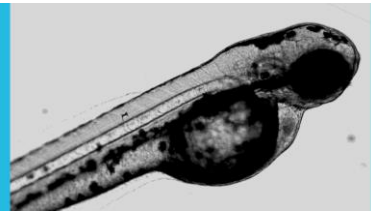
TRANSCRIPTOMIC RESPONSES TO SEXUAL ENDOCRINE ACTIVE SUBSTANCES IN ZEBRAFISH EMBRYO



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Fraunhofer IME

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Background

Chemical interference with the hormone system of an organism is of utmost concern, because of its long-lasting effects on populations

→ EDCs are substances or mixtures of chemicals that interfere with the normal function of the endocrine system & trigger adverse development, reproductive and immune effects to an exposed organism

[In past years] OECD TGs for accessing potential endocrine disrupters via perturbed endocrine modalities such as:

- Estrogen
- Androgen
- Thyroid hormones
- Steroidogenesis

→ leading to reproductive impairment

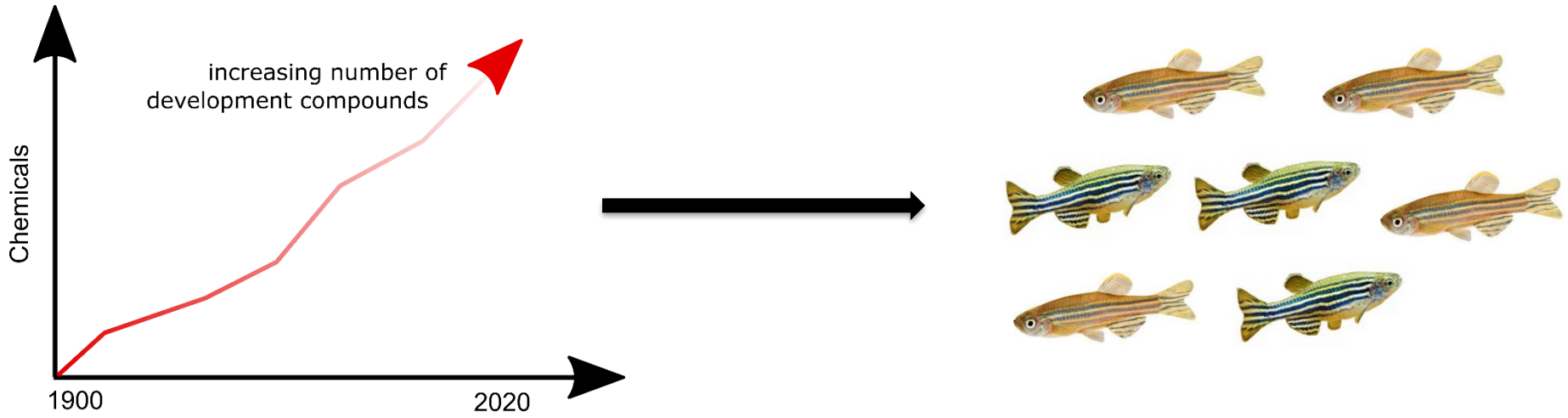
Ayobahan et al., 2019. https://doi.org/10.1038/s41598-019-43089-7

Background

Current standardized in vivo assays for evaluating the impact of endocrine active substances on reproduction in fish such as:

- Fish Short Term Reproduction Assay (FSTRA)(OECD TG 229)
- 21-day Fish Assay (OECD TG 230)
- Fish sexual development test (FSDT) (OECD TG 234)
- Medaka Extended One-Generation Reproduction Test (MEOGRT) (OECD TG 240)
- Zebrafish extended one-generation reproduction test (ZEOGRT) (draft OECD TG)

are expensive, both in terms of resources and animal use!



The required number of fish needed for this test strongly contrasts the 3R principle of:

