Key aspects of the information that GRG submitted to address EFSA's request for additional information in the frame of EU glyphosate active ingredient approval renewal, according to Regulation (EC) No 1107/2009

Glyphosate Renewal Group

Regulation (EC) No 1107 /2009, EFSA request for additional information

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Key aspects in the area of Ecotoxicology

- **Re-evaluation of grass residue decline data**
- **Terrestrial amphibian and reptile risk assessment**
- **Reliability of available non-target arthropod study data**
- **Bees and a revised contaminated water risk assessment**
- **Non-target terrestrial plant risk assessment and phyto-toxicity**
- **Relevance of 7-day chronic fish test with *Brachydanio rerio***
- **New ecotoxicology studies available for sediment dwellers, algae and aquatic plants, conducted with technical material, AMPA and the representative formulation.**

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Summary

The following document provides an update to the overview document of the main points relating to the Ecotoxicological risk assessment for glyphosate and the Annex I renewal dossier submission made by the Glyphosate Renewal Group (GRG, the applicant)[1].

The previous version of this document provided the applicant's opinion of the ecotoxicological evaluation made by the RMS (the Assessment Group for Glyphosate (AGG) that consists of the competent authorities of France, Hungary, Netherlands and Sweden) as it appeared in the draft renewal assessment report (dRAR). This included indications of the areas where the applicant agreed with the evaluation of the RMS and pointed towards how the applicant intended to respond to issues raised in the dRAR.

Since the commenting by Member States, registrants and the general public have commented on the dRAR for glyphosate. An additional information request was received from EFSA on 14th March, 2022 in accordance with Article 13 (3) of Commission Implementing Regulation (EU) No 844/2012, and relates to the following areas;

- Information relevant for harmonized classification and labelling in accordance with Regulation (EC) No 1272/2008.

- New study reports from laboratory studies conducted to support the Ecotoxicological risk assessment.

- Position papers to further support and elucidate on the risk assessment presented in the submitted dossier were indicated as either a data gap in the dRAR or on specific request by EFSA from the 14th March additional information request.

- Tier II summaries of public literature papers already submitted and new public literature papers considered as relevant to the evaluation by the RMS were also requested to be submitted in the interests of complete transparency.

- Some study information, e.g., summaries for studies already summarised in the dRAR, have been updated to reflect - as necessary, changes resulting from the RMS' evaluation or comments, for the technical product (CA) and the representative formulation (CP) parts of the ecotoxicology dossier.

    - For example, in the bird study summary presented under Vol 3 (CA), 8.1.1.3/005 'Sub-chronic toxicity and reproduction to birds' study by [redacted]

[1] See the GRG website (www.glyphosate.eu), under "Transparency" – "Scientific Dossier" – "GRG Position on Scientific Topics" – "Effects on Non-Target Species", where the GRG provided brief summaries of different elements of the dRAR, including indications of how the GRG is intending to respond to the topics raised by the RMS, where appropriate. The full position document covering key areas of the ecotoxicological risk assessment can be accessed via this link.

(1978), in the additional information request, EFSA requested information relating to the frequency of diet renewal, parameters subject to statistical analysis and details of how the endpoint based on mg/kg diet were converted into daily dose values. These have now been provided in an updated study summary.

The following contains targeted summaries of information pertinent to the risk assessment and builds upon the transparency document previously uploaded by the applicant onto the Glyphosate transparency website (see footnote on previous page).

Re-evaluation of residue decline data for grasses

The grass residue decline data used to support the mammalian chronic risk assessment, were evaluated by the RMS in the dRAR in Volume 3 (CP), point B.9.2.2.3. Whilst these data were considered supportive of a DT50 shorter than 10 days (default DT50 used in bird and mammal chronic risk assessment), the RMS requested that these data be re-analysed. Therefore, the available decline data were re-analysed and a kinetics re-analysis report (KCP 10.1.2.2_[redacted]_2022_CEA376_GRG.pdf, submitted during the 'stop of the clock' (SOTC) phase of the evaluation.

The kinetic re-analysis of the grass residue data was conducted using the latest evaluation approaches. A position paper on the re-analysed kinetics data (KCP 10.1.2.2_Anonymous_2022_113898-159_GRG.pdf) was also submitted.

In total, grass residue decline data from 21 historical decline studies were re-evaluated. The conclusions from the re-analysis of these data were that despite the historical nature of the data, that includes a mix of GLP and non-GLP grass residue trial data, collectively, the number and range of DT_{50} values support – in a compelling weight of evidence, a DT_{50} value was established considerably below the default value of 10 days in the region of 2.8 days, (with DT_{50} values shorter than 2.8 days also being supported) for the decline of glyphosate on grasses. These data are considered relevant to the risk assessment and can be used to refine the residues per unit dose (RUD) on food items in the diet of birds and mammals used in the risk assessment.

- *Additional Work Proposal*

In support of the existing grass residue decline dataset (considered acceptable for use in risk assessment according to EFSA (2015)) and to acknowledge the historical nature of the available grass residue trial data, as well as to address potential uncertainties associated with extrapolating residue data for grasses to broadleaf plant species (monocotyledonous (grasses) and dicotyledonous (broadleaf) plants), additional plant residue-decline trials have been initiated by the applicant in 2022. Both grasses and broadleaf plants are being applied with the representative formulation at a representative application rate, in multiple (10) residue decline trials being conducted at Northern / Central and Southern European locations (reflecting the European residue zones) at geographically and climatically distinct locations. These new data

will be used in combination with the existing residue decline data for grasses, to support bird and mammal risk assessments.

Reduced application rate considerations in mammalian risk assessment calculations.

The applicant was requested by EFSA to provide further information to support the assumption that 10% of the full field application rate is appropriate in the risk assessment for use of glyphosate containing herbicides on invasive species. In response to the additional information request from EFSA, the applicant submitted a revised risk assessment for applications made to control invasive species (KCP 10.1_anonymous_2022_113898-192_GRG.pdf). The assessment considers the impact of a directed / targeted application made to mid-late growth stage invasive plant species, on species occurring in the understorey of the targeted invasive plant. Assumptions over the 10% reduction in the application rate being applicable to the mammalian risk assessment, are based on a lower risk of 'off-target' movement due to the controlled nature of the application.

