A PROPOSED OECD TEST GUIDELINE FOR THE SUBMERGED, SEDIMENT-ROOTED MACROPHYTE, *MYRIOPHYLLUM*

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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 chemistries 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 EC_{50} values are > 1 mg ai/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, isoproturon, trifluralin (Table 2)

Test species : *M. spicatum* and *M. aquaticum* (Table 3)

Table 1: Method for *Myriophyllum* species

Protocol	OECD draft protocol (Maltby *et al,* 2010) [1]
Test system	Plant pots in glass test vessels (minimum volume of 2 L; Figure 1)
Sediment	Artificial sediment (OECD 219) with added N and P nutrients
Media	Smart and Barko medium at pH 7.5
Application	Via water column (method can be adapted for sediment application)
Test design	5 concentrations plus untreated control, each with 3 replicate test vessels containing one plant pot of 3 shoots
Test conditions	20 ± 2°C with 16/8 hour photoperiod (160 $\mu E \cdot m^{-2} \cdot s^{-1}$)
Test duration	3 to 7-day acclimation phase followed by 7-day *(M. aquaticum*) or 14-day (*M. spicatum*) exposure phase
Biological assessments	Shoot length on minimum of 4 occasions Fresh and dry weight at beginning and end of test
Analytical measurements	Water sampled for analysis of test substance concentration at beginning and end of test
Environmental assessments	pH, DO and temperature recorded at beginning, middle and end of test
Endpoints	EC_{50} based on increase in biomass and growth rates

[1] Maltby, L., Arnold, D., Arts, G., Davies, J., Heimbach, F., Pickl, C., Poulsen, V. (eds.), (2010). Aquatic Macrophyte Risk Assessment for Pesticides. Guidance from the AMRAP workshop in Wageningen (NL), 14-16 January 2008.

Table 2: Test substance details

Test substance	Mode of action	Mean K_{oc} (g/L)	Water solubility (mg/L)
2,4-D (pre-test only)	Synthetic auxin	56	~23000
3,5-DCP	Non-specific	n.d.	~3000
Isoproturon	PSII inhibition	122	~70
Trifluralin	Microtubule inhibition	>6000	0.2 - 0.4

Table 3: Comparison of *Myriophyllum* species

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

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

Table 4: EC_{50} values for 3,5-DCP (pre-test data)

Measurement parameter		*M. aquaticum* Mean EC_{50} and range (mg ai/L)	*M. spicatum* Mean EC_{50} and range (mg ai/L)
Shoot length	growth rate	7.3 (4.8 – >10.6)	6.9 (5.0 – 8.1)
	yield	5.2 (4.8 – >10.6)	6.2 (3.9 – 7.4)
Fresh weight	growth rate	5.8 (2.8 – 9.8)	6.8 (4.0 – 8.8)
	yield	4.4 (2.8 – >10.6)	7.1 (4.0 – 9.7)
Dry weight	growth rate	2.7 (1.3 – 5.3)	5.1 (3.3 – 6.2)
	yield	3.3 (1.2 – 4.9)	5.7 (3.2 – 9.4)

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Table 5: Participating laboratories

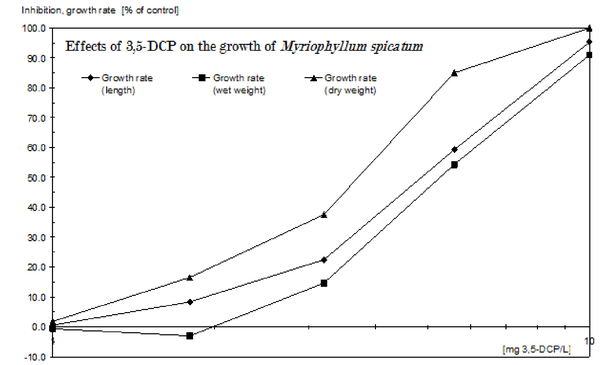
Laboratory	Pre-test	Final ring-test
Alterra	X	X
BASF	X	X
Bayer Crop Science	X	X
Biochem Agrar	X	X
Chemex	X	X
ECT Oekotoxikologie GmbH	-	X
Eurofins-GAB	X	X
Fraunhofer-IME	-	X
Harlan	-	X
IBACON	X	X
Mississippi State University	X	-
Noack	-	X
Smithers Viscient	X	X
UBA, DE	X	X
University of Serbia	-	X
US Army Corps of Engineers	X	-

- did not participate; X participated

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



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.