Comments made on the document "Glyphosate_RAR_14_Volume_3CA_B6.7 - B6.10_2021-08-10", section B.6.10.1 Literature search

Générations Futures maked the count of the percentage of the public literature studies taken into account in the RAR/CLH report of glyphosate. The results are the followings:

For the toxicity endpoints, on the 1550 public studies found in the search:

- 76.9% (1192) are considered as non relevant
- 5% (79) are considered as relevant and providing data for establishing or refining risk assessment parameters (category A)
- 4% (63) are considered as relevant, classified in category A and reliable with restrictions
- 0.7% (11) are considered as relevant, classified in category A and reliable without restriction

In other words, about 95% of the public toxicity studies on glyphosate are deemed non relevant, not useful for the assessment or not reliable.

If we consider the whole toxicity and ecotoxicity studies and the studies used for the ED assessment, only 0.4% (30) of the total number of studies found in the literature search (7188) are considered as relevant and reliable without restriction.

In consequence, the public literature had no weight in the whole studies weight of evidence assessment.

These numbers are facts and raise many questions. Is it normal that the differences between academic science and regulatory science being so huge? What is the purpose of the academic science and knowledge if it can't be applied? Are the OECD compliant studies the only ones who can be used for regulatory purpose?

Beyond these figures, Générations Futures also have specific comments and questions:

1/ Comments made on the section 4.1 Rapid assessment (Page 775/1011):

Can you please provide more explanations regarding the following reasons used for excluding literature studies just by reading the title and abstract:

1-1.-*"Abstracts referring to a conference contribution that does not contain sufficient data"*

Many (> 80) conference contributions have been excluded for this reason. However, many times no abstract is available, just the title is (For instance: Mrzyk Inga, 2017. *An extended one-generation reproductive toxicity study of plant protection product containing glyphosate on rats - Androgen- and estrogen-dependent endpoints*). Then, how is it possible to say that the level of details is not sufficient? Plus, the fact that no sufficient data is available should be treated as a reliability criteria and not as a relevance criteria. The entire text from conferences contributions therefore need to be assessed before saying that theses conferences abstract are not relevant. **Générations Futures therefore asks for considering all conferences contributions as relevant at this stage and requested to be further examined.**

We remind that the conference contributions are considered as relevant source of data according to the EFSA guidance "*Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009*" (EFSA Journal 2011;9(2):2092, p. 7/49 and 17/49):

*"Scientific peer-reviewed open literature is literature that has been through a peer-review process. In this EFSA Guidance, peer review is defined as the critical assessment of manuscripts (e.g. draft journal articles, reports, or **scientific conference abstracts**) prior to publication"*

"Examples of sources of scientific peer-reviewed open literature are represented by:
*• Bibliographic databases which record documents such as journals, reports, **conference proceedings** and books;*
*• Sources other than bibliographic databases, such as reference lists of full-text journal articles (e.g. reviews); journals' tables of contents; or **websites of conferences or organisations**."*

1-2. "Study design, test system, species tested, exposure route etc are not relevant for the European regulatory purposes"

In order to be transparent, can you please explain in details which study design, test system, species tested etc are not relevant for European regulatory purposes?

Générations Futures wishes to point out that many recent studies (for instance, *Caballero et al. J Unexplored Med Data 2018; 3: 4* and *Alzualde et al. Neurotoxicology and Teratology 70 (2018) 40–50*) has revealed the relevance of aquatic species, in particular fishes, for the human assessment. This would be particularly right for the genotoxicity endpoint. ANSES (one of the 4 RMS) itself acknowledges this in the document "Inception impact assessment related to the revision of CLP Regulation - ANSES comments": "*The use of animal data outside the rodent model should be discussed in particular for mutagenicity. There would be a huge potential impact on animal experimentation and this would make it possible to take into account environmental data (example: mutagenicity observed in fish), by aligning CLP with the One Health concept "*
Therefore, all studies performed with glyphosate on aquatic species (at least on fishes) should be considered as relevant for the human effect and risk assessment.

Regarding the relevant routes of exposure, Générations Futures highlights that all exposure routes may give relevant information, even if this information is only supportive. This is well explained in the Guideline on the application of the CLP criteria, version 5.0, 2017 p.384/647 with the example of the carcinogenicity endpoint:
"*Sometimes other non-physiological routes are used, such as intra-muscular, sub-cutaneous, intra-peritoneal and intra-tracheal injections or instillations. Findings from studies using these routes may provide useful information but should be considered with caution*".
We therefore ask for not excluding after the rapid assessment the studies performed using other routes of exposure than the oral dermal and inhalation ones.

