Title

Glyphosate TC: A Study on the Toxicity to the Rooted Aquatic Macrophyte *Myriophyllum spicatum*

- Final Report -

Test Item

Glyphosate TC
Specification Number: 102000037890

Data Requirements / Guidelines

OECD Guideline No. 239 (September 2014),
EU Directive 91/414/EEC Regulation 1107/2009 (Europe), (Nov. 2009)

Study Completion Date:

2022-01-27
(YYYY-MM-DD)

Authors

[redacted]

Test Facility
ECT Oekotoxikologie GmbH
Böttgerstr. 2-14
D-65439 Flörsheim/Main
Germany

Sponsor
Bayer Agriculture BV
Scheldelaan 460/Haven 627
2040 Antwerp
Belgium
(in conjunction with the Glyphosate Renewal Group)

Test Site
CIP – Chemisches Institut Pforzheim GmbH
Schulberg 17
75175 Pforzheim
Germany

Study ID
21P1MW

Activity ID
EBGL0420

Original 1/2

The following statement is only intended and valid for submission which are subject to the United States - Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)

Statement of No Data Confidentiality Claims

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that the information not designated as within the scope of FIFRA, Section 10(d)(1)(A)(B), or (C) in USA and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g) in USA.

However, these data are the property of the Glyphosate Renewal Group and, as such, are considered to be a trade secret and confidential for all purposes other than compliance with FIFRA 10 in USA.
Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any country other than the USA.

Submitter Signature: [redacted] Date: 2022-01-28 (YYYY-MM-DD)

Typed Name of Submitter: [redacted]
(on behalf of the Glyphosate Renewal Group)

Typed Name of Company: Bayer AG, Crop Science Division

The above statement supersedes all other statements of confidentiality that may occur elsewhere in this report.

1 Good Laboratory Practice Compliance Statement

I, the undersigned Study Director, hereby certify that this study was performed in compliance with Good Laboratory Practice Principles (GLP) specified in the most recent edition of:

- OECD (1998).The OECD Principles of Good Laboratory Practice (1998) (as revised in 1997). OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring No.1. ENV/MC/CHEM(98)17. Organisation for Economic Co-Operation and Development, Paris.
- OECD (2002). The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies (June 25, 2002). OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring No. 13. Consensus Document of the Working Group on Good Laboratory Practice. ENV/JM/MONO(2002)9. Organisation for Economic Co-Operation and Development, Paris.
- Chemikaliengesetz in der Fassung der Bekanntmachung vom 28. August 2013 (BGBl. I S. 3498, 3991), das zuletzt durch Artikel 1 des Gesetzes vom 27. Juli 2021 (BGBl. I S. 3274) geändert worden ist. Anhang 1 (zu § 19a Abs. 1) Grundsätze der Guten Laborpraxis (GLP). [German Chemicals Act dated 28. August 2013, Federal Law Gazette, Germany (BGBl) p. 3498, 3991; as amended on July 27, 2021 (BGBl. I S. 3274). Appendix I (rel. to §19a (1), Principles of Good Laboratory Practice].

and latest OECD relevant guidelines available at the start of the study.

It is certified that this study was conducted according to the procedures described herein and that this report represents a complete, true and accurate representation of the study and its results.

Study Director: [redacted] Date: 2022-01-27
(YYYY-MM-DD)

Test Facility ECT Oekotoxikologie GmbH

The following signature(s) are relevant to fulfil EPA requirements (no OECD GLP relevance).

Representative
of the Sponsor/Submitter: [redacted] Date: 2022-01-28
Typed Name of Submitter: [redacted] (YYYY-MM-DD)
(on behalf of the Glyphosate
Renewal Group)

2 Certification of Authenticity

ECT Oekotoxikologie GmbH certifies that the study "Glyphosate TC: A Study on the Toxicity to the Rooted Aquatic Macrophyte *Myriophyllum spicatum*" was conducted according to the procedures described herein and that this report represents a complete, true and accurate representation of the study and its results.

Study Director: [redacted] Date: 2022-01-27
(YYYY-MM-DD)

Head of Test Facility: [redacted] Date: 2022-01-22
(YYYY-MM-DD)

3 Quality Assurance Statement

Study number: 21P1MW

Study title: Glyphosate TC: A Study on the Toxicity to the Rooted Aquatic Macrophyte *Myriophyllum spicatum*

Study plan, experimental phase and report of this study were inspected/audited by the quality assurance unit. The inspection/audit results were reported to study director and test facility management. The dates are given below:

Phase inspected / audited	Date of QAU audit/inspection	Date of reports to study director and test facility management
Study Plan	October 14, 2021	October 14, 2021
Experimental Phase	November 03, 2021	November 03, 2021
Amendment 1	December 01, 2021	December 01, 2021
Amendment 2	January 20, 2022	January 20, 2022
Draft Report	December 15, 2021	December 15, 2021
Final Report	January 27, 2022	January 27, 2022

I hereby declare that the reported results reflect the raw data obtained during the study as far as can be reasonably established.

Quality Assurance: [redacted] Date: 2022-01-27
(YYYY-MM-DD)

The lead QAU was located at ECT Oekotoxikologie GmbH. CIP - Chemisches Institut Pforzheim GmbH provided the QAU at the test site concerned with the analytical part of the study (see analytical part of the report).

4 Test Facility GLP Certificate

[redacted]

HESSEN

Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

[redacted]

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

[redacted]

ECT Oekotoxikologie GmbH
Böttgerstraße 2-14
65439 Flörsheim

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and adress)

Prüfungen nach Kategorien/Areas of Expertise
(gemäß/according ChemVwV-GLP Nr. 5.3/OECD guidance)

4 Ökotoxikologische Prüfungen zur Bestimmung der Auswirkungen auf aquatische und terrestrische Organismen
5 Prüfungen zum Verhalten im Boden, im Wasser und in der Luft, Prüfungen zur Bioakkumulation und zur Metabolisierung

4 Environmental toxicity studies on aquatic and terrestrial organisms
5 Studies on behaviour in water, soil and air; bioaccumulation

20./21. April 2021, 04. Mai 2021
Datum der Inspektion/Date of Inspection
(Tag Monat Jahr/day month year)

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP- Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Im Auftrag

[redacted] 26. Juli 2021

(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)

HESSISCHES MINISTERIUM FÜR UMWELT, KLIMASCHUTZ, LANDWIRTSCHAFT UND VERBRAUCHERSCHUTZ

Hessisches Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz,
Mainzer Straße 80, D 65189 Wiesbaden
(Name und Adresse der GLP-Überwachungsbehörde/Name and address of the GLP Monitoring Authority)

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5 Information on Sponsor, Test Facility and Test Site

5.1 Name and Address of the Sponsor

Bayer Agriculture BV
Scheldelaan 460/Haven 627
2040 Antwerp
Belgium

Sponsor's representative:
[redacted]

5.2 Name and Address of the Test Facility and the Study Director

ECT Oekotoxikologie GmbH
Böttgerstr. 2 - 14
65439 Flörsheim am Main
Germany

Head of Test Facility:
[redacted]

Study Director:
[redacted]

5.3 Name and Address of the Test Site for Analysis and the Principal Investigator

CIP - Chemisches Institut Pforzheim GmbH
Schulberg 17
75175 Pforzheim
Germany

Test Site Management:
[redacted]

Principal Investigator:
[redacted]

6 Dates

Initiation and Completion Dates of the Study:

Study Initiation Date:	October 25, 2021
Experimental Starting Date (biological phase):	October 25, 2021
Experimental Completion Date (biological phase):	November 17, 2021
Study Completion Date:	January 27, 2022

7 Records and Archiving

List of records to be retained:

- Study plan, including all amendments.
- Raw data specific to this study: all notes, measurement data and observations.
- Raw data non-specific to this study:
  - Documents on test equipment,
  - Temperature control,
  - Culture of test system,
  - Consumption of the test item.
- Standard operating procedures.
- Final report including all amendments.
- Originals or copies of relevant communication.
- Quality assurance reports of in-life inspections and audits (e.g. of reports).

All and paginated raw data of the study in respect of which Bayer AG, Crop Science Division has requested performance pursuant to the directives on "Good Laboratory Practice" (GLP), shall be sent to Bayer AG, Crop Science Division within 8 weeks after finalization of the study (Development, Global Regulatory Affairs, 40789 Monheim, Germany) for archiving according to GLP.

The receiving address will be:

Bayer AG
Central GLP Archive Monheim
Building 6100, B1.41
Alfred-Nobel-Strasse 50
40789 Monheim am Rhein
Germany

Archiving of facility, QA and personnel records as well as an authenticated copy of study-specific raw data relevant to the biological phase will be done at ECT Oekotoxikologie GmbH.

Archiving of facility, QA and personnel records as well as an authenticated copy of study-specific raw data relevant to the analytical phase will be done at CIP - Chemisches Institut Pforzheim GmbH.

All raw data (including authenticated copy of study-specific raw data) archived by ECT will be handled as laid down by the actual requirements of the German Chemicals Act. A sample of the test item will be archived at ECT. Upon completion of the study, any additional unused test item will be disposed in accordance with currently applicable local legislation.

8 Distribution of the Final Report

Sponsor	Electronic Copy & Original
Study Director (ECT)	Original
Principal Investigator (Test Site)	Electronic Copy

9 Summary

Report:	[redacted]: "Glyphosate TC: A Study on the Toxicity to the Rooted Aquatic Macrophyte *Myriophyllum spicatum*" Source: ECT Oekotoxikologie GmbH, unpublished report No.: 21P1MW January 27, 2022
Guideline:	OECD (2014). OECD Guideline for the Testing of Chemicals, No. 239, "Water-Sediment Myriophyllum spicatum Toxicity Test", adopted September 26, 2014. Organisation for Economic Co-Operation and Development, Paris.
GLP:	Yes (certified laboratory)
Dates of experimental work:	October 25, 2021 – November 17, 2021 (biological phase)

Test item:

- Name: Glyphosate TC
- CAS number: 1071-83-6

Material and methods:

Test organism:	*Myriophyllum spicatum*
Test medium:	Smart & Barko medium
Endpoints:	NOEC/LOEC; EC_x
Biological parameters:	Inhibition of growth in relation to control (shoot length & biomass)
Test duration:	14 days
Temperature	20±2°C (target): 19.8–21.8 °C (manual measurement; n = 90); 18.5–19.4 °C (automatic measurement in test medium; n = 1002)
Light regime:	16/8 hours light/dark cycle
Light intensity (target):	140±20 µE $m^{-2}s^{-1}$
Test units:	2 L beaker glass covered by watch glasses
Test concentrations (nominal):	1.58, 5.00, 15.8, 50.0, 158 and 500 mg test item/L. 1.52, 4.80, 15.2, 48.0, 152 and 480 mg Glyphosate a.e./L
No. of replicates in the control:	6
No. of replicates per test concentration:	4
Renewal of test solution during exposure:	None (static system)
Chemical analysis of test concentrations:	At days 0 and 14 during exposure period
Data evaluation:	Williams-t-test; 3-parametric normal CDF

a.e.: acid equivalent, see also section 13.1.

The biological phase of the study was conducted at ECT Oekotoxikologie GmbH, Flörsheim am Main, Germany. Samples of test solutions were analysed at CIP - Chemisches Institut Pforzheim GmbH, Pforzheim, Germany, under the responsibility of the principal investigator for analysis.

Findings:

Analytical findings

Samples of overlying water, pore water and sediment of all test concentrations taken on days 0 and 14 were analysed by HPLC-MS/MS to quantify the concentrations of the test item by analysis of Glyphosate acid equivalent (a.e.).

Table 1: Summary of the analytical results as mass balance of overlying water, pore water and sediment.

Nominal concentration	Test period	Overlying water	Pore water	Sediment	Total
[mg Glyphosate a.e./L]	[d]	[%][a]	[%][b]	[%][c]	[%]
Control	0	<LOD	<LOD	<LOD	–
1.52	0	92.1	<LOQ	<LOD	92.1
4.80	0	108	0.2	<LOD	108.2
15.2	0	89.5	0.1	<LOD	89.6
48.0	0	86.5	0.2	<LOD	86.7
152	0	87.4	0.2	<LOQ	87.6
480	0	92.9	0.1	0.2	93.2
Control	14	<LOD	<LOD	<LOD	–
1.52	14	79.7	0.9	<LOD	80.6
4.80	14	98.5	1.1	8.2	107.8
15.2	14	77.5	0.9	5.6	84.0
48.0	14	78.7	1.1	6.7	86.5
152	14	86.3	1.3	1.7	89.3
480	14	92.7	1.3	1.8	95.8

for more detail see also section 19.3 and the analytical phase report.
[a] related to the nominal amount of Glyphosate per test vessel
[b] related to the real amount of pore water per test vessel of initial nominal / test vessel
[c] related to the real amount of sediment (dw) per test vessel

Table 2: Summary of analytical results: geometric mean measured concentrations in overlying water expressed in mg Glyphosate a.e./L.

Nominal concentration [mg test item]	Nominal concentration [mg Glyphosate a.e./L]*	Geometric mean of measured concentrations in overlying water [mg Glyphosate a.e./L]
Control	Control	--
1.58	1.52	1.30
5.00	4.80	4.96
15.8	15.2	12.7
50.0	48.0	39.6
158	152	132
500	480	445

* based on a purity of 96.0% of the test item.

Biological findings

Statistically significant effects on the growth of Myriophyllum spicatum could be determined following application of the test item to the water phase of a sediment-water system. For the parameters total shoot length (yield) and biomass$_{wet\ weight}$ (yield and growth rate) the lowest NOEC was determined to be 4.96 mg Glyphosate a.e./L based on geometric mean measured concentrations.

Effective concentrations after 14 days of exposure were determined due to a clear concentration-response relationship for total shoot length and biomass$_{wet\ weight}$ (yield and growth rate).
For the parameter total shoot length (TSL), EC_{50} values of 51.5 mg Glyphosate a.e./L for yield$_{TSL}$ and 208 mg Glyphosate a.e./L for growth rate$_{TSL}$ were determined based on geometric mean measured concentrations.
For the parameter biomass (wet weight), EC_{50} values of 55.9 mg Glyphosate a.e./L for yield-biomass$_{wet\ weight}$ and 163 mg Glyphosate a.e./L for growth rate-biomass$_{wet\ weight}$ was determined based on geometric mean measured concentrations.
For the parameter biomass$_{dry\ weight}$, EC_{50} values could not be determined since the slope of the relationship was found to be not significant, no EC and confidence limits are provided.

A summary of the results is presented in Table 3 and Table 4 which presents endpoints based on nominal and geometric mean measured concentrations in [mg Glyphosate a.e./L].

Table 3: Summary of test results after the exposure period of 14 days based on statistical evaluation of biological results as nominal concentrations.

Parameter	Endpoint based on nominal concentrations, 0–14d [mg Glyphosate a.e./L]**				
	EC_{10}	EC_{20}	EC_{50}	NOEC	LOEC
Yield (Total Shoot length)	6.95 (3.90-12.4)*	14.7 (8.63-25.6)	61.7 (33.3-117)	4.80	15.2
Growth rate (Total Shoot length)	47.1 (23.5-94.2)	81.6 (41.8-157)	234 (108-516)	15.2	48.0
Yield (Biomass$_{dry\ weight}$)	n.d. (n.d.–n.d.)	n.d. (n.d.–n.d.)	n.d. (n.d.–n.d.)	≥480	>480
Growth rate (Biomass$_{dry\ weight}$)	n.d. (n.d.–n.d.)	n.d. (n.d.–n.d.)	n.d. (n.d.–n.d.)	≥480	>480
Yield (Biomass$_{wet\ weight}$)	3.49 (0.818-14.9)	9.51 (2.34-38.6)	64.6 (11.8-348)	4.80	15.2
Growth rate (Biomass$_{wet\ weight}$)	6.46 (2.17-19.2)	20.4 (6.99-60.2)	185 (46.5-701)	4.80	15.2

* 95% lower and upper confidence limits;
** Endpoints were given based on the a.e. content of the test item (96.0%).

Table 4: Summary of test results after the exposure period of 14 days based on statistical evaluation of biological results as geometric mean measured concentrations of Glyphosate.

Parameter	Endpoint based on geometric mean measured concentrations, 0–14d [mg Glyphosate a.e./L]				
	EC_{10}	EC_{20}	EC_{50}	NOEC	LOEC
Yield (Total Shoot length)	5.26 (2.88-9.64)*	11.5 (6.60-20.5)	51.5 (27.1-100)	4.69	12.7
Growth rate (Total Shoot length)	38.2 (18.4-79.4)	68.3 (33.7-137)	208 (92.3-479)	12.7	39.6
Yield ($Biomass_{dry\ weight}$)	n.d. (n.d.–n.d.)	n.d. (n.d.–n.d.)	n.d. (n.d.–n.d.)	≥445	>445
Growth rate ($Biomass_{dry\ weight}$)	n.d. (n.d.–n.d.)	n.d. (n.d.–n.d.)	n.d. (n.d.–n.d.)	≥445	>445
Yield ($Biomass_{wet\ weight}$)	3.00 (0.715-12.6)	8.19 (2.11-32.9)	55.9 (10.5-303)	4.69	12.7
Growth rate ($Biomass_{wet\ weight}$)	5.41 (1.82-16.1)	17.4 (1.82-51.4)	163 (41.2-626)	4.69	12.7

* 95% lower and upper confidence limits

Validity of the test:

All validity criteria were fulfilled as required by the study plan:

The mean total shoot length and mean total shoot fresh weight in control plants should be at least double during the exposure period. - mean total shoot length in control plants - mean total shoot fresh weight in control plants	 **5.2 fold** **4.0 fold**
The control plants must not show any visual symptoms of chlorosis and should be visibly free from contaminations by other organisms. No algae and/or bacterial films should be visible on the plants, at the surface of the sediment and in the test medium.	**No symptoms of chlorosis, free from contamination**
The mean coefficient of variation for yield based on shoot fresh weight in the control should not exceed 35% between replicates.	**13.5%**

The study is therefore considered to be valid.

10 Title

"Glyphosate TC: A Study on the toxicity to the Rooted Aquatic Macrophyte, *Myriophyllum spicatum*"

11 Test Guideline

This study was designed to comply with the following test guideline:

- OECD (2014). OECD Guideline for the Testing of Chemicals, No. 239, "Water-Sediment Myriophyllum spicatum Toxicity Test", adopted September 26, 2014. Organisation for Economic Co-Operation and Development, Paris.

12 Nature and Purpose of the Study

The aim of this study was to determine the vegetative effects of the test item on the rooted aquatic macrophyte *Myriophyllum spicatum*. To achieve this aim, individual shoot apices of healthy plants potted in artificial sediment containing additional nutrients were exposed in a sediment-water system to a series of test item concentrations applied to the water phase of the sediment-water system. Test vessels without the addition of the test item served as controls. The test organisms were exposed (submersed) to the systems for a period of fourteen days which was considered to be sufficient to assess the impact of the test item on growth of the aquatic macrophyte, *Myriophyllum spicatum*. At the end of the exposure period, the plants were harvested, and growth-related biological parameters were determined.

Endpoints based on length, biomass and visual detrimental effects (e.g. necrosis, chlorosis, development abnormalities) were assessed in comparison to a control. Parameters were the total shoot length of plants, the biomass determined for wet and dry plants, and the derived parameters growth rate and yield.

The preferred endpoint of this type of study was the EC_x (e.g. EC_{50}, EC_{20}, EC_{10}). The lowest observed effect concentration (LOEC) and hence the no observed effect concentration (NOEC) were determined.

To verify the nominally applied concentrations in the sediment-water system samples were taken from the overlying water, sediment and pore water and analytically measured. The samples were transferred to the analytical test site (address see section 5.3) and analytically measured for test concentrations under the responsibility of the principal investigator. The Study Director (ECT) ensures the integration of the biological and chemical-analytical phase of the study.

The requirement of the study is based on:

- COMMISSION REGULATION (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union L 93/1

13 Identification of Test Item and Reference Item

13.1 Test Item

The test item was Glyphosate TC. All data were provided by the sponsor, except where indicated otherwise.