Targeted and directed application of pesticides used to control invasive plants, is also a consideration for other types of use, where targeted applications are required, e.g., weed control on railways where applications are normally made a ground directed using a directed application 'stream' as opposed to a droplet spray – which may result in off-target movement. In the presented risk assessment for invasive species, a worst-case scenario is presented whereby, the max application rate is used in the risk calculation and the impact on species beneath the canopy is assessed. An acceptable risk assessment is achieved based on the expected level of spray interception by the canopy of the target plant, supporting a low exposure risk to mammals feeding on plants in the understorey area.

Concerning the effects of removing invasive species from the agricultural and non-agricultural landscape, is considered to be beneficial to biodiversity, as invasive plant species tend to dominate, where they occur, especially in areas with minimal disturbance that permits the longer-term development of significant rhizome reserves, such as those associated with invasive grasses and knotweeds that are rhizome-forming species, e.g., cooch grass and Japanese knotweed, forming dense mono-specific stands and below ground, where they can have extensive lateral rhizomes and long tap roots e.g., knotweeds. Overall, the dense canopy cover and extensive network of adventitious root and rhizomes prevents the establishment and growth of other plant species that includes indigenous species. Chemical removal of the canopy of invasive species, can lead to the recovery and growth of indigenous plant species.

Spray-train application to control unwanted vegetation along railway tracks

An acceptable risk assessment for the application of glyphosate along railway tracks to control unwanted vegetation was concluded in the dRAR. However - EFSA proposes that the specific use on railways, using spray trains is to be subject of an expert meeting during the peer review period.

Common Vole Population modelling

Common vole (*Microtus arvalis*) population modelling has been conducted by the applicant that analyses the potential impact of glyphosate application in orchard and vines (to control unwanted vegetation in the plantation).

These data are now submitted (KCP 10.1.2.2_Anonymous_2022_113898_159_GRG.pdf) in response to the additional information request from EFSA

Deterministic assessments of exposure were performed based on a single yearly application of 2.88 kg/ha and a twice-yearly application of 2.16 kg/ha. A further assessment was performed based on three applications per year, for both application rates using an exposure multiplication factor (EMF) of 3. In addition, two vole dietary scenarios were simulated; the first being where the animals only obtain food from a treated area, and the second where only 50% of the area is treated, which effectively simulates a band application – which is typical of herbicide use in orchards and vineyards, where applications are targeted around the base of trees within the tree rows leaving the area between tree rows untreated.

Effects data from rodent toxicology studies performed with glyphosate, were used to determine the nature of effects on the modelled populations. For all application scenarios, the model simulation ran for 10 years of continuous year on year application events.

The population modelling demonstrates that a significant decline in the population density of common voles in orchards and vineyards would not be expected at the applications proposed for the Annex I renewal. Single application simulations at rates up to 8.64 kg/ha resulted in <10% reductions in population densities that recovered within a year of application. For the twice-yearly application, simulations at rates up to 12.96 kg/ha resulted in <15% reductions in population density, with population density also recovering within a year of application. Therefore, as vole population density recovery occurred within 1 year for all of the modelled exposure scenarios, adverse long-term effects on vole populations would not be expected.

For the single application scenario at 2.88 kg/ha/annum

Simulated vole population densities were not affected by treatment considering all simulated scenarios (single rate with 100% or 50% of total area treated) which means that the daily mean population density for replicate treatment simulations was always within the normal operating

range of the corresponding replicate control simulations throughout the entire period. The largest relative difference in daily mean population density observed between the treatment and control, did not exceed -5%. The time until recovery for all scenarios for the single application scenarios was zero days after the simulated year on year application event ceased. For the three in year applications at both 2.99 and 2.16 kg /ha, (EMF=3) similar results were achieved for all treatments applications made in April – July. For the time-period between January and October, there was a temporary treatment related reduction in population density detected, although this was <10% compared to the corresponding replicate control simulations, there was no apparent effect on the vole population at the commencement of crop treatment in subsequent years. On cessation of a 10-year period of continuous year on year application events considering the 3-fold increased application rates, recovery of population densities was achieved 188 days following the final application when made in January, and 87 days when the final application was made in October.

For the twice-yearly application scenario at 2 x 2.16 kg/ha/annum

Simulated vole population densities were again not affected by treatment considering all simulated scenarios (twice-yearly rate with 100% or 50% of total area treated) with daily mean population density for replicate treatment simulations always being within ca. 10% of the corresponding replicate control simulations throughout the entire period. The largest relative difference in daily mean population density observed between the treatment and control was -10.3%. The time until recovery for all scenarios for the twice-yearly application scenario was zero days after the simulated year on year application event ceased. For the three-fold increase in application rates (EMF=3) treatment related reductions in population density were detected between January and April and between October and January. The largest relative difference in population between the replicate treatment and control simulations was -15%. In common with the single application scenario, all detected effects disappeared within one annual cycle i.e., before subsequent application events in the following year. On cessation of a 10-year period of continuous year on year application events considering the 3-fold increased application rates, recovery of population densities was achieved after 102 days for the January - April scenario and 265 days for the October – January scenario.

For all modelled exposure simulations, reductions in population densities were only detected in the 3-fold application scenarios, where rates approaching an order of magnitude higher than those proposed on the current GAP table.

The modelling reports and the literature supporting the parametrisation of the population model has also been submitted as part of the stop of the clock submission, along with a review of the modelling approach in accordance with the recommendations of the EFSA PPR Panel Opinion on Good Modelling Practice (EFSA PPR Panel, 2014). Detailed study summaries of eight reports that comprise the population modelling study have also been submitted.

