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Usefulness of the transgenic *cyp19a1b*-GFP zebrafish model to refine the zebrafish embryo acute toxicity test (ZFET)

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❖ Background and Objectives

The zebrafish embryo has emerged as a popular model as it provides a small *in vivo* experimental system with a diversity of applications including screening of chemicals [1]. The validation and the adoption of a test guideline (TG) for the zebrafish embryo toxicity test (FET) further promoted the use of the zebrafish model for assessing the hazards of chemicals into a regulatory context [2,3]. Recently, the EASZY assay was validated and the corresponding TG is under discussion for its adoption within OECD. EASZY allows to sensitively quantify the estrogenic activity of chemicals acting on the glial cell-specific expression of the ER-regulated *cyp19a1b* gene. FET and EASZY covered the same stages of development but are performed either on wild-type (FET) or transgenic *cyp19a1b*-GFP (EASZY) embryos [4]. **Herein, our objective was to evaluate the usefulness of using *cyp19a1b*-GFP model for combining the assessment of toxicity and endocrine potency of test chemicals in the FET [3].**

❖ Materials and Methods

① **Selected Chemicals:** 11 chemicals were selected from the FET validation study [5] as they cover a diversity of usage and a wide range of toxicity. In addition, 4-tert-octylphenol (4-tert-OP) was selected for its known estrogenic activity.

② **FET tests** were performed on wildtype AB strain (wt) and tg(*cyp19a1b*-GFP) (tg) embryos according to the TG 236 [3].

