A PROPOSED OECD TEST GUIDELINE FOR THE SUBMERGED, SEDIMENT-ROOTED MACROPHYTE, MYRIOPHYLLUM
Jo Davies, Syngenta, UK, Peter Dohmen, BASF, Germany and Gertie Arts, Alterra, The Netherlands.

Introduction
Currently, the risk of herbicides to aquatic plants is evaluated from data for four algal species and a single macrophyte species, in the US, and from data for two algal species and one macrophyte species, in the EU. In both cases, the standard macrophyte test species is Lemna gibba or L. minor. In the EU, concern has been expressed that risk assessments based on Lemna species may underestimate toxicity of some chemicals to other macrophyte species, particularly sediment-rooted species. Recent guidance published from the SETAC workshop on Aquatic Macrophyte Risk Assessment for Pesticides (AMRAP) proposed that data for a rooted macrophyte species may be required under the following circumstances:

- If Lemna is known not to be sensitive to the mode of action.
- If Tier 1 Lemna and algal EC50 values are > 1 mg a.i./L.
- If partitioning to sediment is a concern.

The submerged, dicotyledonous species, Myriophyllum, was selected by AMRAP participants as a suitable test species in light of prior experience and its known sensitivity to some herbicide chemistries. A protocol was then developed for validation in a pre-test (11 laboratories) and final ring-test (14 laboratories) as shown in Tables 1 and 5.

Method
Test substances : 2,4-D, 3,5-DCP, isoprotron, trifurilurin (Table 2)
Test species : M. spicatum and M. aquaticum (Table 3)

Table 1: Method for Myriophyllum species

<table>
<thead>
<tr>
<th>Protocol</th>
<th>OECD draft protocol (Maltby et al, 2010)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test system</td>
<td>Plant pots in glass test vessels (minimum volume of 2 l; Figure 1)</td>
</tr>
<tr>
<td>Sediment</td>
<td>Artificial sediment (OECD 219) with added N and P nutrients</td>
</tr>
<tr>
<td>Media</td>
<td>Smart and Barlo medium at pH 7.5</td>
</tr>
<tr>
<td>Application</td>
<td>Via water column (method can be adapted for sediment application)</td>
</tr>
<tr>
<td>Test design</td>
<td>5 concentrations plus untreated control, each with 3 replicate test vessels containing one plant pot of 3 shoots</td>
</tr>
<tr>
<td>Test conditions</td>
<td>20 ± 2°C with 16/8 hour photoperiod (160 μE·m⁻²·s⁻¹)</td>
</tr>
<tr>
<td>Test duration</td>
<td>3 to 7-day acclimation phase followed by 7-day (M. aquaticum) or 14-day (M. spicatum) exposure phase</td>
</tr>
<tr>
<td>Biological assessments</td>
<td>Shoot length on minimum of 4 occasions</td>
</tr>
<tr>
<td>Analytical measurements</td>
<td>Water sampled for analysis of test substance concentration at beginning and end of test</td>
</tr>
<tr>
<td>Environmental assessments</td>
<td>pH, DO and temperature recorded at beginning, middle and end of test</td>
</tr>
<tr>
<td>Endpoints</td>
<td>EC50 based on increase in biomass and growth rates</td>
</tr>
</tbody>
</table>


Table 2: Test substance details

<table>
<thead>
<tr>
<th>Test substance</th>
<th>Mode of action</th>
<th>Mean Kow (g/L)</th>
<th>Water solubility (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-D (pre-test only)</td>
<td>Synthetic auxin</td>
<td>56</td>
<td>~23000</td>
</tr>
<tr>
<td>3,5-DCP</td>
<td>Non-specific</td>
<td>n.d.</td>
<td>~3000</td>
</tr>
<tr>
<td>Isoprotron</td>
<td>Pn inhibition</td>
<td>122</td>
<td>~70</td>
</tr>
<tr>
<td>Trifurilurin</td>
<td>Microtubule inhibition</td>
<td>&gt;6000</td>
<td>0.2 - 0.4</td>
</tr>
</tbody>
</table>

Table 3: Comparison of Myriophyllum species

<table>
<thead>
<tr>
<th>Species</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. aquaticum</td>
<td>- easy to handle and propagate</td>
<td>- limited prior experience</td>
</tr>
<tr>
<td></td>
<td>- rapid growth with biomass doubling in 5-7 days</td>
<td>- availability is limited in some countries (UK)</td>
</tr>
<tr>
<td></td>
<td>- short test duration (7 days)</td>
<td>- submerged and emergent leaves</td>
</tr>
<tr>
<td></td>
<td>- readily available during winter months</td>
<td>- relative sensitivity of different leaf forms is uncertain</td>
</tr>
<tr>
<td>M. spicatum</td>
<td>- extensive prior experience</td>
<td>- longer test duration (14 days)</td>
</tr>
<tr>
<td></td>
<td>- forms only submerged leaves</td>
<td>- sensitive to algal growth</td>
</tr>
<tr>
<td></td>
<td>- biomass doubling in 10-14 days</td>
<td>- availability may be limited over winter</td>
</tr>
</tbody>
</table>

Figure 1: Myriophyllum spicatum test at US Army Corps of Engineers

Table 4: EC50 values for 3,5-DCP (pre-test data)

<table>
<thead>
<tr>
<th>Measurement parameter</th>
<th>M. aquaticum Mean EC50 and range (mg a.i/L)</th>
<th>M. spicatum Mean EC50 and range (mg a.i/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot length</td>
<td>growth rate</td>
<td>7.3 (4.8 – 10.6)</td>
</tr>
<tr>
<td></td>
<td>yield</td>
<td>5.2 (4.8 – 10.6)</td>
</tr>
<tr>
<td>Fresh weight</td>
<td>growth rate</td>
<td>5.8 (2.8 – 9.8)</td>
</tr>
<tr>
<td></td>
<td>yield</td>
<td>4.4 (2.8 – 10.6)</td>
</tr>
<tr>
<td>Dry weight</td>
<td>growth rate</td>
<td>2.7 (1.3 – 5.3)</td>
</tr>
<tr>
<td></td>
<td>yield</td>
<td>3.3 (1.2 – 4.9)</td>
</tr>
</tbody>
</table>

Results
- Pre-test results indicate that both species show similar sensitivity to 3,5-DCP and 2,4-D with low variability between laboratories (Figure 2, Table 4) 
- Shoot length, fresh weight and dry weight provide consistent and reproducible measures of effect.
- Quality of plant material is critical for achieving reproducible results.

Figure 2: Example concentration response curve

Acknowledgements
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Next Steps
- OECD test guideline project is underway.
- Analyses of final ring-test data are in progress.
- Final ring-test results will be reported over next 6 months.