-	Test item name:	Glyphosate TC
-	CAS number:	1071-83-6
-	Synonyms:	MON 77973
-	Batch information:	Lot Number: AZM30338TO (Orion Lot: 11493945)
	Specification No.:	102000037890
-	Sample identification code, as given in certificate of analysis:	TOX 22000-00
-	Purity (Glyphosate):	96.0 wt % (a.e. on dry basis)
-	Appearance:	Consistency: Powder Color: White Ordor: Pungent
-	Density:	1.704 g/cm^3
		0.56 g/cm^3; (loose bulk density)
-	pH	2.25
-	Solubility in water:	11.05 wt % 10.1 g/L
-	Storage requirements:	Room temperature (10–25°C)
-	Stability under correct storage conditions (expiry date):	2023-03-19 (YYYY-MM-DD)

a.e.: acid equivalent

13.2 Reference Item and Results of Reference Testing

A reference test using 3,5-dichlorophenol as reference item was performed in November/December.

Result: Growth rate (total shoot length) EC_{50} (0–14 days): 5.50 mg/L (4.33–6.94 mg/L; 95%-CL)

This result is in accordance with the range given in the ring test report; Ratte, M., Ratte, H. (2014) mentioned in OECD guideline 239, the E_rC_{50} (72h)-values for 3,5-dichlorophenol obtained from different laboratories should be 4.3–6.3 mg/L. Therefore, the results of this reference test are acceptable and the test conditions are reliable.

14 Test Methods

14.1 Characterisation of the Test System (Test Organism) Species

Species:	*Myriophyllum spicatum*
Supplier:	ibacon GmbH Germany
Date of purchase:	September 02, 2021

14.2 Justification for Selection of the Test System

The water plant *Myriophyllum spicatum* was chosen as a representative freshwater plant. The selection of the test system is based on the test guideline and the higher-tier requirement of:

- Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union L 93/1.

14.3 Test organism Delivery and Pre-Culture Conditions

Ibacon GmbH delivered sterile plants from laboratory culture of the test organism *Myriophyllum spicatum* in September 02, 2021. Since then, the plants were cultivated at ECT Oekotoxikologie GmbH.
A sterile pre-culture was cultivated in modified Andrews medium. The sterile plants were transferred in glass tanks, containing Smart & Barko medium and artificial sediment 21 days before the start of the exposure period. The plants were held in a temperature-controlled room at a temperature of 20±2 °C with a photo period of 16 h light (140 ± 20 µE $m^{-2}s^{-1}$) and 8 h dark; type: Philips Son-T Pia 400 “warm white”.

14.4 Test Units

2 L beaker glasses covered by watch glasses were used as test vessels. Test vessels were labelled with the study number, the concentration level and a specific code for each replicate.

14.5 Test Medium

Smart and Barko medium (Smart and Barko 1985) was used as the overlying water. The preparation of Smart & Barko medium is described in the ECT SOP A2.34, which is briefly described below:

$CaCl_2 \times 2\ H_2O$	91.7	mg/L
$MgSO_4 \times 7\ H_2O$	69.0	mg/L
$NaHCO_3$	58.4	mg/L
$KHCO_3$	15.4	mg/L

The required volume of Smart & Barko medium was prepared within three months before use. The physical-chemical characteristics of the Smart & Barko medium were determined according to the test guideline.

14.6 Formulated Sediment

The composition of the formulated sediment was according to OECD Guideline 239 (OECD 2014) and as shown in Table 5.

Table 5: Formulated sediment according to OECD Guideline 239 (OECD 2014).

Constituent	Characteristics	% of sediment dry weight
Peat	Sphagnum moss peat, air dried, no visible plant remains, finely ground (particle size ≤0.5 mm) total organic carbon (TOC): 52% of dry matter Supplier: Albert Thomann GmbH, Thomaflor	4.5
Quartz sand	Grain size: >50% of the particles should be in the range of 50-200 µm Supplier: Quarzwerke GmbH, Quarzsand Frechen F36	75.5
Kaolinite clay	Kaolinite content ≥30% Supplier: Ziegler&Co GmbH, Chinafill 100	20
Organic carbon	based on TOC of peat at 4.5% peat in dry sediment:	2.3
Calcium carbonate	$CaCO_3$, pulverised, chemically pure, in addition to dry sediment	0.4
Deionised Water & nutrient medium	Conductivity ≤10 µS/cm (deionised water), in addition to dry sediment	30–50
Nutrient medium	ammonium chloride NH_4Cl (water solubility 372 g L^{-1} at 20°C)	200 mg/kg dry sediment
	sodium phosphate Na_3PO_4 (water solubility 285 g L^{-1} at 20°C)	200 mg/kg dry sediment

The dry constituents of the sediment were mixed homogeneously prior to mixing the deionised water and the nutrient medium into the sediment. The pH of the final dry sediment mixture was adjusted to 7.4 by addition of calcium carbonate ($CaCO_3$).

The dry sediment mixture was pre-moistened to 40% water content of the sediment dry weight by adding deionised water (4600 g water per 11.5 kg dry sediment). For the final wet sediment containing nutrients, an aqueous nutrient medium (with 2 g/L of ammonium chloride and 2 g/L sodium phosphate) was added to obtain a moisture of 50% in the final mixture. Therefore, 1150 g of the aqueous nutrient medium was added to 11.5 kg dry sediment pre-moistened to 40% of the sediment dry weight.

The moist sediment was prepared two days before the start of the rooting phase. The pH in wet sediment was measured directly in the substrate (pH = 6.8).

14.7 Method for Determination of Shoot Length, Wet Weight and Dry Weight

During the incubation period (exposure period) of fourteen days, the shoot length was measured and recorded on day 0 and at the end of exposure (day 14). In addition, the plants were visually described on day 0, 7 and 14 of exposure.

The dry weight of the plants was determined at day 0 of the test for a representative sample of the rejected plants from the rooting phase. Therefore, fifteen shoots were collected and placed in pre-weighed aluminium weighing boots. Before they dried for 12 hours set at 60°C in a drying cabinet, the wet weight was measured. The dry weights were measured after cooling in a desiccator.

At the end of the exposure period, wet and dry weight of each plant was determined. Therefore, freshly harvested shoots were collected by cutting the shoot at sediment level and the plants were rinsed carefully with water. The plants were placed in pre-weighed aluminium weighing boots and wet weight was measured before they dried for at least 15 hours set at 60°C in a drying cabinet. The dry weights were measured after cooling in a desiccator.

The roots were rinsed carefully with water and visually described.

14.8 Pre-treatment of the Test Item

Prior to start of the test, the test item was stored according to section 13.1.

14.9 Method of Administration and Justification for Selection of the Method

The test organisms were exposed to the test solutions in a static system for 14 days. Healthy freshly clipped shoots within a range of 6±1 cm length were selected.

The method of administration was based on the requirements of the test guideline.

14.10 Frequency and Duration of Administration, Treatment Levels, and Replication

Based on the results of preliminary non-GLP range finding test, the following concentrations in a geometrical series (spacing factor: square-root of ten) were tested in the definitive test:

1.58, 5.00, 15.8, 50.0, 158 and 500 mg test item/L, corresponding to
1.52, 4.80, 15.2, 48.0, 152 and 480 mg Glyphosate a.e./L.

Additionally, plants will be tested under control conditions (test medium only).

Four replicates were used per test item concentration. For the control six replicates were used. Each replicate contained three individual plants.

The test item was applied once into the water phase. The exposure period was fourteen days.

15 Experimental Procedures

15.1 Exposure Conditions

Test organism:	*Myriophyllum spicatum*
Test medium:	Spiked Smart & Barko medium; artificial sediment according to OECD guideline No. 239 (OECD 2014); peat content 5% of sediment dry weight; additional nutrient during test
Control media:	Uncontaminated artificial sediment and Smart & Barko medium
Test duration (exposure):	14 d
Test units:	2 L glass vessels measuring 11 cm in diameter and 24 cm in height, covered by watch glass, within standard plant pot made of polypropylene, diameter on top: 9 cm, diameter bottom: approx. 6 cm, height: approx. 7 cm
Amount of sediment per test vessel:	400 g wet weight
Depth of sediment layer in the test vessel:	5.5 cm
Amount of overlying water per test vessel:	1800 mL
Aeration of test chambers:	No aeration during acclimation and exposure
Nutrient during exposure:	Ammonium chloride and sodium phosphate was added to the sediment to achieve a nutrient content of 200 mg nutrients/kg (dw) for each nutrient
Water renewal:	Static
Sediment conditioning:	None; prior to setup of test vessels
Spiking of overlying water:	Test item dissolved in Smart & Barko medium; test item applied to the water of each test vessel using a mesuring cylinder
Equilibration:	None
Temperature (exposure phase):	19.8–20.8°C (manual measurement in the test vessels) 18.5–19.4°C, mean 18.8°C (automatic measurement in a separate vessel)
pH of the overlying water:	7.7–10.2 (n = 90), see section 19.1
Light regimen:	16 h light: 8 h dark; type: Philips Son-T Pia 400
Light intensity:	135 µE $m^{-2}s^{-1}$ (n = 9); the light intensity across the area was within the range of ±15%
Number of plants per test chamber:	At the beginning of the test: 4 (at day -7); 3 (at day 0)
Number of replicates per test item concentration:	4
Number of replicates in the control:	6
Determination of shoot length:	Days 0 and 14

Determination of wet weight and
dry weight: Days 0 and 14

All data were within the required ranges of the guideline and the study plan, except where indicated otherwise. For measured values, see section 23.1.

15.2 Test Units

Glass beakers (e.g. 2000 mL) covered e.g. with watch glasses were used as test vessels.

15.3 Preparation of the Application Solutions

At the beginning of the exposure period, a stock solution (S1) was prepared in Smart & Barko medium. Therefore, 15.00098 g of the test item were dissolved in 5000 mL of test medium, resulting in a nominal concentration of 3000 mg test item/L. The pH of the stock solution was adjusted from 2.2 to 7.7 using 5 N NaOH and stirred on a magnetic stirrer for 60 minutes at 750 rpm, resulting in a clear solution.

Thereafter the application solutions were prepared by diluting the application solutions (S1) with Smart & Barko medium. All solutions were taken while stirring the solution on a magnetic stirrer and were stirred for 15 minutes on a magnetic stirrer at 750 rpm. The volume of the stock solution (S1) was large enough to prepare all concentration levels of the application solutions and all analytical samples. The following table describes the procedure for preparing the application solutions.

Table 6: Preparation of stock solution (S) and application solutions (AL).

Code of appl. solution (AS)	Nominal concentration of AL [mg test item/L]	Volume [g]	of	Diluted to [mL]
S1/C6-AL	3000	–	–	–
C5-AL	949	632.63	C6-AS	2000
C4-AL	300	200.36	C6-AS	2000
C3-AL	94.9	63.26	C6-AS	2000
C2-AL	30.0	20.09	C6-AS	2000
C1-AL	9.49	6.38	C6-AS	2000

15.4 Preparation of Test Solutions

The application solutions C1-AL to C6-AL were used to prepare the respective test solutions (C1–C6) by diluting the application solution with test medium. From these application solutions an appropriate volume (300 mL application solution, using a measuring cylinder) was mixed with an appropriate amount of test medium (1500 mL of test medium per test vessel). The amount of test medium provided by the application solution was taken into account when preparing the test vessels. To ensure that the test item added to the test medium was evenly distributed, the test medium was mixed thoroughly using a glass rod. The following table describes the procedure for preparing the test solutions and control in each replicate test vessel depending on the concentration of the test item.

Table 7: Preparation of test solutions.

Treatment code	Nominal Concentration in appl. solution (AL) [mg test item/L]	Volume of test medium [mL]	Code of application solution used	Volume of application solution [mL]	Test item applied to each test vessel [mg test item] **
C0*	0.0	1500	medium	300	–
C1	9.49	1500	C1-AL	300	2.85
C2	30.0	1500	C2-AL	300	9.00
C3	94.9	1500	C3-AL	300	28.5
C4	300	1500	C4-AL	300	90.0
C5	949	1500	C5-AL	300	285
C6	3000	1500	C6-AL	300	900

*untreated medium was used for control replicates.
**calculated using nominal concentration in application solution (C1-AL–C6-AL) in mg test item/L, multiplied with the volume of application solution, then divided by 1000.

15.5 Experimental Procedures

Preparation of sediment

- The sediment was prepared as described in section 14.6
- The sediment was distributed to the planting pots. Standard plant pot made of polypropylene were used; diameter on top: 9 cm, diameter bottom: approx. 6 cm, height: approx. 7 cm.
- Each planting pot (glass beaker) was filled with a 5.5 cm layer of nutrient-rich sediment. The sediment layer was covered with a thin layer of ≈ 0.2–0.3 cm of quartz sand.

Establishment phase (root forming period)

- Planting pots (plant pot made of polypropylene) were placed in each glass beaker (2000 mL, measuring 11 cm in diameter and 24 cm in height) and were used for the root forming period and the following exposure period.
- All vessels were labelled with the study number and a specific code for each beaker, and were closed by a watch glass. Replicate test vessels were prepared for each concentration level and the control. Sufficient test vessels were prepared for each concentration level and the control. Additional vessels were prepared to provide replicates to inspect the root growth/development.

- Separate additional vessels of selected concentration levels (see Table 6), and of the control, with planting pot and sediment, were prepared for sampling for later analysis of the test item in overlying water and sediment. These vessels were sampled destructively on day 0 of the test.
- The glass beakers were filled with the test medium (1500 mL), taking into account the volume used for application.
- Healthy shoot pieces from the culture plants were clipped off at a length of 6±1 cm.
- In each planting pot four freshly clipped shoot pieces were planted into each pot containing the sediment such that the lower approx. 3 cm, covering at least two nodes, was within the sediment, and placed into the glass vessels (exposure units).
- Each glass beaker contained one planting pot. The weight of the glass beakers (total weight including planting pot and test medium) was recorded.
- Dry weight of the final sediment (including nutrient medium) was determined.
- At day seven of the establishment phase (root forming period) four plants in spare pots were removed for inspection of root growth. Since root growth was visible (i.e. root tips were visible), the establishment phase was stopped.
- The pre-exposure (root forming period) lasted seven days.

Set-up of the test vessels

- After the establishment phase (root forming period) one of the four plants was removed using steel forceps, to leave three uniform (size, appearance) individuals.
- The glass vessels containing planting pots with three uniform individual plants and test medium were used for later application of the test item.
- All vessels were labelled with a specific concentration level code for each replicate.

Application of the test item and test start (day 0):

- The test item was spiked into the overlying water as described in section 15.3.
- The water level was marked on the outside of each test vessel. Thereafter, the test vessels were incubated at test conditions.
- After spiking, samples for chemical analyses were taken, see section 15.7.
- The water quality parameters in the test vessels were determined, see section 23.1.
- The light intensity was measured.
- Plant biomass, fresh weight and dry weight of the plants (day 0 biomass values) was measured at test initiation. Therefore, fifteen individual homogenous plants, collected from the one of the initially four plants of the pre-exposure planting pots, were taken from the planting pots of the establishment phase beaker and were harvested by cutting the shoot at sediment level and placed in pre-weighed petri-dishes. Before they dried for 13 hours in total at 60°C in a drying cabinet, the wet weight was measured. The dry weights were measured after cooling in a desiccator.

Maintenance (exposure period)

- The shoot length, and the number, length of side shoots per plant was measured and recorded on days 0 and at the end of the exposure period (day 14). Any changes in plant development in comparison to the control (e.g. appearance, necrosis, chlorosis, morphology, root length at the end of the test) were recorded on days 0, 7 and at the end of the exposure period (day 14). Additionally, any significant features of the test medium were recorded.
- The position of side shoots was recorded as an additional observation (side shoot growth below sediment surface).
- Water lost due to evaporation from the test vessels was topped up with deionised water.

End of the exposure period (day 14)

- The plants were observed in order to assess visually any differences compared with the control (e.g. necrosis, chlorosis, root growth/length, development abnormalities and discoloration.
- The water quality parameters in the test vessels were determined, see section 23.1.
- The samples for the analytical determination of the test concentrations were taken according to section 15.7.
- The planting pots designated to access the biological parameter were removed from each test unit and the plants were harvested by cutting the shoot at sediment level.
- The harvested plants were carefully rinsed with water, to remove adhering sediment particles.
- The plants were blotted dry (e.g. paper tissue) and the length of shoots was measured.
- Immediately thereafter the plants were placed in pre-weighed aluminium weighing boots and dried for 24 plus 4 hours at 60°C in a drying cabinet. The dry weight was measured, after cooling in a desiccator, to an accuracy of 0.1 mg.
- The remaining shoot apices below the sediment surface were carefully removed, rinsed with water and the root development compared to the control(s) was determined.
- The remaining test solutions were treated with activated carbon and disposed of.

15.6 Water Quality, Sediment and Environmental Conditions Measurements

After temperature adaptation of the test solutions, the following parameters were measured and recorded for each concentration level and the control in one replicate:

Overlying water:

• Temperature:	At all test vessels of each concentration level and the control on day 0, 7 and 14 of the exposure period; additionally, temperature was recorded in a separate test vessel, once per hour throughout the test;
• Dissolved oxygen content:	At all test vessels of each concentration level and the control on day 0, 7 and 14 of the exposure period;
• pH	At all test vessels of each concentration level and the control on day 0, 7 and 14 of the exposure period;
• Total water hardness:	In the test medium and in one test vessel of the control and the highest concentration at the start and the end of the exposure period.

Sediment:

The wet and dry weight of the sediment was determined for the calculation of moisture content in three sediment samples that was used for the test.

Environmental Conditions:

The light intensity was measured at 9 different positions within the test area.

The temperature in the climate room was recorded once per hour throughout the test.

15.7 Sampling for Chemical Analysis

To verify the nominally applied concentrations, samples were taken from overlying water, sediment and pore water and analytically measured. Samples from the application solutions were taken and were stored as reserve samples. The sampling schedule is shown in Table 8.

Sampling Procedures

- Samples of overlying water will be taken from the test vessels e.g. using glass pipettes. Duplicate samples will be taken from one test vessel designated for analysis.
- For sampling of pore water and sediment, the wet sediment of each beaker was vacuum-filtered, using a glass fibre filter with an average retention capacity of 0.6 µm (Macherey-Nagel, MN 85/70 BF, average retention capacity 0.6 µm).
- The vacuum-filtered sediment samples were homogenised manually, using a stainless steel spatula.
- Samples of selected stock/application solution(s) were taken immediately before or directly after spiking using glass pipettes. These samples (target volume: 20 mL per sample) might be analysed in addition if requested by the sponsor.

The amounts or volumes per sample were:

- Stock/application solution (A): 20 mL per sample
- Overlying water (O): 20 mL per sample
- Pore water (P): 15 mL per sample
- Sediment (S): 20 g wet weight per sample.

The exact volume or weight of each sample were recorded.

Table 8: Proposed sampling schedule for chemical analysis during the test period.

Test Period	S1	Control	C1	C2	C3	C4	C5	C6
At start of exposure period, directly after spiking	A	–	A	A	A	A	A	A
	–	OPS*	OPS*	OPS*	OPS*	OPS*	OPS*	OPS*
At end of exposure period	–	OPS	OPS	OPS	OPS	OPS	OPS	OPS

S1	Stock solution	
C1 – C6	Lowest to highest concentration level	
*	Separate, additional vessels; one vessel per treatment was sacrificed per sampling date; one additional set of vessels (each treatment) was set up as a reserve.	
A	Stock solution/application solution	unicate sample (reserve)
O	overlying water	duplicate samples
P	pore water	duplicate samples
S	sediment	duplicate samples

The duplicate samples were stored as reserve sample of each matrix (sediment, pore water and overlying water), as were the unicate samples of the stock/application solutions: Depending on the results the study director decided after sponsor's approval if reserve samples were analysed.

Samples were transferred to the test site for chemical analysis (address see section 5.3) deep-frozen on dry ice. The dates of transfer of the samples from ECT to the laboratory for chemical analysis were recorded in the raw data.

Analysis was performed according to an analytical method validated by the test site for chemical analysis. Further details describing the analytical procedures are contained in the analytical phase report. The analytical work was reported by the principal investigator and is included in the final report of this study.