1-3. "Publications not dealing with EU representative uses/conditions "

In order to be transparent, can you please explain in details which uses/conditions are not relevant and why?

1-4. ***Générations futures also noticed in the in the list of excluded studies, many studies relating the effect of glyphosate at cellular and molecular levels.***
For example the study of "*Ugarte, Ricardo, 2014. Interaction between glyphosate and mitochondrial succinate dehydrogenase"* However, such information regarding the mechanism of action of the substance should be considered as relevant. This is stipulated in both CLP guidance and the EFSA Journal 2011;9(2):2092, p.47/49: "*Studies that may be useful for the interpretation of other studies present in the dossier, but which do not fall under a particular toxicological endpoint, would be relevant. Examples of such studies are […] studies clarifying the mode of action of the active substance*"

Generations Futures therefore asks for the inclusion after the rapid assessment of all mechanistic studies dealing with effect of glyphosate at cellular and molecular levels. These studies need to be further examined.

2/ Comments on the section 4.2 Detailed assessment (page 776/1011):

Can you please provide more explanations regarding the following reasons used for excluding literature studies after the detailed assessment:

- " Publications dealing with a Roundup formulation that is not the representative formulation for the AIR5 renewal in Europe"

This criteria concerns hundreds of public literature studies. **That's why it should be treated with all the transparency**. As compositions of formulations are confidential, we have no way to verify the fact that formulations used outside the UE are actually not relevant. Which co-formulants are different in comparison with the representative formulation for the AIR5 renewal in Europe? What is the percentage of different co-formulants? What is the (eco)toxicity of these co-formulants?

Did the RMS do the analysis and the comparison of the different formulations? Did a referent document was used for performing such a comparison (for instance the "*Guidance document on significant and non-significant changes of the chemical composition of authorised plant protection products under Regulation (EC) No 1107/2009 of the EU Parliament and Council on placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EE*", SANCO/12638/2011 20 November 2012 rev. 2) ?

3/ Comments on the reliability assessment

This comment regards the reliability assessment performed for all applicant and public literature studies. No reference towards a specific document can therefore be made.

Can you please explain in details the method used for the reliability assessment of the studies? What are the criteria used and **what is the weight of each criteria in the reliability assessment**? A table summarising a list of reliability criteria is present below each public literature study. Can you please explain why this kind of table is not present for any of the applicant study?

We remind here the principle of systematic review laid down in the EFSA guidance (EFSA Journal 2011;9(2):2092, p.11/49) : "*methodological rigour; transparency; and reproducibility*". **This principles should also apply to the reliability assessment of applicant studies**.

Indeed, it is important to have the same assessment for applicant and public literature studies. In this dossier, the reliability assessment of applicant studies seems to be less strict than the one performed for public studies. Many times, the assessment just boils down to saying that applicant studies are GLP compliant and follow the OECD guideline even if some deviations exists. However, in many cases applicant studies do not meet the acceptability criteria of the OECD guideline and are nevertheless considered as reliable or reliable with restrictions. **It is therefore very needed to know in details the criteria used and their corresponding weight in the reliability assessment**.

Conclusion

Considering all the flaws described above regarding the selection of relevant studies and the lack of transparency of the reliability assessment of the studies, Générations Futures considers that the legal requirements regarding the literature search (article 8.5 of the Regulation 1107/2009) are not met.

No decision regarding the classification of glyphosate and its renewal can be made without taking into account all the actual relevant studies.

Comments of Générations Futures on the genotoxicity endpoint

In this document, major points are in **bold** and questions to authorities are highlighted **in blue**.

1/ Comments regarding data obtained in non-standard organisms

In the document "clh_rep_glyphosate_en_202106", section 2.6.4, p177/868, it is stated that "*against the background of an extremely large database using standard test systems (bacteria, mammalian cells and mammals), data obtained in non-standard test systems (e.g. plant, insect, worm, fish etc.) was not considered for classification of health related endpoints even if performed with the active ingredient*."

However, more and more studies (for instance Caballero *et al*. J Unexplored Med Data 2018;3:4 / Alzualde *et al*. Neurotoxicology and Teratology 70 (2018) 40–50) revealed the relevance of aquatic species, in particular fishes, for the human assessment and especially for the mutagenicity assessment.

