Development and use of hepatic AOPs in the SEURAT project cluster

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1. Context of hepatic 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 €)

European Commission

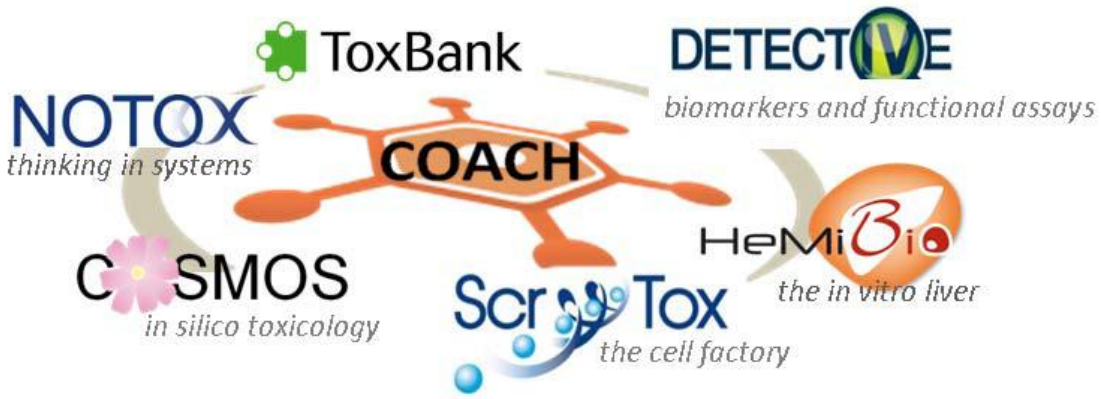
SEVENTH FRAMEWORK PROGRAMME

Cosmetics Europe
the personal care association

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

SEURAT-1

☑ SEURAT 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. Selection of hepatic AOPs

☑ 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

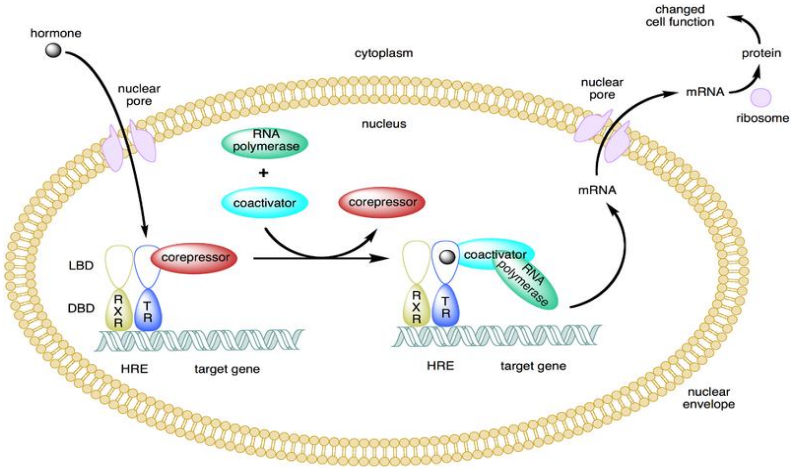
- **The liver is the most frequently targeted organ by cosmetics**
- **Steatosis and cholestasis are prominent forms of liver toxicity induced by cosmetics**
- **Fibrosis identified as additional form of liver toxicity induced by cosmetics**

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

3. Development of hepatic AOPs

☑ Step 1: identification of the MIE and AO

- AOP from liver X receptor activation (MIE) to liver steatosis (AO)
  - ► Liver X receptor (LXR) activation
    - ■ **Type II nuclear receptor**



- ■ **Natural ligands: oxysterols and glucose**
- ■ **Synthetic ligands: T0901317 and GW3965**
- ■ **Target genes**
  - ♦ Carbohydrate response element binding protein (ChREBP)
  - ♦ Sterol response element binding protein 1c (SREBP-1c)
  - ♦ Fatty acid synthase (FAS)
  - ♦ Stearoyl coenzyme A desaturase 1 (SCD1)
  - ♦ Fatty acid uptake transporter (FAT/CD36)

