A pulsed exposure approach to investigate chronic fish developmental stage mortality and its relevance for risk assessment

Richard Maunder[1], David Fazakerley[1], Alastair Lyles[1], Marion Gagniarre[2], Thomas Fruhmann[3], Seamus Taylor[4]

1 Scymaris Ltd., Brixham Laboratory, Freshwater Quarry, Brixham Devon TQ5 8BA UK, 2 Adama Agan Ltd, France, 3 Nufarm GmbH & Co KG, Austria, 4 Adama Agricultural Solutions Ltd UK.

Introduction

The chronic toxicity of pesticides to fish is typically evaluated using standard methods e.g. OECD 210 Test Guideline (TG; Ref 1). In addition, OPPTS 850.1500 guidance (Ref 2) describes the method to conduct the fish life cycle (FLC) test that can be used to detect adverse effects on development, growth and reproduction over an entire life cycle. In some cases, effect endpoints reported in FELS and FLC studies can be inconsistent and further investigation of development growth stage effects may be justified.

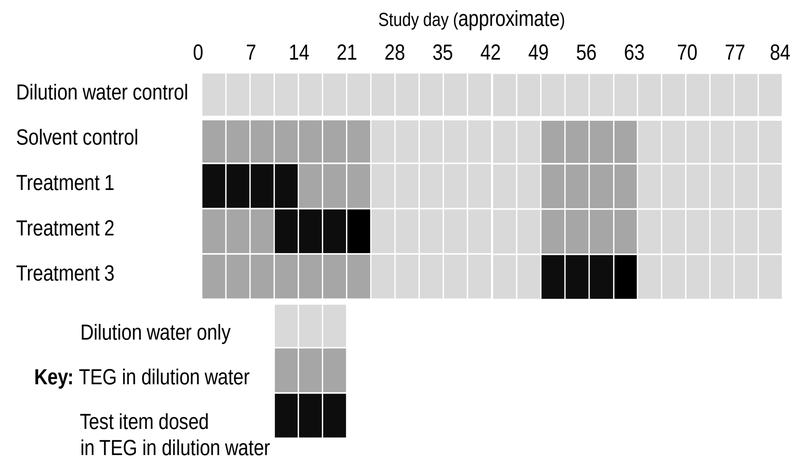
In this project, a bespoke fish study, using pulse-exposures was designed to investigate pesticide toxicity to three different life stages of the medaka *Oryzias latipes*. The test was conducted at Scymaris, Brixham laboratory, UK. A single test item concentration was dosed to three separate experimental groups, each receiving a 15-day pulse exposure (selected to represent worst case modelled exposure events) during a different life stage, with clean water the remaining time. A dilution water control (DWC) and a solvent control (SC) group receiving a matched solvent pulse were concurrently exposed. The three pulse timings were;

Treatment 1: 15 days continuous exposure from egg fertilisation.
Treatment 2: 15 days continuous exposure from egg hatch.
Treatment 3: 15 days continuous exposure from day 39 post-hatch.

The exposure design is shown pictorially in Figure 1:

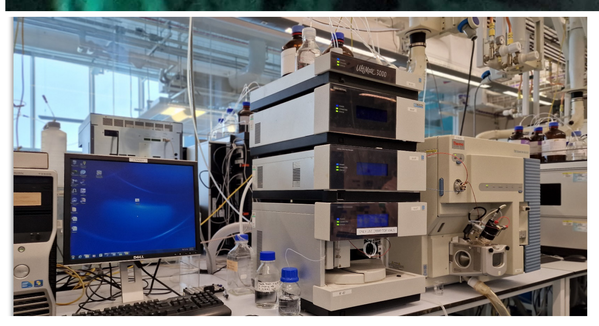
The design allowed the targeting of defined life stages that may be sensitive to exposure, namely embryo development (Treatment 1), early life post-hatch (Treatment 2) and adult sexual differentiation (Treatment 3). The endpoints measured were successful hatch, survival post-hatch and growth at test end (wet weight and total length). The test was designed to end when fish were considered reproductively mature based on criteria in OECD 240 Test Guideline (Ref 3).

Figure 1. Pulse exposure design for the treatments and controls



Species: Medaka *Oryzias latipes*.
Egg source: Scymaris lab. adult broodstock

The species is representative of a widely distributed freshwater fish and is recommended by the OECD 210 TG (Ref 1).



Analytical Chemistry

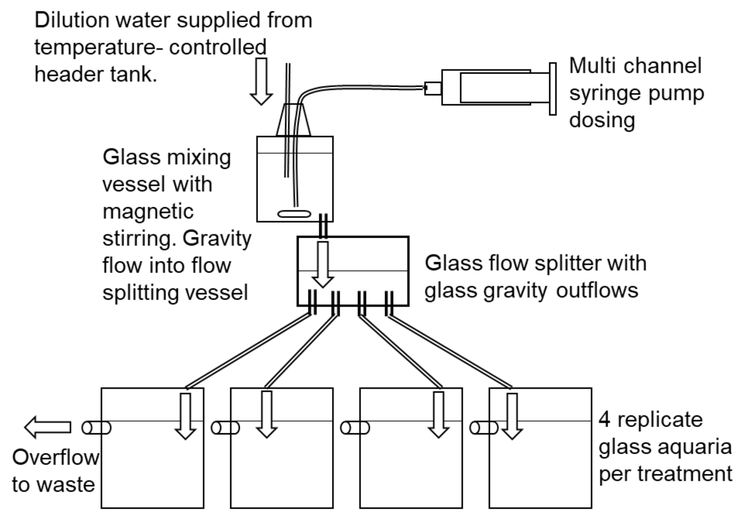
An LC-MS/MS method to determine the concentration of the test item in the test dilution water was validated to SANCO/3029/99 rev. 4 (Ref 4). The test item-specific method had an LOQ of 1.0 µg/L.

