Can we use kinetic data to estimate chronic effect levels for exposure situations different from laboratory EDC testing?

Matthias Teigeler[1], Christoph Schäfers[1]

[1] Fraunhofer IME, Auf dem Aberg 1, 57392 Schmallenberg, Germany
E-mail contact: matthias.teigeler@ime.fraunhofer.de

1. Introduction

To assess the effect of endocrine disrupting substances on fish populations, life cycle studies are performed to record parameters with population relevance for further use in risc assessment. If intrinsic data is of main interest e.g. for substance regulation, the respective tests are performed under flow through conditions. If a more realistic scenario should be adressed, a static approach with single peak exposure might be an appropriate design. Especially static systems are useful to focus on sustainability of effects and to differentiate between effect setting and manifestation [1].

In our study, two life cycle tests were performed using an anti-estrogen, considering both flow-through and static peak exposure design. Effect threshold concentrations of both test designs were compared [2].

In a further step, we tried to derive a linkage between endocrine exposure and resulting effects using a kinetic approach. The calculation was based on available data on lipophility and time weighted average exposure concentrations taken from the static FFLC. The results were used to estimate an appropriate concentration range for the study on intrinsic toxicity.

A further aim was to validate the relation of the exposure linked effect levels via kinetics.

2. Materials and methods

Two life cycle tests were performed using zebrafish (*Danio rerio*). As a test substance the selective estrogen receptor modulator Fulvestrant was chosen. Beside a flow-through FFLC tests with zebrafish [2], we performed an FFLC simulating worst case peak exposure by using static artificial sediment/water systems (270 L) with three life stages of zebrafish (fertilized eggs, juveniles, spawning adults), separated by stainless steel nets. The fish groups including their filial generation were investigated during declining exposure. Measured effects were related to initial concentrations and to time weighted average concentrations during defined life stages. In both tests, hatch, survival and growth of F0 and F1 early life stages, juvenile growth, time to first spawning, fecundity, fertility, sex ratio, length and weight of F0 adults served as endpoints.

Figure 1: Static water sediment system Fraunhofer IME

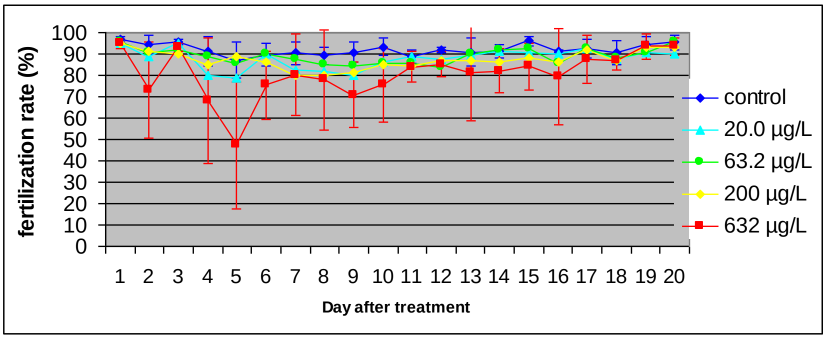

Figure 2: FFLC in a static water sediment system Effect on fertilisation rate [%] after single peak exposure

For estimation of effect levels related to decreasing test substance concentrations we used the approach as used to calculate bioaccumulation factors in fish according to OECD 305 [3]. Different assumptions were made.

The fertilisation success was found to be the most sensitive population relevant endpoint in the static test. Fertility was recorded daily, thus, data was available on effect duration and the timepoint of highest effect intensity. A daily uptake as well as the daily depuration was calculated considering the exponentially decreasing concentrations. The geometric mean of the substance concentrations in fish of each day in relation to the previous day were determined. The depuration constant k2 was calculated using available data on lipophility of the substance. The uptake constant k1 was determined by iterative fitting of the concentration curve against the observed effect levels taken from the static FFLC and also from a pre-test.

3. Results and discussion

In the peak exposure test, the only effect was a temporary one on fertilization rate in the adult fish at 632 µg/L (initial concentration) on days 4 to 6. The DT_{50} in the water column was measured to be approximately 0.5 d.

Using the kinetic approach, a LOEC was estimated to be around 16 µg/L, the effect threshold was determined to be approx. 11 µg/L. Based on these findings, the concentration range for the flow through study could be outlined. We chose Fulvestrant concentrations of 40, 13, 4.0 and 1.3 µg/L in the flow through FFLC test.

The most sensitive effects in the flow through study were a reduction of reproductive (reduced fecundity and fertility at 16 µg/L (LOEC) and a reduction of the survival rate of the F_1 generation (LOEC = 16 µg/L).

4. Conclusions

Based on the kinetic approach, it was possible to estimate effect levels using data on dissipation behavior and lipophility. Data on substance dissipation characteristics and effect duration and intensity could be derived from a test in a static water sediment system.

If otherwise intrinsic data is available, this approach can be a useful tool to assess effect levels after short term exposure based e.g time weighted average concentrations. A broad range of applications is conceivable. Beside the assessment of short term exposure with plant protection products, also the assessment of effects on fish passing short term peaks of substance e.g. from sewage treatment plant effluents, is possible.

5. References

[1] Teigeler M, Schäfers C, Duis K, Knacker T. 2012. Development and validation of a Fish Full Life Cycle (FLC) Test for the OECD test guideline programme - Experimental clarification of mode of action-specific endpoints relevant for regulation (translated from German). Report for the German UBA. FKZ 3709 65 405

[2] Knacker T, Boettcher M, Rufli H, Frische T, Stolzenberg HC, Teigeler M, Zok S, Braunbeck T, Schäfers C. 2010. Environmental effect assessment for sexual-endocrine disrupting chemicals – fish testing strategy. Integrated Environmental Assessment and Management, 6(4):653-662.

[3] OECD (2012). Guidelines for testing of chemicals. Draft TG 305. BIOACCUMULATION IN FISH: AQUEOUS AND DIETARY EXPOSURE. OECD, Paris.