ANNEX II

Assessment of the grounds for the review of Commission Implementing Decision (EU) 2021/61[1] authorising the placing on the market of products containing, consisting of or produced from genetically modified maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and genetically modified maize combining two, three or four of the single events MON 87427, MON 87460, MON 89034, MIR162 and NK603 pursuant to Regulation (EC) No 1829/2003[2]

1. Risk assessment conducted by EFSA and the applicant

1.1 Molecular characterisation and assessment of open reading frames

1.1.1. Claim that EFSA did not take into account all the relevant data

In section 2.1.1 of your request, you claim that the European Food Safety Authority (EFSA) did not take into account all the relevant data required by Annex II to Commission Implementing Regulation (EU) No 503/2013[3] for the assessment of open reading frames ('ORFs')[4] in genetically modified (GM) maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 ('GM stack maize'). In particular, you claim that (1) EFSA assumed that the proteins that might emerge from these ORFs would raise no safety concerns and it did not carry any further investigations; (2) it did not assess other gene products, such as non-coding RNA ('ncRNA') from ORFs; (3) although EFSA identified a putative peptide which might emerge from one of the ORFs, sharing similarity to known allergens, the applicant did not provide data of the absence of this peptide in the GM stack maize; and (4) EFSA did not take into consideration the potential synergistic or antagonistic effects resulting from the combination of the transformation events.

As regards your first and second claims, according to the *EFSA Guidance for risk assessment of food and feed from genetically modified plants*[5], which implements the requirements set out in Regulation (EU) No 1829/2003 and in Commission Implementing Regulation (EU)

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1 OJ L 26, 26.1.2021.

2 Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (OJ L 268, 18.10.2003, p. 1–23).

3 Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006 (OJ L 157, 8.6.2013, p. 1–48).

4 Regulation (EU) No 503/2013 defines "open reading frames" as "any nucleotide sequence that contains a string of codons that is uninterrupted by the presence of a stop codon in the same reading frame) created as a result of the genetic modification either at the junction sites with genomic DNA or due to internal rearrangements of the insert(s)" (see part II, point 1.2.2.2.2, (f) of Annex II).

5 EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011. *Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants*. EFSA Journal 2011;9(5): 2150, 37 pp. https://doi.org/10.2903/j.efsa.2011.2150.

No 503/2013, new peptides, including proteins, likely to be produced from ORFs are assessed, while ncRNA is not assessed unless justified by the nature of the insert[6].

As already stated in Section 3.1.1 of the EFSA *Scientific advice to the European Commission on the internal review submitted under Regulation (EC) No 1367/2006 on the application of the provisions of the Aarhus Convention against the Commission Implementing Decision (EU) 2015/687 to authorise genetically modified oilseed rape MON 88302*[7], EFSA recognises that there is evidence in the peer-reviewed scientific literature for ncRNA to be produced from different sequences of the insert. This ncRNA would be unintended and linked to the genetic transformation. In the case at hand, possible unintended effects in the GM stack maize, resulting from the potential production of such ncRNA, were covered by a comprehensive comparative analysis, consisting of compositional and agronomic/phenotypic characterisation of the GM stack maize as explained in Section 3.4.2 of the *EFSA Scientific Opinion on the assessment of genetically modified maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003* (hereafter 'EFSA scientific opinion on the GM stack maize')[8].

With regard to your third claim, the Commission would like to note that new putative peptides with significant similarities to allergens are assessed, for their likelihood of expression (transcription and translation) based on sequence characteristics (promoter, translation codon), according to the criteria set by WHO[9], the EFSA *Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed*[10] and the EFSA *Guidance for risk assessment of food and feed from genetically modified plants*[11]. It is true that one of the ORFs of GM maize MON 89034 might produce a single putative peptide sharing similarity to known allergens. Because this single putative peptide exceeded the allergenicity assessment threshold, EFSA assessed it in its scientific opinion on GM maize MON 87427 x MON 89034 x NK603[12]. However, because this ORF is located within the transcriptional unit of

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6 According to part II, section 1.2.2.3, point (e) of Annex II to Regulation (EC) 503/2013.

7 EFSA, 2015. *Scientific advice to the European Commission on the internal review submitted under Regulation (EC) No 1367/2006 on the application of the provisions of the Aarhus Convention against the Commission Implementing Decision 2015/687 to authorise genetically modified oilseed rape MON88302*. EFSA supporting publication 2015:EN-864. 44 pp..

8 EFSA GMO Panel, Naegeli H, Bresson J-L, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Moreno FJ, Mullins E, Nogué F, Rostoks N, Sànchez Serrano JJ, Savoini G, Veromann E, Veronesi F, Álvarez F, Ardizzone M, De Sanctis G, Fernandez Dumont A, Gennaro A, Gómez Ruiz J A, Lanzoni A, Papadopoulou N and Paraskevopoulos K, 2019b. *Scientific Opinion on the assessment of genetically modified maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003* (application EFSA-GMO-NL-2016-134). EFSA Journal 2019;17 (8):5774, 36 pp. https://doi.org/10.2903/j.efsa.2019.5774.

9 FAO/WHO, 2001. *Evaluation of allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Food Derived from Biotechnology*, 22-25 January 2001. Food and Agriculture organisation of the United Nations (FAO), Rome, Italy.

10 EFSA GMO Panel, 2010b. *Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed*. EFSA Journal 2010;8(7):1700, 168 pp. https://doi.org/10.2903/j.efsa.2010.1700.

11 See *supra* footnote 5.

the Cry2Ab2 coding sequence and it is in the same orientation but in a different reading frame and it does not contain any in-frame translational start codon, EFSA concluded that the expression of this ORF in maize is highly unlikely[13]. The results from the assessment of the GM stack maize by EFSA confirmed that none of the putative translation products of any newly created ORFs with relevant similarities to toxins or allergens are likely to be produced.

