Advancing the zebrafish embryo test for estrogen receptor agonist screening using aquatic exposure and micro-injection

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Abstract

Fish (embryo) toxicity test guidelines are mostly based on aquatic exposures. However, in some cases, other exposure routes can be more practical and relevant. Micro-injection into the yolk of fish embryos could offer a particular advantage for administering hydrophobic compounds, such as many endocrine disruptors.

Single dose micro-injection was compared to continuous aquatic exposure in terms of compound accumulation and biological responses. The potent estrogen receptor (ER) agonist 17α-ethinyl estradiol (EE2) was used as a model compound for this part of the study. First, the optimal solvent and droplet size were selected, and needle variation was assessed. Next, biological endpoints were evaluated. The accumulated internal dose of EE2 decreased over time in both exposure scenarios. Estrogen receptor (ER) activation, which was evaluated using transgenic zebrafish (*5xERE:GFP*), was concentration/injected dose dependent, increased daily and was related to *esr2b* transcription. *Vtg1* (vitellogenin) and *cyp19a1b* transcription was induced in both scenarios, but the *cyp19a1b* transcription pattern differed between exposure routes. Injection caused an increase of *cyp19a1b* transcripts from 48 hours post fertilization (hpf) onwards, while after aquatic exposure the main increase occurred between 96 and 120 hpf. Some malformations only occurred after injection, while others were present in both exposure scenarios.

The aim of the second part of the study was to validate the use of ER activation and *vtg 1* and *cyp19a1b* gene transcription as biomarkers, also for less potent ER agonists. Furthermore, protein measurements of vitellogenin were performed to evaluate vitellogenin as a biomarker on a higher level compared to gene transcription. For this purpose 4-tert-octylphenol (an ER agonist with moderate potency) and bisphenol A (a weak ER agonist) were used in addition to EE2. We conclude that responses can differ between exposure routes and therefore micro-injection is not a direct substitute for, but can be complementary to aquatic exposure. Nevertheless, *vtg1* and *cyp19a1b* transcription, ER activation and vitellogenin protein measurements are suitable biomarkers for ER agonist screening using zebrafish embryos in both exposure scenarios.