**Myriophyllum spicatum toxicity test: Design and first results of an inter laboratory ring test using a sediment-free test system.**

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**Introduction**

The eco toxicological laboratory of the German Federal Environment Agency has organized an inter laboratory ring test in order to investigate and to optimize a new test method with the dicotyledonous water milfoil Myriophyllum spicatum which has been established at the German Federal Environment Agency (Maletzki et al. 2010).

Typical characteristics of the test system include:
- sediment-free, plants grown one by one and under sterile conditions
Twelve laboratories participated in the ring test running from October 2010 to April 2011. The ring test aimed at:
1. investigating the practicability and reproducibility of the sterile, sediment-free test system,
2. identifying the most appropriate endpoints reflecting different modes of action of the test items and
3. optimizing and standardizing the test method in order to obtain a efficient test system for
dicotyledonous macrophytes.

In a first step the general practicability of the test was investigated and the valid control results were evaluated concerning the intra- and inter-laboratory reproducibility and variability of the test system and endpoints.

**Material and Method**

**Test conditions:**
- 14 days exposure, plants grown one by one, sterile medium replaced after 7 days,
- at 23±2°C, alternating 16:8 hour light/dark phases, light intensity 100-150 µE m-2s-1

**Test items:**
- 2,4-dichlorophenoxyacetic acid (2,4 D, auxine herbicide, growth inhibitor)
- Isoproturon (IP, photosynthesis inhibitor).

**Toxicity parameters:**
- NOEC, ECx and 95%CI

**Statistical design:**
- Control with 10 replicates, 8 treatments with 5 replicates, each

**Response variables:**
- Measured: shoot length (SL), fresh weight (FW), dry weight (DW), number of whorls (W), number and length of lateral branches (LB, LBL), number and length of roots (R, RL)

**Calculated:**
- Total shoot length (TSL), total root length (TRL), Yields (YSL, YFW, YDW), Growth Rates (GSL and G1TL)

**Validity criteria:**
1. shoot length of the control plants should be at least doubled within test duration
2. number of non-sterile control replicates must not exceed 50%
3. number of non-sterile replicates per treatment must not exceed 50%
4. Definition of non-sterility: visible turbidity

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**Results**

**Data base**

<table>
<thead>
<tr>
<th>Test item</th>
<th>Total number of replicates</th>
<th>Number of tests valid</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,5-DOP</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>2,4-D</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>IP</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
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<td>25</td>
</tr>
</tbody>
</table>

**Practicability**

<table>
<thead>
<tr>
<th>Number of replicates</th>
<th>Number of control replicates kept sterile</th>
</tr>
</thead>
<tbody>
<tr>
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<td>5</td>
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<tr>
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</tr>
<tr>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

**Reproducibility of response variables**

**Variability of response variables**

**Discussion and Conclusion**

A sediment-free test system was chosen with a low degree of methodical and analytical complexity to get toxic threshold and effect concentrations being independent of the water-portion of the test item. Also, a direct comparison of the results obtained with the duckweed Lemna spicata (OECD 221) is possible.

**Practicability:** Although 11 of the 12 participants didn’t manage to perform Myriophyllum spicatum culturing under sterile conditions previously, a critical number of non-sterile replicates (>50 %, see validity criteria) only occurred in one control data set (of 30 controls cultured) and in 14 treatment data sets (of 240 cultured). With respect to the latter it still remains to be clarified whether the turbidity in higher concentrations is due to plant degradation, i.e. to a toxic effect rather than to contamination.

Summarizing, even under the assumption that in some laboratories tests have been repeated to fulfill the validity criteria only occurred in one control data set (of 30 controls cultured) and in 14 treatment data sets (of 240 cultured). With respect to the latter it still remains to be clarified whether the turbidity in higher concentrations is due to plant degradation, i.e. to a toxic effect rather than to contamination.

**Response variables variability and reproducibility:** For all variables the inter-laboratory variability clearly exceeds the intra-laboratory variability. This indicates effects of (slightly) varying test conditions (irradiation, temperature...) and variations in handling (e.g. fresh weight measurement) Although this does not necessarily affect the toxicological results (yet to be evaluated). It appears that the test procedure and conditions could be optimized in order to further reduce the inter- and intraindividual variability.

Except for the number and length of lateral branches (LB, LBL), in all remaining variables the intra-laboratory coefficients of variation ranged from 10 to 30%. Thus the given statistical design allows to detect effects in the range of 15% to 35%. LB and LBL are seen as not appropriate as separate response variables, but are essential to calculate total shoot length and therefore are worth to be measured.

Concluding, the comprehensive data set of endpoints under control conditions, obtained from an inter-laboratory ring test, allowed to demonstrate the general practicability of the test system and to show an acceptable reproducibility.

The final evaluation will include a more detailed assessment of the test system with respect to test conditions, validity criteria, appropriate endpoints, sensitivity to various test items and reproducibility of toxicological parameters (NOEC, ECx).

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**Acknowledgment**

Special thanks to our colleagues in the twelve laboratories who generated the raw data for this presentation.

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**References**

- OECD Guideline for testing of chemicals, No. 221, Lemna sp. Growth Inhibition Test, Adopted 23 March 2006