

06 October 2023

Ref. BU/MT/TM/ct-OC-2023-29840988

Claire Bury
Deputy Director-General for Food Sustainability
Directorate-General for Health and Food Safety
European Commission

Subject – Request for a statement on the report by Générations Futures alleging that EFSA and ECHA disregarded certain evidence on glyphosate in their assessments

Dear Ms Bury,

On 25 September 2023 you requested EFSA and ECHA to respond to the points raised by Générations Futures in two publications from September 2023.

Our experts have carefully analysed the points raised and conclude, on this basis, that the findings in the publications do not have any impact on the overall assessment and conclusions adopted by EFSA and ECHA (ECHA's RAC opinion published in July 2022 and the EFSA conclusions published in July 2023).

ECHA and EFSA are fully committed to transparency in our processes. In line with this commitment, we have already publicly addressed many of the questions included in the publications.

For your reference, we are pleased to provide further details about our assessments, previous public responses and other issues raised in the publications in the annex to this letter.

We trust that this information is useful to you and your services and we remain available to provide continued support on this file.

Yours sincerely,

Bernhard Url
Executive Director
European Food Safety Authority

Sharon McGuinness
Executive Director
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Annex

ECHA Responses to the findings of the publication by Générations Futures

In the publication the findings of INSERM under six headings are compared with those of ECHA's Risk Assessment Committee (RAC).

RAC's independent experts assessed a large number of scientific studies and submissions from interested parties against criteria in the EU's classification, labelling and packaging regulation¹. They used a weight of evidence assessment in accordance with the CLP regulation to assess hazards including mutagenicity, reproductive toxicity, neurotoxicity and cancer. This means that well carried out and standardised studies generally are given greater weight in the overall assessment. Please note that in accordance with the relevant regulations (CLP and plant protection products), endocrine disrupting properties were assessed solely by EFSA.

All available evidence was carefully examined to arrive at the conclusion that glyphosate does not possess hazardous properties warranting classification for these hazards. No relevant findings were dismissed. RAC maintained its previous classification of glyphosate for serious eye damage and toxicity to aquatic life.

INSERM provided comments during the Consultation of the CLH report addressing the hazard classes carcinogenicity, germ cell mutagenicity, reproductive toxicity, respiratory sensitisation, STOT SE, STOT RE. The dossier submitter and RAC provided specific responses to these in the Response to comments document (published on the ECHA website at [COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION \(europa.eu\)](https://echa.europa.eu/consultation-clh-2022)²).

In addition, ECHA has previously commented publicly on some of the issues raised. For example

- ECHA's response to a report by HEAL.
- ECHA reply to MEP Bas Eickhout, on the issue of "two missing genotoxicity OECD studies".
- ECHA's dedicated webpage on glyphosate³ includes these detailed responses together with other relevant information.

The RAC opinion⁴ along with its supporting documentation was published on the ECHA website on 5 July 2022. Given the vast amount of information covered by the opinion and the public interest in the process, ECHA also published an "Explanatory note"⁵ to accompany the RAC opinion on glyphosate.

The issues raised in the publication are addressed below in the order in which they appear.

¹ Regulation (EC) No 1272/2008.

² <https://echa.europa.eu/documents/10162/8f8b6a87-8bd8-3cdd-0f70-587fbfb41beb>

³ <https://echa.europa.eu/hot-topics/glyphosate>

⁴ <https://echa.europa.eu/nl/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e185e41a77>

⁵ <https://echa.europa.eu/documents/10162/9a6bdbf8-0d3c-c029-8256-2112189a6f85>

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1. Genotoxicity and Oxidative stress

As regards the concern that key tests were not conducted due to a statement in the RAC opinion referring to the absence of specific assays in relevant target organs (OECD TG 489 “the comet assay” and OECD TG 488 “TGR”), firstly, it should be noted that the CLH process assesses available data – there is no mechanism to generate additional information. Secondly, please note that ECHA has addressed these particular issues in a letter to Bas Eickhout MEP, who raised this in the Exchange of views on 11 July 2022. ECHA addressed these concerns in our letter as follows:

“The statement quoted from the opinion related to the Comet assay and Transgenic rodent (TGR) somatic and germ cell gene mutation assays which are two particular assays among many other lines of evidence potentially informing a classification. The opinion noted the absence of these assays/studies in relevant tissues, but also noted that the biological importance of such DNA lesions (i.e., as identified from these assays) in relation to mutagenicity is equivocal, therefore the fact that some studies of this type were not included is not crucial for the conclusion”

And

“the data available for evaluation of germ cell mutagenicity is extensive and includes studies covering bacterial and mammalian cell in vitro mutagenicity assays as well as in vivo mammalian mutagenicity assays and even some human data. Furthermore, according to the opinion, the data includes studies of sufficient reliability and relevance to allow a robust evaluation, especially in the perspective of the requirements of the CLP Regulation. In RAC’s view, the data were sufficient to arrive at a robust conclusion without these assays/studies.”