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

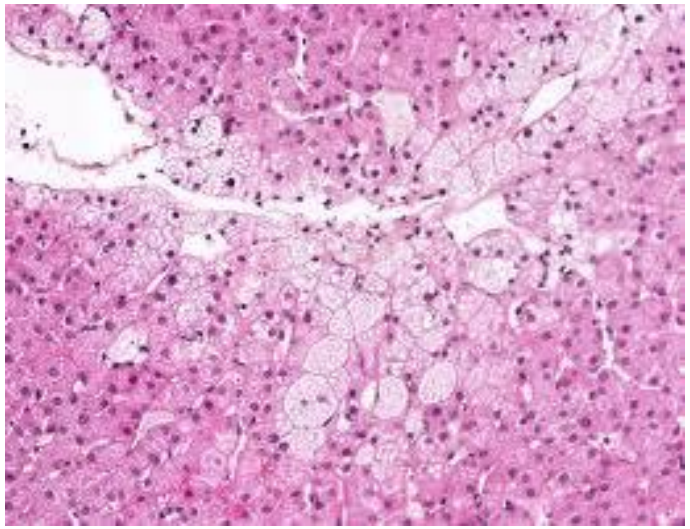
► Liver steatosis

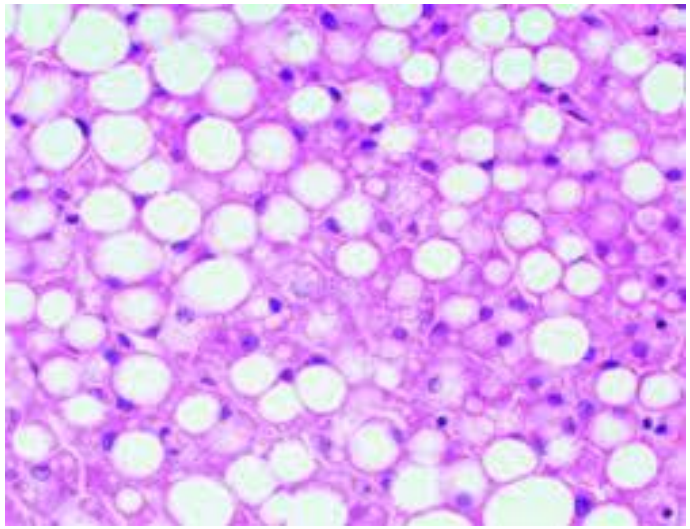
■ Definition

- ♦ Fatty change/fatty degeneration/adipose degeneration/fatty liver
- ♦ Abnormal retention of lipids (triglycerides) in hepatocytes
- ♦ Increase of liver weight by 5-10%