Exposure Design

The test was run at a nominal temperature of 25±2°C, with a photoperiod of 16 hours light:8 hours of dark with 20 minute dawn/dusk transition. The pH, dissolved oxygen and temperature of the test solutions was measured at weekly intervals. Each treatment comprised 4 replicates of 20 fish, (total of 80 fish per treatment).

Fertilised eggs were impartially selected from a pool of eggs that originated from 13 broodstock groups. It proved challenging to hatch the medaka eggs under the flow-through design; we encountered issues with fungus infecting the eggs prior to hatch. Methylene Blue is recommended by the OECD when attempting to hatch medaka eggs (Ref 5) and in other species (Ref 6). We investigated dosing methylene blue into the flow-through tanks but found the most effective use was by maintaining a continuous concentration of methylene blue (2.125 mg/L) within a semi-static exposure with 3 renewals per week. The pulse dose for the egg fertilisation (Treatment 1) was therefore conducted under a semi-static design, with the fry being transferred to the flow-through tanks once hatched. The Treatment 2 and 3 pulse doses were conducted under flow-through conditions throughout.

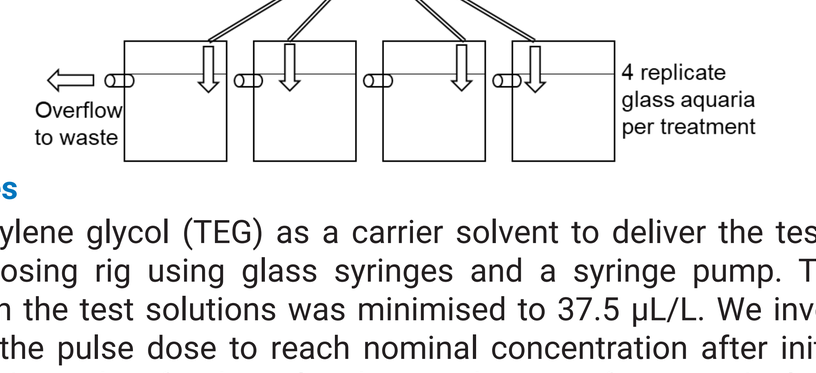
Figure 2. Pulse exposure design for the treatments and controls



Pulse exposures

We used Triethylene glycol (TEG) as a carrier solvent to deliver the test item to the flow through dosing rig using glass syringes and a syringe pump. The final TEG concentration in the test solutions was minimised to 37.5 µL/L. We investigated the time taken for the pulse dose to reach nominal concentration after initiation of the dosing and the time taken for the pulse dose to decrease from nominal down to <LOQ at pulse end. The pulse exposure dosing could be easily added or removed, as required, from the mixing cells, see Figure 2.

Figure 3. Exposure profiles derived from analytical data. A single mean value is plotted when there are multiple results from the same day (eg. semi static / flow through). The previously measured concentration is re-plotted when there is a gap.



Results

Analytical Chemistry The measured chemistry data sets showed a reliable pulse-exposure was achieved for all three exposures, summarised in (Figure 3). The ramp up and ramp down dynamics were shown to always occur within a 24 h period. The use of a mixed semi-static and flow-through exposure design was successfully implemented.

Validity Criteria The validity criteria set for the control fish in the study were based on those in the OECD 210 TG (Ref 1) but also included some detail from the OECD 240 TG (Ref 3) due to the extended duration. All validity criteria were met and the study was considered valid. The use of methylene blue facilitated a very high level of successful hatch (97 and 95% in DWC and SC groups).

Fish Weights The test end date was based on the fish age considered to be sexually mature in OECD TG 240 (Ref 3), para 21; minimum 12 weeks post fertilisation (wpf) and weight ≥250 mg males and ≥300 mg females. In this test, after 11 wpf, it was observed that most groups were starting to spawn in the tanks, while the DWC fish at this stage were still below the specified weights. Upon further investigation, the stated weights are thought to be for fish of 15 wpf (Ref 3, Annex 4) so the test finished on day 84 post fertilisation (=12 wpf). Ideally the fish would have been sampled prior to spawning to avoid the excess variation in weights of females caused by release of eggs.

Conclusions

The pulse exposure design was successful in producing a reliable dose and is practical to manage in the laboratory. The use of methylene blue as an egg fungicide is effective and simple to under semi-static conditions.

The pulse dose approach can be recommended to allow a more thorough assessment of the pesticide toxicity under environmentally realistic exposure scenarios. The study findings promote the use of pulse exposures in environmental risk assessment.

References (1) OECD (2013) Test No. 210: Fish, Early-life Stage Toxicity Test. (2) US EPA (1996) Ecological Effects Test Guidelines OPPTS 850.1500 Fish Life Cycle Toxicity Test. (3) OECD (2015), Test No. 240: Medaka Extended One Generation Reproduction Test. (4) SANCO/3029/99 rev.4 (11/07/2000) - Residues: guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (Part A, section 4) and Annex III (Part A, Section 5) of Directive 91/414. (5) OECD (2022) Test no. 251: Rapid Androgen Disruption Activity Reporter (RADAR) Assay. (6) OECD (2011) Test No. 234: Fish Sexual Development Test.