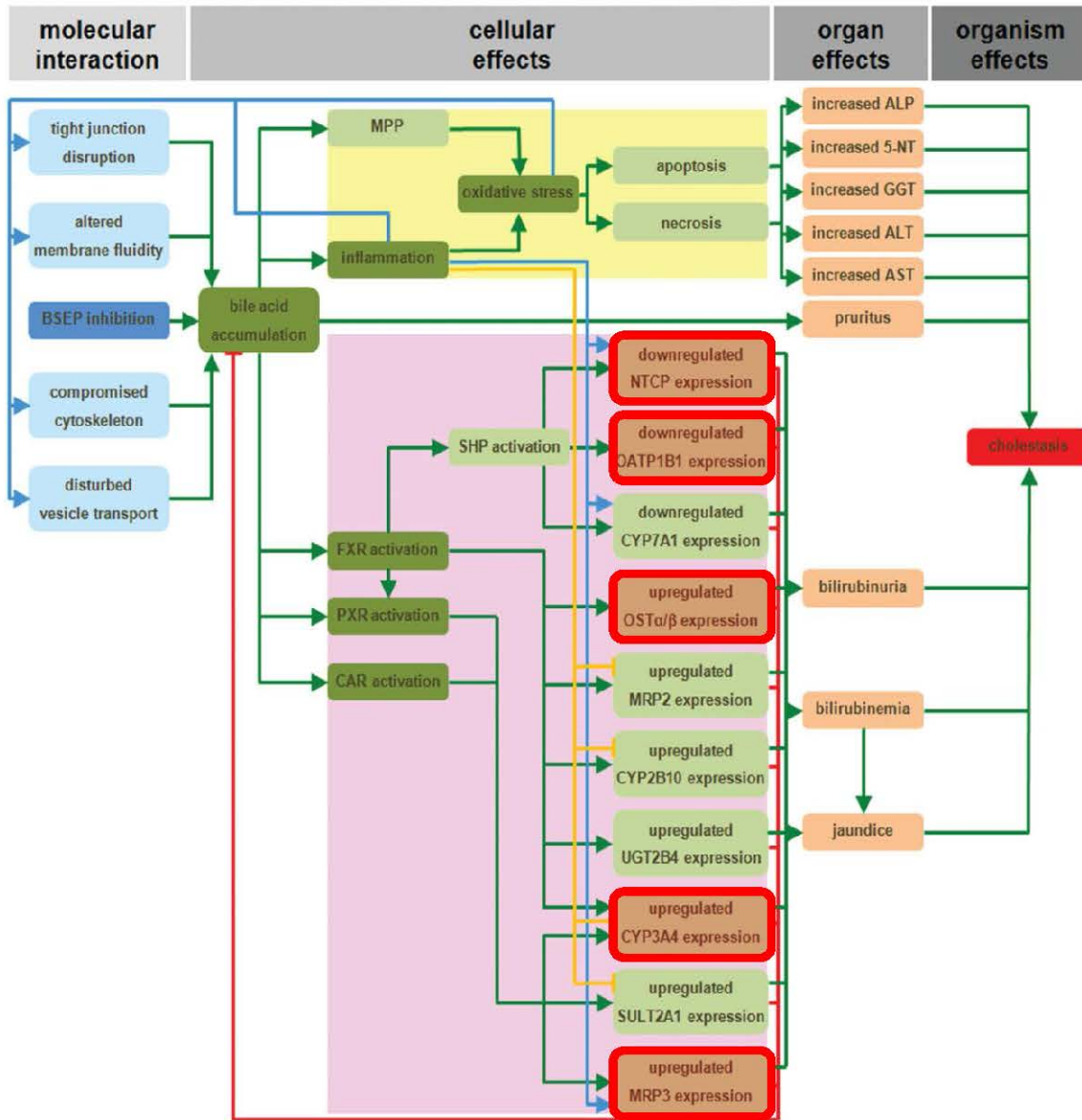
■ HepaRG Bosentan vs CTL ■ hSKP-HPC Bosentan vs CTL



- 5 genes correctly modulated

Gene	HepaRG vs. control	Prediction
<i>SLCO1B1 (OATP1B1)</i>	-2.59	Correct
<i>OST<math>\alpha</math></i>	2.97	Correct
<i>ABCC2 (MRP2)</i>	1.27	Correct
<i>UGT2B4</i>	-5.15	Wrong
<i>CYP3A4</i>	5.05	Correct
<i>ABCC3 (MRP3)</i>	1.78	Correct





- **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

## 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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