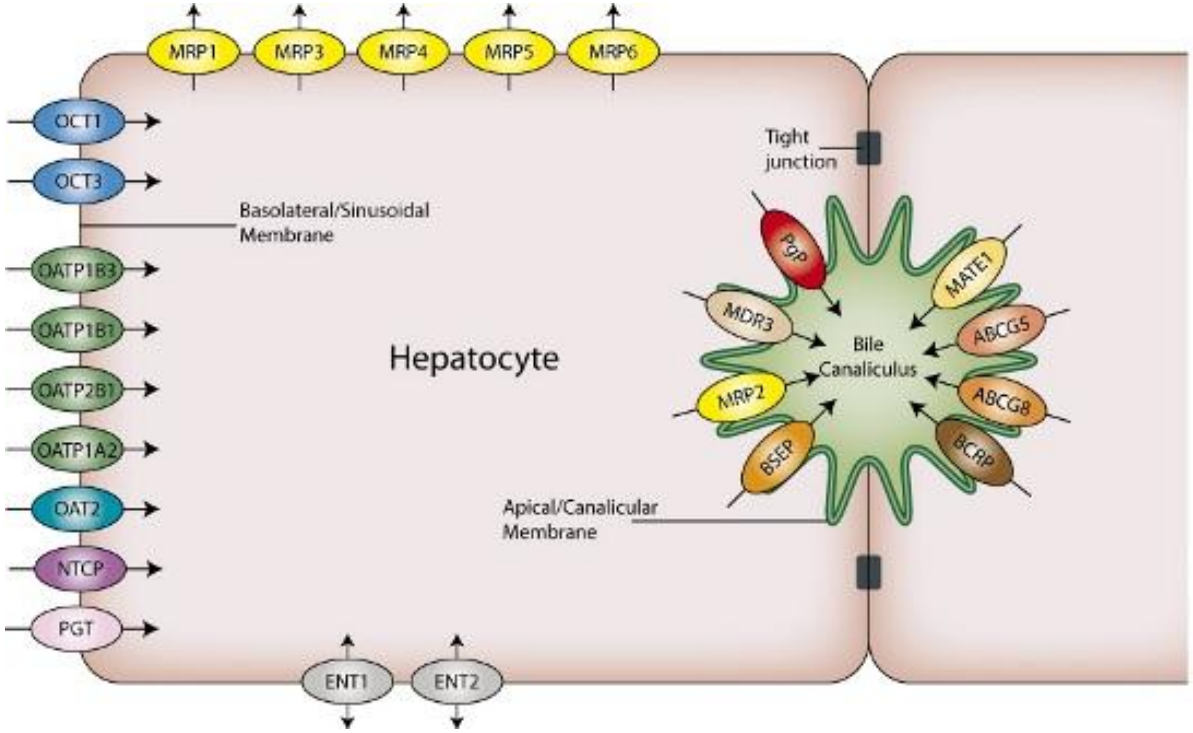
■ Types

- ♦ Microvesicular: small lipid droplets/no displacement of nucleus
- ♦ Macrovesicular: large lipid droplets/displacement of nucleus





- **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**

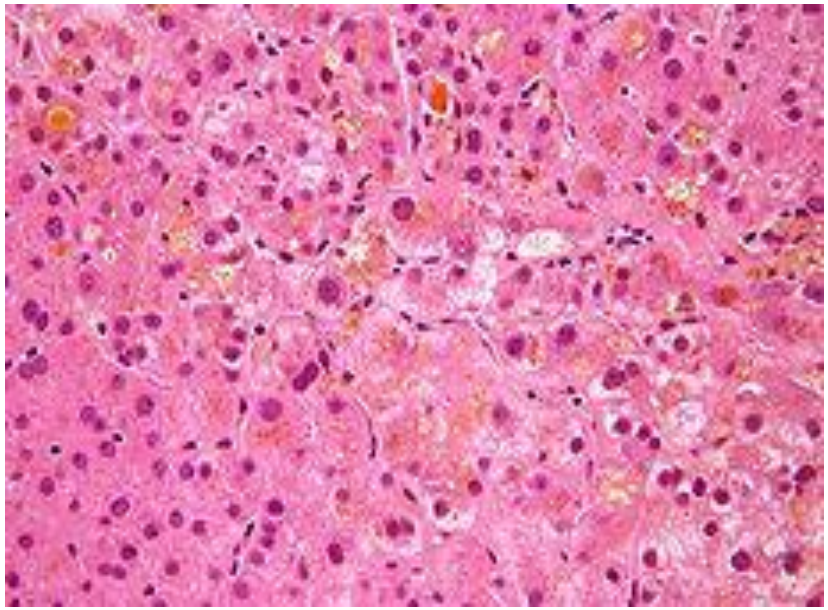
► Cholestasis

■ Definition

- ♦ Impairment of bile flow from liver to duodenum
- ♦ Accumulation of bile plugs in canalicular areas or bile ducts

