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RiskAOP

Adverse outcome pathway guided hazard assessment of mitochondrial uncouplers: a tiered testing strategy using zebrafish *in vitro* assays and embryo toxicity assays

Background

Oxidative phosphorylation (OXPHOS) is the primary metabolic process that produces energy to drive many processes in living cells. Oxidative phosphorylation mainly takes place in mitochondria, where electrons are transferred from electron donors to electron acceptors through a series of redox reactions that produces energy that is used to form ATP. Chemicals that cause mitochondrial dysfunction, by uncoupling OXPHOS (referred to as uncouplers) and disrupting the production of ATP can lead to adverse effects of regulatory concern (e.g. growth inhibition, reduced survival).

Currently, in ecotoxicity assessments there is a need for a transition from traditional animal toxicity tests to alternative testing strategies which is encouraged by various chemical regulatory frameworks (e.g. REACH). This study aims to utilize the adverse outcome pathway (AOP) concept and animal alternatives to develop a tiered testing strategy for hazard assessment of mitochondrial uncouplers. For this purpose, a suite of high-throughput zebrafish *in vitro* assays and embryo toxicity assays have been developed, with the guidance from the newly published AOP linking the uncoupling of OXPHOS to growth inhibition, via a reduction of ATP pool and cell proliferation (OECD project #1.92, AOPWiki, AOP #263).

Approach

- Exposure of **zebrafish cells** (ZF-L) and **embryos** (0 – 96 hpf) to known mitochondria uncoupler CCCP
- *In vitro* and *in vivo* assays for each key event defined in AOP 263

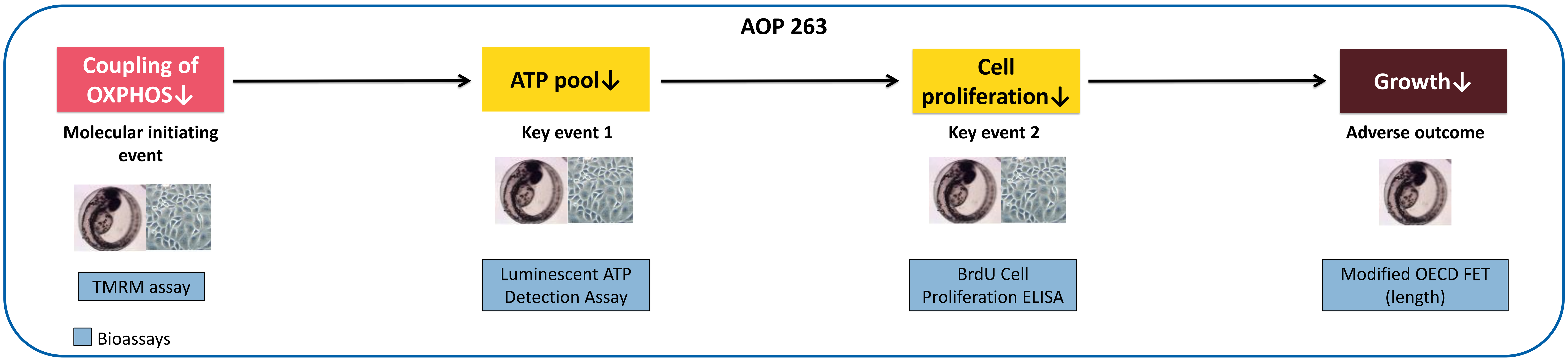

Fig. 1. AOP 263-guided experimental design.