As regards **Oxidative stress**, and the claim that oxidative stress was not adequately taken into account during the assessment of ECHA’s RAC.

Firstly, it is useful to explain that in the context of the CLP criteria, the primary source of evidence to inform on classification is presence of genotoxic effects or enumeration of tumours in animal studies and determination of their level of statistical significance. Many other factors can be taken into consideration, including mode of action/mechanistic considerations. Oxidative stress is a mechanism that can lead to genotoxic effects or tumour formation and therefore falls into the latter category as a factor that can be taken into consideration when assessing genotoxic effects or tumour incidences.

ECHA’s independent assessment is based on a large number of scientific studies designed to examine the hazardous properties of glyphosate, including whether it causes cancer. All available evidence was carefully examined to arrive at a conclusion. No relevant findings were dismissed. Tumour incidences and gene mutations in the available studies were examined in detail and the conclusion was that there was no convincing evidence that glyphosate induces tumours or genotoxic effects.

In the absence of clear evidence of mutations or tumours linked to glyphosate, evidence that glyphosate causes oxidative stress is not relevant for the conclusion. Findings of oxidative stress in a study are not on their own sufficient for classification. In particular,

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potential mode of action considerations arising from one study cannot provide support in the absence of convincing evidence for genotoxicity or carcinogenicity in another study.

2. Reprotoxic effects and endocrine disrupting effects

The assessment of RAC against the criteria for Reproductive toxicity is clearly set out in the RAC opinion. Endocrine disrupting properties were out of scope of RAC's assessment but assessed by EFSA. Please refer to the *EFSA Responses to the findings of the publication by Générations Futures* further down.

3. Mitochondrial toxicity

Mitochondrial toxicity is an indicator for potential toxicity, and could result in a number of different adverse effects, but observed mitochondrial toxicity does not mean that a toxic effect will actually occur. Mitochondrial toxicity is rather in the category of a "mode of action" or in other words a potential explanation for effects seen in toxicity studies. Mitochondrial toxicity is not a hazard class under CLP and therefore this type of information can only be used to understand why toxic effects would occur, rather than leading to a conclusion on its own.

In the absence of clear evidence of effects relevant to a specific hazard class under CLP, information on mitochondrial toxicity on its own cannot be definitive for classification.

As explained in the RAC opinion, all relevant data was thoroughly checked and weighed against the CLP criteria. The data was compiled by the dossier submitter; there was additional information added during ECHA's open consultation and RAC delivered its opinion weighing all of the information available. Mitochondrial toxicity was not definitive in any of the assessments made against the CLP criteria, hence it is not prominently mentioned in the RAC opinion.

We note that the studies referred to by INSERM in the context of mitochondrial toxicity are ecotoxicity studies and that Glyphosate retained its classification as toxic to aquatic life.

4. Epigenetic effects

Similar to the above, epigenetic effects fall into the category of "mode of action" or a potential explanation for effects seen in toxicity studies. In the absence of clear evidence of effects relevant to a specific hazard class under CLP, information on epigenetic effects can support the assessment, but cannot be definitive for classification.

As explained in the RAC opinion, all relevant data was thoroughly checked and weighed against the CLP criteria. The data was compiled by dossier submitter; there was additional information added during ECHA's open consultation and RAC delivered its opinion weighing all if the information available.

Information on epigenetic effects was not definitive in any of the assessments made against the CLP criteria, this is covered in the RAC opinion on page 54 under "*Mechanistic studies from public literature*".

5. Microbiota

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Effects on microbiota are not assessed under the CLP criteria. Please refer to the *EFSA Responses to the findings of the publication by Générations Futures* below.

6. Neurotoxicity

RAC's assessment of neurotoxic effects under relevant CLP hazard classes such as Specific target organ toxicity (single and repeat exposure, STOT SE and RE) and Developmental toxicity is clearly set out in the RAC opinion. The claim in the publication that nine studies were excluded from the assessment as part of the renewal process is addressed by EFSA below.

ECHA conclusions

We trust that the above information helps to reassure you and the public that the latest assessment of glyphosate by ECHA's Risk Assessment Committee was robust and complete within the framework of the criteria for classification under the CLP regulation.

The RAC, composed of independent experts from all EU Member States, has a long history of rigorous assessments against the criteria set out under the CLP Regulation. The integrity of RAC as the competent body to opine on hazard classification is well established with 550 opinions adopted to date. Substances routinely receive recommendation from RAC for the most severe of classifications. To date, 145 different substances have received recommendations for the most severe hazard classification as CMR category 1. This demonstrates that the system works to deliver scientifically reliable and legally sound opinions, to better inform on the hazards of chemicals and allow actions on the most harmful chemicals to mitigate risks.

