Eliminating Stressors in Laboratory Tests with Corixidae

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Cambridge Environmental Assessments
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INTRODUCTION

- Standard laboratory effect studies with invertebrates operate under optimal laboratory conditions specified in relevant OECD guidelines, which are generally 'sterile' and void of natural refuge and food
- When using feral non-standard organisms, these conditions can cause unforeseen stress, thus potentially affecting the outcome of acute toxicity tests for use in the regulatory risk assessment of plant protection products (PPPs)
- Cambridge Environmental Assessments, have recently attempted to develop methods to reduce laboratory stressors for tests conducted with Corixidae
- This allowed subsequent tests to be tailored to the requirements of the species, increasing survival rate and therefore providing more robust and reliable results

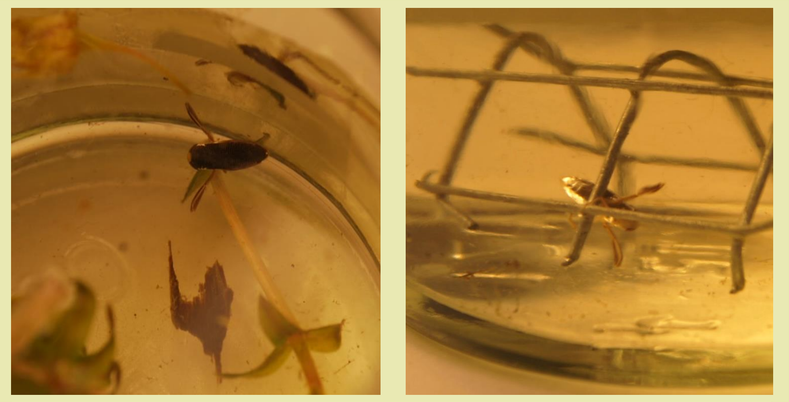

FIGURE 2. Observations of Corixidae behaviour showed that grasping objects was a requirement to maintain submersion (left). In laboratory bioassays, plants were replaced with more chemically inert materials, such as metal mesh (right) to eliminate the stress of increased swimming when the test vessel is empty.

Optimal conditions (OECD 202): *filtered water, 18-22°C, pH: 6-9, dissolved oxygen: >60%, 16hr light-8hr dark, 500-1000 Lux.*

METHOD

- **Initial test:** simple bioassay (e.g. as per OECD 202) (Fig. 1)
- Required to for derivation of EC_x values following exposure to an insecticide
- Adults collected February-March from CEA (ADAS UK Ltd) mesocosm facility
- Acclimatisation period in the laboratory 1-3 days prior to testing; healthy individuals used

Problem
- Observations indicated Corixidae were distressed
- Unable stay submerged
- High mortality

Research objective
- Mitigate stress by providing refuge for grasping (Fig. 2)
- Different substrates tested

Trial 1 findings
- String or metal mesh provided = 30% lower immobility than control
- Corixidae appeared distressed by movement
- Mortality still high (Fig. 3)

Research objective
- Mitigate stress by reducing visibility and light intensity
- Naturally found in groups therefore grouping regimes trialled (Fig. 4)

Trial 2 findings
- 100% survival achieved when individuals grouped, provided with mesh and covered (Fig. 5)
- Repeat tests (metal mesh trial 1 and control trial 2) yielded different results

FIGURE 1. Standard laboratory bioassays, unnatural conditions which may cause non-chemical stress and affect results of immobility observations.

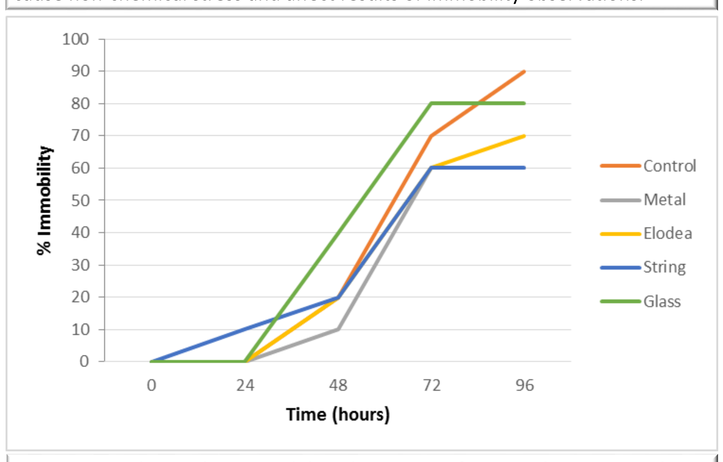

FIGURE 3. Trial 1 results - Immobility observations for Corixidae over a 96 hour laboratory bioassay using a range of substrates; metal mesh, a stem of Elodea, a piece of string and a glass slide (10 replicates per treatment).

