

WP015 Life-stage-specific endpoints for estrogen disruptor screening in the zebrafish embryo

L. Vergauwen, University of Antwerp / Zebrafishlab / Dept Veterinary Sciences & SPHERE Dept Biology; E.D. Michiels, J. Periz Stanacev, University of Antwerp / Zebrafishlab / Dept Veterinary Sciences; A. Van Nuijs, University of Antwerp / Toxicological Center; A. Covaci, University of Antwerp / Toxicological Center; S.J. Van Cruchten, University of Antwerp / Applied Veterinary Morphology / Dept Veterinary Sciences; D. Knapen, University of Antwerp / Zebrafishlab / Dept Veterinary Sciences

New and improved approaches are needed to meet evolving regulatory requirements to evaluate ecotoxicity of chemicals worldwide, while at the same time reducing the use of laboratory animals. In this context, the fish embryo offers particular advantages since in several geographic regions, it is not protected under laboratory animals legislations. Additionally, an OECD test guideline for the Fish Embryo Acute Toxicity (FET) test (TG 236) is already available and can be used as a basis to add relevant exposure methods and biological endpoints for specific purposes. The use of fish embryos instead of adult fish can be considered from different perspectives. Either the fish embryo is used as a model for adult fish and adult endpoints are somehow translated and applied in embryos, or embryo-specific endpoints are studied. In this study we used endocrine disruptor screening in the zebrafish embryo as a case study and we investigated the potential of adding life-stage-specific exposure methods and endpoints. The presence of the yolk in early embryos results in an additional exposure route, corresponding to maternal transfer in a real-life scenario. We explored the use of micro-injection into the yolk as a way to dose chemicals, including hydrophobic compounds, to developing embryos. Although time- and dose-dependent estrogen receptor activation profiles were highly similar, we found evidence that the dynamics of molecular processes differed between micro-injection and the traditional aquatic exposure method, causing disruptions of development at distinct life stages and leading to different adverse effects. Adding a typical endpoint for estrogen disruption, vitellogenin protein levels, that is generally used in later life stages, was possible but seemed to provide limited sensitivity in embryos compared to adults or juveniles. Development is tightly regulated by hormones and thus highly sensitive to endocrine disruption. Here we show that neuromast development is indeed very sensitive to estrogen disruption, comparable to endpoints used in adult fish. In conclusion, when using fish embryos as alternatives to adult fish in toxicity testing, one can either choose to apply endpoints that are well-accepted in later life stages, or one can try to find new, embryo-specific endpoints, which may be more relevant and additionally may increase sensitivity.