



European
Commission

Description of 2 Prototype Modes-of-Action related to Repeated Dose Liver Toxicity

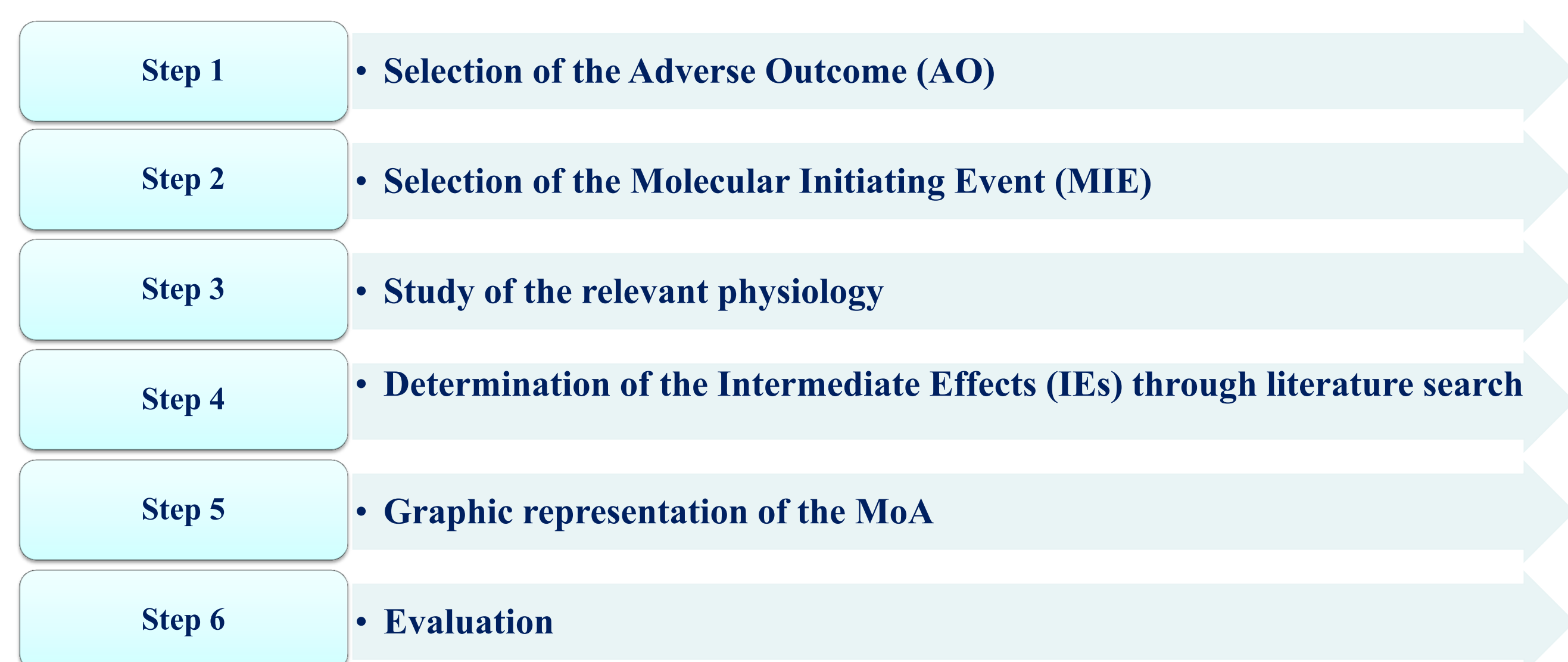
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Introduction

A Mode of Action (MoA) is a sequence of events, starting from a chemical interaction with a biological system at the molecular level leading to perturbation of biological functions at different levels (organelle, cellular, tissue, organ) that might result in adverse health effects. The elucidation of MoAs aims to the understanding of the mechanism that it is underlined behind an adverse effect and it will be crucial for the paradigm shift from conventional animal testing to the use of alternative methods for the prediction of xenobiotics' toxicity. The gathering of mechanistic information can form the basis for chemical categorisation aiming on prediction of adverse outcome based on MoA and the further development of integrated testing strategies combining computational and in vitro methods. Our goal is to use of the elucidated MoAs as a basis for alternative testing strategies and 3R methods development as a contribution to the integrated efforts within SEURAT-1 towards a MoA based safety assessment for repeated dose systemic toxicity.