Terrestrial Amphibian and Reptile risk assessment considerations

No additional studies have been conducted by the applicant on amphibians, beyond the existing developmental- amphibian metamorphosis assay (AMA), that was conducted and submitted with the original dossier submission, to support a lack of endocrine disruption concerns. In this study larvae of the African clawed toad (*Xenopus laevis*) were exposed to glyphosate in water concentrations up to 90 mg glyphosate a.e. (acid equivalent)/L, with no effects on larval survival and development. The exposure concentration in the study, far exceeds expected surface water concentrations (FOCUS Step 1 predicted exposure concentrations in surface water (PECsw)) following a 1.8 kg a.e./ha application, which in the current dossier achieves an expected PECsw value of 105 µg a.e./L. This value is nearly 850 times lower than the highest exposure concentration in the amphibian metamorphosis study, where no effects were observed. Aquatic stage amphibians are considered covered by the aquatic risk assessment, as amphibian larvae are generally not considered more sensitive than fish.

In the additional information request, the applicant was asked by the RMS to provide a risk assessment for reptiles and terrestrial phase amphibians. A qualitative and semi-quantitative assessment for illustrative purposes was submitted in response to this request, as there is no agreed guidance currently available at the EU level, on how to conduct such an assessment. As poikilotherms, amphibians and reptiles have low energy requirements and low food intake rates (FIR), and the risk assessment for reptiles is considered therefore covered by the assessment for birds, as metabolic rates / FIRs for birds are much higher than reptiles. The reptiles and amphibians, that are both opportunistic feeders, a low FIR means that dietary exposure to glyphosate is not the main exposure route. Therefore, contact exposure as terrestrial stage amphibians and reptiles move through the landscape is the most relevant exposure route. The contact / dermal route of exposure is most relevant for amphibians that have highly permeable skins, which is not the case for reptilian skin, which is poorly penetrable. Therefore, the applicant submitted an assessment where the focus is dermal exposure of terrestrial phase amphibians, which for the reasons described, would be protective of reptiles.

Amphibians are mostly associated with field margins / adjacent wooded areas to application sites and occur in areas where more extensive cover prevails. The assessment considers amphibians exposure in both ‘in-field’ and ‘off-field / off-target’ areas. Surrogate toxicity values for amphibians were determined using product LC_{50} values for fish, and bioconcentration factors, to derive internal doses expected within a fish. Interspecies Correlation Equations were then used to determine an expected LD_{50} toxicity value for a terrestrial phase amphibian. Using allometric equations based on body weight and surface area of a small terrestrial phase amphibian, a worst-case dermal exposure value was then determined. This considered interception of the application spray by plant canopies in the assessment, as small terrestrial phase amphibians are more likely found below the canopies of plants in both in-field and off-field areas. The toxicity value and the level of anticipated exposure were then compared.

Based on direct application (in-field) and exposure via a drift rate (off-field), the worst-case dermal exposure concentrations were at least 10 times lower than the derived LD_{50} value for the small terrestrial phase amphibian and a low exposure risk to terrestrial phase small amphibians was concluded. Although the assessment is qualitative / semi-quantitative, it does reflect a state of the current scientific art approach to a risk assessment for amphibians, that is relevant to the submission, where overall, a low exposure risk to terrestrial phase amphibians, that may also be protective of reptiles may be concluded.

Fish chronic (7 day) exposure study with Brachydanio rerio

Concerning the chronic fish study conducted with *Brachydanio rerio*, (see dRAR Volume 3, CA 8.2.2.1/002). The RMS indicated that the study was considered relevant and reliable for use in risk assessment, and the achieved NOEC of 1 mg/L was used in the aquatic risk assessment. However, the applicant has presented compelling arguments against the use of this study in risk assessment, due to the very unreliable nature of the study, due to many inconsistencies between the report and the raw data which is presented as an appendix in the study report. The inconsistencies relate to both the test design and to the study conduct. A key area of uncertainty relates to exposure confirmation in the test system, as test media were not analysed during the 168-h duration of the test. In addition, there is no analytical details presented in the report or the raw data, such as evidence of an actual analytical method and whether a method was validated appropriately for use.

The report describes the study as having been conducted using a semi-static test design, whilst the raw data supports a static test design, whilst the raw data presents only a single occasion of stock solution and test media preparation. This is a critical point, as the RMS's position that an appropriate level of exposure was maintained for the study duration is based on the assumption that the study was conducted using a semi-static test design, and that the results in the report for the periodical analysis of stock solutions supports exposure in the test media. Based on the stock solution and test media preparation details in the raw data, the study was conducted using a static test design and therefore the exposure concentrations cannot be confirmed as having been maintained for the study duration.

Further areas of uncertainty include the report indicating the chronic fish test having been conducted according to a Brazilian chronic fish testing method issued by the Brazilian regulatory authorities 'IBAMA". The official method used states that fish should be fed during this seven-day test and that fish < 24 hours old should be used. There is no record of the fish having been fed during the exposure part of the study and it is not possible to determine the age of the fish used in the test. The raw data appended to the study report, states only that fish 'larvae' were added. The official IBAMA method also indicates that during the test, the fish should have been offered food on a daily basis using 'freshly hatched brine shrimp larvae'. The report states that the fish were not fed during the test with no evidence of feeding recorded in the raw data. Starvation cannot therefore be excluded as having influenced the observations recorded and the overall outcome of the study.

Concerning observed sub-lethal effects during the study, the additional information request received from EFSA (14th March 2022) included a request for ECx values based on the extent of lethargy observed in the chronic fish study. A position paper (KCA 8.2.2.1_anonymous_2022_113898-162_GRG.pdf.) has been submitted as part of the response by the applicant, stating that ECx values for lethargy cannot be determined for the chronic fish study conducted with *Brachydanio rerio* as the study is considered unreliable. This conclusion is further supported by the lack of information in the final report and in the raw data appended to the final report. Lethargic behaviour is scored qualitatively based on the presence or absence of lethargic fish in each of the treatment groups. The actual number of fish exhibiting lethargic behaviour in each of the three replicate test vessels maintained for each treatment group was not scored. It is therefore not possible to provide ECx values based on extent of lethargy in each of the tested concentrations.

