Glyphosate Renewal Group’s (GRG) Comments on the Scientific Committee on Health, Environmental and Emerging Risks’ (SCHEER) Preliminary Opinion on "Draft Environmental Quality Standards for Priority Substances under the Water Framework Directive" for Glyphosate, adopted on 30 September

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Glyphosate Renewal Group

OWNERSHIP STATEMENT

Glyphosate draft candidate priority substance dossier – Comments regarding developments in EQS setting following SCHEER opinion of 30 September 2022

Comments of the GRG

In the draft dossier for the candidate priority substance glyphosate (GLY), a range of environmental quality standards (EQS) have been proposed, including specifically the acute maximum allowable concentration (MAC) and the chronic annual average (AA) as endpoints for freshwater and marine waterbodies. In addition, a quality standard for surface water (SW) abstracted for drinking water (QSdw,hh) has been suggested by the expert reviewers. The following information should be considered in the discussion of the setting of EQS values:

A. Clarity – As endorsed by SCHEER[1], the definition of a $QS_{dw,hh}$ is specific to surface water abstracted for drinking water. It does not replace ecological endpoints such as the freshwater EQS-AA or EQS-MAC. These may be applied to all surface waterbodies as part of an ecosystem risk assessment. Each EQS/QS is applied in risk assessments in support of different protection goals.

B. Requirement - The introduction of a $QS_{dw,hh}$ is not required for many MS as they abstract little to no surface water as raw water for the production of drinking water. In the EU, the majority of large water supplies are sourced from groundwater, ranging from 12% in IE to 100% in AT, likewise, the majority of small supplies also exploit groundwater[2]. This is in agreement with the assessment that ~75% of EU inhabitants rely on groundwater for drinking water[3] (see appendix 1 for more detail, an extract of [redacted] *et al.*, 2022[4]). Being a MS-specific issue, it should be left to each MS to define a $QS_{dw,hh}$ for drinking water abstraction points if regarded as necessary given the current regulations that allow for such MS level action.

C. Definition – In TGD 27[5] on the setting of EQS values the following is noted: "*A treatment factor should be applied to the drinking water threshold so that the $QS_{dw,hh}$ relates to the 'raw' water (i.e. it is an 'environmental' standard). Drinking water thresholds and treatment processes used to achieve them should be taken into account in determining quality standards for water abstraction resources. This should have regard to Article 7 of the WFD with reference* ***where appropriate*** *to simple treatment"*.

   The setting of a treatment factor to the lowest common denominator of 'simple treatment' at an EU scale appears not be appropriate when considering that most MS manage the quality of their drinking water in conjunction with adequate water treatment so that quality standards are finally met at the tap of the consumer. In effect, this typically involves very high rates of GLY removal

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[1] SCHEER (Scientific Committee on Health, Environmental and Emerging Risks), Preliminary Opinion on Draft Environmental Quality Standards for Priority Substances under the Water Framework Directive - glyphosate, 30 September 2022

[2] European Commission (EC, 2016). Synthesis Report on the Quality of Drinking Water in the Union examining the Member States' reports for the 2011-13 period, foreseen under Article 13(5) of Directive 98/83/EC. COM(2016) 666 final. 16pp plus Country Reports and Small Supply Summaries. COM(2019) 128 Final. 13pp.

[3] European Commission (EC, 2008). Groundwater Protection in Europe. The New Groundwater Directive – Consolidating the Eu Regulatory Framework. 36pp.

[4] [redacted] *et al*., Glyphosate (GLY) and the primary metabolites Amino methyl phosphonic acid (AMPA) and Hydroxy methyl phosphonic acid (HMPA): Public monitoring data assessment and interpretation, Glyphosate Renewal Group, EnSa-22-0033, 2022.

[5] EC (European Commission), 2018. Technical Guidance for Deriving Environmental Quality Standards (TGD). Common Implementation Strategy for the Water Framework Directive. Guidance Document No.27 Updated version 2018.

(>90%) by water treatment trains. The latter are already in place for other purposes (see appendix 2, an extract of [redacted] *et al.*, 2022). Using the lowest or average treatment factor is problematic as the $QS_{dw,hh}$ would be set too low for MS with a higher removal rate already present. For example, a 50% removal rate would result in a $QS_{dw,hh}$ of 0.2 µg/L while 90% removal would allow for 1 µg/L $QS_{dw,hh}$. In that the Directive allows MS to establish lower $QS_{dw,hh}$ on a national basis but not a higher value, it would be more appropriate to set the EU-level $QS_{dw,hh}$ at a value arising from a 99% glyphosate removal rate (i.e., 10 µg/L) with a minimum of 0.125 µg/L (based on a 20% removal rate) and allow each individual MS to determine the value they will use. It is clear that using the worst-case removal value sets an overly precautionary QS for all MS and limits their water management options.

