Development of an -omics based detection tool to discriminate between endocrine-mediated activity and systemic toxicity of substances

Steve Ayobahan[1,2,*], Elke Muth-Kohne[1], Matthias Teigeler[1], Matthias Kotthoff[1], Stefan Kalkhof[3], Christoph Schäfers[1], Henner Hollert[2]

[1]Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany

[2]Institute of Environmental Research (Biology V), RWTH Aachen, Germany

[3]Department of Bioanalytics, University of Applied Sciences Coburg, Germany

Contact: steve.ayobahan@ime.fraunhofer.de

Introduction

A mechanistic identification of the events preceding changes in vitellogenin expression is crucial to differentiate systemic toxicity from endocrine modes-of-action (MoA). An adverse outcome pathway (AOP) framework for endocrine MoAs shows that reduced vitellogenin concentrations lead to reduced fecundity and consequently, to a declining trajectory at the population level. Substances with other MoAs, exerting systemic toxicity, might also result in reduced vitellogenin, and thus lead to an identical adverse outcome (AO), although the molecular initiating event (MIE) is different. An improvement in determination tools is thus needed to ensure a reliable discrimination of endocrine disruptors from chemical substances with other MoAs in order to avoid regulatory action and a further elongated higher-tier testing.

Objectives

- Development of an -omics based method for discriminating altered vitellogenin expression as a result of systemic toxicity and endocrine disruption.

Experimental set-up

- The setup is an extension of Fish Short Term Reproduction Assay (FSTRA) OECD, 229 guideline by molecular methods in order to obtain more information on the MoAs and the AOP of a given substance.
- After 21 days exposure of zebrafish to fadrozole and acetaminophen, we intend isolating the liver and gonads for proteomics and qPCR analysis.

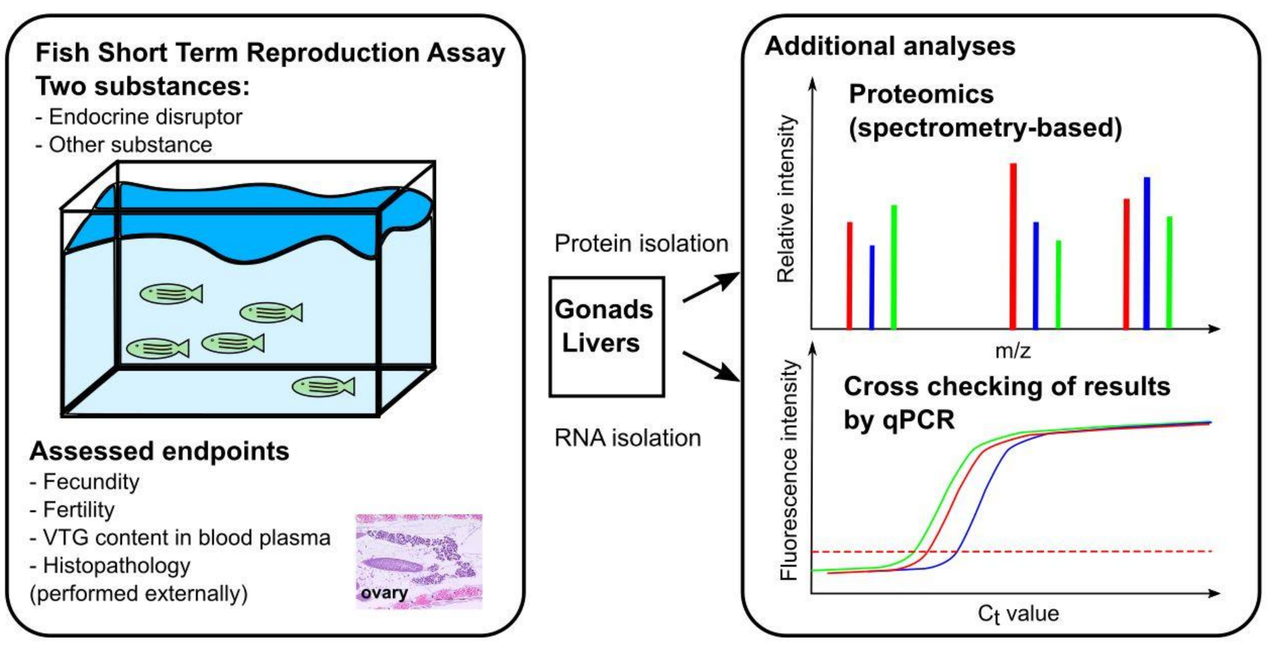
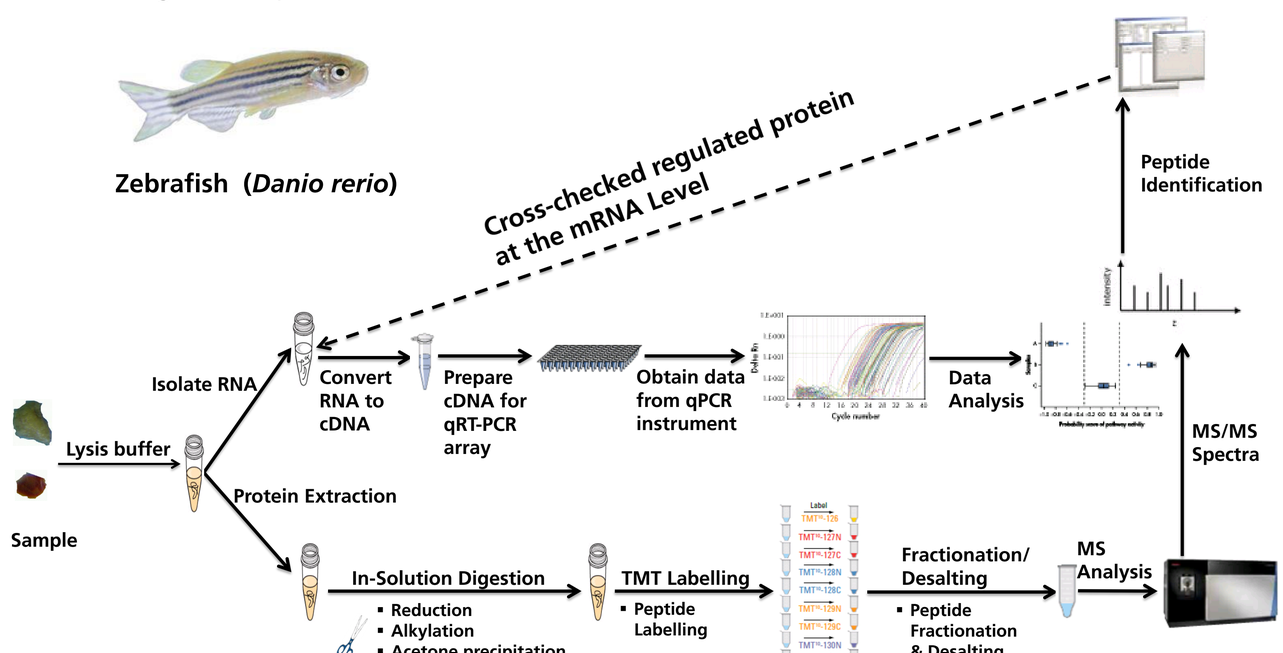