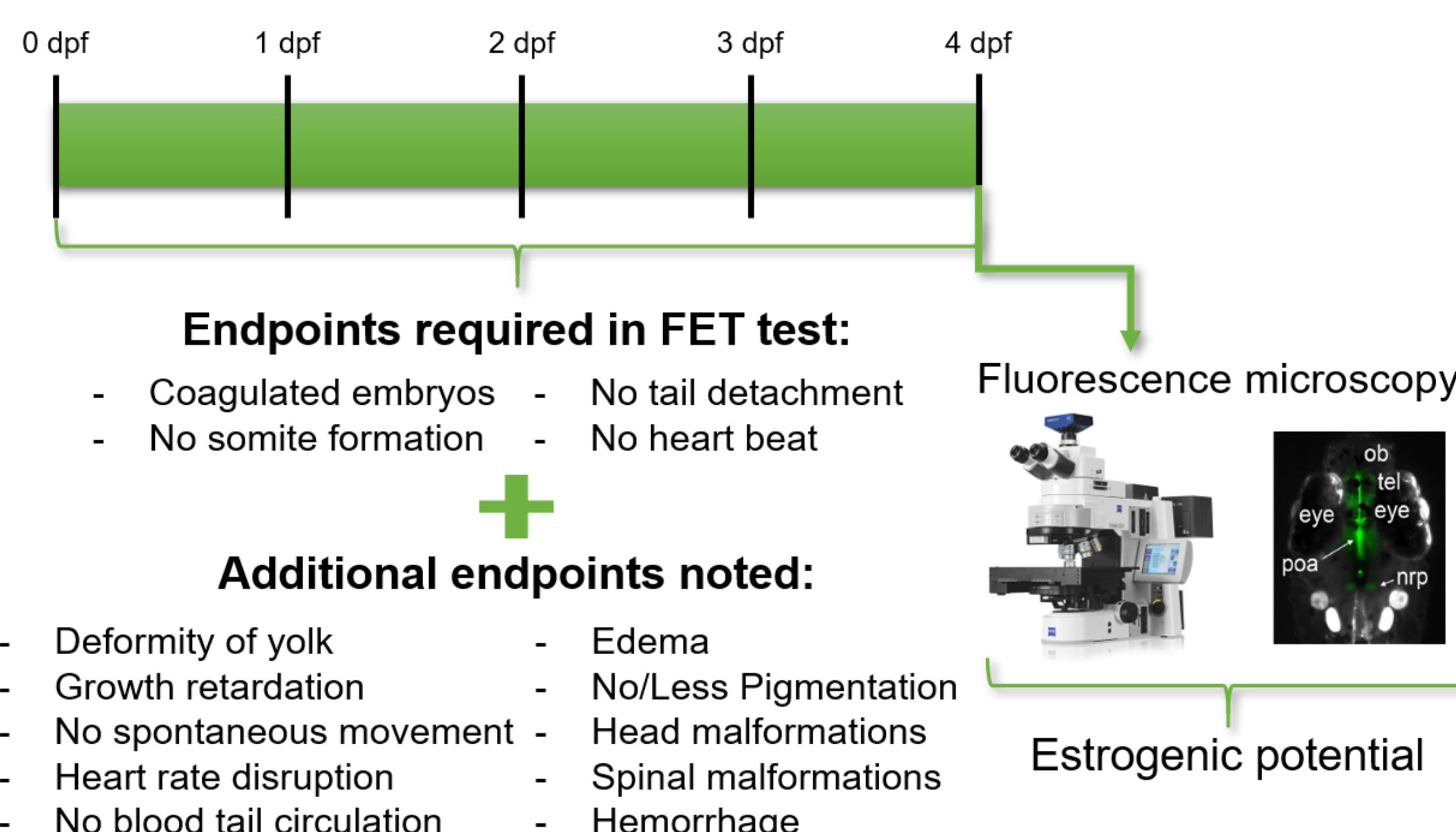


Figure 1: Protocol for exposition to chemicals and endpoints studied

③ **Toxicity and developmental effects:** LC50, using the four endpoints required in the FET test, were derived using the Hill model for each tested chemical in wt and tg embryos; data were then compared with those from the OECD validation study [2] and literature data for 4-tert-OP [5-7]. For the developmental effects, LOECs were determined for each additional endpoint recorded and chemicals tested in wt and tg.

④ **Fluorescence imaging** was performed at the end of the FET test with the *cyp19a1b*-GFP embryos exposed to non-toxic concentrations.

