A PROPOSED OECD TEST GUIDELINE FOR THE SUBMERGED MACROPHYTE, *MYRIOPHYLLUM,* IN A WATER-SEDIMENT SYSTEM

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Introduction

- Previously, the risk of herbicides to aquatic plants has been evaluated from data for four algal species and a single macrophyte species (*Lemna* sp.) , in the US, and from data for two algal species and one macrophyte species (*Lemna* sp.) in the EU.
- New regulation in the EU will require data for an additional macrophyte species. Specifically, the Draft EFSA Guidance Document on tiered risk assessment for aquatic organisms indicates that data for a rooted macrophyte species may be required for substances where:
  - standard *Lemna* and algal EC_{50} values are > 1 mg ai/L.
  - partitioning to sediment is a concern.
- The submerged, dicotyledonous species, *Myriophyllum*, has been identified as a suitable, alternative test species in light of prior experience and its known sensitivity to some herbicide chemistries.
- A protocol for testing two species of *Myriophyllum* in a water-sediment system was developed by an international work group and ring-tested in 15 laboratories in 2011 (Tables 1 & 2).
- In total, 51 toxicity tests were performed and the results , as well as the subsequent protocol recommendations, are summarised here.

Table 1: Ring-test Details

Test species

M. aquaticum

M. spicatum

Test substances

- 3,5, dichlorophenol (3,5 DCP)
- Isoproturon (IP)
- Trifluralin (TF)

Participating laboratories

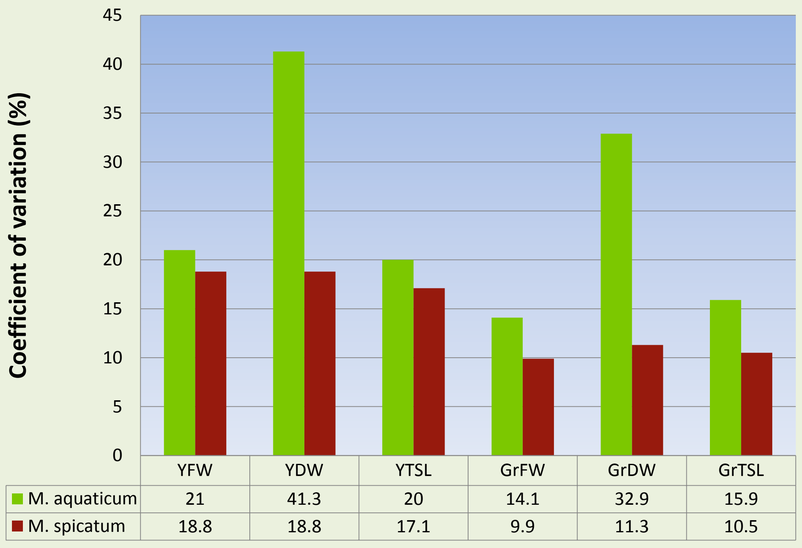
- Alterra, Wageningen, NL
- BASF SE, Limburgerhof, DE
- Bayer CropScience LP, Stilwell, USA
- Biochem Agrar GmbH, Gerichshain, DE
- Chemex Environmental International Ltd, Cambridge, UK
- Dr U Noack Laboratorien, Sarstedt, DE
- ECT Ökotoxikologie GmbH, Flörsheim, DE
- Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, DE
- Federal Environmental Agency, Berlin, DE
- Fraunhofer IME, Schmallenberg, DE
- Harlan Laboratories Ltd, Itingen, CH
- IBacon GmbH, Rossdorf, DE
- Institute of Industrial Organic Chemistry, Pszczyna, PL
- University of Novi Sad, SRB
- Smithers Viscient, Wareham, USA

Table 2: Test 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	Untreated control with 6 replicate test vessels and 5 concentrations ,each with 3 replicate test vessels; each replicate test vessel contains 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 establishment (i.e. rooting) phase followed by 7-day *(M. aquaticum*) or 14-day (*M. spicatum*) exposure phase
Biological assessments	Shoot length and number of lateral branches on 3 or 4 occasions, depending on species. 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} and NOEC values based on yield (Y) and growth rate (Gr) derived from assessments of total shoot length (TSL), shoot dry weight (DW), shoot fresh weight (FW) and number of lateral branches (LB)
Validity criteria for stats analyses [2]	CV of <35% for YFW and minimum growth rate of 0.07 d^{-1} for FW in control plants

[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.
[2] Ratte, M. and Ratte H. T. (2012) Myriophyllum Toxicity Test, Results of a ring-test using *M. aquaticum* and *M. spicatum* grown in a water-sediment system.

