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 residues

- **Extraction efficiency**
- **Storage stability in plant matrices**
- **Storage stability in animal matrices**
- **Metabolism in plants**
- **Metabolism in livestock**
- **Relevance of glyphosate trimesium studies**
- **Residues in primary crops**
- **Residues in livestock commodities**
- **Nature of residues in processed commodities**
- **Residues in rotational crops**
- **Residues in honey**

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Summary

In accordance with Regulation (EC) No 1107/2009, a dossier for the renewal of the approval of glyphosate was submitted by the Glyphosate Renewal Group (GRG, the applicant) to the Assessment Group for Glyphosate (AGG) on 8 June 2020. AGG consists of the competent authorities of France, Hungary, The Netherlands and Sweden that jointly act as the Rapporteur Member State (RMS) for Glyphosate. After a thorough evaluation of the dossier, AGG released a draft Renewal Assessment Report (dRAR) on 15 June 2021.

Comments on the dRAR were collected from other Member States, EFSA, GRG and other interested parties through a public consultation from 23 September 2021 to 22 November 2021. Based on the dRAR and the received comments, EFSA identified the need for additional information in various technical areas, including residues. The applicant was given the opportunity to address this EFSA request during a one month "stop-the-clock" period (which ended on 14 April 2022). The information that was provided by the applicant during the stop-the-clock was evaluated by the AGG and included, as appropriate, in an updated version of the dRAR. It will be further discussed during expert meetings in the context of the peer review.

The purpose of this document is to provide a summary of the information provided by the applicant during the stop-the-clock to address the EFSA requests concerning residues. More specifically this information was about:

- The extraction efficiency of the various residue analytical methods for plant and animal matrices (new study submitted)
- The storage stability studies in plant and animal matrices
- The plant and livestock metabolism studies
- The possibility to use data generated with the glyphosate trimesium variant to support the use of other variants (i.e. glyphosate acid, glyphosate potassium, glyphosate ammonium, glyphosate isopropyl ammonium, glyphosate dimethyl ammonium)
- The residues in primary crops (representative uses)
- The residues in livestock commodities
- The nature of residues in processed commodities
- The residues in rotational crops (new study submitted)
- The residues in honey (new study submitted)

For more details, please refer to the documents submitted in the context of the stop-the-clock.

1. Extraction efficiency

Plant matrices and honey

According to SANTE/2017/10632 rev. 4 the extraction efficiency has to be evaluated based on information from metabolism studies performed with radio-labelled compounds. In most cases in the plant metabolism studies for glyphosate, more than 70% of TRR was extracted either with water as the only extraction solvent or with 0.1% aqueous formic acid in methanol (96/4, v/v) as extraction solvent. In the metabolism studies in which hydrophobic solvents (e.g. chloroform) were used in addition to aqueous solvents, glyphosate and its metabolites were mainly found in the aqueous phase as they are only poorly soluble in hydrophobic solvents. Hence, it may be concluded that acidified water allows extracting the majority of the TRR and is the preferred extraction solvent for glyphosate and its metabolites.

In the storage stability studies in plant commodities and in the residue studies, glyphosate and its metabolites were extracted with acidified water. In some methods dichloromethane was added in the first step of sample preparation for clean-up purposes. In order to evaluate the impact of this clean-up procedure on the extraction efficiency for glyphosate and its metabolites, the applicant conducted a cross-validation study which was submitted during the stop-the-clock. Since the extraction efficiency has to be investigated on incurred residues, the selection of plant matrices was constrained by the availability of samples with incurred residues > LOQ. The tested plant matrices included dry matrices (cereal grain and straw, dry bean seed), matrices with a high oil content (oilseed rapeseed) and matrices with a high water content (sugar beet leaves). In addition, the extraction efficiency in honey was investigated.

The extraction efficiency of 0.1% formic acid (method ME-2015-01) was compared with the extraction efficiency of 0.1% formic acid/dichloromethane (methods AG-ME-1294-01, ME-2000-01 and GRM067.01A) and the extraction efficiency of a (1/1, v/v) mixture of water and 1% formic acid in methanol (EU multi-residue method QuPPe-PO for small polar molecules). The method ME-2015-01 was taken as the reference method (with an extraction efficiency of 100%) since it uses an extraction solvent similar to that of most metabolism studies.
Overall, when compared with the method ME-2015-01, the methods AG-ME-1294-01 and QuPPe-PO showed satisfactory extraction efficiencies (i.e.≥ 70%) for glyphosate and AMPA in cereal (grain and straw), bean (seed), oilseed rape (seed), sugar beet (leaves) and honey. In deviation to this, the QuPPe-PO method showed extraction efficiencies ≤ 70% for glyphosate in bean (seed) and for AMPA in one sample of sugar beet (leaves). A modified QuPPe-PO method with an increased solvent / sample ratio was also tested for bean (seed) and showed extraction efficiencies ≥ 70%. Furthermore, it was not possible to assess the extraction efficiency of the various methods for AMPA in bean (seed) and honey since the residues of AMPA in the available samples were below the limit of quantification (LOQ) of 0.025 mg/kg.

In conclusion, the study demonstrated that the addition of dichloromethane for clean-up during the extraction step has no significant effect on the extraction efficiency for the residues of glyphosate and AMPA in plant matrices and honey. Hence, the storage stability and residue studies in which a combination of 0.1% formic acid and dichloromethane was for extraction and clean-up can be considered valid.

