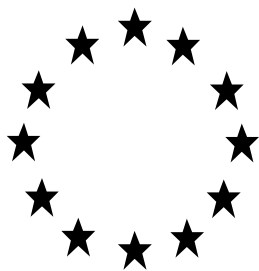


Revised Addendum following the Evaluation of Confirmatory data after approval



ISOXABEN

Summary and Conclusion Volume 3 Annex B.9 Ecotoxicology

Applicant: Dow AgroSciences
Rapporteur Member State: Sweden

February 2014

Volume 1

Level 1: Statement of subject matter and purpose for which the monograph was prepared

Level 2: Reasoned statement of the overall conclusions drawn by the Rapporteur Member State

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Background

Commission Directive 2011/32/EU (8 March 2011) to include isoxaben in Annex I to Directive 91/414/EEC (later transferred to Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011) contains a provision that Member States shall request the submission of confirmatory information on the potential risk to aquatic organisms. This provision relates to a concern raised in the EFSA conclusion on the peer review of isoxaben (EFSA Journal 2010; 8(9): 1714), that the risk assessment for aquatic macrophytes could not be concluded due to uncertainty “... whether the available standard toxicity data on the macrophyte species (*Lemna*) represents the most sensitive species,...”. Because of this, EFSA concluded that “Further data on macrophyte toxicity are required to enable a full assessment of the risk to aquatic organisms”. Specifically, EFSA stated that “The risk to aquatic macrophytes needs to be addressed further to consider concerns regarding exposure route and sensitivity of dicots” and that “concerns remain regarding the potential effects to dicot macrophyte species via root uptake”. To address the uncertainty associated with the representativeness of the toxicity endpoint for *Lemna* to other macrophyte species, the notifier provided a study on the toxicity of isoxaben to a representative dicotyledonous aquatic plant (*Myriophyllum spicatum*). This study is evaluated below.

Summary and conclusions of the Evaluation

It was concluded during the peer review of isoxaben that further evidence was required to show that *Lemna sp* represents the most sensitive species and that the aquatic risk assessment cannot be finalized. To address this concern, the applicant submitted a sediment-water test on the dicotyledon *M. spicatum* with special regards on addressing the sensitivity of a rooted macrophyte in comparison to the morphologically reduced species *Lemna sp.* as well as potential risks considering exposure both via the water phase and the sediment phase.

The applicant submitted a justification for using a sediment-water test to address the concerns regarding the risk to aquatic macrophytes. The RMS agrees on the suitability of the chosen test method. Further information on the test method is presented in section B.9.2.8.1.

In a 14-day *Myriophyllum spicatum* growth inhibition test with isoxaben total shoot length was found to be the most sensitive parameter with EC₅₀ values for yield and growth rate of 67.6 and 126 µg a.s./L, respectively. In terms of the NOEC, yield dry weight was the most sensitive parameter with significant inhibition relative to the pooled controls at all concentrations (NOEC < 11.5 µg/L). Yield and growth rate of fresh weight were unaffected at concentrations of 32.0 and 97.1 µg/L (NOEC). The NOEC for shoot length (both yield and growth rate) and dry weight growth rate was 11.5 µg/L. Resulting endpoints are presented in Table 1.

Table 1. Summary of test results of the growth test with *Myriophyllum spicatum*. Effective concentrations (EC50) and their 95 %-confidence limits (CI), LOEC and NOEC values in terms of geometric mean measured concentrations of the test item after a 14 - day exposure period.