Results

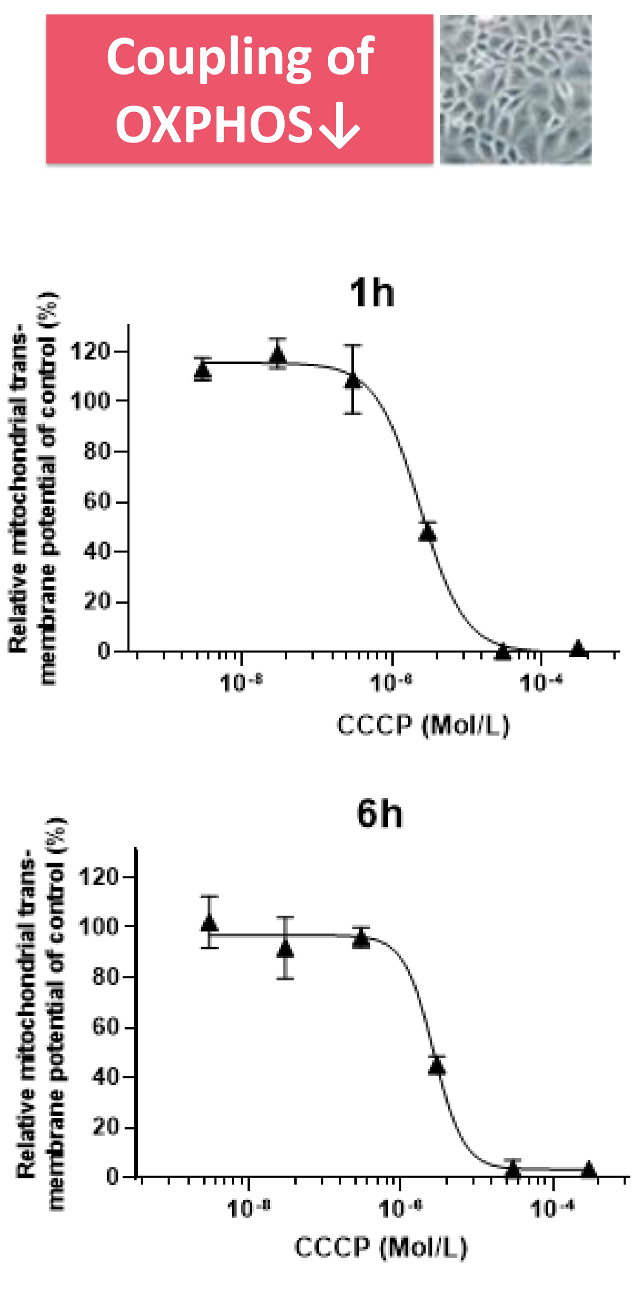

Fig. 2. Non-linear regression analysis of mitochondrial transmembrane potential relative to control in ZF-L cells exposed to CCCP after 1 and 6h.

