Development of AOPs and testing strategies for EDCs and mixtures promoting MASLD

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INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common liver disorder found in a quarter of the world's population. Growing evidence suggests that exposure to **endocrine disrupting chemicals (EDC)** can promote the initiation and/or progression of MASLD, either directly or by exacerbating the effects of a high-fat diet, genetics and/or lifestyle factors (Figure 1).

The newly launched Horizon Europe project ***EDC-MASLD*** (2024-2029, Grant ID: 101136259, Web: https://edc-masld.eu/, Coordinator: Örebro University, Sweden) aims to elucidate the impact of EDCs on the initiation and progression of MASLD, utilizing cutting-edge systems biological/toxicological approaches.

One of the goals of EDC-MASLD is to develop A**dverse Outcome Pathways (AOP)** and **Integrated Testing Strategies (ITS)** for efficient identification and assessment of EDCs promoting MASLD.

MATERIALS AND METHODS

New AOPs will be assembled based on a collection of existing knowledge through systematic review, relevant key events and relationships in the AOPWiki, in-house data, and systems biological understanding (e.g., genome-scale metabolic model) of the progression of MASLD (Figure 1). More focus will be placed on the identification of key cascades in the progressive stage of MASLD following hepatic steatosis. Weight of evidence will be assessed according to OECD's guidance document on AOP development. The chemical applicability domain will be defined by identifying EDCs affecting the same MIE or KEs in the MASLD AOPs, based on existing information from screening programs such as the Tox21. Bioinformatics will be used to identify similarities of major genes and proteins across biological systems and define the biological applicability of the AOPs. Quantitative AOPs will be constructed for selected chemicals and *in vitro* to *in vivo* extrapolation (IVIVE) will be performed to translate dose and effects across biological scales.

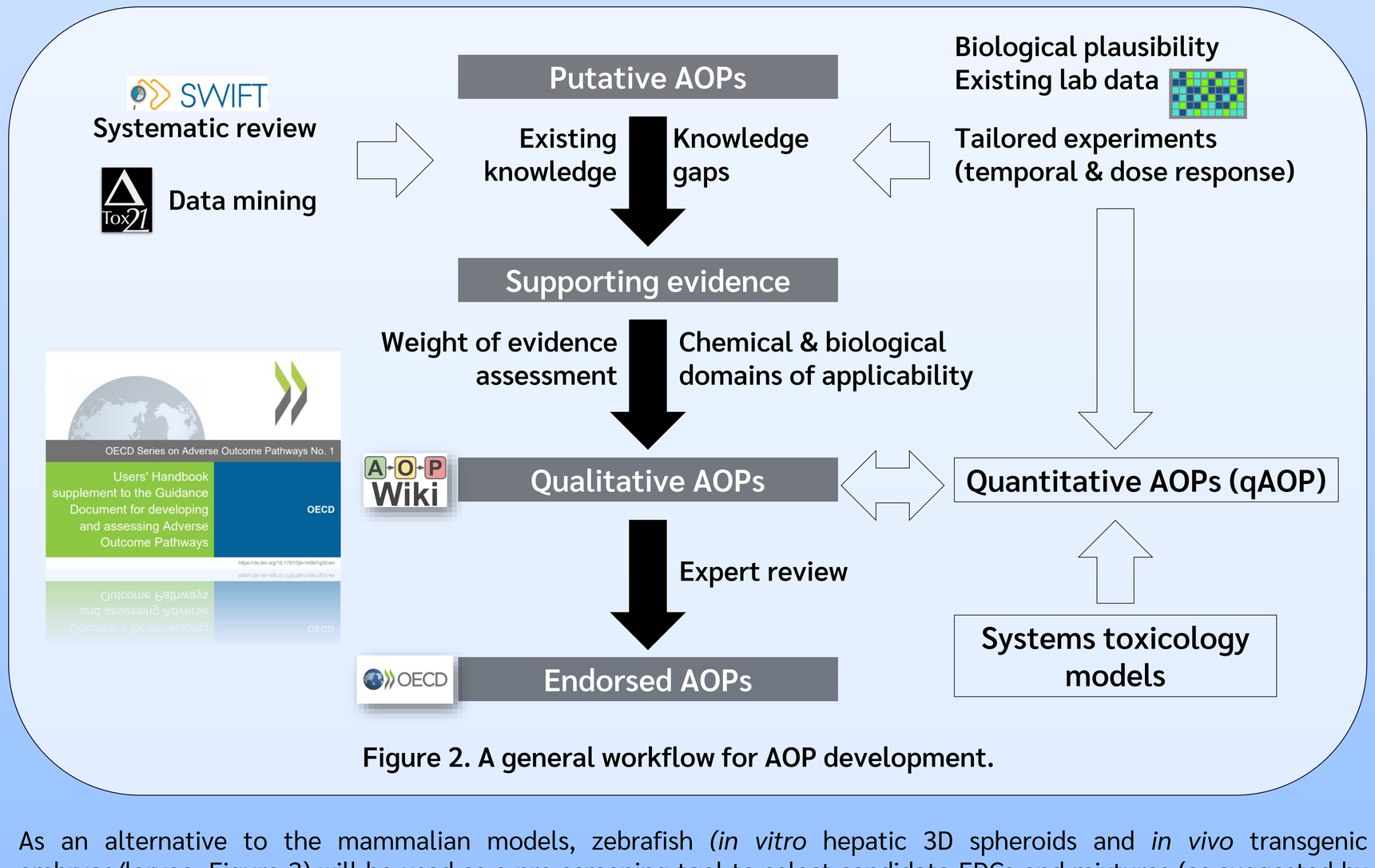

Figure 2. A general workflow for AOP development.

