

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**

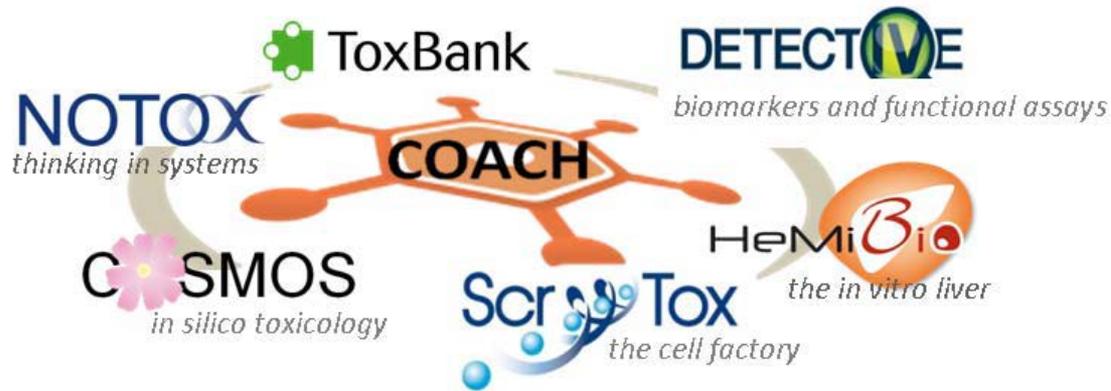
1 January 2011 - 31 December 2015

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**

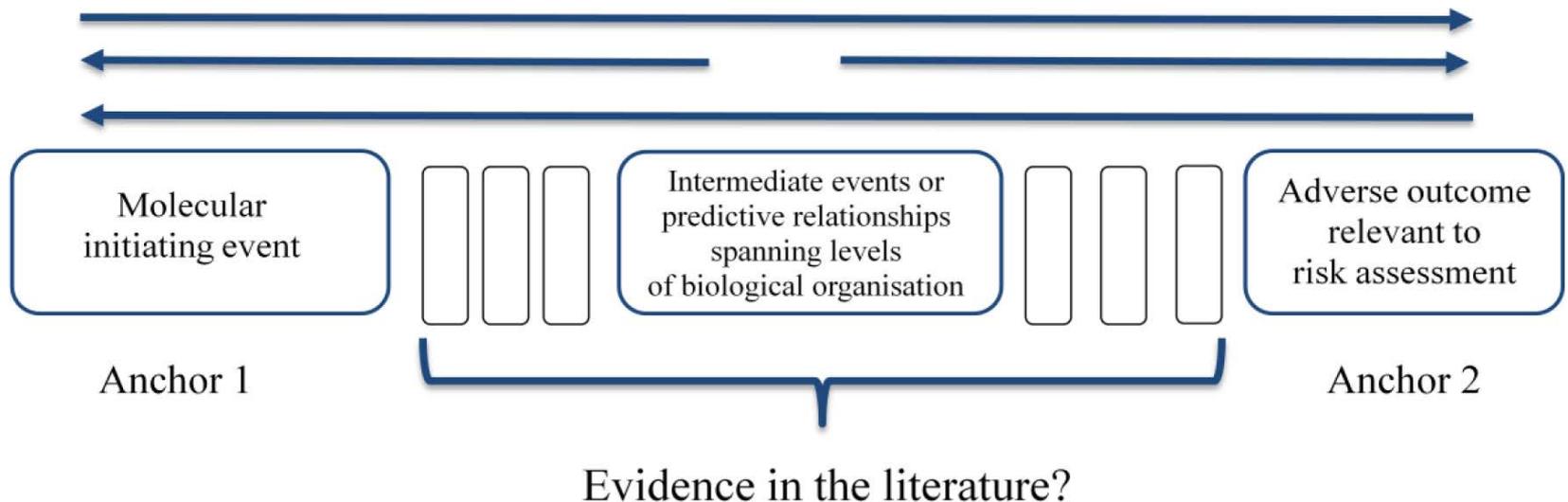
● **The liver is the most frequently targeted organ by cosmetics**