D. Water quality - Article 7.3 of WFD: *"Member States shall ensure the necessary protection for the bodies of water identified with the aim of avoiding deterioration in their quality in order to reduce the level of purification treatment required in the production of drinking water... "*. The potential occurrence of GLY in raw drinking water does not automatically trigger the necessity for additional treatment of surface water abstracted for production of drinking water:

- No evidence has been identified that water companies have to specifically treat raw surface water abstracted for drinking water due to GLY residues, as GLY trace residues are readily removed by existing treatment steps already in place to improve the microbial status of water quality, like bank filtration and chlorination (see appendix 2).

- There are strong indications that there is no longer a necessity to set removal requirements specific for plant protection products. This is concluded from latest examples given in reports from the association of Dutch water companies (RIWA): in October 2021, RIWA published their Rhine catchment 2020 annual report[6]. In Chapter 2, the report describes a 'Removal Requirement Index', comprising the number and quantity of substances that drinking water companies need to remove to meet the Dutch legal obligations for clean and wholesome drinking water. For the monitoring station Lobith (river Rhine at the German/Netherlands border) the index is given for individual substances, including GLY. The conclusion on page 89 is: *"Since 2015, glyphosate, isoproturon, TCA and the sum of the pesticides no longer have a removal requirement for drinking water purification. In 2020, the removal requirement for the substance group plant protection products, biocides and their metabolites was in fact zero."*

- The available data from public monitoring and rates of drinking water compliance (see appendix 3) indicate a very high compliance rate to the EU drinking water trigger at the tap of the consumer[7]. Exceedances of the threshold by GLY residues occurred occasionally at very low concentrations[8] and are well below science-based threshold values for human safety[9].

E. Section 7.1 Acute Aquatic Ecotoxicity, SCHEER question the reliability scores given to acute ecotoxicity data apparently conducted with the active substance. This relates to Tables 10.1.1 and 10.1.4 of the draft JRC EQS dossier. The scoring for these studies from the public literature reflects the quality of the study, which for the most part, were conducted without analytical confirmation and not in accordance with a recognized test guideline. In some cases, the active

[6] https://www.riwa-rijn.org/wp-content/uploads/2021/10/RIWA-2021-EN-Anual-Report-2020-The-Rhine.pdf
[7] > 99.9% for unaggregated and >99.84% for available aggregated datasets
[8] Maximum concentration in all public monitoring datasets is 1.79 µg/L (recorded in FR).
[9] Lifetime health-based ADI (average daily intake) concentration of 1500 µg/L

substance / commercial product tested - column entry, identifies the substance tested as an active substance, whilst in the '% active substance' column entry, purity values that substantially lower than 95%, e.g., 40 and 41% purity are stated, which appear to reflect active substance concentrations in products and not conducted with technical substance, where the purity would be expected to be >90%. Therefore, there are uncertainties relating to the test substances used in the literature papers, which appears to have been considered in the reliability score.

- Concerning the acute endpoints considered relevant for QS setting, the exclusion of the endpoints generated in the Roshon (1997) work is considered appropriate, as there is no analytical confirmation in the aquatic plant studies conducted on glyphosate in Roshon (1997). SCHEER identify that the next most appropriate acute aquatic endpoint to be for *Chlorella vulgaris* in Ma (2002), noting the endpoint (EC50= 4.7 mg/L) was a nominal concentration. There appears also to be no analytical confirmation of the exposure concentration in this work, and exclusion of the value would also be considered appropriate.

- Due to the lack of analytical confirmation of exposure concentrations in both Roshon (1997) and Ma (2002), it is also considered appropriate to exclude endpoints from these studies, from the probabilistic approach to setting of the $MAC\text{-}QS_{fw\text{-}eco}$.

- The next lowest aquatic endpoint for algae from Table 10.1.1 of the JRC EQS dossier, was achieved for the alga *Skeletonema costatum*, in [redacted] (1996) with an EC50 value of 13.5 mg a.e./L achieved. The reliability of this study was evaluated by the RMS for the Annex I renewal. JRC gave this study a reliability score of 1 (valid).

F. In section 7.2 of the SCHEER review (Chronic Aquatic Ecotoxicity) the Roshon (1997) data are discussed as being relevant for the derivation of the $AA\text{-}QS_{fw\text{-}eco}$ using the deterministic approach. However, previously in Section 7, SCHEER state they disagree with the use of the data in Roshon (1997) as there is no analytical confirmation of exposure concentrations in the studies conducted with glyphosate. These data should therefore not be considered in the derivation of $AA.QS_{fw\text{-}eco}$.

G. In Section 4 of the SCHEER review, specific questions are addressed by SCHEER relating to aquatic plants, where uncertainties relating to endpoints achieved in the Roshon (1997) work are discussed. The Roshon (1997) data should be excluded from consideration due to the lack of analytical confirmation of exposure concentraton, as previously discussed. In addition, in the aquatic plant study conducted by [redacted] (2012) using *Myriophyllum aquaticum* exposed to the active substance – this study was suggested as an alternative for endpoint selection. However, SCHEER state, that this study was not cited in the list of references.

