

Effects on Non-Target Species

The Glyphosate Renewal Group (GRG) submitted a 180,000-page dossier for consideration by the **Rapporteur Member State (RMS) Assessment Group on Glyphosate (AGG)** (consisting of France, Hungary, The Netherlands and Sweden) seeking renewal of the active substance Glyphosate in the EU (Annex I). The AGG has evaluated this dossier and released a consultation version of their assessment (the draft Renewal Assessment Report: dRAR-approximately 11,000 pages). A brief summary of salient points relating to the ‘Effects on Non-Target Species’ sections of the dRAR – specifically to the 846 - page Volume 3 Part B.9 (AS) for the active substance and to the 471 page Volume 3 Part B.9 (PPP) for the formulated plant protection product with the code MON 52276, sections of the dRAR are presented here, including an indication of how the GRG is intending to respond to the issues raised by the AGG, where appropriate.

Summary of effects on bird and other terrestrial vertebrates

Higher Tier Refinement of the mammalian long-term risk assessments considering residue decline kinetics data for grass:

The residue decline data for grasses used to refine the higher tier long-term mammal risk assessment are being re-analysed by the GRG using the latest state of current knowledge kinetics analysis, to determine suitable DT₅₀ values for use in risk assessment, used to modify the default fTWA and MAF factors used in the risk calculations. The GRG are committed to supporting the grass residue decline dataset further by conducting additional analysis of the residue data and to conduct further residue decline study work as necessary to support the residue decline refinements applied in the current long-term mammalian risk assessment.

Ecotoxicological studies considering bird acute, short-term, sub-chronic and reproductive toxicity of glyphosate and its’ metabolite AMPA were assessed for validity by the RMS to ensure they met current and relevant guidelines. This included all studies previously evaluated in either the monograph (2001) or the RAR from the last renewal (2015). The RMS presented extensive study re-evaluations of all studies conducted and these are presented in Volume 3CA, Section B.9.1 of the dRAR

Acute toxicity to birds:

The GRG supports the RMS evaluation in the dRAR relating to the acute toxicity to birds.

The RMS have agreed with the previous acute bird endpoint position stated in the EFSA Journal 7(12): 1438 (2009) to use the extrapolated LD₅₀ endpoint of 4334 mg/kg bw in the risk assessment. This was based on all study endpoints being >2000 mg/kg bw and that there were several studies without mortality at the highest level. In the acute oral toxicity study with the metabolite, AMPA, this achieved similar acute toxicity as the parent, with an LD₅₀ of >2250 mg/kg bw.

Short-term dietary toxicity to birds:

The GRG supports the RMS evaluation in the dRAR relating to the short-term dietary toxicity to birds.

Although this is not a current data requirement under Regulation 1107/2009, based on the endpoints achieved in the short-term dietary studies all being greater than the highest doses tested (between >1511 mg/kg bw and >1715 mg/kg bw) the RMS have concluded, after short-term dietary endpoint conversion to daily dose values and comparison with acute study endpoints, the route of exposure, i.e., (dietary vs. oral gavage exposure does not lead to an increased severity of effects.

Sub-Chronic & reproductive toxicity to birds:

The GRG supports the RMS evaluation in the dRAR relating to the sub-chronic and reproductive toxicity to birds.

Multiple bird reproductive studies submitted by the GRG, have been re-evaluated by the RMS who noted that the validity of the bird reproductive study in which the previously agreed reproductive endpoint NOAEL of 96 mg/kg bw/day was achieved (EFSA Journal 7(12): 1438, 2009)) could not be

confirmed. Therefore, the RMS concluded the reproductive NOAEL to be 116 mg/kg bw/day derived in a study with mallards which is used in the risk assessment.

Public domain literature relating to potential effects of herbicides containing glyphosate:
The available regulatory studies submitted by the GRG where birds are either exposed to glyphosate via the diet on a short term (8 days) and on a long term (20 weeks) basis have been performed with many parameters evaluated including clinical observations and gross pathological observations at termination of the studies. In the longer-term studies, reproductive performance assessments including egg production, No. of viable embryos and hatchling survival are recorded. Feed consumption and body weight change of parents and hatchlings are also evaluated. In the gross necropsy assessments - moulting flight feathers, air sacs, liver, spleen, egg yolks, ovary and testes are evaluated. These studies which comprise 5 x dietary, 4 x reproduction studies, concluded that all birds appeared normal throughout the studies and all findings were considered unrelated to the exposure to glyphosate. Neither male or female birds were concluded to be more sensitive than the other.

The multiple regulatory guideline studies, both short term and reproduction studies results in LD₅₀s ranging from >1012.4 to >1715.2 mg glyphosate/kg bw/day, and reproduction NOELs ranging from 96.3 to 201.1 mg glyphosate/kg bw/day in the dietary and reproduction studies respectively. As the birds were exposed without food options available in cages, the endpoints generated in the studies are considered highly conservative in comparison with public domain literature.

Acute toxicity to mammals:

The GRG supports the RMS evaluation in the dRAR relating to the acute toxicity to mammals.