■ Types

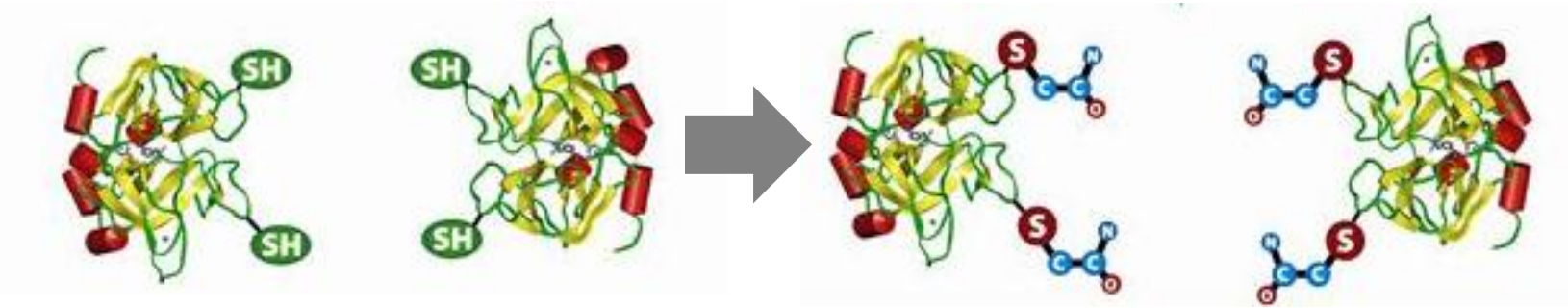
- ♦ Intrahepatic: blockage inside the liver
- ♦ Extrahepatic: blockage outside the liver



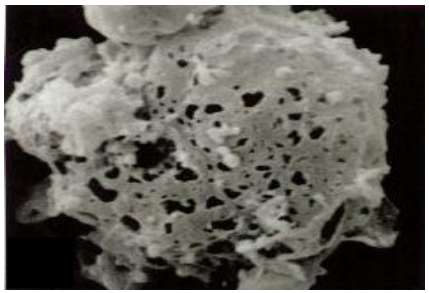
AOP from protein alkylation (MIE) to liver fibrosis (AO)

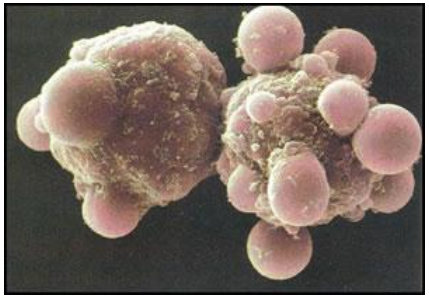
► Protein alkylation

- **Addition of alkyl groups**



- **Consequences**
  - ♦ Structural and functional cell injury
  - ♦ Imbalanced reduction-oxidation reactions
- **Outcome**
  - ♦ Necrotic cell death
  - ♦ Apoptotic cell death