- reduction
- replacement and
- refinement of animal experiments

Naidu et al., 2021 https://doi.org/10.1016/j.envint.2021.106616

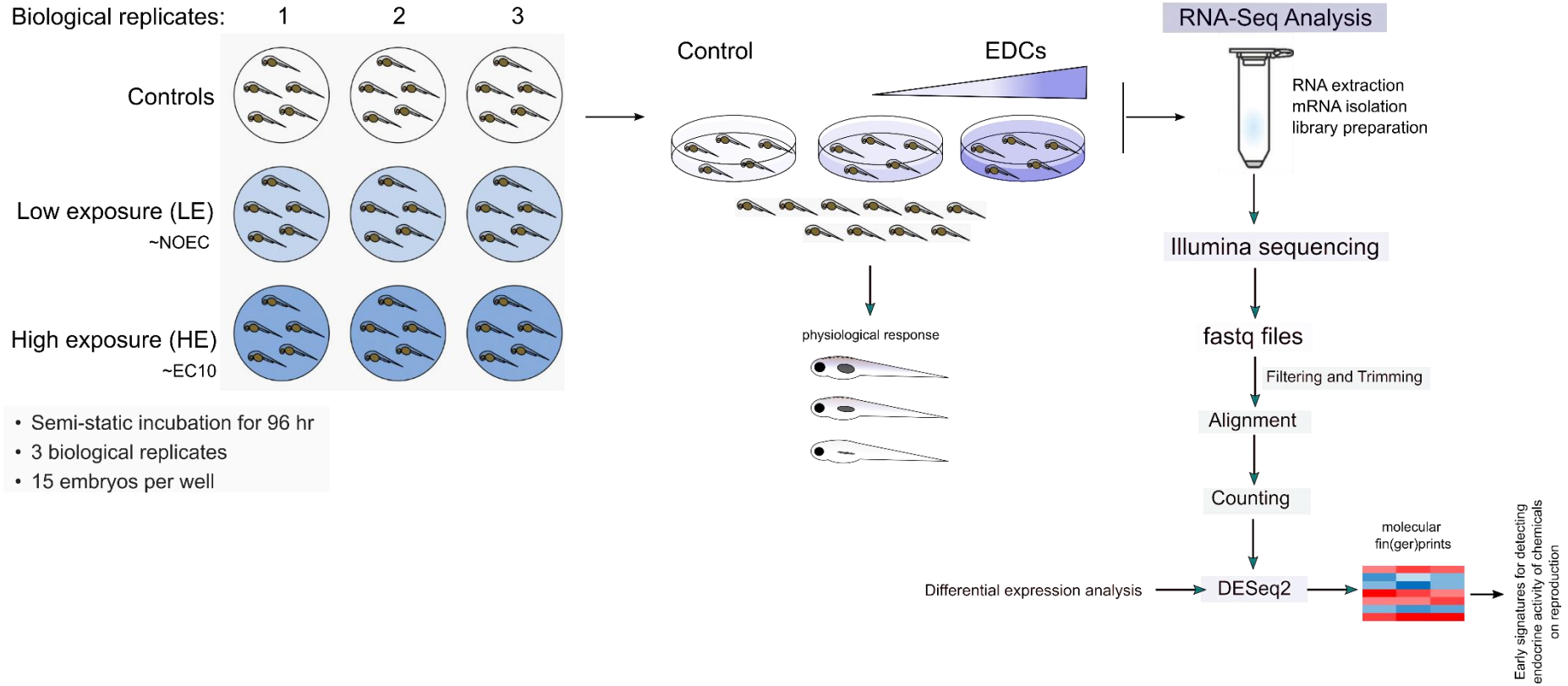
Test strategy

Test strategy

Objective

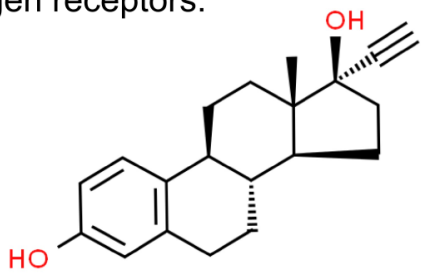
- To identify robust and reliable MoA-specific early signatures for detecting endocrine activity of chemicals on reproduction.

Experimental design

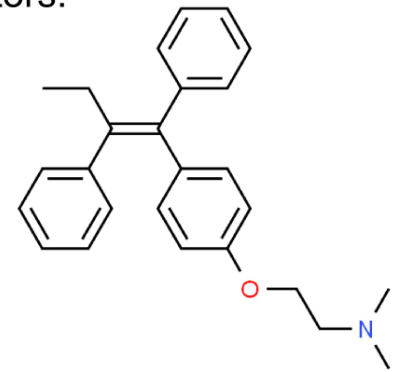


Endocrine disrupting substances with a known Mode of Action (MoA)

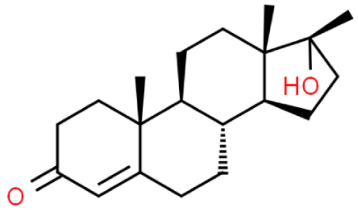
Ethinylestradiol stimulates the development and maintenance of female sex characteristics by binding to oestrogen receptors.



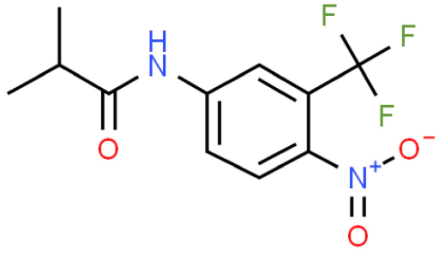
Tamoxifen inhibits the binding of estradiol to estrogen receptors.



Methyltestosterone is an androgen receptor agonist that stimulates the development and maintenance of masculine characteristics by binding to androgen receptors.



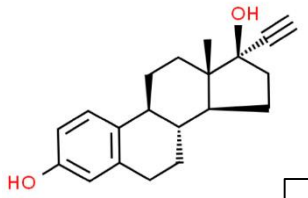
Flutamide iinhibits or antagonises the biosynthesis or actions of androgens.



Estrogen & Androgen targeting EDCs!