With regard to your fourth claim, contrary to your allegations, the possible impact of the combination of the transformation events on the integrity of each of them, on the expression levels of the newly expressed proteins and on the biological functions conferred by the individual inserts were considered by EFSA and reflected in section 3.4.1 of its scientific opinion on the GM stack maize, in line with the requirements laid down in part II, point 1.2.2.4. of Annex II to Regulation (EU) 503/2013. In that regard, the sequencing of the events in the GM stack maize and an updated bioinformatics analysis confirmed previous results from the EFSA Scientific opinions on the GM single maize[14], indicating that no known endogenous genes were disrupted by any of the inserts and no new significant similarities of the newly expressed proteins and ORFs to toxins and allergens were identified. Considerations on the assessment of ORFs were previously discussed by EFSA, in section 3.1.1 of its scientific advice on the internal review concerning GM oilseed rape MON 88302, referred to above[15]. In the present case, EFSA assessed the sequences of the events in the GM stack maize and found them identical to the sequences originally reported for the single events, thus confirming that the integrity of these events was maintained in the stack.

In view of the above considerations, your claims on this point must be rejected as unfounded.

1.1.2. Claim that confidential treatment of DNA sequencing information is in contradiction with EU legislation

With regard to the DNA sequences encoding the newly expressed proteins included in the application for authorisation, you claim that confidential treatment of that information is in contradiction with the EU legislation requiring public access to all risk relevant information, and that lack of access to such data prevents the carrying out of independent risk research of the specific peptide with allergenic potential.

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[12] EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Guerche P, Jones H, Manachini B, Messéan A, Nielsen EE, Nogué F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Gennaro A, Neri FM and Paraskevopoulos K, 2017. *Scientific Opinion on application EFSA-GMO-BE-2013-117 for authorisation of genetically modified maize MON 87427 × MON 89034 × NK603 and subcombinations independently of their origin, for food and feed uses, import and processing submitted under Regulation (EC) No 1829/2003 by Monsanto Company*. EFSA Journal 2017;15(8):4922, 26 pp. https://doi.org/10.2903/j.efsa.2017.4922.

[13] See *supra* footnote 12, section 3.2.

[14] EFSA Scientific opinion on GM maize MON 87427 (https://doi.org/10.2903/j.efsa.2015.4130), MON 87460 (https://doi.org/10.2903/j.efsa.2012.2936), MON 89034 (https://doi.org/10.2903/j.efsa.2008.909), MIR162 (https://doi.org/10.2903/j.efsa.2012.2756) and NK603 (https://doi.org/10.2903/j.efsa.2009.113).

[15] See *supra* footnote 7.

It is correct that, in its application for authorisation, the applicant indicated its wish to have the sequencing information to be treated as confidential on the basis of Article 30(1) of Regulation (EC) No 1829/2003, on the ground that that its disclosure would significantly harm its competitive position. In particular, the applicant explained that this information constituted its secret know-how resulting from investments in research and innovation and a determining factor of its competitive position, and that its disclosure would cause it substantial harm by allowing competitors to copy its products.

In that regard, it should be noted that Article 30(2)(a) of Regulation (EC) No 1829/2003, as amended by Article 2(10) of Regulation (EU) 2019/1381 on the transparency and sustainability of the EU risk assessment in the food chain[16], recognises DNA sequence information (except for sequences used for the purpose of detection, identification and quantification of the transformation event) as information to which confidential treatment may be granted, where its disclosure is demonstrated by the applicant to potentially harm its interests to a significant degree. Although Regulation (EU) 2019/1381 does not apply to the application for authorisation in question (which dates from before 27 March 2021), it does acknowledge the potentially sensitive nature of DNA sequencing information for the applicant.

Based on the above considerations, the Commission is of the view that the confidentiality grounds submitted by the applicant constitute a verifiable justification of its request for confidentiality treatment of DNA sequencing information in accordance with Article 30(1) of Regulation No 1829/2003 as applicable.

Therefore, your claim must be rejected.

1.2 Impact of environmental factors, agricultural practice and genetic backgrounds on gene expression, plant composition and phenotypic characteristics

As a preliminary remark, this section addresses the claims made under sections 2.1.3 and 2.2 of your request. Those claims, which are of a similar nature, have been grouped under the type of information concerned, i.e. data on environmental factors and stress conditions, data on herbicide application rates, data on stacking and genetic backgrounds and data from compositional analysis.

According to those claims, the data presented by the applicant do not satisfy the requirements of Regulation (EU) 503/2013, based on the following grounds: (1) the field trials were not conducted in all relevant regions where the GM stack maize will be cultivated and no extreme weather conditions were taken into account; (2) the field trials did not take into account current agricultural management practices; (3) the field trials only included one transgenic stacked variety, and (4) the data from compositional analysis showed the need for further investigations.

[16] Regulation (EU) 2019/1381 of the European Parliament and of the Council of 20 June 2019 on the transparency and sustainability of the EU risk assessment in the food chain and amending Regulations (EC) No 178/2002, (EC) No 1829/2003, (EC) No 1831/2003, (EC) No 2065/2003, (EC) No 1935/2004, (EC) No 1331/2008, (EC) No 1107/2009, (EU) 2015/2283 and Directive 2001/18/EC (OJ L 231, 6.9.2019, p. 1).

1.2.1 Data on environmental factors, stress conditions and their impact on gene expression and on plant composition and phenotype

In sections 2.1.3.1 and 2.2.1 of your request, you allege that the data presented by the applicant were insufficient to conclude on the impact of environmental factors and stress conditions on gene expression, plant composition and the biological characteristics of the plant, as the GM stack maize should have been subject to field trials under more extreme climate conditions and stressors such as drought.

In that regard, you refer to a number of publications. Among others, you refer to a paper by Tritkova *et al*. (2015)[17], concerning the impact of climate conditions on the content of the *Bacillus thuringiensis* ('Bt') protein in the plant tissue. Furthermore, you refer to publications by Wang *et al*. (2014)[18], Yang *et al*. (2017) [19], Fang *et al*. (2018) [20], Beres *et al*. (2018)[21] and Beres (2019)[22], showing effects of the EPSPS enzyme, one of the newly expressed protein from the GM stack maize, on plant growth hormone metabolism and common breeding parameters. On that basis, you claim that interference in the plant metabolism might cause changes in gene activity, and that extreme weather conditions can cause unexpected stress reactions in GM plants expressing additional EPSPS enzymes. In that regard, you claim that the concentration of EPSPS enzyme in the GM stack maize is higher compared to the parental plants and that, therefore, even if no such effects were observed in the parental plants, the likelihood of interaction of the EPSPS enzyme with the plant growth hormone metabolism is higher in the stack.