Parameter	[µg a.s./L]		
	EC ₅₀ (95% CI)	LOEC	NOEC
Total shoot length, yield	67.6 (58.7 – 77.9)	32.0	11.5
Total shoot length, growth rate	126 (110 – 145)	32.0	11.5

Fresh weight, yield	156 (117 – 208)	97.1	32.0
Fresh weight, growth rate	217 (158 – 292)	320	97.1
Dry weight, yield	93.4 (60.8 – 143)	11.5	< 11.5
Dry weight, growth rate	250 (166 – 401)	32.0	11.5

The lowest 14 day E_yC_{50} and E_rC_{50} (67.6 µg/L and 126 µg/L, based on shoot lengths) is approximately 6 and 11 times higher than the lowest 7d E_bC_{50} (11µg a.s./L) derived from the study by Putt (2003) on *Lemna gibba* used in the risk assessment on aquatic macrophytes in the DAR (2006) and the Final Addendum to the DAR and the Additional Report (2010). The Lemna endpoint of 11µg a.s./L was based on biomass (dry weight). As a comparison, the 14 day E_yC_{50} based on dry weight for *M. spicatum* was 93.4 µg a.s./L. Hence, it can be concluded that the aquatic risk assessment based on *Lemna gibba* also is protective to rooted aquatic macrophytes. In the test on *M. spicatum*, plants were exposed both via overlying water and via root uptake.

It is the opinion of the RMS that the concerns regarding the risk assessment of aquatic macrophytes has been adequately addressed.

B.9 Ecotoxicology

B.9.2.8 Effects on Aquatic macrophytes

B.9.2.8.1 Test strategy

The following justification was submitted by the applicant regarding the suitability of test method for measuring the toxicity of isoxaben to the aquatic macrophytes *Myriophyllum sp.* with special regards to address the concerns regarding exposure route and sensitivity of dicots:

“To address the uncertainty associated with the representativeness of the toxicity endpoint for Lemna to other macrophyte species, the toxicity of isoxaben to a representative dicotyledonous aquatic plant (*Myriophyllum spicatum*) has been determined. Two test methods for measuring the toxicity of herbicides to *Myriophyllum sp.* are currently being evaluated to determine their suitability as regulatory tests; a water-only test (Federal Environment Agency (UBA) method) and a water-sediment test (SETAC AMEG method). The latter method was initiated following recommendations arising from the SETAC AMRAP Workshop in 2008. The main differences between the water-only and water-sediment test methods are:

- (a) presence or absence of roots at exposure initiation,
- (b) presence or absence of artificial sediment,
- (c) presence or absence of sucrose in the test medium,
- (d) choice of test species (*M. spicatum* or *M. aquaticum*)

Regarding point (a), it should be noted that a plant rooting phase only precedes the exposure phase using the water-sediment method, consequently rooted plants are exposed from test initiation. In contrast, in the water-only method, freshly cut shoot tips without roots are exposed in the water phase at test initiation. Due to the lack of sediment in the water-only method (point (b)), the addition of sucrose is required for growth, indicating changed plant physiology, and a need to supplement photosynthesis (point (c)); this also means that as the roots develop in the water-only method they are exposed for 16 hours each day to irradiation. It is uncertain how this (unnatural) combination of sucrose in the test media and 16 hours irradiation of developing roots might influence plant metabolism, growth, shoot and root development. In contrast, the water-sediment method approximates more natural growth conditions (plants rooted in sediment), and the sediment can act as a possible sink for toxic substances and serve as an additional exposure route via root uptake. Finally, the choice of test species in the AMRAP method (point (d)) impacts the choice of test duration, as well as plant growth and validity criteria, e.g. species-specific growth rates, appearance of side shoots and available endpoints.

All of these factors were considered prior to selecting the appropriate test method to be used for isoxaben.

As part of the ongoing evaluation of the two test methods, inter-laboratory ring-tests have been conducted. The findings for the water-only evaluation have been reported by Maletzki (2011) and the findings for the water-sediment method have been reported by Ratte & Ratte (2012). A comparison of the methods and results from

both inter-laboratory ring-tests indicates that plant performance (as measured by control growth rate and variability) was best for *M. spicatum* using the water-sediment method, followed by *M. spicatum* using the water-only method. Also, it was observed that regular lateral branch production occurred for *M. spicatum* in the water-sediment test system, contributing approximately 50% of total shoot length. In contrast, in the water-only test system, *M. spicatum* only produced lateral branches infrequently, possibly due to the restricted space in the test tubes. As a result, considerable production of lateral branches by *M. spicatum* resulted in 4-5 fold higher absolute yield levels of weight and length as compared to the water-only test.