Animal matrices

In the livestock metabolism studies with glyphosate, AMPA and/or *N*-acetyl glyphosate, the residues were extracted using water, either alone or in combination with chloroform: 0.2 N HCl, 0.1 M HCl/chloroform or water/chloroform. Comparison between the results obtained in the different studies indicates that the pH of the extraction solvent or the presence of chloroform did not (as such) significantly impact the extraction efficiency.

The metabolism studies using water/chloroform (no acid) for extraction resulted in recoveries greater than 70 % of the TRR for all tissues (except fat which had low residue levels). These recoveries were similar if not better than the extraction recoveries obtained using 0.1M HCl/chloroform for extraction, demonstrating that pH is not a critical factor in extraction efficiency for these matrices. Furthermore 0.2 N HCl was used for extraction in the livestock metabolism studies for *N*-acetyl glyphosate, which is expected to behave similarly to glyphosate and AMPA.

The fact that the pH of the extraction solvent does not significantly impact the extraction efficiency may be accounted for by the fact that in a very large range of pH values both glyphosate and AMPA are present as ionic species. The exact nature of the ionic species depends on the pH-value but, by nature, all these ionic species are highly soluble in aqueous solutions due to their polar nature. Hence, the pH of the extracting solution has little effect on preferential partitioning of glyphosate and AMPA from tissues into the aqueous phase. This behaviour is further supported by the fact that the n-octanol : water partition coefficients (log Pow) do not change significantly with pH.

When chloroform was used as a partitioning solvent in the animal metabolisms studies, glyphosate and metabolites were highly recovered in the aqueous phase. Hence, it is considered that aqueous extraction is sufficient to extract the majority of glyphosate and its metabolites and acidified water is the preferred extraction solvent. Therefore, it may be concluded that (like for plant commodities) the extraction efficiency is not affected by the addition of halogenated organic solvents (like chloroform or dichloromethane) during extraction for concurrent clean-up by liquid/liquid partitioning.

In the feeding studies and in the related storage stability studies, water, water/chloroform, 0.1 N HCl or 0.1% formic acid/methanol (96/4, v/v) were used as extraction solvents. The extraction efficiency of the solvents is supported by the livestock metabolism studies, which used similar extraction solvents and confirmed that water (or aqueous media) allow extracting the major part of parent glyphosate and its metabolites.

2. Storage stability in plant matrices

In the context of the stop-the-clock, the applicant submitted the interim report for an additional storage stability study for AMPA in protein containing matrices. The study shows that AMPA is stable for at least 12 months.

Further information was provided by the applicant regarding the preparation of the spiked samples for the storage stability testing of glyphosate and AMPA. In the most cases the samples were spiked and stored as homogenates. Only in two studies, the samples were stored either as whole commodity or as chopped material (oilseed rape seeds/linseeds (CA 6.1/007) and wheat/rye grain (CA 6.1/011) or straw (CA 6.1/011), respectively).
In the residue trials, the field samples were first stored deep-frozen as whole commodity, then homogenised at the analytical laboratory and further stored as a homogenate. Since in accordance with OECD guideline 506, a homogenate is likely to represent a worst case versus the use of a whole commodity, storage stability data conducted in homogenised samples are considered appropriate to cover the storage period of the field samples stored as whole commodity.

In the study CA 6.1/012 low procedural recoveries and low stored recoveries (outside the acceptable range of 70-110%) were observed for glyphosate in maize grain at 6, 9, 12 and 18 months. No further information is given in the report, justifying or clarifying the low procedural recoveries and low stored recoveries. The result of the stored recoveries corrected for the procedural recoveries demonstrate a plausible curve from 0 to 31 months after storage. Moreover, the storage stability for the 24-month interval can be regarded as proven, as the procedural recovery was acceptable and, based on the amount of glyphosate recovered in the stored fortified samples, the decline (if any) was less than 30 % of the nominal fortification level and less than 30 % of the initial value at day 0.

For some storage stability studies a mixed spiking solution was used for glyphosate, *N*-acetyl-glyphosate and AMPA. Although this represents a deviation from OECD Test Guidance 506, the storage stability data for each analyte shows that there is no significant change in the concentration of any of the analytes. Hence, transformation from one compound to another is very unlikely and the studies can be regarded as reliable.

Conclusion

Since storage stability data are available for glyphosate in each of the five categories of plant matrices (high water content, high oil content, high protein content, high starch content, and high acid content). These data show that glyphosate is stable for at least 18 months in all plant commodities. It is noted that “other matrices” are an exception since stability of residues was only demonstrated for 12 months in this group. However, those matrices do not belong to one of the five ‘standard’ categories as defined by OECD Guideline 506 and, therefore, the corresponding data are not considered for extrapolation.
Storage stability data are also available for AMPA in each of the five categories of plant matrices. These data show that AMPA is for at least 12 months in all plant commodities except for “other matrices”.
N-acetyl-glyphosate is demonstrated to be stable for 12 months in forage (maize, soybean), maize grain, soybean seed, maize stover and soybean hay. Since only single crops were investigated per category of matrices, no general extrapolations can be made.
N-acetyl-AMPA is stable in forage for 18 months, in maize for 23 months and soybean seed for 18 months. In dry matrices, *N*-acetyl-AMPA was stable for 23 months in maize stover and 18 months in soybean hay. Since only single crops were investigated per category of matrices (two crops in category high water), no general extrapolations can be made.

3. Storage stability in animal matrices

In the context of the stop-the-clock, the applicant submitted additional summaries for the storage stability data included in two of the animal feeding studies (CA 6.1/018 and CA 6.1/019). Based on the obtained results, glyphosate, *N*-acetyl glyphosate, AMPA and *N*-acetyl AMPA were shown to be stable upon frozen storage in homogenised liver, kidney, fat, and muscle for the maximum period tested of approximately 3 months.