MoA development



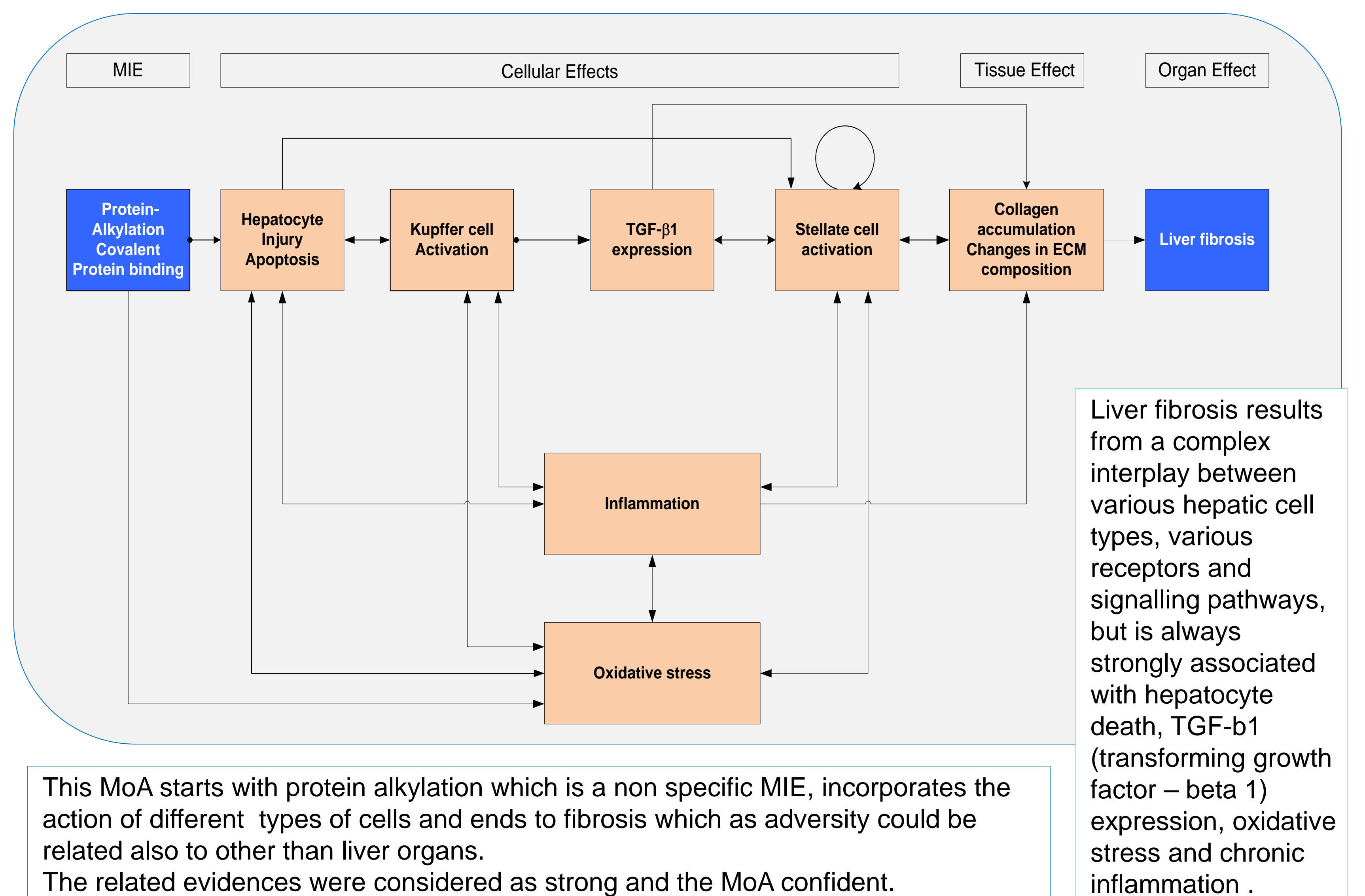
In the current study two adversities were considered named liver fibrosis and liver steatosis covering a big part of the liver repeated dose toxicity cases. The selection of liver as target organ was based on the SEURAT-1 priorities.

For fibrosis protein alkylation was chosen as the MIE because it is common to the two chemicals that have been chosen by the SEURAT Gold Compound Working Group as reference chemicals for liver fibrosis.

In relation to steatosis, nuclear receptor (NR) binding was chosen due to the globally increased concern in relation to chemicals that act on the endocrine system through such molecular initiating events which could be possibly considered as endocrine disrupting chemical (EDCs). From the six NRs that were initially considered as being involved in steatosis formation, liver X receptor (LXR) was chosen since the SEURAT Gold Compound Working Group proposed the LXR agonist T0901317 as reference chemical for liver steatosis.

