Mathieu VINKEN, Ph.D., E.R.T.

Department of Toxicology
Vrije Universiteit Brussel
Brussels-Belgium
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 €)

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

www.seurat-1.eu
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

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.
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.
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

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

Landesmann et al. (2012) JRC scientific and policy report.
Step 3: data linkage and representation

AOP from LXR activation to liver steatosis

Landesmann et al. (2012) JRC scientific and policy report.
AOP from BSEP inhibition to cholestasis

AOP from protein alkylation to liver fibrosis

Landesmann et al. (2012) JRC scientific and policy report.
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?

OECD (2012) Proposal for a template and guidance on developing and assessing the completeness of adverse outcome pathways.
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

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

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

- Department of Toxicology, Vrije Universiteit Brussel, Belgium
- Department of Pathology, University of Sao Paulo, Brazil
- University Hospital of the Vrije Universiteit Brussel, Belgium
- Foundation for Research Support of the State of Sao Paulo, Brazil
- Fund for Scientific Research Flanders, Belgium
- FP7/Cosmetics Europe projects HeMiBio and DETECTIVE
- European Research Council (ERC-StG) project CONNECT