For the storage stability of glyphosate and AMPA further information on the study design with regard to the preparation of the spiked samples was provided by the applicant. It was shown that in the storage stability studies for various animal matrices (CA 6.1/015 and CA 6.1/016) the samples for day 0 recoveries and the samples for storage were prepared from one batch.
Further information was provided by the applicant regarding the preparation of the spiked samples for the storage stability testing. Similar to plants, sample work-up procedures for animal matrices included homogenisation prior to freezing.

In the livestock feeding studies CA 6.4.1/002 and CA 6.4.2/003, animal matrices were stored homogenised, thus, in line with the spiked storage samples of the freezer storage stability studies.
In the livestock feeding studies CA 6.4.1/003 and CA 6.4.2/002, fat, muscle, liver and kidney samples were first stored as whole commodity, and then homogenised and stored as homogenised samples after shipment.
In the study CA 6.4.1/003, egg samples were stored as whole commodity during the complete storage period.
Overall, the storage conditions of the samples from the feeding studies are considered sufficiently covered by the available freezer storage stability studies, as in accordance with OECD guideline 506, a homogenate is likely to represent a worst case versus the use of a whole commodity. Since the storage stability of glyphosate and AMPA residues in animal matrices has been investigated in homogenised samples, the storage stability was, therefore, proven for animal tissues and eggs stored as whole commodity or homogenate.
The sample work-up (e.g. spiking of homogenised or whole specimen) used in the storage stability studies as well as additional information on the extraction procedure is provided in updated study summaries.

Conclusion

In animal matrices, glyphosate is demonstrated to be stable in eggs for maximally 14 months, since a decline was observed after 25 and 28 months to 32%. Furthermore, the stability of glyphosate was demonstrated for 22 months in milk, for 26 months in pigs tissues, for 24-25 months in ruminant and poultry tissues, except for poultry kidney, where stability was investigated up to 13 months only.
AMPA is demonstrated to be stable in eggs for a maximum of 14 months, in milk for at least 16 months, in pig tissues for 26 months, except for fat where decline was observed after 15 months. In ruminant and poultry tissues, AMPA is stable for 24-25 months, except for poultry kidney, where stability was investigated up to 13 months only.
N-acetyl glyphosate and *N*-acetyl AMPA were shown to be stable in liver, kidney, fat, and muscle for the maximum period tested of approximately 3 months.

4. Metabolism in plants

A large number of metabolism studies conducted with glyphosate in conventional crops are included in the dossier; seven in fruits (three acceptable and four classed as supporting), two in root and tuber vegetables (one classed as supporting and one considered not acceptable), four in cereals and grass crops (one acceptable and three classed as supporting), two in pulses and oilseeds (one acceptable and one classed as supporting) and two in miscellaneous crops (one in coffee considered acceptable and one in sugar cane classed as supporting). Studies were assessed as only supportive where they were judged to be lacking sufficient information on the identification of the metabolites, or where in some cases the relevant RAC was not investigated. Nonetheless, these supportive studies provide limited but useful information on the uptake and metabolism of glyphosate in plants.

Although the representative crops proposed for the renewal of approval of glyphosate are all conventional crops, metabolism studies conducted with glyphosate in genetically-modified crops are included in the dossier for completeness and because they can provide metabolism information also relevant to conventional crops. Six studies, all acceptable, are provided for the EPSPS or EPSPS/GOX modification in a range of crops, and three studies, all acceptable, for the GAT modification in cereals and oilseeds.

Other than for use on railways and spot treatment of invasive weeds, the maximum application rate supported is 1.44 kg a.s./ha. The metabolism studies in conventional crops are made at (often significantly) higher rates previously common for glyphosate and therefore represent worst-cases for residues of glyphosate and any metabolites. The metabolism studies in modified crops are generally made at lower rates closer to the proposed representative uses.

If only the fully-acceptable studies are considered, these cover four crop categories: fruit (citrus and grapes), cereals and grass crops (wheat), pulses and oilseeds (soya bean) and miscellaneous crops (coffee), and include both soil and foliar applications at rates sufficient to encompass the representative uses.

Taken together, these studies demonstrate a consistent pattern in the metabolism of glyphosate. The representative uses are all soil-directed and under those conditions, there is only low uptake of glyphosate from the soil, though sometimes sufficient to give measurable TRRs in plant parts. Some of the metabolism studies used other application methods (hydroponics or foliar application) to facilitate the identification or characterisation of residues. Three studies in different crop groups (grape, wheat and coffee) used foliar application of glyphosate and again showed similar overall metabolic profiles.
Several of the plant metabolism studies were conducted using glyphosate as its trimesium salt. Although the trimesium salt is no longer a relevant supported variant of glyphosate, these studies are relevant to support the representative uses of glyphosate; this question is discussed in more detail below.

