Abstract

Dynamics of fluorescence intensity of transgenic zebrafish (5xERE:GFP) exposed to 17α-ethinylestradiol: comparison between 2 exposure routes

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Pharmaceutical companies have to perform an environmental risk assessment for every drug that is launched on the market. The mandatory tests for potential endocrine disrupting (ED) compounds require a lot of time and laboratory animals, which is not consistent with the 3R principle. Therefore, the goal of this study is to develop a zebrafish embryo test, which is not considered an animal test according to European regulations. However, it is often difficult to expose fish aquatically to ED pharmaceuticals because of their lipophilicity. Nano-injection is therefore proposed as an alternative exposure route because the yolk of zebrafish embryos contains many lipids, and this route mimics maternal transfer. To use nano-injection as an alternative it needs to be characterised and compared to the classical exposure route via water, because it is generally known that toxicity can depend on the exposure route. In this part of our study 17α-ethinylestradiol (estrogen receptor (ER) agonist) was chosen as a model compound to compare binding to the ER between both routes over time.

Transgenic (*5xERE-GFP*, obtained from the Carnegie Institution of Washington) zebrafish embryos, expressing GFP upon ER binding, were aquatically exposed or injected. 0.5 nl of 17α-ethinylestradiol dissolved in dimethyl sulfoxide was injected. Dimethyl sulfoxide was also used as vehicle control. Afterwards, the embryos were monitored until 120 hours post fertilization. Each day, spectrofluorometric measurements were used to compare the time-dependent dynamics of ER binding in both routes.

Throughout the 5 day period, we observed fluorescent signals in the same order of magnitude in embryos from both routes, indicating that 17α-ethinylestradiol was still active 5 days after injection. The fluorescent intensity increased every day after both aquatic exposure and nano-injection, corresponding to the increase of *esr2b* mRNA levels during normal development. Furthermore, within each day, a concentration dependent increase was observed.

The dynamics of ER binding are similar between both exposure routes, suggesting that other mechanisms (e.g. uptake in different organs) are responsible for the differences in morphological and physiological effects. Nano-injection can be used as an alternative exposure route but with precaution.