ECHA remains committed to open, transparent discussion and resolution of any lingering concerns.

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EFSA Responses to the findings of the publication by Générations Futures

In the responses provided by EFSA, reference is made to the final Renewal Assessment Report (RAR, 2023), to the Peer Review Report with the supporting published documentation from the Pesticides Peer Review Experts' meetings, and to the EFSA conclusions (EFSA, 2023). All the cited documents are publicly available at the following links:

- **EFSA Conclusion** published on 26 July 2023 (EFSA Journal 2023;21(7):8164): <https://www.efsa.europa.eu/en/efsajournal/pub/8164>
- Background documents, comprising of the **final RAR** (2023) and the **Peer Review Report**: <https://open.efsa.europa.eu/study-inventory/EFSA-Q-2020-00140>

The issues raised in the publication by Générations Futures are addressed below in the order in which they appear.

1. Genotoxicity and Oxidative stress

Claims stated in the report by Générations Futures:

- INSERM: Takes into account 18 academic studies and indicates that glyphosate can induce oxidative stress and genotoxic damage which can lead to the appearance of mutations and cancers.
- ECHA/EFSA: Do not retain any academic study for their evaluation and consider that glyphosate possibly induces oxidative stress but is not genotoxic.

Response from EFSA:

EFSA agrees with the response from ECHA. EFSA would like to add that EFSA did not identify in its Conclusion data gaps on the assessment of genotoxicity and/or oxidative stress for the active substance and the formulation for the representative uses. The Weight of Evidence (WoE) approach for genotoxicity on glyphosate during the peer review included more than 70 studies (regulatory and public literature studies) assessed as acceptable, supplementary or supportive (see Renewal Assessment Report at Table 2.0.5.2-3 and Table 2.0.5.2-4 (RAR, Volume 1, 2023)).

As regards oxidative stress, EFSA concluded that glyphosate may induce oxidative stress as shown in some *in vitro* and *in vivo* studies, but increased oxidative stress was not consistently demonstrated in the available studies (EFSA, 2023).

The "Annexes Bibliographiques" (Générations Futures, 2023) did not include any new list of studies on genotoxicity and oxidative stress. In addition, all the points raised by Générations Futures, 2023 on genotoxicity and oxidative stress (as potential mode of action for genotoxicity) and by the French National Institute of Health and Medical Research (Inserm) were already *considered* during the peer review process (see data

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requirements 2.3 and 2.4, respectively, identified following the public consultation)⁶ and they do not *change* the overall assessment and conclusions adopted by EFSA.

2. Reprotoxic effects and endocrine disrupting properties

Claims stated in the report by Générations Futures:

- INSERM: Takes into account 21 academic studies and emphasizes the endocrine disruption potential of glyphosate.
- EFSA/ECHA: Retain only 1 academic study for their evaluation but consider that glyphosate has no endocrine effect.

Response from EFSA:

Regarding the assessment of the endocrine disruption properties, a large data set was considered: for the human health assessment a total of 122 studies were included in the WoE: **71** *in vivo* (see Table 1⁷ for complete references), **31** *in vitro* (see Table 2⁷ for complete references), **18** human observation studies (see Table 3⁷ for complete references) and **2** *in silico* studies (see Table 4⁷ for complete references) were included in the WoE; for ecotoxicology, a total of **85**⁸ studies were included in the assessment. Only *in vivo* data providing evidence on potential adversity and *in vivo/ex vivo* endocrine activity were available with non-mammalian species. The evidence included in Inserm report (2021)⁹ was also considered as part of the body of evidence assessed for human health and ecotoxicology.

Regarding the three studies indicated as not cited in the RAR by Générations Futures, EFSA noted that they were all instead considered in the final RAR (see Appendix A, **Table A1**).

With regard to the assessment of the endocrine disruption (ED) potential of glyphosate, it was performed in line with the ECHA/EFSA guidance (2018)¹⁰. In determining whether glyphosate interacts with the oestrogen, androgen and steroidogenesis (EAS) and thyroid (T) mediated pathways, the number and type of effects induced, and the magnitude and pattern of responses observed across studies were considered. The assessment is

⁶ Refer to the Peer Review Report in the Open EFSA under 'Supporting documents' under EFSA Question number EFSA-Q-2020-00140: <https://open.efsa.europa.eu/study-inventory/EFSA-Q-2020-00140>); see Part 4 (Evaluation Table section 2 following comments by public).

