COMPARISON OF ENDOCRINE EFFECTS IN DIFFERENT LIFE STAGES OF ZEBRAFISH EXPOSED TO MASCULINIZING SUBSTANCES IN VARYING LIFE CYCLE EXPOSURE SCENARIOS

Sven Kroesen[1], Matthias Teigeler[1], Elke Eilebrecht[1], Eric Bruns[2], Christoph Schäfers[1]

[1] Fraunhofer Institute for Molecular Biology and Applied Ecology/IME, Schmallenberg, Germany
[2] Bayer AG – Crop Science, Monheim, Germany
Contact: sven.kroesen@ime.fraunhofer.de

Background

Fish populations represent an important part in ecosystems and fish are accordingly tested for short- and longterm constraints during the authorization process of plant protection products. Of high concern are effects upon the endocrine system as they may alter *e.g.* the reproduction or sex ratio.
Despite a long history of research there is still debate about the applicability of the concept of dose-response relationships and threshold values to endocrine effects. To test if effects are comparable in different exposure scenarios and doses, zebrafish were exposed to Tamoxifen citrate, an anti-estrogenic substance, under pulsed *(see Fig. 1)* and continuous exposure.

Test systems

1. Static Fish Full Life Cycle (FLCT) test in a water-sediment system:

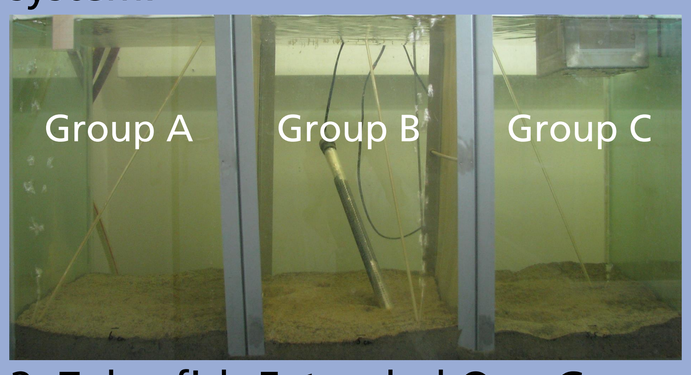

Figure 1: Three life stages (A: eggs, B: juveniles, C: adults) were pulse-exposed in concentrations from 125 – 1000 µg/l (Test 1) and 12,5 – 125 µg/l (Test 2)

2. Zebrafish Extended One Generation Reproduction Test (ZEOGRT):
Adult zebrafish were exposed under flow-through conditions (0,2 – 20 µg/l). Exposure continued over the life course of the F_1- until hatch of the F_2-generation.

Results

- Exposure to Tamoxifen citrate resulted in a sex ratio shift towards male fish in both test designs
- Two pulse exposure studies were performed, covering together a range from 12,5 – 1000 µg/l
- The NOEC for sex ratio of these two runs was determined in the second pulsed exposure study to be 39,5 µg/l (group B, *see Fig. 2*)
- Time-dependent internal concentrations based on uptake and elimination rates estimated from substance properties were calculated for MATCs (*see Fig. 3*)
- Calculation of time weighted internal concentrations considering the sensitive developmental time windows resulted in similar internal concentrations under constant and peak exposure (*see Tab. 1*)

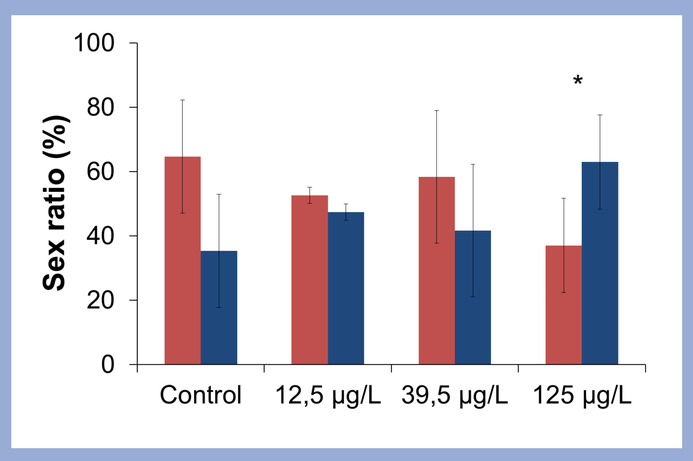

Figure 2: Sex ratio in fish introduced as juveniles (B) was shifted towards males (blue) after pulsed exposure.