You further support your claim by asserting that, due to the particular genetic modification of this GM stack maize, it has to be expected that it will be grown under drought conditions to an extent most of the parental GM plants were not tested, and that it is a first time that a combination of events is meant to be grown under drought conditions.

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[17] Trtikova M, Wikmark OG, Zemp N, Widmer A and Hilbeck A, 2015.Transgene expression and Bt protein content in transgenic Bt maize (MON 810) under optimal and stressful environmental conditions. PLoS ONE, 10(4): e0123011.

[18] Wang, W., Xia, H., Yang, X., Xu, T., Si, H.J., Cai, X.X., Wang, F., Su, J., Snow, A.A., Lu, B.-R. (2014) A novel 5-enolpyruvoylshikimate-3-phosphate (EPSP) synthase transgene for glyphosate resistance stimulates growth and fecundity in weedy rice (Oryza sativa) without herbicide. New Phytol, 202(2): 679-688. https://doi.org/10.1111/nph.12428.

[19] Yang, X., Li, L., Jiang, X., Wang, W., Cai, X., Su, J., Wang, F., Lu, B.-R. (2017) Genetically engineered rice endogenous 5-enolpyruvoylshikimate-3-phosphate synthase (epsps) transgene alters phenology and fitness of crop-wild hybrid offspring. Sci Rep, 7(1): 1-12. https://doi.org/10.1038/s41598-017-07089-9.

[20] Fang, J., Nan, P., Gu, Z., Ge, X., Feng, Y.-Q., Lu, B.-R. (2018) Overexpressing exogenous 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) genes increases fecundity and auxin content of transgenic Arabidopsis plants. Front Plant Sci, 9: 233. https://doi.org/10.3389/fpls.2018.00233.

[21] Beres, Z.T., Yang, X., Jin, L., Zhao, W., Mackey, D.M., Snow, A.A. (2018) Overexpression of a native gene encoding 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) may enhance Fecundity in Arabidopsis thaliana in the absence of glyphosate. Int J Plant Sci, 179(5):390–401. https://doi.org/10.1086/696701.

[22] Beres, Z.T. (2019) Ecological and evolutionary implications of glyphosate resistance in Conyza canadensis and Arabidopsis thaliana. Dissertation presented in partial fulfillment of the requirements for the degree Doctor of Philosophy in the graduate school of the Ohio State University. http://rave.ohiolink.edu/etdc/view?acc_num=osu1555600547328876.

Based on the above arguments, you conclude that the field trials should also have been done under severe drought conditions, with and without irrigation, with and without application of the intended herbicide, and that therefore the data provided were insufficient to conclude on the impact of environmental factors on gene expression, plant composition and agronomic and phenotypic characteristics of the GM stack maize.

It should be firstly noted that, since the receiving environments are highly diverse and dynamic over time, it is considered unfeasible, in practice, to assess GM events under all possible receiving environments. Therefore, applicants must select sufficiently different locations to capture the environmental variability within the set of possible receiving environments in which the GM stack maize may be cultivated. In the case at hand, EFSA considered that the experimental design and the tested materials for this GM stack maize were adequate to identify possible unintended changes introduced with the genetic modifications.

Regarding the findings reported by Trtikova *et al*. (2015), EFSA has already assessed them in the past[23]. The authors based their findings on their experiments with plants grown in controlled environments, claiming that genetic background and environmental conditions, especially abiotic environments, could affect Cry1Ab transgene expression and Bt protein levels in GM maize MON810. EFSA is of the opinion that, taking those findings into account, the risk assessment conclusions and risk management recommendations on all Cry1Ab-expressing Bt-maize events remain valid and applicable.

As regards the findings reported by Wang *et al*. (2014), Yang *et al*. (2017), Fang *et al*. (2018), Beres *et al*. (2018) and Beres (2019), these were observed in rice, *Arabidopsis* and *Conyza canadensis* and not in maize. In any case, as highlighted in Vila-Aiub *et al*., 2019[24], reports on benefits on common breeding parameters from EPSPS overexpression in transgenic events need to be further validated before it can be confirmed that this remarkable finding is solely due to the intended glyphosate resistance. In addition, the agronomic and phenotypic characterisation included the assessment of common breeding parameters and revealed no biologically relevant differences between the GM stack soybean and its conventional counterpart.

Regarding the other publications you are referring to concerning the impact of climate conditions on protein expression levels, EFSA recognises that there is evidence in the peer-reviewed scientific literature suggesting that stressful conditions could in some instances be a factor influencing protein expression levels[25]. However, the possible consequences for protein

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[23] EFSA, 2015. *Relevance of a new scientific publication (Trtikova et al., 2015) on previous EFSA GMO Panel conclusions on the risk assessment of maize MON 810 and other Cry1Ab-expressing Bt-maize events*. EFSA supporting publication 2015:EN-878. 11 pp..

[24] Vila-Aiub M, Yu Q, Powles S, 2019. Do plants pay a fitness cost to be resistant to glyphosate? New Phytologist, 223(2): 532-547. https://doi.org/10.1111/nph.15733

[25] EFSA, Neri FM, Afonso A, De Sanctis G, Devos Y, Fernandez Dumont A, Lanzoni A and Papadopoulou N, 2021. *Technical and scientific assistance on the internal review under Regulation (EC) No 1367/2006 of the Commission's decisions authorising the placing on the market of genetically modified soybean MON 87751 x MON 87701 × MON 87708 x MON 89788 (application EFSA-GMO-NL-2016-128), maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and subcombinations (application EFSA-GMO-NL-2016-134) and maize MON 87427 × MON 89034 × MIR162 × MON 87411 and subcombinations (application EFSA-GMO-NL-2017-144)*. EFSA supporting publication 2021:EN-6590. 182 pp. https://doi.org/10.2903/sp.efsa.2021.EN-6590.

expression levels are unpredictable and may result in either higher or reduced protein expression levels (Hendawey, 2009[26]; Merewitz *et al*., 2011[27]; Parvaiz, 2014[28]).

