Omics-based fingerprinting of androgen disruption in zebrafish embryos

S.U. Ayobahan[1], J. Strompen[1], C. Schäfers[2], E. Eilebrecht[2], S. Eilebrecht[1]

[1] Fraunhofer Attract Eco'n'OMICs, Fraunhofer Institute for Molecular Biology and Applied Ecology, Schmallenberg, Germany

[2] Department of Ecotoxicology, Fraunhofer Institute for Molecular Biology and Applied Ecology, Schmallenberg, Germany

Email contact: steve.ayobahan@ime.fraunhofer.de

Introduction

Endocrine disruptors (EDCs), which are capable of disrupting an organism's endocrine system, are undeniably an increasing concern owing to their developmental and reproductive health impact both in human and the environment. Screening for EDC properties, particularly of active ingredients of pesticides, biocides, or complex mixtures of contaminants, involves a diverse range of species and endpoints that are costly in terms of both resources and animal use. To this end, we applied transcriptomics to identify molecular signatures of androgen hormone signaling interference that underpin apical effects in zebrafish (*Danio rerio*) embryos.

In this study, zebrafish embryos were exposed to different sublethal concentrations of the androgenic steroid trenbolone and the antiandrogenic herbicide linuron as model substances to detect enhanced and suppressed androgen hormone signaling pathways in a modified zebrafish embryo toxicity test (zFET).

Methods

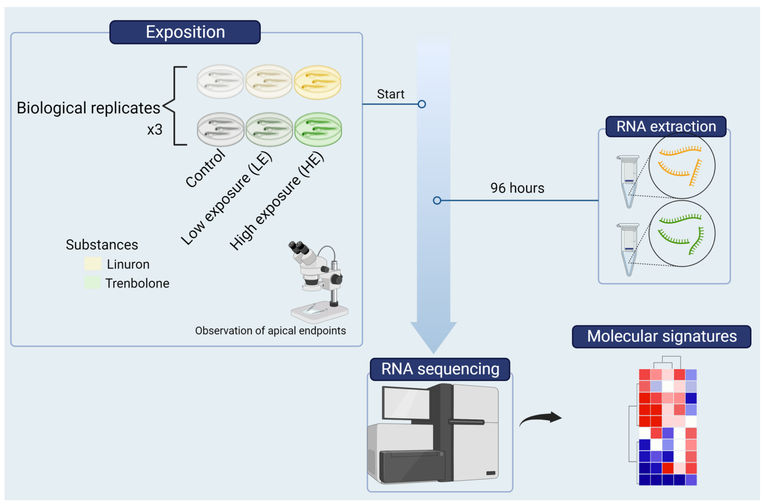

Figure 1: *Experimental design. Created using Biorender.com*

A modified version of the OECD 236 fish embryo toxicity test was used to detect substance-induced gene expression changes at sublethal concentrations. Fertilized zebrafish eggs at approximately 3 hours post-fertilization (hpf) were exposed to different sublethal concentrations of linuron and trenbolone (Figure 1). Sublethal test concentrations were established in preliminary experiments. Embryos were exposed in glass Petri dishes pre-incubated with the test solution overnight. The medium was changed at 48 hpf. At 24, 48 and 96 hpf, embryos were visually inspected for physiological effects.

At 96 hpf, total RNA was extracted from 10 individuals and subjected to Illumina TruSeq library preparation for mRNA enrichment followed by sequencing on an Illumina NovaSeq 6000 system. mRNA-Seq reads were mapped to the zebrafish genome (version GRCz11) and feature mapped reads counted using STAR. Based on three biological replicates per condition, differentially expressed genes (DEGs, BH padj ≤ 0.05 & abs (apeglm shrunk log2FC) > 0.1) were identified using DESeq2.

