Targeted vs. non-targeted identification of endocrine disruptors by gene expression analysis as an additional ecotoxicological endpoint in fish studies: Challenges and opportunities

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When investigating potentially endocrine-disrupting substances using existing test guidelines, it is important to evaluate rapid response indicative parameters in addition to population-relevant endpoints. They can provide important information about the underlying modes of action (MoA). A promising approach is the investigation of molecular endpoints, as they react very quickly to pollutants. However, gene expression analyses by qPCR revealed some limitations, as a targeted analysis of pre-selected genes may hinder the identification of endocrine active substances with unknown MoA. More comprehensive approaches, such as transcriptomics (e.g. by RNAseq), would allow the identification of MoA-specific expression patterns as molecular fingerprints, serving as data base for substances with unknown endocrine activity.

Gene expression analyses can be implemented at different time points during chronic fish studies without the need for further test animals

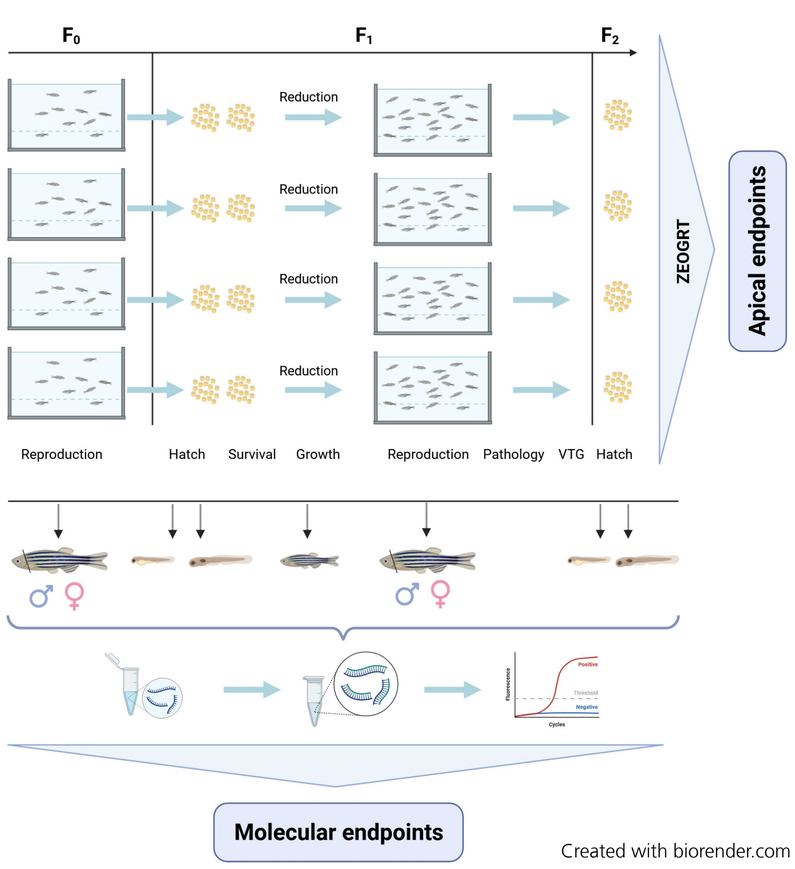

Figure 1: Schematic representation of the in-life phase of the zebrafish EOGRT combined with gene expression analyses by qPCR.

Endpoint	Tamoxifen-citrate	Prochloraz
F_0 egg number	↓ (LOEC 6.3 µg/L; not significant at concentrations applied in gene expression analysis)	Not significant
F_1 sex ratio (%males)	↑ (LOEC 2.0 µg/L)	↑ (LOEC 100 µg/L)
F_1 egg number	↓ (LOEC 6.3 µg/L; not significant at concentrations applied in gene expression analysis)	↓ (LOEC 100 µg/L)
F_0 VTG females	↓ (LOEC 6.3 µg/L; not significant at concentrations applied in gene expression analysis)	↓ (LOEC 320 µg/L; not significant at concentrations applied in gene expression analysis)
F_0 VTG males	↑ (LOEC 2.0 µg/L)	Not significant
F_1 VTG females	↓ (LOEC 0.63 µg/L)	↓ (LOEC 100 µg/L)
F_1 VTG males	Not significant	Not significant
Cyp19a1b (female-enriched)	↑	(↓)
Vtg1 (female-enriched)	(↑) Not significant	(↓)
Dmrt1 (male-enriched)	↑	↑
Amh (male-enriched)	↑	↑
Sox9a (male-enriched)	↑	Not analysed
Foxl2a (female-enriched)	↑ (significant increase based on one outlier in controls)	Not analysed
Ihß (female-enriched)	Not analysed	Not significant
Zp3a.1 (female-enriched)	Not analysed	(↓)

The integration of gene expression analyses into the ZEOGRT approach detected changes at different life stages, which fit comparably well to the expected changes for a substance acting as aromatase inhibitor, while the results for an estrogen receptor modulator were less predictable. It was observed that the expression of selected genes highly depends on the life stage and the chosen tissue; for example, expression changes in head tissue might be of less relevance for sex-specific genes; this observation is supported by the literature.