⁷ Refer to the supporting documents of the ED assessment available in the Peer Review Report in the Open EFSA under 'Supporting documents' under EFSA Question number EFSA-Q-2020-00140: <https://open.efsa.europa.eu/study-inventory/EFSA-Q-2020-00140>); refer to Part 3, Peer Review Report_Glyphosate_Annexes: TC 84, Annex 1. EFSA ED WG advice human health (7. ED WG WoE assessment of the ED WG glyphosate)

⁸ Refer to the supporting documents of the ED assessment available in the Peer Review Report in the Open EFSA under 'Supporting documents' under EFSA Question number EFSA-Q-2020-00140: <https://open.efsa.europa.eu/study-inventory/EFSA-Q-2020-00140>); refer to Part 3, Peer Review Report_Glyphosate_Annexes: TC 84, Annex 2. EFSA ED WG advice Non-target organisms (Appendix A – Protocol; ED assessment ecotoxicology)

⁹ <https://www.inserm.fr/wp-content/uploads/inserm-expertisecollective-pesticides2021-synthese-va.pdf>

¹⁰ ECHA (European Chemicals Agency) and EFSA (European Food Safety Authority) with the technical support of the Joint Research Centre (JRC), Andersson N, Arena M, Auteri D, Barmaz S, Grignard E, Kienzler A, Lepper P, Lostia AM, Munn S, Parra Morte JM, Pellizzato F, Tarazona J, Terron A and Van der Linden S, 2018. Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. EFSA Journal 2018;16(6):5311,135 pp. <https://doi.org/10.2903/j.efsa.2018.5311>. ECHA-18-G-01-EN.

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therefore providing a WoE analysis of the potential interaction of glyphosate with the EAS and T signaling pathways using the available evidence in the dataset.

The full body of evidence is listed in the final RAR (2023), while the detailed assessment is comprehensively and transparently reported in the supporting published documentation of the Pesticides Peer Review Experts' meeting TC 84 (see Annexes 1 and 2 to Peer Review Meeting Report)¹¹. The methodology used to conclude on the WoE analysis is described in the published protocols, which also include the criteria used and the outcome of the Risk of Bias analysis performed for the full body of evidence, including the ones reported by Générations Futures. The WoE analysis was conducted with the support of the EFSA ED working group (WG) which provides technical advice to the peer review on the interpretation of the data related to the ED assessments, in particular in case of complex or controversial scientific issues. The final RAR includes all the available and assessed evidence while the Peer Review Report (together with its Annexes) contains all the details of the WoE analysis carried out by the EFSA ED WG.

3. Mitochondrial toxicity

Claims stated in the report by Générations Futures:

- INSERM: Observes mitochondrial toxicity of glyphosate and highlights a possible link with neurodegenerative pathologies.
- ECHA/EFSA: Do not evaluate mitochondrial toxicity.

Response from EFSA:

EFSA agrees with the response provided by ECHA regarding the interpretation of data on mitochondrial toxicity. EFSA further highlights that in the frame of the pesticide risk assessment, while considering the relevance of mitochondrial perturbation as relevant mechanistic information, mitochondrial toxicity is not an apical endpoint but is an intermediate key event that, if sufficiently perturbed in terms of time and concentration response, has the potential to lead to an adverse outcome.

EFSA noted that across the full body of evidence, including *in vivo* experimental animal studies conducted up to the maximum tolerated/administrable dose and of different durations, and human epidemiological studies exploring/assessing apical endpoints and diseases for which mitochondrial toxicity could be an intermediate mechanistic key event, no clear evidence of adversity primarily ascribable to mitochondrial toxicity was observed. Evidence of adversity, where noted, was taken into account in the derivation of human toxicological reference values as part of the standard risk assessment process. In addition, it is worth noting that the use of non-mammalian methods/systems for the assessment of mitochondrial effects is of uncertain relevance for mammalian species and additional research is needed.

¹¹ available in the Peer Review Report in the Open EFSA under 'Supporting documents' under EFSA Question number EFSA-Q-2020-00140: <https://open.efsa.europa.eu/study-inventory/EFSA-Q-2020-00140>); refer to Part 3_Peer Review Report_Glyphosate_Annexes: TC 84

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The studies included in the Inserm report (2021)¹² have been performed with zebra fish (*Danio rerio*) (Lopes et al., 2018, Pereira et al., 2018), duckweed (*Lemna minor L.*) (Gomes et al., 2016) and on the nematode *Caenorhabditis elegans* (Bailey et al., 2018).

EFSA noted that two out of four studies mentioned in the report were evaluated and included in the updated RAR in the ecotoxicology section. The two studies not included in the RAR were excluded by the applicant based on the rapid title/abstract screening. Full consideration of these studies is included in Appendix A of this statement, **Table A2**.

Regarding the ecotoxicology risk assessment, as explained above, the effects reported in these studies are not relevant for such assessment (mitochondrial toxicity cannot be extrapolated to an effect at the population level, which is the one relevant for the ecotoxicological risk assessment). In addition, the effects reported were observed at tested concentrations much higher than the endpoints included in the EFSA Conclusion, driving the risk assessment for both aquatic and soil organisms.

4. Epigenetic effects

Claims stated in the report by Générations Futures:

- INSERM: Takes into account 5 academic studies and observes an epigenetic mode of action of glyphosate based herbicides (GBH).
- ECHA/EFSA: Do not evaluate epigenetic effects.