The number and range of available crop metabolism studies are sufficient and reliable to support the representative uses. In particular, the submitted data are sufficient to derive appropriate and reliable residue definitions for glyphosate in conventional crops according to the supported uses. While the metabolism studies in CP4 EPSPS modified crops provide information that is also relevant to conventional crops, more caution is required when extrapolating the results of metabolism studies with GOX- or GAT-modified crops to conventional crops since these modifications are specifically intended to alter the metabolism of glyphosate by inducing the formation of herbicidally inactive derivates or degradates. Whereas *N*-acetyl glyphosate and *N*-acetyl AMPA are major metabolites in GAT-modified

crops, they are not major metabolites in conventional or CP4 EPSPS-modified crops. Overall, the studies with genetically modified crops confirm that, besides parent glyphosate, AMPA is the only metabolite likely to be present in significant amounts in conventional crops. Furthermore, in practice no GAT-modified crops are grown anywhere in the world, and from February 2022 no GAT-modified crops are authorised for food and feed purposes in the EU. For those genetically-modified glyphosate-tolerant crops that are relevant in trade (i.e. EPSPS modified crops) it does not seem justified to include the *N*-acetyl metabolites in the residue definition.
The available metabolism and residues data, including on modified crops, support the proposed residue definitions for conventional crops as:

for monitoring: glyphosate
for risk assessment: sum of glyphosate and AMPA, expressed as glyphosate

The available plant metabolism data are extensive, covering four crop categories and showing similar metabolic behaviour in each. Since the only genetically-modified glyphosate-tolerant crops that are relevant in trade are the EPSPS modified crops, which show metabolic behaviour of glyphosate consistent with conventional crops, general residues definitions can be proposed as above, applicable to other crop groups and to include enforcement of MRLs in imported crops.

5. Metabolism in livestock

Revised and expanded summaries have been provided for the animal metabolism studies, including details of standards used, analytical procedures applied and extraction schemes.

In one poultry study (CA 6.2.2/004), matrices with lower residues received less extensive fractionation than matrices with higher residues and the measured ratios of residues in the fractions with high residues were used to estimate ratios of residues in the fractions that were not further fractionated. Specifically, the measured ratio of glyphosate, AMPA and unknown 1 in fraction 13 of liver and thigh muscle was used to calculate the occurrence of glyphosate, AMPA and unknown 1 in other fractions (i.e. fractions 7, 13, 14, 28 and 29 for liver and fractions 7, 9, 11, 13, 14, 19, 21 and 22 for thigh muscle).

Further information has been provided on the conjugates found in poultry matrices after dosing with glyphosate trimesium and comparisons made with the potential formation of conjugates in ruminant tissue in the metabolism study where ruminants were fed with glyphosate trimesium. In the poultry metabolism study dosed with glyphosate trimesium (CA 6.2.2/004) extraction of residues was performed using different ratios of 0.1M HCl/chloroform (v/v). In the ruminant metabolism study dosed with glyphosate trimesium (CA 6.2.3/002), kidney, liver and muscle were extracted with 0.1 N aqueous HCl, while fat was extracted with water and chloroform, and milk (protein pellet) was extracted with chloroform. The extraction conditions applied in both studies were therefore similar but not identical.
Since conjugates were found in the poultry study, it can be concluded that the extraction conditions were not sufficient to (completely) cleave conjugates. Generally, a weak acid with extraction times of only a few minutes would not be expected to provide conditions to completely cleave polar conjugates. The report contains no details of any attempt to further investigate these conjugates. It cannot be excluded that conjugates were also present in the ruminant study; the unknown material which was not well-retained in the HPLC method applied could include conjugated material. No attempt to further investigate this unknown material is reported. Both studies are GLP compliant and it can be assumed that the reports accurately reflect the findings of the studies. Therefore, it is accepted that conjugates were found but not identified in the poultry study and may have been present but were not found in the ruminant study.

Considering the differences of extractability observed in the poultry metabolism study with glyphosate trimesium and studies with glyphosate/AMPA 9:1, it is observed that, though similar, the extraction solvents were not quite the same, the weak acid used in the glyphosate trimesium study being more polar than pure water as used in the studies with glyphosate/AMPA 9:1. Furthermore, the mixing ratios were different. Breast muscle in the glyphosate trimesium study was extracted with 0.1M HCl/chloroform in three different ratios (5:2, 2:1 and 15:7, v/v), while in the studies with glyphosate/AMPA 9:1 the extraction solvent was water/chloroform 1:1 (v/v). In the studies with glyphosate/AMPA 9:1, egg white was not extracted at all because residues in the egg white were low. Extractability from egg yolk was about 81-90%, which is comparable with the extractability of about 90% obtained in the study with glyphosate trimesium. The observed differences in extractability are deemed not to be significant given these differences in experimental conditions.

Although OECD guideline 505 says that Generally the feeding of mixtures is not recommended and needs a specific rationale, in the case of the animal metabolism studies in poultry and ruminants dosed with glyphosate/AMPA 9:1, it is noted that these studies were conducted before guideline 505 became applicable, that the ratio of 9:1 approximates typical residues found in crop residue trials and, as noted above, the metabolic pathways and distributions in the mixture studies are generally similar to those in

studies conducted with glyphosate only. Glyphosate accounted for the main part of radioactive residues in all studies. Furthermore, AMPA was identified as a major metabolite in several commodities. Therefore, it can be concluded that the use of a mixture did not have an impact on the observed nature of the residues. It can be reasonably expected that this is also true for the magnitude of the residue.

For obvious reasons of animal welfare, no comparative studies on the effects, if any, of different salts on the metabolism in animals are available. Glyphosate is, or has been, available with a number of different counter-ions. However, in general, for ionic substances, the uptake and behaviour of one ion are not affected by the nature of the counter-ion. Specifically, the trimesium cation has no influence on the uptake of glyphosate by plants and there is no reason to suppose that this cation has any effect on the behaviour of the glyphosate anion in animals. In particular, during passage through the animal digestive system and tissues, the glyphosate anion and its cation (whichever ion that might be) will be completely dissociated. There are effectively four metabolism studies for glyphosate in poultry and four in ruminants, one in each species with the trimesium salt and three with other salts. There are no evident differences in the identified metabolites, pathways or abundances of metabolites between the studies conducted with the trimesium salt and those conducted with other salts. The metabolism studies conducted using the glyphosate trimesium salt are therefore applicable and should be considered in the context of the current submission to assess the metabolism in animals of the active substance (glyphosate acid) and the isopropylammonium salt in the representative formulation.

