Testing the emergent macrophyte, *Glyceria maxima*, in a water-sediment system

Results of a ring-test with Isoproturon

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Introduction

- Regulation in the EU requires data for a rooted macrophyte species for some herbicidal compounds. Specifically, the EFSA Guidance Document on tiered risk assessment for aquatic organisms indicates that data for a rooted macrophyte species may be required where:
  - terrestrial plant data indicates a high selectivity for monocot or dicot species.
  - standard Lemma and algae test species are not sensitive to the mode of action (e.g. $EC_{50} > 1$ mg a.i./L).

- partitioning to sediment is a concern.

- The dicot species, *Myriophyllum spicatum*, and the monocot species, *Glyceria maxima* have been identified as alternative test species in light of prior experience and known sensitivity to some chemistries.

- OECD Test Guideline 239 for *Myriophyllum* in a water-sediment test system has been adapted for *Glyceria maxima* (reed sweet grass) and the modified protocol has been ring-tested using the protocol in Table 1 in several laboratories in the EU and the US.

Objectives of Isoproturon Ring-Test

The ring-test was designed to establish the following test parameters:

1. Test duration, i.e. the duration of time required to achieve a doubling in control biomass

2. Test design, i.e. replication required to achieve acceptable control coefficients of variation

Thirteen laboratories participated in the ring test, generating between 10 to 11 control datasets and 5 to 8 datasets for estimation of effects of isoproturon at 14 and 21 days.

Key Results

**Growth of control plants (Figure 3)**

- Control plants achieved >2-fold increase in FW, DW and TLL within the minimum 14-day test duration.
- Doubling time for all growth parameters increased with increasing test duration due to a slower growth rate between days 14 and 21. This trend may be caused by nutrient limitations.

**Variability in control plants (Figure 3)**

- Yield CoVs are higher than growth rate CoVs.
- For growth rate, CoVs for most endpoints were <35% while for yield only TLL has a CoV of <35%.
- Day 14 repeatability CoVs are typically higher than Day 21 values while a clear relationship was not observed between test duration and reproducibility.
- High CoVs are typically correlated with larger plant size and high variability at test initiation.

**Sensitivity to Isoproturon (Figure 4)**

- Mean $EC_{50}$ values based on different growth variables were not significantly different.
- Yield $EC_{50}$s tended to be lower than growth rate $EC_{50}$s.

**Key points to be revised in protocol**

- More stringent recommendations will be made regarding the size of plants at test initiation.
- Environmental conditions may be modified to promote growth rates.
- Maximum / minimum water-sediment ratios will be defined.
- Test duration will be set according to doubling time in controls and is likely to be 14 days.
- Validity criteria are likely to set as follows (to be confirmed):
  - Maximum variability of 35% for growth rates and for yield leaf length
  - Minimum growth rates of 0.070 d⁻¹ for fresh weight and leaf length

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- Test items are supplied by Bayer AG (isoproturon) and BASF (imazapyr).