All available acute oral toxicity data for mammals (rodents) were re-evaluated by the RMS comparing the results, the study test designs and the nature and duration of clinical sublethal effects observed. Clinical signs were observed in less than 50% of the test animals and were considered slight in nature and transient, with recovery within 24 hours in most cases. These data were used to determine a geometric mean endpoint for the acute mammal risk assessment, firstly according to species (mouse or rat) and then combined, to give an overall geometric mean endpoint of 3447 mg/kg bw for use in the acute mammal risk assessment. This endpoint replaces the >2000 mg/kg bw endpoint used in the acute risk assessment in the last AIR renewal (EFSA Journal 7(12): 1438, 2009).

Long term toxicity to wild mammals:

The GRG supports the RMS evaluation in the dRAR relating to the long-term toxicity to wild mammals.

The multiple long-term developmental toxicity studies in rabbits were re-evaluated by the RMS, to inform on endpoint selection for use in the long-term mammalian risk assessment. In line with the approach proposed by the GRG, the highest NOAEL below the lowest relevant LOAEL for both maternal and developmental long-term effects in rabbits, was determined to be a NOAEL of 100 mg/kg bw/day, an endpoint achieved in two rabbit developmental toxicity studies (Moxon, 1996 and Hojo, 1995). This endpoint is proposed to replace the lower endpoint of 50 mg/kg bw/day, used in the long-term mammalian risk assessment during the last renewal.

Effects on other terrestrial vertebrate wildlife: Amphibian and Reptiles:

The GRG supports the RMS request for additional information relating to the potential for effects on amphibian and reptiles, whilst maintaining that there is no specific regulatory guidance on how such an assessment should be performed within the context of an Annex I renewal.

In the evaluation by the RMS, further consideration of the exposure risk from glyphosate to amphibians and reptiles was requested. Therefore, the GRG present an updated risk assessment in the dRAR commenting, that considers the useful advice and recommendations available in the EFSA Scientific opinion on the state of the science on pesticide risk assessment for amphibians and reptiles (EFSA Journal, 2018, 16(2): 5215), as suggested by the RMS in the dRAR. Therefore, a qualitative and semi-quantitative risk assessment using methodologies related to physiology, energy budgets and internal body burdens in amphibians and reptiles has been prepared by applying allometric equations to derive toxicity endpoints.

Further Amphibian and reptile assessment: As poikilotherms, amphibians and reptiles have low energy needs and low food intake rates (FIR). Field metabolic rates reptiles are lower than for birds (¹Nagy 1987) and the risk assessment for reptiles is therefore considered protected by the bird risk assessment, as bird metabolic rates and FIRs are much greater than reptiles, which gives an increased dietary exposure potential. Dermal contact is a relevant exposure route for terrestrial phase amphibians that have permeable skins and is considered protective of reptiles that have a poorly penetrable skin. As ‘generally’ amphibian larvae are not considered more sensitive than fish, aquatic stage amphibians are covered by the aquatic risk assessment. Therefore, a qualitative assessment focusing on dermal exposure of terrestrial phase amphibians, which are mostly associated with field margins / adjacent wooded areas to application sites where more extensive cover prevails (Paton & Egan (2002)), is appropriate. As a foliar applied herbicide, interception by the plant canopy reduces the amount of product reaching the under-storey, where the amphibians would take refuge, plant interception (70%) is also considered in the qualitative assessment.

As limited data are available on terrestrial phase amphibian toxicity, the fish LC₅₀ for the product (MON 52276) (>277 mg a.e./L) can be used to determine a fish internal dose (LD₅₀) by multiplying the endpoint by the bioconcentration factor (log K_{ow} for glyphosate is < 1, therefore BCF considered in assessment is 1) achieves an LD₅₀ of 277 mg/kg bw. By considering the interspecies correlation estimation approach (ICE - ³Raimondo et al, 2017 & ⁴Raimondo et al., 2010)) and the highly significant positive correlation between internal doses (LD₅₀ mg/kg) for amphibians and fish (⁵Weltje et al., 2017), it is possible to determine an LD₅₀ for amphibians, by substituting the fish LD₅₀ value into the ICE equation (Eq 1. LD₅₀ replaces [LC_{50,fish}*BCF_{4d}]) to achieve an amphibian LD₅₀ of 203 mg/kg, that can be used in a qualitative assessment.

$$\text{Eq. 1: } \log[\text{LD}_{50,\text{amphibian}}] = 0.852 * \log[\text{LC}_{50,\text{fish}} * \text{BCF}_{4d}] + 0,226$$

Allometric equations convert potential dermal exposure value (based on applied rate) to a body exposure value (mg/kg bw) based on the exposed body surface and body weight that relate exposed skin area to body length & weight (based on the methods described in Weltje et al., 2017). The bodyweight of a small amphibian or reptile of 1.4 g (US EPA, T-HERPS model) was used, with the product application rate (kg/ha) being converted to a ‘per cm² rate’ and used to determine a worst-case dermal exposure concentration. An assessment factor of 5 is used as the original fish product LC₅₀ is a greater than / unbounded value.