Further details have been provided on the attempted identification and characterisation of an unknown metabolite observed at 32% TRR (0.021 mg eq/kg) in milk in the goat metabolism studies conducted with glyphosate/AMPA 9:1 (CA 6.2.3/003 and CA 6.2.3/004). The unknown residue eluted in the void volume during the cation exchange HPLC analysis and represented 23.5 - 31.6 % TRR (0.005 - 0.021 mg eq/kg). Details of the identification/characterisation attempts for this unknown are reported in study CA 6.2.3/004 and summarised here.
The unknown was separated from the glyphosate and AMPA contained in the milk by column chromatography using an Fe(III)-impregnated chelating resin column. While glyphosate and AMPA were retained on this column at pH 2.1, the unknown residue was not retained and was collected in the initial wash. The eluant containing the unknown was neutralised, filtered and lyophilised. A portion of the lyophilised residue was soluble in methanol (72.3 -76.3 %). The methanol solution was desalted by passage through a strong cation exchange column and the eluant allowed to stand for several days, forming a white crystalline precipitate. The precipitate was found to contain greater than 70% of the radioactivity initially present in the methanol solution. The precipitate was submitted to gel filtration Fast Protein Liquid Chromatography (FPLC) to further purify and size the unknown residue in milk. The fractions were analysed by liquid scintillation counting with a mixture of four protein standards of varying molecular weight. The molecular weight of the unknown could be estimated by retention time relative to the protein standards. Analysis of the solubilised precipitate by gel filtration chromatography demonstrated that the radioactivity eluted as a band corresponding to a molecular weight of less than 6,500 Daltons. The precipitate was also subjected to strong acid digestion (6N HCl, 110 °C, 24 h), producing a black charred residue, indicative of the presence of sugars. Extraction of the charred residue with 0.2N citrate buffer pH 2.2 failed to recover any radioactivity, indicating that the radioactivity was bound to the charred residue. However, amino acid analyses of the citrate buffer extract detected the presence of a significant amount of amino acids. These data suggested that the unknown residue was associated with low molecular weight proteins or glycoproteins.

6. Relevance of glyphosate trimesium studies

Glyphosate is acidic and is often formulated as a salt for ease of application and handling. The glyphosate [N-(phosphonomethyl)glycine] anion is paired with various cations as its counter-ion, one of which, historically, was the trimethylsulfonium (trimesium) ion. Glyphosate is no longer used or applied as the trimesium salt; glyphosate salts relevant to the current submission are: isopropylamine-salt, potassium-salt, ammonium-salt and dimethylammonium-salt.

In general, for ionic substances, the uptake and behaviour of one ion are not affected by the nature of the counter-ion; in the context of glyphosate salts, the cation and anion will dissociate under conditions typical of spraying/application and plant biochemistry. Specifically, the trimesium cation has no influence on the uptake of glyphosate by plants and there is no reason to suppose that this cation has any effect on the behaviour of the glyphosate anion in animals. In particular, during passage through the animal digestive system and tissues, the glyphosate anion and its cation (whichever ion that might be) will be completely dissociated.
At the request of EFSA the GRG provided and reviewed the following publications during the stop-the clock :

- Li et al., 2005, "Influence of formulation and glyphosate salt on absorption and translocation in three annual weeds," Weed Science 53(2), 153-159, (1 March 2005)
- Satchivi et al. “Absorption and translocation of glyphosate isopropylamine and trimethylsulfonium in Abutilon theophrasti and Setaria faberi”, Weed Science 48:675–679 (2000)

These two papers provide experimental support to the theoretical considerations of ionisation and dissociation and show that in practice the type of cation employed in a glyphosate formulation, including the trimesium cation, does not affect the uptake of the glyphosate anion by plants, as the observed differences (if any) are minor and more likely to be the result of differences in the formulation components than the type of salt.

There are four metabolism studies for glyphosate in poultry and four in ruminants, one in each species with the trimesium salt of glyphosate, and three with other test items, including glyphosate, a mixture of the glyphosate and AMPA sodium salts or the sodium salt of *N*-acetyl glyphosate. There are no evident differences in the identified metabolites and pathways between the studies conducted with the trimesium salt and those conducted with the other test items. The observed differences in residue levels may be explained by the different dose levels, different study durations, by the nature of the anion (e.g. *N*-acetyl glyphosate vs. glyphosate) as well as the normal variability between individuals.
Based on the above, it is concluded that the plant and animal metabolism studies conducted using the glyphosate trimesium salt are applicable to understanding the metabolism of glyphosate and its isopropylammonium salt and should be considered in the context of the current submission. The same applies to other types of studies conducted with the glyphosate trimesium salt (e.g. storage stability studies or field residue studies). These studies also provide relevant information to support the representative uses of glyphosate as its isopropylammonium salt.

7. Residues in primary crops

In the dRAR of August 2021 the RMS expressed reservations about the validity of some of the submitted residue trials due to concerns about the extraction efficiency of the used residue analytical methods and/or about the storage stability of AMPA. As mentioned earlier in this document, supplementary data on both topics were submitted in the context of the stop-the-clock. These supplementary data are expected to address the concerns raised by the RMS and to confirm the validity and reliability of the respective residue studies.