Fish chronic (85 day) fish early life stage study with Oncorhynchus mykiss

EFSA requested the applicant to submit EC_{10} calculations for the fish early life stage test conducted with trout. The applicant has submitted a statistical re-evaluation (See document KCA 8.2.2.1_[redacted]2022_113898-009_GRG.pdf.) of the study and a position paper (See document KCA 8.2.2.1_[redacted] 2022_113898-009_GRG.sum.docx.) on the outcome of the analysis that confirms the relevance of the existing NOEC of 9.63 mg a.e./L for use in the risk assessment. The re-analysis of the data confirmed that an EC10 value generated based on the available data would be unreliable. The reasons for this have been added to an updated study summary and also submitted (see document; KCA 8.2.2.1-001_[redacted]_2010-1005.029-GTF-sum.docx).

Since the above narrative on the fish early life stage test was prepared, EFSA have evaluated the trout fish early life stage test and conclude in the commenting tables that the variability observed in the parameters measured during the ELS study, were **not** sufficient to exclude the use of the trout early life stage test from the chronic fish risk assessment. The applicant has submitted a further statement concerning the achieved NOAEC concentration of 9.63 mg a.e./L in the trout ELS, where a further analysis of the variability observed in the study was conducted, and that where, the applicant concludes the study as valid, relevant and reliable for use in the chronic fish risk assessment. It is still the contention of the applicant that the trout early life stage test is the most relevant chronic fish endpoint for use in the chronic fish risk assessment.

Submitted Ecotoxicology Studies

Additional aquatic ecotoxicological studies have been conducted and are now submitted as part of the additional information request response received from EFSA on 14th March, 2022. The studies were either in the process of being conducted at the time of dossier submission or have been conducted in response to questions raised in the dRAR by the RMS during commenting.

Additional studies have been conducted with sediment dwellers, algae and aquatic plants, conducted with either the technical material, AMPA (major metabolite of glyphosate) or with the representative formulation. These studies were conducted in response to questions raised by the RMS during commenting and to ensure that studies satisfied the most recent test guideline validity criteria.

The study reports are available on request through the applicant website (www.glyphosate.eu). Tier II study summaries for each study were also prepared and submitted as part of the additional information request response.

Overall - the endpoints achieved in these additional ecotoxicity studies conducted with aquatic taxa exposed to either the technical material, AMPA or to the representative formulation, further support the low exposure risk to aquatic organisms following application of glyphosate made according to the proposed uses table.

New Tier II summaries or updates to the existing Tier II summaries in the dRAR were prepared for all new studies conducted and the corresponding risk assessments in CP B.9.4 Risk Assessment for aquatic organisms, has also been updated.

Studies with Chironomus riparius - the sediment dwelling organism

Relevant dossier Section	Species & study type	Test substance	Endpoint type	Endpoint
KCA 8.2.5.3-002	*Chironomus riparius* sediment dweller - **spiked water** (OECD 219)	AMPA	Emergence ratio and developmental rate NOEC and LOEC values:	NOEC = 512 mg AMPA/L LOEC >512 mg AMPA/L
Note:	The achieved endpoints (nominal measured concentrations as water concentrations were > 80 and <120% of nominal). Relative to the highest predicted surface water concentration (PECsw) in the aquatic risk assessment for AMPA, presented in the dRAR (Table B.9.3.3-9 = 111.02 µg/L – FOCUS Step 1), the NOEC value achieved in the *Chironomus riparius* spiked water study (1000 mg test item /L) is nearly 4600 times higher, supporting a very low exposure risk to sediment dwelling organisms for exposure to AMPA.			
KCA 8.2.5.4-002	*Chironomus riparius* sediment dweller - **spiked water** (OECD 219)	AMPA	Emergence ratio and developmental rate NOEC and LOEC values:	NOEC ≥349.2 mg AMPA /kg dry sediment (sediment only) NOEC >786.5 mg AMPA /kg dry sediment (whole system)

KCA 8.2.5.3-001	*Chironomus riparius* sediment dweller - **spiked water** (OECD 219)	Glyphosate technical	Emergence ratio and developmental rate NOEC and LOEC values:	NOEC =1000 mg/kg dry sed LOEC >1000 mg/kg dry sed
Note:	Measured concentrations in water were >80 and <120% of nominal. Relative to the highest predicted surface water concentration (PECsw) in the aquatic risk assessment in the dRAR (Table B.9.3.3-9 = 167.2 µg/L – FOCUS Step 1), the NOEC value achieved in the *Chironomus riparius* spiked water study (1000 mg test item /L) is nearly 6000 times higher, supporting a low exposure risk conclusion for sediment dwelling organisms.			
KCA 8.2.5.4-001	*Chironomus riparius* sediment dweller - **spiked sediment** (OECD 218)	Glyphosate technical	Emergence ratio; NOEC and LOEC:	NOEC =740 mg / kg dry sed LOEC >740 mg / kg dry sed
			Developmental rate NOEC and LOEC	NOEC = 154 mg / kg dry sed LOEC >303 mg / kg dry sed
Note:	Endpoints based on geometric mean measured values The lowest NOEC value achieved in the *Chironomus riparius* spiked sediment study (154 mg test item /kg dry sediment, is equivalent to a field application rate of > 100 kg /ha, which is more than 35 times higher than the highest application rate proposed on the current use table, further supporting a low exposure risk conclusion for sediment dwelling organisms.			

Sediment fraction M3.3 (known as 'Unknown 1') risk to sediment dwelling organisms.

An updated risk assessment has been submitted in an additional document (KCP 10 Anonymous_2022_113898-238_GRG), to support a low exposure risk to aquatic organisms from exposure to an unknown sediment fraction M3.3 (known as 'Unknown1').

No ecotoxicological endpoints are available for 'Unknown1', therefore, in the risk assessment presented in the above document the toxicity of this sediment fraction is assumed to be 10 times more toxic than AMPA as a worst case.