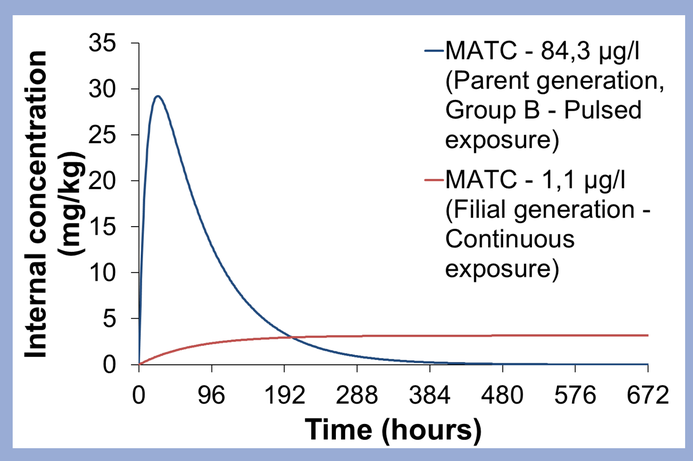

Figure 3: MATCs for the endpoint sex ratio were transversed into internal concentrations for both study designs.

Table 1: MATCs for the endpoint sex ratio, observed life stages, assessed time frames and corresponding internal concentrations

Exposure	MATC (µg/l)	Life stage	Age (dpf)	Study day	$C_{internal}$ (mg/kg)
Continuous	1,1	F_1	28-70	28-70	3,15
Pulsed	84,3	Group B	28-70	0-42	2,95

Discussion & Perspective

- Shift of sex ratio towards male fish was observed in both study designs
- Estimation of internal concentrations in suspected sensitive time frames resulted in highly comparable values
- Time weighted average approach for sensitive time windows will be applied to other study endpoints
- Study results will also be compared with other existing data pairs (flow-through vs. peak exposure) focusing on masculinizing modes of action. Evaluation of the additional data sets should reveal whether the comparability of internal concentrations within sensitive time windows can be generalized

Acknowledgments

The continuous exposure experiment was sponsored by the German **Umweltbundesamt (UBA)**, Dessau-Roßlau, Germany, in the OECD validation process of the ZEOGRT study design. The latter test design is presented in detail by Matthias Teigeler, Fraunhofer IME, Schmallenberg, Germany, at this conference. The studies (FLCT and ZEOGRT) were sponsored independently.

Session: Novel Developments in Testing Chemicals for Endocrine Disrupting Properties (P)
Poster, Exhibition hall, ID We200
Wednesday May 29th, 2019, 8:30 AM

Comparison of endocrine effects in different life-stages of zebrafish exposed to anti-estrogenic/androgenic substances in varying life-cycle exposure scenarios

Authors: Sven Kroesen, Matthias Teigeler, Elke Eilebrecht, Eric Bruns, Christoph Schäfers

Keywords: Endocrine Disruptors, Fish Full Life-Cycle Test, Variable Exposure Scenarios, Risk Assessment

During the authorization process of plant protection products a series of toxicity testing has to be performed to ensure an acceptable risk of use for humans and the environment. In these assessments fish populations represent an important spot in ecosystems and are tested for short- and long-term constraints accordingly. Of high interest in the procedure of testing are effects upon the endocrine system as they may alter e.g. the reproductive fitness of adult fish or their progeny.

Recently, scientific criteria for the identification of endocrine disruptors (EDs) have been set up by the EC. But despite a long history of research there is still a debate about mechanisms of action of EDs affecting the legislative regulation. These uncertainties arise mainly from the occurrence of non-monotone concentration-response relationships and the applicability of the concept of effect thresholds.

To answer these questions we exposed zebrafish (*D. rerio*) during different phases of their life-cycle to an anti-estrogenic substance, tamoxifen citrate, in both a pulsed and a permanent exposure test setup. The pulsed exposure experiments were performed using a spiked water-sediment system, in which three different developmental stages were exposed in parallel. A concentration-dependent mortality occurred in a first experiment (125 µg/L – 1000 µg/L). Nonetheless, in concentrations not affected by mortality and thus no subject to systemic toxicity a shift of the sex ratio towards males was observed. As no NOEC could be established for this sensitive endpoint, the experiment was replicated applying a lower concentration range (12.5 µg/L – 125 µg/L). Similar effects were observed when fish were constantly exposed to the test substance following the protocol of the Zebrafish Extended One Generation Reproduction Test (ZEOGRT), currently undergoing the OECD guideline validation process. Adult fish were exposed to concentrations ranging from 0.2 µg/L to 20 µg/L during their reproductive phase, followed by life-long exposure of their offspring (F1) until hatching of the F2-generation. The shift to male animals was conclusively observed in fish of the F1-generation.

Further results from this project will deliver additional information with respect to type and time of onset of effects. The data will be complemented with available data for other EDs (anti-estrogenic/androgenic) using similar testing approaches but covering differences in bioavailability prior to test item degradation.