All samples taken from the test and stored at ECT (except the archived sample, see section 7) will be disposed upon completion of the study, unless the sponsor has indicated prior to study completion in written form to arrange for continued storage of the above-mentioned items.

16 Data Assessment and Statistical Evaluation

16.1 Calculation of Yield, Growth Curves and Geometric Mean Measured Concentrations

16.1.1 Calculation of Yield

Yield is the value of a measurement variable to express shoot length or biomass at the end of a test period minus the measurement variable at the start of the test period.

The yield was calculated on the basis of changes in shoot length, and in addition, on the basis of changes in biomass (dry weight and wet weight) in the controls and in the test item treatment groups at several time points (e.g. end of the exposure period). For each test concentration and control, a mean value was calculated for yield along with variance estimates.

The percentage inhibition in yield ($\%I_y$) was calculated for each treatment replicate as follows:

$$\%Iy = \frac{bc - bt}{bc} x100$$

where: $\%I_y$ is the percent inhibition in average yield, b_C is the mean value for biomass in the control and b_t is the mean value for biomass in the treatment group. The same equation was used to calculate yield based on shoot length (b_C, equivalent to mean value for shoot length in the control and b_t, equivalent to the mean value for shoot length in the treatment group).

16.1.2 Calculation of Growth Curves

The average specific growth rate μ was calculated on the basis of changes in the logarithms of shoot length, and in addition, on the basis of changes in the logarithms of biomass (dry weight and wet weight) over time (expressed per day) in the controls and each treatment group according to the following equation:

$$\mu_{i-j} = ln(N_j) - ln(N_i) / t$$

where $\mu i\text{-}j$ is the average specific growth rate from time i to j, Ni is the measurement variable in the test or control vessel at time i, Nj is the measurement variable in the test or control vessel at time j and t is time period from i to j.

For each test concentration and control, a mean value for growth rate along with variance estimates was calculated. The percentage inhibition of average specific growth rate for each treatment replicate (%Ir) was calculated according to the equation:

$$\%Ir = \frac{\mu c - \mu t}{\mu c} x100$$

where: $\%I_r$ is the percent inhibition in average specific growth rate, μ_C is the mean value for μ in the control and μ_t is the mean value for μ in the treatment group.

16.1.3 Calculation of Geometric Mean Meausred Concentrations

The mean measured concentration at each concentration level over the whole exposure period was calculated according to the equation:

$$geometric\ mean = \sqrt[n]{(x_1 * x_2 * x_3 \dots x_n)}$$

where
x = terms (concentration measured at a data point of one concentration level)
n = number of terms (x) that are multiplied

16.2 Statistics

The data were evaluated on normal distribution by Shapiro-Wilk´s Test and for homogeneity of variances by Levene´s Test and a trend analysis by contrast (monotonicity of concentration response). Williams' Multiple sequential t-test were used to calculate whether there were significant differences between the growth of plants in the controls and the plants exposed to the test item concentrations.

To determine the effect concentrations (EC_x-values), 3-parameter normal CDF and Probit analyses were used.

The statistical software package ToxRat 3.3.0 Professional (ToxRat Solutions GmbH, Naheweg 15, D-52477 Alsdorf) was used for these calculations.

Details are described in section 24.

17 Frequency of the Test

The definitive test (biological phase) was performed once from October 25, 2021 (start of rooting phase) to November 17, 2021 (end of exposure period).

18 Deviations from the Study Plan or the Guideline

Deviating from the study plan and the guideline the overall pH range in the control replicates exceeded the required range of ≤1.5 pH units by 0.8 pH units during the exposure phase. Additionally, a pH increase greater than 1.5 units was observed for all treatment levels, for details see section 23. It is likely that the increase of pH was not substance related since the stock solution was adjusted to 7.7 at exposure start, see section 15.3, to prevent a pH related substance effect at day 0. The pH of the controls showed similar values in comparison with the treatment levels, see section 23 Table 41, at the end of the test. It is known that plant growth can cause a pH increase, which is the probable explanation for the observed pH increase during the test. This is supported by the fact that the control and the lower treatment levels (C1 and C2; 1.58 and 5.00 mg test item/L) showed similar pH increase and yet no difference in plant growth. Therefore, the deviation of more than 1.5 pH units is expected to have no influence on the integrity of the study and its biological results as all validity criteria, see section 20,were met. This is in agreement with the guideline, OECD 239:
“The pH of the control medium should not increase by more than 1.5 units during the test. However, deviation of more than 1.5 units would not invalidate the test when it can be shown that the validity criteria specified previously are met.”

Deviating from the study plan the total organic carbon content (TOC) was not determined based on DIN EN 12879 (2001). Instead, the TOC was calculated using supplier specifications. The TOC of the sediment of the test was calculated to be 2.3% based on TOC (52%) of peat at 4.5% peat in dry sediment. It is expected that this deviation has no influence on the integrity of the study results. vanish

There were no further deviations from the study plan and the test guideline.

19 Results

The definitive test was performed from November 03, 2021 (start of exposure period), to November 17, 2021 (end of exposure period). In section 19.1, the data on physical-chemical parameters are summarised. In section 19.2, the biological results are presented. Details are given in section 23.

19.1 Test Conditions, Summary of Physical-Chemical Parameters

Details of data concerning physical-chemical parameters measured at each date are tabulated in section 23. Summary tables were compiled from these data and are shown in the following.

Table 9: Characteristics of Smart & Barko medium used for the test; prepared on October 05, 2021.

pH	Temperature [°C]	Total Hardness [°dH]	Total Hardness [mg/L $CaCO_3$]*	Conductivity [µS/cm]
7.9	20.4	5.0	89.3	298

* Total hardness in mg/L $CaCO_3$ was calculated by multiplication of the hardness-value in °dH by 17.86.

Summary of the physical-chemical parameters measured during the test period:

Minimum minimum value
Maximum maximum value
N number of measurements

Table 10: Oxygen concentrations [% of air saturation value, ASV], summary.

Oxygen concentrations [% of air saturation value, ASV]	Minimum	Maximum	N
in all treatment levels and control (day 0)	97	105	30
in all treatment levels and control (day 7)	150	202	30
in all treatment levels and control (day 14)	182	239	30

Raw data of the definitive test are described in detail in section 23.

Table 11: Oxygen concentrations [mg/L], summary.

Oxygen concentrations [mg/L]	Minimum	Maximum	N
in all treatment levels and control (day 0)	8.4	9.2	30
in all treatment levels and control (day 7)	13.1	18	30
in all treatment levels and control (day 14)	16.2	21.5	30

Raw data of the definitive test are described in detail in section 23.

Table 12: pH values in the overlying water, summary.

Nominal concentration [mg test item/L]	Minimum	Maximum	Difference	N
Control	7.9	10.2	2.3	18
1.58	7.9	10.1	2.2	12
5.00	7.9	10.2	2.3	12
15 8	7 7	10 0	2 3	12
50.0	7.8	10.0	2.2	12
158	7.8	9.8	2.0	12
500	7.8	9.5	1.7	12

Raw data of the definitive test are described in detail in section 19.1.

The overall pH range in the control replicates exceeded the required range of ≤1.5 pH units by 0.8 pH units during the exposure phase and a pH increase greater than 1.5 units was observed for all treated levels for details see section 23. This pH increase in the controls and treatment levels is expected to have no influence on the integrity of the study results, since the growth of the treated plants at the lower concentration levels (e.g. C1 and C2; 1.58 and 5.00 mg test item/L) showed normal growing compared to the controls.

Table 13: Temperature in the test vessels [°C], summary.

Nominal concentration [mg test item/L]	Minimum	Maximum	Difference	N
Control [a]	18.5	19.4	0.9	1002
Control	19.9	20.8	0.9	18
1.58	20.3	20.8	0.5	12
5.00	20.1	21.0	0.9	12
15.8	20.3	21.4	1.1	12
50.0	20	21.8	1.8	12
158	19.8	21.1	1.3	12
500	20.3	21.0	0.7	12

[a] Temperature data obtained from online measurement in one separate test vessel during the whole test period, including rooting phase.
Raw data of the definitive test are described in detail in section 23.

The temperature was within the required range of 20±2°C throughout the exposure period as required in the guideline and the study plan.

Total Hardness

The total hardness in the control, the lowest and the highest test concentration level range between 6.0 and 7.6°dH throughout the exposure period, corresponding to 107.2 and 135.7 mg/L $CaCO_3$, calculated by multiplication of the hardness-value in °dH by 17.86.

Light Intensity of the Test Area

The light intensity over the incubation area was measured twice during the test period at six different positions each:

Minimum value:	122 µE $m^{-2}s^{-1}$
Maximum value:	154 µE $m^{-2}s^{-1}$
Mean value:	134.7 µE $m^{-2}s^{-1}$
Number of measurements:	9
Lower range (mean value -15%):	114.5 µE $m^{-2}s^{-1}$
Upper range (mean value +15%):	154.9 µE $m^{-2}s^{-1}$

The light intensity over the incubation area was within ±15% from the average light intensity as required in the guideline and the study plan.

Characteristics of the formulated sediment:

The pH as measured in the final pre-sediment (with nutrient medium; day -7) was 6.8.

The water content of the sediment (with nutrient medium) was measured on day -7 of the test in order to calculate the organic carbon content (Total Organic Carbon; TOC). The dry-to-wet-weight ratio was determined to be 0.680 (n = 3) in the final sediment with nutrient solution.

The total organic carbon content (TOC) of the sediment of the test was calculated to be 2.3% based on TOC (52%) of peat at 4.5% peat in dry sediment.

19.2 Biological Data (exposure phase)

Number of Shoots/Plants (Mortality)

At the end of the exposure phase (day 14) no necrotic plants were observed throughout the concentration levels.

Table 14: Number of healthy plant shoots and percent of necrotic plants (dead plants) at the end of the exposure phase.

Nominal concentrations [mg test item/L]		Control	1.58	5.00	15.8	50.0	158	500
14 d Exposure period	Replicates:	6	4	4	4	4	4	4
	Total number of plants:	18	12	12	12	12	12	12
	Mortality in %	0	0	0	0	0	0	0

Total Shoot Length

Table 15: Total Shoot length, TSL [cm]; during exposure phase:
mean values, standard deviations and coefficient of variation of the replicate total shoot length for various experimental test intervals inspected.

Nominal concentration [mg test item/L]		Control	1.58	5.00	15.8	50.0	158	500
0 d	Replicates:	6	4	4	4	4	4	4
	Mean:	6.82	6.66	6.52	5.95	5.45	5.04	5.80
	Std.Dev.:	0.646	1.595	0.981	0.52	0.733	2.194	0.813
	% CV:	9.5	24.0	15.1	8.7	13.5	43.5	14.0
14 d	Replicates:	6	4	4	4	4	4	4
	Mean:	35.33	37.54	34.27	28.86	22.85	13.43	9.24
	Std.Dev.:	4.604	6.381	4.469	3.904	1.831	2.015	1.491
	% CV:	13.0	17.0	13.0	13.5	8.0	15.0	16.1

Std.Dev.: standard deviation; % CV: coefficient of variation in percent

Biomass (dry weight)

Table 16: Biomass$_{(dry\ weight)}$ [mg per replicate];during exposure phase:
mean values, standard deviations and coefficient of variation of the replicate biomass for experimental test intervals inspected.

Nominal concentrations [mg test item/L]		Control	1.58	5.00	15.8	50.0	158	500
0 d	Mean*:	23.48	23.48	23.48	23.48	23.48	23.48	23.48
14 d	Replicates:	6	4	4	4	4	4	4
	Mean:	65.37	64.73	60.32	56.82	61.12	59.92	59.68
	Std.Dev.:	3.308	9.785	6.117	3.844	4.417	3.349	8.313
	% CV:	5.1	15.1	10.1	6.8	7.2	5.6	13.9

Std.Dev.: standard deviation; % CV: coefficient of variation in percent
* mean value of representative group on day 0

Biomass (wet weight)

Table 17: Biomass$_{(wet\ weight)}$ [mg per replicate]; during exposure phase: mean values and standard deviations of the replicate biomass for experimental test intervals inspected.

Nominal concentrations [mg test item /L]		Control	1.58	5.00	15.8	50.0	158	500
0 d	Mean*:	190.34	190.34	190.34	190.34	190.34	190.34	190.34
14 d	Replicates:	6	4	4	4	4	4	4
	Mean:	756.76	795.74	721.09	593.93	511.47	388.91	328.21
	Std.Dev.:	76.678	163.554	102.415	94.360	33.615	33.630	47.334
	% CV:	10.1	20.6	14.2	15.9	6.6	8.7	14.4

Std.Dev.: standard deviation; % CV: coefficient of variation in percent
* mean value of representative group on day 0

19.2.1 Inhibition of Yield (total shoot length, TSL)

Table 18: Inhibition of yield (total shoot length, TSL); during exposure phase: values of the response variable y and %inhibition (%I_y) as computed from the raw data for experimental test intervals inspected.

Nominal concentrations [mg test item/L]	0–14 d			
	Mean y	Std.Dev. y	n	%I_y
Control	28.51	4.307	6	
1.58	30.88	5.227	4	-8.32
5.00	27.76	3.635	4	2.64
15.8	22.91	3.683	4	19.65
50.0	17.40	1.316	4	38.97
158	8.39	1.522	4	70.57
500	3.44	0.919	4	87.93

-% inhibition: increase in yield relative to that of control

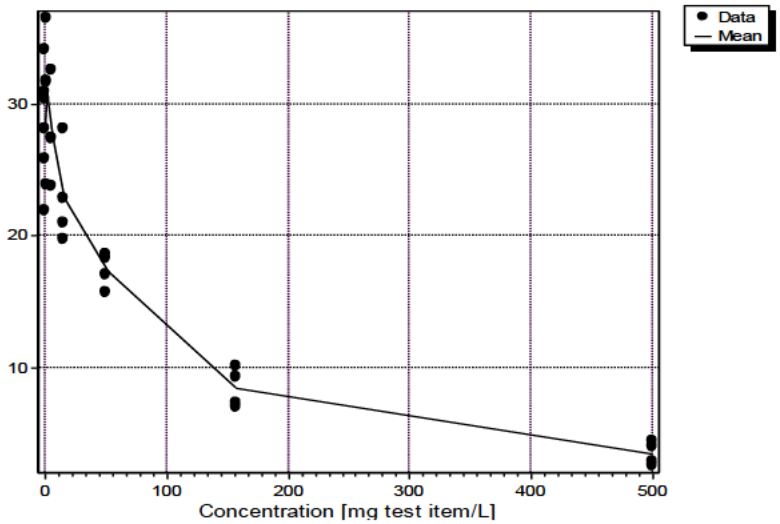

Figure 1: Yield (total shoot length) of *Myriophyllum spicatum* as observed under presence of the test item after 14 d.

Table 19: Results of yield (total shoot length); during exposure phase: selected effective concentrations (EC_x) and threshold concentrations (NOEC/LOEC) at day 14 based on geometric mean measured and nominal concentrations in [mg Glyphosate a.e./L].

Parameter	geometric mean measured concentrations	nominal concentration
	[mg Glyphosate a.e./L]	[mg Glyphosate a.e./L]
EC_{10}	5.26 (2.88-9.64)	6.95 (3.90-12.4)
EC_{20}	11.5 (6.60-20.5)	14.7 (8.63-25.6)
EC_{50}	51.5 (27.1-100)	61.7 (33.3-117)
NOEC	4.96	4.80
LOEC	12.7	15.2

19.2.2 Inhibition of Growth rate (total shoot length, TSL)

Table 20: Inhibition of growth rate (total shoot length); during exposure phase: values of the response variable μ and %inhibition (%I_r) as computed from the raw data for experimental test intervals inspected.

Nominal concentrations [mg test item/L]	0–14 d			
	Mean μ	Std.Dev.	n	%I_r
Control	0.117	0.0087	6	
1.58	0.124	0.0115	4	-5.89
5.00	0.119	0.0054	4	-1.20
15.8	0.113	0.0081	4	4.06
50.0	0.103	0.0060	4	12.45
158	0.077	0.0347	4	34.21
500	0.033	0.0067	4	71.76

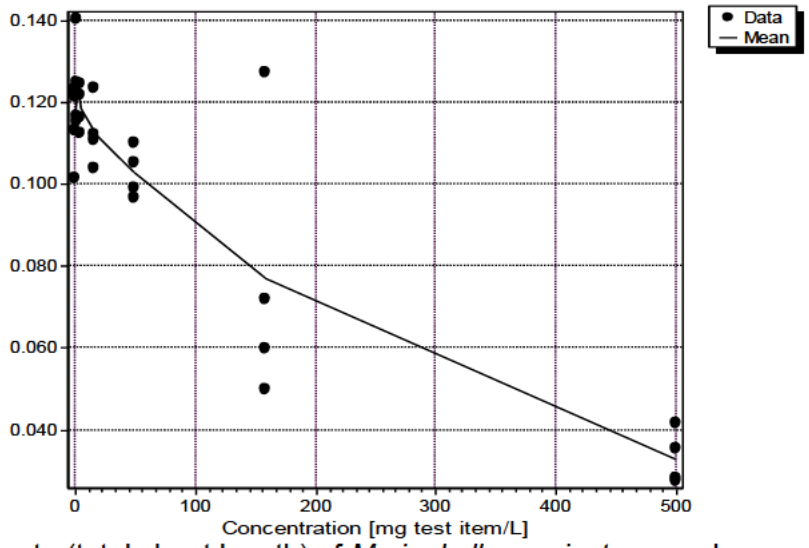

Figure 2: Growth rate (total shoot length) of *Myriophyllum spicatum* as observed under presence of the test item after 14 d.

Table 21: Results of growth rate (total shoot length); during exposure phase: selected effective concentrations (EC_x) and threshold concentrations (NOEC/LOEC) for day 0–14, based on geometric mean measured and nominal concentrations in [mg Glyphosate a.e./L].

Parameter	geometric mean measured concentrations	nominal concentrations
	[mg Glyphosate a.e./L].	[mg Glyphosate a.e./L].
EC_{10}	38.2 (18.4–79.4)	47.1 (23.5-94.2)
EC_{20}	68.3 (33.7–137)	81.6 (41.8-157)
EC_{50}	208 (92.3–479)	234 (108-516)
NOEC	12.7	15.2
LOEC	39.6	48.0

19.2.3 Inhibition of Yield (Biomass$_{dry\ weight}$)

Table 22: Inhibition of yield (biomass$_{dry\ weight}$); during exposure phase:
values of the response variable y and %inhibition (%I$_y$) as computed from the raw data for experimental test intervals inspected.

Nominal concentrations [mg test item/L]	0–14 d			
	Mean y [mg]	Std.Dev. y [mg]	n	%Iy
Control	41.89	3.308	6	
1.58	41.25	9.785	4	1.53
5.00	36.84	6.117	4	12.07
15.8	33.34	3.844	4	20.40
50.0	37.64	4.417	4	10.16
158	36.44	3.349	4	13.02
500	36.20	8.313	4	13.58

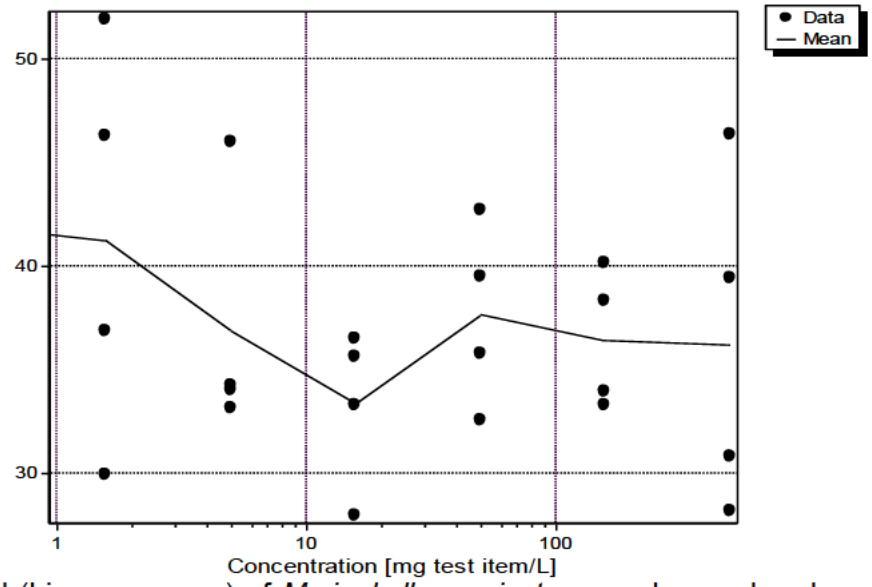

Figure 3: Yield (biomass$_{dry\ weight}$) of *Myriophyllum spicatum* as observed under presence of the test item after 14 d.