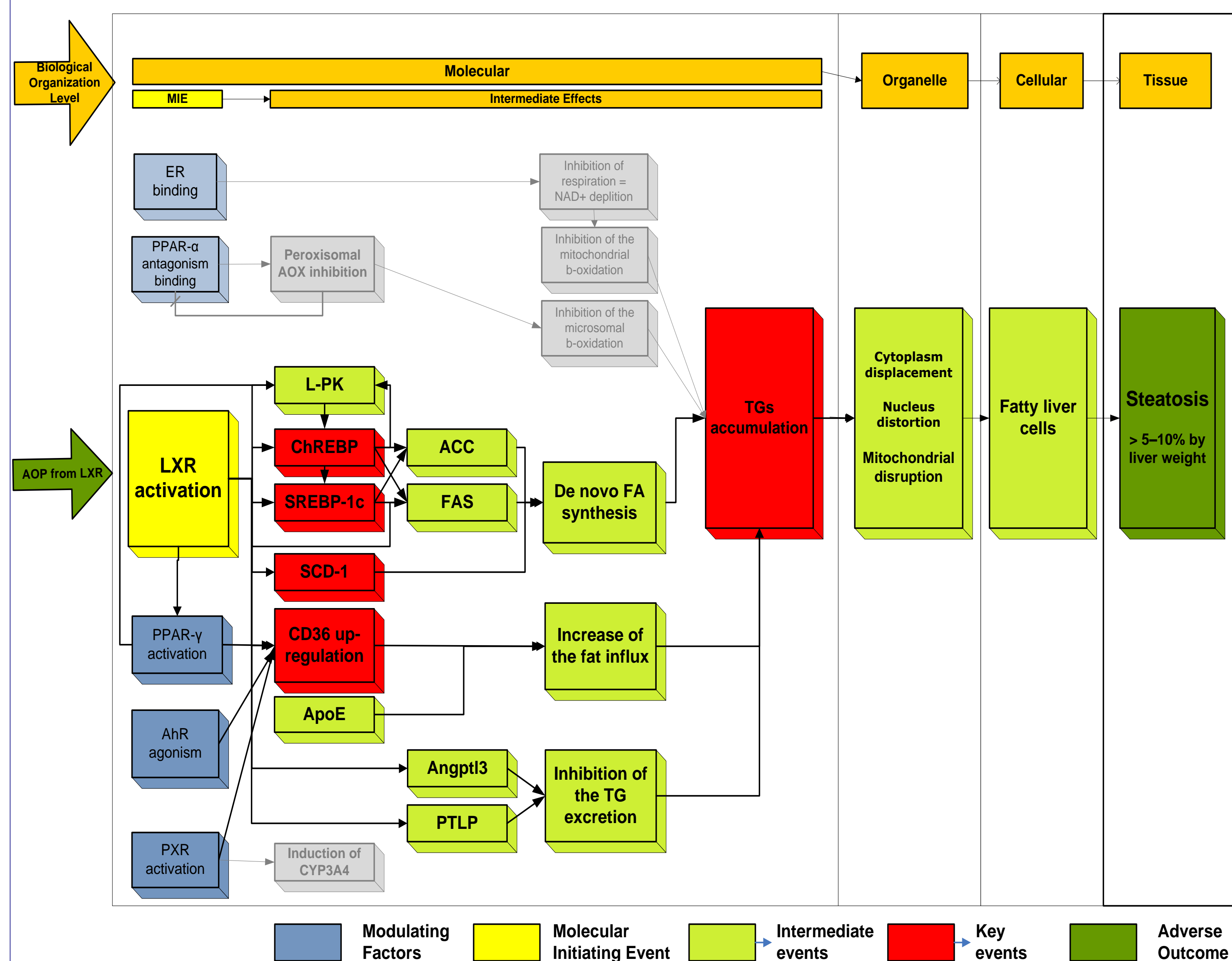
As a final step evaluation of the MoAs was made according to the OECD 2012 guidance (e.g evaluation of consistency, plausibility, relevance and temporal concordance). The presentation tried to follow the OECD draft template as close as possible.

From Protein Alkylation to Liver Fibrosis



This MoA starts with protein alkylation which is a non specific MIE, incorporates the action of different types of cells and ends to fibrosis which as adversity could be related also to other than liver organs.
The related evidences were considered as strong and the MoA confident.

From LXR Activation to Liver Steatosis



This MoA starts with LXR activation which is a specific MIE, incorporates the action of only hepatocytes and ends to steatosis which is mainly a liver adversity.

The related evidences were considered as strong and the MoA confident.

Steatosis despite non adverse per se can lead to development of steatohepatitis, fibrosis, cirrhosis and ultimately in liver failure. Steatosis is the output of the disturbance on the homeostasis of hepatic lipids which depends on the dynamic balance of 4 pathways:

1. fatty acid (FA) uptake,
2. de novo FA synthesis,
3. β-oxidation inhibition and
4. TG (VLDL) secretion

Nuclear receptors activation is known that can cause liver steatosis. LXR activation can cause liver steatosis through 3 from the 4 above mentioned pathways.