Despite the differences in test methods, for the test materials used in both ring-tests, one (3,5-DCP) resulted in similar toxicity to *M. spicatum* in both test methods, while for the other (isoproturon) *M. spicatum* exposed in the water-only test system was less sensitive than when exposed in the water-sediment test system (though there was limited data available). Therefore, based on the ring-testing information, there was no indication that results obtained for *M. spicatum* using a water-sediment method will significantly differ from those obtained using a water-only method. Indeed since toxicity was lower in the water-only method for isoproturon, a systemic herbicide which is taken up by roots in terrestrial plants, there is little reason to believe that uptake of isoxaben, a non-systemic herbicide, via the roots of aquatic plants is likely to be significant relative to shoot uptake. These findings were taken into consideration when considering the appropriateness of the test method used to evaluate isoxaben.

Based on the information available, and because the aim of the test with isoxaben was to consider exposure route and sensitivity of *Myriophyllum sp.*, the water-sediment method with *M. spicatum* was chosen since:

- (a) test duration for *M. spicatum* is longer (14 days) than *M. aquaticum* (7 days), and so represents a worse-case; *M. spicatum* performance (control growth and variability) was better than *M. aquaticum* in the water-sediment ring-test; *M. spicatum* performance was better in the water-sediment ring-test than in the water-only test,
- (b) a well developed root system is present at initiation of exposure using the water-sediment method, thus maximizing the opportunity for root uptake, unlike the water-only test,
- (c) plant roots are exposed under relevant growing conditions in the water-sediment method, rather than irradiated in water,
- (d) potential interference by sucrose on plant metabolism and growth is avoided by using the water-sediment method

Finally, in the regulatory context the water-only method may be considered to represent a Tier 1 screening test, while the water-sediment method may be considered a Tier 2 test incorporating more realistic growth conditions for a rooted macrophyte species.”

RMS comments and conclusion:

In previous communication with the applicant, the RMS suggested a water only test as a first tier for better comparison of the sensitivity of *Myriophyllum sp.* and *Lemna gibba*. Although no direct comparison of the

sensitivity of the two species can be made from the test method finally chosen, the RMS considers the water-sediment test a more conservative approach since the endpoint derivation from the water-sediment test is based on exposure from the water phase only. In reality, the effects may have been caused by the additive exposure from the water phase and the sediment phase. Therefore, to address the regards concerning the relative sensitivity of *Myriophyllum sp* and *Lemna sp.*, the RMS considers the water-sediment test to be appropriate.

The second concern identified was potential effects to dicot macrophyte species via root uptake. This concern is adequately addressed by a sediment-water test as suggested by the applicant.

A concern raised by the RMS regarding test methodology is the qualitative judgement of root development, since classification into categories such as “very good root development”, “moderate root development” or “few roots” is somewhat subjective, a quantitative measure of root length would have been preferable. In the study on isoxaben evaluated below, there are indications that root development is the less sensitive parameter. Therefore, the RMS considers a qualitative judgement is sufficient to determine the root development following exposure from isoxaben to *Myriophyllum sp*.

Overall, the RMS considers the water-sediment test to be appropriate to address the concerns raised in the EFSA conclusion on the peer review of isoxaben (EFSA Journal 2010; 8(9): 1714) regarding the risk assessment for aquatic macrophytes.

B.9.2.8.2 Toxicity studies in aquatic plants

Reference:	Wenzel A (2013) Effect of Isoxaben on the growth of <i>Myriophyllum spicatum</i> in the presence of sediment with exposure via the water phase.
Dow AgroScience Study No.:	120580
Guideline:	Aquatic Macrophyte Risk Assessment for Pesticides. Chapter 5.2: Development of a proposed test method for the rooted aquatic macrophyte, <i>Myriophyllum sp.</i> , p. 47-63. SETAC Europe Workshop AMRAP Wageningen (The Netherlands), January 2008.
GLP:	Yes
Previous evaluation:	Submitted for the purpose to address requirement for confirmatory information (Regulation (EU) No 540/2011)
Material and methods:	
Test material:	Isoxaben
Lot/Batch No:	0041; TSN 100774
Purity:	91.7%
Species:	<i>Myriophyllum spicatum</i> , Haloragaceae, Dicotyledonous.
Sediment:	Standard artificial sediment: 5.02% sphagnum peat (2 ±0.5% OC in the final sediment; 20.0% kaolin clay; 75% quartz sand; deionised water to obtain a moisture of about 30%)