For the compositional and agronomic/phenotypic characterisation, the applicant selected field trial sites located in major maize producing areas of the United States, and each of these sites reflect different meteorological and agronomic conditions under which the crop is to be grown. EFSA considered that the meteorological and agronomic variability at the sites selected for the compositional and agronomic/phenotypic characterisation of the application were able to ensure a sufficient range of environmental and agronomic conditions reflecting those under which the GM stack maize might be cultivated in practice.

Regarding your claims on drought conditions, it is true that one of the events of the GM stack maize, GM maize MON 87460, expresses the CSPB protein which helps reducing the yield loss caused by drought stress. In the EFSA opinion on the GM maize MON 87460[29], a comparative analysis was specifically conducted for this event under water-limited conditions and other stressful conditions. Under these conditions, GM maize MON 87460 showed enhanced agronomic performance characteristics and some differences in composition in comparison with its conventional counterpart. However, the differences observed were not unexpected and EFSA concluded that they did not raise safety concerns.

In addition, no safety issue concerning the five single maize events was identified by the updated bioinformatics analyses, nor reported by the applicant since the publication of the EFSA scientific opinions on those single maize events. Therefore, EFSA considered that its previous conclusions on the safety of the single maize events remained valid. EFSA considered that, taken together, all the data in the dossier were sufficient to conclude on the absence of interactions between the events (including the newly expressed proteins) that would raise safety concerns in GM stack maize.

In conclusion, considering that there was no indication of an interaction between the events as described in section 3.4.1.4 of the EFSA opinion, and EFSA did not identify any safety concern during its assessment of GM maize MON 87460, EFSA considered that it was not necessary to request the inclusion of field trials under drought conditions for the GM stack maize.

In view of the above considerations, your allegations on this point must be rejected.

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[26] Hendawey MH, 2009. Effect of salinity on proteins in some wheat cultivars. Australian Journal of Basic and Applied Sciences 3:80-88.

[27] Merewitz EB, Gianfagna T, Huang B, 2011. Protein accumulation in leaves and roots associated with improved drought tolerance in creeping bentgrass expressing an iptgene for cytokinin synthesis. Journal of Experimental Botany 62:5311-5333.

[28] Parvaiz A, 2014. Legumes under environmental stress: yield, improvement and adaptations. John Wiley & Sons. ISBN 978-1-118-91708-4.

[29] EFSA GMO Panel, 2012. *Scientific Opinion on an application (Reference EFSA-GMO-NL-2009-70) for the placing on the market of genetically modified drought tolerant maize MON 87460 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto*. EFSA Journal 2012;10(11):2936, 42 pp. https://doi.org/10.2903/j.efsa.2012.2936.

1.2.2 Data on herbicide application rates and their impact on gene expression and on plant composition and agronomic and phenotypic characteristics

In sections 2.1.3.2 and 2.2.2 of your request, you claim that the field trials for the GM stack maize did not take into consideration current agricultural management practices. You claim that, due to increased weed pressure, the GM stack maize will be exposed to much higher dosage and repeated spraying of glyphosate. You claim that this should have been considered because higher rates of herbicide application can influence the expression of the transgenes or other genome activities of the plants as well as the plant composition and its biological characteristics.

Similar criticisms on the non-representativeness of the herbicide regime applied on herbicide tolerant GM plants have been previously rebutted in Section 3.1.2.1 of the EFSA *assessment of the outcomes of the project "Risk Assessment of Genetically Engineered Organisms in the EU and Switzerland" (RAGES)*[30].

Part II, sections 1.3.1 and 1.3.3 of Annex II to Regulation (EU) No 503/2013 require that herbicide tolerant GM plants are exposed to the intended herbicide. In line with those provisions, the application of the intended herbicide in the field trials for the comparative assessment of herbicide tolerant GM plants is a mandatory requirement of the EFSA *Guidance for risk assessment of food and feed from genetically modified plants*[31]. Later on, in the EFSA *Guidance on the agronomic and phenotypic characterisation of GM plants*[32], the GMO Panel provided further clarifications on the type of information that applicants should report with regard to the application of the complementary herbicides (e.g. timing, dose, volumes, coadjuvants) to ensure a proper evaluation of their correct application.

In the field trials for comparative analysis of herbicide tolerant GM plants, the intended herbicides are to be kept at a similar application rate across sites, to ensure comparability between locations, while the combinations of conventional herbicides applied at the selected sites are to reflect different weed management practices, chosen to maintain the weed pressure under control. EFSA verifies that the timing and rate of the applied intended herbicides are in line with the recommendations of the manufacturers. This information is routinely verified by EFSA and specifically discussed in the section of its scientific opinions on management practices.

In the case of the GM stack maize in question, which is tolerant to glyphosate-based herbicides, the plots containing the GM stack maize were exposed to the intended herbicide[33]. The glyphosate treatment was conducted at standard doses and timing and in accordance with the manufacturer's recommendations. On that basis, EFSA concluded that the tested materials in the GM stack maize application were in line with the requirements of Regulation (EU) No 503/2013

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[30] EFSA, Gennaro A, Álvarez F, Devos Y, Fernandez Dumont A, Gómez Ruiz JÁ, Lanzoni A, Paoletti C, Papadopoulou N, Raffaello T, Waigmann E, 2020a. Assessment of the outcomes of the project "Risk Assessment of Genetically Engineered Organisms in the EU and Switzerland" (RAGES). EFSA supporting publication 2020:EN-1890. 31 pp.

[31] See *supra* footnote 5.

[32] EFSA GMO Panel, 2015a. *Guidance on the agronomic and phenotypic characterisation of genetically modified plants*. EFSA Journal 2015;13(6):4128, 44pp. doi:10.2903/j.efsa.2015.4128.