ANSES itself acknowledges this during the public consultation for the revision of the CLP regulation (Inception impact assessment related to the revision of CLP Regulation – ANSES comments): "*The use of animal data outside the rodent model should be discussed in particular for mutagenicity. There would be a huge potential impact on animal experimentation and this would make it possible to take into account environmental data (example: mutagenicity observed in fish), by aligning CLP with the One Health concept*".

Taking into account the actual relevance of aquatic species for mutagenicity assessment, Générations Futures therefore asks for the integration of all mutagenicity data obtained in fishes for the mutagenicity assessment. No decision can be made regarding the genotoxicity potential of glyphosate without considering these data.

2/ Comments on the reliability assessment of applicant studies and weight of evidence assessment

Can you please explain in details the method used for the reliability assessment of the studies? What are the criteria used and what is the weight of each criteria in the reliability assessment?
A table summarising a list of reliability criteria is present below each public literature study. **Can you please explain why this kind of table is not present for any of the applicant study?**
Indeed, it is important to have the same assessment for applicant and public literature studies. In this dossier, the reliability assessment of applicant studies seems to be less strict than the one performed for public studies. Many times, the assessment just boils down to saying that applicant studies are GLP compliant and follow the OECD guideline even if some deviations exists. However, in many cases applicant studies do not meet the acceptability criteria of the OECD guideline and are nevertheless considered as reliable or reliable with restrictions. 2 examples of this situation are described below for in vitro chromosome aberration tests and in vivo micronucleus tests

<u>First example: In the report N°CTL/P/6050</u>, the following deviations were noted (Document "Glyphosate_RAR_11_Volume_3CA_B-6.4_2021-08-10", p109/396):

- "*Only 200 instead of 300 cells in metaphase were evaluated per condition for test item treated cultures and only 25 metaphases were evaluated for the corresponding positive control*"
- "Data on the laboratory's historical control range were not provided.

However, the number of cells in metaphase analysed and the inclusion of HCD are part of the acceptability criteria of the OECD guideline 473 (Paragraph 43: "*Acceptance of a test is based on the following criteria: The concurrent negative control is considered acceptable for addition to the laboratory historical negative control database as described in paragraph 39. Concurrent positive controls (see paragraph 26) should induce responses that are compatible with those generated in the*

historical positive control data base and produce a statistically significant increase compared with the concurrent negative control. […] Adequate number of cells and concentrations are analysable").
Therefore 3 out of 6 acceptability criteria of the OECD guideline are not met.
In addition, many other deviations are noted: "*There are no information on numerical chromosome aberrations given (polyploidy index could not be determined). Positive controls were only included for the 20 hour sampling time point. A short-term exposure in the absence of metabolic activation as recommended by OECD 473 (2016) was not included. Although pH changes were observed, there was no attempt to buffer the pH change. Acceptance criteria were not specified and evaluation criteria were inconsistent with test guideline OECD 473 (2016)*".

Despite all of this, the study is considered by the RMS as acceptable with restrictions. These deviations are considered as "*minor deviations"* and *"not considered to affect the validity or integrity of the data*" (clh_rep_glyphosate_en_202106, p216/868). **As 3 acceptability criteria are not met (and many other deviations exist), can you please explain why these deviations are considered as minor and not affecting the validity or integrity of data?**
In its overall conclusion on *in vitro* genotoxicity tests (clh_rep_glyphosate_en_202106, p219/868), RMS say that "*Further, the majority of in vitro chromosomal aberration tests and micronucleus tests were negative All studies performed according to GLP resulted in negative findings. Several in vitro indicator tests gave positive results for induction of SCE and DNA strand breaks (comet assay) mainly at cytotoxic concentrations but a negative result for induction of DNA repair (UDS). However, for all these studies several methodological shortcomings were identified. Thus, it is concluded that no obvious mutagenicity/genotoxicity could be evidenced for glyphosate based on acceptable in vitro data*."
This is forgetting that 1/ **compliance with GLP is NOT an indicator to be used for the reliability assessment and 2/ that these GLP studies present major deficiencies as they do not meet the half of acceptability criteria of the OECD guideline.**

