Nano-injection in the zebrafish embryo as an alternative exposure route for environmental risk assessment of endocrine disrupting pharmaceuticals

Michiels Ellen D. G., University of Antwerp (Presenting author)
Vergauwen Lucia, University of Antwerp
Corremans Christoph, University of Antwerp
Van Cruchten Steven J., University of Antwerp
Knapen Dries, University of Antwerp

Pharmaceutical companies are required 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 it mimics maternal transfer. To use nano-injection as an alternative exposure route it needs to be characterised and compared to the classical exposure route via water. As a case-study 5 ED pharmaceuticals with each a different mode of action (MoA) were chosen (i.e., estrogen receptor (ER) agonism and antagonism, androgen receptor (AR) agonism and antagonism and aromatase inhibition).
Zebrafish embryos were injected with 0.5 nl of one of the pharmaceuticals or with dimethyl sulfoxide (vehicle control). After injection, the embryos were monitored until 120 hours post fertilization. Using a profile based on multiple biological responses both exposure routes were compared.
Transgenic (5xERE-GFP, obtained from the Carnegie Institution of Washington) exposed embryos gave a significantly higher fluorescent signal compared to controls and with a comparable order of magnitude between both routes, indicating that 17α-ethinylestradiol (ER agonist) was still active 5 days after injection. Nano-injection or aquatic exposure also caused morphological and physiological effects after exposure to 17α-ethinylestradiol (ER agonist) or to 17β-trenbolone (AR agonist). This was the case for both routes but the effects were different. After exposure to fulvestrant (ER antagonist) or flutamide (AR antagonist) only mortality or no effects at all were observed. Letrozole (aromatase inhibitor) only caused physiological effects for both routes.
Nano-injection can be used as an alternative exposure route because effects were detectable and pharmaceuticals were still active after 5 days. In general, the effects caused by the pharmaceuticals were comparable between both exposure routes but detailed effects were different.

Keywords: zebrafish embryo, endocrine disruption, pharmaceuticals, nano-injection

Contact: ellen.michiels@uantwerpen.be