Response from EFSA:

EFSA agrees with the response by ECHA. EFSA would like to add that Générations Futures (2023) quoted five publications on glyphosate and/or glyphosate-based formulations and claimed that one publication (i.e. Ben Maamar et al., 2020) was not cited in the regulatory file. However, this publication was submitted by the applicant and assessed by the Rapporteur Member States (RMS) (RAR, 2023, Vol 1., page 683 and Vol 3). The RMS considered the study not acceptable (less relevant / unreliable). EFSA agreed with the RMS' assessment.

All the five publications on epigenetics quoted by Générations Futures were already considered during the peer review process (RAR, 2023, Vol 1 and 3) and contributed to the overall assessment and conclusions adopted by EFSA. In addition, EFSA would like to highlight that, based on the current state of knowledge, considering that standardised regulatory guidance and/or established harmonised criteria are currently not available for the assessment of epigenomic modifications, no definitive conclusions can be drawn from this type of studies.

5. Effects on microbiota

Claims stated in the report by Générations Futures:

¹² <https://www.inserm.fr/wp-content/uploads/inserm-expertisecollective-pesticides2021-synthese-va.pdf>

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- INSERM: Takes into account 7 academic studies showing a dysregulation of the microbiota and is concerned that these effects are not taken into consideration in the evaluation.
- ECHA/EFSA: Do not take into account any academic studies in their assessments.
- Générations Futures considered the conclusions of EFSA on the possible effects of microbiome as contradicting.

Response from EFSA:

In the area of mammalian toxicology, **57** public literature studies on the gut microbiome, its perturbations and consequence for human and animal (livestock and pets) health were identified and assessed. The full information on the identified studies and methodology for their appraisal is reported in the supporting published documentation of the Pesticides Peer Review Experts' meeting TC 80 (see Annex to the Peer Review meeting report)¹³.

In the area of ecotoxicology, EFSA considered **36** public literature studies investigating effects on soil microbiota and gut microbiota of non-target organisms. The full information on the identified studies and methodology for the appraisal of the studies is reported in the supporting published documentation of the Pesticides Peer Review Experts' meeting TC 82 (see Annex to the Peer Review meeting report)¹⁴.

All the studies indicated as not cited in the RAR by Générations Futures were assessed. Five were assessed in the context of mammalian toxicological assessment, and one in the context of the ecotoxicological assessment. One paper does not refer to microbiome and it is considered unrelated to the topic (see Appendix A – **Table A.3**).

To further clarify its conclusion reached with regard to possible effects on the microbiome, EFSA highlights that:

- EFSA acknowledges that investigations on the microbiome(s) are currently not part of the regulatory requirements for plant protection products; there are no guidelines and harmonised criteria for the assessment of the effects of pesticides on the gut microbiome and subsequent health consequences. However, EFSA reiterates that the current toxicological reference values for glyphosate have been derived based on a robust data package and are protective towards all the observed adverse effects, including those that could be secondary to gut microbiome perturbation, under the current state of knowledge;
- EFSA acknowledges that the field of microbiome research has evolved rapidly over the last years and could play an important role in various areas of EFSA's scientific assessments. In June 2020, EFSA published an editorial (Merten et al, 2020),¹⁵ highlighting that gut microbiome research is expected to play a relevant role in

¹³ available in the Peer Review Report in the Open EFSA under 'Supporting documents' under EFSA Question number EFSA-Q-2020-00140: <https://open.efsa.europa.eu/study-inventory/EFSA-Q-2020-00140>; refer to Part 3 – TC 80_Peer Review Report_Glyphosate_Annexes. Refer to Annex 9.

¹⁴ available in the Peer Review Report in the Open EFSA under 'Supporting documents' under EFSA Question number EFSA-Q-2020-00140: <https://open.efsa.europa.eu/study-inventory/EFSA-Q-2020-00140>; refer to Part 3 – TC 82_Peer Review Report_Glyphosate_Annexes. Refer to Annex - Ecotoxicology Microbiome Study Evaluation.

¹⁵ Merten C, Schoonjans R, Di Gioia D, Pelaez C, Sanz Y, Maurici D, Robinson T, 2020. Editorial: Exploring the need to include microbiomes into EFSA's scientific assessments. EFSA Journal 2020;18(6):e18061, 7 pp. <https://doi.org/10.2903/j.efsa.2020.e18061>

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regulatory science and that further research is needed to enhance the understanding of the toxicological significance of microbiome-mediated metabolism of chemicals. To start building this capacity, EFSA launched a thematic grant in March 2020 (GP/EFSA/ENCO/2020/02) on this topic to collaborate with EU Member States and to identify indications for future EU research agendas with a focus on specific needs from a risk assessment perspective; reports are expected to become publicly available in the first quarter of 2024.