- For information - this study was part of the submitted dossier for the re-registration of glyphosate onto Annex I in the EU. In the evaluation by the RMS, the study by [redacted] (2012) conducted using the active substance, was considered to be invalid due to an incorrect plant density in the replicate exposure vessels. The registrant (GRG) therefore conducted a guideline complaint aquatic plant study according to OECD 239, 'Water-sediment *Myriophyllum spicatum* toxicity test', where plants were exposed to the active substance ([redacted] & [redacted] 2022) in a study, where all validity criteria in the test guideline (OECD 239) were satisfied and the study was considered valid, which is also the conclusion drawn by the RMS, in the evaluation of the new *Myriophyllum spicatum* study. The 14-d ErC_{50} value achieved for biomass wet weight was 163 mg a.e./L and for total shoot length, the ErC50 value was 208 mg a.e./L. The endpoints achieved in the study are considered appropriate alternative endpoints for consideration when establishing a QS for surface water.

- A summary of the [redacted] (2022) *Myriophyllum spicatum* study is presented in Appendix 3 of this document.

H. In section 4, specific questions are addressed by SCHEER relating to acute and chronic fish tests. Reference is made to an acute and a chronic study conducted with the zebrafish (*Danio rerio*). It is highlighted by SCHEER that they were unable to identify the acute study conducted with *Danio rerio* based on the citation in the JRC dossier (CLH, 2016).

- This appears to be a citation error, as both the acute and chronic studies were conducted by [redacted] in 2000, and both appear in the dossier submitted to support the re-registration of glyphosate onto Annex I.

- The acute study may be found in the current Annex I re-registration draft RAR, in section Part B9 (AS) Ecotoxicology data, under section point KCA 8.2.1-015, where the current RMS consider the study as 'supportive' due to uncertainties associated with the analytical verification conducted during the 96-hour test. The acute study endpoint from this study does appear in the current list of endpoints in EFSA (2015) Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate (EFSA Journal2015; 13(11):4302).

I. Concerning the chronic fish study using zebrafish (*Danio rerio*), SCHEER also the citation for this study as CLH (2016). This study was conducted by the same study director who performed the acute zebrafish study described above. The chronic study may also be found in the current Annex I re-registration draft RAR in section Part B9 (AS) Ecotoxicology data, under section point KCA 8.2.2.1/002.

- The registrant believes that due to many uncertainties associated with the chronic fish study conduct and reporting, that it is not a valid study and should not be used in risk assessment.

- The uncertainty associated with the validity of the chronic zebrafish study ([redacted] 2000), relates to the test design, which is reported to have a semi-static test design, whilst raw data appended to the final report, supports only a static test design, with only one occasion of stock and test media preparation recorded. Furthermore, there are no analytical method details reported and reported analytical results are for periodic analyses of concentrated stock solutions prepared at test start, and not for test media. These data do not permit confirmation of exposure concentration for the 7-day duration of the study.

J. Within the available regulatory dataset, submitted to support the Annex I re-registration, onto Annex I in the EU, additional chronic fish data / endpoints are available, that includes a fish early life stage (ELS) test by [redacted] (2010) conducted with rainbow trout (*Oncorhynchus mykiss*), a fish full life cycle (FFLC) study by Anonymous (1975) and a fish short term reproduction assay (FSTRA) by [redacted] (2012) - both conducted with fathead minnows (*Pimephales promelas*). Summaries for these studies are presented in the draft RAR, in Part B9

(AS) Ecotoxicology data, under section points KCA 8.2.21-001, KCA 8.2.2.2-001 and KCA 8.2.3-001 respectively, where across all three studies, no effects were observed.

- In the fish ELS study with rainbow trout, no significant effects on hatching success, survival, growth and development were observed following an 85-day exposure period to glyphosate at concentrations upto 9.63 mg a.e/L.

- In the FFLC study with fathead minnow, no significant effects on survival, growth and reproduction were observed following a 255-day exposure period to glyphosate at concentrations upto 25.7 mg/L.

- In the FSTRA, after a 21-day exposure to glyphosate at concentrations upto 33 mg a.e./L, there were no effects on survival, growth and reproduction, nor were there any effects on secondary sex characteristics, gonadsomatic index (GSI), plasma concentrations of vitellogenin (VTG) or gonad histopathology in the exposed fish.

Appendix 1 – Sources of Raw Water used for Drinking Water (from [redacted] *et al*., 2022)

The sources of water abstracted for the production of drinking water are summarised in Table 1 and Table 2. This indicates that the majority of large water supplies are sourced from groundwater, ranging from 12% in IE to 100% in AT. While the data for small water supplies is not as complete (as MS were not obliged to provide these data) it indicates that the majority of small supplies exploit groundwater. This is in agreement with the assessment that ~75% of EU inhabitants rely on groundwater for drinking water (EC, 2008).