[33] Section 3.4.2.4 of EFSA Scientific opinion on the GM stack maize (see *supra* footnote 8).

as well as with the EFSA *Guidance on the agronomic and phenotypic characterisation of genetically modified plants*[34]. The Commission agrees with that assessment.

In view of the above considerations, your allegations on this point must be rejected.

1.2.3 Impact of genetic backgrounds on gene expression and on plant composition and agronomic and phenotypic characteristics

In sections 2.1.2.3 and 2.2.3 of your request, you claim that EFSA should have requested additional data from several varieties of GM maize, including those cultivated in South America, because the genomic background of the variety can influence the expression of the inserted genes or the concentration of the additional proteins present in the GM plants. On that basis you claim that the data provided were insufficient to conclude on the impact of the genetic background on gene expression and therefore also on plant composition and agronomic and phenotypic characteristics.

Regarding your claim on the impact of genetic background on gene expression, the Commission would also like to note that EFSA acknowledges, in the technical report *Relevance of a new scientific publication (Trtikova et al., 2015) on previous EFSA GMO Panel conclusions on the risk assessment of maize MON 810 and other Cry1Ab-expressing Bt-maize events*[35], that in order to expand the range of receiving environments, the use of more than one genetic background represents a valuable solution. However, EFSA considered adequate the experimental design and the tested materials provided by the applicant and, for that reason, it did not require the use of additional genetic background, to identify possible unintended changes introduced with the genetic modification.

In addition, for the protein expression study, the applicant selected field trial sites located in major maize producing areas of the United States, and each of these sites reflects different meteorological and agronomic conditions under which the crop is to be grown. This was documented in the field production data provided by the applicant, which included information on the meteorological and agronomic conditions. EFSA considered that the meteorological and agronomic variability at the sites selected for the protein expression data were able to ensure a range of environmental and agronomic conditions reflecting those under which the GM stack maize might be cultivated in practice.

In view of the above, your claims of the impact of the genetic background on plant composition and agronomic and phenotypic characteristics are consequently unfounded.

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[34] See *supra* footnote 32.

[35] See *supra* footnote 23.

1.2.4 Relevance of data from other events and previous applications

In section 2.1.2.4 of your request, you claim that data on the expression of the Vip3Aa20 protein from GM maize MIR162 from previous applications show a wide difference in gene expression of that protein. In your view, these differences might be caused by genetic instability, varietal backgrounds, agricultural practices, environmental factors or stacking. You quote several publications, in particular by Mesnage *et al*. (2016)[36] and Ben Ali *et al*. (2020)[37], that, according to you, show unintended changes in the overall proteome or the metabolome of transgenic maize linked to the expression of Bt and EPSPS proteins. Furthermore, you claim that the genetic elements can interact with each other and impact plant composition and biological characteristics and that, therefore, an assessment of the interactions between the genome and the environment would have been necessary.

Regarding your claim on the expression of the Vip3Aa20 protein, one of the newly expressed proteins, the Commission would like to note that the submitted data and analysis on the protein expression levels, including those of Vip3Aa20, in the GM stack maize are in line with the EFSA *Guidance for risk assessment of food and feed from genetically modified plants* and the GMO legislation[38]. EFSA considered that those data were sufficient to conclude that there is no indication of interactions between the events that would affect the levels of the newly expressed proteins due to the combination of the events to produce the GM stack maize. In addition, in the frame of the applications for the single events, no safety concerns were identified for the newly expressed proteins. When comparing the data from the GM stack maize with the data from the single events provided in those applications, EFSA concluded that the variability in the protein expression levels, including those of Vip3Aa20, is within the expected natural variability[39], due to genetic background or environmental stress. In addition, it is, in any case, within the safety margins considered by EFSA in the food and feed safety assessment.

Regarding the stability and integrity of the events, the Commission would like to recall that during its risk assessment, EFSA assessed the sequences of the events (inserts and their flanking regions) in the GM stack maize. The sequence of the events in the GM stack maize was found to be identical to the sequences originally reported for the single events, thus confirming that the integrity of these events was maintained in the GM maize stack. Furthermore, the stability of the single events and the integrity of the combined events in the GM stack maize were also confirmed and supported EFSA conclusions on protein safety[40].

Regarding the publication by Ben Ali *et al*. (2020), a working group of the EFSA GMO Panel reviewed this scientific publication. EFSA concluded that the experimental design and the results

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[36] Mesnage, R., Agapito-Tenfen, S.Z., Vilperte, V., Renney, G., Ward, M., Séralini, G.E., Nodari, R.O., Antoniou, M.N. (2016) An integrated multi-omics analysis of the NK603 Roundup-tolerant GM maize reveals metabolism disturbances caused by the transformation process. Sci Rep, 6: 37855. https://doi.org/10.1038/srep37855.

[37] Ben Ali, S.E., Draxler, A., Poelzl, D., Agapito-Tenfen, S., Hochegger, R., Haslberger, A.G., Brandes, C. (2020) Analysis of transcriptomic differences between NK603 maize and near-isogenic varieties using RNA sequencing and RT-qPCR. Environ Sci Eur, 32(1): 1-23. https://doi.org/10.1186/s12302-020-00412-8.

[38] Part II, section 1.2.2.3 of Annex II of Regulation (EU) 503/2013.

[39] Section 3.4.1.3 of the EFSA scientific opinion on the GM stack maize (see *supra* footnote 8).

[40] Section 3.4.1.2 of the EFSA scientific opinion on the GM stack maize (see *supra* footnote 8).

provided in the paper could not prove that the differences in gene expression are caused by the insertion of the event examined in that paper (GM maize NK603), rather than being merely a perturbation caused by environmental factors. The publication by Mesnage *et al*. (2016) was also assessed by EFSA in 2017[41], and it concluded that it did not reveal any new information that would invalidate the previous conclusions made on GM maize NK603[42].