❖ Results

① Lethal effects (LC50) are similar for wt and tg embryos

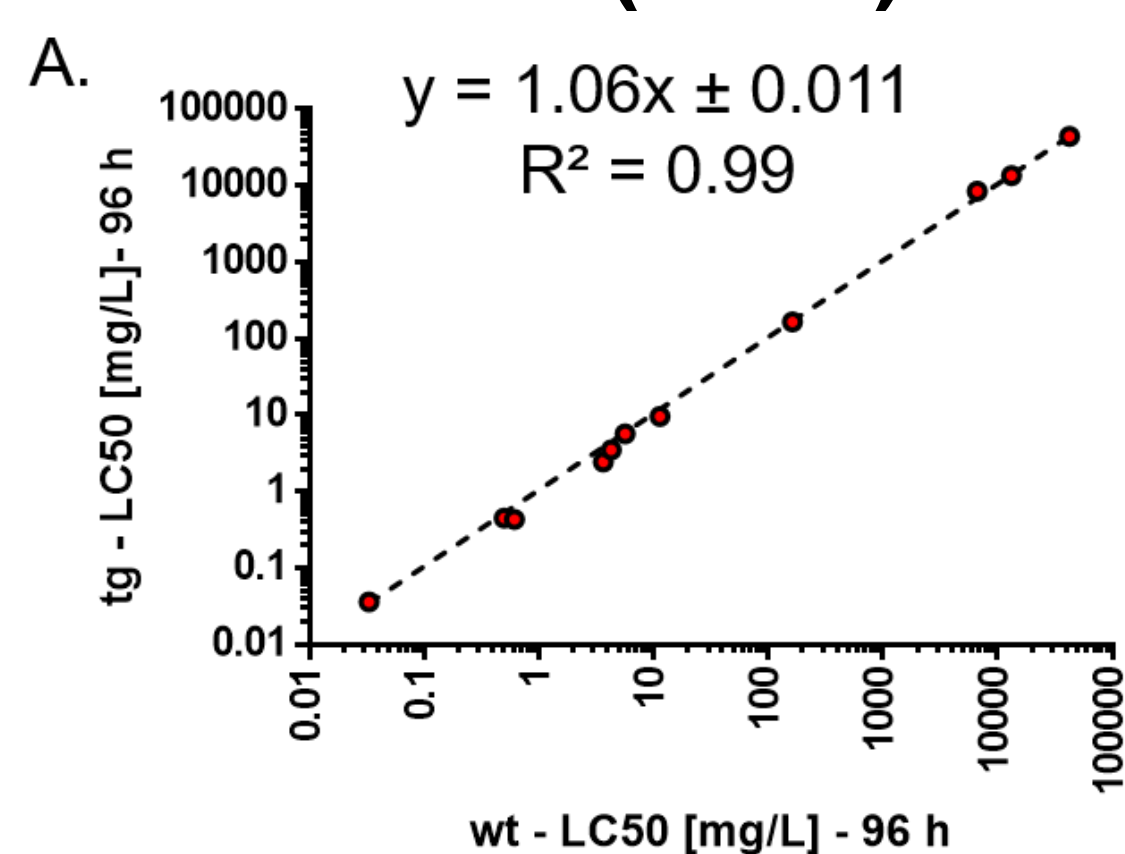
