Can gene expression be used to detect endocrine activity in zebrafish embryos?

Y.Streltsova[1], S.U.Ayobahan[2], H.Reinwald[1], E.Eilebrecht[2], H.Hollert[3], S.Eilebrecht[2], T.Pamminger[1]

COOPERATION PARTNERS:

JOHANN WOLFGANG GOETHE UNIVERSITÄT FRANKFURT AM MAIN · Fraunhofer IME · UFZ · BAYER

Yana Streltsova
PhD candidate
Early Environmental Safety
Bayer Crop Science
yana.streltsova@bayer.com

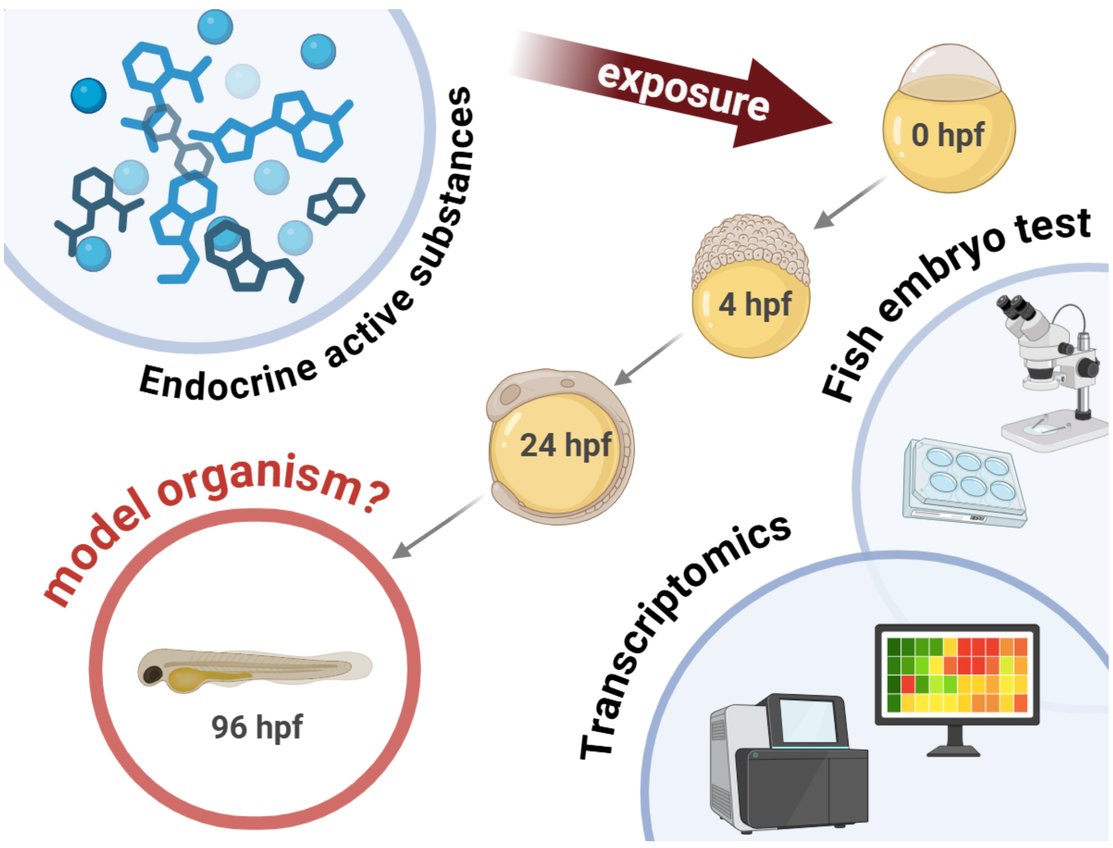

Figure 1: *Graphical abstract. Figure created in BioRender.*

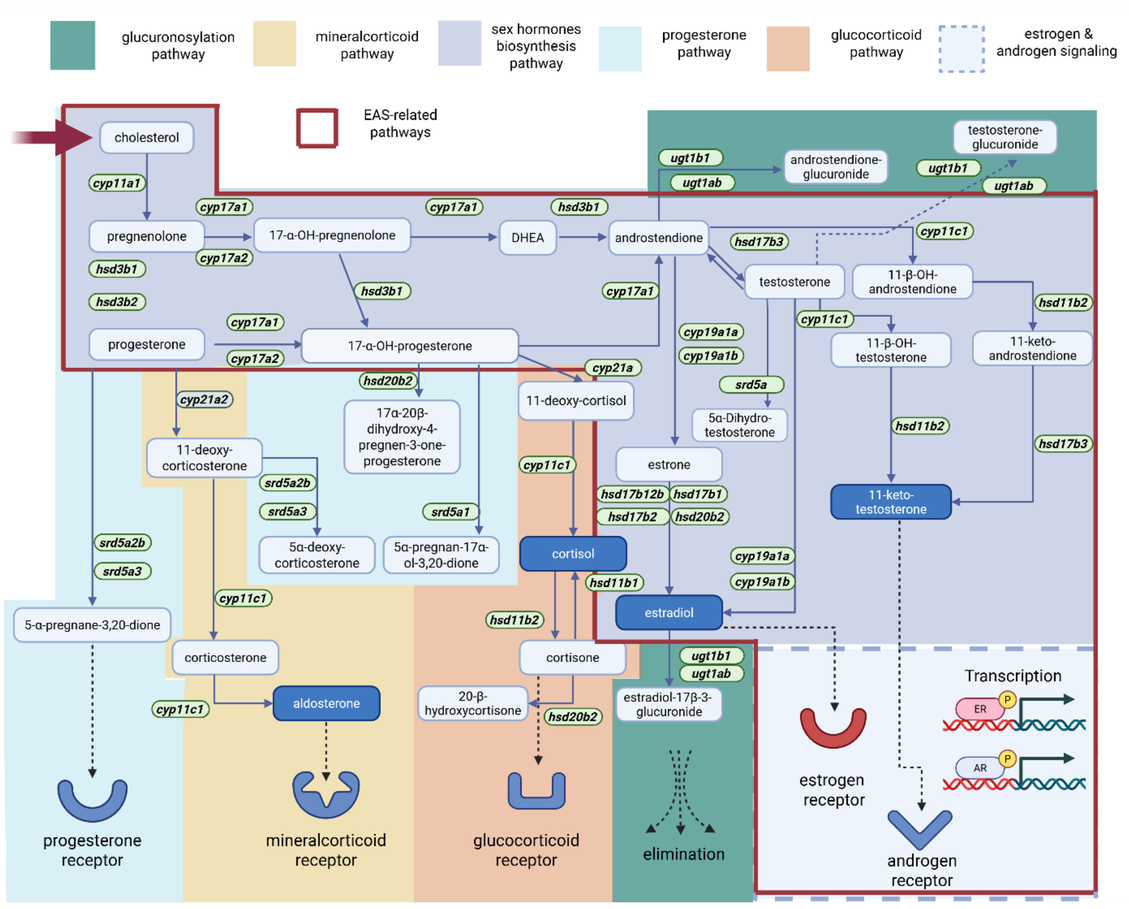

Figure 2: *Simplified steroidogenesis pathway in zebrafish derived from the KEGG database (dre00140).* The primary sex endocrine-related metabolic pathway is highlighted in the red box. *Figure created in BioRender.*

