LRI-ECO35:

Interference of hepatotoxicity with endocrine activity in fish

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Background

Within the scope of recent OECD test protocols for screening for endocrine activity in fish, **vitellogenin (VTG)** has been established as a major **marker for the diagnosis of endocrine disruption in fish**. A reduction of VTG production in female fish may be associated with, e.g., an interference of steroidogenesis, whereas an increase of VTG production in males is regarded indicative of the presence of estrogen-receptor agonists. However, **the production of VTG may not only be modified by typical endocrine-related pathways, but also through non-endocrine-mediated processes**. In particular, liver toxicity, i.e. the toxicant-induced impairment of liver structure and function, can influence the VTG biomarker. A false VTG result in the endocrine disruptor screening assays would trigger very labour-, time- and cost- intensive higher tier-testing, as it would increase the number of fish used in animal experiments. **Therefore, an intimate understanding of the interplay between primary endocrine-related and non-endocrine-related pathways is crucial for the avoidance of false-positive diagnoses.**

Aims

The new LRI project is driven by the hypothesis that **hepatotoxicity may positively or negatively interfere with VTG production**, which is used as a key marker of endocrine activity in current OECD test guidelines 229, 230 and 234.

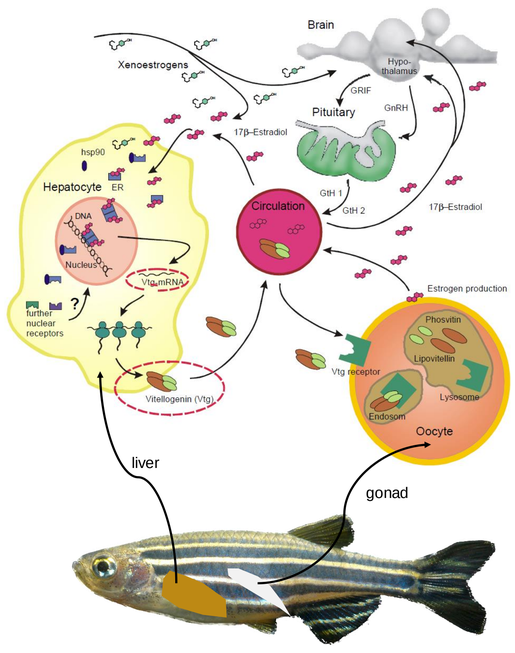
Thus, the present project was designed to:

1. identify scenarios, where liver toxicity may affect, through a non-endocrine mode of action, the induction, synthesis and secretion of VTG from hepatocytes in small fish models;
2. develop a set of diagnostic tools to distinguish liver toxicity-mediated modulation of the VTG biomarker in fish, from endocrine effects.

The information obtained from the project will be utilized in the context of the Adverse Outcome Pathway (AOP) framework to attempt to develop an AOP for liver toxicity-mediated modulation of the VTG biomarker in fish.

Experimental approach

- **Zebrafish** (*Danio rerio*), one of the most commonly used small fish model species, will be used to address the above-mentioned aims
- **OECD guideline 229 (**"Fish Short Term Reproduction Assay") will be used as experimental exposure scenario, which requires that adult fish will be exposed to the test compounds for 21 days
- **Hepatotoxicants** with different modes of action will be applied to induce different effects on the liver of exposed fish:
  a) General hepatotoxicants causing liver damage
  b) Hepatotoxicants interfering with hepatocellular protein and lipid synthesis
  c) Hepatotoxicants interfering with hepatic energy metabolism
  d) Hepatotoxicants interfering with hepatocellular VTG synthesis through a crosstalk of various signaling pathways



Hepatotoxicants

Acetaminophen: alterations of cellular liver structure causing general pathological liver damage, which might inhibit VTG synthesis

Isoniazid: metabolized by hepatic *N*-acetyltransferase and cytochrome P450 2E1 to form hepatotoxic metabolites that might inhibit VTG synthesis

Valproate: interference with protein or lipoprotein synthesis and secretion pathways of the liver cell, which would also inhibit VTG synthesis

DNOC: uncoupler – energetic futile cycling through interference with mitochondrial ATP production, resulting in inhibition of VTG synthesis

Microcystin-L: increases phosphorylation of proteins in liver cells by inhibition of cytoplasmic phosphatase activities, which might also inhibit VTG synthesis

→ Impaired VTG synthesis in the liver

Endpoints

- endpoints required by OECD TG 229 (optionally also TG 234)
- hormone (E2) levels to assess possible indirect effects on VTG synthesis, which are not mediated through liver toxicity
- VTG ELISA in males and females
- VTG RT-PCR (absolute quantification with standard curves for all sub-forms)
- histopathology of liver and gonads in males and females to assess liver structural toxicity
- serum liver toxicity markers (alanine aminotransferase) to assess liver functional toxicity
- transcriptomic analyses (microarrays) of genes involved in energy metabolism
- respirometric measurements of the energy budget to assess liver functional toxicity

Acknowledgements

LRI-ECO35 Research Liaison Team (RLT):
Grace Panter (Syngenta), James Wheeler (Dow AgroScience), Arnd Weyers (Bayer), Malyka Galay-Burgos & Alan Poole (ECETOC), Bruno Hubesch (CEFIC)