Finally, regarding the interactions between the genome and the environment, it is true that it may occur that specific environmental conditions and agricultural practices in the field could impose stress on plants. However, according to EFSA, such conditions can be temporary. The possible consequences for protein content are unpredictable and may result in either higher or reduced protein levels (Hendawey, 2009; Merewitz *et al*., 2011; Parvaiz, 2014). EFSA also considers that protein expression variability in the field will be balanced by the fact that some plants will have higher protein content, and this will be compensated for by plants expressing less protein (Section 3.2.1 EFSA, 2015[43]). Overall, a weight of evidence approach is considered by EFSA, assessing the variability in protein expression levels in conjunction with the protein function, the dietary exposure and the outcome of the toxicological studies, in order to conclude on protein safety.

Based on the grounds above, your allegation on this point must be rejected.

1.2.5 Data from compositional analysis

In section 2.2.4 of your request you claim that only data from a low number of agronomic parameters, as required by the EFSA *Guidance on the agronomic and phenotypic characterisation of genetically modified plants*, were subjected to statistical analysis and that those data showed significant differences in the GM stack maize compared to their conventional counterpart. On that basis, you claim that EFSA should have requested much more data.

According to EFSA, the statistical outcomes for the treated and untreated GM stack maize compared to the conventional counterparts were not expected to be identical because the statistical analysis was carried out on experimental data with a limited (albeit large) sample size. Furtermore, differences in outcome due to natural background variability were expected. In that regard, the pattern of significant differences (and magnitude thereof) has to be considered in order to determine whether there could be an indication of an altered metabolism[44]. Such pattern is systematically considered by EFSA in its assessment.

As regards the compositional analysis of this specific case, EFSA found that the effective number of significant results is much lower than what you claim in your request. Even if changes

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[41] EFSA , 2017. *Relevance of a new scientific publication (Mesnage et al., 2016) on previous EFSA GMO Panel conclusions on the risk assessment of maize NK603*. EFSA supporting publication 2017:EN-878. 10 pp https://doi.org/10.2903/sp.efsa.2017.EN-1249.

[42] Section 4 of the EFSA supporting publication, see *supra* footnote 412.

[43] See *supra* footnote 8.

[44] EFSA GMO Panel *(2010), Statistical considerations for the safety evaluation of GMOs. EFSA Journal, 8: 1250. https://doi.org/10.2903/j.efsa.2010.1250*.

were observed in the level of 17 amino acids (32% of the analytes in grain), those were highly correlated with the change in the level of a single analyte, namely the "crude protein in grain". In addition, the test of equivalence for those compounds showed that they were within the range of natural variability. Regarding all the agronomic and phenotypic characteristics, the test of equivalence showed that those of the GM stack maize were within the range of natural variability, with only two exceptions (days to 50% silking and final stand count). EFSA further assessed those characteristics and found no environmental safety concerns. Hence, in conclusion, EFSA considered that the number of significant differences per se was not a reason for concern[45].

In view of the above considerations, your allegations on this point must be rejected.

1.3 Toxicity

1.3.1 No testing of the whole stacked plant in spite of findings from molecular characterisation and comparative approach

In section 2.3.1 of your request, you claim that significant changes were identified in the plant composition and agronomic characteristics, which would, according to your claim, require feeding study with the GM stack maize.

Regarding the lack of animal studies testing the toxicity of the whole food and feed, the Commission would like to note that they are not required by Regulation (EU) No 503/2013 for stacked transformation events obtained by conventional crossing of GM plants containing a single transformation event, unless the specific hypotheses mentioned in the second paragraph of section 1.4.4.1 of part II of Annex II to the Regulation are identified. EFSA has confirmed that no specific hypotheses were identified by the EFSA GMO Panel requiring animal feeding studies were identified by EFSA to conclude on the safety assessment of this GM stack maize[46].

However, as required in part II, section 1.4.4.1 of Annex II of Regulation (EU) No 503/2013, animal feeding studies on the parental lines were re-scrutinised in the context of the GM stack maize application dossier with regard to their adherence to the methodology, and clarification questions were asked to the applicant, as necessary. EFSA confirmed that the studies adhered to the legal requirements and that they did not identify adverse effects[47]. Interpretation of the results of these studies by Member States were duly taken into consideration and assessed by EFSA in the context of the assessment of the single events[48].

In view of the above considerations, your allegations on this point must be rejected.

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[45] Section 3.4.4.1 of the EFSA scientific opinion on the GM stack maize (see *supra* footnote 8).

[46] Section 3.4.3.3 of the EFSA scientific opinion on the GM stack maize (see *supra* footnote 8).

[47] Section 3.4.3.3 of the EFSA scientific opinion on the GM stack maize (see *supra* footnote 8).

[48] Appendix A of EFSA technical and scientific assistance on the internal review request (see *supra* footnote 24).

1.3.2 Claims regarding the toxicity of the Bt proteins

In section 2.3.2 of your request, you claim that the assessment of the toxicity of Bt proteins alone or in combination with other stressors was not sufficient. In particular, you consider that the assessment of the potential synergistic or combinatorial effects of Bt proteins was not sufficient considering the uncertainties on the mode of action of these proteins, their differences with the natural templates and the potential stressor effects of herbicides on herbicide tolerant crops, leading to higher levels of expression of such proteins.

You also claim that EFSA did not carefully examine publications by MacIntosh *et al*. (1990)[49] and by Mesén-Porras *et al*. (2020)[50], and other publications showing potential synergistic effects between Bt proteins and other compounds present in the plant such as protease inhibitors and strongly enhancing their toxicity.

In addition, you claim that the concentration of insecticidal proteins is much higher in gluten meal produced from the GM stack maize compared to the kernels of the GM stack maize.

Regarding the toxicity of Bt proteins alone or in combination with other stressors, EFSA has previously assessed the publications cited in your request in its *Scientific advice on the internal review under Regulation (EC) No 1367/2006 of the Commission's decision authorising the placing on the market of genetically modified maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and subcombinations*[51]. It found that those publications do not invalidate its assessment of the safety of Bt proteins (alone or in combination) in food and feed from the assessed GMO.

Regarding, in particular, the publications by MacIntosh *et al*. (1990) and Mesén-Porras *et al*. (2020), a working group organised by the EFSA GMO Panel recently discussed them and concluded that the findings on Bt proteins of those publications do not raise concerns for human and animal health[52].