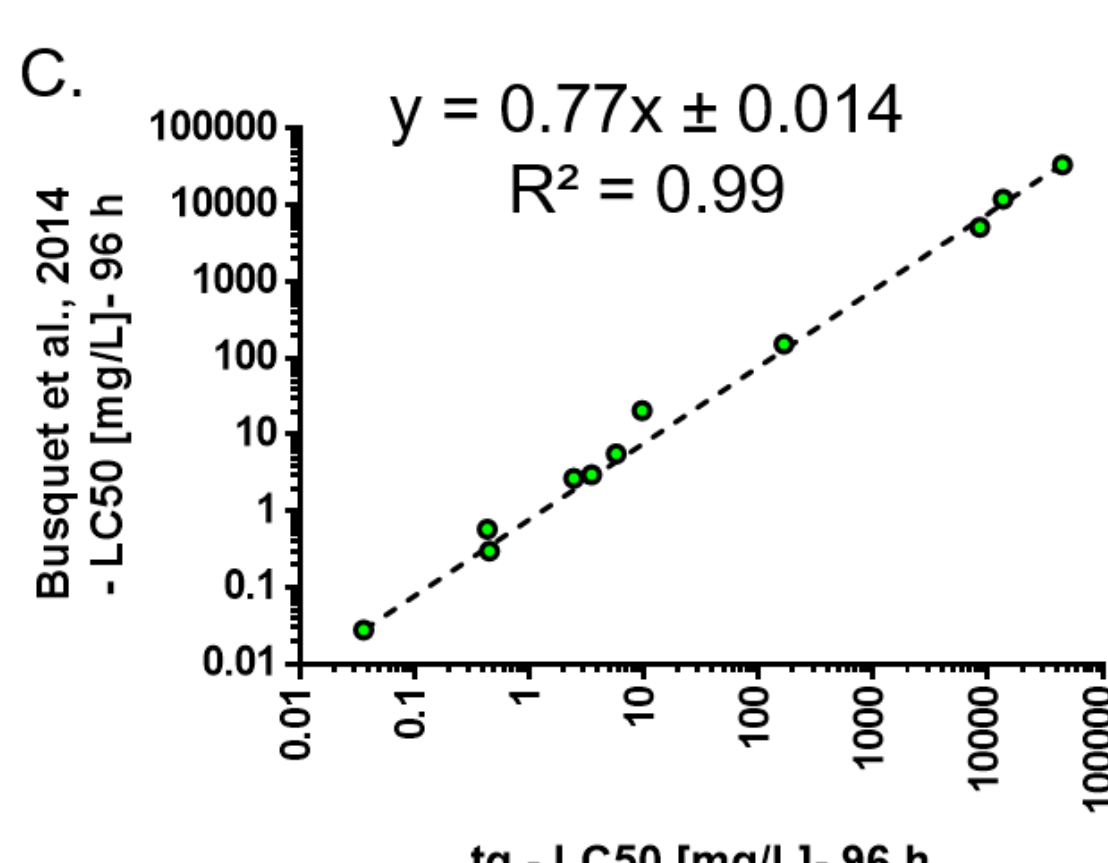
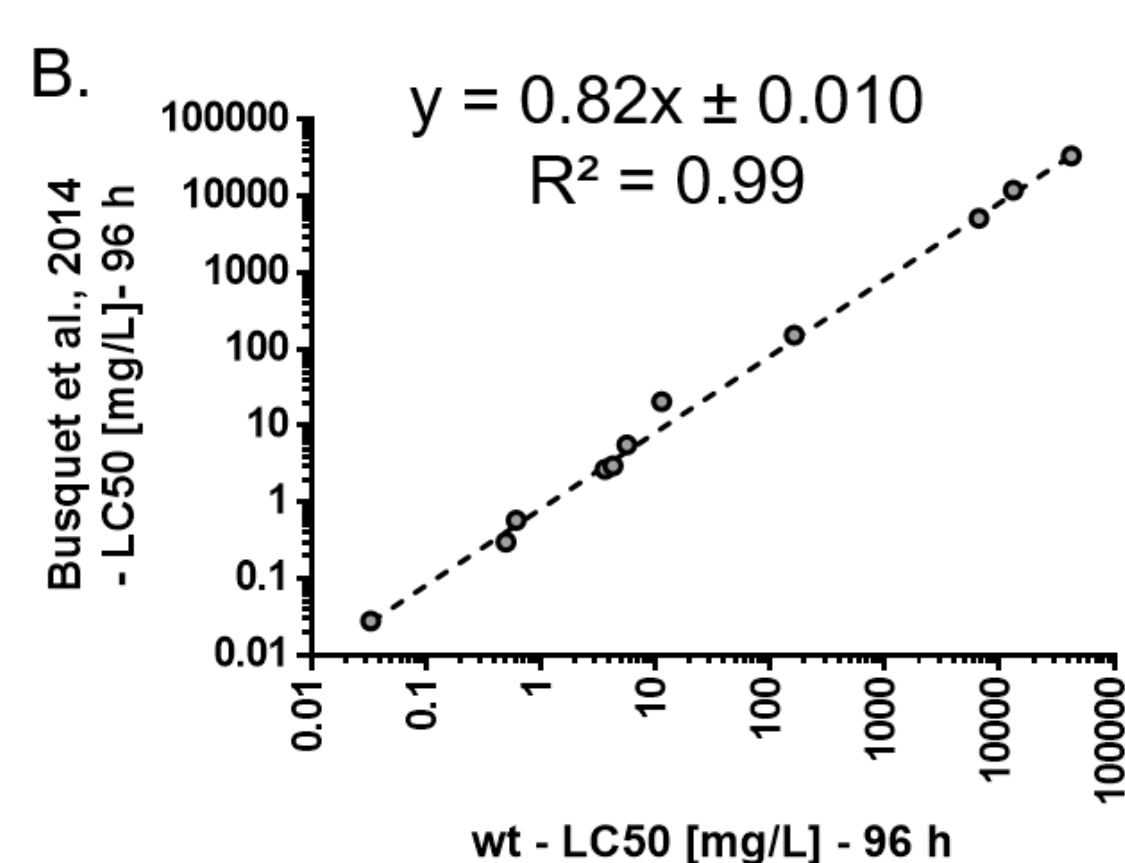