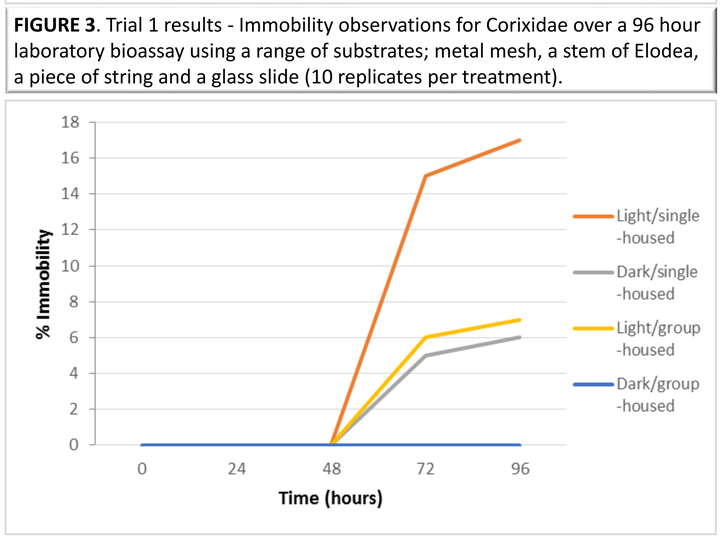

FIGURE 5. Trial 2 results - Immobility observations for Corixidae over a 96 hour laboratory bioassay under different light and grouping regimes. 'Dark' vessels were covered with a layer of foil to reduce startling from movement. Group-housed vessels consisted of five individuals per replicate (2 replicates; 10 replicates for single-housed treatments).

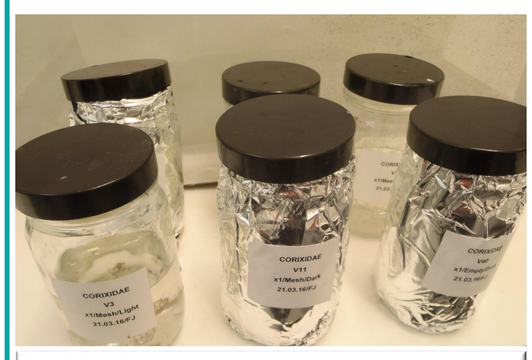

FIGURE 4. Trials to tailor laboratory tests to eliminate potential stressors, such as light levels, startling from movement and absence of refugia.

RESULTS

- Using findings from laboratory trials to reduce stressors, a bioassay using an insecticde was conducted
- Optimal conditions specified by OECD guidelines were met, though results were better without light/visibility
- Control immobility was reduced and the test was valid
- Ec_x values were reliably calculated (Fig. 6)

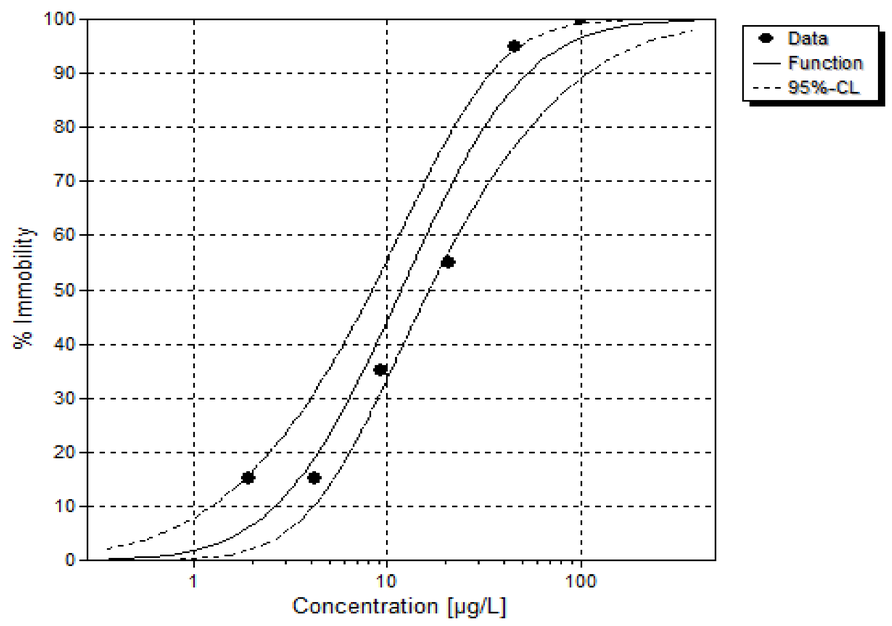

FIGURE 6. Regression curve with 95% confidence limits (CL) for Corixidae immobility using a pesticide in an acute laboratory bioassay. EC_x values based on % immobility were reliably calculated following trials to eliminate stressors.

CONCLUSIONS & RECOMMENDATIONS

- Corixidae are naturally found amongst vegetation in high numbers (Fig. 7) and are easily distressed
- Results were improved by tailoring laboratory tests to meet basic requirements
- Repeat tests using the same regime gave different results, showing that cause of stress and immobility is multifactorial
- More research needed to cover potential differences, including age, season, species or other stressors such as noise
- Knowledge gained can be used to develop standard operating procedures for testing with highly sensitive organisms for risk assessment of PPPs

FIGURE 7. Natural habitat of Corixidae collected from the CEA (ADAS UK Ltd) mesocosm facility.

REFERENCES

- OECD (2004), *Test No. 202: Daphnia sp.* Acute Immobilisation Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.
- OECD (2011), *Test No. 235: Chironomus sp.,* Acute Immobilisation Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.