The updated aquatic risk assessment demonstrates an extremely low risk to aquatic organisms from exposure to 'Unknown 1', where the highest PEC/RAC ratio achieved was 0.099, achieved based on modelled surface water and sediment concentrations achieved at FOCUS Step 1, across all uses on the GAP table. This confirms the low exposure risk to sediment dwelling organisms from this sediment fraction.

Studies with the rooted aquatic macrophyte Myriophyllum spicatum

Relevant dossier Section	Species & study type	Test substance	Endpoint type	Endpoint
KCA 8.2.7	*Myriophyllum spicatum* – Rooted aquatic macrophyte study (OECD 238)	Glyphosate technical	14 day Inhibition of growth rate; ErC50:	ErC50 (biomass wet/wt) = 163 mg a.e./L ErC50 (shoot length) = 208 mg a.e./L
Note:	Endpoints based on mean measured concentrations.			

In addition to the new technical material study conducted with *M. spicatum*, the RMS requested information to address the risk to macrophytes via overspray with the active substance and the representative formulation. A statement was prepared by the applicant that informs on the lack of risk assessment guidance for such an assessment. An assessment based on overspray of the active substance does not reflect the application situation in the field, where application over water is not a proposed use for the Annex I renewal.

Studies with the floating aquatic macrophyte Lemna gibba

Relevant dossier Section	Species & study type	Test substance	Endpoint type	Endpoint
KCA 8.2.7	*Lemna gibba* – floating aquatic macrophyte study (OECD 221)	Glyphosate technical	7 day Inhibition of growth rate; E_rC_{50}:	ErC50 (biomass wet/wt) = 28.7 mg a.e./L
Note:	Endpoints based on mean measured concentrations.			

Studies with algae – multiple species

Relevant dossier Section	Species & study type	Test substance	Endpoint type	Endpoint
KCA 8.2.6.2	*Rhaphidocelis subcapitata* (OECD 201) freshwater alga toxicity study	AMPA	72-hour inhibition of yield and growth rate (E_yC_{50} & E_rC_{50})	72-h E_yC_{50} = 28.7 mg AMPA/L 72-h E_rC_{50} = 31.9 mg AMPA/L
Note:	Endpoints based on mean measured AMPA concentrations.			
KCA 8.2.6.2	*Skeletonema costatum* (OECD 201) marine alga toxicity study	AMPA	72-hour inhibition of yield and growth rate (E_yC_{50} & E_rC_{50})	No inhibition in yield or growth rate. E_yC_{50} & E_rC_{50} both considered to be > 85.9 mg AMPA/L

Note:	85.9 mg AMPA/L was the highest measured concentration of AMPA measured during the 72-hour test.			
KCA 10.2.1/007	**Skeletonema costatum* (OECD 201) marine alga toxicity study	MON 52276	72-hour inhibition of yield and growth rate (E_yC_{50} & E_rC_{50})	72-h E_yC_{50} = 9.2 mg a.e./l 72-h E_rC_{50} = 21.4 mg a.e./L
Note:	Endpoints expressed in terms of product = 25 & 58 mg product/L			
KCP 10.2.1/008	*Rhaphidocelis subcapitata* (OECD 201) freshwater alga toxicity study	MON 52276	72-hour inhibition of yield and growth rate (E_yC_{50} & E_rC_{50})	72-h E_yC_{50} = 37.7 mg a.e./L 72-h E_rC_{50} = 103.9 mg a.e./L
Note:	Endpoints expressed in terms of product = 102.4 & 282.2 mg product/L			
*Final report available on request. Final Report was only available after GRG had submitted the additional information to EFSA, which included an interim study findings summary.				

Ecotoxicology Position papers

In addition to the formal responses to the additional information requests from EFSA, the applicant has prepared several ecotoxicology position papers, which include further information to support existing risk assessments and positions presented by the applicant in the submitted dossier. This includes for example, recalculation of endpoint and revised exposure modelling values. The following are summaries of some of the key position papers submitted by the applicant to EFSA.

Aquatic endpoint recalculations

In addition to the further position papers supporting existing applicant positions on the outcome of many aquatic studies and their relevance for use in the aquatic risk assessment, the additional information request by EFSA (March 2022) included a number of requests for statistical re-evaluations of existing aquatic study data, to ensure expectations of current data requirements were met. For example, to include timepoint specific ECx values (e.g., 72 and 96 hr EC_{10}, EC_{20} and EC_{50} values in algal studies) and where geometric mean measured concentrations are considered necessary.

In all cases where statistical re-calculation of endpoints has been conducted, the outcome of the risk assessment for those taxa are unchanged relative to the conclusions drawn in the dRAR by the RMS. Statistical re-calculations that resulted in minor changes to endpoints are reflected in updated study summaries, which have also been submitted as part of the additional information request.

The applicant's glyphosate aquatic dataset is quite large and reflects work conducted by multiple registrants over many years. In some cases, the additional information requested by

EFSA are not available due to the expiry of archiving periods and raw data being disposed of in-line with agreed protocols. There are very few cases of this occurring, and endpoints achieved in these studies have no impact on the aquatic risk assessment.

Bees –Contaminated water risk assessment using revised PEC_{sw} and PEC_{puddle} values

The applicant was requested to provide a further risk assessment information for 'puddle water' (PEC_{puddle}) relating to the exposure assessment to bees, based on the concentration of the runoff water according to the EFSA's 2013 bee guidance document and not based on FOCUS PEC_{sw} from the runoff scenarios. In addition, the applicant was requested to also update risk assessment for surface water consumption by bees, if the related PEC_{sw} values changed – based on the request to update FOCUS modelling, that was also part of the additional information request from EFSA.

Consequently, a further statement was prepared and submitted as part of the Ecotoxicology additional information request (KCP 10.3.1_anonymous_2022_113898-199_GRG.pdf), using revised PEC_{sw} and PEC_{puddle} values, with corresponding data files submitted as part of the Environmental Fate additional information request (KCP 9.2.5_anonymous_2022_113898-034, 113898-035 and 113898-036).