As an alternative to the mammalian models, zebrafish *(in vitro* hepatic 3D spheroids and *in vivo* transgenic embryos/larvae, Figure 3) will be used as a pre-screening tool to select candidate EDCs and mixtures (as suggested by human cohort studies) for in-depth assessment.

Definitive screening design (DSD), a statistical Design of Experiment (DoE) approach will be employed to design the mixtures and maximally reduce the testing needs. Mixture toxicity assessment using the classical concentration addition (CA) and independent action (CA) models will be performed based on the screening data to identify possible additive or interactive effects of EDC mixtures on the progression of MASLD.

RESULTS & CONCLUSION

The new AOP network is currently under development and will be submitted to the AOP repository AOPWiki (https://aopwiki.org/). The project will generate comprehensive knowledge on the chemical-mediated progression of MASLD and supply useful tools for future regulation of EDCs.

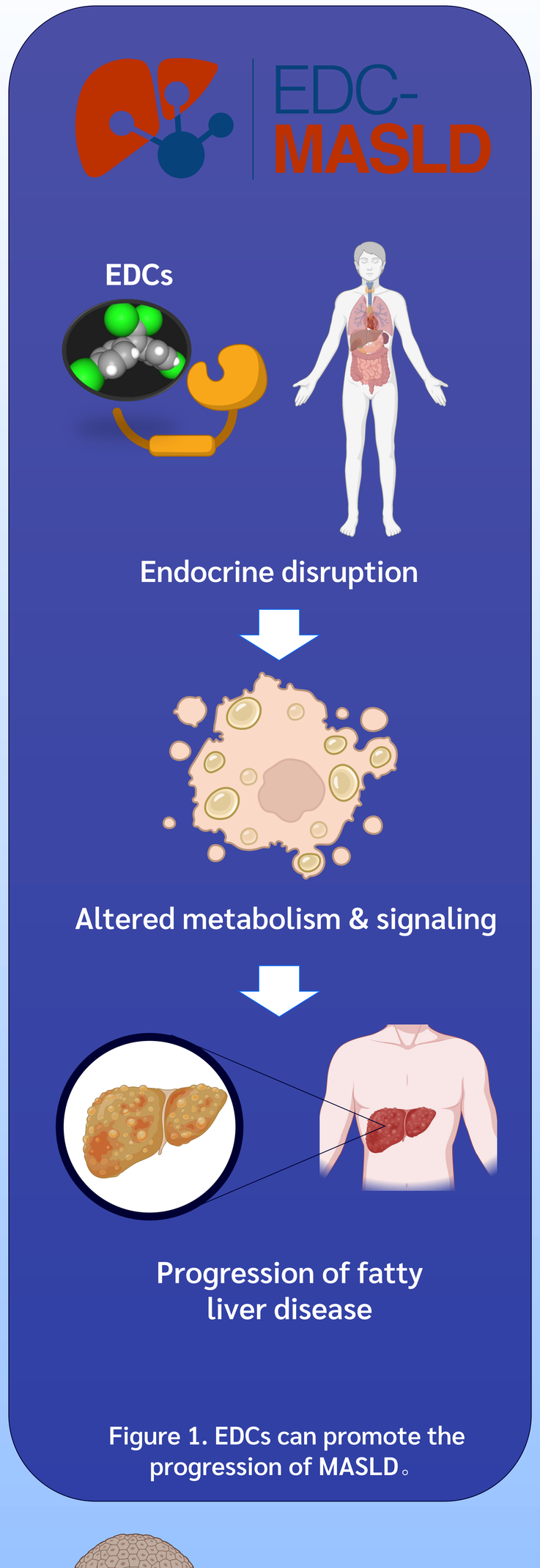

Figure 1. EDCs can promote the progression of MASLD。

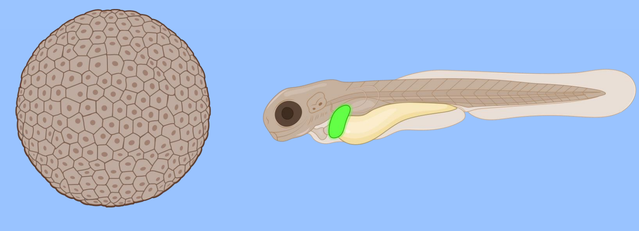

Figure 3: Zebrafish hepatic (ZF-L) 3D spheroid (left) and transgenic larvae (right) as alternative models for screening EDCs relevant for MASLD.

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