Next steps: From MoA to toxicity prediction

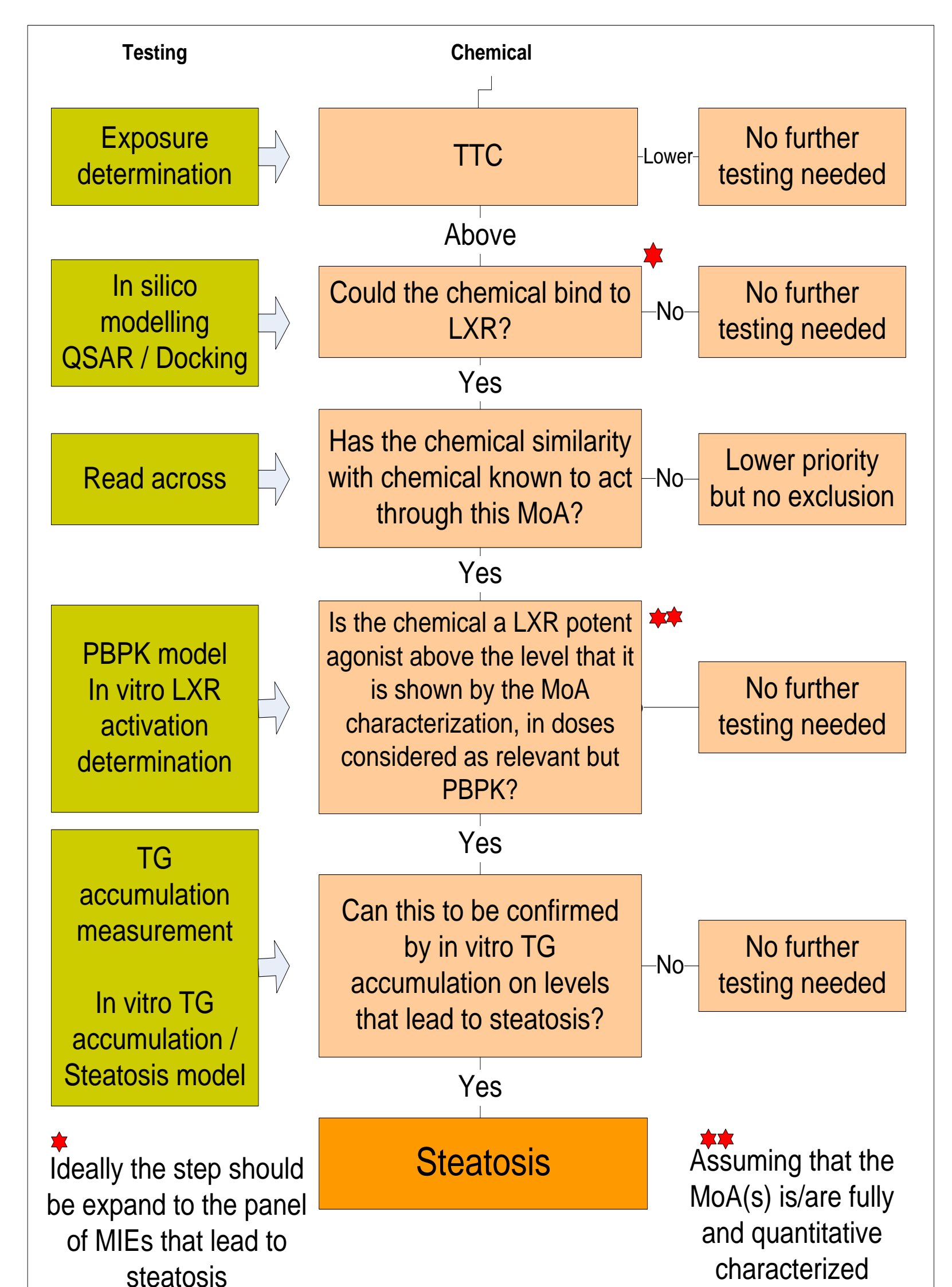
1. Quantitative characterization of the MoA

2. Prediction Strategy

After the appropriate characterization of a MoA is it possible combining in silico and in vitro methods to conclude to toxicity prediction without the use of animal testing.

Here we represent an example based on the LXR activation – Steatosis MoA. The prediction goal is to determine if a specific chemical can cause steatosis through LXR activation at doses relevant to real exposure scenaria.

The strategy could be expanded to incorporate more MIEs that lead to steatosis.



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