Based on an application rate of 2.88 kg/ha rate and assuming 70% plant interception, achieves an amphibian body exposure value of 18.8 mg/kg bw. For the **off-field / off-target** assessment, additionally, a 2.77% drift value is considered, that achieves an amphibian (body burden) exposure value of 1.74 mg/kg bw. The corresponding TER values achieved for dermal exposure of terrestrial phase amphibians, for exposure both **in-field** and **off-field / off-target**, when compared to the amphibian LD₅₀ of 203 mg/kg b, are **11** and **117** respectively, which both exceed the TER assessment factor. Thus, it may be qualitatively concluded that the risk to amphibians from dermal exposure to MON 52276, applied according to the GAP, is low / acceptable

¹ Nagy K.A.(1987) Field Metabolic Rate and Food Requirement Scaling in Mammals and Birds. Ecological Monographs, Vol 57(2): pp 111-128.

² Paton P. W. C and Egan R. S (2002) Strategies to maintain pond-breeding amphibians on golf courses. USGA Turfgrass and Environmental Research Online. Vol 1(20) pp 1-7

³ Raimondo S., Mineau P. and Barron M. G. (2007) Estimation of chemical toxicity to wildlife species using interspecies correlation models Environ. Sci. Technol., 41 (2007), pp. 5888-5894

⁴ Raimondo S., Jackson C. R. and Barron M. G. (2010) Influence of taxonomic relatedness and chemical mode of action in acute interspecies estimation models for aquatic species Environ. Sci. Technol., 44 (2010), pp. 7711-7716

⁵ Weltje L., Janz P and Sowig P. (2017) An interspecies correlation model to predict acute dermal toxicity of plant protection products to terrestrial life stages of amphibians using fish acute toxicity and bioconcentration data Chemosphere Volume 189, December 2017, Pages 619-626

Summary of effects on aquatic organisms

Chronic fish endpoint selection for risk assessment:

The GRG maintains the position that the chronic fish study, an embryo / sac-fry assay, conducted with *Brachydanio rerio* (see dRAR Volume 3, CA 8.2.2.1/002) is unreliable and should be excluded from qualitative or quantitative use as an endpoint for risk assessment and hazard classification. Further information supporting this conclusion is presented here.

The RMS selected an endpoint (1 mg a.e./L) for use in the chronic fish risk assessment from a chronic (168 hour) exposure study using ⁶zebrafish. To support selection of this endpoint, the RMS refer to the results of chemical analysis performed on concentrated stock solutions (analysed at 0, 24, 72 and 120 hours after test start (the test media were not analysed) as being adequate to confirm exposure in the test media over the entire study duration (168 hours). This was based on the assumed stability of the test substance in the test media given the reported test design – described in the report as a semi-static test with 48-hour test media renewal interval.

The GRG disagrees with this conclusion as the study was conducted using a static test design with no test media renewal. In addition, the available analytical data, based on concentrated stock solution analysis, are not considered supportive of adequate exposure over the entire test duration. Further details are presented below.

The study report contains all the raw data recorded during the study. This includes a concentrated stock solution preparation record on one occasion at the start of the test, and a test media preparation record for only a single occasion at the start of the test.

- If the test was conducted using a semi-static test design, there would be a record of test media preparation for each occasion of test media renewal in the raw data, which is not the case. Therefore, as the raw data supports only a single occasion of test media preparation, the GRG conclude the test was conducted as a static test without test media renewal.

The water quality raw data record supports a static test design having been employed in this definitive study, as there are water quality measurements recorded for only a single set of replicate vessels on each day of the test.

- If the test was conducted using a semi-static test design, there would be a record of the water quality measurements performed for freshly prepared test media prior to fish transfer on each occasion of test media renewal, which is not the case. This would be expected to ensure equivalency of water quality prior to fish transfer from the expired media to freshly prepared test media. Fish transfer times would also have been recorded in the raw data to confirm fish transfer to freshly prepared test media.

*Concerning the chemical analysis conducted during the chronic fish study with *Brachydanio rerio*:* The GRG agree with the RMS, that having appropriate analytical data that supports test organism exposure over the entire exposure period is critical when selecting ecotoxicological endpoints for use in regulatory risk assessment.

The GRG considers that the analytical data are not supportive of adequate test media exposure concentrations having been achieved for the duration of the 168-hour test, due to the uncertainties associated with the conduct of the study in the following areas:

- The chronic fish test with *B. rerio* was conducted using a static test design.
- Exposure concentrations in the test vessels cannot be confirmed. Only concentrated stock solutions were analysed – the test media were not analysed.

⁶ [REDACTED] (2000) Chronic Toxicity of Glifosate Tecnico [REDACTED] to zebrafishlarvae (*Brachydanio rerio*) dossier location reference: MCA Volume 3 CA Part B-9, Data point Volume 3, CA 8.2.2.1-002; page 144 (dRAR).

- Measurement units for the stock solution chemical analysis are not recorded in the raw data.
- The concentrated stock solutions chemical analysis at 0, 24, 72 and 120 hours do not support the entire exposure period (168 hours).
 - o If there were no other uncertainties with the study – the longest exposure period that could theoretically be covered by the stock solution analytical data would be 120 hours.
 - o There is a 48-hour period between 120 and 168 hours (test end) that is not supported by chemical analysis of either test media or concentrated stock solutions. This coincides with a period of increasing mortality during the test.