6. Neurotoxicity

Claims stated in the report by Générations Futures:

- INSERM: Takes into account academic studies and indicates that GBH as well as glyphosate alone modify the concentrations of several neurotransmitters in different regions of the brain in rodents.
- ECHA/EFSA: Do not retain any academic studies for their evaluation and affirm that there is insufficient evidence for an effect of glyphosate and GBH on neurotransmitters.

Response from EFSA:

The set of studies considered in the renewal assessment of glyphosate includes a package of regulatory neurotoxicity studies performed in rodents (one acute and two sub-chronic neurotoxicity studies in rats) and one delayed polyneuropathy study (delayed neurotoxicity study in domestic hens), in agreement with the data requirement as set out in Commission Regulation (EU) No 283/2013. As indicated in the EFSA Conclusion (EFSA, 2023), no indication of neurotoxicity potential of glyphosate was present from the above-mentioned studies.

In addition, 56 public literature studies were identified and considered in a WoE approach: 22 *in vivo*, 6 *in vitro*, 19 epidemiological studies, 5 reviews and 4 others (e.g. neuroimaging, etc.).

Amongst these, 9 studies were considered for autism (1 *in vivo*, 1 review and 7 epidemiological studies), 13 studies for Parkinson's disease (2 *in vivo*, 1 *in vitro*, 8 epidemiological studies, 1 review and 1 neuroimaging study), 9 *in vivo* studies for neurotransmitters, 12 developmental neurotoxicity (DNT) studies (7 *in vivo*, 2 reviews, 3 others), 2 epidemiological studies for amyotrophic lateral sclerosis and 11 studies on other neurotoxicity endpoints (3 *in vivo*, 5 *in vitro*, 1 review and 2 epidemiological studies). The list of the studies assessed including the study appraisal and WoE methodology are described in the supporting published documentation of the Pesticides Peer Review Experts' meeting TC 80 (see Annex 7 to the Peer Review meeting report TC 80)¹⁶.

Générations Futures lists nine studies on neurotoxicity included in the Inserm report. According to Générations Futures, none of these studies were taken into account in the regulatory dossier: they were either deemed irrelevant for the regulatory assessment, or

¹⁶ available in the Peer Review Report in the Open EFSA under 'Supporting documents' under EFSA Question number EFSA-Q-2020-00140: <https://open.efsa.europa.eu/study-inventory/EFSA-Q-2020-00140>; refer to Part 3_Peer Review Report_Glyphosate_Annexes. TC 80. Refer to Annex 7.

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were excluded from the literature review after rapid screening at title/abstract level, or not included at all. Contrary to this allegation, all the studies mentioned by Générations Futures are included in the RAR, as shown in Appendix A (**Table A4**).

EFSA conclusions

Taking into account the considerations reported above, it is concluded that all the relevant evidence was examined in the current peer review process and no relevant findings were dismissed. Therefore, the points raised in the report by Générations Futures do not change the overall assessment and conclusion adopted by EFSA.

Furthermore, EFSA would like to reiterate that the peer review on glyphosate is the most comprehensive and transparent assessment carried out for a pesticide active substance in the EU. The assessment took into account about 2,400 studies related to human and animal health or the environment, including 700 published papers. It involved dozens of scientists from EFSA and approximately 90 experts from 27 national public authorities across the EU.

Since 2003, EFSA has been responsible for the EU peer review of the pesticide risk assessment for active substances used in plant protection products. This task is carried out by EFSA's Pesticides Peer Review Unit, in close cooperation with EU Member State competent authorities, following procedures that are set out in the applicable legislations and according to the latest scientific standards and methods. The EFSA independent scientific advice is submitted to risk managers for their decisions on regulatory matters, including the approval of active substances. It is worth noting that, over the years, this process has led to the non-approval of hundreds of harmful substances, which are no longer approved in the EU (see EU Pesticide database for further details: <https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances>).

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Appendix A

TABLE A. 1 REPROTOXIC EFFECTS AND ENDOCRINE DISRUPTION PROPERTIES

Study	Title	Comment by GF ^a	EFSA reply ^b
Cai W, Ji Y, Song X, et al., 2017. Environ. Toxicol. Pharmacol.; 55: 148-55	Effects of glyphosate exposure on sperm concentration in rodents: A systematic review and meta-analysis.	'étude non citée dans le dossier'	Study assessed in the RAR (2023) – Vol.1, page 493
Dallegrave E, Mantese FD, Oliveira RT, et al., 2007. Arch Toxicol; 81: 665-73	Pre- and postnatal toxicity of the commercial glyphosate formulation in Wistar rats.	'étude non citée dans le dossier'	Study assessed in the RAR (2023) – Vol.1, page 677
Anifandis G, Katsanaki K, Lagodoti G, et al., 2018. Int J Environ Res Public Health; 15: 1117	The effect of glyphosate on human sperm motility and sperm DNA fragmentation.	'étude non citée dans le dossier'	Study assessed in the RAR (2023) – Vol.1, page 666

^a Comments are reported as original citations from the Générations Futures report, when available. When necessary, additional notes are reported between brackets.