The ecotoxicology statement (KCP 10.3.1_anonymous_2022_113898-199_GRG) contains two sets of bee contaminated water – risk assessment tables. The first are based on the worst-case environmental fate input parameters (see KCP 9.2.5_anonymous_113898-034_GRG) and the second are the environmental fate input parameters based on the new kinetic evaluations (see KCP 9.2.5_anonymous_113898-035_GRG) as requested by EFSA in the additional information request.

The revised PEC_{sw} and PEC_{puddle} values used in the revised contaminated water bee risk assessment are;

1. PEC_{sw} – worst case value at Step 3 = 69.948 µg a.e./L - based on new kinetic evaluations / environmental fate parameters

2. PEC_{puddle} – worst case value = 48.031 µg a.e./L - based on new kinetic evaluations / environmental fate parameters

3. PEC_{sw} – worst case value at Step 3 = 63.272 µg a.e./L - based on worst case environmental fate input parameters

4. PEC_{puddle} – worst case value = 37.139 µg a.e./L - based on worst case environmental fate input parameters

The new / revised PEC_{SW} and PEC_{puddle} values are lower than the values originally submitted, and a low exposure risk to bees from contaminated water remains the conclusion, which aligns

with the conclusion by the RMS in the dRAR. The statement prepared by the applicant does include a complete contaminated water bee risk assessment based on the new PEC values, which confirms a low risk to bees from consumption of contaminated water.

Bees and the metabolite AMPA

A further statement was prepared and submitted by the applicant on the relevance of the metabolite AMPA for bees (KCP 10.3.1_anonymous_2022_113898-205_GRG). This statement confirms that based on recent rotational crop residue data also submitted in response to the additional information request from EFSA), that assuming residues of the metabolite AMPA measured in wheat grain material (despite non-relevance to bees) as a surrogate for worst-case residue concentrations in pollen and nectar in succeeding crops, the ETRs achieved for the metabolite exposure to forager and larval honey bees and to adult bumble bees, considering both acute and chronic exposure (honeybee) and acute exposure (bumble bee) never exceed the EFSA thresholds indicating an acceptable risk to bees from the metabolites.

As part of the applicant's response to the additional information request from EFSA, an interim report for a limited field rotational crop study was submitted, where bare soil was applied with glyphosate and AMPA at nominal rates of 3.18 kg glyphosate/ha and 2.86 kg AMPA/ha, selected to cover the possible plateau levels of glyphosate and AMPA residues in soil after repeated use at the maximum yearly rate for many years. Crops were sown in the applied soils after nominal plant-back intervals ranging from 27 to 332 days post application. A full and complete understanding of the residues detected in the edible parts of the sown crops is currently ongoing. In most cases, the levels of glyphosate and AMPA detected are <0.05 mg/kg. However, in some cases, levels of AMPA exceed 0.05 mg/kg and are higher than glyphosate, which is not consistent with available monitoring data. Based on the EU pesticide residue monitoring data for 2011-2017 (downloadable from the Zenodo website, one file per Member State), the detection rate for AMPA in the edible parts of crops is extremely low, with AMPA detected in only 79 out of nearly 36,000 samples, with only 14 samples exceeding a detection level for AMPA of 0.05 mg/kg, and in all cases, when AMPA was detected, the glyphosate levels were higher. Nevertheless, multiple additional field rotational crop trials have been initiated in accordance with the Guidance Document on Residues in Rotational Crops (OECD (2018) Series on Pesticides No. 97 and Series on Testing and Assessment No. 279) using a number of additional crop types and these trials are currently ongoing.

Terrestrial arthropods – endpoint recalculations and study reliability assessment

The applicant was requested to submit an estimate of the LR_{50} value from an extended laboratory study conducted with *Typhlodromus pyri* (CP 10.3.2.2/003) exposed to MON 52276. The applicant prepared and submitted a statement (KCP 10.3.2.2_anonymous_2022_113898-198_GRG.pdf) at the stop-of-the-clock phase of the evaluation in response, which presents an estimated LR50 endpoint of 4.44 L/ha. This value was calculated by linear interpolation between the 3 and 6 L/ha rates used in the extended study. The estimate was calculated using the 'FORECAST' function in Microsoft Excel for Microsoft 360 MSO (16.0).

In addition, for an extended laboratory assay performed using *Aleochara bilineata* (CP 10.3.2.2/007), a further statement was prepared (KCP 10.3.2.2_anonymous_2022_113898-201_GRG.pdf) and submitted by the applicant on the reliability of the study given that no assessment of reproduction could be made in the reference group due to high mortality and that a sufficiently high effect on reproduction could not be shown due to the 100% mortality in the reference item treatment. The strong effect on survival in the reference item treatment demonstrates that the individuals were clearly exposed to the chemical and fully sensitive to the type of application and in the substrate used in this study.

In the *Aleochara sp.* chronic test guideline (Grimm, 2000) a test is considered valid if the average number of beetles emerging from fly pupae (reproductive capacity) in the control group, is above 400. In this study, the reproductive capacity of the control group, showed a robust performance with a mean number of F_1 progeny (beetles emerging from fly pupae) per arena of 862.5, and a standard deviation of 66.8 leading to a CV = 7.7%, which substantially exceeds the test guideline validity criteria for control performance.

In addition, the test guideline also states with respect to the validity criteria relating to reproductive capacity in the reference item treatment, that a minimum of 50% reduction in reproductive capacity relative to the control should be achieved. With 100% reduction in reproductive capacity relative to the control having been achieved in the reference item group, and as an upper limit of the % reduction in reproductive capacity in the reference group, is not stated in the guideline, both criteria are considered to have been satisfied, and the test is considered valid and therefore relevant for use in risk assessment.

Non-target terrestrial plants – endpoints based on phytotoxicity

In the additional information request received from EFSA (14th March, 2022) the applicant was requested to derive ER_{50} values based on the phyto-toxicity observations in a vegetative vigour – non target terrestrial plant study (CA 8.6.2/001) and to amend the corresponding Tier II summary in the dRAR. A statement was prepared by the applicant in response to this request (KCA 8.6.2_anonymous_2022-113898-196.GRG) and submitted in response.