Table 23: Results of yield (biomass$_{dry\ weight}$); during exposure phase: selected effective concentrations (EC$_x$) and threshold concentrations (NOEC/LOEC), based on geometric mean measured and nominal concentrations in [mg Glyphosate a.e./L].

Parameter	geometric mean measured concentrations	nominal concentrations
	[mg Glyphosate a.e./L]	[mg Glyphosate a.e./L]
EC$_{10}$	n.d.	n.d.
EC$_{20}$	n.d.	n.d.
EC$_{50}$	n.d.	n.d.
NOEC	≥445	≥480
LOEC	>445	≥480

n.d.: not determined

19.2.4 Inhibition of Growth Rate (Biomass$_{dry\ weight}$)

Table 24: Inhibition of growth rate (biomass$_{dry\ weight}$); during exposure phase: values of the response variable µ and %inhibition (%I) as computed from the raw data for experimental test intervals inspected.

Nominal concentrations [mg test item/L]	0–14 d			
	Mean µ	Std.Dev. µ	n	%Ir
Control	0.073	0.0036	6	
1 58	0 072	0 0110	4	1 71
5.00	0.067	0.0069	4	8.12
15.8	0.063	0.0050	4	13.77
50.0	0.068	0.0052	4	6.67
158	0.067	0.0040	4	8.53
500	0.066	0.0099	4	9.50

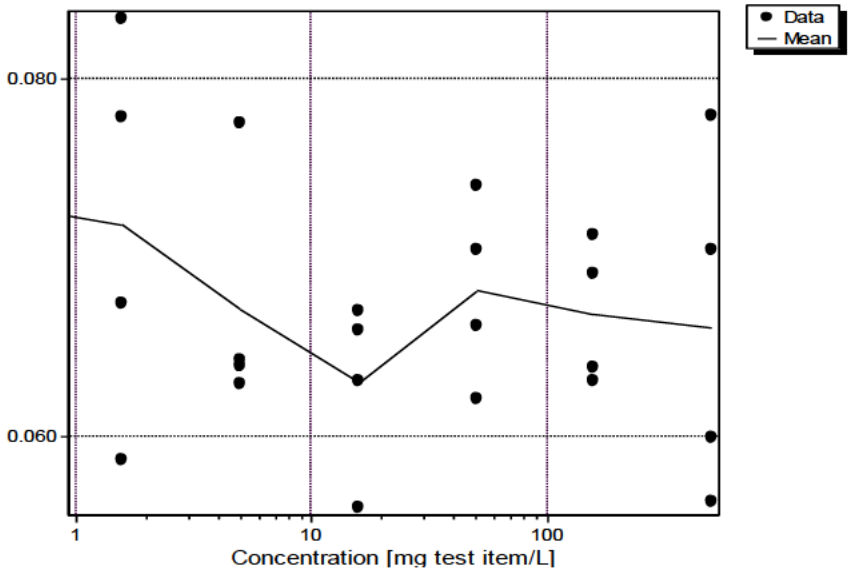

Figure 4: Growth rate (biomass$_{dry\ weight}$) of *Myriophyllum spicatum* as observed under presence of the test item after 14 d.

Table 25: Results of growth rate (biomass$_{dry\ weight}$); during exposure phase: selected effective concentrations (EC_x) and threshold concentrations (NOEC/LOEC), based on geometric mean measured and nominal concentrations in [mg Glyphosate a.e./L].

Parameter	geometric mean measured concentrations	nominal concentrations
	[mg Glyphosate a.e./L]	[mg Glyphosate a.e./L]
EC_{10}	n.d.	n.d.
EC_{20}	n.d.	n.d.
EC_{50}	n.d.	n.d.
NOEC	≥445	≥480
LOEC	>445	≥480

n.d.: not determined

19.2.5 Inhibition of Yield (Biomass$_{wet\ weight}$)

Table 26: Inhibition of yield (biomass$_{wet\ weight}$); during exposure phase:
values of the response variable y and %inhibition (%I) as computed from the raw data for experimental test intervals inspected.

Nominal concentrations [mg test item/L]	0–14 d			
	Mean y [mg]	Std.Dev. y [mg]	n	%Iy
Control	566.42	76.678	6	
1.58	605.40	163.554	4	-6.88
5.00	530.75	102.415	4	6.30
15.8	403.58	94.360	4	28.75
50.0	321.13	33.615	4	43.30
158	198.57	33.630	4	64.94
500	137.87	47.334	4	75.66

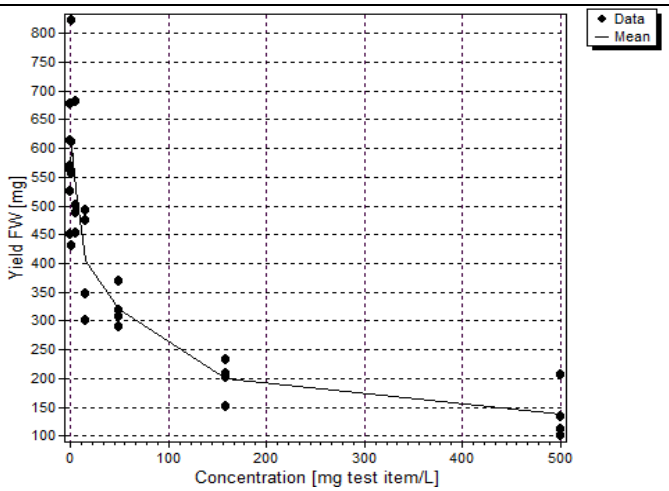

Figure 5: Yield (biomass$_{wet\ weight}$) of *Myriophyllum spicatum* as observed under presence of the test item after 14 d.

Table 27: Results of yield (biomass$_{wet\ weight}$); during exposure phase: selected effective concentrations (EC_x) and threshold concentrations (NOEC/LOEC), based on geometric mean measured and nominal concentrations in [mg Glyphosate a.e./L].

Parameter	geometric mean measured concentrations	nominal concentrations
	[mg Glyphosate a.e./L]	[mg Glyphosate a.e./L]
EC_{10}	3.00 (0.715012.6)	3.49 (0.818-14.9)
EC_{20}	8.19 (2.11–32.9)	9.51 (2.34-38.6)
EC_{50}	55.9 (10.5–303)	64.6 (11.8-348)
NOEC	4.96	4.80
LOEC	12.7	15.2

19.2.6 Inhibition of Growth Rate (Biomass$_{wet\ weight}$)

Table 28: Inhibition of growth rate (biomass$_{wet\ weight}$); during exposure phase: values of the response variable µ and %inhibition (%I) as computed from the raw data for experimental test intervals inspected.

Nominal concentrations [mg test item/L]	0–14 d			
	Mean µ	Std.Dev. µ	n	%Ir
Control	0.098	0.0073	6	
1.58	0.101	0.0145	4	-2.84
5.00	0.095	0.0096	4	3.71
15.8	0.081	0.0115	4	18.00
50.0	0.070	0.0046	4	28.27
158	0.051	0.0064	4	48.28
500	0.038	0.0098	4	60.93

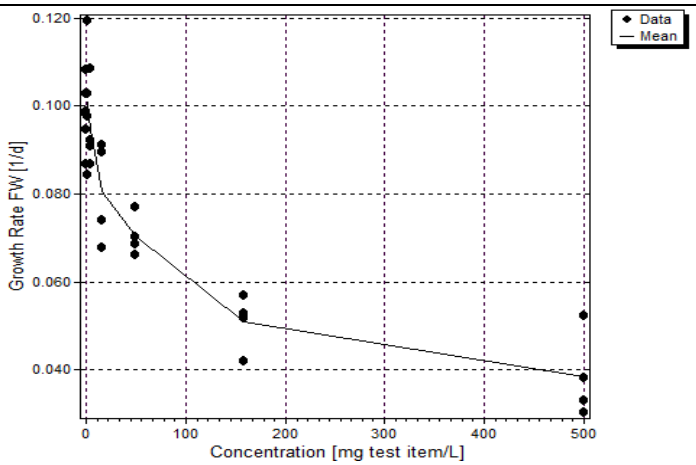

Figure 6: Growth rate (biomass$_{wet\ weight}$) of *Myriophyllum spicatum* as observed under presence of the test item after 14 d.

Table 29: Results of growth rate (biomass$_{wet\ weight}$); during exposure phase: selected effective concentrations (EC_x) and threshold concentrations (NOEC/LOEC), based on geometric mean measured and nominal concentrations in [mg Glyphosate a.e./L].

Parameter	geometric mean measured concentrations	nominal concentrations
	[mg Glyphosate a.e./L]	[mg Glyphosate a.e./L]
EC_{10}	5.41 (1.82–16.1)	6.46 (2.17-19.2)
EC_{20}	17.4 (5.95–51.4)	20.4 (6.99-60.2)
EC_{50}	163 (41.2–626)	185 (46.5-701)
NOEC	4.96	4.80
LOEC	12.7	15.2

19.2.7 Root development

Control:
Root growth was observed for all plants in the controls.

Treatments:
The harvested plants on day 14 (end of the exposure period) showed clear effects on root development at concentration ≥15.8 mg test item/L. For all other treatment levels no distinct differences compared to the control treatment were observed.

19.2.8 Observations

Control:
No other adverse effects were observed for the plants in the controls (e.g. no visual symptoms of chlorosis and visibly free from contaminations by other organisms, no algae and/or bacterial films was observed).

Treatments:
During the exposure phase (day 0 to 14) clear concentration-related adverse visual effects were observed at the tested concentration range. At day 7 reduced growth at concentrations ≥158 mg test item/L were determined and in addition at 500 mg test item/L the plants showed deformed development of plants lying healthy green on sediment surface. At day 14 the deformed development of plants which lied on side of the sediment surface but looked healthy green were still observed growth at concentrations ≥158 mg test item/L

For details, see section 23.

19.3 Analytical Results, Summary

Samples taken from this study were analysed for concentrations of the active substance by HPLC-MS/MS

Sample preparation:

Water (overlying water, pore water):	Samples were analysed directly. If necessary, dilutions were prepared in test medium.
Sediment:	Samples were extracted with sodium hydroxide solution, filtered with a single-use filtration unit (0.45 µm Nylon) and an aliquot of the final extract was mixed with 100 µL formic acid and 200 µL internal standard solution.
Final analysis:	HPLC MS/MS (monitoring two mass transitions: 168.0-->150.0 (quantification) and 168.0-->63.0 (confirmation)
Limit of quantification:	Water: LOQ was 0.15 mg a.e. /L Sediment: LOQ was 1 mg a.e. /kg
Limit of detection:	Water: LOD was defined as lowest detectable concentration and set to 30 % of the LOQ, i.e. 0.045 mg/L Sediment: LOD was defined as lowest detectable concentration and set to 30 % of the LOQ, i.e. 0.3 mg/kg

The concentrations of the test item measured in the total sediment-water system were expressed as mg a.e./L and as mg a.e./kg dry sediment.

A summary of the measured concentrations and recovery of the test item in the test system is given in Table 30 and Table 32.

Detailed analytical methods and results are presented in the analytical phase report.

Table 30: Summary of analytical results: Concentrations of test item, a.e. and % of nominal a.e. measured in overlying water and pore water.

Nominal concentration [mg test item/L]	Nominal concentration [mg Glyphosate a.e./L]*	Test period [d]	Measured concentration in overlying water [mg Glyphosate a.e./L]	% of nominal a.e. [%]	Measured concentration in pore water [mg Glyphosate a.e./L]
Control	Control	0	< LOD	n.a.	<LOD
1.58	1.52	0	1.40	92.1	<LOQ
5.00	4.80	0	5.20	108	0.505
15.8	15.2	0	13.6	89.5	0.299
50.0	48.0	0	41.5	86.5	2.89
158	152	0	133	87.4	8.96
500	480	0	446	92.9	30.3
Control	Control	14	< LOD	n.a.	<LOD
1.58	1.52	14	1.21	79.7	0.431
5.00	4.80	14	4.73	98.5	1.50
15.8	15.2	14	11.8	77.5	4.18
50.0	48.0	14	37.8	78.7	15.5
158	152	14	131	86.2	53.6
500	480	14	445	92.7	209

* based on a purity of 96.0% of the test item
LOQ: 0.15 mg/L
LOD: 0.045 mg/L
n.a.: not applicable

Table 31: Summary of analytical results: Geometric mean measured concentrations in overlying water (day 0 – 14) expressed in mg a.e./L.

Nominal concentration [mg test item/L]	Nominal concentration [mg Glyphosate a.e./L]*	Geometric mean of measured concentrations in overlying water [mg Glyphosate a.e./L]
Control	Control	–
1.58	1.52	1.30
5.00	4.80	4.96
15.8	15.2	12.7
50.0	48.0	39.6
158	152	132
500	480	445

* based on a purity of 96.0% of the test item

Table 32: Summary of analytical results: Concentrations of test item, a.e. and % of nominal applied a.e. measured in the sediment.

Nominal concentration [mg test item/L]	Nominal concentration [mg Glyphosate a.e./L]*	Test period [d]	Measured concentration in sediment [mg Glyphosate a.e./kg dry sediment]
Control	Control	0	<LOD
1.58	1.52	0	<LOD
5.00	4.80	0	<LOD
15.8	15.2	0	<LOD
50.0	48.0	0	<LOD
158	152	0	<LOQ
500	480	0	7.65
Control	Control	14	<LOD
1.58	1.52	14	<LOD
5.00	4.80	14	2.60
15.8	15.2	14	5.63
50.0	48.0	14	21.2
158	152	14	17.1
500	480	14	56.6

* based on a purity of 96.0% of the test item
LOQ: 1 mg/kg
LOD: 0.3 mg/kg

The measured concentrations in the overlying water at start of the exposure period ranged from 86.5–108% of nominal a.e. concentrations, which confirms the correct application of the test item. At the end of the test the measured concentrations in the overlying water ranged from 77.6–98.5%. Therefore the measured concentrations were not within 20% of the nominal a.e. concentrations, therefore, the biological endpoints are calculated based on nominal a.e. and on geometric mean values of the measured a.e. concentrations in the overlying water.

Details are given in the analytical phase report.

Table 33: Mass balance of analytical results

Nominal concentration		Test period	Overlying water			Pore water				Sediment			Total
			measured concentration of Glyphosate			measured concentration of Glyphosate				measured concentration of Glyphosate			
[mg Glyphosate a.e./L]	[mg Glyphosate a.e./tv][a]	[d]	[mg a.e./L]	[mg a.e./tv][a]	[%][a]	[mg a.e./L]	Volume of pore water [mL]	[mg a.e./tv][c]	[%][c]	[mg a.e./kg dw sed]	[mg a.e./tv][d]	[%][d]	[%]
Control	Control	0	<LOD[1]	–	–	<LOD[1]	44.30	–	–	<LOD[2]	–	0	–
1.52	2.74	0	1.4	2.52	92.1	<LOQ[1]	41.80	n.a.	n.a.	<LOD[2]	n.a.	n.a.	92.1
4.80	8.64	0	5.2	9.36	108	0.505	39.00	0.0197	0.2	<LOD[2]	n.a.	n.a.	108.2
15.2	27.4	0	13.6	24.5	89.5	0.299	50.00	0.0150	0.1	<LOD[2]	n.a.	n.a.	89.6
48.0	86.4	0	41.5	74.7	86.5	2.89	50.60	0.146	0.2	<LOD[2]	n.a.	n.a.	86.7
152	274	0	133	239	87.4	8.96	49.10	0.440	0.2	<LOQ[2]	n.a.	n.a.	87.6
480	864	0	446	803	92.9	30.3	40.80	1.24	0.1	7.65	2.08	0.2	93.2
Control	Control	14	<LOD[1]	–	–	<LOD[1]	55.98	–	–	<LOD[2]	–	0	–
1.52	2.74	14	1.21	2.18	79.7	0.431	59.63	0.0257	0.9	<LOD[2]	n.a.	n.a.	80.6
4.80	8.64	14	4.73	8.51	98.5	1.5	65.10	0.0977	1.1	2.6	0.707	8.2	107.8
15.2	27.4	14	11.8	21.2	77.5	4.18	58.70	0.245	0.9	5.63	1.53	5.6	84.0
48.0	86.4	14	37.8	68	78.7	15.5	63.52	0.985	1.1	21.2	5.77	6.7	86.5
152	274	14	131	236	86.3	53.6	64.10	3.44	1.3	17.1	4.65	1.7	89.3
480	864	14	445	801	92.7	209	55.50	11.6	1.3	56.6	15.4	1.8	95.8

[1] LOQ: 0.15 mg/L; LOD: 0.045 mg/L.
[2] LOQ: 1 µg/kg; LOD: 0.3 µg/kg
[a] related to nominal amount of Glyphosate per test vessel (1800 mL)
[b] related to the real amount of pore water per test vessel (see respective column) of initial nominal / test vessel (tv)
[c] related to the real amount of sediment (dw) per test vessel (400 g wet weight sed. (dry-to-wet-ratio 0.680) /test vessel = 272 g sediment (dw))

Details are given in the analytical phase report.

20 Validity Criteria

The following criterion was fulfilled as required by the guideline:

The mean total shoot length and mean total shoot fresh weight in control plants should be at least double during the exposure period. - mean total shoot length in control plants - mean total shoot fresh weight in control plants	 **5.2 fold** **4.0 fold**
The control plants must not show any visual symptoms of chlorosis and should be visibly free from contaminations by other organisms. No algae and/or bacterial films should be visible on the plants, at the surface of the sediment and in the test medium.	**No symptoms of chlorosis, free from contamination**
The mean coefficient of variation for yield based on shoot fresh weight in the control should not exceed 35% between replicates.	**13.5%**

The study is therefore considered to be valid.

21 Conclusions

Analytical findings

Samples of overlying water, pore water and sediment of all test concentrations taken on days 0 and 14 were analysed by HPLC-MS/MS to quantify the concentrations of the test item by analysis of the acid equivalent (a.e.).

Table 34: Summary of the analytical results as mass balance of overlying water, pore water and sediment.

Nominal concentration	Test period	Overlying water	Pore water	Sediment	Total
[mg Glyphosate a.e./L]	[d]	[%][a]	[%][b]	[%][c]	[%]
Control	0	<LOD	<LOD	<LOD	–
1.52	0	92.1	<LOQ	<LOD	92.1
4.80	0	108	0.2	<LOD	108.2
15.2	0	89.5	0.1	<LOD	89.6
48.0	0	86.5	0.2	<LOD	86.7
152	0	87.4	0.2	<LOQ	87.6
480	0	92.9	0.1	0.2	93.2
Control	14	<LOD	<LOD	<LOD	–
1.52	14	79.7	0.9	<LOD	80.6
4.80	14	98.5	1.1	8.2	107.8
15.2	14	77.5	0.9	5.6	84.0
48.0	14	78.7	1.1	6.7	86.5
152	14	86.3	1.3	1.7	89.3
480	14	92.7	1.3	1.8	95.8

for more detail see also section 19.3 and the analytical phase report.
[a] related to the nominal amount of Glyphosate per test vessel
[b] related to the real amount of pore water per test vessel of initial nominal / test vessel
[c] related to the real amount of sediment (dw) per test vessel

Table 35: Summary of analytical results: geometric mean measured concentrations in overlying water expressed in mg Glyphosate a.e./L.