Regarding your claim on gluten meal, the Commission would like to recall that gluten meal is a by-product of the processing of maize kernels into their different components, and that it is used for animal consumption. It is true that the protein content in gluten meal produced by the GM stack maize is higher when compared to the content in that maize kernel, but this was taken into

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[49] MacIntosh, S.C., G.M. Kishore, F.J. Perlak, P.G. Marrone, T.B. Stone, S.R. Sims, and R.L. Fuchs. 1990. Potentiation of Bacillus thuringiensis insecticidal activity by serine protease inhibitors. J. Agric. Food Chem. 38:1145-1152. doi:10.1021/jf00094a051.

[50] Mesen-Porras, E., Dahdouh-Cabia, S., Jimenez-Quiros, C., Mora-Castro, R., Rodriguez, C. and Pinto-Tomas, A. 2020. Soybean protease inhibitors increase Bacillus thuringiensis subs. israelensis toxicity against Hypothenemus hampei. Agronomia Mesoamericana, vol.31, n.2, pp.461-478. ISSN 2215-3608. http://dx.doi.org/10.15517/am.v31i2.36573.

[51] EFSA , 2019. *Scientific advice on the internal review under Regulation (EC) No 1367/2006 of the Commission's decision authorising the placing on the market of genetically modified maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and subcombinations*. EFSA supporting publication 2019:EN-1603. 25 pp. doi:10.2903/sp.efsa.2019.EN-1603.

[52] Minutes of the 122nd Meeting of the Working Group on Food and Feed Safety, available at https://www.efsa.europa.eu/sites/default/files/wgs/gmo/wg-applications-foodfeed-2018-2021.pdf.

account in the dietary exposure estimates[53]. EFSA concluded, in section 3.4.3.5 of its scientific opinion on the GM stack maize, that the dietary exposure assessment did not raise safety concerns.

In view of the above considerations, your allegations on this point must be rejected.

1.3.3 Claims concerning the immunogenicity of the Bt proteins

In section 2.3.3 of your request, you claim that EFSA did not consider the potential enhancement of toxic or immunogenic effects caused by interaction of Bt proteins with plant components in food and feed products derived from the GM stack maize and that the GM stack maize needs to be much more carefully risk assessed for its impact on the immune system compared to genetically engineered plants producing just one Bt protein.

Furthermore, you claim that the safety assessment of this GM stack maize should have included animal feeding studies on the whole food and feed to investigate long-term organ toxicity, immune responses and impact on the gut microbiome, also taking into account combinatorial effects and mixed toxicity.

EFSA conducted its assessment of the combination of the events in line with the requirements of part I, section 2.2 (c) of Annex II to Regulation (EU) No 503/2013 and the *EFSA GMO Panel approach to the risk assessment of sub-combinations as required by Implementing Regulation (EU) No 503/2013*[54]. With regard in particular to the assessment of the individual proteins newly expressed in the GM stack maize, elements considered included, among others, updated bioinformatic searches for their homology to toxic proteins, updated literature searches and an over-conservative exposure assessment in both humans and animals, as provided section II. points 1.2.2 and 1.5.1 of Annex II to Regulation (EU) No 503/2013[55].

Regarding potential immunogenic effects of Bt proteins, EFSA previously published comprehensive scientific reports addressing similar criticism to the EFSA assessments of GM plants and the potential effects of Bt proteins on the immune system[56,57,58]. In those scientific

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[53] Section 3.4.3.5, point *Animal dietary exposure* of the EFSA scientific opinion on the GM stack maize (see *supra* footnote 8).

[54] Annex I to the minutes of the 115th EFSA GMO Panel plenary meeting, available at https://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf.

[55] EFSA GMO Panel, Naegeli H, Bresson J-L, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Moreno FJ, Mullins E, Nogué F, Rostoks N, Sanchez Serrano JJ, Savoini G, Veromann E, Veronesi F and Fernandez Dumont A, 2021. Statement on in vitro protein digestibility tests in allergenicity and protein safety assessment of genetically modified plants. EFSA Journal 2021;19(1):6350, 16 pp. https://doi.org/10.2903/j.efsa.2021.6350.

[56] EFSA GMO Panel, Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Guerche P, Jones H, Manachini B, Messéan A, Nielsen EE, Nogué F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Eigenmann P, Epstein M, Hoffmann-Sommergruber K, Koning F, Lovik M, Mills C, Moreno FJ, van Loveren H, Selb R and Fernandez Dumont A, 2017b. Guidance on allergenicity assessment of genetically modified plants. EFSA Journal 2017;15(5):4862, 49 pp. https://doi.org/10.2903/j.efsa.2017.4862.

reports, EFSA did not find indications that Bt proteins in the GM stack maize might act as adjuvants with the potential to enhance a specific immunoglobulin E response and to favour the development of an allergic reaction. Furthermore, as none of the newly expressed proteins in the assessed GM stack maize showed potential for allergenicity, considering current knowledge, no reasons for concern regarding the simultaneous presence of these newly expressed proteins in the GM stack maize were expected.

In conclusion, the Commission is of the view that EFSA assessment of this GM stack maize fulfils the requirements of the GMO legislation as regards evaluation of potential synergistic or antagonistic effects resulting from the combination of the transformation events in the GM stack maize, in particular with regard to potential toxicity and adverse health effects from the consumption of food and feed derived from it. In addition, in relation to allergenicity and immunogenicity, EFSA performed its risk assessment according to part II, section II, point 1.5 of Annex II to Regulation (EU) No 503/2013 and its *Guidance for risk assessment of food and feed from genetically modified plants*, the principles of which are aligned with the *Codex Alimentarius* (2009)[59].

In view of the above considerations, your claims on this point must be dismissed.

1.3.4. Effects from residues of intended herbicides and their mixed toxicity

In section 2.3.4 of your request, you recall that residues from spraying were considered by EFSA to be outside its remit, and claim that without a detailed assessment of these residues, no conclusion can be drawn on the safety of the imported products.

You state that it should be taken into account that EFSA, in its review of the maximum residue level (MRL) for glyphosate, explicitly stated that no conclusion can be drawn on the safety of residues from spraying with glyphosate occurring in genetically engineered plants made resistant to this herbicide.