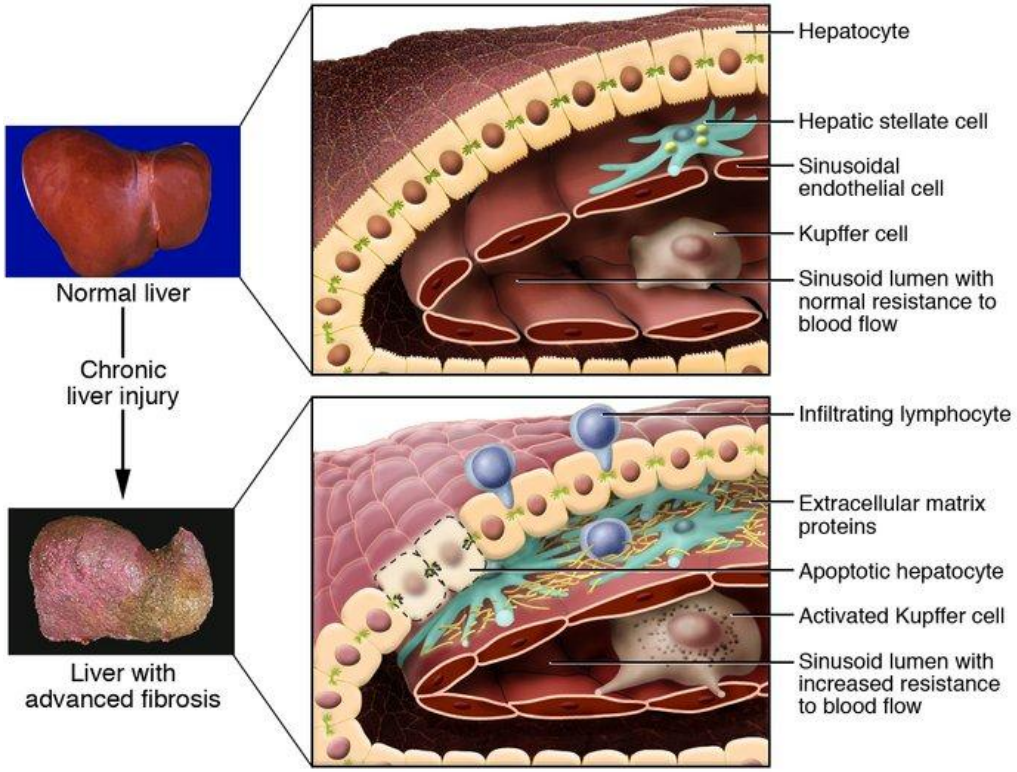


► Liver fibrosis

■ Definition

- ♦ Wound healing response to a variety of chronic injuries
- ♦ Scar formation/deposition of extracellular matrix components
- ♦ Reversible process

■ Consequence: structural and functional deterioration



☑ Step 2: identification of the intermediate steps and key events

- **AOP from LXR activation to liver steatosis**
  - ► **Intermediate steps**
    - ■ **Molecular level**
      - ♦ Activation of ChREBP expression
      - ♦ Activation of SREBP-1c expression
      - ♦ Activation of FAS expression
      - ♦ Activation of SCD1 expression
      - ♦ Activation of CD36 expression
      - ♦ *De novo* fatty acid synthesis
      - ♦ Increased fatty acid influx from peripheral tissues
    - ■ **Organelle level**
      - ♦ Cytoplasm displacement
      - ♦ Nucleus distortion
      - ♦ Mitochondrial disruption
    - ■ **Cellular level: fatty liver cells**
  - ► **Key event (molecular level): triglyceride accumulation**

Landesmann *et al.* (2012) JRC scientific and policy report.
Vinken (2013) Toxicology 312: 158-165.

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.