^b Details on the outcome of the appraisal and assessment of the studies are reported in the Renewal Assessment Report unless otherwise stated.

TABLE A. 2 MITOCHONDRIAL TOXICITY

Study	Title	Comment by GF ^a	EFSA reply ^b
Pereira et al., 2018. Chemosphere (2018), Vo. 209, p. 353-362	Low-concentration exposure to glyphosate-based herbicide modulates the complexes of the mitochondrial respiratory chain and induces mitochondrial hyperpolarization in the <i>Danio rerio</i> brain	'Étude exclue de la revue de la littérature du dossier réglementaire dès lecture du résumé'	Study assessed, see RAR (2023): Volume_3CA_B-9_ecotoxicology_appendix literature search, pp. 1742 See also Table A.4
Lopes et al., 2018. Ecotoxicol Environ Saf 2018; 162: 201-7	Toxicity induced by glyphosate and glyphosate based herbicides in the zebrafish hepatocyte cell line (ZF-L)	'Étude pas prise en compte par le demandeur'	Study assessed in the RAR (2023) Vol 3CA B9, table B.9.11.1.4-2 Publications excluded from the risk assessment after detailed assessment of full-text documents, pp. 700

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Study	Title	Comment by GF ^a	EFSA reply ^b
<p>Bailey et al., 2018. Environ Toxicol Pharmacol 2018; 57: 46-52</p>	<p>Chronic exposure to a glyphosate-containing pesticide leads to mitochondrial dysfunction and increased reactive oxygen species production in <i>Caenorhabditis elegans</i></p>	<p>'étude non citée dans le dossier réglementaire'</p>	<p>The study was excluded by the applicant from the literature search based on title and abstract rapid screening.</p> <p><i>EFSA has considered the full paper in the context of the current statement as follows:</i> Based on the effects observed on the investigated parameters (i.e., cellular level), the study is considered of low relevance since the type of investigated endpoints cannot be translated into an effect relevant at the level of population (population abundance and survival). The study also lacks in analytical verification of the test item, which can negatively impact on the overall reliability. It should be noted that the lowest tested concentration was 2.7% glyphosate. While it is unclear if this percentage is expressed as weight/weight or weight/volume, when expressed in more standard units, it translates into concentrations around 30 g/L. This concentration is about 30'000 times higher than the endpoint currently driving the risk assessment.</p>
<p>Gomes et al., 2016. Environ. Pollut. Nov; 218:402-409</p>	<p>Oxidative stress in duckweed (<i>Lemna minor L.</i>) induced by glyphosate: Is the mitochondrial electron transport chain a target of this herbicide?</p>	<p>'D'autres études sur la toxicité mitochondriale (non citées par l'Inserm) ont été exclues de la revue de la littérature dès lecture des résumés'</p>	<p>The study was excluded by the applicant from the literature search based on title and abstract.</p> <p><i>EFSA has considered the full paper in the context of the current statement as follows:</i> Based on the effects observed on the investigated parameters (i.e., cellular level), the study is considered of low relevance since the type of investigated endpoints cannot be translated into an effect relevant at the level of population (population abundance and survival). The study also lacks in analytical verification of the test item, which can negatively impact on the overall reliability.</p>

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			Furthermore, the relevant endpoint for the risk assessment considers 50% effect (i.e. EC ₅₀). While a comparable endpoint was not reported in this study, the available plots show that for either photosynthesis, respiration, or chlorophyll, a 50% reduction was not achieved even at 500 mg/L, i.e. 500 times the concentration driving the risk assessment.

^a Comments are reported as original citations from the Générations Futures report, when available. When necessary, additional notes are reported between brackets.

^b Details on the outcome of the appraisal and assessment of the studies are reported in the Renewal Assessment Report unless otherwise stated.