Test concentrations

Estrogen agonists

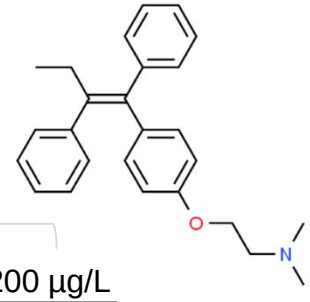


Ethinylestradiol

0	20	200 µg/L
Control	Low exposure	High exposure

Nominal conc.

Estrogen antagonists

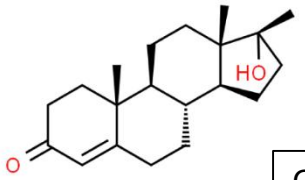


Tamoxifen

0	50	200 µg/L
Control	Low exposure	High exposure

Estrogen targeting EDCs!

Androgen agonists

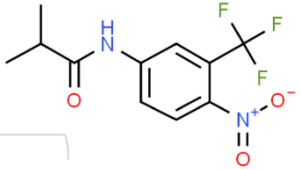


Methyltestosterone

0	1600	3200 µg/L
Control	Low exposure	High exposure

Nominal conc.

Androgen antagonists



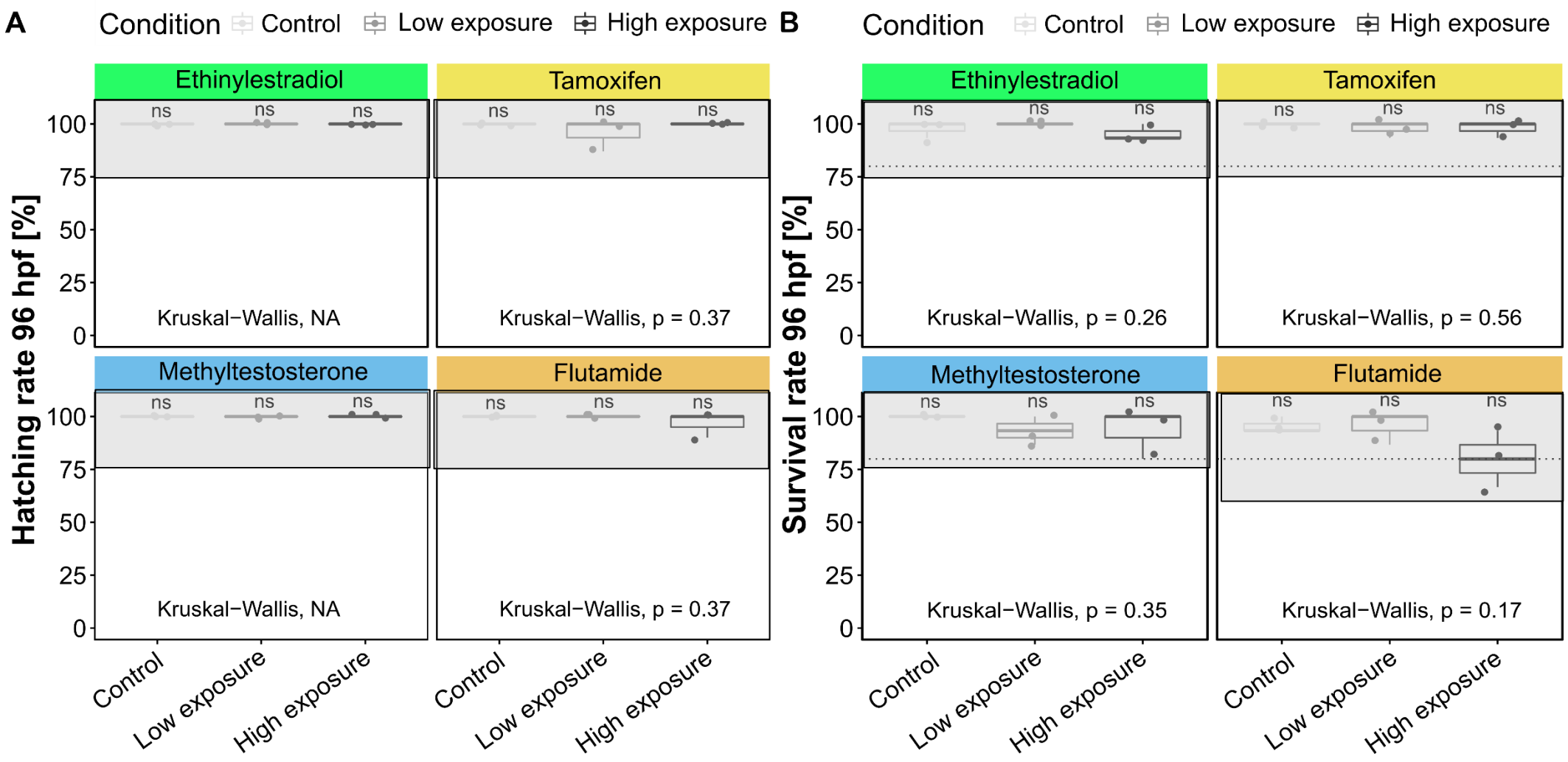
Flutamide

0	50	500 µg/L
Control	Low exposure	High exposure

Androgen targeting EDCs!