The statement describes in detail that deriving ER_{50} values based on qualitative empirical data, such as the '0-5' point phyto-toxicity scoring system used in the report is not appropriate. The scoring for each treatment group is subjective and actual quantitative measure of phyto toxic effects were not determined in a quantifiable and statistically robust way. Additionally, as phyto-toxicity scores are not continuous data, they are under no circumstance appropriate for estimating ER_{50} values using standard statistical approaches. The statement also highlights that there is no guidance available to quantitatively conduct phytotoxicity assessments for use in regulatory risk assessment. The statement also confirms the most relevant study for use in the non-target terrestrial plant risk assessment is the valid vegetative vigour study (CA 8.6.2/005) conducted in 2021 with the representative formulation due to the older vegetative vigour study

being considered invalid due to the illumination under which plants in the study were maintained being lower than required according to the test guideline.

The submitted non-target terrestrial plant risk assessment was conducted according to standard approaches, using both deterministic and probabilistic risk assessments. A multiple application factor (MAF) of one was used in accordance with the outcome of the Pesticide Peer Review panel meeting No. 133 (Sept. 2015). Based on the lowest endpoint achieved in the 2021 vegetative vigour study, an acceptable deterministic risk assessment is achieved for all uses when mitigation measures are included (in-field buffer in combination with spray drift reduction measures). Based on the valid endpoints for 10 plant species achieved in the 2021 vegetative vigor study, it is appropriate to address the variability associated with extrapolation of laboratory endpoints to the field, by performing a probabilistic risk assessment and to derive an HR_5 value using a species sensitivity distribution (SSD) curve, constructed from the available endpoints. In this case, the probabilistic risk assessment demonstrates that an acceptable risk to plants in off-target areas may be achieved without mitigation measures. Of note is that the HR_5 value (78.3 g a.e./ha [CI 52.3-98.9]) achieved is slightly higher than the lowest ER_{50} value in the study (ER_{50} = 69.87 g a.e./ha, tomato shoot fresh weight), which indicates that very sensitive species were tested which increases the protectiveness of the derived endpoints.

Biodiversity - information relating to non-target terrestrial organisms (flora & fauna)

The applicant was requested to provide a revised biodiversity assessment, considering indirect effects, and addressing the concerns of EFSA and the Member States. Specifically, EFSA requested the following;

- The applicant should provide a revised biodiversity assessment, considering indirect effects, addressing the concerns of EFSA and the Member States. In addressing this data requirement, the applicant should consider the following:

- The data collection should be done in a systematic manner and the information structured appropriately. This should be done considering the proceeding bullet points.

- The direct effects on the target weeds (including the impact on the seed bank), non-target plants, non-target arthropods and bees should be quantified. Note that such quantification of direct effects is not addressed simply by the outcome of the standard risk assessment. A conclusion of low risk does not necessarily translate into ‘no effect’. Such quantification should consider the magnitude and duration of the impact in a spatial and temporal context. The quantification of the direct effects should then inform the extent of potential indirect effects via trophic interactions.

- The assessment of biodiversity should be done to address all representative uses. Nevertheless, it may be more practical to focus the biodiversity assessment for a few representative scenarios (defined considering the GAP).

- If proposed, specific mitigation should be linked to the representative uses. It is also suggested that the applicant demonstrates how both specific and general mitigation addresses the identified risks. Please note that only mitigation proposed by the applicant or RMS can be considered as part of the assessment. See reporting table ecotoxicology (other) 5(435).

Therefore, the applicant has prepared a revised biodiversity assessment (KCP 10_anonymous_2022_113898-227.GRG) and a revised GAP table (Anonymous_2022_113898-166_GAP table_GRG.pdf) that includes a provision for biodiversity.

The goals of the revised biodiversity report were:
- To Provide background and context on aspects of crop production and biodiversity related to glyphosate.

- Provide a revised biodiversity assessment for glyphosate that principally informs on potential indirect effects through trophic interactions in aquatic and terrestrial environments.

Included in the revised report are the five dimensions relating to the development of specific protection goals e.g., level of biological organisation, endpoint, duration, magnitude and scale of effect, where both temporal and spatial elements are considered for each individual taxonomic group.

- Provide concrete standard and non-standard risk mitigations for representative uses that are protective of biodiversity from direct and indirect effects are also presented in the report.

- Different mitigation types are presented for each of the proposed uses.

- A statement requiring risk managers in individual Member States to compensate for indirect effects to biodiversity from the use of glyphosate has been added to the GAP table, with specific considerations added for each of the proposed uses.

- A worked example of a biodiversity assessment quantifying effects on arthropods other than bees, pollinators (that are dependent on targeted vegetation) and implications to the in-field weed seedbank, and the cascade effect on bird populations in-field is also presented in the revised report (Chapter 4).

Other pertinent information requested as part of the Additional Information Requests

The additional information request included further information requests relating to references used in the biodiversity assessment, and to references used in the endocrine risk assessment for which further references and updates to the endocrine risk assessments tables were prepared and submitted in response to the EFSA request.

References

1. KCP 10.1.2.2 [redacted] 2022_CEA2376 _GRG.pdf - response to Request ECOTOXICOLOGY No. 9, KCP 10.1.2.2 [redacted] 2022 Glyphosate: foliar residue kinetics for use in bird and mammal risk assessment.

2. KCP 10.1.2.2_Anonymous_2022_113898-159_GRG.pdf – response to Request ECOTOXICOLOGY No. 9, CP 10.1.2.2 residue decline data and population modelling that relates to the Common vole population modelling for applications made in orchard plantations.

3. KCP 10.1_anonymous_2022_113898-192_GRG.pdf - response to Request ECOTOXICOLOGY 10, CP 10.1 Effects on birds and other terrestrial vertebrates invasive species, where a refined risk assessment for mammals for applications made to control invasive species

4. KCA 8.2.2.1_anonymous_2022_113898-162_GRG.pdf - response to Request ECOTOXICOLOGY No. 34. Evaluation of chronic Fish Study, where the request for ECx values for the study to provide information on the extent of lethargy at each of the tested concentrations are discussed.

