Development of a liver transgenic zebrafish line (*vtg1:mCherry*) for the detection of estrogenic substances

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Introduction

A wide range range of synthetic and natural compounds can disrupt the endocrine system, thus adversely affect key physiological processes. Some hormonal substances act as analogues of natural estrogens and disrupt estrogen regulated processes.

Chemical structure of estrogenic substances is highly diverse, and additional compounds in the sample might also affect overall estrogenicity, so the full estrogenic impact of an environmental sample can only be revealed by a complex series of *in vitro* and *in vivo* ecotoxicological tests.

Our aim was to establish and characterize a transgenic zebrafish line (*vtg1:mcherry*) that - in the presence of estrogenic substances - produces red fluorescent protein in the liver.

Materials and methods

The line was generated by using the Tol2 transposon-based genomic integration system. The mCherry fluorescent protein coding region was built downstream to the promoter of the zebrafish *vitellogenin-1* gene (Figure 1.). Fragments were cloned into the construct with the Gateway site-specific recombination technique. Normally, *vitellogenin* genes are only expressed in adult females, and not or at very low levels in larvae and males, however the expression gets activated in the presence of estrogens. In the transgenic line, along with endogenous *vitellogenin*, fluorescent protein gene expression is expected to be induced in a concentration dependent manner.

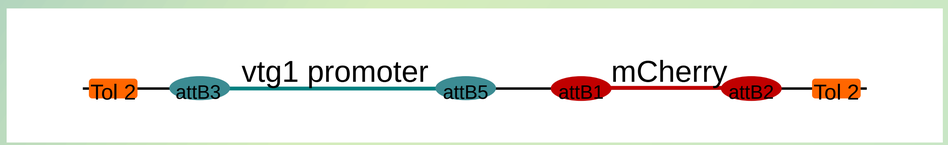

Figure 1. The transgene construct

Results

Seven generations of the line have been established, and embryos were exposed to different estrogenic substances (F4) and wastewater samples (F7) from 3 to 5 days post fertilisation (dpf). Fluorescence in embryos (Figure 2.) could be detected upon treatment with 17-ß-estradiol (E2) from a concentration of 100 ng/L, 17-α-ethynilestradiol (EE2) from 1 ng/L, zearalenone (ZEA) from 100 ng/L and bisphenol-A (BPA) from 1 mg/L respectively (Figure 3.).

