Pulsed exposure of fish at sensitive life stages: The ‘worst case’ challenge.

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Introduction & Objectives

Refined exposure tests have become part of the regulation framework for plant protection products in the EU [1]. A pulse dose test can be used to address areas of risk that cannot be satisfied with the standard suite of aquatic toxicity tests. A pulse dose considers situations where the expected exposure events in the field are significantly shorter than in the standard laboratory tests.

How do we manage to cover exposure profiles from multiple scenarios within one test?

In this study, the aim was simulate a realistic profile to cover a large number of scenarios, by considering the following parameters:
- maximum exposure (pulse) concentration
- adequate number of pulses
- sufficient duration of pulses
- appropriate interval between pulses.

Approach

Three different life stages of rainbow trout (*Oncorhynchus mykiss*) were exposed to nine pulses of the test chemical. To set these pulses as sharp as possible, the fishes were transferred from treatment vessels to untreated vessels at each time of pulse application [Figures 1, 2, 3]. All vessels, including controls, were kept under flow through conditions. The concentrations of the test chemical were measured at start and end of each pulse event.
Fertilised eggs, newly hatched fry [Figure 4] and juveniles, already swimming up, were exposed. Glass aquaria with a total volume of 30 L were used. The evaluation of biological effects (hatch, survival and growth) was based on mean measured concentrations measured for the test substance pulses and could be compared with the predicted environmental concentrations based on FOCUS modeling simulations.

Conclusion

In contrast to a continuous exposure, the procedure of several pulse applications may have an impact and possible impairment of the sensitive stages. However, it was demonstrated that the performance of the life stages exposed was acceptable and conforms to quality criteria set by the test guidelines (OECD, USEPA). The test design was shown to provide a suitable approach to address a very complex exposure regime to cover the ‘worst case’ when a typical laboratory exposure is unrealistic.

***Figure 1**: Flow through setup, including exposure and transfer vessels*

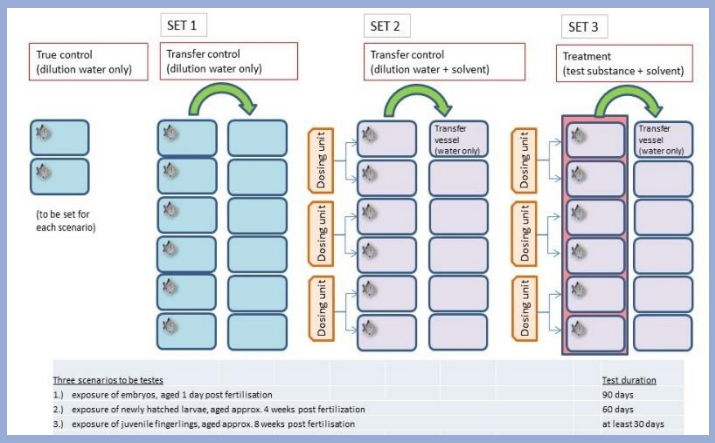

***Figure 2**: Setup of pulsed exposure study with Rainbow trout (Oncorhynchus mykiss)*

***Figure 3**: Chamber to keep and transfer fish larvae and alevins*

***Figure 4**: Newly hatched trout alevins*

References

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

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Refined exposure tests have become part of the regulation framework for plant protection products in the EU (EFSA Aquatic Guidance Document 2013). A pulse dose test can be used to address areas of risk that cannot be satisfied with the standard suite of aquatic toxicity tests. A pulse dose considers situations where the expected exposure events in the field are significantly shorter than in the standard laboratory tests. However, the challenge is often to cover exposure profiles from multiple scenarios within one test. Therefore, the maximum exposure (peak) concentration, the number of peaks, the duration of the peaks, and the interval between peaks are considered to simulate a realistic profile covering a large number of scenarios.

In this study, three different life stages of rainbow trout (*Oncorhynchus mykiss*) were exposed to nine pulses of the test chemical. To set these pulses as sharp as possible, the fishes were transferred from treatment vessels to untreated vessels at each time of pulse application. All vessels, including controls, were kept under flow through conditions. The concentrations of the test chemical were measured at start and end of each pulse event.

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In contrast to a continuous exposure, the procedure of several pulse applications may have an impact and possible impairment of the sensitive stages. However, it was demonstrated that the performance of the life stages exposed was acceptable and conforms to quality criteria set by the test guidelines (OECD, USEPA). The test design was shown to provide a suitable approach to address a very complex exposure regime to cover the 'worst case' when a typical laboratory exposure is unrealistic.