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



	YFW	YDW	YTSL	GrFW	GrDW	GrTSL
M. aquaticum	21	41.3	20	14.1	32.9	15.9
M. spicatum	18.8	18.8	17.1	9.9	11.3	10.5

Figure 2: Mean Coefficients of Variation (%) for repeatability for control plants in valid datasets

Table 3: Mean EC_{50} values (mg/L) for valid datasets

Test substance	Assessment parameter	*M. aquaticum*			*M. spicatum*		
		7-d EC_{50}	n	95% PI	14-d EC_{50}	n	95% PI
3,5-DCP	YDW	-	0	-	5.0	5	2.3 – 10.7
	GrDW	3.7	1	n.a.	4.8	4	2.4 – 9.7
	YFW	4.6	2	3.6 – 5.8	4.7	6	2.0 – 10.7
	GrFW	5.2	2	2.8 – 9.7	5.3	5	2.5 – 11.2
	YTSL	5.7	5	3.1 – 10.6	5.3	5	2.9 – 9.8
	GrTSL	6.1	5	3.5 – 10.8	6.3	6	3.9 – 10.1
Isoproturon (IP)	YDW	0.12	1	n.a.	0.052	4	0.016 – 0.168
	GrDW	0.13	1	n.a.	0.074	4	0.034 – 0.161
	YFW	0.86	2	0.59 – 1.25	0.083	6	0.024 – 0.280
	GrFW	1.18	2	1.09 – 1.27	0.164	6	0.046 – 0.581
	YTSL	0.37	3	0.16 – 0.84	0.140	6	0.054 – 0.362
	GrTSL	0.51	3	0.13 – 1.91	0.315	6	0.117 – 0.849
Trifluralin (TF)	YFW	-	-	-	0.319	2	0.104 – 0.978
	GrFW	-	-	-	0.840	1	n.a.
	YTSL	0.82	3	0.16 – 4.1	0.165	3	0.115 – 0.236
	GrTSL	0.75	1	n.a.	0.314	3	0.203 – 0.485

n.a. not applicable
- not determined due to lack of valid datasets (3,5 DCP) or the absence of a consistent dose response (TF)

Key Findings

Performance of control plants and reproducibility (Figure 2)

- Analyses of all datasets indicated that 86% of *M. spicatum* tests and 50% of *M. aquaticum* tests met the preliminary validity criteria for analysis.
- The valid datasets showed control CVs of up to ca. 20%, although *M. aquaticum* CVs were >30% for DW variables. The great variability seen for *M. aquaticum* was attributed to poor growth in some tests.
- Lateral branches were formed in *M. spicatum* but infrequently in *M. aquaticum*, prohibiting the derivation of endpoints based on this parameter for *M. aquaticum* .
- Generally, both species allowed statistical testing at minimal detectable differences of up to 30%, with the exception of the DW variable for *M. aquaticum.*

Sensitivity to 3,5-DCP, Isoproturon and Trifluralin (Table 3)

- Both species were sensitive to all test substances although DW in both species and FW in *M. aquaticum* did not show a conclusive response to TF. This lack of consistent sensitivity may be attributed to the poor water solubility of TF (0.22 mg/L).
- Both species showed similar sensitivity to 3,5-DCP while *M. spicatum* was more sensitive than *M. aquaticum* to IP and TF.
- Systematic differences between Gr and Y endpoints based on DW, FW or TSL were not apparent, except for the finding that Gr endpoints tended to be higher than Y endpoints.
- Growth rate and Y based on lateral branch formation (*M. spicatum*) were not typically the most sensitive endpoints for any test substance.
- Root development was affected by all test substances in both species. Assessments of *M. aquaticum* root length indicated that root endpoints showed similar sensitivity to shoot endpoints for 3,5-DCP (n=2) but were less sensitive than shoot endpoints for IP (n=1).

Recommendations included in the Updated Draft OECD TG

Steps to improve test performance, reproducibility and efficiency

- Use uniform, healthy plant material and verify root formation prior to test initiation.
- Extend the exposure phase for *M. aquaticum* to 10 days if required to achieve doubling.
- Use submerged *M. aquaticum* shoot material , if possible, to initiate test cultures.
- Reduce the number of assessments during the test period, i.e. 3 measurements of TSL for both species.

Validity criteria

- Total shoot length and shoot fresh weight in control plants must at least double within the test duration.
- The mean coefficient of variation for Yield, based on measurements of shoot FW, DW and length, in the control cultures must not exceed 35%.

Recommended Endpoints

- The primary recommended endpoints will be based on growth rate. Options are included for the estimation of yield-based endpoints.
- Growth rate and yield endpoints will be derived from TSL, DW and FW.

Status of Draft OECD TG for *Myriophyllum* in a water-sediment system

- The Draft TG has been updated to include these recommendations and has been circulated to all participants and interested parties for comment.
- The updated TG will be made available to OECD during May 2013.

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