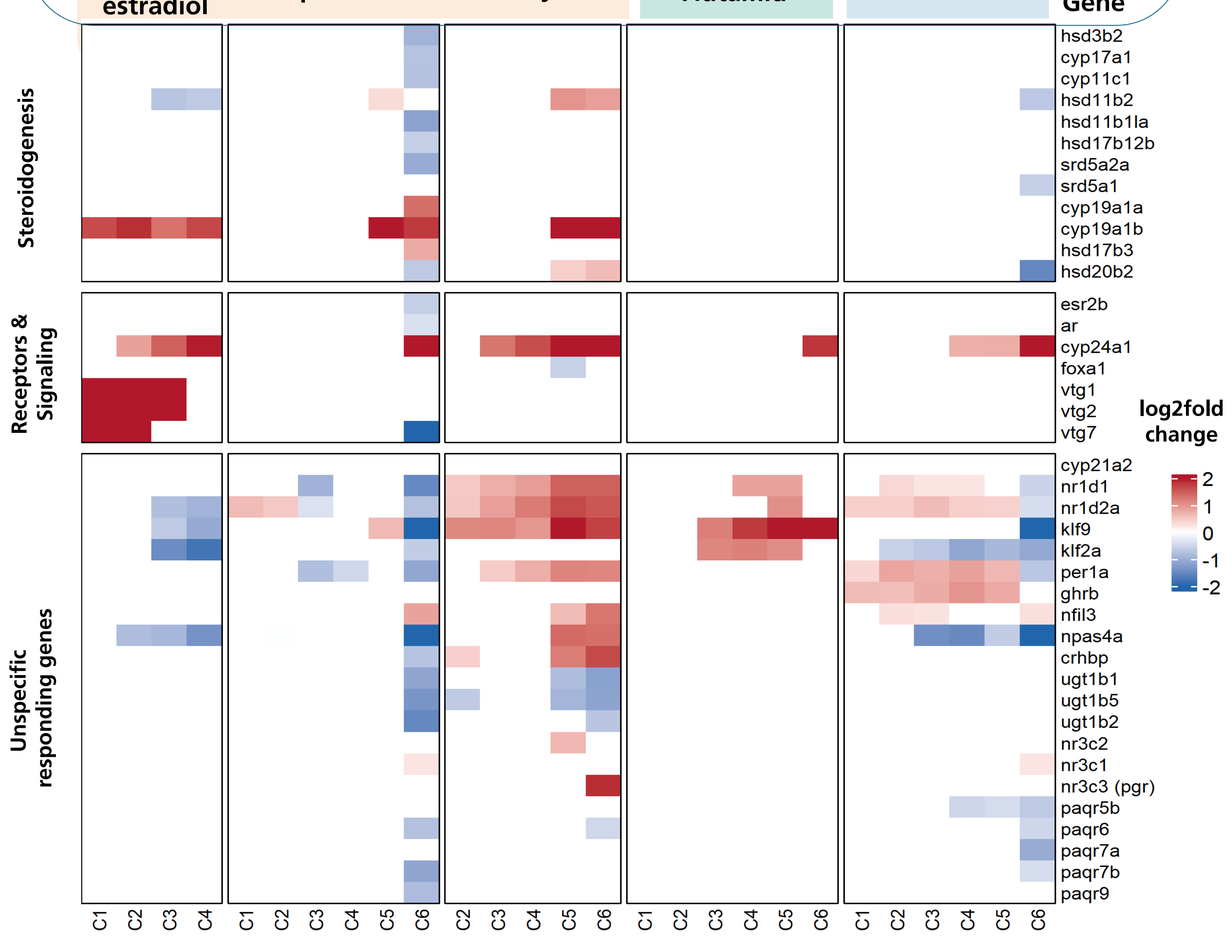

Figure 3: *Transcriptional response (log2fold changes, pagj <0,005) of endocrine-related gene sets across test substances. Genes are grouped into functional categories including EAS-related steroidogenesis, receptors & signaling (genes containing estrogen-responsive elements) and unspecific responding genes.*

ABSTRACT

Endocrine disrupting compounds (EDCs) interfere with hormonal systems and pose risks to wildlife and human health. To be classified as EDCs, substances must show endocrine activity at relevant targets and cause adverse, population-relevant effects. Current regulatory testing relies largely on *in-vivo* assays in adult vertebrates, raising ethical concerns regarding animal welfare. Consequently, there is increasing momentum to apply the 3Rs principles and develop alternative, non-animal-based methods. This study investigates zebrafish embryos as a potential alternative model to assess endocrine activity across estrogenic, androgenic, and steroidogenic pathways. To this end, we evaluated five well-characterized endocrine-disrupting compounds across a broad concentration range (six concentrations) using a 96 hours post fertilization (hpf) zebrafish embryo assay. Resulting significantly differentially expressed genes (DEGs) were analyzed and compared in a mode-of-action-specific manner to identify potential endocrine-related transcriptional fingerprints.

TEST SUBSTANCES

Estrogen agonists			Androgen-antagonist	Steroidal modulator
Ethinyl-estradiol	Bisphenol A	Methoxychlor	Flutamid	Prochloraz

SUMMARY

DEGs category	Estrogen agonists	Androgen-antagonist	Steroidal modulator
Steroidogenesis	*cyp19a1b*	-	*hsd20b2*
Receptors & signaling	*vtg cyp24a1*	*cyp24a1*	*cyp24a1*
Unspecific responding genes	-	*klf9 nr1d1 klf2a*	*klf9 klf2a paqr*

Across all tested compounds, a relatively strong non-specific response was observed. However, all estrogen agonists induced a significant upregulation of ***cyp19a1b***. For prochloraz, responses were primarily detected at the secondary level, particularly involving progesterone-related genes (***paqr5a, hsd20b2***), whereas flutamide mainly elicited non-specific signals, but also showed an upregulation of the ERE-responsive gene ***cyp24a1*** at the highest concentration.

OUTLOOK

To further evaluate the specificity of the observed transcriptional fingerprints, additional experiments with reference compounds are planned. These will include substances without a primary endocrine mode of action, such as the anti-epileptic drug **carbamazepine** and the non-steroidal anti-inflammatory drug **diclofenac.**

In addition, well-characterized endocrine-active compounds will be tested to refine and validate mode-of-action signatures, including androgen agonists (**methyltestosterone, trenbolone**), estrogen antagonists (**tamoxifen, fulvestrant**), and steroidogenesis modulators (**letrozole, ketoconazole**). This approach aims to strengthen the predictive power and specificity of gene expression-based fingerprints for the identification and classification of endocrine disruptors.

1 Bayer AG Crop Science, Monheim, Germany,
2 Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany,
3 Goethe-University Frankfurt am Main, Germany