Nominal concentration [mg test item]	Nominal concentration [mg Glyphosate a.e./L]*	Geometric mean of measured concentrations in overlying water [mg Glyphosate a.e./L]
Control	Control	--
1.58	1.52	1.30
5.00	4.80	4.96
15.8	15.2	12.7
50.0	48.0	39.6
158	152	132
500	480	445

* based on a purity of 96.0% of the test item

Biological findings

Statistically significant effects on the growth of Myriophyllum spicatum could be determined following application of the test item to the water phase of a sediment-water system. For the parameters total shoot length (yield) and biomass$_{wet\ weight}$ (yield and growth rate) the lowest NOEC was determined to be 4.96 mg Glyphosate a.e./L based on geometric mean measured concentrations.

Effective concentrations after 14 days of exposure were determined due to a clear concentration-response relationship for total shoot length and biomass$_{wet\ weight}$ (yield and growth rate).
For the parameter total shoot length (TSL), EC_{50} values of 51.5 mg Glyphosate a.e./L for yield$_{TSL}$ and 208 mg Glyphosate a.e./L for growth rate$_{TSL}$ were determined based on geometric mean measured concentrations.
For the parameter $_{biomass wet\ weight}$, EC_{50} values of 55.9 mg Glyphosate a.e./L for yield-biomass$_{wet\ weight}$ and 163 mg Glyphosate a.e./L for growth rate-biomass$_{wet\ weight}$ was determined based on geometric mean measured concentrations.
For the parameter biomass$_{dry\ weight}$, EC_{50} values could not be determined since the slope of the relationship was found to be not significant, no EC and confidence limits are provided.

A summary of the results is presented in Table 36 and Table 37 and, which presents endpoints based on nominal concentrations and mean measured concentration.

Table 36: Summary of test results after the exposure period of 14 days based on statistical evaluation of biological results as nominal concentrations.

Parameter	Endpoint based on nominal concentrations, 0–14d [mg Glyphosate a.e./L]**				
	EC_{10}	EC_{20}	EC_{50}	NOEC	LOEC
Yield (Total Shoot length)	6.95 (3.90-12.4)*	14.7 (8.63-25.6)	61.7 (33.3-117)	4.80	15.2
Growth rate (Total Shoot length)	47.1 (23.5-94.2)	81.6 (41.8-157)	234 (108-516)	15.2	48.0
Yield (Biomass$_{dry\ weight}$)	n.d. (n.d.–n.d.)	n.d. (n.d.–n.d.)	n.d. (n.d.–n.d.)	≥480	>480
Growth rate (Biomass$_{dry\ weight}$)	n.d. (n.d.–n.d.)	n.d. (n.d.–n.d.)	n.d. (n.d.–n.d.)	≥480	>480
Yield (Biomass$_{wet\ weight}$)	3.49 (0.818-14.9)	9.51 (2.34-38.6)	64.6 (11.8-348)	4.80	15.2
Growth rate (Biomass$_{wet\ weight}$)	6.46 (2.17-19.2)	20.4 (6.99-60.2)	185 (46.5-701)	4.80	15.2

* 95% lower and upper confidence limits;
** Endpoints were given based on a purity of 96% of the test item

Table 37: Summary of test results after the exposure period of 14 days based on statistical evaluation of biological results as geometric mean measured concentrations of Glyphosate.

Parameter	Endpoint based on geometric mean measured concentrations, 0–14d [mg Glyphosate a.e./L]				
	EC_{10}	EC_{20}	EC_{50}	NOEC	LOEC
Yield (Total Shoot length)	5.26 (2.88-9.64)*	11.5 (6.60-20.5)	51.5 (27.1-100)	4.96	12.7
Growth rate (Total Shoot length)	38.2 (18.4-79.4)	68.3 (33.7-137)	208 (92.3-479)	12.7	39.6
Yield ($Biomass_{dry\ weight}$)	n.d. (n.d.–n.d.)	n.d. (n.d.–n.d.)	n.d. (n.d.–n.d.)	≥445	>445
Growth rate ($Biomass_{dry\ weight}$)	n.d. (n.d.–n.d.)	n.d. (n.d.–n.d.)	n.d. (n.d.–n.d.)	≥445	>445
Yield ($Biomass_{wet\ weight}$)	3.00 (0.715-12.6)	8.19 (2.11-32.9)	55.9 (10.5-303)	4.96	12.7
Growth rate ($Biomass_{wet\ weight}$)	5.41 (1.82-16.1)	17.4 (1.82-51.4)	163 (41.2-626)	4.96	12.7

* 95% lower and upper confidence limits;

The data are described in detail in section 23 and 24.

22 References

German Chemicals Act dated 28. August 2013, Federal Law Gazette, Germany (BGBl) p. 3498, 3991. Appendix I (rel. to §19a (1), Principles of Good Laboratory Practice.

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23 Biological Raw Data

23.1 Physico-chemical parameters

Table 38: Raw data: physico-chemical parameters measured during the test; Oxygen concentrations [mg/L].

Time		Conc. [mg test item/L]						
[d]	Replicate	Control	1.58	5.00	15.8	50.0	158	500
0	A	9.2	9.0	8.7	8.9	8.6	8.8	8.5
0	B	8.8	9.1	9.0	8.8	8.4	8.8	8.9
0	C	8.9	9.2	9.1	8.6	8.6	8.4	8.6
0	D	9.1	8.9	8.9	8.9	8.7	8.6	8.9
0	E	9 1						
0	F	8.9						
7	A	15.9	16.9	16.1	15.6	16.4	14.7	15.7
7	B	15.1	16.0	16.1	15.9	14.4	15.9	15.6
7	C	16.1	13.1	16.8	14.7	14.8	18.0	16.4
7	D	16.7	16.6	14.2	15.4	16.2	15.2	14.9
7	E	15.6						
7	F	14.9						
14	A	21.5	19.7	19.9	21.1	20.5	16.8	16.2
14	B	19.6	19.6	19.1	19.4	18.7	18.6	17.5
14	C	16.6	21.2	20.1	19.5	18.1	19.0	17.9
14	D	20.6	20.9	21.4	20.2	19.8	18.2	16.4
14	E	20.0						
14	F	18.6						

Table 39: Raw data: physico-chemical parameters measured during the test; Oxygen concentrations [% of air saturation value, ASV].

Time		Conc. [mg test item/L]						
[d]	Replicate	Control	1.58	5.00	15.8	50.0	158	500
0	A	105	102	100	103	99	101	97
0	B	101	104	103	102	97	101	102
0	C	102	105	103	99	98	97	99
0	D	105	102	102	102	100	98	101
0	E	103						
0	F	102						
7	A	181	192	183	176	189	165	180
7	B	172	182	184	182	169	181	178
7	C	184	150	192	169	170	202	186
7	D	191	190	162	178	185	173	169
7	E	178						
7	F	170						
14	A	239	220	222	237	230	188	182
14	B	218	218	215	218	211	210	198
14	C	218	236	226	219	204	214	202
14	D	229	234	237	227	222	205	185
14	E	223						
14	F	206						

Table 40: Raw data: physico-chemical parameters measured during the test; Temperature in the test vessels [°C].

Time		Conc. [mg test item/L]						
[d]	Replicate	Control	1.58	5.00	15.8	50.0	158	500
0	A	20.3	20.3	20.3	21.0	20.6	20.4	20.5
0	B	20.4	20.4	20.4	20.8	20.9	20.6	20.3
0	C	20.8	20.6	20.1	20.3	20.0	20.5	20.7
0	D	20.7	20.7	20.2	20.4	20.4	20.2	20.3
0	E	19.9						
0	F	20.5						
7	A	20.6	20.4	20.4	20.3	21.1	19.8	20.7
7	B	20.3	20.3	20.5	20.6	21.8	20.3	20.4
7	C	20.6	20.6	20.6	21.2	20.9	19.8	20.3
7	D	20.8	20.8	20.3	21.4	20.5	20.4	20.3
7	E	20.7						
7	F	20.4						
14	A	20.5	20.6	20.5	20.9	21.0	21.1	20.8
14	B	20.5	20.5	21.0	21.0	21.0	20.9	21.0
14	C	20.4	20.4	20.9	21.0	20.9	20.9	21.0
14	D	20.6	20.6	20.1	20.8	20.9	20.7	20.9
14	E	20.5						
14	F	20.1						

Table 41: Raw data: physico-chemical parameters measured during the test; pH values in the overlying water.

Time		Conc. [mg test item/L]						
[d]	Replicate	Control	1.58	5.00	15.8	50.0	158	500
0	A	8.0	8.0	8.0	7.7	7.9	7.9	7.8
0	B	7.9	7.9	8.0	7.9	8.0	7.8	7.9
0	C	7.9	7.9	8.1	7.9	7.8	8.0	7.9
0	D	8.0	8.0	7.9	8.0	7.8	7.8	7.8
0	E	7.9						
0	F	8.0						
7	A	9.3	9.2	9.1	9.0	9.1	8.6	8.7
7	B	9.0	8.9	9.1	9.1	8.9	8.8	8.5
7	C	9.0	8.8	9.1	8.9	8.8	8.7	8.6
7	D	9.1	9.0	9.0	9.1	8.8	8.6	8.5
7	E	9.1						
7	F	8.8						
14	A	9.5	10.1	10.0	9.8	10.0	9.7	9.4
14	B	9.9	10.0	10.0	9.9	9.9	9.7	9.5
14	C	10.0	10.1	10.2	10.0	9.7	9.6	9.4
14	D	10.2	9.9	10.1	9.9	9.6	9.8	9.5
14	E	10.1						
14	F	10.1						

It is expected that the increase of pH was not substance related after 14 day of exposure since the stock solution was adjusted to 7.7, see section 15.3, to vanish a pH related substance effect at day 0 and the pH of the controls showed similar pH values in comparison with the treatment levels, see Table 41, at the end of the test. Rather, it is known that *Myriophyllum* growth cause a pH increase during plant growth, which is expected to be responsible for the strong pH increase. In addition, the controls and the lower treatment levels (C1 and C2; 1.58 and 5.00 mg test item/L) showed no difference in plant growth. Therefore, the deviation of more than 1.5 pH units is expected to have no influence on the integrity of the study. This is in agreement with the guideline OECD 239: According to the OECD 239 guideline pH deviations of more than 1.5 units do not invalidate the test when the validity criteria are met (for validity criteria see chapter 20).

Table 42: Raw Data: Light intensity in µE $m^{-2}s^{-1}$; measured twice on day 0 of the test.

	µE $m^{-2}s^{-1}$	
Day 0	122.54	143.28
	122.21	151.58
	124.55	153.61
	125.11	
	131.64	
	137.41	
Minimum value	114.46	
Maximum value	154.86	
Mean value	134.66	
Number of measurements	9	

23.2 Biological data

Table 43: Mean total shoot lengths [cm] per replicate during exposure phase.

Treatment [mg test item/L]	Control	1.58	5.00	15.8	50.0	158	500
0 d	6.83	5.17	5.80	6.40	5.00	6.90	5.07
	7.43	8.87	6.10	6.13	4.67	5.83	5.80
	6.80	6.67	6.20	6.07	5.93	5.57	6.93
	6.97	5.93	7.97	5.20	6.20	1.87	5.40
	5.60						
	7.27						
Mean:	6.82	6.66	6.52	5.95	5.45	5.04	5.80
Std.Dev.:	0.646	1.595	0.981	0.520	0.733	2.194	0.813
n:	6	4	4	4	4	4	4
CV:	9.5	24.0	15.1	8.7	13.5	43.5	14.0
14 d	37.70	36.83	33.13	27.40	23.30	13.83	9.07
	41.57	45.33	33.50	28.93	20.37	15.93	8.60
	37.17	38.23	29.93	34.17	22.97	12.87	11.37
	28.80	29.77	40.53	24.93	24.77	11.10	7.93
	31.40						
	35.33						
Mean:	35.33	37.54	34.27	28.86	22.85	13.43	9.24
Std.Dev.:	4.604	6.381	4.469	3.904	1.831	2.015	1.491
n:	6	4	4	4	4	4	4
CV:	13.0	17.0	13.0	13.5	8.0	15.0	16.1

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation

Table 44: Yield (total shoot length) during exposure phase.

Treatment [mg test item/L]	Control	1.58	5.00	15.8	50.0	158	500
0 d	0	0	0	0	0	0	0
14 d	30.87	31.67	27.33	21.00	18.30	6.93	4.00
	34.13	36.47	27.40	22.80	15.70	10.10	2.80
	30.37	31.57	23.73	28.10	17.03	7.30	4.43
	21.83	23.83	32.57	19.73	18.57	9.23	2.53
	25.80						
	28.07						

Treatment [mg test item/L]	Control	1.58	5.00	15.8	50.0	158	500
Mean:	28.51	30.88	27.76	22.91	17.40	8.39	3.44
Std Dev	4 307	5 227	3 635	3 683	1 316	1 522	0 919
n:	6	4	4	4	4	4	4
CV:	15.1	16.9	13.1	16.1	7.6	18.1	26.7

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation

Table 45: Mean growth rate per day (total shoot length) during exposure phase.

Treatment [mg test item/L]	Control	1.58	5.00	15.8	50.0	158	500
0 d	0	0	0	0	0	0	0
14 d	0.122	0.140	0.124	0.104	0.110	0.050	0.042
	0 123	0 117	0 122	0 111	0 105	0 072	0 028
	0.121	0.125	0.112	0.123	0.097	0.060	0.035
	0.101	0.115	0.116	0.112	0.099	0.127	0.027
	0.123						
	0.113						
Mean:	0.117	0.124	0.119	0.113	0.103	0.077	0.033
Std.Dev.:	0.0087	0.0115	0.0054	0.0081	0.0060	0.0347	0.0067
n:	6	4	4	4	4	4	4
CV:	7.4	9.3	4.6	7.2	5.9	44.9	20.1

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation

Table 46: Mean biomass [mg dry weight per replicate] of *Myriophyllum spicatum* during exposure phase.

Treatment [mg test item/L]	Control	1.58	5.00	15.8	50.0	158	500
0 d	23.48	23.48	23.48	23.48	23.48	23.48	23.48
	23.48	23.48	23.48	23.48	23.48	23.48	23.48
	23.48	23.48	23.48	23.48	23.48	23.48	23.48
	23.48	23.48	23.48	23.48	23.48	23.48	23.48
	23.48						
	23.48						
Mean:	23.48	23.48	23.48	23.48	23.48	23.48	23.48
Std.Dev.:	0.000	0.000	0.000	0.000	0.000	0.000	0.000
n:	6	4	4	4	4	4	4
CV:	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14 d	65.00	60.33	56.63	59.10	62.97	63.67	62.90
	70.13	75.40	57.47	60.00	59.27	57.40	51.67
	65.17	69.80	57.70	56.77	56.03	61.80	69.87
	60.87	53.40	69.47	51.43	66.20	56.80	54.30
	67.93						
	63.13						
Mean:	65.37	64.73	60.32	56.82	61.12	59.92	59.68
Std.Dev.:	3.308	9.785	6.117	3.844	4.417	3.349	8.313
n:	6	4	4	4	4	4	4
CV:	5.1	15.1	10.1	6.8	7.2	5.6	13.9

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation

Table 47: Yield (Biomass, dry weight, mg dry weight per replicate) during exposure phase.

Treatment [mg test item/L]	Control	1.58	5.00	15.8	50.0	158	500
14 d	41.52	36.85	33.15	35.62	39.49	40.19	39.42
	46.65	51.92	33.99	36.52	35.79	33.92	28.19
	41.69	46.32	34.22	33.29	32.55	38.32	46.39
	37.39	29.92	45.99	27.95	42.72	33.32	30.82
	44.45						
	39.65						
Mean:	41.89	41.25	36.84	33.34	37.64	36.44	36.20
Std.Dev.:	3.308	9.785	6.117	3.844	4.417	3.349	8.313
n:	6	4	4	4	4	4	4
CV:	7.9	23.7	16.6	11.5	11.7	9.2	23.0

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation

Table 48: Mean growth rate per day (Biomass, dry weight, mg dry weight per replicate) during exposure phase.

Treatment [mg test item/L]	Control	1.58	5.00	15.8	50.0	158	500
14 d	0.073	0.067	0.063	0.066	0.070	0.071	0.070
	0.078	0.083	0.064	0.067	0.066	0.064	0.056
	0.073	0.078	0.064	0.063	0.062	0.069	0.078
	0.068	0.059	0.077	0.056	0.074	0.063	0.060
	0.076						
	0.071						
Mean:	0.073	0.072	0.067	0.063	0.068	0.067	0.066
Std.Dev.:	0.0036	0.0110	0.0069	0.0050	0.0052	0.0040	0.0099
n:	6	4	4	4	4	4	4
CV:	4.9	15.3	10.3	7.9	7.6	6.0	14.9

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation

Table 49: Mean biomass [mg wet weight per replicate] of *Myriophyllum spicatum* during exposure phase.

Treatment [mg test item/L]	Control	1.58	5.00	15.8	50.0	158	500
0 d	190.34	190.34	190.34	190.34	190.34	190.34	190.34
	190.34	190.34	190.34	190.34	190.34	190.34	190.34
	190.34	190.34	190.34	190.34	190.34	190.34	190.34
	190.34	190.34	190.34	190.34	190.34	190.34	190.34
	190.34						
	190.34						
Mean:	190.34	190.34	190.34	190.34	190.34	190.34	190.34
Std.Dev.:	0.000	0.000	0.000	0.000	0.000	0.000	0.000
n:	6	4	4	4	4	4	4
CV:	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14 d	754.10	747.37	642.50	536.63	509.07	398.70	324.27
	866.90	1012.90	692.30	665.63	481.03	421.87	290.83
	715.57	802.20	678.10	682.07	496.93	393.00	396.07
	641.10	620.50	871.47	491.37	558.87	342.07	301.67
	759.43						
	803.43						
Mean:	756.76	795.74	721.09	593.93	511.47	388.91	328.21
Std.Dev.:	76.678	163.554	102.415	94.360	33.615	33.630	47.334
n:	6	4	4	4	4	4	4
CV:	10.1	20.6	14.2	15.9	6.6	8.7	14.4

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation

Table 50: Yield (Biomass, wet weight, mg wet weight per replicate) during exposure phase.

Treatment [mg test item/L]	Control	1.58	5.00	15.8	50.0	158	500
14 d	563.76	557.03	452.16	346.29	318.73	208.36	133.93
	676.56	822.56	501.96	475.29	290.69	231.53	100.49
	525.23	611.86	487.76	491.73	306.59	202.66	205.73
	450.76	430.16	681.13	301.03	368.53	151.73	111.33
	569.09						
	613.09						
Mean:	566.42	605.40	530.75	403.58	321.13	198.57	137.87
Std.Dev.:	76.678	163.554	102.415	94.360	33.615	33.630	47.334
n:	6	4	4	4	4	4	4
CV:	13.5	27.0	19.3	23.4	10.5	16.9	34.3

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation

Table 51: Mean growth rate per day (Biomass, wet weight, mg wet weight per replicate) during exposure phase.

Treatment [mg test item/L]	Control	1.58	5.00	15.8	50.0	158	500
14 d	0.098	0.098	0.087	0.074	0.070	0.053	0.038
	0.108	0.119	0.092	0.089	0.066	0.057	0.030
	0.095	0.103	0.091	0.091	0.069	0.052	0.052
	0.087	0.084	0.109	0.068	0.077	0.042	0.033
	0.099						
	0.103						
Mean:	0.098	0.101	0.095	0.081	0.070	0.051	0.038
Std.Dev.:	0.0073	0.0145	0.0096	0.0115	0.0046	0.0064	0.0098
n:	6	4	4	4	4	4	4
CV:	7.5	14.3	10.2	14.3	6.5	12.5	25.6

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation

Table 52: Visual observations during exposure phase.

Treatment [mg test item/L]	Control	1.58	5.00	15.8	50.0	158	500
0 d	G	G	G	G	G	G	G
7 d	G	G	G	G	G	G, RG	Def, RG
	G	G	G	G	G	G, RG	Def, RG
	G	G	G	G	G	G, RG	Def, RG
	G	G	G	G	G	G, RG	Def, RG
	G						
	G						
14 d	G	G	G	G	AW, Chl	AW, Chl, RG	AW, Def, RG
	G	G	G	G	AW, Chl	AW, Chl, RG	AW, Def, RG
	G	G	G	G	AW, Chl	AW, Chl, RG	AW, Def, RG
	G	G	G	G	AW, Chl	AW, Chl, RG	AW, Def, RG
	G						
	G						

G: Healthy
Def: Plants lie on their side but look still healthy & green
Chl: light chlorotic
RG: reduced growth
AW: algae growth (Only slight algae growth was observed. Green algae were visible at the water surface but did not cover the whole water surface. The plants were algae free).