AOP from protein alkylation to liver fibrosis

► Intermediate steps/key events at the cellular level

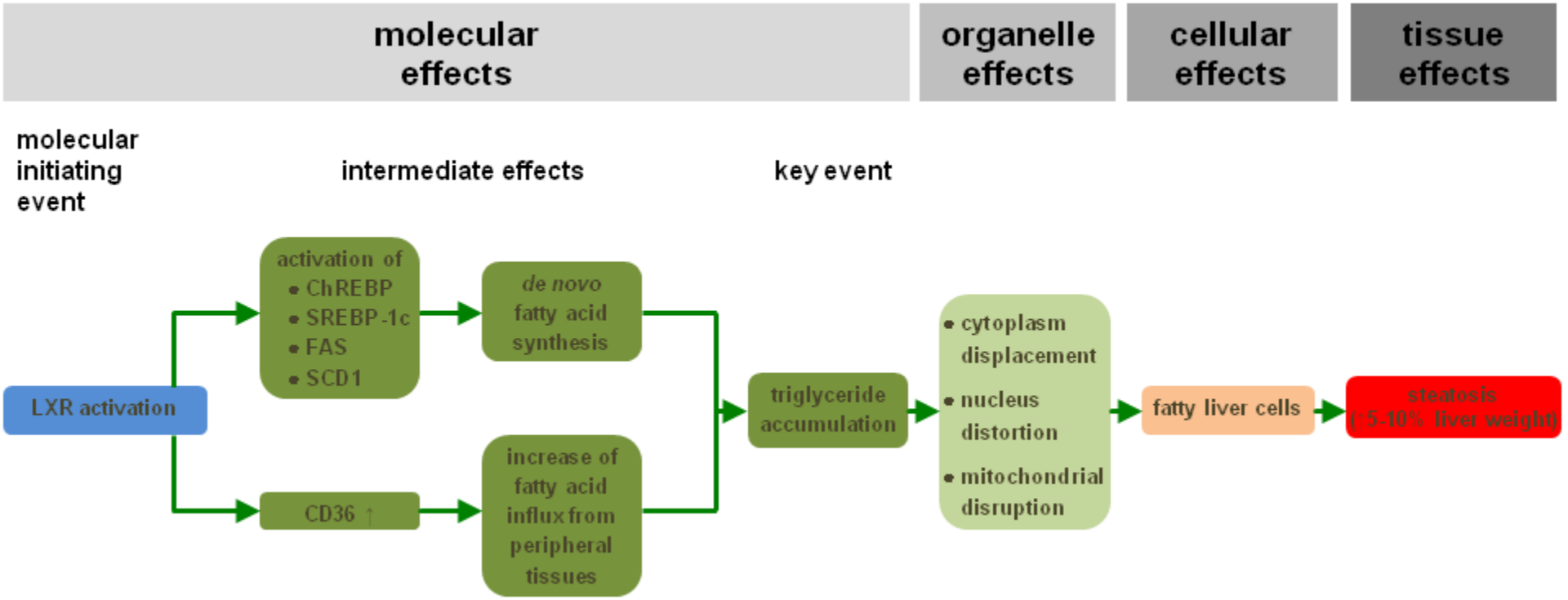
- **Hepatocyte injury/cell death**
- **Activation of Kupffer cells**
- **Inflammation**
- **Oxidative stress**
- **Activation of transforming growth factor beta 1 (TGF-β1)**
- **Activation of stellate cells**