Fig.1: The Adverse Outcome (AO) of two substances with either endocrine disrupting or hepatotoxic properties will be determined by a standard FSTRA according to the OECD TG 229 with zebrafish (Danio rerio). The Key Events (KE) will be identified by –omics approaches including proteomics and qPCR of liver and gonads

Omics Workflow

- Extraction of Protein and RNA sample from a single zebrafish tissue.
- In-solution digestion.
- TMT labeling.
- Fractionation and desalting using SCX-C18 and C18 stage tips.
- Peptides resolubilization into HPLC dilution solvent for MS analysis.
- Regulated protein will be cross-checked at the mRNA level.



Adverse Outcome Pathway

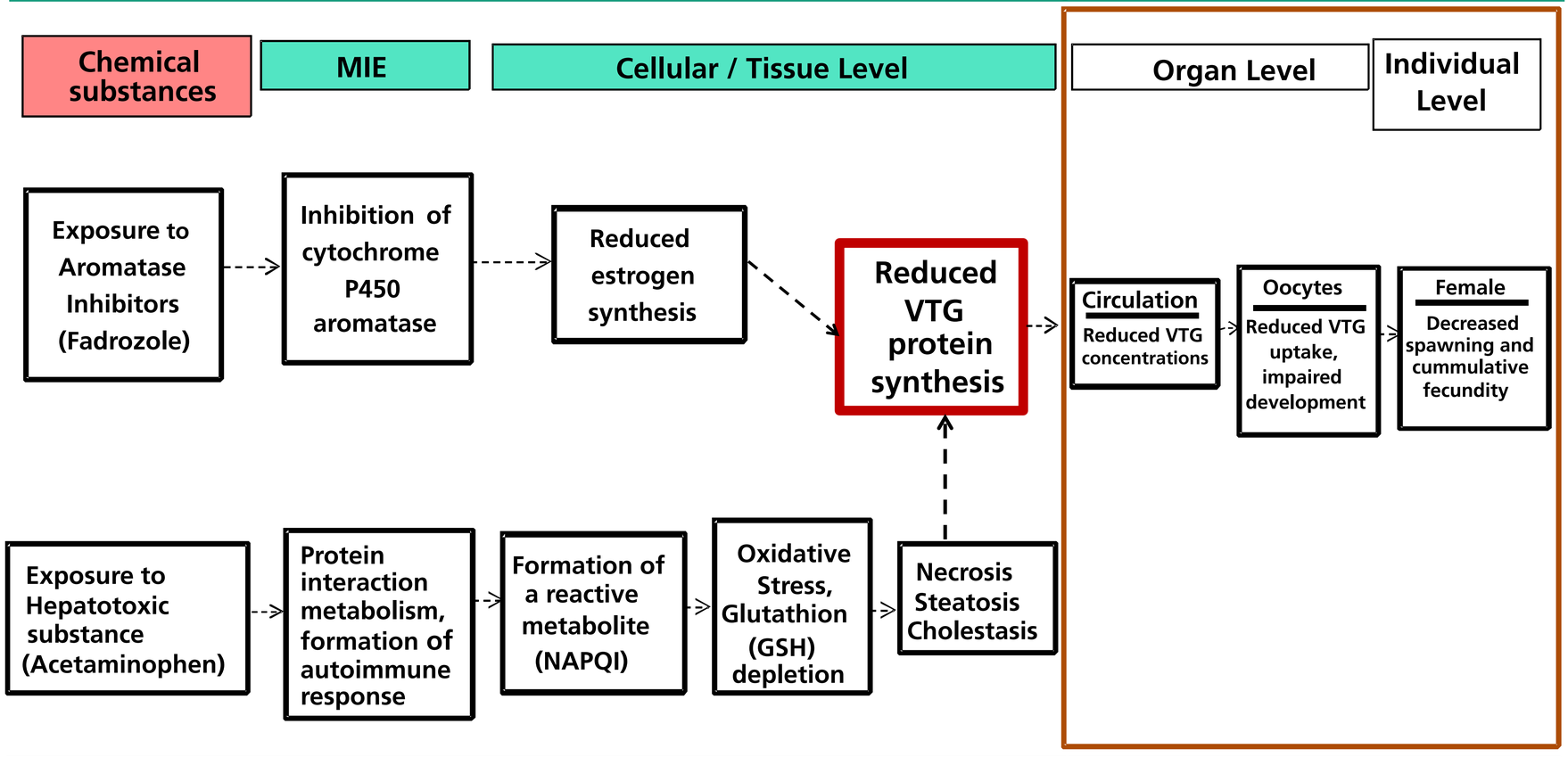
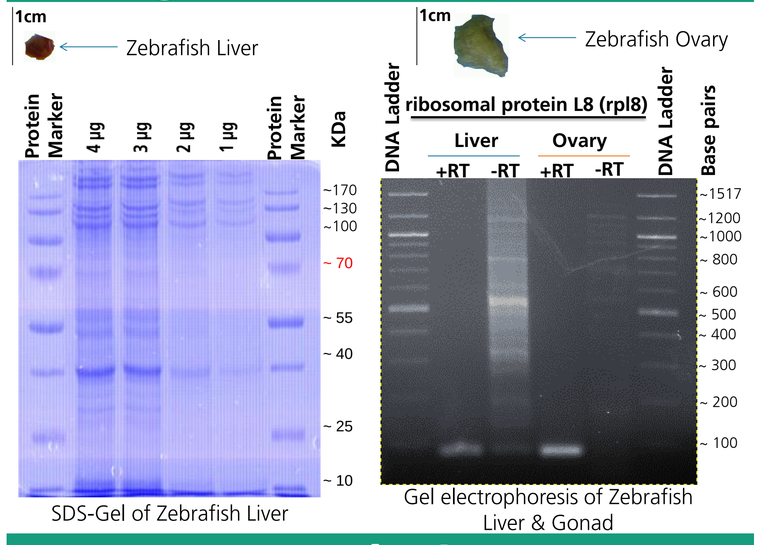


Fig. 2:The Adverse Outcome Pathway (AOP) of an aromatase inhibitor compared to hepatotoxic substance. Both AOPs share an identical adverse outcome as well as regulation of vitellogenin (VTG) synthesis, while diverging Key Events (KE) could not be detected by standard tests.

Single Tissue Preparation



Conclusion

- These improved approaches will allow identification and validation of specific modes-of-action (MoAs).
- Discrimination between different MoAs, which could not be distinguished by standard approach due to an identical adverse outcome.

DAAD Deutscher Akademischer Austauschdienst German Academic Exchange Service