24 Statistical Analysis of Raw Data

The following software packages were used:

ToxRat Professional 3.3.0, ToxRat Solutions GmbH, Alsdorf, Germany
Excel 2013, 2016 & 365 (Version: 2111), Microsoft GmbH, Unterschleißheim, Germany

Statistical Analysis of Raw Data of Nominal Concentrations (Definitive Test)

24.1 Yield (total shoot length), Yield TSL

Effective concentrations (EC_x) for yield TSL at 14 d

Results of the the 3-param. normal CDF based on nominal concentrations in mg a.e./L.

Estimated parameters of the 3-param. normal CDF

Table 53: Estimated parameters of the 3-param. normal CDF with yield TSL at 14 d:
Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	29.621	1.285	26.984	32.258	23.049	<0.0001
b1	0.842	0.122	0.591	1.093	6.877	<0.0001
b2	0.740	0.052	0.634	0.846	14.278	<0.0001

Stop Reason = Converged (Optimization method: Levenberg-Marquardt)

R^2: 0.996; adjusted R^2: 0.996
Residual standard error: 0.24357
Akaike Criterion (AIC): 108.076
Shapiro Wilk`s test on normal distribution of residuals: p = 0.9261.

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Table 54: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with yield TSL at 14 d:
Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	251.46	2	125.73	2119.338	<0.0001
Residuals	1.60	27	0.06		
- Lack of Fit	0.11	4	0.03	0.411	0.799
- Pure Error	1.49	23	0.06		
Total	252.45	29			

Since p(F|Regression) <= 0.05, a significant amount of variance is explained by the regression model..Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit.

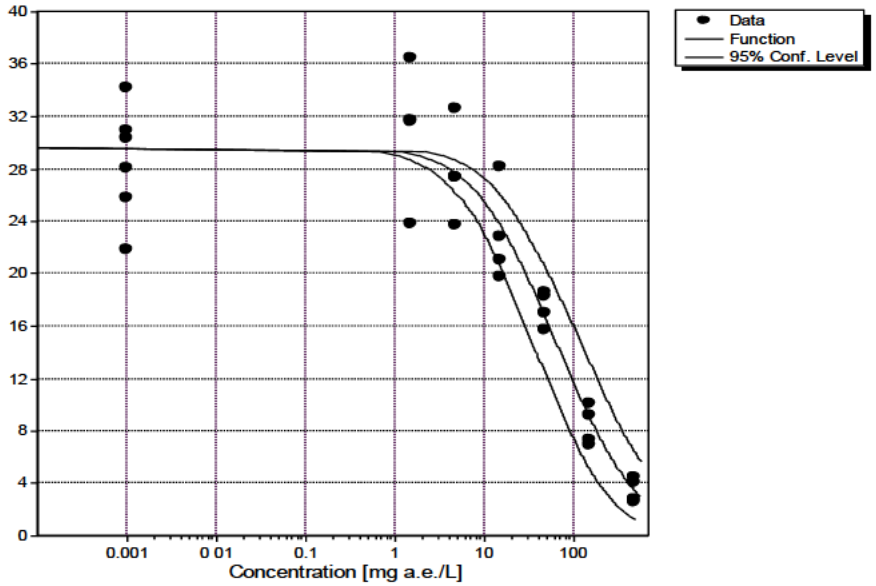

Figure 7: Concentration-effect curve showing the influence of the test item on yield TSL of the introduced Myriophyllum spicatum as observed after 14 d.

Point estimates from the 3-param. normal CDF

Table 55: Point estimates from the 3-param. normal CDF with yield TSL at 14 d: Selected effective concentrations (ECx) of the test item; cl: confidence limit.

Parameter	E_yC_{10}	E_yC_{20}	E_yC_{50}
Measured Conc. [mg Glyphosate a.e./L]	6.946	14.699	61.675
Lower 95%-cl	3.896	8.628	33.324
Upper 95%-cl	12.383	25.551	116.531

cl: confidence limit; n.d.: not determined

The confidence limits of the EC10 used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ECx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs).

Results of the the 3-param. normal CDF based on geometric mean measured concentrations in mg a.e./L.

Estimated parameters of the 3-param. normal CDF

Table 56: Estimated parameters of the 3-param. normal CDF with yield TSL at 14 d:
Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std Err	95%LCL	95%UCL	t	p(t)
b0	29.860	1.336	27.120	32.600	22.359	<0.0001
b1	0.721	0.128	0.459	0.984	5.636	<0.0001
b2	0.773	0.054	0.663	0.883	14.435	<0.0001

Stop Reason = Converged (Optimization method: Levenberg-Marquardt)

R^2: 0.997; adjusted R^2: 0.997
Residual standard error: 0.24385
Akaike Criterion (AIC): 108.385
Shapiro Wilk`s test on normal distribution of residuals: p = 0.8610.

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Table 57: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with yield TSL at 14 d:
Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	252.29	2	126.15	2121.411	<0.0001
Residuals	1.61	27	0.06		
- Lack of Fit	0.12	4	0.03	0.453	0.769
- Pure Error	1.49	23	0.06		
Total	253.02	29			

Since p(F|Regression) <= 0.05, a significant amount of variance is explained by the regression model..Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit.

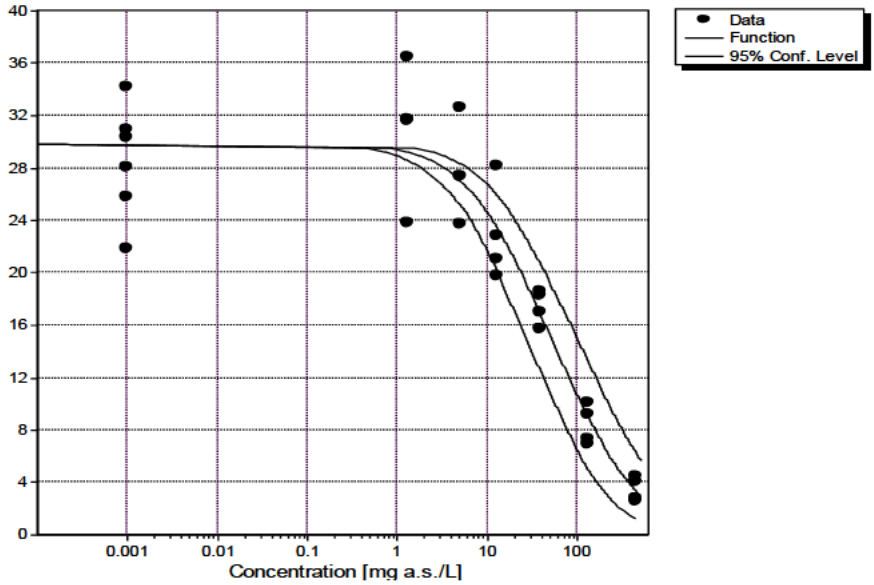

Figure 8: Concentration-effect curve showing the influence of the test item on yield TSL of the introduced Myriophyllum spicatum as observed after 14 d.

Point estimates from the 3-param. normal CDF

Table 58: Point estimates from the 3-param. normal CDF with yield TSL at 14 d: Selected effective concentrations (ECx) of the test item; cl: confidence limit.

Parameter	E_yC_{10}	E_yC_{20}	E_yC_{50}
Measured Conc. [mg Glyphosate a.e./L]	5.264	11.515	51.487
Lower 95%-cl	2.875	6.603	27.066
Upper 95%-cl	9.636	20.533	100.112

cl: confidence limit; n.d.: not determined

The confidence limits of the EC10 used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ECx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs).

Threshold concentrations (NOEC) for yield TSL at 14 d

Statistical characteristics of the sample

Table 59: Statistical characteristics:
Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits

Measured Conc. [mg Glyphosate a.e./L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s (X)	95%l	95%u
Control	28.51	29.22	21.83	34.13	6	4.307	15.10	1.758	6.20	23.99	33.03
1.30	30.88	31.62	23.83	36.47	4	5.227	16.90	2.613	8.50	22.57	39.20
4.96	27.76	27.37	23.73	32.57	4	3.635	13.10	1.817	6.50	21.97	33.54
12.7	22.91	21.90	19.73	28.10	4	3.683	16.10	1.841	8.00	17.05	28.77
39.6	17.40	17.67	15.70	18.57	4	1.316	7.60	0.658	3.80	15.31	19.49
132	8.39	8.27	6.93	10.10	4	1.522	18.10	0.761	9.10	5.97	10.81
445	3.44	3.40	2.53	4.43	4	0.919	26.70	0.459	13.30	1.98	4.90

Normality check was passed (p > 0.01). (Shapiro-Wilk's Test on Normal Distribution)

Variance homogeneity check was passed (p > 0.01). (Levene´s Test on Variance Homogeneity (with Residuals).

The analysis of contrasts revealed a linear trend, thus the selected Williams test was performed.

Williams Multiple Sequential t-test Procedure

Table 60: Williams Multiple Sequential t-test Procedure with yield TSL at 14 d:
Comparison of treatments with "Control" by the t test procedure after Williams with yield TSL at 14 d: Significance was Alpha = 0.0500, one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: μ1 = μ2 = ... = μk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered.

Measured Conc. [mg Glyphosate a.e./L]	Mean	s	df	LhM	%MDD	t	t*	Sign.
Control	28.51	3.424						
1.30	30.88	3.424	23	30.88	-13.29	1.07	-1.71	-
4.96	27.76	3.424	23	27.76	-13.83	-0.34	-1.78	-
12.7	22.91	3.424	23	22.91	-14.02	-2.53	-1.81	+
39.6	17.40	3.424	23	17.40	-14.09	-5.03	-1.82	+
132	8.39	3.424	23	8.39	-14.15	-9.1	-1.83	+
445	3.44	3.424	23	3.44	-14.16	-11.34	-1.83	+

+: significant; -: non-significant

A NOEC of 4.96 mg Glyphosate a.e./L based on geometric mean measured concentrations is suggested by the program.

24.2 Mean growth rate (total shoot length), Mean growth rate TSL

Results of the the 3-param. normal CDF based on nominal concentrations in mg a.e./L.

Estimated parameters of the 3-param. normal CDF

Table 61: Estimated parameters of the 3-param. normal CDF with growth rate TSL at 14 d: Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	0 118	0 005	0 108	0 128	24 321	0 0001
b1	1 673	0 147	1 372	1 974	11 397	0 0001
b2	0 543	0 082	0 375	0 710	6 648	0 0001

Stop Reason = Converged (Optimization method: Levenberg-Marquardt)

R^2: 0.948; adjusted R^2: 0.944
Residual standard error: 0.00232
Akaike Criterion (AIC): -220.306
Shapiro Wilk`s test on normal distribution of residuals: p = 0.0006.

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Table 62: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with yield TSL at 14 d:
Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with growth rate TSL at 14 d: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	0.003	2	0.001	256.189	<0.0001
Residuals	0.000	27	0.000		
- Lack of Fit	0.000	4	0.000	0.156	0.958
- Pure Error	0.000	23	0.000		
Total	0.003	29			

Since p(F|Regression) <= 0.05, a significant amount of variance is explained by the regression model..Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit.

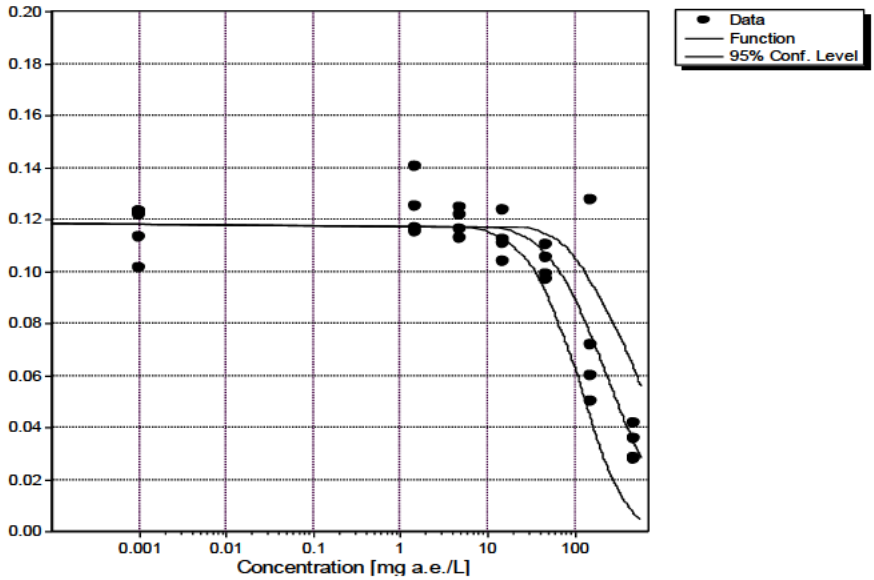

Figure 9: Concentration-effect curve showing the influence of the test item on growth rate TSL of the introduced Myriophyllum spicatum as observed after 14 d.

Point estimates from the 3-param. normal CDF

Table 63: Point estimates from the 3-param. normal CDF with growth rate TSL at 14 d: Selected effective concentrations (ECx) of the test item; cl: confidence limit.

Parameter	E_rC_{10}	E_rC_{20}	E_rC_{50}
Nominal concentration [mg Glyphosate a.e./L]	47.087	81.593	233.561
Lower 95%-cl	23.536	41.842	108.235
Upper 95%-cl	94.204	157.434	516.299

cl: confidence limit; n.d.: not determined

The confidence limits of the EC10 used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ECx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs).

Results of the the 3-param. normal CDF based on geometric mean measured concentrations in mg a.e./L.

Estimated parameters of the 3-param. normal CDF

Table 64: Estimated parameters of the 3-param. normal CDF with growth rate TSL at 14 d: Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std Err	95%LCL	95%UCL	t	p(t)
b0	0.118	0.005	0.108	0.128	24.104	<0.0001
b1	1.582	0.155	1.264	1.900	10.206	<0.0001
b2	0.574	0.086	0.397	0.750	6.679	<0.0001

Stop Reason = Converged (Optimization method: Levenberg-Marquardt)

R^2: 0.948; adjusted R^2: 0.944
Residual standard error: 0.00232
Akaike Criterion (AIC): -220.393
Shapiro Wilk`s test on normal distribution of residuals: p = 0.0004.

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Table 65: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with yield TSL at 14 d:
Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with growth rate TSL at 14 d: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	0.003	2	0.001	256.745	<0.0001
Residuals	0.000	27	0.000		
- Lack of Fit	0.000	4	0.000	0.148	0.962
- Pure Error	0.000	23	0.000		
Total	0.003	29			

Since p(F|Regression) <= 0.05, a significant amount of variance is explained by the regression model..Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit.

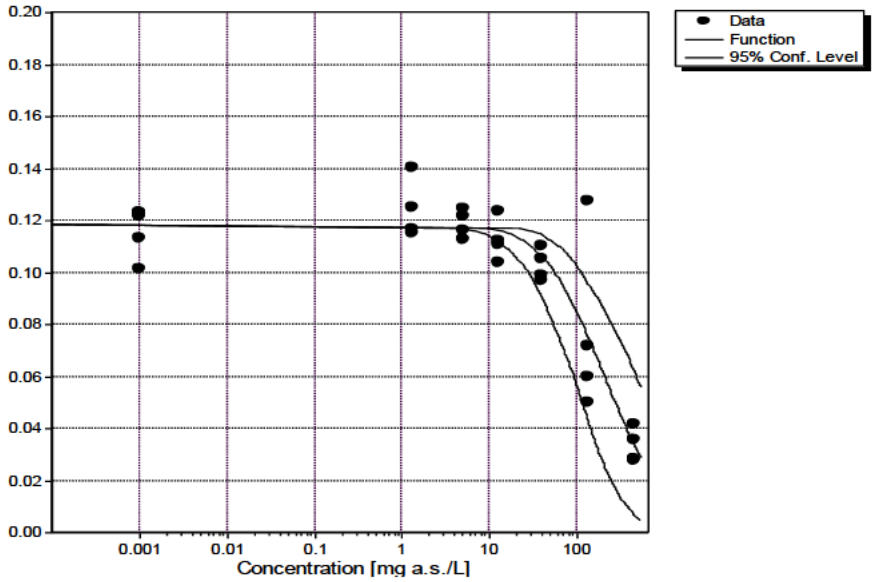

Figure 10: Concentration-effect curve showing the influence of the test item on growth rate TSL of the introduced Myriophyllum spicatum as observed after 14 d.

Point estimates from the 3-param. normal CDF

Table 66: Point estimates from the 3-param. normal CDF with growth rate TSL at 14 d: Selected effective concentrations (ECx) of the test item; cl: confidence limit.

Parameter	E_rC_{10}	E_rC_{20}	E_rC_{50}
Nominal concentration [mg Glyphosate a.e./L]	38.191	68.285	207.552
Lower 95%-cl	18.362	33.740	92.264
Upper 95%-cl	79.433	136.661	479.476

cl: confidence limit; n.d.: not determined

The confidence limits of the EC10 used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ECx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs).

Threshold concentrations (NOEC) for mean growth rate (total shoot length) at 14 d

Statistical characteristics of the sample

Table 67: Statistical characteristics:
Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Measured Conc. [mg Glyphosate a.e./L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s (X)	95%l	95%u
Control	0.117	0.122	0.101	0.123	6	0.0087	7.40	0.0035	3.00	0.108	0.126
1.30	0.124	0.121	0.115	0.140	4	0.0115	9.30	0.0058	4.60	0.106	0.143
4.96	0.119	0.119	0.112	0.124	4	0.0054	4.50	0.0027	2.30	0.110	0.127
12.7	0.113	0.111	0.104	0.123	4	0.0081	7.20	0.0041	3.60	0.100	0.125
39.6	0.103	0.102	0.097	0.110	4	0.0060	5.90	0.0030	2.90	0.093	0.112
132	0.077	0.066	0.050	0.127	4	0.0347	44.90	0.0173	22.50	0.022	0.132
445	0.033	0.032	0.027	0.042	4	0.0067	20.10	0.0033	10.00	0.023	0.044

Normality check was failed (p <= 0.01). (Shapiro-Wilk's Test on Normal Distribution)

Variance homogeneity check was passed (p > 0.01). (Levene´s Test on Variance Homogeneity (with Residuals).

The analysis of contrasts revealed a linear trend, thus the selected SD Jonckheere-Terpstra test was performed.

Step-down Jonckheere-Terpstra Test Procedure

Table 68: Step-down Jonckheere-Terpstra test procedure with growth rate TSL at 14 d:
Step-down test to detect a trend in decreasing median effects on growth rate TSL (Alpha is 0.0500; one-sided smaller); Med: median, n: sample size; J: test statistic; J*: standardized J (in case a value for J* is shown for a treatment, the large-sample approximation was calculated (= sum of all replicates N > 11)); p(J): probability that the observed trend could be due to chance; Ho is accepted, if p(J) > Alpha. Note that the step-down test terminates after the first non-significant treatment is encountered

Measured Conc. [mg Glyphosate a.e./L]	Mean	Med	n	J	J*	p(J)	Sign.
Control	0.117	0.122	6				
1.30	0.124	0.121	4	8.0		0.2381	-
4.96	0.119	0.119	4	2.0	0.120	0.4531	-
12.7	0.113	0.111	4	26.0	1.030	0.1525	-
39.6	0.103	0.102	4	90.0	2.600	0.0046	+
132	0.077	0.066	4	136.0	3.050	0.0011	+
445	0.033	0.032	4	240.0	4.340	<0.0001	+

+: significant; -: non-significant.

A NOEC of 12.7 mg Glyphosate a.e./L based on geometric mean measured concentrations is suggested by the program.

24.3 Yield (biomass$_{dry\ weight}$), Yield DW

Effective concentrations (EC_x) for yield (biomass$_{dry\ weight}$) at 14 d

The following non-linear regression procedure stands as an example since all other tried statistical non-linear regression procedure led to similar results.