Table 1: Summary of the water sources used for drinking water production (2010) in each country (EC, 2016), including surface water (SW), groundwater (GW) and mixed/other sources. Small supplies are (<1000 m^3/day). Other sources include seawater, bank infiltration and artificial groundwater recharge.

Country	Small Water Supply Zone Source			Large Water Supply Zone Source		
	% SW	% GW	% Mixed/ Other‡	% SW	% GW	% Mixed/ Other‡
AT		100			100	
BE		>80		35	65	
BG		>84		63	37	
CY	Mix	Mix	Mix	21	23	56
CZ	Some	Mostly	Few	47	29	24
DE		87		26	74	
DK		100			100	
EE		100		35	65	
EL		95		65	35	
ES	71			70	29	1
FI		>95		45	41	14
FR		>80		29	49	22
HU		>90		8	35	57
IE	Mix	Mix	Mix	88	12	
IT		Mostly		18	80	2
LT		100			93	7
LU	50	50		59	51	
LV		100		22	64	14
MT		100			44	56
NL		100		39	54	7
PL		>96		35	65	
PT	Some	Mostly	Some	39	35	26
RO		>80		67	30	3
SE	NS	NS	NS	24	51	25
SI	>52			31	69	
SK		>85		15	85	
UK	NS	NS	NS	48	19	33
EU	**Some**	**Mostly**	**Few**	**36**	**50**	**14**

Table 2: Summary of freshwater sources, including surface water (SW) and groundwater (GW), exploited for household supply (EUROSTAT, 2021 – Table ENV_WAT_ABS).

Country	**Year to which data relates**	**% SW**	**% GW**
Belgium	2015	36.9	63.1
Bulgaria	2018	46.4	53.6
Czechia	2018	52.2	47.8
Denmark	2018	1.3	98.7
Germany	2016	30.9	69.1
Estonia	2017	46.1	53.9
Ireland	2017	83.3	16.7
Greece	2018	55.5	44.5
Spain	2016	66.5	33.5
France	2017	32.9	67.1
Croatia	2018	8.8	91.2
Italy	2018	15.1	84.9
Cyprus	2018	55.4	44.6
Latvia	2018	30.1	69.9
Lithuania	2018	0.2	99.8
Luxembourg	2016	47.7	52.3
Hungary	2018	40.9	59.1
Malta	2018	0.0	100.0
Netherlands	2018	37.2	62.8
Austria	2016	0.0	100.0
Poland	2018	27.1	72.9
Portugal	2017	64.3	35.7
Romania	2017	56.9	43.1
Slovenia	2018	0.8	99.2
Slovakia	2018	16.0	84.0
Finland	2014	35.0	65.0
Sweden	2015	77.4	22.6
Iceland	No Data	No Data	95.0†
Norway	2018	85.6	14.4
Switzerland	2018	20.9	79.1
United Kingdom	2014	68.1	31.9

† EEA, 2012

Appendix 2 – Removal of Glyphosate and AMPA by Water Treatment Processes ([redacted] *et al.*, 2022)

Summary

For drinking water derived from surface water, there is almost always water treatment processes applied to generate the drinking water. The prevalence across the EU of the chemical treatment processes, can be inferred from a publication (van der Hoek *et al*., 2014[10]). This paper was the result of a survey carried out amongst the members of the European Federation of National Associations of Water and Wastewater Services. This organisation covered 23 EU MS's and 405 million European citizens. The report indicates that the vast majority of raw water sources for drinking water production (88%) are subject to disinfection.

Further, almost all the raw water taken from surface water is subject to disinfection; and where surface water is disinfected, chlorine disinfection is applied to a minimum of 62% of the raw water. Glyphosate and AMPA are known to be transformed by the most common disinfection methods. Transformation products appear to be small molecules, often similar or identical to those found from natural sources.
Other chemical treatment processes are often applied (either for disinfection or for the explicit removal of micro-pollutants), and low chemical processes are also very frequently applied. Monitoring data is usually only available for raw water, before any water treatment processes have been applied, but for contextualising monitoring data, the effects of these processes should be included. Removal rates for glyphosate and AMPA, for various water treatment processes, have been discussed above, and are summarised in Table 3.

Table 3: Summary of removal rates for glyphosate and AMPA following removal processes

Treatment Process	Glyphosate removal (%)	AMPA removal (%)
Bank and dune filtration	20 - >95	25 - >95
Aluminium coagulant and clarification	15 - 40	20 - 85
Iron coagulant and clarification	40 - 70	20 - 85
Activated carbon adsorption	10 - 90	20 - 70
Chlorination	71 - >99	40 - >95
Chlorine dioxide	17 - 93	>99
Ozonation	60 - >99	25 - 95

In addition to disinfection processes, bank filtration can be an effective process for removal of glyphosate and AMPA from water, when sufficient residence time within soil/sediment occurs to allow the normal aerobic/anaerobic soil degradation processes to progress to their full extent (total mineralisation). Generally, drinking water treatment processes are carefully controlled, and the characteristics of a specific source raw water needs to be known – as the water treatment process train needs to be optimised to ensure that quality standards are met at the tap of consumers. Consequently, where glyphosate or AMPA are known to be present in the raw water, the drinking water treatment train can be optimised, where necessary, to ensure removal of these substances below the required threshold values, and therefore, there is a low risk of exceeding the relevant thresholds in drinking water of 0.1 µg/L for glyphosate and 10 µg/L for AMPA, nor for exceeding the life-time health-based ADI concentrations of 1500 µg/L for GLY and 3960 µg/L for AMPA.