► Intermediate steps/key events at the tissue level

- **Collagen accumulation**
- **Changes in extracellular matrix composition**

☑ Step 3: data linkage and representation

- AOP from LXR activation to liver steatosis



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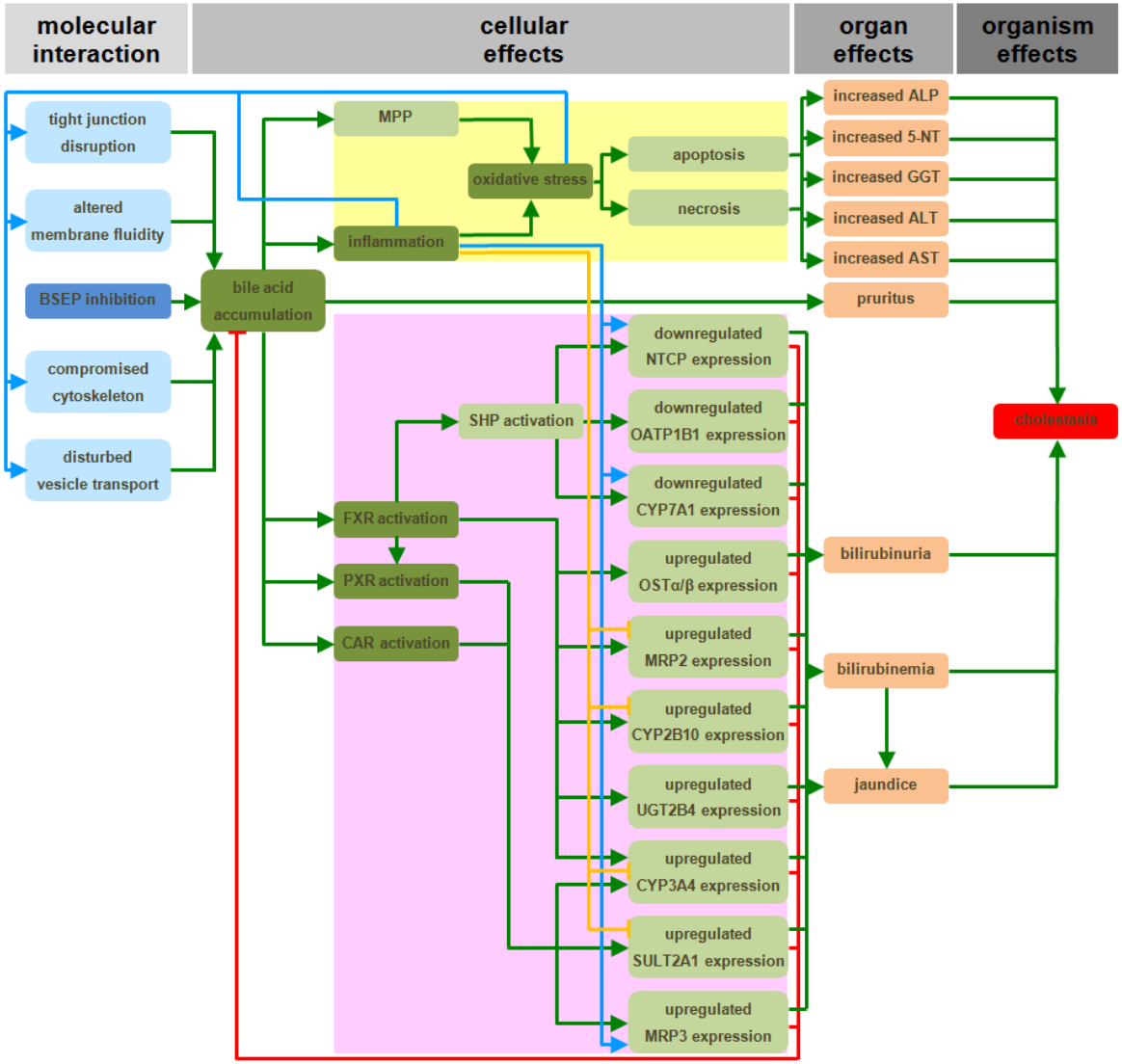
AOP from BSEP inhibition to cholestasis



