# The impact of synthetic progestins on fish populations:
# Results from a Zebrafish EOGRT with Dienogest

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## Introduction & objectives

The release of endocrine active substances (EAS) into the environment can have effects on the aquatic community. Due to a very direct route of exposure and the similarity of the endocrine systems in vertebrates, fish are in the main focus of environmental science and research.
The release of human and veterinary pharmaceuticals is steadily increasing. Moreover, the progress in drug development results in the design of new and more specific acting and potent molecules. There are already several known substances, which act at very low concentration levels and finally cause adverse effects in aquatic organisms.
The regulation of pharmaceuticals considers the impact on the environment. However, the standard Environmental Risk Assessment (ERA) procedure is not able to cover all Mode of Actions (MoA). Beside others, EAS need a tailored risk assessment strategy. Currently, only for estrogen, androgen and aromatase inhibitors, MoA tailored ERA strategies are available.
A current project of the German Environment Agency (UBA) aims to develop tailored risk assessment strategies for the groups of progestins and glucocorticoids. Effects on aquatic vertebrates, invertebrates and sediment dwellers will be recorded within this project, and should build a database for developing this adapted assessment strategy. For fish, a Zebrafish extended one generation reproduction test (ZEOGRT) was conducted, as the endpoints in this test can address all potential and relevant MoA. Here, we present first results of the project, for which dienogest was chosen as a model substance for progestins.

## ZEOGRT with Dienogest

SETUP The study followed a draft test protocol for a two generation test with zebrafish (*Danio rerio*), including the continuous exposure of:
- a parental generation (adult fish),
- a full filial 1 (F1) generation
- a filial 2 (F2) generation (hatch only).

Zebrafish were exposed to 3.2, 10, 32, 100 and 320 ng dienogest/L.

RESULTS The results showed no impact of dienogest exposure to reproduction capability of the parental generation. However, a clear reduction of F1 post hatch survival was found (figure 2).
A significant reduction of F1 fertilization success was detected (figure 3). An effect on sex ratio of F1 fish was not evident. Mean control sex ratio was 61 % males to 39% females.
F2 hatching success was significantly reduced. Biomarker measurements and histological examinations are still pending, and will eventually complete the evaluation of reproductive effects.

## Conclusion

The available results confirm the applicability of the chosen study protocol and already show high sensitivity of fish to progestins. As next step, the sensitivity of fish will be compared to other aquatic organisms, like daphnids, snails and sediment-dwellers. This will provide the basis for a tailored risk assessment strategy for progestins.

***Figure 1**: Technical setup of Zebrafish extended one generation reproduction test (ZEOGRT) – Test vessel and fry chambers*

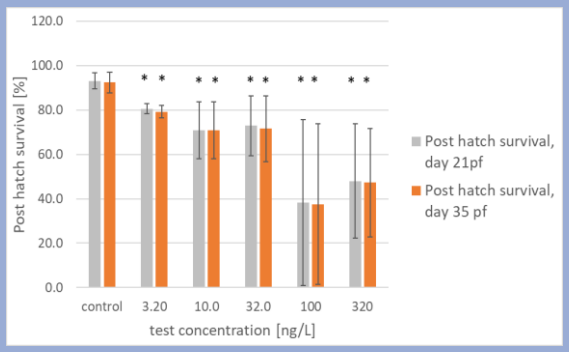


***Figure 2**: ZEOGRT with Dienogest: Post hatch survival of F1 Generation*

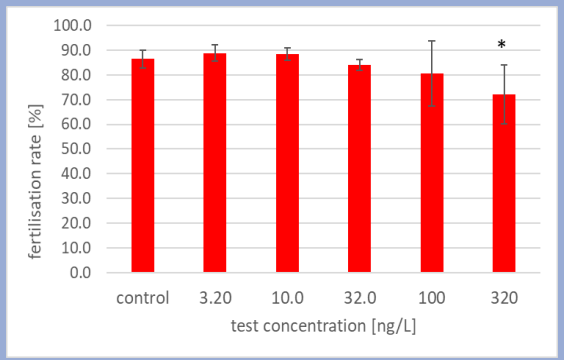


***Figure 3**: ZEOGRT with Dienogest: Fertilisation rate of F1 Generation*

For more technical details of ZEOGRT procedure, see also Poster WE 196.

## Acknowledgements

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The intake of endocrine acting substances (EAS) can represent a relevant threat scenario for the aquatic environment. Due to a very direct route of exposure, fish populations are recently in the main focus of environmental science and research.

The release of human pharmaceuticals and veterinary drugs is steadily increasing. Moreover, the progress in pharma science aiming to design new active pharmaceutical ingredients (API) results in the development of sufficiently stable, specific and potent molecules. There are many examples for substances acting already at very low concentration levels and finally causing adverse effects in the aquatic environment, expecially on fish populations.

The regulation of pharmaceuticals increasingly considers the impact on the environment. A current project of German Environment Agency (UBA) aims to develop a tailored risk assessment strategy for the group of new synthetic progestins and glucocorticoids.

Effects on aquatic vertebrates, invertebrates and sediment dwellers should build a data base for developing this adapted assessment strategy.

Here we present results from a Zebrafish extended one generation reproduction test (ZEOGRT). The synthetic progestin dienogest was chosen as test substance.

The study followed a new test protocol for a two generation test with zebrafish (*Danio rerio*), including the continuous exposure of a parental generation (adult fish), a full filial 1 ($F_1$) generation and finally a filial 2 ($F_2$) generation (hatch only).

The results showed no impact of dienogest exposure to reproduction capability of the parental generation. However, a clear reduction of $F_1$ post hatch survival was found.

Further endpoints to be assessed will be life stage specific growth and survival rates, reproduction in terms of egg number and fertility for $F_1$, and sex ratio.

The available results confirmed the applicability of the chosen study protocol and already show an increased sensitivity of fish compared to other inhabitants of the aquatic environment, also exposed to progestins.