

DEVELOPMENT OF A CHRONIC LEMNA ASSAY TO SUPPORT REGULATORY ENDPOINTS FROM MESOCOSM STUDIES WITH HERBICIDES



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Introduction

Lemna sp. are free-floating macrophytes used in Tier 1 assessments of ecotoxicity for chemicals with a herbicidal mode of action. Recent experience has demonstrated that *Lemna* is not well suited for inclusion in higher tier static mesocosm studies due to its need to derive nutrients solely from the water column and its inability to grow in competition with macrophytes rooted in the sediment when dissolved nutrients (principally soluble reactive phosphorus) are limited. As a result, a chronic laboratory assay to assess the growth of *Lemna* sp. was developed to run alongside a higher tier mesocosm study with a herbicide. The results of this highlighted some potential drawbacks to long term laboratory testing of *Lemna* and highlighted the need for further testing to be conducted to try and determine the causes of the issues identified.

Methods (main laboratory assay)

- The assay was based upon OECD guideline 221
- Study duration was 118 days
- During the study the same original culture was used throughout the exposure period
- No fronds were replaced unless they were of extremely poor health or dead
- Water collected from the mesocosm study provided the test media
 - This was filter sterilised (0.2 µm cellulose nitrate) prior to the addition of nutrients (SIS growth media)
- 4 treatments x 3 replicates were employed for testing
- Semi-static exposure design was used with media changed 3 times a week
- Measurements included the following:
 - *Lemna* frond number
 - *Lemna* health
 - Plant number

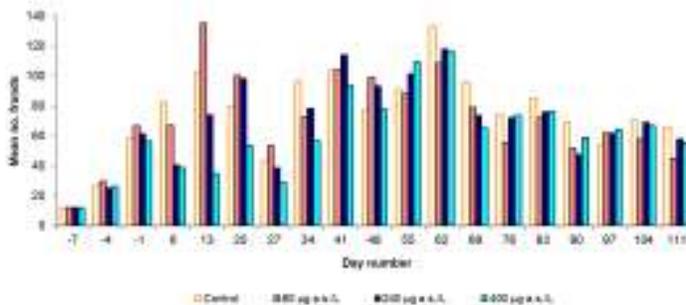


Figure 1. Mean No. of fronds counted during the main laboratory assay

Results (main laboratory assay)

- Recovery was observed in all treatments by Day 41 (Figure 1)
- However, gibbosity (swollen, overlapping fronds, Figure 2) occurred occasionally in dose groups and also in the controls causing variable frond numbers towards the latter stages of the test
- A literature review highlighted prolonged exposure to ethylene, a component of the SIS nutrients added to the pond water as a possible cause of gibbosity along with low genetic diversity and a high growth rate
- Based on these results a further study was conducted to investigate whether the gibbosity observed was a result of exposure to the test item or from the findings highlighted in the literature review



Figure 2. Gibbosity in test cultures during the main test

Methods and Results (investigative test)

- Duplicate cultures not previously exposed to the test item were maintained, one from the original 'old' master culture maintained alongside the chronic laboratory bioassay and one using 'fresh' lemna sourced from our mesocosm facility
- Gibbosity, health and frond number were assessed
- Gibbosity was observed in both cultures but the effect was slightly delayed in the 'new' culture
- Health measurements revealed shortness of roots, frond size reduction and discoloration



Figure 3. Gibbosity 'old' vs. 'new' cultures

Discussion

- The gibbosity observed during the main laboratory assay was the same as that observed during the investigative test (Figures 2 and 3)
- Low genetic diversity may be the cause of the gibbosity observed
- A correlation between plant number and gibbosity suggest that plant number is not a suitable assessment of recovery in chronic laboratory assays
- This study illustrated that the method can successfully establish whether recovery of *Lemna* is possible principally by through assessment of frond number

Conclusion and recommendations

Based on the results obtained from both laboratory assays the following recommendations should be taken into account when designing laboratory assays for *Lemna* sp.

1. Laboratory bioassays should be used to assess the recovery potential of *Lemna* in higher tier mesocosm studies
2. Strength of nutrient growth media should be reduced for long term assays to avoid gibbosity caused by ethylene
3. Chronic endpoints should be based on frond number only
4. Similar techniques may aid higher tier testing of other macrophytes

Reference: OECD 221 (2006), OECD guidelines for the testing of chemicals. *Lemna* growth inhibition test.