Development of Adverse Outcome Pathways as a tool for understanding and analysis of nanoparticle-induced liver toxicity

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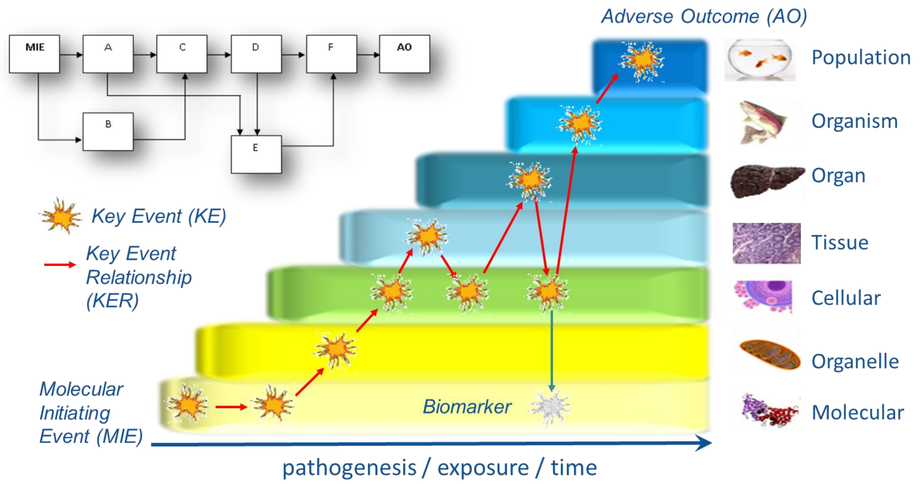
The Adverse Outcome Pathway (AOP) concept

An AOP is a conceptual construct that portrays existing knowledge concerning the link between a **Molecular Initiating Event (MIE)** and an **Adverse Outcome (AO)**, by capturing the sequential chain of causally-linked **Key Events (KEs)** at different levels of biological organisation. AOPs are increasingly developed as useful tools in human risk assessment and to reduce the reliance on animal testing. They are a practical solution to the integration, curation and dissemination of toxicological knowledge and a tool to bring systems biology thinking into mainstream biomedical and toxicological research. One AOP consists of a single MIE and AO, but can have multiple KEs that can furthermore be shared amongst different AOPs. Moreover, one MIE might lead to various AOs. AOPs can help identifying knowledge gaps and inform the design of suitable testing strategies. Following OECD guidance, specific AOPs were recently developed for hepatotoxic events such as fibrosis, steatosis or cholestasis, based on effects induced by chemicals.

Exemplary flow scheme of a typical AOP, starting from the Molecular Initiating Event, inducing a variety of Key Events and resulting in a specific Adverse Outcome.



AOPs – Relevance in Nanotoxicology

The use of nanoparticles (NP) in foods and food products is rapidly growing, and the consumer is progressively exposed to these materials via ingestion. An increasing number of studies currently address the kinetics and adverse effects of ingested NP, with the liver being one of the major target organs. Although significant work has been done to understand basic mechanisms of toxicity, little has been done to integrate our understanding of primary mechanisms into AOP thinking and thus better support regulatory decision making.
Development of nano-specific AOPs could help structure our basic knowledge, provide overviews of existing information and facilitate identification of knowledge gaps to inform and optimise future testing strategies.

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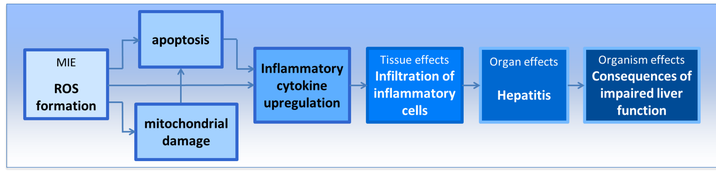
Objectives

- Development of AOPs specific to NP-induced liver toxicity, using case studies (e.g. hepatitis induction by TiO_2)
- Applying AOP-derived concepts on mechanisms of toxicity of known NP to unknown NP, thus facilitating concerted selection of relevant research questions
- Validation of test systems for hazard identification based on *in vitro* assays that reflect KEs within an AOP, using high throughput screening (HTS) and high content analysis (HCA)
- Acquired knowledge on toxicity of unknown materials will further feed back into the AOP development

Challenges and chances specific to nanoparticle-AOP development

- No availability of human data (for liver toxicity)
- Properties of tested nanomaterials can alter hugely even for the same material
  example: TiO_2 → rutile versus anatase, particle size, charge, SSA,… → small NP (5nm) might lead to a different AO than larger NP (100nm)
- Studies are difficult to compare in terms of treatment protocols, tested endpoints, tested species, cell types,…
- Initial fate of nanomaterials is often unknown (protein coating, dissolution etc prior to reaching the target organ → do they act as "nanomaterials"? → challenge for defining the relevant MIE)
- Relevance of barriers

→ Joint efforts and increased awareness of scientists can lead to more harmonisation and value of studies for future AOP developments



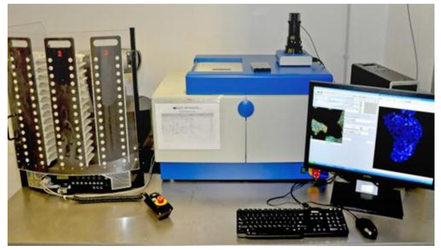
Putative AOP for a TiO_2-induced adverse liver outcome, towards the development of hepatitis: the formation of reactive oxygen species (ROS) can trigger various cellular effects (mitochondrial damage, apoptosis induction), induces inflammatory key events and eventually leads to hepatitis and respective organism effects.

Designing an AOP based testing strategy

HTS and HCA are used to identify nano-specific adverse effects associated with AOP-derived KEs:

In an initial phase and within the framework of the FP7 **NanoMILE project**, a wide range of carefully selected known and new NP is used to detect KEs such as mitochondrial damage, apoptosis induction and cell toxicity. To this end, differentiated human hepatic HepaRG cells are used. This information will directly feed back into further AOP developments.

Automated cell seeding, treatment and staining using a HTS platform (Hamilton)



Automated image acquirement and analysis using cellomics (Cellomics ArrayScan VTI HCS Reader)