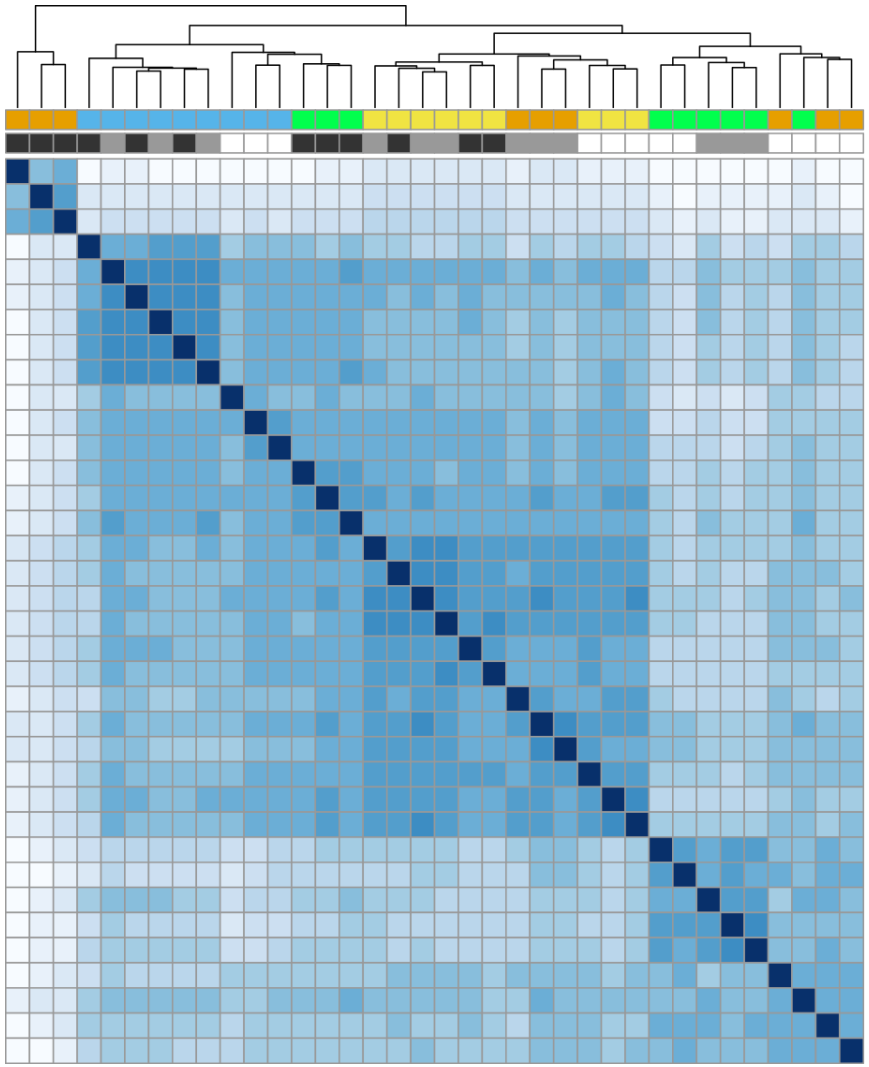
Result - Physiological effects

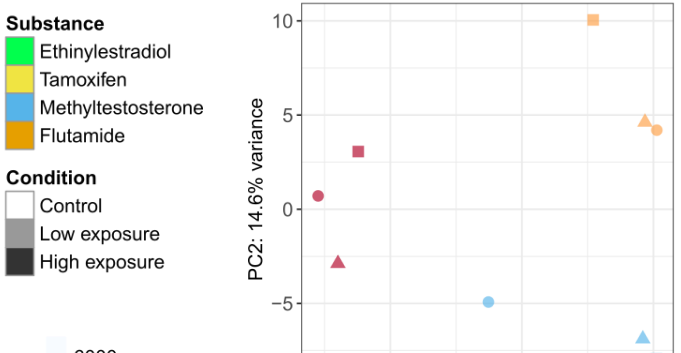
Physiological responses at 96 hpf

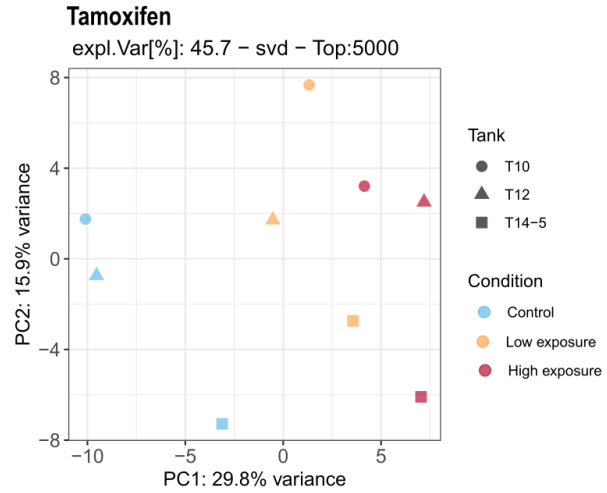


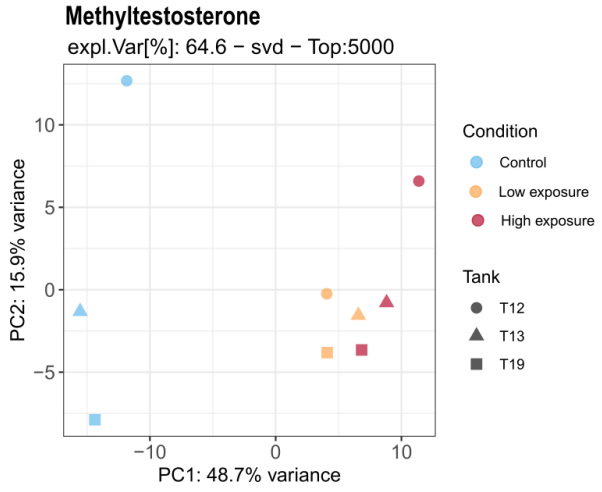
Transcriptomics Results

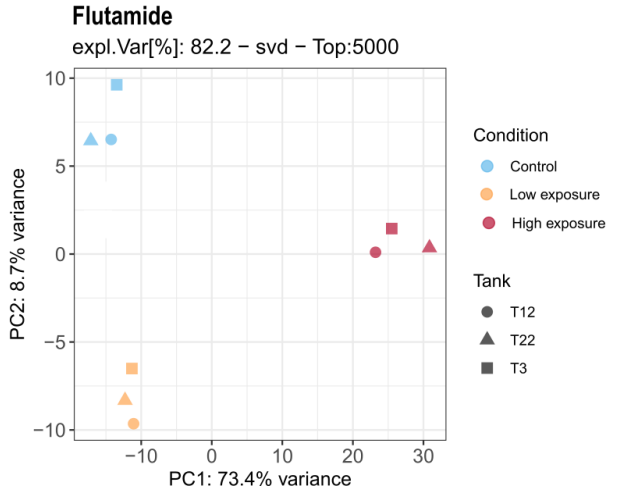
Sample Clustering and Principal Component Analysis