Concerning the results of chemical analysis of concentrated stock solution's, the difference (deflection) between the measured and nominal concentrations achieved for the two stock solutions (used to prepare the test media) exceed nominal test media concentrations. The following assumes the units of measurement for the results of chemical analysis recorded in the as 'mg/L'.

- The 100 mg/L stock solution was used to prepare test media at 0.32, 0.56, 1.0 and 3.2 mg/L, and the 1000 mg/L stock solution was used to prepare test media at 5.6, 10 and 32 mg/L.
- The results of the chemical analysis of the stock solutions on four occasions of analysis (0, 24, 72 and 120 hours) recorded in the raw data respectively were:

100 mg/L	= 97.98, 101.38, 97.0 and 91.72 mg/L.
1000 mg/L	= 1054.62, 1005.05, 982.77 and 988.84 mg/L.
- For the 100 mg/L stock solution, deflections from nominal were 2.02, 1.38, 3.0 and 8.28 mg/L respectively, that all exceeded the three lowest nominal test media concentrations (prepared from this stock) on all sampling occasions, and the highest nominal test media concentration at 120 hours, where the measured concentration declined by 8.3%.
- For the 1000 mg/L stock solution, deflections from nominal were 54.62, 5.05, 17.23 and 11.16 mg/L respectively, which also exceeded the three lowest nominal test media concentrations (prepared from this stock) on all sampling occasions, and the highest nominal test media at 120 hours. The greatest decline in the 1000 mg/L stock solution concentration was achieved at 72 hours.
- The measured stock solution concentrations do not support adequate exposure in the test media, as the deflections in the measured concentrations from nominal, for both stock solutions, exceeded all nominal test media concentrations on three of the four occasions of analysis and on one occasion at the highest nominal test media concentration prepared from each stock solution.

Further discussion of the uncertainties associated with the study conduct and design are presented in detail in the GRG commenting, and include points that relate to the test design, the age and developmental stage of the fish larvae used, feeding, fish loading rates and water quality.

Given the uncertainties associated with the test design, that clearly indicate that the toxicity results achieved in this test, should not be considered representative of the sensitivity of zebrafish to glyphosate. Critically, the uncertainty associated with the test design and the chemical analysis, confirms that adequate maintenance of test media concentrations for the 168-hour duration of the test cannot be confirmed / supported. Together, these points make the outcome of the study unreliable and not relevant for use in regulatory risk assessment, and nor should the study be used in hazard classification as these data are not scientifically robust.

The Applicant therefore maintains the position that the chronic fish study with *B. rerio* (CA 8.2.2.1/002) is not reliable for use in risk assessment and should be excluded from qualitative or quantitative use as an endpoint for risk assessment and hazard classification.

Multiple chronic fish studies are submitted to support the Annex I re-registration discussed further below. In the opinion of the GRG, these studies are considered to be valid for use in risk assessment and do support a lack of long-term effects to fish following chronic exposure to glyphosate. Of these chronic studies, the study with the lowest achieved NOEC and therefore most relevant for risk assessment is the fish early life stage test conducted with rainbow trout which is discussed further below.

Fish Full Life Cycle test:

The GRG agrees with the RMS, that the fish full life cycle study does not show any evidence for effects, even at the highest exposure concentration (25.7 mg a.e./L) following long-term exposure to glyphosate and the achieved endpoint is considered valid for use in risk assessment.

The RMS evaluation of the fish full life cycle study conducted with fathead minnows (Vol 3 Part B9, CA 8.2.2.2/001) states the study to be informative only and not sufficiently reliable to set a robust endpoint. The GRG considers the study to be valid for use in risk assessment. An RMS concern relates to methods of chemical analysis used in the study, where a phosphate specific assay was used, to quantify ortho-phosphate and total phosphorous in the test media and then corrected achieved values for background levels of phosphate. The remaining mg/L phosphorous, was attributable to glyphosate in the test system. The report contains a detailed explanation on the Method 155-71W (1973) that was used, with orthophosphate analysis conducted using spectrophotometric analysis. The orthophosphate assay relies on a simple reaction to form blue phosphomolybdenum complex that is then quantified at 880 nm using a standard curve. The second analyses for total phosphorous, used an acid digestion method, that converts total phosphate in the sample to phosphate, reacts with molybdate and antimony ions to form a blue colour, and then reduced with acid and read spectrophotometrically at 660 nm. The report contains a step-by-step instruction how the samples for total phosphate were digested, with the method being based on a Technicon Industrial Method 327 74W (1974). These assays are still in use today, although it is accepted that the specificity and accuracy of analytical methods used to detect glyphosate have advanced since the study conduct in 1975. All samples were analysed in triplicate and quantified using standard curves based on optical density with glyphosate calibration solutions, which are also described in the report. The level of detail provided in the report is considered sufficient to support the analysis of test media during the fish full life cycle study.