Figure 2: Correlation between (A) wild-type and transgenic LC50(96h) and (B-C) with LC50(96h) of the validation study [2].



② Developmental effects (LOECs) are analogous between wt and tg embryos

Table 2: LOECs reported for each chemicals in wt and tg.

Chemicals	LOECs (mg/L)		Endpoints occurring at these LOECs
	AB strain	<i>cyp19a1b</i> -GFP	
Methylmercury (II) chloride	0.00625	0.00625	Hemorrhage
Triclosan	-	-	-
4,6-Dinitro-o-cresol	0.32	0.32	Growth retardation, Spinal malformations
3,4-Dichloroaniline	3	1	Edema
2,4-Dinitrophenol	2.5	2.5	Growth retardation, Spinal malformations
Prochloraz	8	8	Edema, Blood tail circulation, Pigmentation, Spinal malformations, Hemorrhage
1-Octanol	2.5	2.5	Spinal malformations
Carbamazepine	107	76.5	Hemorrhage
Sodium chloride	4 000	4 000	Spinal malformations
Ethanol	5 300	5 300	Edema
Dimethyl sulfoxide	30 000	30 000	Spinal malformations
4-tert-octylphenol	0.25	0.25	Spinal malformations

③ Detection of estrogenic activity of chemicals using tg(*cyp19a1b*-GFP) embryos

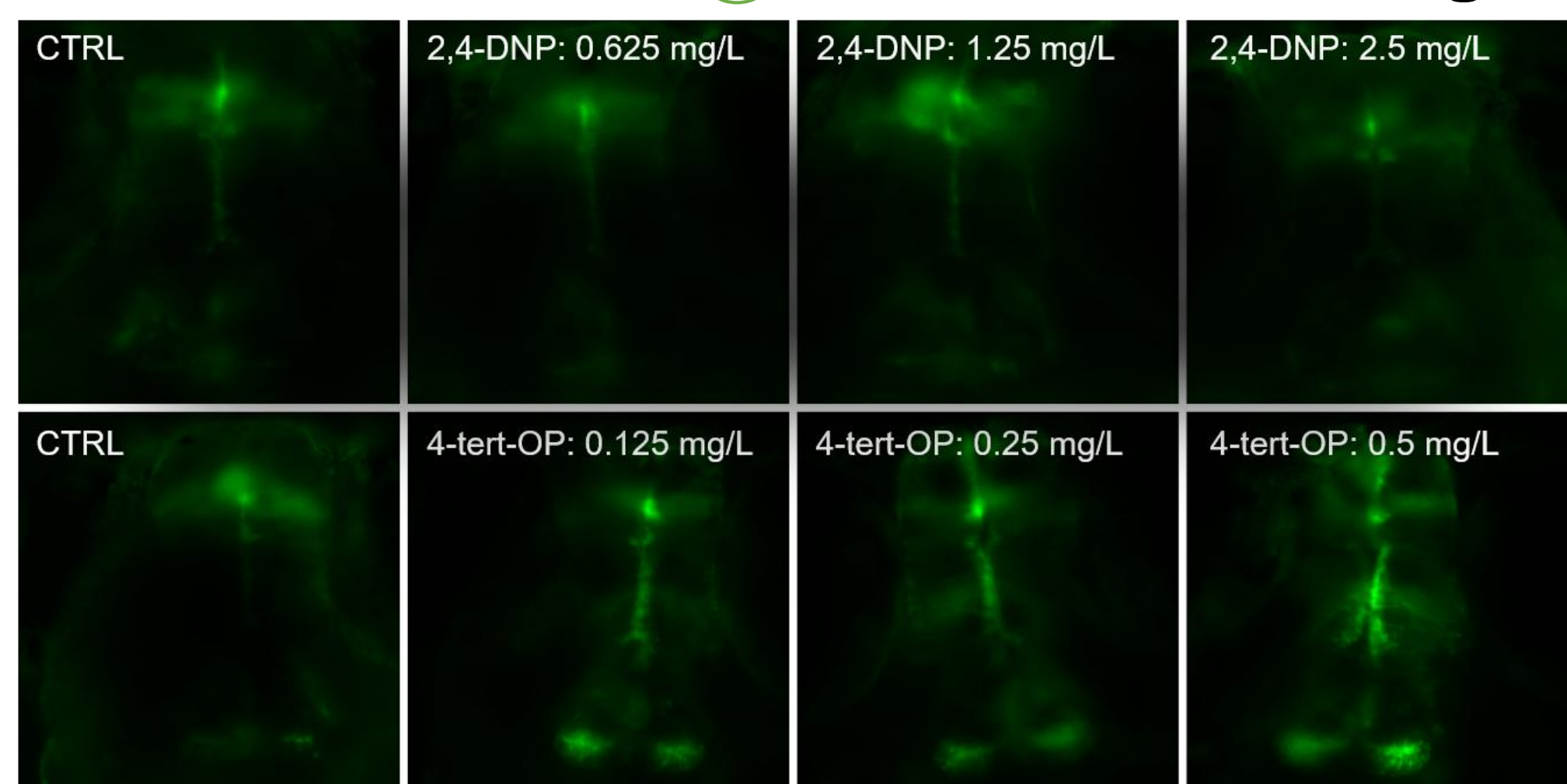


Figure 3: Photomicrographs of *cyp19a1b*-GFP transgenic zebrafish embryos solvent control (CTRL) and exposed to 2,4-DNP (inactive) and to 4-tert-OP (active); head was photographed using X10 objective.

