The Extended one generation reproduction test with zebrafish (ZEOGRT), First results from a validation study with tamoxifen-citrate

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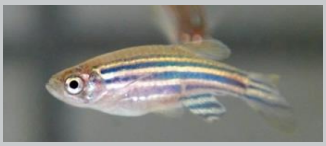

Introduction & objectives

After acceptance and enforcement of the criteria for identification of endocrine disruptors (ED) for plant protection products and biocides in the EU, there is increasing need for valuable testing strategies and methods. In this context, fish populations are considered as main targets of endocrine acting compounds in the aquatic environment.
In 2015, OECD adopted a protocol for an Extended one generation reproduction test (EOGRT) with Medaka (*Oryzias latipes*). This test protocol includes the exposure of a parental generation (adult fish), a full Filial 1 (F1) generation (from egg to adult) and a Filial 2 (F2) generation (until hatch of embryos).
As this protocol was designed and validated for a single test species, there was an initiative from Germany to develop a similar test approach considering zebrafish (*Danio rerio*) as a further species (figure 1).

The development of the Zebrafish EOGRT protocol was placed on the OECD workplan in 2016.
The ZEOGRT protocol considers:
- true replication of test tanks
- no tissue samplings during the test
- focus on more species-appropriate settings.

Due to the lack of a gene locus defining the genetic sex, a genetic sex determination is excluded from the Zebrafish procedure.

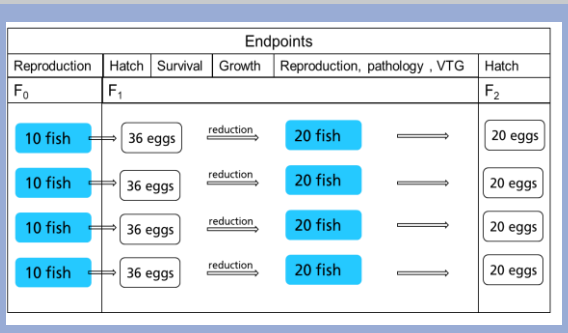

***Figure 1**: Test design of Zebrafish extended one generation reproduction test (ZEOGRT)*

ZEOGRT with Tamoxifen-Citrate

First results from a validation study with tamoxifen citrate (0.20, 0.63, 0.2, 6.3 and 20 µg/L), a known estrogen receptor antagonist, are available.
The data obtained were in line with literature data for zebrafish and other fish species. Comparable sensitivity was observed for e.g. reproduction parameters in terms of fecundity and fertility.
A shift in sex ratio towards an increased number of males (figure 2) was observed and defined as the most sensitive endpoint with population relevance. Biomarker measurements revealed a decrease of vitellogenin concentrations in female blood plasma samples (figure 3). Decreased survival rates for juvenile fish of F1 and F2 generation showed corresponding effects levels. It can be postulated, that these effects were the result of an impacted egg quality.

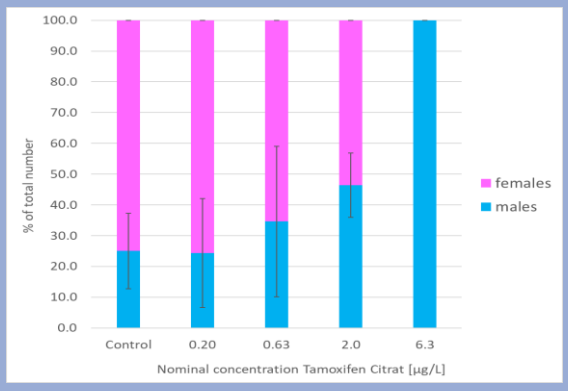

***Figure 2**: ZEOGRT with Tamoxifen citrate: F1 sex ratio*

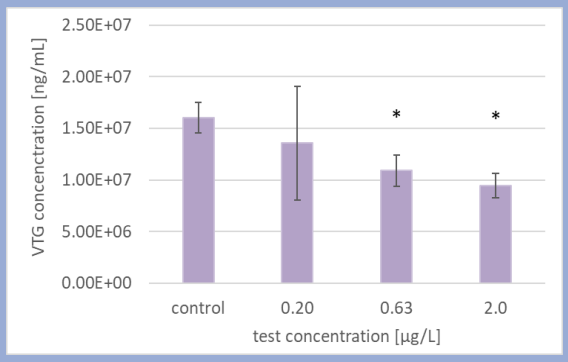

***Figure 3**: ZEOGRT with Tamoxifen citrate: F1 Vitellogenin females*

Conclusion

The results confirmed the applicability of the test protocol. The data obtained is valuable to identify sensitive endpoints and gain mechanistical information used for ED assessment.

Acknowledgements

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The development of the Zebrafish EOGRT protocol was placed on the OECD workplan in 2016.

The protocol considers the timeline as developed for the Medaka EOGRT but adds some method adjustments like true replication of test tanks, absence of tissue samplings during the test and the focus on more species-appropriate settings. Due to the lack of a gene locus defining the genetic sex, a genetic sex determination is excluded from the Zebrafish procedure.

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