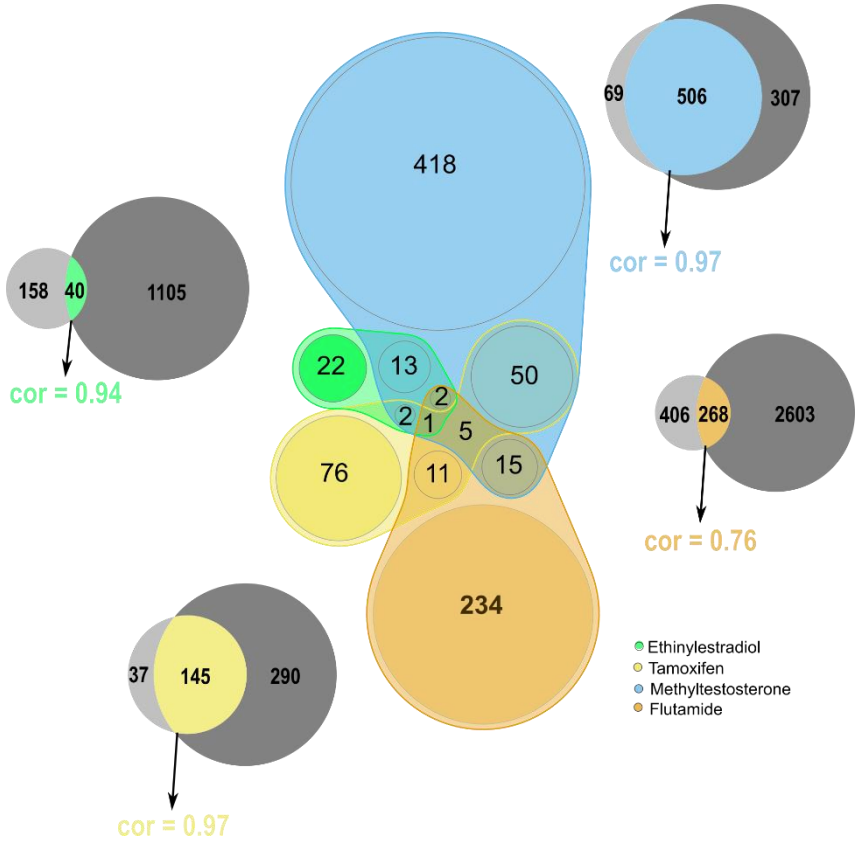


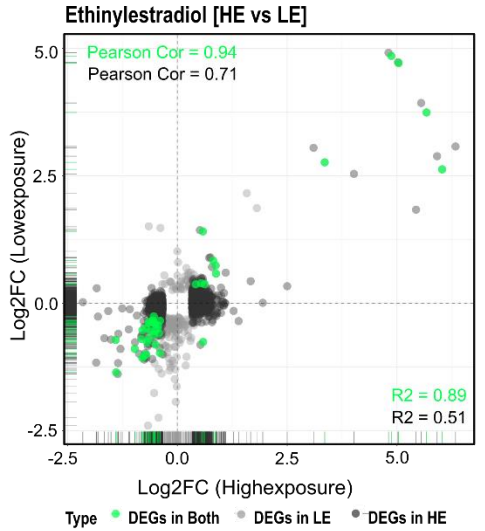


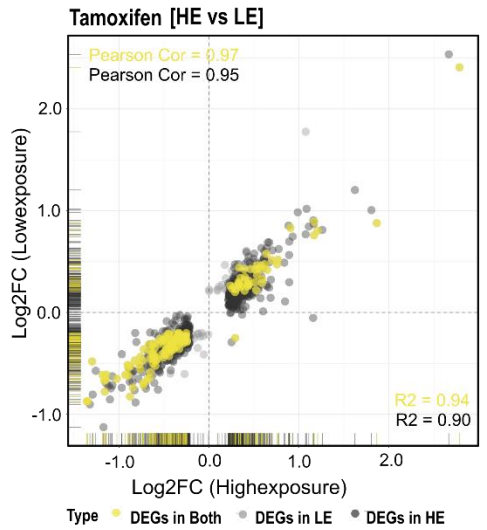


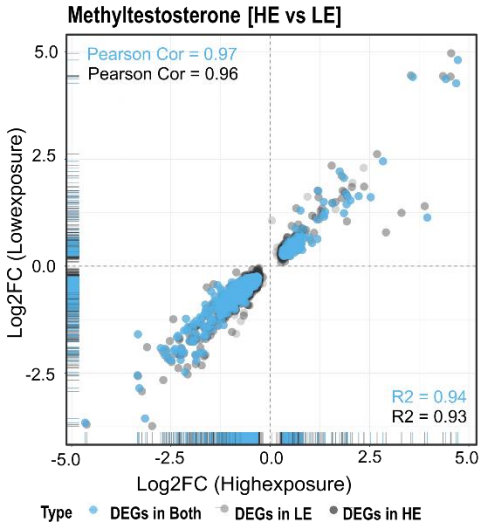
DEGs correlation for each EDCs

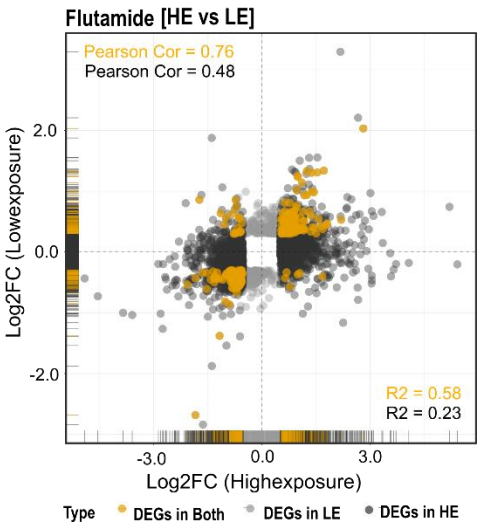
Strong and positive correlation!!!



