

# The informative value of transcriptomics in combination with the fish embryo test

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## 1. Introduction

In aquatic ecotoxicology, the fish embryo toxicity test FET can be considered as one of the most promising animal alternative [1] methods. Internationally, considerable endeavours have been made to bring the FET to acceptance as a valid alternative method to the fish acute toxicity test (OECD203). However, proof-of-principle studies have repeatedly shown that the FET can deliver more than just acute toxicity data, in particular when new and especially molecular endpoints are included in the test. Notably postgenomic approaches, like transcriptomics using microarrays or qPCR, and fluorescent microscopy-based morphometrical analyses, are well suited to evaluate subacute or teratogenic effects mechanistically due to their high sensitivity and information rich data. They are also excellent tools for the identification of effect-specific markers, which prove very beneficial for (pre-)screening applications or for the prioritization of chemicals according to their hazard potential.

## 2. Materials and methods

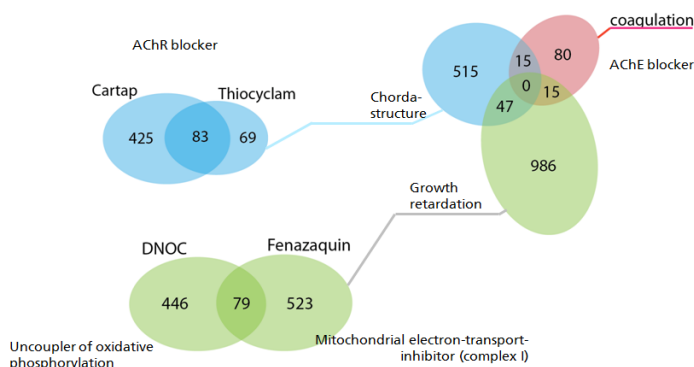
We conducted gene expression studies with zebrafish and medaka embryos after exposure (for 48 hpf to 120 hpf and 7 days, respectively) to different groups of chemicals. For each chemical, we conducted a standard FET (48h), and for the insecticides also extended FETs (120h) in 96-well plates. Embryos exposed to a selection of the tested substances were subjected to whole-genome expression analysis (using the Agilent<sup>®</sup> Zebrafish Gene Expression Microarrays) or quantitative PCR. To exclude non-specific stress responses and to find MoA-specific effects on gene expression, embryos were exposed to concentrations at EC<sub>10</sub>, EC<sub>10</sub>/2 and in some cases at EC<sub>20</sub> level for the gene expression experiments.

## 3. Results and discussion

Presented will be the results of our studies on endocrine disruptors and insecticides. Estrogenic and anti-androgenic compounds (ethinylestradiol-EE<sub>2</sub>, flutamide, genistein [2], bisphenol A-BPA, linuron, prochloraz) were studied in both species; effects of insecticide were evaluated in zebrafish only. Functional analysis of regulated genes revealed that compounds suspected of endocrine activity in fish were able to disrupt endocrine system related pathways already in the embryos. Often, even the mode of endocrine disruption could be inferred from the transcriptome response. Insecticides like cartap or fenazaquin, affected the transcriptome very specifically, and the intersection of regulated genes was comparably larger for insecticides of similar than of dissimilar MoA.

### 3.1. Gene response to insecticide exposure

Thirteen insecticides with known acute adult fish LC<sub>50</sub> values were selected for testing, representing four different modes of action. Gene expression experiments were conducted subsequently. All chemicals induced differential gene expression at the EC<sub>20</sub>. Insecticides classified as 'energy metabolism modulators' by IRAC displayed a higher number of differentially expressed genes than those classified by 'nerve action'. Intersections revealed that insecticides with functionally similar MoAs share a higher number of differentially expressed genes than those with dissimilar ones (Figure 1). Functional analysis showed that regulated genes at 48h relate to molecular functions, which can be linked to the morphological effects and to the given MoA of the pesticide.



**Figure 1: The figure shows that insecticides of different MOAs induce distinct morphological phenotypes and that this reflects on their gene responses. The more functionally similar the MOAs are, the more regulated genes (depicted as numbers in the ovals of the Venn diagrams) are shared (proportionally).**

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### 3.2. Gene response to EDC exposure

Responsive pathways comprised endocrine relevant pathways in both species and were found for all chemicals. Estrogenic modes of action were clearly discernible, and the transcriptome of the tested substances showed patterns indicating either a predominantly estrogenic or antiandrogenic activity. In medaka embryos, we examined endocrine modes of action-related genes by quantitative RT-PCR. The response to the estrogens in medaka was similar to zebrafish. For the antiandrogenic substances, however, medaka provided differing information on the mechanisms of action to zebrafish, like the regulation of genes encoding for known androgenic markers and receptors.

## 4. Conclusions

For insecticides as well as EDCs, the inclusion of transcriptomics data in the analysis of the FET proved very beneficial. Different MoA insecticides causing distinct morphological phenotypes, also exhibited very specific transcriptome responses. EDCs of estrogenic and antiandrogenic activity also demonstrated very distinctive patterns of transcriptome regulation, and many known estrogenic or androgenic markers for fish already responded in the embryos. Preliminary data for androgenic and antiestrogenic synthetic substances indicate that these also evoke MoA-specific transcriptome responses in the embryos, thus allowing us to identify such EDCs with the FET. Our data also suggest that for EDC screening assays, a combined zebrafish-medaka FET may provide complementary data and is therefore worth considered. Overall, the results of our investigations strengthen the evidence that the FET has excellent potential as an alternative testing and screening assay. It combines the read-outs of several mechanism-specific *in-vitro* tests in one with the additional benefit of a whole organism approach.

## 5. References

- [1] European Parliament. 2010. Directive 2010/63/EU on the protection of animals used for scientific purposes.
- [2] Schiller V, Wichmann A, Kriehuber R, Muth-Köhne E, Giesy JP, Hecker M, Fenske M. 2013. Studying the effects of genistein on gene expression of fish embryos as an alternative testing approach for endocrine disruption. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 157(1): 41-53

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