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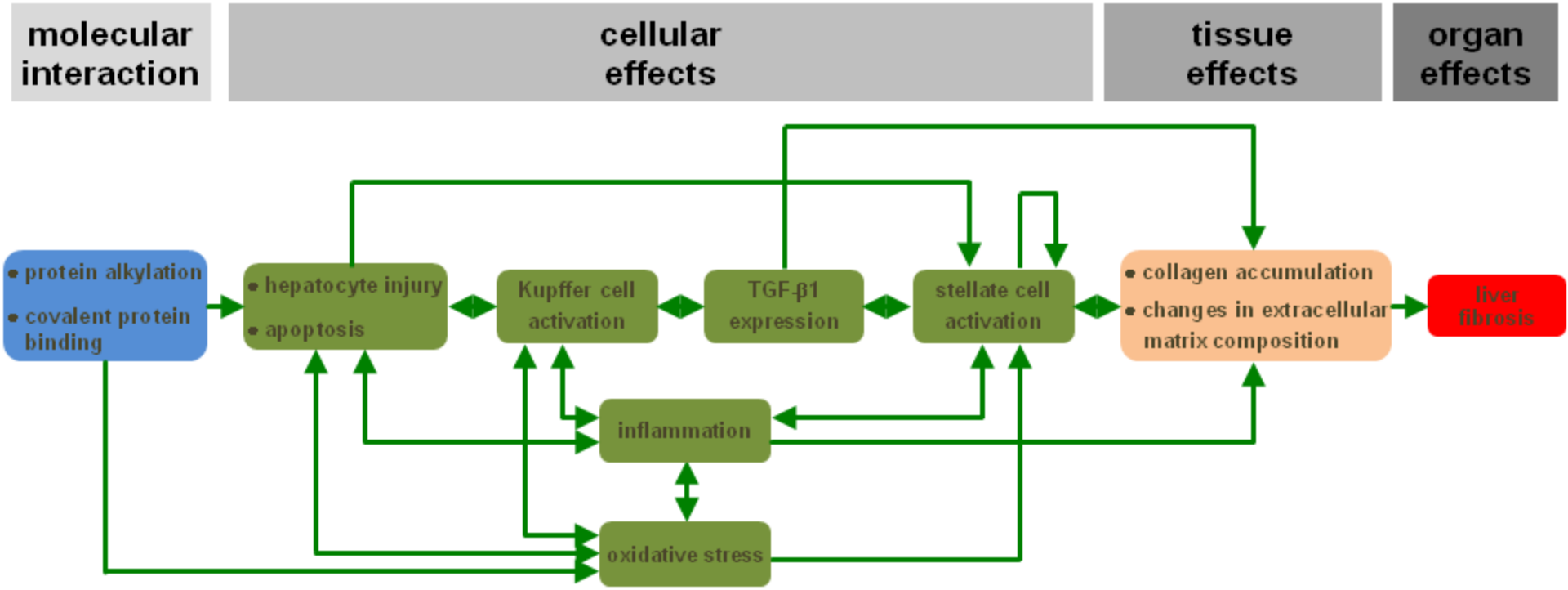
AOP from protein alkylation to liver fibrosis



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Vinken (2013) Toxicology 312: 158-165.

4. Evaluation of hepatic AOPs

☑ 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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Vinken (2013) Toxicology 312: 158-165.

5. Use of hepatic AOPs

☑ Chemical categorization/grouping

- Focus on MIEs
- Establishment of (quantitative) structure-activity relationships
- Basis for read-across approaches and *in silico* modeling
- Examples

  ► Drug-induced liver steatosis

  - LXR activation
  - Contributing structural determinants: phenyl rings, chloro groups and methyl moieties

  ► Drug-induced cholestasis

  - BSEP inhibition
  - Contributing structural determinants: esters bound to a carbon atom of heterocyclic groups
  - Counteracting structural determinants: hydroxyl groups bound to aliphatic carbon atoms

Vinken (2013) Toxicology 312: 158-165.

☑ Identification of biomarkers and test development

- **Focus on MIEs and key events**
- **Establishment of mechanistic *in vitro* methods**
- **Characterization of *in vivo*-relevant biomarkers**
- **Examples**
  - ► **Drug-induced liver steatosis**
    - ■ **LXR activation: gene reporter constructs**
    - ■ **Activation of CD36 expression: enzyme-linked immunosorbent assay**
  - ► **Drug-induced cholestasis**
    - ■ **BSEP inhibition**
      - ♦ Cholyl-lysyl-fluorescein assay
      - ♦ Vesicular transport assay
      - ♦ BSEP ATPase assay
    - ■ **Nuclear receptor activation: gene reporter constructs**

Vinken (2013) Toxicology 312: 158-165.

6. Follow-up and perspectives

☑ Fine-tuning and optimization of hepatic AOPs

- Using chemicals with clear-cut (drugs) and promiscuous (cosmetics) toxicity profiles
- Testing of robustness/flexibility of MIEs and key events
- Identification of novel AOP components
- Quantification of AOPs

☑ Utilization of hepatic AOPs

- Biomarker identification
- *In vitro* test development
- Other uses/purposes?

☑ Integration and continuation of hepatic AOP research

- Participation in the development of the AOP wiki
- Opportunities for AOP research in European H2020 program (2014-2020)

7. Acknowledgements

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