Both, non-targeted and targeted gene expression analyses provide a number of advantages and limitations

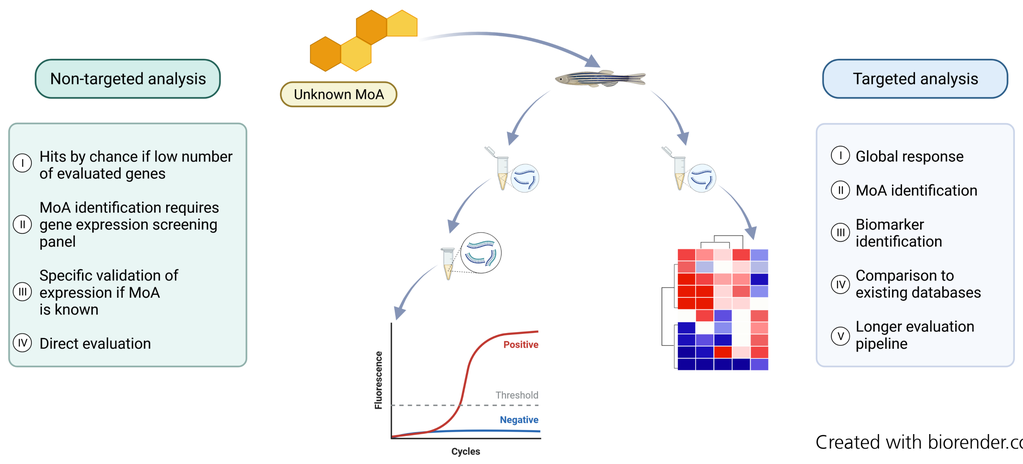

Figure 2: Comparison of non-targeted vs. targeted gene expression analysis in combination with chronic fish studies with the aim to provide a causal link between MoA and adverse effect

Targeted analysis of pre-selected genes may hinder the identification of endocrine active substances with unknown MoA. Transcriptomics would allow the identification of MoA-specific expression patterns as molecular fingerprints, serving as database for substances with unknown endocrine activity. Those non-targeted analyses of gene expression further allow the identification of MoA-specific gene expression biomarkers.

A tiered approach allows early identification of endocrine disruptors while considering animal welfare aspects.

Tier 1 will be represented by the identification of gene expression biomarkers by transcriptomics during a screening approach with embryonic fish. Within a second tier, i.e. chronic fish studies like the FELS test will be conducted coupled with an integrated qPCR analyses of biomarkers identified in the first tier.

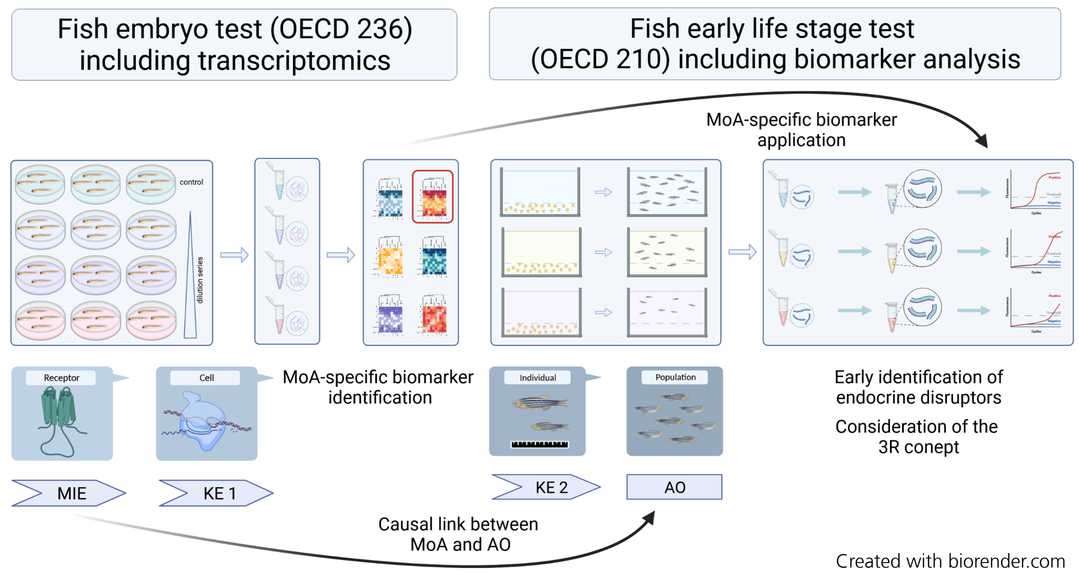

Figure 3: Schematic representation of a potential tiered approach for early and animal-saving endocrine disruptor identification

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