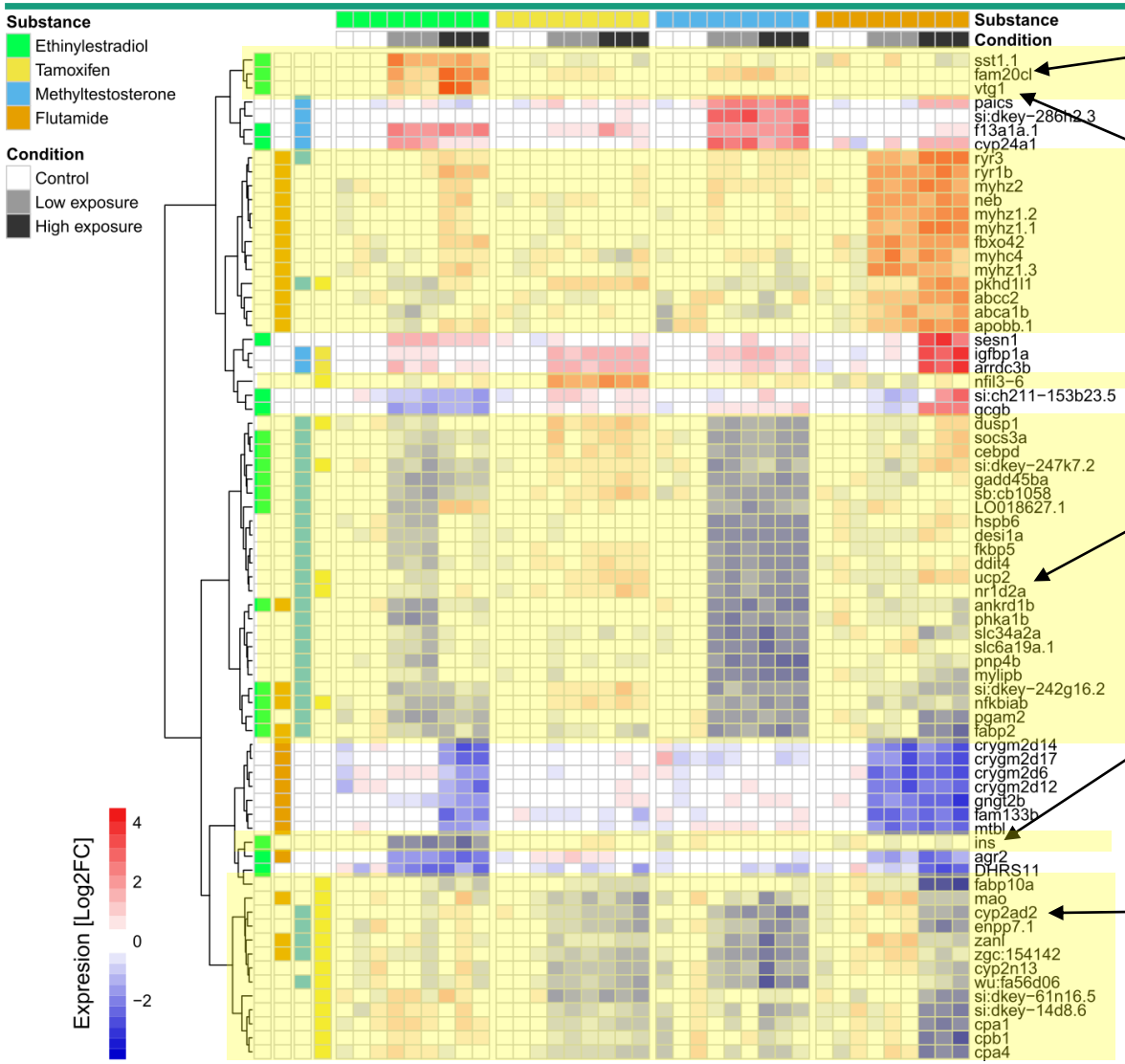








Biomarker candidates for each test substance



fam20cl: Family with sequence similarity 20 member C,-like

Involved in cellular response to estrogen stimulus.

vtg1: vitellogenin 1

Exhibits antioxidant activity. Involved in cellular response to estrogen stimulus; response to estradiol; and response to xenobiotic stimulus