The US EPA have also evaluated the fish full life cycle study (MRID 00108171/1975) as part of the current registration review of glyphosate and concluded that the study met the test guideline requirements. The test was conducted to the recommended bioassay procedure for fathead minnow chronic tests issued by the National Water Quality Laboratory in Duluth, Minnesota (EPA (1971) and compared to the current EPA (1996) test guideline for life cycle testing in fish, OPPTS 850.1500 Fish Life cycle toxicity, and there were no deviations from the guideline. The study has been previously considered acceptable at EU level. In the US EPAs 2020 Interim Registration Review Decision for glyphosate, the achieved 25.7 mg/L chronic fish full life cycle study endpoint from this study is used in risk assessment. The GRG therefore consider the study to be acceptable for use in risk assessment to support a lack of long-term effects following exposure to glyphosate under worst case exposure conditions.

Bioconcentration Study in Fish:

The GRG agree that a fish bioconcentration study with glyphosate is not triggered based on the low partition coefficient (Log Pow = -6.28 at 25°C at pH7). Consequently, the risk of bioaccumulation and / or biomagnification through the food chain is low.

A valid fish bioconcentration study has been submitted by the GRG to support the Annex I renewal, which supports the RMS conclusion, achieving a very low bioconcentration factor (BCF) value of 1.1. This value further supports the acceptable bioaccumulation and / or biomagnification risk through the food chain but also supports a low chronic risk potential to fish following a long-term continuous exposure to glyphosate. Fish were exposed for an extended period to glyphosate at 12 mg a.e./L in the BCF study, which was approximately 50% of the chronic fish endpoint achieved in the fish full life cycle study (NOEC = 25.7 mg a.e./L).

Full Fish Early Life Stage Test:

The GRG submitted a valid and scientifically robust fish early life stage test conducted with rainbow trout according to the OECD 210 test guideline, that is considered the most appropriate chronic fish study for use in the risk assessment.

The 85-day fish early life stage test was conducted using rainbow trout (*Oncorhynchus mykiss*) at nominal exposure concentrations up to 10 mg/L with two replicates per treatment group in accordance with the OECD 210 (1992) fish early life stage test guideline. The test was fully valid according to the validity criteria of the 1992 version of the test guideline, that related to control hatching success, post hatching survival, water quality measurements and appropriate analytical verifications having been made. The RMS did agree that the test also satisfied the validity criteria of the current 2013 version of the OECD 210 fish early life stage test guideline, against which the RMS completed their evaluation of the study.

The RMS noted that two replicates per treatment group and not four were used, which is required based on OECD 210 (2013) test guideline. Therefore, the robustness of the study endpoints were questioned and the RMS concluded the study was **valid** but not reliable for risk assessment due to observed variability in measured parameters.

The RMS reference Annex 5 of the current OECD 210 (2013) test guideline, which requests test labs to provide a demonstration of Its ability to meet the power requirement either by conducting its own power analysis or by demonstrating that the coefficient of variation (CV) for each response does not exceed the 90th percentile of CVs used in the development of the TG.

Despite no concentration dependent effects being observed in the study, an analysis of the variability in the measured parameters has been conducted by the GRG and presented in detail in the GRG comments to the dRAR. An overview of the findings of the analysis are presented here, that indicate that the study is sufficiently robust for use in risk assessment. Repeating a valid vertebrate study to meet the requirements of an updated test guideline, without considering in detail, the validity of the existing study, is from an ethical perspective not an option in the opinion of the GRG.

The analysis conducted by the GRG, demonstrates that the CVs for the relevant responses have been substantially achieved to meet the recommendations in Annex 5 of test guideline OECD 210 (2013), to demonstrate that the study is sufficiently robust to determine statistical significance for the tested parameters.

Despite some minor exceedances of the target 90th percentile CV values from Annex 5 of the OECD 210(2013) test guideline, it was shown that the CVs for the most sensitive and relevant parameters (total fish length and wet weights) were fulfilled for the relevant 'between' replicate CV values.

For the parameter fish wet weight it could be shown that the slight deviation from the 90th percentile CV values for 'between' replicates in the highest test concentration of 10.0 mg a.e./L was likely the result of differences in fish densities between the two replicates due to lower hatched fry in one of the replicates (as the lower rate was isolated to a single replicate at the highest treatment level, it was not attributable to glyphosate exposure). The reduced fish density in one replicate may have led to larger and heavier fish in one replicate compared to the other. By comparing the variation in the mean values

achieved for each of the measured growth parameters (length, wet and dry weights) across the treatment groups and comparing these with the control group, the maximum percentage difference in the mean values across all parameters, is <6%, further supporting the absolute lack of observed effects in the study.

In a worst-case approach, if the variability in the highest test concentration is omitted due to variability an overall NOEC value based on the next lower test concentration of 3.125 mg a.e./L is absolutely relevant to be used. It is stressed that based on the weight-of-evidence provided above, lowering of the endpoint is not required, since the entire data set based on the most relevant endpoints for this study type as described further in the commenting, is considered sufficiently robust. The reduced endpoint is only presented as an alternative worst-case approach. Repetition of this vertebrate study is not considered justified due to ethical animal welfare reasons and the GRG conclude that the submitted trout early life stage test is valid and sufficiently robust for use in risk .