The non-linear regression procedure was terminated without achieving convergence due to mathematical problems.
Other fittings were checked for acceptance and the increase in the number of optimization cycles both did not lead to convergence

Results of the 3-param. normal CDF analysis based on nominal concentrations in mg a.e./L.

Estimated parameters of the 3-param. normal CDF

Table 69: Estimated parameters of the 3-param. normal CDF with yield DW at 14 d:
Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with yield DW at 14 d: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	103.108	54.857	-9.450	215.666	1.880	0.0355
b1	-54.849	20.449	-96.808	-12.891	-2.682	0.0062
b2	34.196	12.377	8.800	59.591	2.763	0.0051

Stop Reason = Converged (Optimization method: Levenberg-Marquardt)

R^2: 0.140; adjusted R^2: 0.076
Residual standard error: 5.77829
Akaike Criterion (AIC): 141.686
Shapiro Wilk`s test on normal distribution of residuals: p = 0.2718.

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Table 70: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with yield DW at 14 d:
Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	146.51	2	73.25	2.194	0.131
Residuals	901.49	27	33.39		
- Lack of Fit	103.45	4	25.86	0.745	0.571
- Pure Error	798.04	23	34.70		
Total	1046.47	29			

Since p(F|Regression) > 0.05, the amount of variance explained by the regression model is NOT significant. Therefore, confidence limits cannot be provided. Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit..

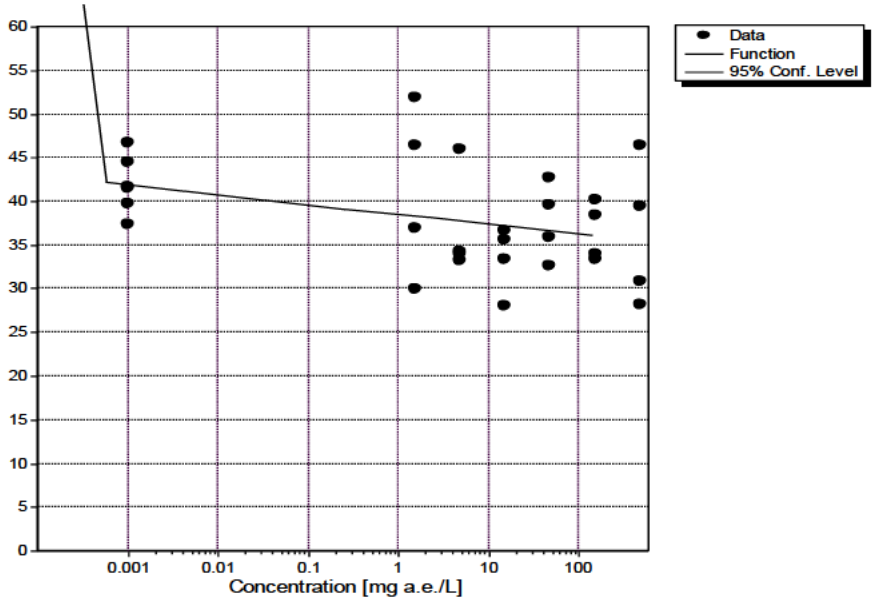

Figure 11: Concentration-effect curve showing the influence of the test item on yield DW of the introduced *Myriophyllum spicatum* as observed after 14 d.

<u>Point estimates from the 3-param. normal CDF</u>

Table 71: Point estimates from the 3-param. Normal CDF with yield DW at 14 d: Selected effective concentrations (ECx) of the test item.

Parameter	E_yC_{10}	E_yC_{20}	E_yC_{50}
Nominal concentration [mg Glyphosate a.e./L]	n.d.	n.d.	n.d.
Lower 95%-cl	n.d.	n.d.	n.d.
Upper 95%-cl	n.d.	n.d.	n.d.

cl: confidence limit; n.d.: not determined

Since the slope of the relationship was found to be not significant, no EC and confidence limits are provided.

Results of the 3-param. normal CDF analysis based on geometric mean measured concentrations in mg a.e./L.

Estimated parameters of the 3-param. normal CDF

Table 72: Estimated parameters of the 3-param. normal CDF with yield DW at 14 d:
Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with yield DW at 14 d:
Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	101.978	54.812	-10.487	214.443	1.861	0.0369
b1	-53.447	20.237	-94.969	-11.924	-2.641	0.0068
b2	33.526	12.182	8.531	58.522	2.752	0.0052

Stop Reason = Converged (Optimization method: Levenberg-Marquardt)

R^2: 0.140; adjusted R^2: 0.077
Residual standard error: 5.77731
Akaike Criterion (AIC): 141.676
Shapiro Wilk`s test on normal distribution of residuals: p = 0.2691.

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Table 73: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with yield DW at 14 d:
Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	147.02	2	73.51	2.202	0.130
Residuals	901.19	27	33.38		
- Lack of Fit	103.15	4	25.79	0.743	0.572
- Pure Error	798.04	23	34.70		
Total	1046.47	29			

Since p(F|Regression) > 0.05, the amount of variance explained by the regression model is NOT significant. Therefore, confidence limits cannot be provided. Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit..

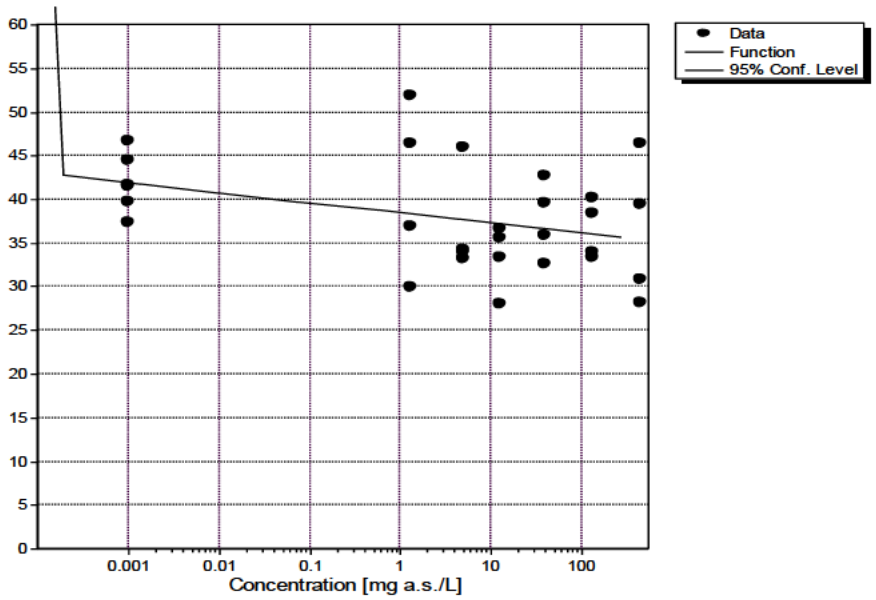

Figure 12: Concentration-effect curve showing the influence of the test item on yield DW of the introduced *Myriophyllum spicatum* as observed after 14 d.

Point estimates from the 3-param. normal CDF

Table 74: Point estimates from the 3-param. Normal CDF with yield DW at 14 d: Selected effective concentrations (ECx) of the test item.

Parameter	E_yC_{10}	E_yC_{20}	E_yC_{50}
Nominal concentration [mg Glyphosate a.e./L]	n.d.	n.d.	n.d.
Lower 95%-cl	n.d.	n.d.	n.d.
Upper 95%-cl	n.d.	n.d.	n.d.

cl: confidence limit; n.d.: not determined

Since the slope of the relationship was found to be not significant, no EC and confidence limits are provided.

Threshold concentrations (NOEC) for yield (biomass$_{dry\ weight}$) at 14 d

Statistical characteristics of the sample

Table 75: Statistical characteristics with yield DW at 14 d:
Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Measured Conc. [mg Glyphosate a.e./L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s (X)	95%l	95%u
Control	41.89	41.60	37.39	46.65	6	3.308	7.90	1.351	3.20	38.42	45.36
1.30	41.25	41.59	29.92	51.92	4	9.785	23.70	4.893	11.90	25.68	56.82
4.96	36.84	34.10	33.15	45.99	4	6.117	16.60	3.059	8.30	27.10	46.57
12.7	33.34	34.45	27.95	36.52	4	3.844	11.50	1.922	5.80	27.23	39.46
39.6	37.64	37.64	32.55	42.72	4	4.417	11.70	2.208	5.90	30.61	44.66
132	36.44	36.12	33.32	40.19	4	3.349	9.20	1.675	4.60	31.11	41.77
445	36.20	35.12	28.19	46.39	4	8.313	23.00	4.156	11.50	22.98	49.43

Normality check was passed (p > 0.01). (Shapiro-Wilk's Test on Normal Distribution)

Variance homogeneity check was passed (p > 0.01). (Levene´s Test on Variance Homogeneity (with Residuals).

The analysis of contrasts revealed a linear trend (p <= 0.05), thus the selected Williams test was performed.

Williams Multiple Sequential t-test Procedure

Table 76: Comparison of treatments with "Control" by the t test procedure after Williams with yield DW at 14 d:
Significance was Alpha = 0.0500, one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: μ1 = μ2 = ... = μk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered

Measured Conc. [mg Glyphosate a.e./L]	Mean	s	df	LhM	%MDD	t	t*	Sign.
Control	41.89	5.890						
1.30	41.25	5.890	23	41.25	-15.56	-0.17	-1.71	-
4.96	36.84	5.890	23	36.84	-16.2	-1.33	-1.78	-
12.7	33.34	5.890	23	35.91	-16.41	-1.57	-1.81	-
39.6	37.64	5.890	23	35.91	-16.49	-1.57	-1.82	-
132	36.44	5.890	23	35.91	-16.57	-1.57	-1.83	-
445	36.20	5.890	23	35.91	-16.58	-1.57	-1.83	-

+: significant; -: non-significant

A The NOEC appears to be higher than or equal 445 mg Glyphosate a.e./L based on geometric mean measured concentrations.

24.4 Mean growth rate (biomass$_{dry\ weight}$), Mean growth rate DW

Effective concentrations (EC_x) for mean growth rate DW at 14 d

The following non-linear regression procedure stands as an example since all other tried statistical non-linear regression procedure led to similar results.

The non-linear regression procedure was terminated without achieving convergence due to mathematical problems.
Other fittings were checked for acceptance and the increase in the number of optimization cycles both did not lead to convergence

Results of the 3-param. normal CDF analysis based on nominal concentrations in mg a.e./L.

Estimated parameters of 3-param. normal CDF

Table 77: Estimated parameters of the 3-param. normal CDF with growth rate DW at 14 d:
Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	0.129	0.162	-0.202	0.461	0.800	0.2154
b1	-45.668	68.107	-185.412	94.076	-0.671	0.2541
b2	38.260	17.496	2.361	74.160	2.187	0.0188

Stop Reason = Converged (Optimization method: Levenberg-Marquardt)

R^2: 0.144; adjusted R^2: 0.080
Residual standard error: 0.00665
Akaike Criterion (AIC): -264.360
Shapiro Wilk`s test on normal distribution of residuals: p = 0.3949.

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Table 78: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with yield DW at 14 d:
Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with growth rate DW at 14 d: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	0.000	2	0.000	2.267	0.123
Residuals	0.001	27	0.000		
- Lack of Fit	0.000	4	0.000	0.711	0.593
- Pure Error	0.001	23	0.000		
Total	0.001	29			

Since p(F|Regression) > 0.05, the amount of variance explained by the regression model is NOT significant..Therefore, confidence limits cannot be provided..Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit.

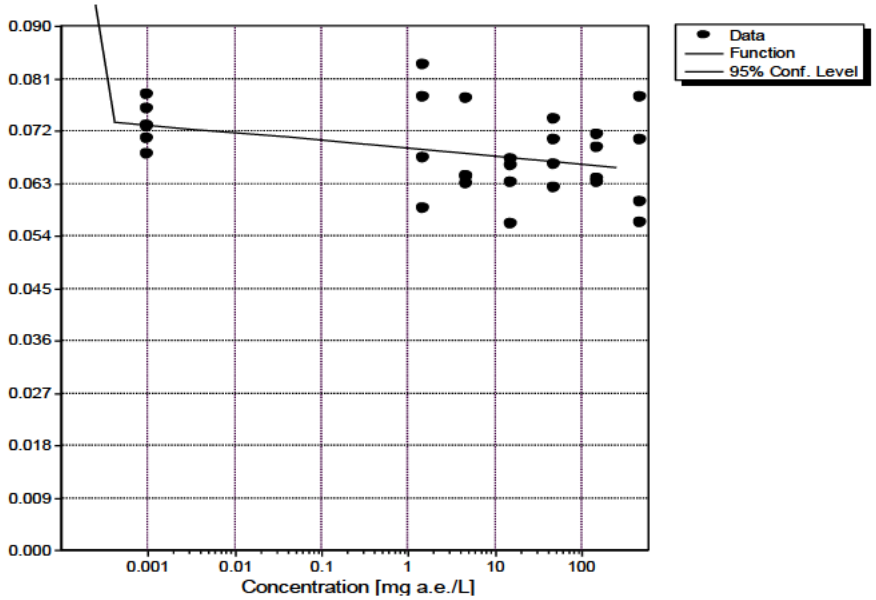

Figure 13: Concentration-effect curve showing the influence of the test item on growth rate DW of the introduced *Myriophyllum spicatum* as observed after 14 d.

Point estimates from the 3-param. normal CDF

Table 79: Point estimates from the 3-param. normal CDF with growth rate DW at 14 d: Selected effective concentrations (ECx) of the test item; cl: confidence limit.

Parameter	E_yC_{10}	E_yC_{20}	E_yC_{50}
Nominal concentration [mg Glyphosate a.e./L]	n.d.	n.d.	2313.350
Lower 95%-cl	n.d.	n.d.	n.d.
Upper 95%-cl	n.d.	n.d.	n.d.

cl: confidence limit; n.d.: not determined.

Since a signifcant lack of fit was found, it is recomended to try other dose/response functions.

Results of the 3-param. normal CDF analysis based on geometric mean measured concentrations in mg a.e./L.

Estimated parameters of 3-param. normal CDF

Table 80: Estimated parameters of the 3-param. normal CDF with growth rate DW at 14 d: Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	0.130	0.162	-0.203	0.463	0.800	0.2152
b1	-45.832	67.122	-183.554	91.891	-0.683	0.2503
b2	38.088	17.251	2.692	73.485	2.208	0.0180

Stop Reason = Converged (Optimization method: Levenberg-Marquardt)

R^2: 0.144; adjusted R^2: 0.081
Residual standard error: 0.00665
Akaike Criterion (AIC): -264.369
Shapiro Wilk`s test on normal distribution of residuals: p = 0.3876.

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Table 81: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with yield DW at 14 d:
Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with growth rate DW at 14 d: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	0.000	2	0.000	2.275	0.122
Residuals	0.001	27	0.000		
- Lack of Fit	0.000	4	0.000	0.709	0.594
- Pure Error	0.001	23	0.000		
Total	0.001	29			

Since p(F|Regression) > 0.05, the amount of variance explained by the regression model is NOT significant..Therefore, confidence limits cannot be provided..Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit.

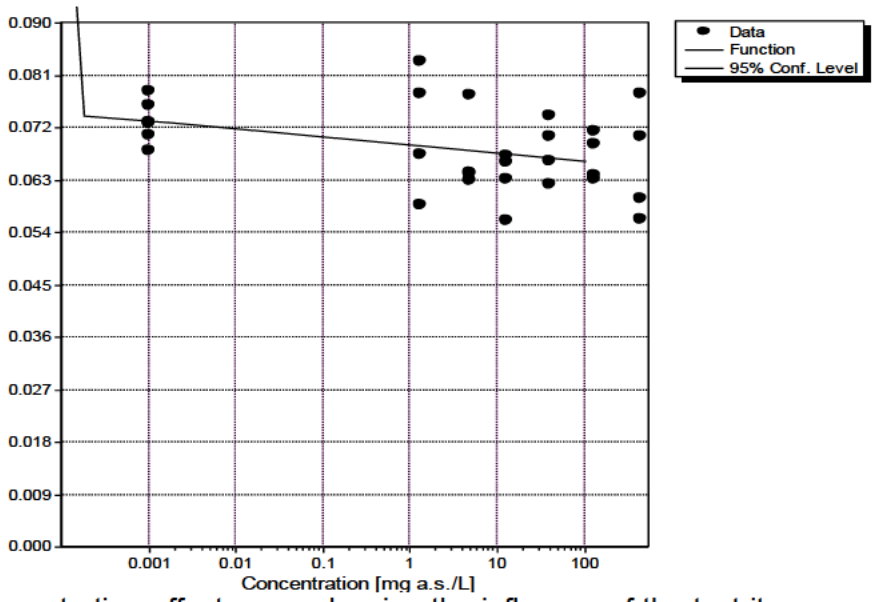

Figure 14: Concentration-effect curve showing the influence of the test item on growth rate DW of the introduced *Myriophyllum spicatum* as observed after 14 d.

Point estimates from the 3-param. normal CDF

Table 82: Point estimates from the 3-param. normal CDF with growth rate DW at 14 d: Selected effective concentrations (ECx) of the test item; cl: confidence limit.

Parameter	E_yC_{10}	E_yC_{20}	E_yC_{50}
Nominal concentration [mg Glyphosate a.e./L]	n.d.	n.d.	955.883
Lower 95%-cl	n.d.	n.d.	n.d.
Upper 95%-cl	n.d.	n.d.	n.d.

cl: confidence limit; n.d.: not determined

Since a signifcant lack of fit was found, it is recomended to try other dose/response functions.

Threshold concentrations (NOEC) for mean growth rate DW at 14 d

Statistical characteristics of the sample

Table 83: Statistical characteristics with growth rate DW at 14 d:
Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits

Measured Conc. [mg Glyphosate a.e./L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s (X)	95%l	95%u
Control	0.073	0.073	0.068	0.078	6	0.0036	4.90	0.0015	2.00	0.069	0.077
1.30	0.072	0.073	0.059	0.083	4	0.0110	15.30	0.0055	7.60	0.054	0.089
4.96	0.067	0.064	0.063	0.077	4	0.0069	10.30	0.0035	5.20	0.056	0.078
12.7	0.063	0.064	0.056	0.067	4	0.0050	7.90	0.0025	3.90	0.055	0.071
39.6	0.068	0.068	0.062	0.074	4	0.0052	7.60	0.0026	3.80	0.060	0.076
132	0.067	0.066	0.063	0.071	4	0.0040	6.00	0.0020	3.00	0.060	0.073
445	0.066	0.065	0.056	0.078	4	0.0099	14.90	0.0049	7.50	0.050	0.082

Normality check was passed (p > 0.01). (Shapiro-Wilk's Test on Normal Distribution)

Variance homogeneity check was passed (p > 0.01). (Levene´s Test on Variance Homogeneity (with Residuals).

The analysis of contrasts revealed a linear trend (p <= 0.05), thus the selected Williams test was performed.

Williams Multiple Sequential t-test Procedure

Table 84: Comparison of treatments with "Control" by the t test procedure after Williams with growth rate DW at 14 d:
Significance was Alpha = 0.0500, one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: μ1 = μ2 = ... = μk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered

Measured Conc. [mg Glyphosate a.e./L]	Mean	s	df	LhM	%MDD	t	t*	Sign.
Control	0.073	0.0068						
1.30	0.072	0.0068	23	0.072	-10.29	-0.28	-1.71	-
4.96	0.067	0.0068	23	0.067	-10.71	-1.35	-1.78	-
12.7	0.063	0.0068	23	0.066	-10.86	-1.6	-1.81	-
39.6	0.068	0.0068	23	0.066	-10.91	-1.6	-1.82	-
132	0.067	0.0068	23	0.066	-10.96	-1.6	-1.83	-
445	0.066	0.0068	23	0.066	-10.97	-1.6	-1.83	-

+: significant; -: non-significant.

A The NOEC appears to be higher than or equal 445 mg Glyphosate a.e./L based on geometric mean measured concentrations.

24.5 Yield (biomass$_{wet\ weight}$), Yield FW

Results of the 3-param. normal CDF analysis based on nominal concentrations in mg a.e./L.