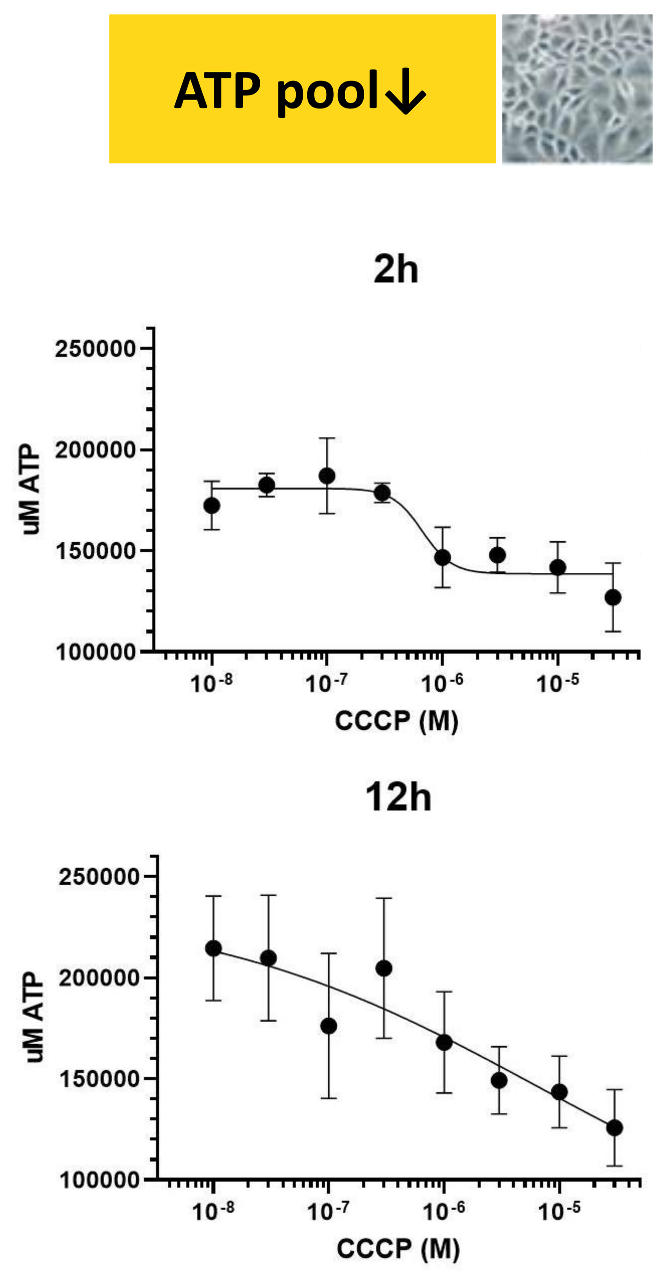

Fig. 3. Non-linear regression analysis of ATP content in ZF-L cells exposed to CCCP after 2 and 12h.