[10] Van der Hoek, JP., Bertelkamp, C., Verliefde, ARD. and Singhal, N. 2014. "Drinking water treatment technologies in Europe: state of the art – challenges – research needs" Journal of Water Supply: Research and Technology, 63.2, 124-130

Appendix 3 – Drinking Water Compliance ([redacted] *et al*., 2022)

Headline Results

Across all countries the GLY public monitoring dataset compiled comprised >9 500 samples collected from >3 700 sampling sites (see Table 4). Given the limited size of the dataset and the limited number of countries from which it was sourced, a combined European dataset was not created.

Compliance with the drinking water threshold of 0.1 µg/L was high (99.90%) given few exceedances (~0.10%). All 5 samples in SE that are ≥ 0.1 µg/L come from 5 apparently untreated sources (2 drilled wells, 2 dug wells, 1 unspecified GW source; see Table 5). Only 1 site has more than a single sample to assess if exceedance was systematic and for that dug well a further sample 7 weeks later was <LOD. All exceedances are old (≤2007) and significant strides in groundwater protection have been made in SE since the introduction of the water protection regulations in 2004 such that these exceedances do not reflect the current state of the GW environment in SE. The 2 sites in IE that ≥ 0.1 µg/L were <LOQ 2-5 months later in the next sample. Only 1 of the DE sites had a second sample and was <0.1 µg/L the following month.

Maximum concentrations were 0.61 µg/L in DE, 0.186 µg/L in IE , <LOQ (0.025 µg/L) in SK and 0.17 µg/L in SE. These are well below the life-time ADI based concentration of 1500 µg/L. In addition, GLY exceedances extracted from aggregated data in official reports (see Table 6) range between 0.00% in AT and 0.29% in ES with an average of <0.16% of samples ≥ 0.1 µg/L. Maximum concentrations were up to 1.79 µg/L (recorded in FR). This value is well below the life-time ADI based concentration of 1500 µg/L.

Table 4: Summary of the unaggregated drinking water (DrW) data for glyphosate (GLY) sourced from Germany, Ireland and Sweden

GLY								
Country	**DE**		**IE**		**SE**		**SK**	
Threshold	**DrW: 0.1 µg/L**	**LTHAC: 1500 µg/L**	**DrW: 0.1 µg/L**	**LTHAC: 1500 µg/L**	**DrW: 0.1 µg/L**	**LTHAC: 1500 µg/L**	**DrW: 0.1 µg/L**	**LTHAC: 1500 µg/L**
Number of sites	16	16	1027	1027	2335	2369	337	337
Number of samples	18	18	2215	2215	6917	7135	404	404
Number of samples > threshold	3	0	2	0	5	0	0	0
% of samples > threshold	16.7	0.00	0.09	0.00	0.072	0.0	0.0	0.0
Number of sites > threshold	3	0	2	0	5	0	0	0
% of sites > threshold	18.8	0.0	0.2	0.0	0.21	0.0	0.0	0.0
Number of consecutive samples > threshold	0	0	0	0	0	0	0	0
% of samples that are consecutive samples > threshold	0.0	0.0	0.00	0.0	0.00	0.0	0.0	0.0
Max number of samples > threshold at a single site	1	0	1	0	1	0	0	0
Max number of consecutive samples > threshold at a single site	1	0	1	0	1	0	0	0

NA – No data available
LTHAC - lifetime health-based ADI concentration

Table 5: Summary of all samples at sampling sites where glyphosate (GLY) concentrations are ≥ 0.1 µg/L

Sampling Site ID	Water Type	Date	GLY Concentration µg/L
SE-SLU_0002220	Drinking water from drilled well	28/05/2007	**0.11**
SE-SLU_0002234	Drinking water from drilled well	07/01/2004	**0.1**
SE-SLU_0002293	Drinking water from dug well	27/06/2006	**0.17**
SE-SLU_0002293	Drinking water from dug well	02/08/2006	<LOD
SE-SLU_0002297	Drinking water from dug well	21/11/2001	0.021
SE-SLU_0002372	Drinking water from dug well	02/01/2006	<LOD
SE-SLU_0002380	Drinking water from dug well	17/01/2006	**0.12**
SE-SLU_0002400	Drinking water from dug well	10/01/2006	<LOD
SE-SLU_0002857	Drinking water from ground water unspecified	12/12/2007	**0.1**
SE-SLU_0002984	Treated drinking water from surface water from ground water	06/10/2004	<LOD
SE-SLU_0002984	Treated drinking water from surface water from ground water	19/10/2005	<LOD
SE-SLU_0002984	Treated drinking water from surface water from ground water	12/12/2005	<LOD‡
SE-SLU_0002984	Treated drinking water from surface water from ground water	17/10/2006	<LOD

