DEVELOPMENT OF A HIGHER-TIER, INDOOR MICROCOSM STUDY USING *CHIRONOMUS RIPARIUS*

Zoe Jones[1#], Hanna Schuster[1], Marie Brown[1], Jamie Smith[1], Nadine Taylor[1], Gwendolin Kraetzig[2], Seamus Taylor[2].
[1]Cambridge Environmental Assessments, Boxworth, UK; [#]Corresponding author: Zoe.Jones@cea-res.co.uk [2]ADAMA Deutschland GmbH.

CEA 20 YEARS | 2001 - 2021
CAMBRIDGE ENVIRONMENTAL ASSESSMENTS

ADAMA

INTRODUCTION

The larval stages of *Chironomus riparius* reside in the sediment of freshwater habitats such as ponds, rivers and lakes. They are considered as a key indicator species for ecosystem health and commonly utilised as a test species in standard, regulatory ecotoxicology studies for effect assessment of formulated plant protection products (PPPs) and their active substances.

Tier I and Tier II ecotoxicity tests are typically conducted under worst case conditions of exposure using axenic populations of a single (usually juvenile) age group. It is generally accepted that endpoints from these studies are conservative and lack a degree of environmental realism, and therefore context is required when extrapolating to real-world situations. For example, standard ecotoxicity studies do not consider species interactions and an organism's ability to recover and/or recolonise following a perturbation to their environment. Higher tier ecotoxicological testing and bespoke study designs are able to incorporate these factors, enabling refinement of effect endpoints to be used in environmental risk assessment.

The study presented here was designed to simultaneously expose incorporate several life-stages of *C. riparius* in an attempt to determine baseline mortality and emergence data in a microcosm setting. A representative insecticide was used to test the feasibility of the experimental design in a 28 day single pulsed exposure, focusing on the rate of emergence over time for this mixed-age-group population.

Table 1: Test conditions

Water:	Natural pond water (filtered at 30 µm)
Sediment:	Lake sediment; exhausted in lab for at least 30 days.
Exposure regime:	Single application, water spiked, static
Exposure duration:	28 Days
Test vessels:	3 L glass beakers; 1.5 L pond water + 0.5 L sediment
Water Temperature (°C)	20°C ± 2°C
pH:	6-9
DO_2 (%ASV):	>60% ASV (Air Saturation Value), provided by gentle aeration
Photoperiod:	16h light: 8h dark
No. control groups:	1
No. treatment groups:	2 (5 µg a.s./L and 12.5 µg a.s./L of a representative insecticide)
Replicates per group:	3
Validity Criteria	At least 70% emergence of LS3 control based on OECD 218[#]
Organisms (larvae) per replicate:	90 larvae: 30 small larvae (2 – 3 days old = LS1), 30 medium larvae (12 - 15 days old = LS2), 30 large larvae (20 + days old and before pupae phase = LS3)

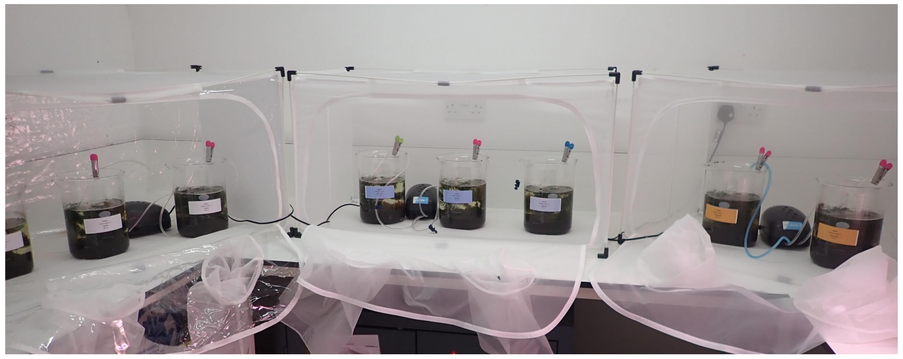
TEST SYSTEM, DOSING AND ASSESSMENT

This test was modified based on the current OECD 218[#] guidance. A mixed population of larval stages (Table 1) of *C. riparius* were exposed to two concentrations of a representative insecticide for 28 days.

The test vessels were set up with exhausted sediment and overlying water and allowed to settle for 48 h. After 48 h the mixed larval population of *C. riparius* were added to the test system. After a further 24 h (to allow for air exchange between the water and sediment component and enable the system to have sufficient levels of dissolved oxygen) the overlying water in the vessels was spiked with the relevant dose of the test item. Adult emergence was assessed over 28 days.

DESTRUCTIVE SAMPLING

At study termination, the microcosms were destructively sampled to assess survival of the non-hatched larvae.



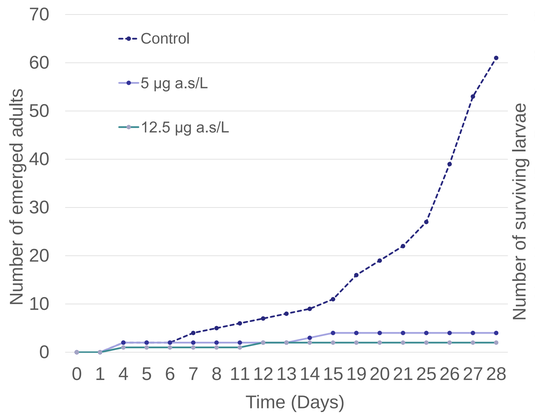


Figure 1: Number of emerged *C. riparius* after 28 days of exposure

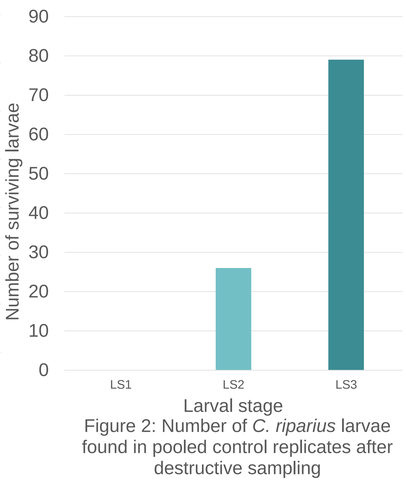


Figure 2: Number of *C. riparius* larvae found in pooled control replicates after destructive sampling

RESULTS AND DISCUSSION

- The data from the control vessels showed emergence in both the top two life stages. The targeted validity criterium was met (> 70 % emergence of for the oldest larval stage (LS3) after 28 days). The LS1 larvae developed to LS2 and LS3.
- This study has confirmed the feasibility of using this test design for non-standard higher tier testing and that the imposed validity criteria for emergence in the control group is achievable.
- In this preliminary trial the concentrations of the representative insecticide induced high mortality. Therefore, no dose response endpoints can be determined for survival or emergence in this dataset. Further investigations, with an increased number or treatments and/or alternative test items are required

CONCLUSION

The study design was successful in determining a baseline level of mortality and emergence in different larvae stages *C. riparius* under control conditions. This is useful to support the assessment of population effects. As such, the system could also be applicable for use with other sediment dwelling species and could potentially be extended to include reproduction and the impact on the next generation. may

Further work is needed, using other representative toxicants and species, to address if valid endpoints can be statistically derived and thus prove to be an acceptable approach for higher tier ecotoxicity testing.

REFERENCE [#]OECD Guidelines for Testing of Chemicals, No. 218: "Sediment-Water Chironomid Toxicity Test Using Spiked Sediment", adopted, 2004.