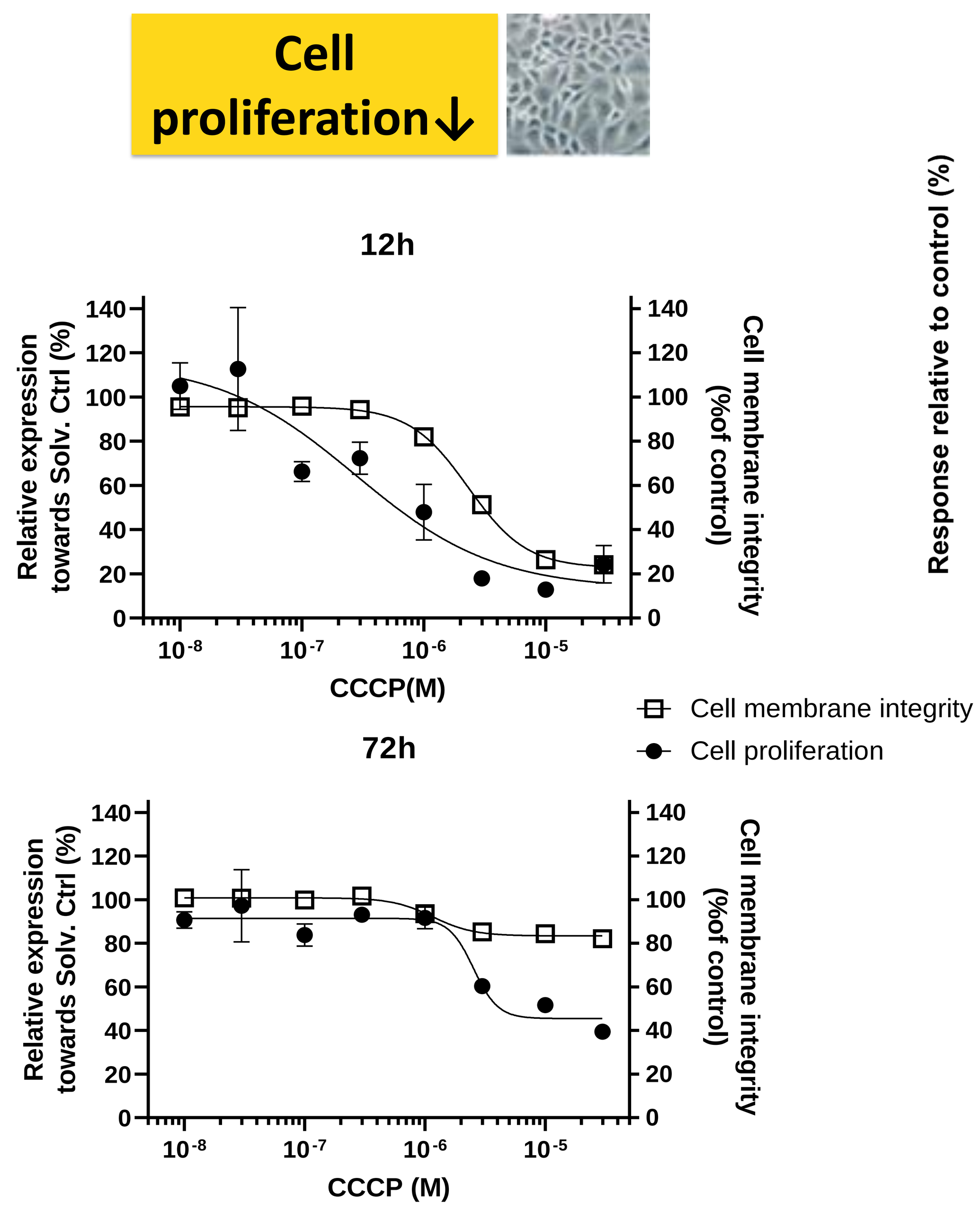

Fig. 4. Non-linear regression analysis of cell proliferation and cell membrane integrity in ZF-L cells exposed to CCCP after 12 and 72h.

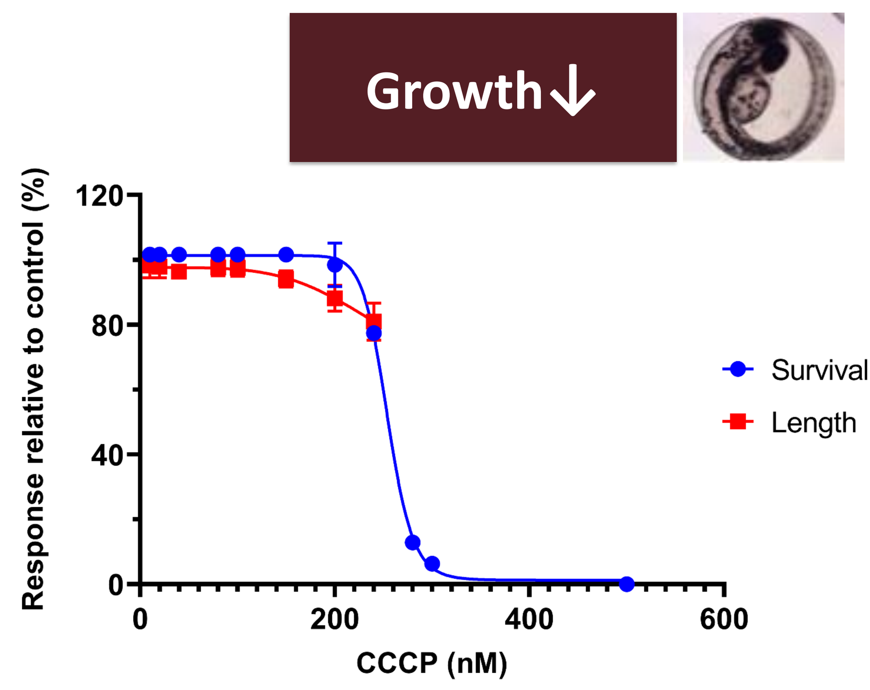

Fig. 5. Non-linear regression analyis of survival and length in zebrafish larvae exposed to CCCP for 96h

Fig. 6. Zebrafish larvae exposed for 96h to A) Solvent control (0.1% DMSO) or B,C) 200nM CCCP

Future perspectives

- *In vivo* bioassays for molecular initiating event and 2 key events of AOP 263
- Standardization of *in vivo* bioassays
- Chemical analysis
- *In vitro* to *in vivo* (IVIVE) extrapolation and Physiologically Based Toxicokinetic (PBTK) model

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