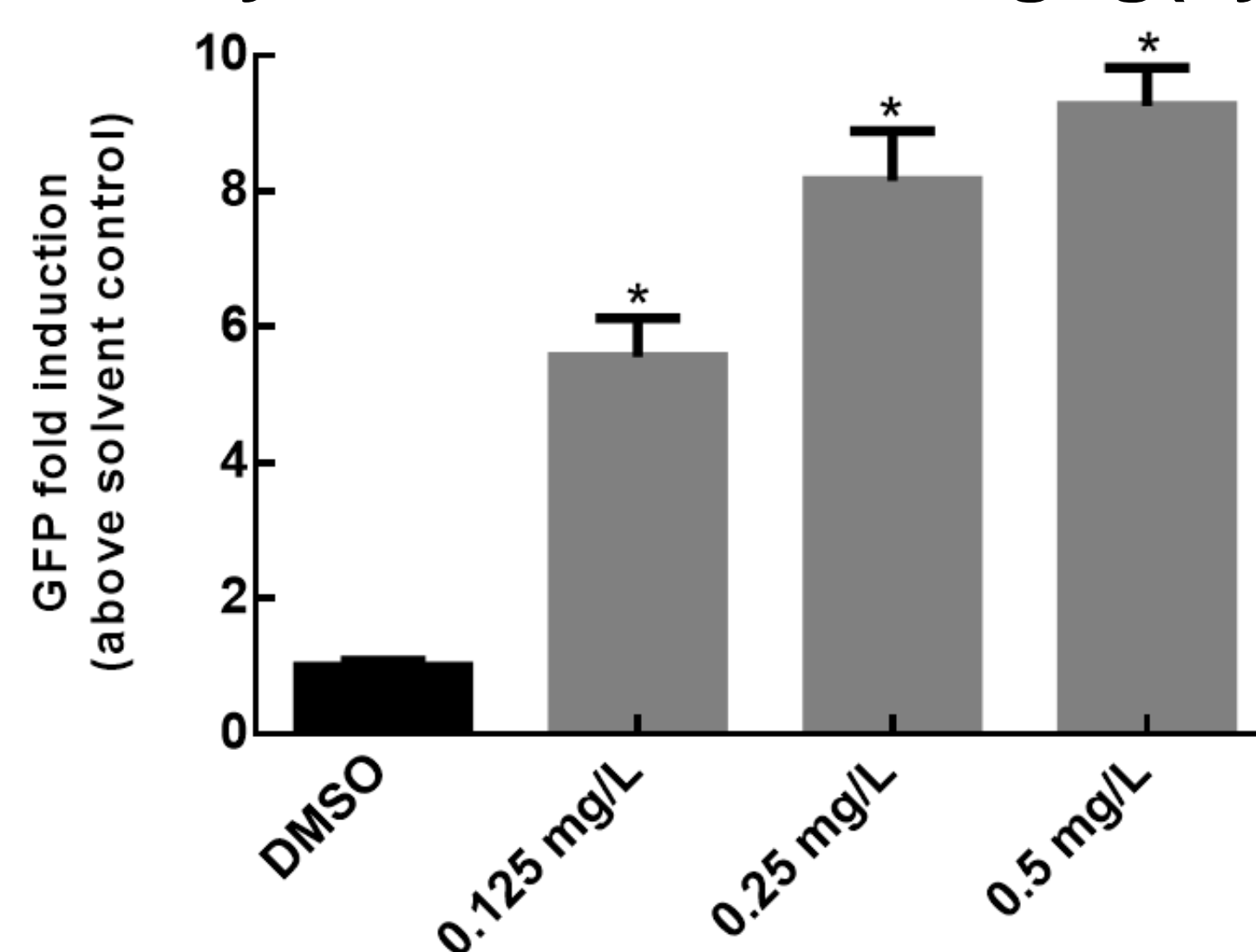


Figure 4: GFP induction at 96h after exposure to 4-tert-OP, data are represented as mean ± SEM.

Substance	Estrogenic activity
4,6-Dinitro-o-cresol	inactive
2,4-Dinitrophenol	inactive
Prochloraz	inactive
1-Octanol	inactive
Carbamazepine	inactive
4-tert-octylphenol	active

Table 3: Identification of estrogenic and non-estrogenic chemicals at the end of the FET test based on fluorescence imaging.

❖ Conclusions & Perspectives

- Comparison of the acute and developmental toxicity data revealed that both **wildtype** and **transgenic** embryos have **similar sensitivity** to chemicals, as shown by **similar toxic and developmental responses**.
- Our data indicates that the ***cyp19a1b*-GFP zebrafish embryo model** can be used in the FET test to assess the **toxicity** of test chemicals, allowing to provide additional preliminary information on the **endocrine potency** of chemicals, thereby **refining** the actual FET assay and enhancing the **hazard assessment**.
- Consequently, realizing a FET test with *cyp19a1b*-GFP transgenic embryos allows to develop **efficient testing strategy**; which is the **combination of FET and EASZY assays**: if GFP is induced at the end of the FET test for non-toxic concentrations of chemicals, a complete EASZY assay is carried out; and if GFP is not induced, EASZY assay is not realized.

❖ References

- [1] Scholz et al., (2008); *ESPR* **15**, 394–404
 [2] Busquet et al., (2014); *RTP* **69**(3), 496-511
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 [4] Brion et al., (2012); *PLoS One* **7**(5), e36069
 [5] Cruz-Li (2004); Thesis, Texas Tech University
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 [7] Wenzel et al., (2001); *Contract* **6**(7920/98), 000015