In the Renewal dossier three types of representative uses are relevant to edible crops :

- Weed control in orchards (citrus, tree nuts, pome fruit, stone fruits, kiwi, banana, olives) and vineyards
- Pre-sowing or pre-planting application in vegetables (root & tuber vegetables, bulb vegetables, fruiting vegetables, brassica, leaf & stem vegetables, sugar beet)
- Inter-row application in vegetables (root & tuber vegetables, bulb vegetables, fruiting vegetables, legume vegetables, leaf vegetables)

For each type of use, several "variants" differing in application rates and/or number of applications are supported. The data provided in the residue section aim at supporting the critical use within each category of representative use, although they do not always exactly match this critical use :

- While the critical use in orchards and vineyards consists in two applications at 1.44 kg ae/ha with a PHI of 7 days, the submitted trials were conducted with a single application at about or above twice this rate (2.88 kg ae/ha). Furthermore, in many cases sampling was performed on the day of application only. The residues were not determined at the PHI of 7 days. Since, based on the metabolism studies, no uptake of residues from the ground is expected in fruit trees, the investigated use (with a single use at or above the maximum yearly application, just before harvest) represents a worst case compared to the critical GAP. And since, in spite of this, the residue trials all showed residues of parent glyphosate and AMPA < LOQ, they adequately support the critical representative use.
- While the critical use for pre-sowing or pre-planting application in vegetables consists in two applications at 1.08 kg ae/ha, the submitted trials were conducted with a single application at about or above twice this rate (2.16 kg ae/ha). As application is conducted at an early stage, leaving enough time for uptake of residues from soil, if any, the investigated use (with a single use at or above the maximum yearly application) is expected to result in similar or higher residues compared to the critical GAP. And since, in spite of this, the residue trials all showed residues of parent glyphosate and AMPA < LOQ, they adequately support the critical representative use.
- The critical use for inter-row application in vegetables consists in one application at 1.08 kg ae/ha until BBCH 20. The submitted trials were conducted at about this rate and, therefore, support the critical GAP.

Owing to the "no residue" situation (i.e. residues are < LOQ) and in line with the approach taken by EFSA in the Article 12 Review of the existing MRLs of glyphosate, the representative uses may be supported based on a limited set of residue trials. In the Article 12 review it was deemed acceptable to extrapolate between all types of top fruits. Furthermore, for the uses in vegetables two trials were deemed appropriate to confirm the no residue situation. Based on this approach, the dRAR of August 2021 includes enough residue trials to support the representative uses. However, in the context of the

stop-the-clock, the applicant submitted 16 supplementary trials for the pre-sowing or pre-planting use in vegetables, all of which showed residues of glyphosate and AMPA < 0.025 mg/kg (LOQ).

8. Residues in livestock commodities

Several livestock feeding studies are available to estimate the transfer of glyphosate-derived residues in food commodities of animal origin:

- A poultry feeding study, a cow feeding study and a pig feeding study in which the animals were dosed with a 9:1 mixture of glyphosate and AMPA.
- A poultry feeding study and a cow feeding study in which the animals were dosed with glyphosate trimesium.
- A poultry feeding study and a cow feeding study in which the animals were dosed with N acetyl glyphosate.

In the dRAR of August 2021 the RMS expressed reservations about the validity of some of these studies due to concerns about the extraction efficiency of the used residue analytical methods and/or about the storage stability of glyphosate and AMPA in animal matrices. Position papers were submitted in the context of the stop-the-clock to address these concerns. Summaries of the provided information and arguments may also be found earlier in this document.

The dietary burden of livestock which results from the representative uses is low since the residues of parent glyphosate and AMPA in primary crops were always below the LOQ (0.05 mg/kg per analyte). Based on the on-going limited field rotational crop study, higher residues can occur in rotational crops, specifically leafy crops and cereals, and these residues also must be considered when evaluating the dietary burden of livestock. Since, pending the conduct of extended field rotational crop trials, the available information on residues in rotational crops is incomplete, the dietary burden of livestock cannot be estimated accurately. However, based on rough estimates, it is obvious that the feeding studies were usually conducted at dose levels that significantly exceed the expected dietary burdens or, when the 1X dose level is below the expected dietary burden, the other dose levels exceed the dietary burden.
Based on the results of the feeding studies and considering the level of exaggeration of these studies with respect to the expected dietary burden, it may be concluded that the representative uses do not lead to residues of glyphosate and AMPA in food of animal origin above the LOQ of the respective feeding studies. Therefore, the representative uses do not make it necessary to amend the existing MRLs for glyphosate in food of animal origin. Furthermore, the residues of glyphosate and AMPA in food of animal origin are safe for consumers.

In the above reasoning, the nature of the residue in feed is not taken into account. Implicitly, it is assumed that all potential residue components (parent glyphosate, AMPA and *N*-acetyl glyphosate) behave in a similar way in the organism of livestock and that the transfer factors derived when administering one of them (e.g. *N*-acetyl glyphosate) may be extrapolated to the two other ones (e.g. glyphosate and AMPA). Based on structural similarities this is a reasonable assumption. However, it's difficult to confirm this assumption based on the feeding studies since, besides the nature of the test item (9:1 mixture of glyphosate and AMPA, glyphosate trimesium or *N*-acetyl glyphosate), these studies differ by a couple of other parameters which may equally impact the transfer factors.

Overall and in spite of some possible deficiencies, the fact that three feeding studies with different test items are available for each poultry and ruminants is considered to allow a robust assessment of the residues in food of animal origin.

9. Nature of residues in processed commodities

Questions were received about the high temperature hydrolysis studies for AMPA & *N*-acetyl AMPA as well as for *N*-acetyl glyphosate.