Results

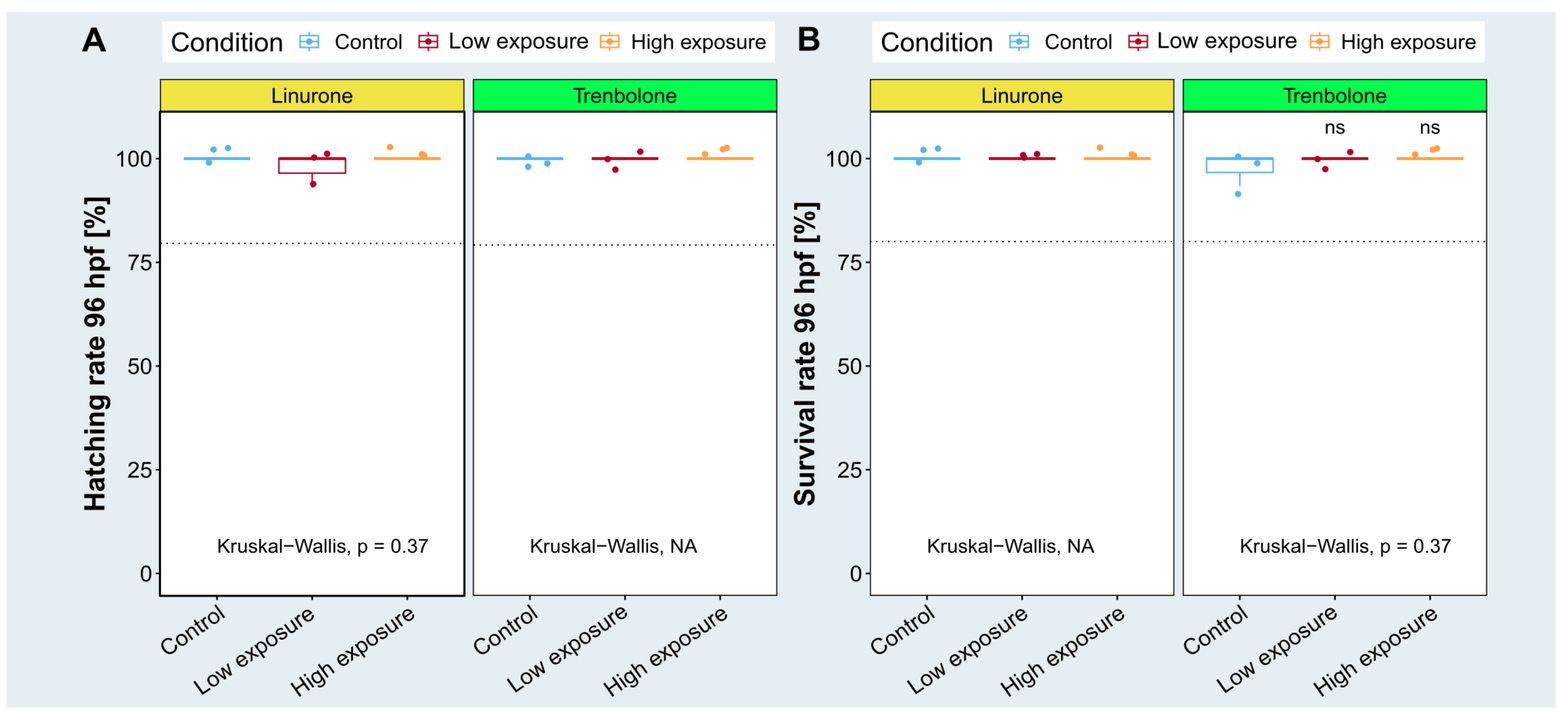

Figure 2: Hatching and survival rates of zebrafish embryos exposed to EDCs that suppress and enhance androgen hormone signaling at 96 hpf. (A) Hatching and (B) survival rates after exposure to nominal test concentrations of 250 & 500 µg/L and 1500 & 3000 µg/L of linurone and trenbolone, respectively, compared to the untreated control. ns = not significant.