Estimated parameters of the 3-param. normal CDF

Table 85: Estimated parameters of the 3-param. normal CDF with yield FW at 14 d:
Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	589.163	31.866	523.780	654.546	18.489	<0.0001
b1	0.543	0.307	-0.087	1.174	1.767	0.0442
b2	0.988	0.190	0.599	1.378	5.203	<0.0001

Stop Reason = Converged (Optimization method: Levenberg-Marquardt).

R^2: 0.838; adjusted R^2: 0.826
Residual standard error: 86.24929
Akaike Criterion (AIC): 303.874
Shapiro Wilk`s test on normal distribution of residuals: p = 0.0344.

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Table 86: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with yield FW at 14 d:
Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	876145.46	2	438072.73	58.889	<0.0001
Residuals	200851.39	27	7438.94		
- Lack of Fit	19521.88	4	4880.47	0.619	0.653
- Pure Error	181329.52	23	7883.89		
Total	1045544.02	29			

Since p(F|Regression) <= 0.05, a significant amount of variance is explained by the regression model..Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit.

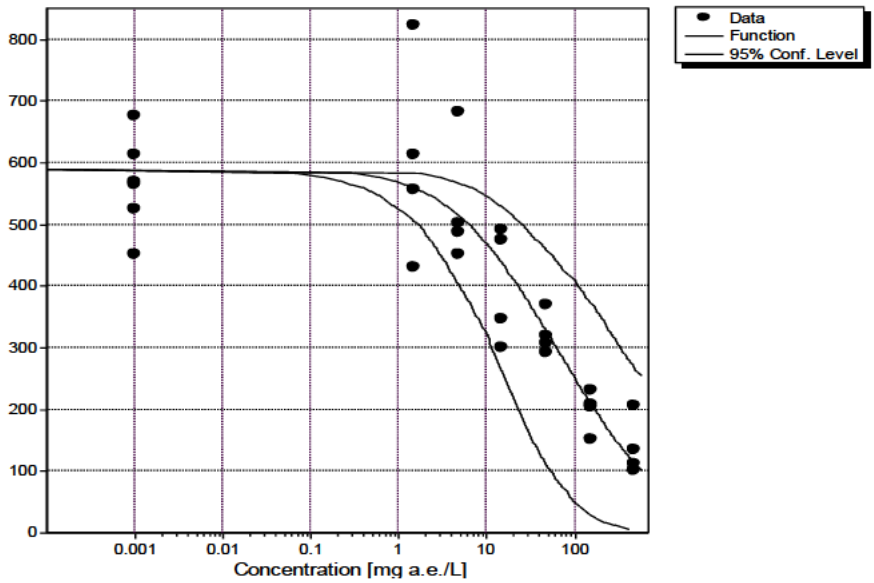

Figure 15: Concentration-effect curve showing the influence of the test item on yield FW of the introduced *Myriophyllum spicatum* as observed after 14 d.

Point estimates from the 3-param. normal CDF

Table 87: Point estimates from the 3-param. normal CDF with yield DW at 14 d: Selected effective concentrations (ECx) of the test item; cl: confidence limit.

Parameter	E_yC_{10}	E_yC_{20}	E_yC_{50}
Nominal concentration [mg Glyphosate a.e./L]	3.493	9.508	64.566
Lower 95%-cl	0.818	2.340	11.764
Upper 95%-cl	14.923	38.550	347.604

cl: confidence limit; n.d.: not determined

The confidence limits of the EC_{10} used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ECx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs).

Results of the 3-param. normal CDF analysis based on geometric mean measured concentrations in mg a.e./L.

Estimated parameters of the 3-param. normal CDF

Table 88: Estimated parameters of the 3-param. normal CDF with yield FW at 14 d:
Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	590.174	32.192	524.120	656.227	18.333	<0.0001
b1	0.477	0.304	-0.146	1.100	1.572	0.0638
b2	0.991	0.189	0.604	1.379	5.249	<0.0001

Stop Reason = Converged (Optimization method: Levenberg-Marquardt).

R^2: 0.840; adjusted R^2: 0.829
Residual standard error: 87.03038
Akaike Criterion (AIC): 304.415
Shapiro Wilk`s test on normal distribution of residuals: p = 0.0299.

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Table 89: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with yield FW at 14 d:
Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	878648.31	2	439324.16	58.002	<0.0001
Residuals	204505.75	27	7574.29		
- Lack of Fit	23176.23	4	5794.06	0.735	0.578
- Pure Error	181329.52	23	7883.89		
Total	1045544.02	29			

Since p(F|Regression) <= 0.05, a significant amount of variance is explained by the regression model..Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit.

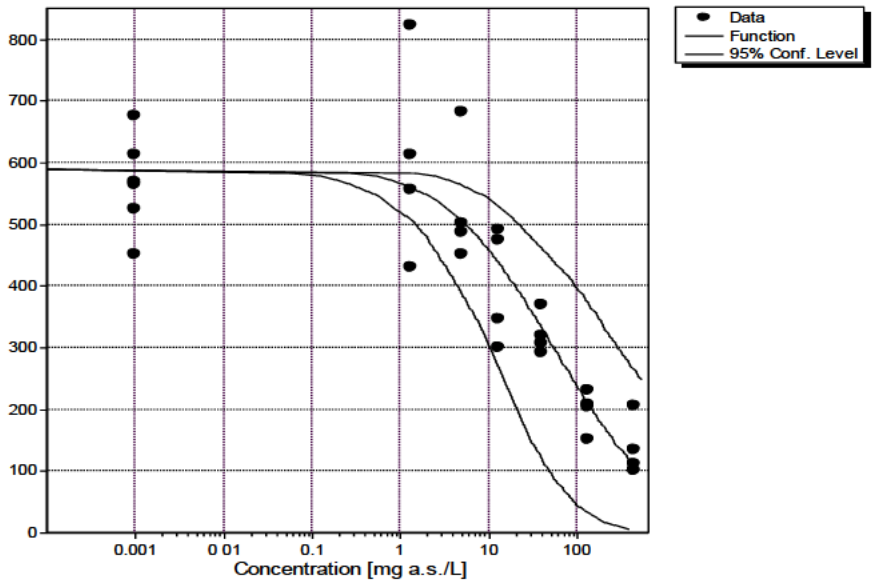

Figure 16: Concentration-effect curve showing the influence of the test item on yield FW of the introduced *Myriophyllum spicatum* as observed after 14 d.

Point estimates from the 3-param. normal CDF

Table 90: Point estimates from the 3-param. normal CDF with yield DW at 14 d: Selected effective concentrations (ECx) of the test item; cl: confidence limit.

Parameter	E_yC_{10}	E_yC_{20}	E_yC_{50}
Nominal concentration [mg Glyphosate a.e./L]	3.002	8.192	55.912
Lower 95%-cl	0.715	2.113	10.540
Upper 95%-cl	12.599	32.928	303.112

cl: confidence limit; n.d.: not determined

The confidence limits of the EC_{10} used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ECx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs).

Threshold concentrations (NOEC) for yield (biomass$_{wet\ weight}$) at 14 d

Statistical characteristics of the sample

Table 91: Statistical characteristics with yield FW at 14 d:
Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Measured Conc. [mg Glyphosate a.e./L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s (X)	95%l	95%u
Control	566.42	566.43	450.76	676.56	6	76.678	13.50	31.304	5.50	485.95	646.88
1.30	605.40	584.44	430.16	822.56	4	163.554	27.00	81.777	13.50	345.15	865.65
4.96	530.75	494.86	452.16	681.13	4	102.415	19.30	51.208	9.60	367.79	693.72
12.7	403.58	410.79	301.03	491.73	4	94.360	23.40	47.180	11.70	253.44	553.73
39.6	321.13	312.66	290.69	368.53	4	33.615	10.50	16.808	5.20	267.65	374.62
132	198.57	205.51	151.73	231.53	4	33.630	16.90	16.815	8.50	145.05	252.08
445	137.87	122.63	100.49	205.73	4	47.334	34.30	23.667	17.20	62.55	213.19

Normality check was passed (p > 0.01). (Shapiro-Wilk's Test on Normal Distribution)

Variance homogeneity check was passed (p > 0.01). (Levene´s Test on Variance Homogeneity (with Residuals).

The analysis of contrasts revealed a linear trend (p <= 0.05), thus the selected Williams test was performed.

Williams Multiple Sequential t-test Procedure

Table 92: Comparison of treatments with "Control" by the t test procedure after Williams with yield FW at 14 d:
Significance was Alpha = 0.0500, one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: μ1 = μ2 = ... = μk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered

Measured Conc. [mg Glyphosate a.e./L]	Mean	s	df	LhM	%MDD	t	t*	Sign.
Control	566.42	88.791						
1.30	605.40	88.791	23	605.40	-17.34	0.68	-1.71	-
4.96	530.75	88.791	23	530.75	-18.06	-0.62	-1.78	-
12.7	403.58	88.791	23	403.58	-18.29	-2.84	-1.81	+
39.6	321.13	88.791	23	321.13	-18.39	-4.28	-1.82	+
132	198.57	88.791	23	198.57	-18.47	-6.42	-1.83	+
445	137.87	88.791	23	137.87	-18.49	-7.48	-1.83	+

+: significant; -: non-significant.

A NOEC of 4.96 mg Glyphosate a.e./L based on geometric mean measured concentrations is suggested by the program.

24.6 Mean growth rate (biomass$_{wet\ weight}$), Mean growth rate FW

Effective concentrations (EC_x) for mean growth rate FW at 14 d

Results of the 3-param. normal CDF analysis based on nominal concentrations in mg a.e./L.

Estimated parameters of the 3-param. normal CDF

Table 93: Estimated parameters of the 3-param. normal CDF with growth rate FW at 14 d:
Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	0.101	0.003	0.094	0.107	30.541	<0.0001
b1	0.810	0.231	0.337	1.284	3.514	0.0008
b2	1.137	0.165	0.798	1.475	6.888	<0.0001

Stop Reason = Converged (Optimization method: Levenberg-Marquardt)

R^2: 0.883; adjusted R^2: 0.875
Residual standard error: 0.00911
Akaike Criterion (AIC): -245.466
Shapiro Wilk`s test on normal distribution of residuals: p = 0.4853.

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Table 94: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with growth rate FW at 14 d:
Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	0.015	2	0.008	91.691	<0.0001
Residuals	0.002	27	0.000		
- Lack of Fit	0.000	4	0.000	0.541	0.707
- Pure Error	0.002	23	0.000		
Total	0.017	29			

Since p(F|Regression) <= 0.05, a significant amount of variance is explained by the regression model. Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit.

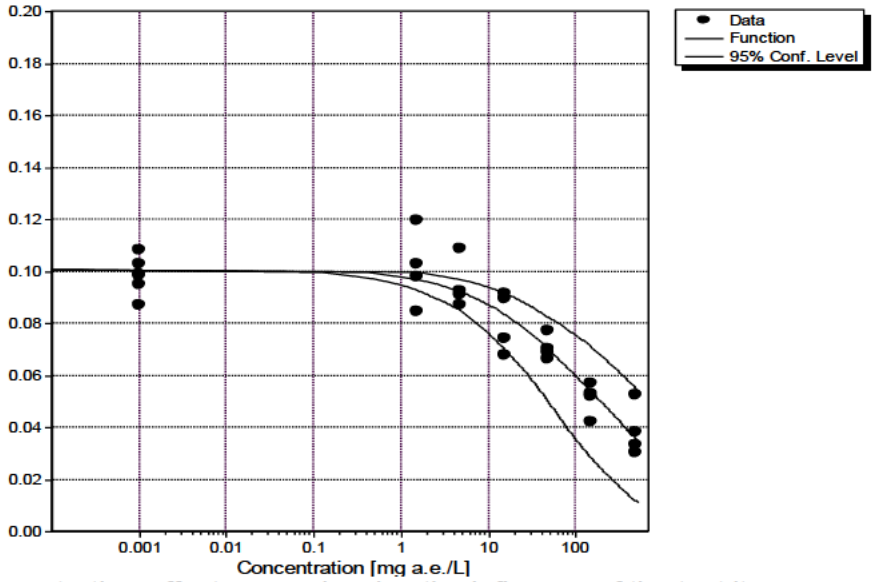

Figure 17: Concentration-effect curve showing the influence of the test item on mean growth rate FW of the introduced *Myriophyllum spicatum* as observed after 14 d.

Point estimates from the 3-param. normal CDF

Table 95: Point estimates from the 3-param. logistic CDF with mean growth rate DW at 14 d: Selected effective concentrations (ECx) of the test item; cl: confidence limit.

Parameter	E_rC_{10}	E_rC_{20}	E_rC_{50}
Nominal concentration [mg Glyphosate a.e./L]	6.463	20.442	185.009
Lower 95%-cl	2.174	6.993	46.477
Upper 95%-cl	19.219	60.192	700.907

cl: confidence limit; n.d.: not determined

The confidence limits of the EC_{10} used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ECx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs).

Results of the 3-param. normal CDF analysis based on geometric mean measured concentrations in mg a.e./L.

Estimated parameters of the 3-param. normal CDF

Table 96: Estimated parameters of the 3-param. normal CDF with growth rate FW at 14 d:
Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std Err	95%LCL	95%UCL	t	p(t)
b0	0.101	0.003	0.094	0.108	30.028	<0.0001
b1	0.733	0.231	0.259	1.207	3.172	0.0019
b2	1.154	0.165	0.815	1.492	6.997	<0.0001

Stop Reason = Converged (Optimization method: Levenberg-Marquardt)

R^2: 0.884; adjusted R^2: 0.875
Residual standard error: 0.00921
Akaike Criterion (AIC): -244.789
Shapiro Wilk`s test on normal distribution of residuals: p = 0.3434.

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Table 97: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with growth rate FW at 14 d:
Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	0.015	2	0.008	89.677	<0.0001
Residuals	0.002	27	0.000		
- Lack of Fit	0.000	4	0.000	0.685	0.610
- Pure Error	0.002	23	0.000		
Total	0.017	29			

Since p(F|Regression) <= 0.05, a significant amount of variance is explained by the regression model. Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit.

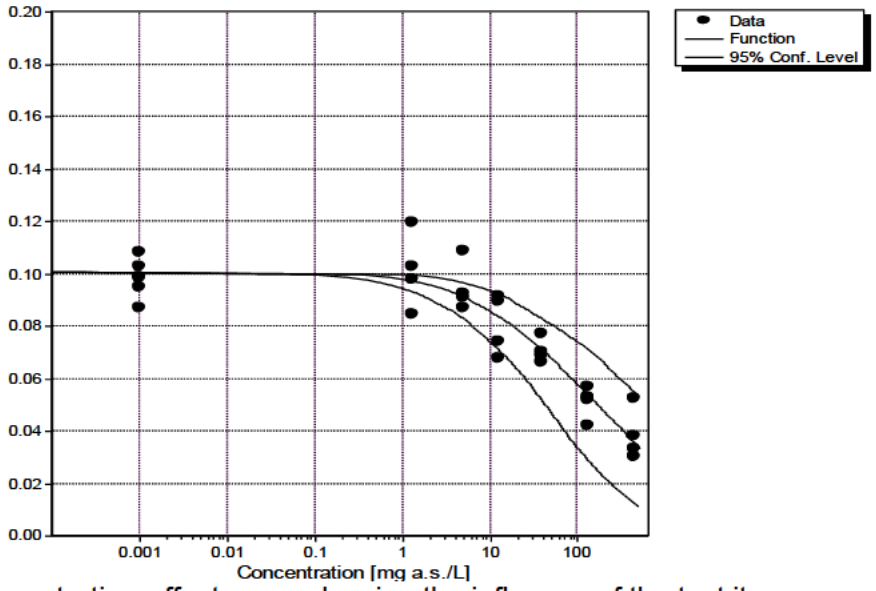

Figure 18: Concentration-effect curve showing the influence of the test item on mean growth rate FW of the introduced *Myriophyllum spicatum* as observed after 14 d.

Point estimates from the 3-param. normal CDF

Table 98: Point estimates from the 3-param. logistic CDF with mean growth rate DW at 14 d: Selected effective concentrations (ECx) of the test item; cl: confidence limit.

Parameter	E_rC_{10}	E_rC_{20}	E_rC_{50}
Nominal concentration [mg Glyphosate a.e./L]	5.409	17.407	162.839
Lower 95%-cl	1.815	5.948	41.234
Upper 95%-cl	16.122	51.351	625.723

cl: confidence limit; n.d.: not determined

The confidence limits of the EC_{10} used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ECx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs).

Threshold concentrations (NOEC) for mean growth rate (biomass$_{wet\ weight}$) at 14 d

Statistical characteristics of the sample

Table 99: Statistical characteristics with growth rate FW at 14 d:
Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Measured Conc. [mg Glyphosate a.e./L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s (X)	95%l	95%u
Control	0.098	0.099	0.087	0.108	6	0.0073	7.50	0.0030	3.00	0.091	0.106
1.30	0.101	0.100	0.084	0.119	4	0.0145	14.30	0.0072	7.20	0.078	0.124
4.96	0.095	0.091	0.087	0.109	4	0.0096	10.20	0.0048	5.10	0.079	0.110
12.7	0.081	0.082	0.068	0.091	4	0.0115	14.30	0.0058	7.10	0.062	0.099
39.6	0.070	0.069	0.066	0.077	4	0.0046	6.50	0.0023	3.30	0.063	0.078
132	0.051	0.052	0.042	0.057	4	0.0064	12.50	0.0032	6.30	0.041	0.061
445	0.038	0.035	0.030	0.052	4	0.0098	25.60	0.0049	12.80	0.023	0.054

Normality check was passed (p > 0.01). (Shapiro-Wilk's Test on Normal Distribution)

Variance homogeneity check was passed (p > 0.01). (Levene´s Test on Variance Homogeneity (with Residuals).

The analysis of contrasts revealed a linear trend (p <= 0.05), thus the selected Williams test was performed.

Williams Multiple Sequential t-test Procedure

Table 100: Comparison of treatments with "Control" by the t test procedure after Williams with growth rate FW at 14 d:
Significance was Alpha = 0.0500, one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: μ1 = μ2 = ... = μk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered

Measured Conc. [mg Glyphosate a.e./L]	Mean	s	df	LhM	%MDD	t	t*	Sign.
Control	0.098	0.0094						
1.30	0.101	0.0094	23	0.101	-10.62	0.46	-1.71	-
4.96	0.095	0.0094	23	0.095	-11.06	-0.6	-1.78	-
12.7	0.081	0.0094	23	0.081	-11.21	-2.9	-1.81	+
39.6	0.070	0.0094	23	0.070	-11.26	-4.56	-1.82	+
132	0.051	0.0094	23	0.051	-11.31	-7.79	-1.83	+
445	0.038	0.0094	23	0.038	-11.32	-9.83	-1.83	+

+: significant; -: non-significant.

A NOEC of 4.96 mg Glyphosate a.e./L based on geometric mean measured concentrations is suggested by the program.

25 Certificate of Analysis of the Test Item

BAYER	**Certificate of Analysis**	**Testing Facility** Bayer CropScience 800 N. Lindberg Blvd St. Louis, MO 63137 314-694-1000
Sample ID:	MON 77973	
Lot Number:	AZM30338TO (Orion Lot: 11493945)	
TOX Number:	TOX 22000-00	
Storage Conditions:	Warehouse (35°-100°F)	
Tests Performed:		
	Content	
Analyte/Test	Method	Result
Glyphosate	ME-1847-02	96.0 wt% (a.e. on dry basis)
NMR Identity	EQ-1818-02	Consistant with Structure
	Physical Properties	
Analyte/Test	Method	Result
Appearance	DIN 10964 [Sensory]	White Powder
pH	CIPAC MT 75.3	2.25
Water	EQ-2204-01	11.05 wt%
Experimental Completion Date:	19-Mar-19	
Expiration Date:	19-Mar-23	
If unknown Expiration Date, enter Reanalysis Date:		
GLP Statement: Data generated in the certification of this material is in compliance with Good Laboratory Practices 40 CFR § 160. Any Exceptions are listed below.		
A retain sample of the test item has been stored in the GLP sample archive.		
Comments:		
Issued By: [redacted] 2021-09-08 [redacted] Study Director		