Second example: In vivo micronucleus (MN) tests (Reports N° 485-1-06-4696, 1479200, 1158500, 2060/014, G12.79/99, /P/4954, 889-MUT.MN, 12324, 23917)
In these studies, three major flaws were noted:
- No evidence for bone marrow exposure has been provided for all these MN studies. However this is a condition to be met in order to say that the test substance is clearly negative. Without this evidence of target exposure, no conclusion can be drawn (see paragraph 3 of the present document)
- None study analyses the sufficient number of cells compared to current recommendations of the OECD guideline 474 (2016) : Instead of 4000 erythrocytes (type of blood cells produced in the bone marrow studied in the test) to analyse, only 2000 erythrocytes, and even 1000 for a study (Report N° G12.79/99), have been studied. However, the number of cells to be analysed is part of the acceptability criteria of the guideline 474 of 2016: "*The following criteria determine the acceptability of the test: […] c) The appropriate number of doses and cells is analysed"*.
- Historical laboratory data was not provided at all for 6/9 studies and partially for 3/9. However, these data are part of the study's acceptability criteria according to OECD guideline 474. More over, **it is highlighted that for public literature, the lack of HCD is considered as a major deficiencies**. For instance, regarding the study of Milic *et al*. , RMS stated that (clh_rep_glyphosate_en_202106, p.222/868): "*Based on the information provided it is not possible to determine whether the acceptance criteria of the assay are met as no information is provided on the compatibility of the positive and negative controls with the laboratory's historical control database. Therefore it is not possible to determine whether the outcome of the Comet assay should be considered positive, negative or equivocal*".

Regarding all these deviations, can you please explain why these studies are considered as reliable or reliable with restrictions?

Despite these major deviations, the studies are considered as acceptable or acceptable with restrictions and they thus weighed a lot in the final weight of evidence assessment. The argument repeated several times "*RMS is of the opinion that these [positive] studies do not alter the conclusion that* <u>*based on the extensive database of guideline compliant in vivo somatic cell mutagenicity studies*</u> *it is concluded that glyphosate is not genotoxic to rodents"* is therefore not correct.

<u>In conclusion</u>, the method used by RMS for reliability assessment seems really unclear as, even with the major deviations detailed above, applicant studies are considered reliable or reliable with restrictions. These major deviations seem to have been forgotten during the whole studies weight of evidence assessment. **Générations Futures therefore asks for clear, transparent and equitable between applicant and public studies, methods of reliability and weigh of evidence assessments. No conclusion regarding the genotoxicity of glyphosate can be drawn without reconsidering the reliability of applicant studies, especially clastogenicity studies.**

3/ Comments on the *in vivo* micronucleus (MN) studies (OECD 474)

Bone marrow exposure has been confirmed only in one study out of the 10 available in vivo MN studies submitted by the applicant and considered as reliable or reliable with restrictions.

As stated in the report (clh_rep_glyphosate_en_202106, p.220/868):), "F*or the in vivo micronucleus studies, target organ exposure to glyphosate should be proven. However, only toxicokinetic studies showing bone marrow exposure are available for the rat but not for mice*" whereas <u>all</u> MN studies have been performed in mice. We remind here the recommendation of the EFSA Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment (EFSA Journal 2011;9(9):2379, p34/69): "***A negative result from an in vivo study would have limited or even no relevance if there was no indication from the study that the test substance reached the target tissue and if there were no other data, e.g. toxicokinetic data, on which such an assumption could be based***"

The first argument used in the report to prove the bone marrow exposure is that it has been proven in one study (out of the 14 performed MN studies). However this study (Report N°14613.402.078.14, 2015) is the only one confidential. Therefore, **Generation Futures asks for this study become available entirely, excepted for the part describing the impurities (the reason why this study is confidential).** This is very important to ensure that the plasma analysis has been well conducted in line with the recommendations laid down in the Scientific opinion "Clarification of some aspects related to genotoxicity assessment" (EFSA Journal 2017;15(12):5113, p12/25) , in particular the following ones:

- *"Is the analytical method appropriate, i.e. can it detect and quantify the actual substances to be measured for the particular evaluation (are the limit of detection, the limit of quantification and the calibrated range reported for the analytical method used and are these parameters considered adequate)?*
- *Are the blood/plasma levels measured within or below the calibrated range?*
- *Are the blood/plasma levels plausible considering, e.g. consistency among animals of the same group (i.e. how large is the variation) and among different sampling times?"*

Moreover, the fact that glyphosate has reached the bone marrow in one study, does not confirm that it has reached it in the 9 other studies.