Nutrient solution:	1 L solution contained 6.66 g NH_4Cl and 15.4 g $\text{Na}_3\text{PO}_4 \times 12 \text{ H}_2\text{O}$ (concentration in the final sediment: 200 mg Na_3PO_4 /kg sediment and 200 mg NH_4Cl /kg sediment)
Growth medium:	Smart & Barko medium was used as liquid growth medium. At test initiation, the pH of the medium was adjusted to a level of 7.5 - 8.0 to allow optimum plant growth (using 0.1 M NaOH).
Experimental setup:	Planting pots were prepared with two filter papers in the bottom of the pot to stop soil loss. A 1 cm layer of the standard sediment (70 g) was added followed by a 4 cm layer of nutrient supplemented sediment (270 g). A second 1 cm layer of standard sediment (100 g) was added followed by a fine layer (approx. 2 mm) of coarse quartz sand in order to reduce suspension of sediment into the water. Five replicate test vessels were used for each treatment group, 10 replicate test vessels for the control group, and 10 replicates for the solvent control group. During exposure phase, each test vessel contained one sediment pot and three plant shoots. <u>Pre-incubation</u> Healthy shoot apices 6 cm (± 0.5 cm) long were abscised from the stock culture plants, patted dry, and individually weighed. Five plants with a uniform weight within a 30 % range (range of 100 – 130 % based on the lowest used weight) were selected and potted 3 cm deep into the sediment in each pot. Shoots were then maintained for 7 days, in aquaria with enough tap water to cover the plants, in order to induce root development. <u>Test initiation</u> After pre-incubation, the pots were relocated from the pre-incubation aquaria to individual 2 L glass beakers. The shoot length above the sediment was measured (day 0) using a ruler inside the test vessels. Two of the five plants per pot were removed to leave three individuals, uniform in size and appearance. Smart & Barko test medium was carefully poured into the beakers via a funnel so as not to disturb the sediment. Each vessel contained 1.8 L of test media at a minimum depth of 12 cm. The water level was marked. The water level was adjusted with distilled water to the marked level when evaporation loss during the test was more than 10%. To obtain the respective mean biomass data for day 0 (test start), five homogeneous plants from each of five additional pots were harvested at test initiation and the fresh and dry weights were determined.
Treatments:	The nominal concentrations of the active substance isoxaben, spaced by a factor of 3.16, were: control, solvent control (100 $\mu\text{L/L}$ DMF), 10.0, 31.6, 100, 316 and 1000 $\mu\text{g a.s./L}$. Treatment were spiked using 100 $\mu\text{L/L}$ DMF.
Controls:	Negative control: Artificial sediment + growth medium Solvent control: Artificial sediment + growth medium + dimethylformamide (DMF)
Duration:	7 days pre-exposure and 14 days exposure phase
Test conditions:	Temperature: $20.7 \pm 0.84^\circ\text{C}$ Light: Cool white fluorescent lighting was used to provide a light intensity between 7609-7824 lux. A light/ dark cycle of 16/8 h was used pH: 9.58-10.2
Observations:	Visual inspections of plant growth, morphological changes/symptoms, or any other unusual observations were recorded on days 5, 10, and 14. Shoot length (cm) was determined on days 7 and 14, from the level of the sediment, using a ruler inserted into the test vessel and stood against the straightened plant. If side shoots were present, their numbers and length were also measured. At test end (day 14), the plants were washed free of sediment. A visual qualitative assessment of the roots was made. Total fresh weight and dry weight (mg) after oven drying at 60°C for 48 h was then determined.
Chemical analysis:	The concentrations of the active substance in the water phase of all test treatments were assessed by chemical analysis (LCMS/MS) at test start and end. Samples from the highest test concentration were additionally taken for analysis at 3 and 7 days. For analysis in sediments, two aliquots of 10 g wet sediments were shaken twice for 30 minutes with 20 mL of methanol:acetic acid (999:1) and afterwards centrifuged at 3000 rpm for 5 min. Prior to LC-MS/MS analysis the samples were centrifuged again for 5 min at 14000 rpm.