Effects on pollinators – The GRG agrees with the RMS, that based on the acceptable acute and chronic risk assessments for bees, as demonstrated in the extensive pollinator risk assessment presented in the dRAR that the exposure risk to bees from glyphosate following application according to the GAP is low / negligible.

To further illustrate the safety of glyphosate to bees, the following information are presented. Since the 2015 RAR, several additional acute and chronic toxicity studies with bees have been completed, submitted, reviewed, and included in the dRAR with a risk assessment for bees (section B.9.6.1). For the 2015 RAR, the risk assessment for bees included acute contact and oral studies for adult honeybees exposed to glyphosate technical, various salts of glyphosate, and the representative formulation. A pollen and nectar residue study that informed dose setting for a semi-field brood study was also conducted and submitted for consideration. The conclusion by the RMS on the risk to bees, based on EPPO guidance in the 2015 RAR was as follows:

“...toxicity studies demonstrate that glyphosate, glyphosate salts and MON 52276 have very low acute and contact toxicity to honeybees with LD50 values around or higher than 100 µg a.s./bee. The calculated HQ values show an acceptable risk for honeybees due to the intended use of the lead formulation MON 52276 according to the label.

Additionally a bee brood study was performed following established methodology which demonstrates that glyphosate poses no chronic risk to bee brood as at worst case field exposure levels.”

The new acute and chronic bee studies followed OECD test guidelines that were validated and approved since the 2012 submission that supported the 2015 RAR.

New studies available for honey bees (chronic larval and adult), bumble bees (acute oral and contact exposure) and solitary bees (acute contact), covered exposure to the different life stages of these species from the active substance glyphosate the representative formulation (MON 52276). The new bee risk assessment in the dRAR considered endpoints for all previously submitted studies that are still concluded to be valid by the RMS, the endpoints from new studies, and addressed studies from the literature. There are no endpoints identified in the open literature by the RMS at this time that may impact the outcome the risk assessment for direct effects to bees.

The dRAR includes an extensive risk assessment for bees to address the relevant exposure scenarios, conducted according to the EPPO and EFSA bee guidance document approaches. Beyond contact exposure and dietary exposure from pollen and nectar consumption, the risk assessment includes the potential for bee exposure to glyphosate via contaminated drinking water such as puddles and guttation water. Based on these assessments the RMS concluded the following in the dRAR

“Glyphosate presents a low acute and chronic exposure risk to honeybees and non-*Apis sp.* bee species when products are applied in accordance with the proposed GAP.” In addition it was concluded “In view of the available information and the outcome of the risk assessment, field studies with honeybees for the representative EU formulation MON 52276 are not considered required.”

However, there are some aspects of the assessment that warrant further comment:

- The RMS doubled the maximum glyphosate residues in nectar and pollen from Thompson (2011, Vol.3 CP 10.3.1.5/001) for use in the bee risk assessment. The RMS justified this approach by stating that the first nectar and pollen samples in the Thompson (2011) study, were collected 1-day post-application. For nectar, the rationale for collecting samples 24 h post-application was as follows. As a foliar systemic herbicide, glyphosate must penetrate through the cuticle of the plant before it can be detected at maximum levels in nectar. For glyphosate residues in nectar, the max value reported by Thompson (2011) was 31.3 mg glyphosate a.e./kg for an application rate of 2.88 kg glyphosate a.e./ha, that translates to a ‘residues per unit dose’ (RUD) value of 10.9 mg a.s./ha (adjusting for a 1 kg a.e./ha rate). This value is of the same magnitude as the 90th percentile RUD for nectar in Appendix F of the 2013 EFSA guidance on risk assessment for bees, where in Appendix F (Table F1), the median, 90th percentile, and max RUD values for nectar are 2.9, 11.2 and 20.7 mg a.s./ha, respectively, for foliar applications.

For additional comparison, Odemer et al., (2020) as part of a study run by JKI measured glyphosate levels in nectar 1 h post-application in *Phacelia tenacetifolia*, the same plant species used by Thompson (2011). The level of residues in nectar at 1 h post-application reported by Odemer et al., (2020) was 24.9 mg a.s./kg for an application rate of 1.8 kg glyphosate a.e./ha. Adjusting to 1 kg a.s./ha results in a RUD of 13.8 mg glyphosate a.e./kg, which is very comparable to the RUD from Thompson (2011) of 10.9 mg glyphosate a.e./kg.

Based on this analysis, the nectar residues reported by Thompson (2011) are conservative in terms of the timing of collection and the maximum residues generated. Therefore, doubling the nectar residue values reported by Thompson (2011) for use in risk assessment as has been done in the risk assessment conducted by the RMS, is not considered necessary. The achieved residues in nectar are considered representative.