Study 1 CA 6.5.1/001:
This study aimed at investigating the stability or possible degradation of AMPA and *N*-acetyl AMPA under hydrolytic conditions representative of the main processing procedures. In accordance with the guideline OECD 507 this type of study is normally conducted with radiolabelled test substances since any degradate representing more than 10% of the initial test item concentration must be identified. However, the hydrolysis study for AMPA and *N*-acetyl AMPA was conducted with cold (non-radiolabelled) test substances. This is because, due to short time frame between the AIR 2 renewal and the submission date of AIR 5, there was not enough time to synthesize the radiolabelled test substances prior to the hydrolysis study. Furthermore, based on the known hydrolytic behaviour of parent glyphosate and considering the chemical structure of the test substances, no significant degradation was expected and, hence, no need to identify degradation products.
Despite the deviation to the test guideline of using unlabelled material, the study can be regarded as valid for the following reasons:

– The analytical method was successfully validated for the determination of AMPA and *N*-acetyl AMPA in buffered solutions. Samples were spiked with the analytes at 2 fortification levels, namely at the LOQ and 22 x LOQ. All average recovery values (mean of 5 replicates per fortification level and analyte) ranged from 95.5% to 105.0% with RSDs from 1.4 to 8.8%.
– No significant change in the concentration of test items, sample weights or pH-values was observed during the hydrolysis tests under conditions representative of pasteurisation (pH 4), baking, brewing and boiling (pH 5) and sterilisation (pH 6). The (apparent) decline of the test item concentration did not exceed 6.9 % (estimated for AMPA under conditions representative of sterilisation). It is concluded that no single degradate representing more than 10% of the initial test item concentration and hence requiring identification was formed during the hydrolysis tests.

Since the analytical method produced accurate and repeatable results and as the decline of the test items (if any) was shown to be less than 10 %, the study is considered to be fit for purpose although it was not conducted with radiolabelled test items.

Study 3, CA 6.5.1/003
This study aimed at investigating the stability or possible degradation of *N*-acetyl glyphosate under hydrolytic conditions representative of the main processing procedures.
Besides the peak of the test item, the chromatograms of the test solutions after hydrolysis at elevated temperature also showed minor peaks which – on average and collectively – did not exceed 4.7% of the applied radioactivity or 0.055 µg/mL. This concentration of 0.055 µg/mL must be put in perspective owing to the high test item concentration of 1.18 µg/mL before hydrolysis.
The same minor peaks were also observed in the chromatograms of the test solutions at pH 4, 5 and 6 which were not heated. Collectively and on average these peaks accounted for no more than 5.5% of the applied radioactivity and no more than 0.065 µg/mL in the unheated test solutions.
Furthermore, similar signals were also present in the chromatogram of the pure test item and, therefore, may correspond to impurities of the test item.

Further identification of the minor peaks was not attempted but it is important to note that collectively and a fortiori individually they represent a very low percentage of the applied radioactivity, for which identification is not required according to the OECD guideline 507.
In summary, the minor peaks observed in the chromatograms of the test item solutions after hydrolysis at elevated temperature were also observed at comparable levels in the unheated solutions and were present in the chromatogram of the pure test item. Hence, they may correspond to impurities of the test item (of slightly lower polarity). Altogether, the peaks did not exceed 5.5% of the applied radioactivity. Therefore, their identification is not triggered by the OECD guideline 507.

10. Residues in rotational crops

Based on the available confined rotational crop studies, the presence of residues of glyphosate and/or AMPA > LOQ in rotational crops cannot be excluded. Therefore, a limited field rotational crop study is triggered.

In the context of the stop-the-clock, the applicant submitted the interim report for a limited field rotational crop study which is currently on-going at two sites (one in Germany and one in Spain). At each site, bare soil was treated at the nominal rates of 3.18 kg ae/ha glyphosate and 2.86 kg/ha AMPA. These application rates were selected to cover the possible plateau level of glyphosate and AMPA residues in soil after repeated use of glyphosate at the maximum yearly rate for many years. Carrots, lettuce and wheat were sown after nominal plant-back intervals of 27±2 days, 135±10 days and 332±10 days. Soil samples (0-30 cm) were taken for analysis shortly before seeding.

The interim report provides the results for the two first plant-back intervals except for the wheat samples of the second plant-back interval in the Spanish trial. On the treated plots :

- No residues of glyphosate or AMPA above the LOQ of 0.025 mg/kg were found in carrots (root and leaves)
- Lettuce head showed residues of glyphosate < 0.025 mg/kg while in one trial AMPA was present at up to 0.049 mg/kg.
- Wheat forage showed residues of glyphosate < 0.025 mg/kg while in one trial AMPA was present at up to 0.039 mg/kg.
- Wheat grain showed residues of glyphosate < 0.025 mg/kg while AMPA was present at levels up to 0.18 mg/kg.
- No residues of glyphosate or AMPA above the LOQ of 0.025 mg/kg were found in wheat straw.

Two control samples were found to contain residues above the LOQ (0.029 mg/kg of glyphosate in a carrot root sample and 0.041 mg/kg of AMPA in a wheat forage sample). The reason for these findings could not be clarified, especially since the corresponding treated samples showed residues below the LOQ.

Based on these results, further field rotational crop trials will be needed to more accurately determine the levels of glyphosate and AMPA residues in the main types of rotational crops. However, based on the residue levels found so far and considering the favourable ADI and ARfD of glyphosate, the residues of glyphosate and AMPA in rotational crops are not expected to adversely impact consumer safety.

