LIFE SCIENCES

HOW DO YOU DO I DETERMINE POTENTIAL FOR TOXICOLOGICAL EFFECTS OF MY PRODUCT?

APPLICATION NOTE

Looking Forward: Invertebrate Acute Toxicity Testing Today and Beyond

J.M. Griebel and D.S. Brougher, EAG Laboratories, Easton, Md., USA

ABSTRACT

Acute aquatic invertebrate toxicology testing has been primarily focused on a few select species that includes daphnia (D. magna), the saltwater mysid (Americamysis bahia), and the eastern oyster (Crassostrea virginica). As more is learned about the properties and environmental consequences (e.g. solubility, stability, degradation and half-life) of today's chemicals a call for more diversified species sensitivity profiles has been requested. EAG Laboratories (formerly Wildlife International Inc.) has been actively working with a number of other important invertebrate species to determine species sensitivity distributions. Protocols for many of these species have often cited the Daphnia guideline for acute testing (1,2). Experience has shown that there are species specific ecological requirements and challenges (e.g. sediment/substrate requirements, cannibalism, feeding during testing) that are not addressed by the daphnia guideline. A need for species specific guidelines appears to be in order. Our recommendation is that the current guidelines for acute toxicity testing with invertebrates be reviewed and updated to accommodate the diversity of organisms being included in risk assessment testing in the future.

INTRODUCTION

In recent years invertebrate toxicity testing has become increasingly important in the registration process for today's modern chemicals. The call for broader species sensitivity distribution testing has become more common place. In 2016 new EPA guidelines (OCSPP) were adopted for use in toxicity testing. However, no new species specific guidelines were adopted. Including the OECD guidelines, current invertebrate guidelines are in place that include daphnia, saltwater mysids, gammarus, and chironomids. The number of invertebrate species being requested for risk assessment studies has steadily increased in recent years. Species tested by EAG include the larval stage freshwater insects (mayfly - Neocloeon triangulifer, caddisfly - Chimarra atterima, stonefly - Soyedina carolinensis), chironomids (C. riparius and C. dilutus), crayfish (Procambarus clarkii), freshwater amphipods (Gammarus pseudolimnaeus, Hyalella azteca), lumbriculus (Lumbriculus variegatus), and the daphnids (D. pulex and Ceriodaphnia dubia).

Absent specific guidance for testing most of these species, protocols for many of these species have cited the daphnia guidelines for acute testing. A number of the species mentioned above have very specific culture requirements requiring modification of the testing regime in order to ensure testing acceptability requirements for control survival are met. Others are wild caught and require specific acclimation procedures prior to use in a test. Sediment/substrate requirements, cannibalism, and whether or not to feed during the test offer additional testing challenges that are not addressed by daphnia guidelines.

Typically the challenge in successful acute testing is ensuring control organisms meet the acceptability requirement for control survival (>90%). Our experience has shown that modifying the test design to meet species specific ecological requirements has improved the success rate with non-typical test species. A need for further species specific guidelines is apparent and would aid in increasing the percentage of studies that meet guideline criteria, while also reducing the amount of tests repeated due to non-treatment related mortality.

METHODS

Acute Testing – typically 48 (daphnia, chironomid, flies) or 96 (mysid, gammarus, crayfish) hour tests.

Test design typically determined by chemical stability

- Can be Static, Static-Renewal (24 or 48 hour renewals) or Flow-through

Species selectivity can also determine study design

- Can the organism survive in a Flow – Through test system?
- May not be able to withstand the high flow rates, turbulence or disturbances incurred with test solution delivery
- Rapid loss of feed as test chamber contents are turned over

May need to consider species behavior when determining study design

- Sediment dwellers really need some type of substrate to be added to the test chamber, even in static or semi-static tests
- Cannibalism – Structure provided in the test chambers (e.g.

pvc tiles) affords protection from aggressive cohorts

- Can the organism survive without feeding?
- Some species simply cannot last 48 or 96 hours without food (ceriodaphnia), or become highly cannibalistic (gammarids, crayfish) when not fed

Species availability and life stage should be considered when determining study type or design

- Many field collected species are only available at certain times during the year (stonefly, caddisfly), or are preferably obtained at specific times (e.g. gammarus – Spring and early Summer)
- Typically immature organisms are preferred so as to avoid biasing the test
  - Chironomids can reproduce asexually and cultures should be “synchronized” prior to testing to reduce the likelihood of them reproducing during the test and confounding the results of the study
  - Oyster spawning invalidates a study if it is observed during a test (results in lack of shell deposition)

OBSERVATIONS

Species requiring substrate:

- **Chironomids:** typically sediment dwellers, a small amount of sand added to the test chamber improves control survival and study success
- **Gammarus/Crayfish:** cannibalistic, small pvc tiles or mesh provided a shelter and /or protection from aggressive cohorts
- **Hyalellla:** small nylon mesh squares added to the test chambers, especially in flow-through studies, are helpful in providing a substrate for those organism that prefer to be “attached “ to the bottom

Age of the organism:

- Younger, uniform age and size is typically best, particularly for cannibalistic species

Feeding during the test:

- Preferred whenever possible, but can lead to dissolved oxygen problems, requiring gentle aeration added to the study. Important for cannibalistic species

CONCLUSIONS

Sponsors requests in recent years would suggest increased demand for a broader range of invertebrate species for testing. The unique requirements of those species, makes adaptation of additional species specific guidelines imperative.

ACKNOWLEDGEMENTS

The authors wish to thank the following personnel for their expertise with these findings: Christian Disque, Lois O’ Boyle, Joshua Rogers, Michelle Stence and Rachel Woodward.

REFERENCES

1. U.S. Environmental Protection Agency. 2016. Series 850–Ecological Effects Test Guidelines, OCSPP 850.1010: Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids.

2. Organisation for Economic Cooperation and Development. 2004. OECD Guidelines for Testing of Chemicals, Guideline 202: Daphnia sp., Acute Immobilization Test. Adopted 13 April 2004.

3. Organisation for Economic Cooperation and Development. 2011. OECD Guideline for the Testing of Chemicals, Guideline 235: Chironomus sp., Acute Immobilization Test. Adopted 28 July 2011.

4. U.S. Environmental Protection Agency. 2016. Series 850-Ecological Effects Test Guidelines, OCSPP 850.1020: Gammarid Amphipod Acute Toxicity Test.