TABLE A.3 EFFECTS ON MICROBIOTA

Study	Title	Comment by GF ^a	EFSA reply ^b
Shehata et al., 2013. Curr. Microbiol 66:350-8	The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro	'étude non citée dans le dossier réglementaire'	Study assessed, see RAR (2023): B.6.8.2.43. pages 346 -350
Ackermann et al., 2015. Curr Microbiol 70: 374-82	The influence of glyphosate on the microbiota and production of botulinum neurotoxin during ruminal fermentation.	'étude non citée dans le dossier réglementaire'	Study assessed, see RAR (2023): B.6.8.2.43 pages 353-354
Lozano et al., 2018. Toxicol Rep; 5: 96-107	Sex-dependent impact of Roundup on the rat gut microbiome	'étude citée mais non prise en compte dans le dossier réglementaire'	Study assessed, see RAR (2023): B.6.8.2.22 pages 135-142 and B.6.8.2.43. pages 351-352
Mao et al, 2018. Environ Health 17: 50	The Ramazzini Institute 13-week pilot study on glyphosate and Roundup administered at human-equivalent dose to Sprague Dawley rats: effects on the microbiome	'étude citée mais non prise en compte dans le dossier réglementaire'	Study assessed, see RAR (2023): B.6.8.2.23.) pages 143-149 and B.6.8.2.43. pages 352-353
Ait Bali et al., 2018. Neurotoxicol	Glyphosate-based herbicide exposure affect gut microbiota, anxiety and	'étude citée mais non prise en compte	Study assessed, see RAR (2023): B.6.8.2.18.) pages 116-122

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Study	Title	Comment by GF ^a	EFSA reply ^b
Teratol 67:44-49.	depression-like behaviours in mice.	dans le dossier réglementaire'	
Ait Bali et al., 2017. Front Behav Neurosci 11: 146	Behavioral and immunohistochemical study of the effects of subchronic and chronic exposure to glyphosate in mice	'Etude non citée dans le dossier réglementaire'	No investigations on the microbiome, study not relevant for microbiome assessment. Considered under the assessment of neurotoxicity.
Motta et al., 2018. Proc Natl Acad Sci USA; 115: 10305	Glyphosate perturbs the gut microbiota of honey bees.	'Etude jugée non fiable dans le dossier réglementaire'	Study assessed, see RAR (2023): Volume_3CA_B-9_ecotoxicology_appendix literature search B.9.3.1.1, pp 200 – 211 The study was considered relevant and reliable during the peer review.

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TABLE A.4 NEUROTOXICITY

Study	Title	Comment by GF ^a	EFSA reply ^b
Hernandez-Plata et al., 2015. Neurotoxicology, 46:79-91.	The herbicide glyphosate causes behavioral changes and alterations in dopaminergic markers in male Sprague-Dawley rats.	' <i>This publication is considered not relevant</i> ' (in the dossier)	Study assessed, see RAR (2023): B.6.7.3.10 pages 137-151.
Cattani et al., 2017. Toxicology, 387:67-80.	Developmental exposure to glyphosate-based herbicide and depressive-like behaviour in adult offspring: implication of glutamate excitotoxicity and oxidative stress.	' <i>Not relevant for the assessment</i> ' (in the dossier)	Study assessed, see RAR (2023): B.6.7.3.11 pages 151-153.
Gallegos et al., 2018. Neurotoxicity research, 34(3):363-374.	Perinatal glyphosate-based herbicide exposure in rats alters brain antioxidant status, glutamate and acetylcholine metabolism and affects recognition memory.	' <i>Not relevant</i> ' (in the dossier)	Study assessed, see RAR (2023): B.6.7.3.12 pages 153-162.
Martinez et al., 2018.	Neurotransmitter changes in rat brain	' <i>Relevant/Reliable with restrictions</i> ' (not commented by	Study assessed, see RAR (2023): B.6.7.3.2 pages 58-65.

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Study	Title	Comment by GF ^a	EFSA reply ^b
Environ Res 161:212-219.	regions following glyphosate exposure.	<i>Générations Futures</i>)	
Yu et al., 2018. Biochem Biophys Res Commun 501(4):838-845.	Circular RNA expression profiles in hippocampus from mice with perinatal glyphosate exposure.	'non pertinente' (in the dossier)	Study assessed, see RAR (2023): B.6.7.3.13 pages 162-171.
Ait Bali et al., 2017. Front Behav Neurosci, 11:146	Behavioural and immunohistochemical study of the effects of subchronic and chronic exposure to glyphosate in mice.	'pas citée' (in the dossier)	Study assessed, see RAR (2023): B.6.7.3.14 pages 171-181.
Ait Bali et al., 2018. Neurotoxicol Teratol 67:44-49.	Glyphosate-based herbicide exposure affect gut microbiota, anxiety and depression-like behaviours in mice.	'The neurobehavioral part of the study is considered unreliable' (in the dossier)	Study assessed, see RAR (2023): B.6.7.3.15 pages 181.
Pereira et al., 2018. Chemosphere 209:353-362.	Low-concentration exposure to glyphosate-based herbicide modulates the complexes of the mitochondrial respiratory chain and induces mitochondrial hyperpolarisation in the Danio rerio brain.	'Exclue de la revue de la littérature après lecture de l'abstract' (in the dossier)	Study assessed, see RAR (2023): B.6.7.3.17 pages 193-206.
Bridi et al., 2017. Toxicol 392:32-39.	Glyphosate and Roundup alter morphology and behaviour in zebrafish.	'Non incluse dans le RAR.'	Study assessed, see RAR (2023): B.6.7.3.16 pages 181-193.

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