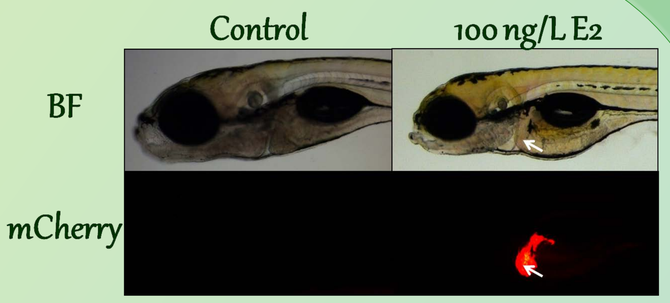

Figure 2. Liver-specific fluorescent signal in 5 dpf *vtg2:mCherry* embryos

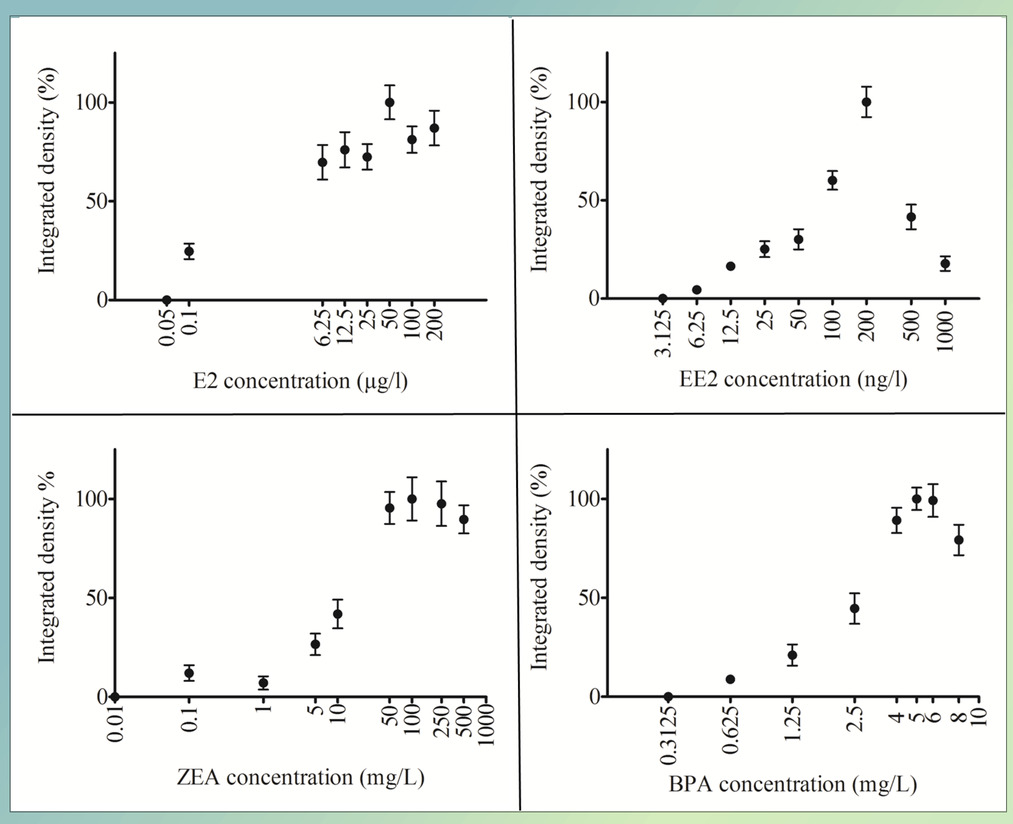

Figure 3. Dose-response curves for exposures to E2, EE2, ZEA and BPA in 5 dpf vtg1:mCherry larvae. Results are expressed as Integrated density generated from signal strength and the size of the affected area

In the adult stage transgene activity appeared to be more sensitive to treatment, with detectable transgene activity from 5 ng/L 17-ß-estradiol concentration (Figure 4.). The line was also suitable for the direct measure of estrogenicity in wastewater samples without sample extraction. Results were confirmed by the bioluminescent yeast estrogen screen (BLYES) (Figure 5.).

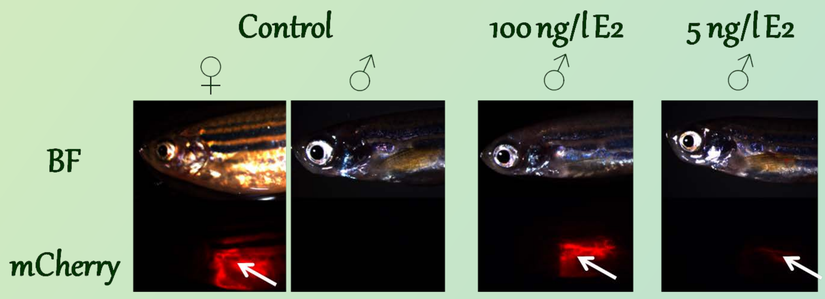

Figure 4. Fluorescent signal in untreated controls (male and female) and 17-ß-estradiol treated males

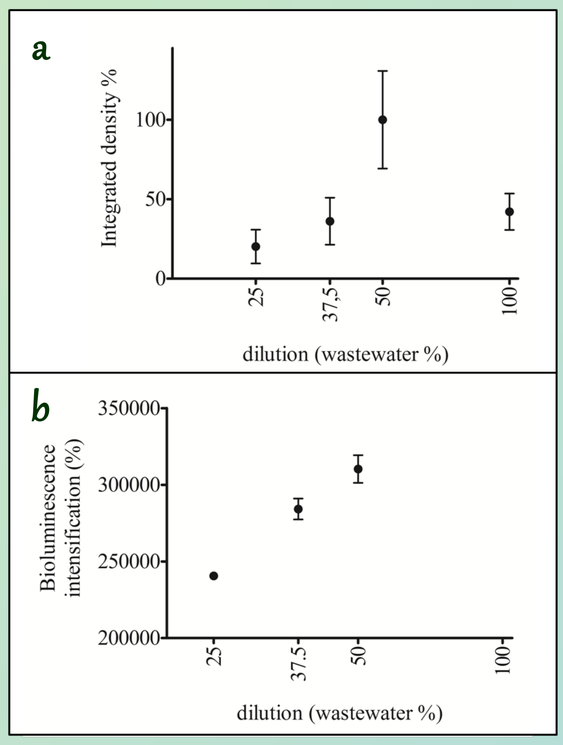

Figure 5. Dose-response curve for wastewater exposure in vtg1:mCherry larvae (a) and BLYES (b)

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