Data analysis

The results for the parameters (growth rate and yield) were subjected to ANOVA. Since there was no difference between the control and solvent control for any parameter, the controls were pooled for comparison to the treatments. When treatment-effects were detected, a Williams' test or Welch t-test were performed to derive the No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC). The EC₅₀ values were calculated by probit analysis modified for continuous data. All statistical analyses were conducted by the computer program ToxRat Professional.

Results:

At test start, the measured test concentrations were 14.0, 38.3, 110, 395 and 885 µg/L (i.e. 88.5 – 140 % of nominal). The highest test concentration of isoxaben was 102 % and 90.3 % of nominal on days 3 and 7, respectively (Table 9.2.8.2-1). By test end (day 14), the measured test concentrations were 9.4, 26.7, 85.5, 259 and 842 µg/L (i.e. 82.1 – 94.4 % of nominal) (Table 9.2.8.2-1). The geometric mean of measured concentrations at day 0 and day 14 for each test concentration was calculated and used for the evaluation of the growth inhibition test. The geometric mean measured concentrations were 11.5, 32.0, 97.1, 320, and 863 µg/L (i.e. 86.3 – 115 % of nominal).

Table 9.2.8.2-1. Measured concentrations of isoxaben in the test medium.

Nominal concentrations [µg/L]	Day 0		Day 3		Day 7		Day 14	
	Measured conc [µg/L]	% of Nominal	Measured conc [µg/L]	% of Nominal	Measured conc [µg/L]	% of Nominal	Measured conc [µg/L]	% of Nominal
Control	< LOQ	-	-	-	-	-	< LOQ	-
Solvent control	< LOQ	-	-	-	-	-	< LOQ	-
10.0	14.0	140	-	-	-	-	9.44	94.4
31.6	38.3	121	-	-	-	-	26.7	84.5
100	110	110	-	-	-	-	85.5	85.5
316	395	125	-	-	-	-	259	82.1
1000	885	88.5	1019	102	903	90.3	842	84.2

Isoxaben was measured in representative sediment samples of all treatment levels at test end. The measured isoxaben concentrations in sediment were 9.41, 45.5, 103, 288 and 782 µg/kg sediment dry matter, respectively, with increasing test concentrations. The total mass of isoxaben in sediment represented 13.0, 23.1, 18.1, 14.2 and 17.2 % of the total initial mass of isoxaben present in the test vessels. By test end, the mass balance of isoxaben including sediment and water column was between 79.8 and 112 % of the mass of isoxaben originally introduced into the test vessels. Concentrations of isoxaben are reported in Table 9.2.8.2-2

Table 9.2.8.2-2. Measured concentrations of isoxaben in the test sediment at day 14.

Measured initial concentrations [µg/L]	Total initial isoxaben mass in test vessel (1.8 L water) [µg]	Mean concentration in sediment [µg/kg dry weight]	Total mean mass in sediment (349.4 g dw sediment) [µg]	% of initial mass in test vessel present in sediment
Control	-	< LOQ ¹	< LOQ ¹	-
Solvent control	-	< LOQ ¹	< LOQ ¹	-
14.0	25.2	9.41	3.29	13.0
38.3	68.9	45.5	15.9	23.1
110	198	102	35.8	18.1
395	711	288	100.7	14.2

Measured initial concentrations [µg/L]	Total initial isoxaben mass in test vessel (1.8 L water) [µg]	Mean concentration in sediment [µg/kg dry weight]	Total mean mass in sediment (349.4 g dw sediment) [µg]	% of initial mass in test vessel present in sediment
885	1593	782	273	17.2

¹ LOQ = 3.15 µg/kg dw

The total recovery of sediments and test media are presented in Table 9.2.8.2-3.