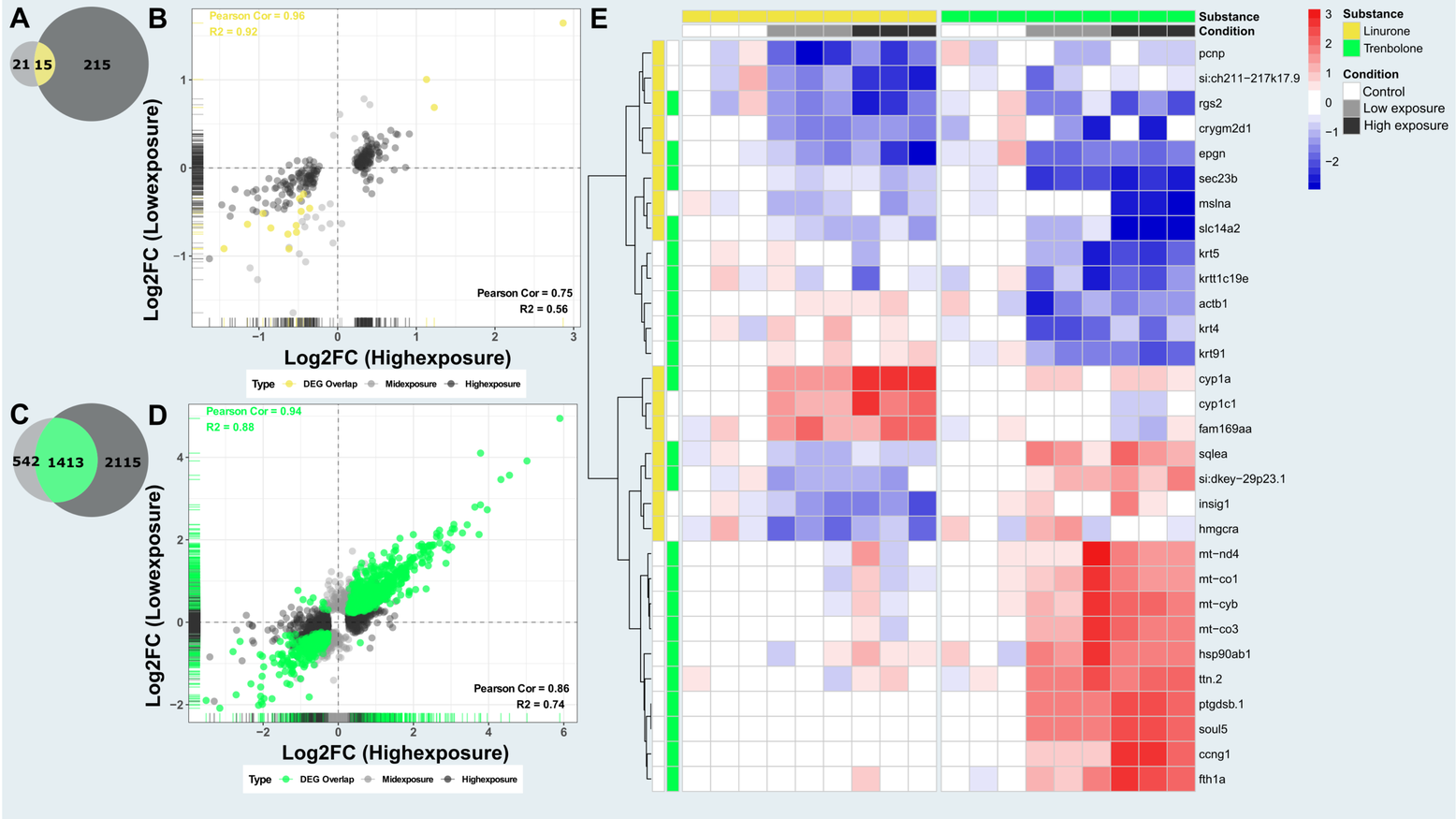

Figure 3: Transcriptomic responses to sublethal concentrations of EDCs that suppress and enhance androgen hormone signaling in zebrafish embryos at 96 hpf. Venn diagram showing the differentially expressed genes (DEGs) and scatterplots comparing the log2-fold change (lfc) values of the observed DEGs upon exposure to low and high sublethal test concentrations of (A, B) linurone and (C, D) trenbolone. (E) Heatmap showing patterns of DEGs. Red colour indicates an increase and blue colour indicates a decrease in gene expression compared to control.

Exposure of zebrafish embryos to linurone and trenbolone for 96 hours did not result in statistically significant changes in hatching rate and mortality in either the control or treatment groups (Figure 2). Although no significant phenotypic changes were observed following zFET endpoints, results from transcriptomic analysis revealed a concentration-dependent increase in the number of differentially regulated genes (DEGs) (Figure 3). Compound-specific molecular fingerprints were defined for each substance, revealing MoA-specific signatures.

Conclusion

Our study shows that omics fingerprints, when integrated with conventional approaches, can significantly improve the identification of endocrine disruptors that suppress and enhance androgen signaling. Future screening approaches using such data will enable the development of more environmentally friendly substances with less impact on the aquatic environment.

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