nr1d2a: nuclear receptor subfamily 1, group D, member 2a

Predicted to have several functions, including nuclear receptor activity; steroid hormone receptor activity; and zinc ion binding activity.

ins: preproinsulin

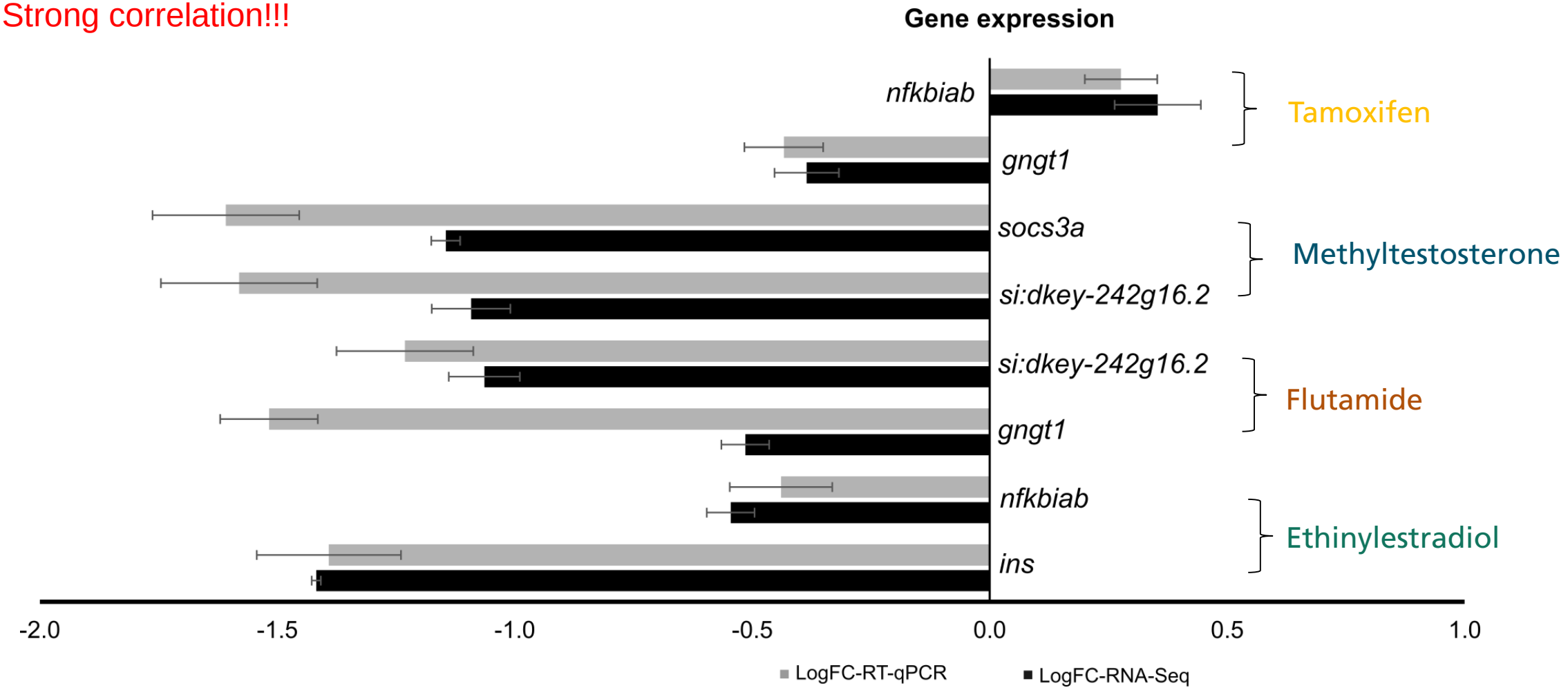
Predicted to have hormone activity. Is expressed in several structures, including blood plasma; dermal bone; digestive system; endocrine cell; and endoderm.