Table 9.2.8.2-3. Total recovery of sediments and test media

Measured initial concentrations [µg/L]	Total initial isoxaben mass in test vessel (1.8 L water) [µg]	Mean concentration in test media at 14 d [µg/L]	Total mean mass in test media (1.8L) [µg]	Mean concentration in sediment at 14 d [µg/kg dry weight]	Total mean mass in sediment (349.4 g dw sediment) [µg]	Total recovery from sediment and test media [%]
Control	-	< LOQ	-	< LOQ ¹	-	-
Solvent control	-	< LOQ	-	< LOQ ¹	-	-
14.0	25.2	9.44	17.0	9.41	3.29	80.6
38.3	68.9	26.7	48.1	45.5	15.9	92.9
110	198	85.5	153.9	102	35.8	95.7
395	711	259	466.2	288	100.7	79.8
885	1593	842	1515.6	782	273	112

¹ LOQ = 3.15 µg/kg dw

Total shoot length was found to be the most sensitive parameter with EC₅₀ values for yield and growth rate of 67.6 and 126.4 µg/L respectively. Yield fresh weight and yield dry weight EC₅₀ values were 156 and 93.4 µg/L respectively. For growth rate the respective EC₅₀ values were 217 and 250 µg/L. All results are presented in Tables 9.2.8.2-4 to 9.2.8.2-7.

In terms of the NOEC, yield dry weight was the most sensitive parameter with significant inhibition relative to the control at all concentrations (NOEC < 11.5 µg/L). Yield and growth rate of fresh weight were unaffected at concentrations of 32.0 and 97.1 µg/L (NOEC). The NOEC for shoot length (both yield and growth rate) and dry weight growth rate was 11.5 µg/L.

Table 9.2.8.2-4. Percent inhibition (means) of shoot length yield and growth rate of *Myriophyllum spicatum*.

Geometric mean measured concentrations [µg/L]	Day 0	Day 14		
	Shoot length [cm]	Shoot length [cm]	Yield shoot length [cm] (inhibition ^a in %)	Growth rate shoot length [cm/day] (inhibition ^a in %)
Control	4.58	32.47	27.893 (-)	0.140 (-)
Solvent control	4.34	32.29	28.053 (-)	0.145 (-)
11.5	4.39	31.78	27.387 (2.1)	0.141 (0.6)
32.0	4.35	25.53	21.180 (24.3)	0.126 (11.5)
97.1	4.85	14.88	10.027 (64.2)	0.079 (44.3)
320	4.25	6.52	2.273 (91.9)	0.030 (78.7)
863	4.11	5.40	1.293 (95.4)	0.019 (86.7)

^a Inhibition in comparison to pooled controls

Table 9.2.8.2-5. Percent inhibition (means) of fresh weight yield and growth rate of *Myriophyllum spicatum*.

Geometric mean	Day 0 ^a	Day 14
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measured concentrations [µg/L]	Fresh weight [mg]	Fresh weight [mg]	Yield fresh weight [mg] (inhibition ^b in %)	Growth rate fresh weight [mg/day] (inhibition ^b in %)
Control	209.7	1011.2	801 (-)	0.112 (-)
Solvent control		1039.8	830 (-)	0.114 (-)
11.5		1113.3	904 (-10.4)	0.119 (-5.5)
32.0		1032.5	823 (-0.9)	0.113 (-0.3)
97.1		796.4	587 (28.1)	0.093 (17.6)
320		368.9	159 (80.5)	0.035 (68.9)
863		253.0	43 (94.7)	0.011 (90.5)

^a Average of 25 representative plants^b Inhibition in comparison to pooled controls**Table 9.2.8.2-6. Percent inhibition (means) of dry weight yield and growth rate of *Myriophyllum spicatum*.**

