

## Population relevant effects on fish – an update on ZEOGRT validation

Matthias Teigeler<sup>1</sup>, Elke Eilebrecht<sup>1</sup>, Christoph Schäfers<sup>1</sup>, Gerd Maack<sup>2</sup>,  
Susanne Walter-Rohde<sup>2</sup>

<sup>1</sup>Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Auf dem Aberg 1, 57392  
Schmallenberg, Germany

<sup>2</sup> Umweltbundesamt (German Environment Agency), Wörlitzer Platz 1, 06844 Dessau-Roßlau, Germany  
E-mail contact: [matthias.teigeler@ime.fraunhofer.de](mailto:matthias.teigeler@ime.fraunhofer.de)

---

In 2015, OECD adopted a protocol for an Extended One Generation Reproduction Test (EOGRT) with Medaka (*Oryzias latipes*) as Test Guideline 240. This test protocol includes the exposure of a parental generation (F<sub>0</sub>), a full Filial 1 (F<sub>1</sub>) generation and a Filial 2 (F<sub>2</sub>) generation.

As this protocol was designed and validated for a single test species only, there was an initiative from Germany at OECD to develop a similar test approach with zebrafish (*Danio rerio*). Data from the initial phase of validation are presented.

The aim of the phase 1 of validation - including the performance of four ZEOGRT studies - was to proof the applicability of the test protocol for the test species zebrafish. The obtained effect concentrations were compared with existing data from the MEOGRT validation studies and from the literature. The studies were conducted with tamoxifen-citrate, prochloraz, dienogest and dexamethasone.

The list of relevant life stages and endpoints included reproduction of the F<sub>0</sub> generation, early life stages, juvenile growth, sexual maturation, and reproduction of the F<sub>1</sub> generation, and the early embryonal stage of the F<sub>2</sub>. Blood plasma samples of the adult fish of both generations were measured for vitellogenin (VTG) concentrations; a histopathological examination of the fish gonads of the adult fish of F<sub>0</sub>- and F<sub>1</sub>- generation was performed.

To ensure consistency with fish test guidelines on endocrine acting properties, the list of test acceptance criteria contains values and ranges for the different parameters regarding test conditions and biological performance, as defined in OECD TG 210, 229, 234 and 240. For nearly all of the biological data obtained in the four studies, compliance with the defined validity criteria was confirmed.

Considering the control variability, the reproduction parameters showed low variability, while sex ratio variability was higher. The growth parameters expressed sex specific differences. The VTG concentrations in females were similar for both F<sub>0</sub> and F<sub>1</sub>, while VTG concentrations in males were at a low level as expected.

The test protocol applied is feasible to detect population relevant endpoints for zebrafish at sufficient sensitivity. The results obtained were in good compliance with available data from the literature. Finally, it can be stated that the ZEOGRT setup is suitable to assess endocrine effects beside receptor modulated mechanisms and steroidogenesis inhibition (EAS).