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

3. AOP development

☑ Step 1: identification of AOP anchors

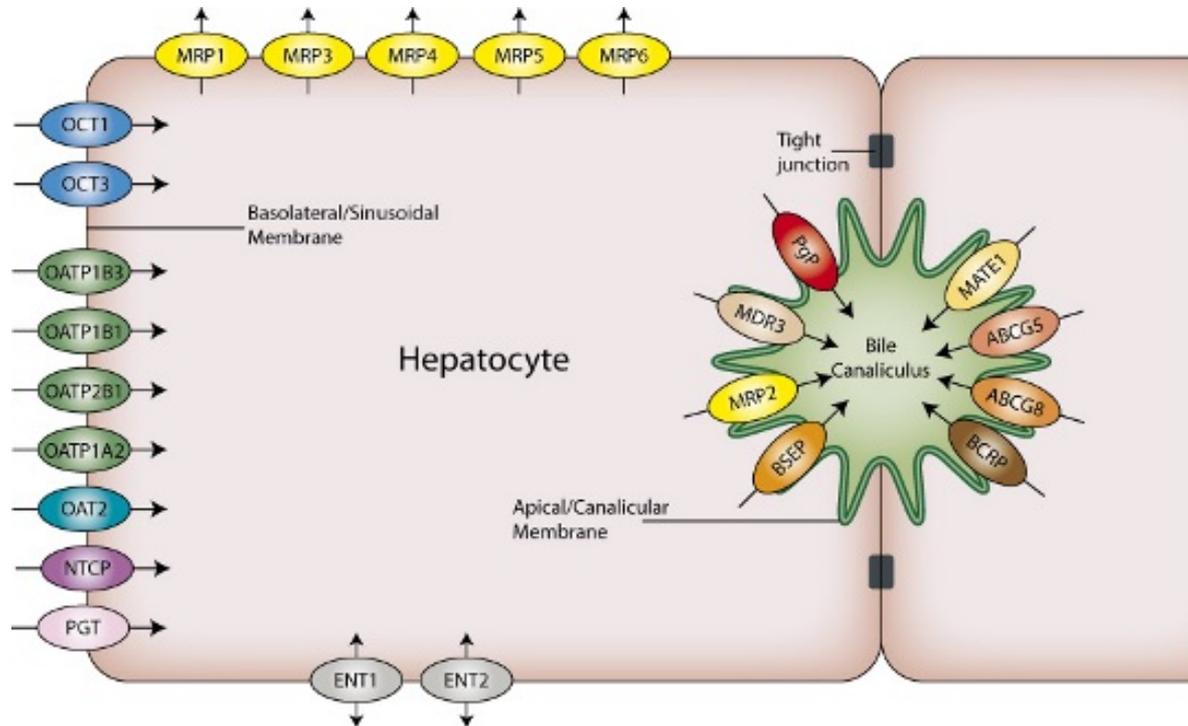
- Adverse outcome (AO): change in morphology or physiology of an organism or system that results in impairment of the functional capacity or the capacity to compensate for stress
- Molecular initiating event (MIE): initial point of chemical-biological interaction within the organism



● **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

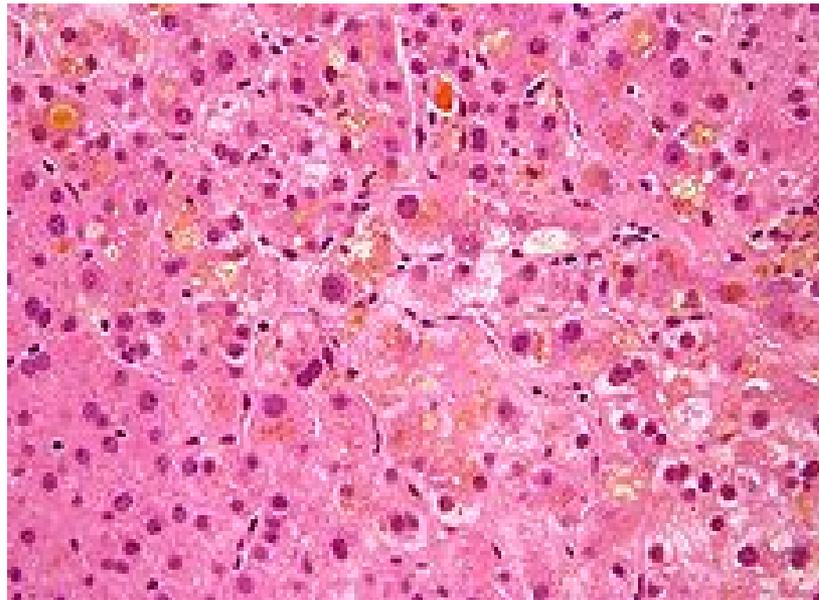
Impairment of bile flow from liver to duodenum