‡ Average of 4 replicate samples

Table 6: Summary of drinking water (DrW) monitoring data aggregated in reports for glyphosate (GLY) and AMPA

Country	Substance	Number of reports identified	Reports with data relating to threshold						Maximum value (µg/L)
			Number of reports	Date range	Number of samples	Threshold (µg/L)	Samples above threshold	% samples above threshold	
AT	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	2	2	2011 - 2017	2020	0.1	0	0.00	NS
CH	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
CY	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
CZ	AMPA	1	0	2019	856	NS	NS	NS	NS
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
BE	AMPA	1	1	2016	1169	0.1	1	0.09	0.087
	GLY	1	1	2016	1157	0.1	2	0.2	0.051
DE	AMPA	3	3	2011-2016	9525	0.1	5	0.05	NS
	GLY	3	3	2011-2014	4531	0.1	9	0.20	NS
DK	AMPA	1	1	2014-2016	1336	0.1	0	0.00	NS
	GLY	1	1	2014-2016	1337	0.1	1	0.07	NS
EE	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
ES	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	10	9	2008-2018	>5313	0.1	>10	0.22/0.29[†]	0.92
EU Transboundary	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
FI	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	1	0	2011	4	0.1	NS	NS	NS
FR[‡]	AMPA TTP	1	1	2016-2020	79011	0.1	56	0.1	1.40
	AMPA TTP	1	1	2016-2020	79011	0.9	1	<0.1	1.40
	AMPA UDI	1	1	2016-2020	7553	0.1	7	0.1	0.88
	AMPA UDI	1	1	2016-2020	7553	0.9	0	0.0	0.88
	GLY TTP	1	1	2016-2020	79035	0.1	34	<0.1	12
	GLY TTP	1	1	2016-2020	79035	0.9	7	<0.1	12
	GLY UDI	1	1	2016-2020	7851	0.1	7	0.1	1.79

Country	Substance	Number of reports identified	Reports with data relating to threshold						Maximum value (µg/L)
			Number of reports	Date range	Number of samples	Threshold (µg/L)	Samples above threshold	% samples above threshold	
	GLY UDI	1	1	2016-2020	7851	0.9	1	<0.1	1.79
HR	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
IE	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	1	0	NA	NA	NA	NA	NA	NS
IT	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
NL	AMPA	11	0	NA	NA	NA	NA	NA	3.0
	GLY	11	0	NA	NA	NA	NA	NA	0.3
NO	AMPA	1	0	2002	NS	NS	NS	NS	0.012
	GLY	1	0	2002	NS	NS	NS	NS	0.026
PT	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
SE	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
SI	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
SK	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
UK	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	9	0	NA	NA	NA	NA	NA	NS

† - Report data includes sample counts and % values – The first value is the average using count data only while the second is the average of report averages
ND – No data identified; NS – Not specified; NA – Not available e.g. not monitored
‡ - The data comes from an assessment of the SISE-EAU database
TTP – Within the treatment plant
UDI – Within the distribution network

Appendix 4 – Study summary - Glyphosate: A Study on the Toxicity to the Rooted Aquatic

Macrophyte *Myriophyllum spicatum* study ([redacted] & [redacted] 2022)

Data point:	CA 8.2.7/010a
Report author	[redacted] & [redacted]
Report year	2022
Report title	Glyphosate TC: A Study on the Toxicity to the Rooted Aquatic Macrophyte Myriophyllum spicatum
Report No	21P1MW
Guidelines followed in study	OECD TG 239 (2014), "Water-Sediment Myriophyllum spicatum Toxicity Test
Deviations from current test guideline	Deviations from 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. As the pH of the controls showed similar values in comparison with the treatment levels and since the stock solution was adjusted to 7.7 at exposure start, it is likely that the increase of pH was not substance related. Therefore, this deviation is expected to have no influence on the integrity of the study and its biological results as all validity criteria were met. This is in agreement with the guideline, OECD 239
Previous evaluation	New study not previously submitted
GLP/Officially recognised testing facilities	Yes GLP
Acceptability/Reliability	Valid.

Executive Summary

The toxicity of glyphosate acid on growth of Myriophyllum spicatum was evaluated in a 14-day static toxicity test performed at concentrations of 1.58, 5.0, 15.8, 50, 158 and 500 mg test item/L, equivalent to 1.52, 4.80, 15.2, 48.0, 152 and 480 mg a.e./L, with 4 replicates per test concentration. Six negative control replicates (Smart & Barko medium) were prepared in parallel.