Geometric mean measured concentrations [µg/L]	Day 0 ^a	Day 14		
	Dry weight [mg]	Dry weight [mg] (inhibition in %)	Yield dry weight [mg] (inhibition ^b in %)	Growth rate dry weight [mg/day] (inhibition ^b in %)
Control	20.1	99.8	79.7 (-)	0.112 (-)
Solvent control		99.8	79.7 (-)	0.112 (-)
11.5		80.9	60.8 (23.7)	0.099 (11.2)
32.0		74.0	53.9 (32.4)	0.093 (17.2)
97.1		67.0	46.9 (41.2)	0.085 (23.9)
320		38.9	18.8 (76.5)	0.043 (61.8)
863		33.0	12.9 (83.8)	0.033 (70.5)

^a Average of 25 representative plants^b Inhibition in comparison to pooled controls**Table 9.2.8.2-7. Root development of *Myriophyllum spicatum* following exposure to isoxaben**

Geometric mean measured concentrations [µg/L]	Root length / development (Code)	Description
Solvent control	-	
11.5	2	moderate roots development
32.0	3	very good roots development, comparable to controls
97.1	S/2	roots are shorter than in the control /moderate roots development
320	S/2	roots are shorter than in the control /moderate roots development
863	S/1	roots are shorter than in the control /few roots

Table 9.2.8.2-8. Summary of test results of the growth test with *Myriophyllum spicatum*. Effective concentrations (EC50) and their 95 %-confidence limits (cl), LOEC and NOEC values in terms of geometric mean measured concentrations of the test item after a 14 - day exposure period.

Parameter	[µg a.s./L]		
	EC ₅₀ (95% CI)	LOEC	NOEC
Total shoot length, yield	67.6 (58.7 – 77.9)	32.0	11.5
Total shoot length, growth rate	126 (110 – 145)	32.0	11.5
Fresh weight, yield	156 (117 – 208)	97.1	32.0
Fresh weight, growth rate	217 (158 – 292)	320	97.1
Dry weight, yield	93.4 (60.8 – 143)	11.5	< 11.5
Dry weight, growth rate	250 (166 – 401)	32.0	11.5

RMS comments and conclusion:

The study was well performed and reported. No specific validity criteria for macrophyte growth tests have been set yet. However, in the draft guideline used it was suggested that for the test to be valid, a biomass increase in

controls (about 100 %), growth throughout the test duration, and maintenance of temperature and pH within a pre-determined range should be fulfilled. The biomass increase in the controls during the 14 day study was above 100% and temperature was kept stable. The test guideline suggests an initial test pH of 7.5-8.0. The actual values were considerably higher (9.90 – 10.24). However, since the growth throughout the test was maintained and the test concentrations in the test medium and sediments were well recorded, the RMS find it unlikely that the elevated test pH would have major impacts on the final results.

The total recovery of isoxaben from sediments and test media ranged between 79.8 to 112 %.

The concerns identified by the RMS in section B.9.2.8.1 regarding the qualitative judgement of root development were considered not to have major influences on the final results. The NOEC based on the qualitative root development was 32 µg a.s./L which was higher than the NOEC based on both dry weight and shoot length. In addition, the NOEC for *Lemna sp.* was determined to be 6.0 µg/L (Putt et al. 2003; DAR isoxaben 2006), a figure 5 times lower than the NOEC for root development suggesting that the risk assessment based on *Lemna gibba* is protective also to rooted macrophytes such as *Myriophyllum sp.*

The RMS concludes that total shoot length was the most sensitive parameter measured, with E_yC_{50} 67.6 µg a.s./L and E_rC_{50} 126 µg a.s./L. Overall NOEC (based on growth rate) was 11.5 µg a.s./L, as determined for total shoot length and dry weight.

B.9.11 References relied on

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner
IIA 8.2.8	Wenzel, A.	2013	Effect of Isoxaben on the growth of <i>Myriophyllum spicatum</i> in the presence of sediment with exposure via the water phase. Dow AgroScience study No 120580. GLP, not published		Dow Agro- Science

*: Data protection claimed by the notifier, no assessment on acceptability at this stage.