- The maximum glyphosate residue in pollen measured by Thompson (2011) over the first day post-application was 629 mg glyphosate a.e./kg for an application rate of 2.88 mg glyphosate a.e./ha, which translates to a RUD of 218 mg glyphosate a.e./kg for an application rate of 1 kg a.s./ha. A comparison to the glyphosate RUDs for pollen in Appendix F of the 2013 EFSA bee risk assessment guidance reports the median, 90th percentile, and maximum residues for pollen from foliar application of 6.1, 51.9 mg, and 149.8 mg a.s./kg, respectively
- For or additional comparison, Odemer et al., (2020) measured glyphosate levels in pollen 1 h post-application to *Phacelia*, the same species used by Thompson (2011). The residue in corbicular pollen 1 h post-application was 615 mg a.s./kg for an application rate of 1.8 kg glyphosate a.e./ha. Adjusting for 1 kg a.s./ha to produce a RUD value results in a value of 342 mg glyphosate a.e./kg. This value is of the same magnitude as reported by Thompson (2011) and could conservatively be used in place of the RUD from Thompson (2011). Simply doubling the pollen residues from Thompson (2011) is not justified since pollen collected for the Day 1 sample represents pollen collected over the first day post-application.

Ideally, the residues for the chronic bee assessment should be based on the time weighted average measured for pollen and nectar samples collected post-application. In addition, the likelihood of workers from a hive collecting enough pollen and nectar at the maximum residue post-application for

a complete brood cycle is unlikely. Rather, the pollen and nectar required by the colony to complete one brood cycle is collected over time and glyphosate levels in nectar and pollen decline over time.

Overall, there was a good agreement between residues measured in larvae on days 4 and 7 in the exposure phase of the study and the effects portion of the study, indicating that the assumptions used for dosing used in the effects portion of the study were robust and valid (Thompson, 2011; Thompson et al., 2014)⁷.

Concerning the acute and chronic laboratory endpoints for honeybees and non-Apis sp. bee species used in the risk assessment:

These endpoints are considered worst-case as they reflect the maximum amount of glyphosate that could be practically added to a 50% sugar solution and to maintain homogeneity. For instance, in the bumble bee acute oral study, bees were fed a 50% sugar solution containing the equivalent of 25 g of product/L (1000 µg pr. / 40 µL), which is far higher than any anticipated concentration in nectar and pollen. At this concentration, bumble bees received an oral dose of 412 µg a.e./bee at which no bees died and is therefore the no observed effect dose (NOED). Based on these data, a definitive LD₅₀ could not be determined, and the LD₅₀ value is considered greater than the highest dose used in the test (i.e. > 412 µg a.e./bee). For the acute bumble bee risk assessment, the NOED value was taken by the RMS as a surrogate for the LD₅₀ value and for the risk assessment. The trigger applied in the EFSA bee guidance risk assessment for acute oral risk to bumble bees is 0.036 meaning that to pass the risk assessment, the tier I exposure estimates must be 27.7-times lower than the dose that would result in 50% of bumble bees. Therefore, the risk assessment by the RMS is highly conservative as the maximum safe is artificially assessed to be 2.28 kg a.s./ha because the assessment is based on a NOED rather than the actual LD₅₀. However, based on the results of the acute bumble bee studies application rates well above 2.28 kg a.s./ha are not expected to pose and acute risk to bees.

For all acute and chronic oral exposure studies, the highest dose tested used in the risk assessments, reflect the maximum amount of glyphosate that could be practically added to the dosing solution, with one exception. The exception is using the EC₁₀ value for the chronic larval study, were there a statistically significant decrease in larval survival at the highest dose tested of . Consequently, the proposed maximum safe use rates achieved in the risk assessments for bees are considered highly protective of bees and biodiversity (Regulation (EU) 2017/2324). The NOED values used in the risk assessments far exceed the anticipated concentration in nectar and pollen following field application at rates proposed on the GAP. Moreover, based on the Terrestrial guidance document SANCO/10329/2002 rev 2 final / EPPO approach there is no risk to bees as all HQ values are below the Annex VI trigger of 50. This is still a highly conservative assessment as all endpoints are NOED rather than true LD₅₀ values.

Testing on Non-target Terrestrial Plants:

In the RMS evaluation of the submitted Non-target terrestrial plant study data, endpoints, the conduct and reporting of vegetative vigour studies are discussed, covering studies conducted on both the technical material and the representative formulation (MON 52276). These are addressed in detail in the commenting but also warrant discussion here;

Concerning presenting ECx estimates based on phytotoxicity scores in non-target terrestrial plant studies - vegetative vigour study conducted using technical material (CA 8.6.2/001):

The GRG maintains a position that endpoints based on a subjectively scored parameters are not appropriate for use in risk assessment.

There are currently no guidance available within guidelines or as an outcome of the Peer Review Experts' meeting 185 on general issues in ecotoxicology (EFSA, 2019) on how to quantitatively

⁷ Thompson HM, Levine SL, Doering J, Norman S, Manson P, Sutton P, von Mérey G. 2014. Evaluating exposure and potential effects on honeybee brood (*Apis mellifera*) development using glyphosate as an example. *Integr Environ Assess Manag.* 10(3):463-70. doi: 10.1002/ieam.1529.

conduct phytotoxicity assessments or how to calculate such an endpoint based on subjective datasets, that can be considered robust for use in regulatory risk assessment.