Test vessels were 2-L beakers, each containing three individual plants potted in individual pots containing artificial sediment. Plant length, fresh weight, dry weight and root length were determined in all vessels. Plant shoot length was determined on days 0 and 14. In addition, any changes in plant development in comparison to the control were recorded on days 0, 7 and 14. At the end of the test all plants were harvested and the length of shoots was measured and the dry weight was determined after cooling in a desiccator.

Test media were analysed for glyphosate acid content at test start and end of exposure of the sedimentwater system. Glyphosate acid was not detected in the control group. The measured concentrations in the overlying water ranged from 77.6–108% of nominal glyphosate acid concentrations and thus not within 20% of the nominal glyphosate acid concentrations, therefore, the biological endpoints are calculated based on geometric mean values of the measured glyphosate acid concentrations in the overlying water.

Reduced growth at concentrations ≥158 mg test item/L were determined at day 7 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. The study fulfilled all validity criteria and was therefore considered to be valid.

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. The 14-d ErC50 value for biomass wet weight was determined to be 163 mg a.e./L and for total shoot length ErC50 208 mg a.e./L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	
Test item:	Glyphosate TC (MON 77973)
Description:	White powder
Lot/Batch #:	AZM30338TO (Orion Lot: 11493945)
Purity:	96.0 wt % (Glyphosate a.e. on dry basis)
2. Vehicle and/or positive control:	3,5-dichlorophenol as reference item was performed in Nov/Dec 2021
3. Test organism:	
Species:	*Myriophyllum spicatum*
Source:	Sterile plants from Ibacon GmbH, Then cultivated at test facility (ECT Oekotoxikologie GmbH).
Replication:	4 replicates per test item concentration and 6 replicates for the control
Test vessel:	2 L glass beakers covered by watch glasses
4. Environmental conditions:	
Growth medium:	Smart & Barko medium
Artificial sediment:	According to OECD guideline No. 239 (OECD 2014) 4.5% peat 20% kaolin clay 75.5% quartz sand 0.4% CaCO3 The dry sediment mixture was pre-moistened to 40% water content of the sediment dry weight by adding deionised water. For the final wet sediment containing nutrients, an aqueous nutrient medium was added to obtain a moisture of 50% in the final mixture. The moist sediment was prepared two days before the start of the rooting phase. Temperature
Temperature:	19.8 – 21.8°C (manual measurement; n =90); 18.5 – 19.4°C (automatic measurement in test medium; n =1002)
Photoperiod:	16 h light/ 8 h dark
Light intensity:	122 – 154 µE m–2 s –1 (mean: 134.7 µE m–2 s –1)
pH	Values recorded at test start in the overlying water Controls = 7.9 – 10.2 1.58 mg/L = 7.9 – 10.1 5 mg/L = 7.9 – 10.2 15.8 mg/L = 7.7 – 10.0 50.0 mg/L = 7.8 – 10.0 158 mg/L = 7.8 – 9.8 500 mg/L = 7.8 – 9.5
Oxygen saturation:	97 – 105% on day 0 150 – 202% on day 7 182 – 239% on day 14
5. Dates of experimental work:	Oct 25th to Nov 17th 2021

A. STUDY DESIGN AND METHODS

Experimental treatments: The toxicity of glyphosate acid on growth of *Myriophyllum spicatum* was evaluated in a 14 day static toxicity test performed at concentrations of 1.58, 5.0, 15.8, 50, 158 and 500 mg test item/L, equivalent to 1.52, 4.80, 15.2, 48.0, 152 and 480 mg a.e./L, with 4 replicates per test concentration. Six control replicates (without test substance) were tested under the same conditions as the test groups. The plants were planted in plastic plant pots (6-8 cm diameter) into sediment and placed in 2 L glass beakers (test vessels with 11 cm diameter) containing 400 g wet weight sediment and 1.8 L overlying water. The test was conducted under static conditions for 14 days. Three plants were added to each test and control replicate. At the beginning of the exposure period, a stock solution was prepared in Smart & Barko medium. Thereafter the application solutions were prepared by diluting the stock solution with Smart & Barko medium. The test item was spiked into the overlying water.

Observations: Inhibition of growth relative to the control in terms of plant shoot length, and the number and length of side shoots per plant were determined on days 0 and at the end of the exposure period at 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). At the end of the test, all plants were harvested and the length of shoots was measured. The dry weight was determined after cooling in a desiccator. Temperature dissolved oxygen and pH were recorded 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. Total water hardness was measured 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. Light intensity was measured twice at test start.

Analytical procedures: For analytical control measurements of the actual concentration of the glyphosate acid equivalent, samples of overlying water, pore water and sediment of all test concentrations taken on days 0 and 14 were analysed by HPLC-MS/MS. Statistical calculations: 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 (EC50,20,10), 3-parameter normal CDF and Probit analyses were used. The statistical software package ToxRat 3.3.0 Professional (ToxRat Solutions GmbH) was used for these calculations.

II. RESULTS AND DISCUSSION

A. FINDINGS

<u>Analytical data</u>: Analytical measurements of the concentration of the glyphosate acid equivalent (a.e.) in the overlying water, pore water and sediment were performed at test start and after 14 days in the control replicates and in all test concentration replicates, as summarised in the table below.