5. KCA 8.2.2.1_[redacted] 2022_113898-009_GRG.pdf – response to request ECOTOXICOLOGY No. 11. A Statistical analysis (non-GLP) of the study 1005.029.321 on the early life stage toxicity of glyphosate acid to rainbow trout.

6. KCA 8.2.2.1-001_[redacted]_2010_1005.029_GTF_sum.docx - response to Request ECOTOXICOLOGY No. 11 – A revised study summary on the fish early life stage test considering the statistical re-analysis of the presented in document identified as 113898-009.

7. KCA 8.2.5.3/001 [redacted] 2022 MON 77973 A study on the toxicity to the sediment dweller *Chironomus riparius* using spiked water. Report No. 20FV2ME

8. KCA 8.2.5.3/002 [redacted] 2022 Sediment-water chironomid toxicity test using spiked water (OECD 219) Effects of aminomethylphosphonic acid (AMPA) on the development of *Chironomus riparius*. Report No. BAY-051/4-23/R/a

9. KCA 8.2.5.4-001 [redacted] 2021 MON 77973 A study on the toxicity to the sediment dweller *Chironomus riparius* using spiked sediment. Report No. 20FV1ME

10. KCA 8.2.5.4/002 [redacted] 2022 Sediment-water chironomid toxicity test using spiked sediment (OECD 218) Effects of aminomethylphosphonic acid (AMPA) on the development of *Chironomus riparius*. Report No. BAY-051/4-23/R

11. KCP 10 _Anonymous _2022_113898-238 _GRG - response to ECOTOXICOLOGY Request No. 32, 47 Update of aquatic risk assessment for sediment dwelling organism from exposure to an unknown metabolite.

12. KCA 8.2.7 [redacted] (2022) Glyphosate TC: A study on the toxicity to the rooted aquatic macrophyte *Myriophyllum spicatum*. Report No. 21P1MW OECD

13. KCA 8.2.7 [redacted] 2021 Glyphosate technical (ASF71W) – Toxicity to the duckweed Lemna gibba under laboratory conditions (static test design). Report No. S21-0068

14. KCA 8.2.6 [redacted] 2021 AMPA: A 72 hour toxicity test with the marine diatom (*Raphidocelis subcapitata*) Report No. EUR-2020-0385

15. KCA 8.2.6 [redacted]. 2021 AMPA: A 72 hour toxicity test with the marine diatom (*Skeletonema costatum*) Report No. EUR-2020-0386

16. KCP 10.2.1 [redacted] 2022 Glyphosate isopropylammonium SL 486 (486 g/L): A study on the toxicity to algae (*Raphidocelis subcapitata*). Report No. EBGL0417

17. KCP 10_Anonymous_2022_13898-218_GRG.pdf - response to ECOTOXICOLOGY Request No. 28 and 45 CP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes - Interim report for *Skeletonema costatum* study with MON 52276, which includes a summary of the interim results for the following algal study; 'Softcheck, K.A 2022 Glyphosate-isopropylammonium SL 486 (486 g/L) – Toxicity to the Marine Diatom, *Skeletonema costatum*. Report No. EBGL0421' as the final report was not available before the deadline for the submission of additional information requested by EFSA. The final report is available on request.

18. KCP 10.3.1_anonymous_2022_113898-199_GRG - response to request ECOTOXICOLOGY 50 CP 10.3.1 Effects on bees relating to recalculated predicted concentrations in contaminated water, and a corresponding revised risk assessment for bees.

19. KCP 9.2.5_anonymous_2022_113898-034 - response to FATE request No. 41. Estimation of concentrations in surface water, and sediment. Relating to the bee contaminated water assessment values based on the new kinetic evaluations environmental fate input parameters.

20. KCP 9.2.5_anonymous_2022_ 113898-035_GRG.pdf - response to FATE request No. 42. Estimation of concentrations in surface water, and sediment. Relating to the bee contaminated water assessment values based on the new kinetic evaluations environmental fate input parameters.

21. KCP 9.2.5_anonymous_2022_113898-036_GRG.pdf – response to FATE request No. 43. Estimation of concentrations in surface water, and sediment. Relating to the bee contaminated water assessment values based on the new kinetic evaluations environmental fate input parameters.

22. KCP 10.3.1_anonymous_2022_113898-205_GRG - response to request ECOTOXICOLOGY 56 CP 10.3.1 Effects on bees relating to the relevance of metabolites for bees.

23. KCP 10.3.2.2_anonymous_2022_113898-198_GRG - response to request ECOTOXICOLOGY 51 CP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods, relating to a recalculated endpoint and reliability evaluation of an extended bioassay on *Typhlodromus pyri*

24. KCP 10.3.2.2_anonymous_2022_113898-201_GRG - response to request ECOTOXICOLOGY 52 CP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods, relating to the reliability of the extended bioassay on *Aleochara bilineata.*

25. KCA 8.6.2_anonymous_2022_113898-196.GRG.pdf - response to request ECOTOXICOLOGY No. 60. CA 8.6.2/001 [redacted] 1994., relating to the request for EC_{50} values on phyto-toxicity.

26. KCP 10_anonymous_2022_113898-227.GRG - A Revised Biodiversity Assessment as requested by the RMS

27. Anonymous_2022_113898-166_GAP table_GRG – Updated and reformatted GAP table Response to requests GENERAL 6 and 7, RESIDUES 20 and ECOTOXICOLOGY 79.

28. Grimm (2000) A test for evaluating the chronic effects of plant protection products on the rove beetle *Aleochara bilineata* Gyll. (Coleóptera:Staphylinidae) under laboratory and extended laboratory conditions (In Candolfi (2000) Guidelines to evaluate side-effects of plant protection products to non-target arthropods, IOBC, BART and EPPO Joint Initiative).