WE215 Development of a zebrafish embryo test for environmental risk assessment of pharmaceuticals with endocrine disrupting properties

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Pharmaceutical companies are obligated to perform an environmental risk assessment for each new drug that is launched on the market. The mandatory tests for potential endocrine disrupting (ED) compounds require a lot of test animals, which is not consistent with the 3R principle. Therefore, the aim of this study is to develop a zebrafish embryo test, which is not considered an animal test according to the European regulation on the use of laboratory animals, and which is capable of detecting and discriminating among 5 endocrine disrupting modes of action (MOAs). The MOAs that will be studied are aromatase inhibition, estrogen receptor (ER) agonism and antagonism, androgen receptor (AR) agonism and antagonism. As a first stage of test development, 1 pharmaceutical per MOA was investigated to map differences among the 5 compounds in zebrafish embryos at the morphological, physiological and molecular level. We are currently exposing transgenic zebrafish embryos of 2 lines to aid in discriminating among the MOAs: *SR4G* (fluorescent signal after glucocorticoid receptor activation) and *5xERE:GFP* (reports estrogen receptor activation). An ontogeny study is ongoing to determine the gene transcription levels of the receptors, biomarkers and enzymes of the steriodogenesis pathway which will be compared to the gene transcription levels of embryos which were exposed to each of the 5 pharmaceuticals. As a model for ER agonism, 17?-ethinylestradiol (EE2) was used. Fulvestrant was used to investigate ER antagonism. 17?-trenbolone and flutamide were respectively the AR agonist and antagonist. Letrozole was used as a model for aromatase inhibition. Both receptor agonists showed some sublethal effects, while no sublethal effects were observed after exposure to flutamide. After EE2 exposure, the embryos showed sublethal, dose-dependent skeletal malformations of the spine. Cranial malformations were only significant around the LC_{50} concentration and above. For 17?-trenbolone heart rate and growth were already significantly decreased at sublethal concentrations. Swim bladder inflation and swimming behaviour were impaired for both agonists at sublethal concentrations. The data available from 5 medicines with 5 different ED MOAs show the potential of a combination of different datatypes to distinguish among the 5 MOAs. In order to establish a consistent profile that can be used for ED screening of medicines, additional compounds will be tested.