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Repeated pulsed exposure in a partial life cycle test with zebrafish: Keep it realistic!

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Introduction & objectives

Refined exposure scenarios can be used to bring more realism into aquatic ecotoxicity testing. The aim is to achieve a more realistic perspective under consideration of the intended (worst-case) application pattern of a specific plant protection product. This option of risk refinement is also reflected in the recent Aquatic Guidance Document [1]. Here we present a partial life cycle test with zebrafish (*Danio rerio*) performed in a static water sediment system under pulsed exposure conditions.

***Figure 1**: Zebrafish (Danio rerio), adult spawners*

Approach

The test design allows addressing effects on different sensitive life stages of fish, successively and multiply exposed to the test item within the same environment. In the first part, adult spawning fish (i.e. the parental generation, F_0) were exposed to 4 pulsed applications at weekly intervals. The performance of the reproduction in terms of egg numbers and fertilisation rate was assessed. The second part was initiated by placing fertilized eggs from the parental groups into the same water sediment systems [Fig. 3]. This F_1 generation was also exposed to 4 pulses of the test item at weekly intervals. Survival and growth of the early life stages were assessed. Other endpoints like endocrine-disruptor effects can be covered by measurement of vitellogenin and histopathological analysis of fish gonads.

Glass aquaria of a total volume of 30 L were used and filled with a layer of artificial sediment to ensure stability of the test system throughout the 9-weeks test period [Fig. 2]. After pulse application, the dissipation of the test substance was analytically monitored. The evaluation of biological effects was based on mean measured as well as on 'area under the curve' concentrations (AUC) of the test substance in order to be able to compare it to predicted environmental concentrations (PECsw, according to FOCUS). The analysis of the AUC as well as of the DT_{50} values showed that dissipation profiles in the test systems were in line with the predicted exposure profiles in the field.

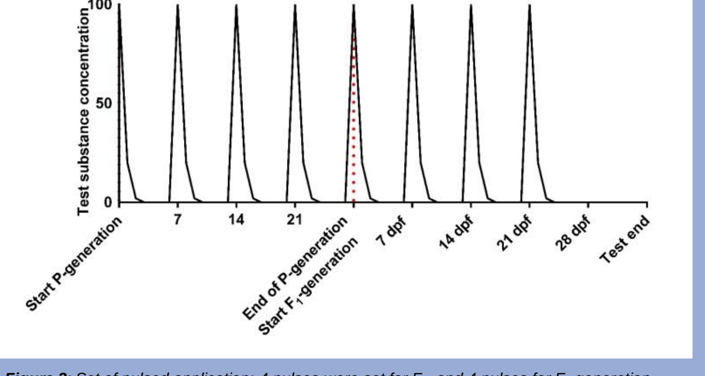

***Figure 2**: Set of pulsed application: 4 pulses were set for F_0, and 4 pulses for F_1 generation*

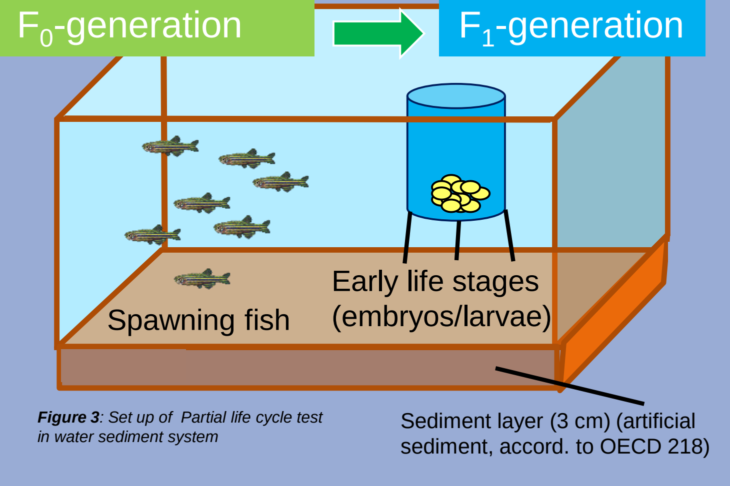

***Figure 3**: Set up of Partial life cycle test in water sediment system*

Conclusion

In contrast to a continuous exposure, the procedure of several pulse applications may have an impact and possible distortion of the static system. However, it was demonstrated that the performance of the F_0 as well as the F_1 generation–meet the quality criteria as set by the official test guidelines (OECD, USEPA). The test design was shown to provide a suitable approach to address both complex exposure regimes and specific endpoint issues.

References

[1] EFSA Aquatic Guidance Document (EFSA Journal 2013;11(7):3290)

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Refined exposure tests can be used to transfer more realism into standardised aquatic ecotoxicity testing. The aim is to achieve a more realistic perspective under consideration of the intended (worst-case) application pattern of a specific plant protection product. This option of risk refinement is also reflected in the recent Aquatic Guidance Document (EFSA, 2013). Here we present a partial life cycle test with zebrafish (*Danio rerio*) performed in a static water sediment system under pulsed exposure conditions.

The test design allows addressing effects on different sensitive life stages of fish, subsequently and multiply exposed to the test item within the same environment. In the first part, adult spawning fish (i.e. the parental generation, F0) were exposed to 4 pulsed applications at weekly intervals. The performance of the reproduction in terms of egg numbers and fertilisation rate was assessed. The second part was initiated by placing fertilized eggs from the parental groups into the same water sediment systems. This F_1 generation was also exposed to 4 pulses of the test item at weekly intervals. Survival and growth of the early life stages were assessed. Other endpoints like endocrine-disruptor effects can be covered by measurement of vitellogenin and histopathological analysis of fish gonads.

Glass aquaria of a total volume of 30 L were used and filled with a layer of artificial sediment to ensure stability of the test system throughout the 9-weeks test period. After pulse application, the dissipation of the test substance was analytically monitored. The evaluation of biological effects was based on mean measured as well as on area under the curve concentrations (AUC) of the test substance in order to be able to compare it to predicted environmental concentrations (PEC_{sw}, calculated with the FOCUS tools). The analysis of the AUC as well as of the DT_{50} values showed that the dissipation profile in the test systems were in line with the predicted exposure profiles in the field.

In contrast to a continuous exposure, the procedure of several pulse applications may have an impact and possible distortion of the static system. However, it was demonstrated that the performance of the parental as well as the filial fish was fine and in line with the quality criteria set by the official test guidelines (OECD, USEPA). The test design was shown to provide a suitable approach to address both complex exposure regimes and specific endpoint issues.