11. Residues in honey

In one of the four honey trials submitted in the renewal dossier, the honey yield was extremely low (6 g). For this reason, the affected trial was not considered valid by the RMS.

In order to fill the data gap, a supplementary honey trial was conducted in 2021 and submitted to the RMS in the context of the stop-the-clock. The trial design was similar to the protocol of the previous trials with a single application to flowering phacelia at the rate of ca. 2160 g ae/ha. However, two duplicate treated plots were established instead of a single treated plot in the previous trials and there was no need to move the hives to a monitoring site to obtain ripe honey. On each treated plot, 50-129 g of ripe honey was collected. The honey samples were found to contain 2.8 4.5 mg/kg of glyphosate and < 0.025 mg/kg of AMPA residues. These results are consistent with those of the three previous (valid) trials, which showed 0.87-6.9 mg/kg of glyphosate and < 0.025 0.028 mg/kg of AMPA.

Based on the four valid trials and according to the OECD MRL calculator, an MRL of 15 mg/kg is derived for parent glyphosate in honey. This value, however, significantly exceeds the levels reported from monitoring analyses, both in the EU and in other geographies. The scenario investigated in the honey trials implies that, for several days, bees exclusively forage on flowering weeds treated with glyphosate. In practice, this scenario has only a low probability to occur, and this may explain - at least in part - the difference between the MRL of 15 mg/kg derived from the honey trials and the residue levels from monitoring analyses.

References

1. KCA 6.1_anonymous_2022_113898-045_GRG.pdf – Response to Request RESIDUES 01, CA 6.1 Storage stability.

2. KCA 6.1_anonymous_2022_113898-038_GRG.pdf – Response to Request RESIDUES 3, CA 6.1 Storage stability (plant).

3. KCA 6.1_anonymous_2022_113898-039_GRG.pdf – Response to Request RESIDUES 04, CA 6.1 Storage stability

4. KCA 6.1_anonymous_2022_113898-040_GRG.pdf – Response to Request RESIDUES 05, CA 6.1 Storage stability (animal)

5. KCA 6.1_KCA 6.4_anonymous_2022_113898-041_GRG.pdf – Response to Request RESIDUES 6, CA 6.1 Storage stability (animal) / CA 6.4 Feeding studies

6. KCA 6.1_anonymous_2022_113898-042_GRG.pdf – Response to Request RESIDUES 8, CA 6.1 Storage stability (maize)

7. KCA 6.1_KCA 4.1.2_[redacted]_2021_S19-22488_GRG.pdf – Storage Stability of AMPA in Dry Bean under Deep Frozen Conditions- Final Interim Report

8. KCA 6.2.1_anonymous_113898-043_GRG.pdf – Response to Request RESIDUES 09, CA 6.2.1 Metabolism, distribution and expression of residues in plants

9. KCA 6.2.1_Li et al_2005_Influence of formulation_LIT.pdf - Influence of formulation and glyphosate salt on absorption and translocation in three annual weeds

10. KCA 6.2.1_Satchivi et al_2000_Absorption and translocation_LIT.pdf - Absorption and translocation of glyphosate isopropylamine and trimethylsulfonium salts in Abutilon theophrasti and Setaria faberi

11. KCA 6.2.2_KCA_6.2.3_anonymous_113898-044_GRG pdf – Response to Request RESIDUES 10, 11, 13, 16, CA 6.2.2 Poultry / CA 6.2.3 Lactating ruminants

12. KCA 6.2.2_KCA_6.2.3_anonymous_113898-046_GRG.pdf – Response to Request RESIDUES 12, CA 6.2.2 Metabolism in poultry / CA 6.2.3 Metabolism in ruminants

13. KCA 6.2.2_anonymous_113898-048_GRG.pdf – Response to Request RESIDUES 14, CA 6.2.2 Poultry

14. KCA 6.2.2_KCA_6.2.3_anonymous_113898-049_GRG.pdf – Response to Request RESIDUES 15, CA 6.2.2 Poultry / CA 6.2.3 Lactating ruminants

15. KCA 6.2.2_KCA 6.2.3_KCA 6.4_anonymous_2022_113898-055_GRG.pdf – Response to Request RESIDUES 21, CA 6.2.2 Poultry / CA 6.2.3 Lactating ruminants / CA 6.4 Feeding studies

16. KCA_6.2.3_anonymous_113898-051_GRG.pdf – Response to Request RESIDUES 17, CA 6.2.3 Lactating ruminants

17. KCA 6.3_anonymous_2022_113898-053_GRG pdf – Response to Request RESIDUES 19, CA 6.3 Magnitude of residues

18. KCA 6.3.1_KCA 6.5.3_anonymous_2022_113898-052_GRG pdf – Response to Request RESIDUES 18, CA 6.3.1 Orchard use/ CA 6.5.3 Processing

19. KCA 6.3.1_anonymous_2022_113898-054_GRG.pdf – Response to Request RESIDUES 20, CA 6.3.1 Orchard use

20. KCA 6.5.1_anonymous_2022_113898-057_GRG.pdf – Response to Request RESIDUES 23, CA 6.5.1 Nature of the Residue

21. KCA 6.6.2_[redacted] et al_2022_S20-01527_GRG.pdf - Determination of the residues of Glyphosate and AMPA in/on soil and the field rotational crop carrot, lettuce and wheat after spray application of Glyphosate and AMPA to bare soil in Germany and Spain - Amendment No.1 to Interim Report –

22. KCA 6.10.1_[redacted]_2021_S21-05797_GRG.pdf - Determination of Residues of Glyphosate in Honey after One Application in *Phacelia tanacetifolia* at One Site in Germany 2021