The second argument used by the RMS to prove bone marrow exposure is "*In addition, several MN studies were conducted using intraperitoneal (IP) injection. While ensuring high systemic exposure, these caused clinical signs of toxicity consistent with systemic exposure. In one of these studies by IP administration, effects were noted which were considered to be due to bone marrow toxicity (report no. 2060/014 (CA 5.4.2/007))"*. However, the IP route of exposure does not include the metabolism of the substance after oral administration. in any case these studies can therefore be used to prove the bone marrow exposure in studies performed *via* the oral route. The relevance of such studies using

the IP route is questionable. These studies are just supportive and can't be used in the argument dealing with bone marrow exposure.

The last argument laid down by the RMS is "*Moreover, as also stated in the previous CLH-report, in a long-term study in rats (report No. 2060-0012, CA 5.5/001) the occurrence of hypoplasia in bone marrow was reported although this latter finding was confined to a very high dose*". However, MN studies have been performed in mice. Therefore, this study cannot neither be used to prove the actual bone marrow exposure in MN studies.

Overall it clearly **can't be concluded that there is sufficient evidence to prove the bone marrow** exposure in MN studies as stated by the RMS *("Overall, there is sufficient evidence to conclude that the target tissue in these studies, namely the bone marrow, was actually exposed to glyphosate. The same conclusion was drawn in the previous CLH report (BAuA, May 2016))*".

Moreover, the absence of evidence of bone marrow exposure is not the only flaws found in the applicant MN studies, as explained in the paragraph 2 of the present document.

Therefore, **the relevance and the reliability of the *in vivo* clastogenic studies submitted by the applicant is very questionable, as stated by the EFSA Journal 2011;9(9):2379. The argument used many times in the report which is that there is an "*extensive database of guideline-compliant in vivo somatic cell mutagenicity studies*" is therefore not correct: there is no extensive relevant and reliable database of *in vivo* somatic cell clastogenicity studies (we remind that the 2 *in vivo* chromosome aberration tests are considered as supportive only).**

It is therefore not possible to say that the glyphosate is not genotoxic (and especially not clastogenic) based on these *in vivo* data. It is very needed to perform another *in vivo* test, conducted with another target tissue than the bone marrow. No decision regarding the classification of glyphosate can be made without another *in vivo* test. This is stated in the EFSA Journal 2011;9(9):2379, p44/69: "*in some cases, a second in vivo test may be necessary on an alternative tissue. The comet assay can be performed in any tissue is therefore considered much more sensible than the micronucleus test"* […]"*The in vivo Comet assay is considered a useful indicator test in terms of its sensitivity to substances which cause gene mutations and/or structural chromosomal aberrations and can be used with many target tissues*".
In its opinion of February 2016 (saisine n°2015-SA-0093) the expert group mandated by Anses (GECU, groupe d'expertise collective d'urgence) acknowledges this (translate from the French) : « *although almost all the in vivo tests lead to results that are not statistically significant,* ***we do not have results of in vivo comet assay, which nevertheless seems to be the most sensitive biological parameter****. Therefore, it might be useful to perform an in vivo comet test on defined target organs (kidney and liver)*», and later in the text : « ***However, the positive results reported require confirmation with an in vivo approach on different tissues such as for example liver and kidney cells****.* ».

it therefore seems obvious that the comet assay is the most relevant *in vivo* test to be conducted. However none in vivo comet assay is available in this dossier. Again, no conclusion regarding the genotoxic potential of glyphosate can be drawn without further testing

Conclusions

Générations Futures asks for not taking any decision regarding the genotoxicity assessment and the renewal of glyphosate without taking into account <u>all</u> the followings:

1/ All actual relevant studies must be included in the literature search (see the document "Comments of Générations Futures on the literature search"

2/ All available data obtained in fishes should be taken into account in the genotoxicity assessment

3/ Applicant data and public literature studies must be assessed for their reliability in a transparent and equitable way. A clear method for the reliability assessment of both industry and public studies and a clear method of the weight of evidence assessment must be provided.

4/ The reliability of applicant studies, especially clastogenicity studies, must be re-considered, taking into account their major deviations (not meting the acceptability criteria of OECD guideline). The relevance of the *in vivo* micronucleus without any convincing demonstration of bone marrow exposure must be questioned.

5/ An *in vivo* comet assay must be conducted on target organs such as kidney or liver