cyp2ad2: cytochrome P450, family 2, subfamily AD, polypeptide 2

Predicted to have heme binding activity; oxidoreductase activity and steroid hydroxylase activity.

DEGs validation with RT-qPCR

Strong correlation!!!



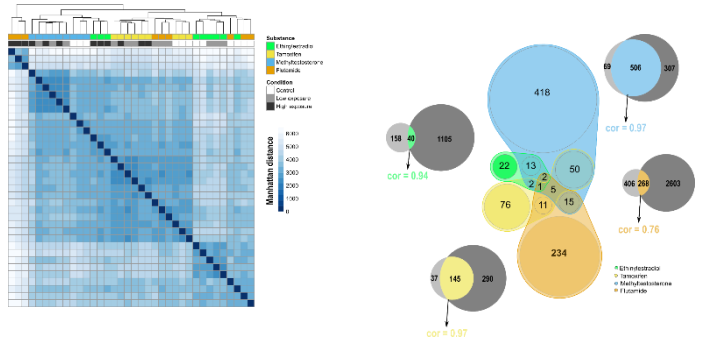
Overrepresentation analysis

Perturbed biological processess for substance prioritization!!

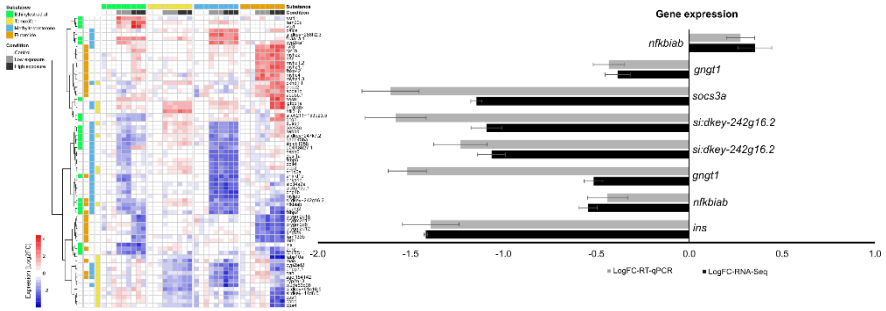
Take home message

Take home message

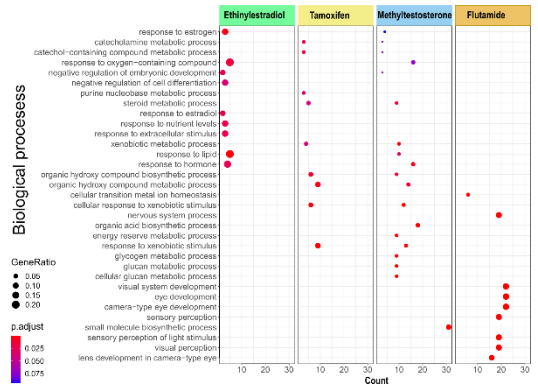
- Our study demonstrates that **omics-methodologies can help to identify biomarker candidates of endocrine disruption**.



- The **identified transcriptome fingerprints** can be utilized for assessing sexual-related impairment by endocrine active agents in zebrafish embryos.



- **Future screening** approaches developed on the basis of such data could enable for an **ab initio development of greener substances with less adverse effects on the aquatic environment**.



Thank you for your attention

The ATTRACT Eco'n'OMICs Group

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- Fatma Marghany (MSc.)
- Alexandra Loll (MSc.)
- Jannis Strompen
- Dr. Steve Ayobahan

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