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

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

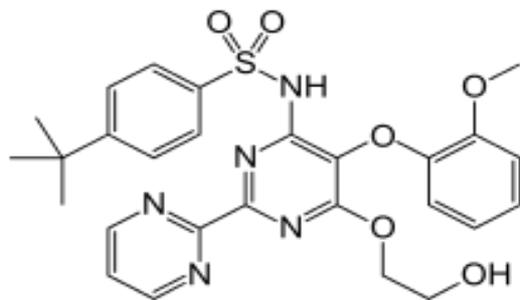
Human primary hepatocytes

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

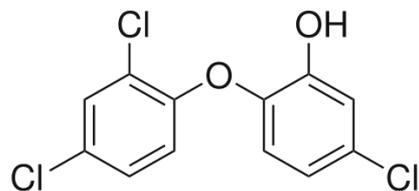
 - Epigenomics

 - Metabonomics

 - Proteomics

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

 - Triclosan: antimicrobial agent in consumer products



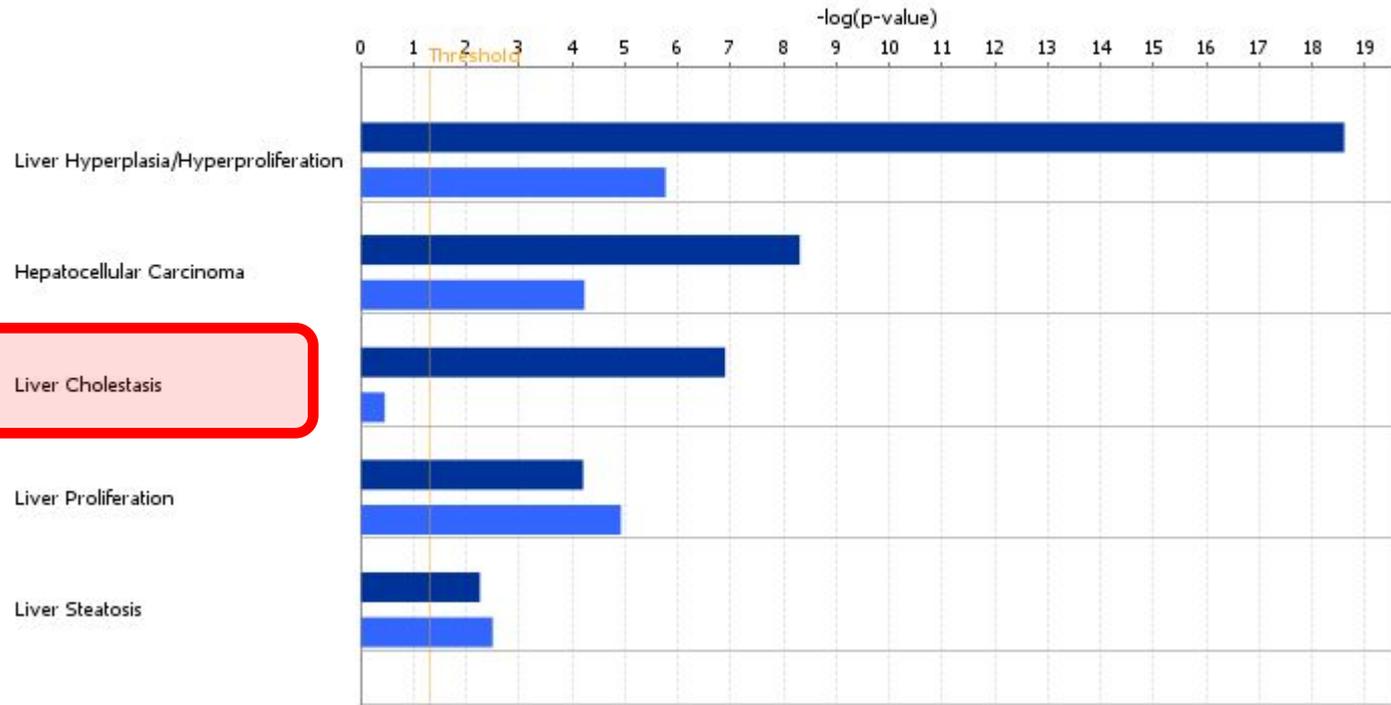
 - 24 hours exposure to 3 concentrations

✓ Preliminary results

- HepaRG cells: 135 μ 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>OSTα</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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