In this vegetative vigour study plant phytotoxicity scores are based on levels of increasing symptomology (0-5), recorded following subjective visual assessment of individual plants within each treatment group compared to a control group of plants by trained personnel. All individual plant phytotoxicity scores are presented in Appendix C of the study report. Proportional values derived of a qualitative ranked (0-5) scoring systems do not provide any indication of natural variability within the test species (i.e. all controls are scored as zero effect). Scoring data are neither continuous data e.g., dry weight, fresh weight and height, nor quantal data like survival. Therefore, they provide the same level of granularity across the entire effects range and are therefore not considered appropriate for estimating ER₅₀ values using standard statistical procedures. If despite the explained uncertainties an ER₅₀ is still required to be derived of the phytotoxicity estimations made in this study, it is suggested to use the rating “3: severe effect with recovery possible” as representing 50% effect. The most sensitive species with regard to phytotoxicity was tomato for which the rating of 3 was given at a test rate of 314 g a.e./ha for most of the tested plants. This rate is higher than the lowest ER₅₀ based on dry weight (146 g/ha for tomato dry weight) and is therefore considered more relevant for the risk assessment. EC_x calculations are therefore not required for the phytotoxicity observations, based on the uncertainties described above and the fact that the lowest relevant endpoint is considered in the RA.

Concerning the vegetative vigour study on the representative formulation from 2013 (CP 10.6.2/002) and the validity concerns relating to light intensity:

The GRG agree with the RMS evaluation that it cannot be excluded that low light intensity influenced the results achieved in the study and therefore request that the study be excluded from the list of endpoints. The RMS conclude in the evaluation in the dRAR that the study should be considered supportive, as the light intensities during the test were upto 50% lower than those recommended in the test guideline. As stated the GRG agree that the uncertainties associated with the low light intensity in the study cannot be resolved by a re-analysis of these data, and influence on the achieved results cannot be excluded, which may have led to under- or over-estimation of effects.

Concerning the robustness of the most recent (2021) vegetative vigour study (CP 10.6.2/005) submitted to support the Annex I renewal:

The GRG agreed that there were reporting concerns relating to data reported for cucumber in the recent (2021) vegetative vigour study (CP 10.6.2/005). Therefore, the GRG have now received an amended GLP report.

The RMS stated that the study was considered valid but that there was uncertainty associated with the data presented in the report for cucumber. The amended final report, that is available through the GRG transparency site, demonstrates the acceptability of the study for all species tested and is completely suitable for use in risk assessment, The endpoints achieved in the original report are not impacted. The data for all 10 species tested are now considered acceptable and the study valid for use in both deterministic and probabilistic risk assessment.

The uncertainty voiced by the RMS over the more recent (2021) vegetative vigour study conducted with the representative formulation, precluded the use of the endpoint data from a probabilistic risk assessment, with the RMS presenting only a non-target terrestrial plant - deterministic risk assessment in the dRAR.

A full probabilistic risk assessment has now been included in the commenting table and the outcome of the assessment is presented in the following table.

Based on the probabilistic risk assessment presented in the dRAR commenting table, the risk to non-target terrestrial plants can be considered acceptable when risk mitigations to protect non target terrestrial plants at the edge of the field are implemented. The risk mitigations are reported in the table below.

Risk mitigation measures for terrestrial non-target plants for the use of MON 52276

GAP number and summary of use	Application rate (g/ha) considered (28 day interval unless otherwise stated)									
	1 × 540	1 × 720	2 × 720	3 × 720	1 × 1080	2 × 1080 ^A	1 × 1440	2 × 1440	1 × 1800	2 × 1800 (90 days apart)
Uses 1a-c: Applied to weeds; pre-sowing, pre-planting, pre-emergence of field crops .		No risk mitigation measures necessary			No risk mitigation measures necessary		No risk mitigation measures necessary			
Uses 2 a-c: Applied to weeds; post-harvest, pre-sowing, pre-planting of field crops .		No risk mitigation measures necessary			No risk mitigation measures necessary		No risk mitigation measures necessary			
Use 3 a-b: Applied to cereal volunteers; post-harvest, pre-sowing, pre-planting of field crops .	No risk mitigation measures necessary									
Use 6 a-b: Applied to weeds (post-emergence) in field crops BBCH < 20		No risk mitigation measures necessary			No risk mitigation measures necessary					
Uses 10 a-c: Applied to couch grass; post-harvest, pre-sowing, pre-planting of field crops		No risk mitigation measures necessary			No risk mitigation measures necessary					
Use 4 a-c: Applied to weeds (post-emergence) below trees in orchards .		No risk mitigation measures necessary			No risk mitigation measures necessary					
Use 5 a-c: Applied to weeds (post-emergence) below vines in vineyards		No risk mitigation measures necessary			No risk mitigation measures necessary					
Use 7 a-b: Applied to weeds (post-emergence) around railroad tracks									No risk mitigation measures necessary	
Use 8 and 9: Applied to invasive species (post-emergence) in agricultural and non-agricultural areas									No risk mitigation measures necessary	

BS = Untreated buffer strip

^A Due to the long spray interval of 28 days this use covers also the following possible application pattern: 2 × 1080 g a.s./ha plus 1 × 720 g a.s./ha (28 day interval between each application)