Table 7: Analytical results as mass balance of overlying water, pore water and sediment.

Nominal [mg a.e./L]	Test start – 0 d				End of test - 14 d			
	Overlying water [%][a]	Pore water [%][b]	Sediment [%][c]	Total [%]	Overlying water [%][a]	Pore water [%][b]	Sediment [%][c]	Total [%]
Control	< LOD[1]	< LOD[1]	< LOD[2]	-	< LOD[1]	< LOD[1]	< LOD[2]	-
1.58	< LOD2	< LOQ[1]	< LOD[2]	92.1	79.7	0.9	< LOD[2]	80.6
4.80	108	0.2	< LOD[2]	108.2	98.5	1.1	8.2	107.8
15.2	89.5	0.1	< LOD[2]	89.6	77.5	0.9	5.6	84.0
48.0	86.5	0.2	< LOD[2]	86.7	78.7	1.1	6.7	86.5
152	87.4	0.2	< LOD[2]	87.6	86.3	1.3	1.7	89.3
480	92.9	0.1	0.2	93.2	92.7	1.3	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 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

The total measured concentrations ranged from 86.7 – 108.2% of nominal at test initiation and ranged from 80.6 – 107.8% of nominal at test termination. The measured concentrations in the overlying water at start of the exposure period ranged from 86.5–108% of nominal glyphosate 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 glyphosate acid concentrations, therefore, the biological endpoints are calculated based geometric mean values of the measured glyphosate a.e. concentrations in the overlying water.

Table 8: Analytical results of overlying water.

Nominal concentration [mg test item/L]	Nominal concentration [mg a.e. /L]*	Geometric mean of measured concentrations in overlying water [mg 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 test item.

The EC50,20.10 and NOEC values after 14 day growth inhibition test are given below based on geometric mean measured concentrations of glyphosate a.e.

Biological data:

Table 9: Inhibition of growth of Myriophyllum spicatum exposed for 14 days compared to control plants

	Glyphosate geometric mean measured concentrations [mg a.e./L]*					
	1.52	**4.80**	**15.2**	**48.0**	**152**	**480**
Inhibition of Yield (total shoot length) (%)	-8.32	2.64	19.65	38.97	70.57	87.93
Inhibition of Growth Rate (total shoot length) (%)	-5.89	-1.20	4.06	12.45	34.21	71.76
Inhibition of Yield (biomass dry weight) (%)	1.53	12.07	20.40	10.16	13.02	13.58
Inhibition of Growth Rate (biomass dry weight) (%)	1.71	8.12	13.77	6.67	8.53	9.50
Inhibition of Yield (Biomass wet weight) (%)	-6.88	6.30	28.75	43.30	64.94	75.66
Inhibition of Growth Rate (Biomass wet weight) (%)	-2.84	3.71	18.00	28.27	48.28	60.93

*Negative values indicate a better growth compared to the control plants

A reference test using 3,5-dichlorophenol as reference item was performed in Nov/Dec 2021. Growth rate (total shoot length) EC50 (0–14 days) was 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 mentioned in OECD guideline 239, the ErC50 (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.

B. OBSERVATIONS

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. During the exposure phase (day 0–14) 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 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. Root growth was observed for all plants in the controls. At the 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. A summary of the endpoints calculated are provided in the table below.

Table 10: 14-day endpoints based on geometric mean measured concentrations.

Parameter	**Endpoint based on geometric mean measured concentrations**, 0–14 d [mg a.e./L]				
	EC_{10}	**EC_{20}**	**EC_{50}**	**NOEC**	**LOEC**
Growth Rate (Total Shoot length)	38.2 (18.4-79.4)	68.3 (33.7-137)	208 (92.3-479)	12.7	39.6
Growth Rate (Biomass dry weight)	n.d.	n.d.	n.d.	≥445	≥445
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
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
Yield (Biomass dry weight)	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

n.d. = could not be determined
*95% lower and upper confidence limits

All validity criteria were fulfilled;

- The mean total shoot length in control plants was 5.2 fold (should be double)
- The mean total shoot fresh weight in control plants was 4.0 fold (should be double)
- The control plants did not show any visual symptoms of chlorosis and were visibly free from contaminations by other organisms.
- The mean coefficient of variation for yield based on shoot fresh weight in the control was 13.5% (should not exceed 35% between replicates)

III. CONCLUSIONS

The toxicity of glyphosate acid on the growth of *Myriophyllum spicatum* was evaluated in a 14 day static sediment-water test system. The test item was spiked into the overlying water according to OECD TG 239 (2014). The biological endpoints were calculated based geometric mean values of the measured glyphosate a.e. concentrations in the overlying water. The resulting 14-d ErC50 value for biomass wet weight was determined to be 163 mg a.e./L and for total shoot length ErC50 208 mg a.e./L. The study is considered valid and reliable for risk assessment purposes.