You also claim that glyphosate is known to cause shifts in the microbial composition and associated microbiomes of plants and animals, thus leading to a specific situation with regard to chronic exposure from food consumption.

The Commission would like to clarify that under Articles 4(1)(a) and 16(1)(a) of Regulation (EC) No 1829/2003, GM food and feed must not have adverse effects on human health, animal health or the environment. However, these conditions for the authorisation of GM food and feed

[57] EFSA (European Food Safety Authority), Dumont AF, Lanzoni A, Waigmann E and Paoletti C, 2018b. Relevance of new scientific information (Santos-Vigil et al., 2018) in relation to the risk assessment of genetically modified crops with Cry1Ac. EFSA supporting publication 2018:EN-1504. 13 pp. doi:10.2903/sp.efsa.2018.EN-1504.

[58] Parenti MD, Santoro A, Del Rio A, Franceschi C, 2019. Literature review in support of adjuvanticity/immunogenicity assessment of proteins. EFSA supporting publication 2019:EN-1551. 68 pp. doi:10.2903/sp.efsa.2019.EN-1551.

under Regulation (EC) No 1829/2003 do not cover the assessment of the potential effects of pesticide residues on human health, including possible cumulative effects.

This has been confirmed by the EU Court of Justice in its judgment of 12 September 2019 in Case C-82/17 P, *TestbioTech and Others v. Commission*[60] (par. 106 and 107), which upheld the General Court's interpretation that the assessment of the effects of pesticide residues on health is not covered by Regulation (EC) No 1829/2003 but by Regulation (EC) No 396/2005 of the European Parliament and of the Council on maximum residue levels (Case T-177/13, par. 233 and 289). The safety of GM food and feed products with a possible presence of pesticide residues is therefore guaranteed by the combined application of Regulations (EC) No 1829/2003 and No 396/2005.

In any event, Regulation (EC) No 396/2005 applies to pesticide residues on all food and feed placed on the market in the EU, including food and feed imported from third countries, and whether they are conventional or GM products. As any other food and feed, GM products placed on the EU market have to comply with the corresponding MRLs under Regulation (EC) No 396/2005.

In addition, in its reasoned opinion on the review of MRLs for glyphosate, EFSA concluded that it was not possible to derive MRLs specific to GM maize expressing EPSPS or GAT protein as no data were reported relating to the GAPs of these GM crops. However, EFSA evaluated a GAP for conventional maize that leads to a higher MRL than the GAP underlying the existing MRL for maize. EFSA concluded that no risk to consumers was identified with the GAP that leads to higher residue levels, therefore the existing MRL is also considered sufficiently protective for consumers. Existing MRLs are fully applicable to both conventional and GM maize and ensure an adequate level of safety.

Finally, regarding your claim that glyphosate has an impact on plants and animal microbiomes, EFSA recently launched a call for a thematic grant on the evaluation of the impact of microbiomes in risk assessment, including gastrointestinal tract microbiomes (human and domestic animals) and environmental microbiomes (plants, wildlife, soil)[61]. The microbiome is also being considered as a possible future scientific theme by the Science Studies and Project Identification & Development Office of EFSA (SPIDO)[62]. Finally, the need to explore the integration of microbiomes in EFSA risk assessment is included in the draft EFSA 2027 strategy, currently under public consultation.

In view of the above considerations, your allegations on this point must be rejected.

1.4 Allergenicity

Firstly, in section 2.4.1 of your request, you claim that EFSA's assessment of the GM stack maize does not fulfil the legal requirements for assessing allergenicity of the source of the

[60] ECLI:EU:C:2019:719

[61] https://www.efsa.europa.eu/en/news/new-grant-opportunity-microbiomes-and-plant-pests.

[62] https://www.efsa.europa.eu/en/events/event/79th-advisory-forum-meeting, item 2.5.

transgene, because the allergenicity of the Bt protein Cry1Ac, which was used as a source for the Cry1A.105 toxin expressed in the GM stacked maize, was not investigated in detail. In support of your claim, you refer to the publication of Santos-Vigil *et al*. (2018)[63], according to which Cry1Ac is thought to be allergenic. You claim that this publication is also relevant in the case of Cry1A.105 and that the EFSA's technical report on this publication[64] is biased.

Secondly, in section 2.4.2 of your request, you claim that potential synergistic effects between Bt proteins and other compounds present in the plant, such as protease inhibitors, were not carefully examined regarding potential adjuvanticity of Bt proteins.

With regard to your first claim, the Commission does not share your views that EFSA's assessment of the Santos-Vigil *et al*. (2018) publication is biased. In that regard, an external report on immunogenicity commissioned by EFSA, Parenti *et al*. (2019), has also published[65]. This report also discusses the adjuvanticity of Bt proteins and concludes that:

"*The adjuvanticity and immunogenicity of Cry proteins in certain experimental conditions seems plausible but due to low dosage, oral route of administration, food and feed processing and digestion, it is unlikely to emerge as a safety issue in food and feed. This assessment is consistent with the assessment by the EFSA GMO panel whereby they concluded that there is not a safety concern for the health of humans or animals that consume food/feed derived from GM plants containing Cry proteins. […]*"[66]

This conclusion also confirms the conclusions on immunogenicity and adjuvanticity of Bt proteins.

In addition, as mentioned above, EFSA has published in the past comprehensive scientific reports addressing similar questions on its assessment of GM plants and the potential effects of Bt proteins on the immune system and has found no reason for concern (see section 1.3.3 above).

It should also be noted that EFSA performed its risk assessment according to relevant guidelines, the principles of which are aligned with the *Codex Alimentarius* (2009).In addition, the assessment of allergenicity of the whole GM stack maize was also considered. Protease inhibitors are compounds naturally occurring in specific crops. In that respect, it is noted that the composition of the GM stack maize was also analysed, and it included an analysis of the Kunitz trypsin inhibitor. Considering all the information available, EFSA considered that there was no evidence that the genetic modification might substantially change the overall allergenicity of the

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63 Santos-Vigil, K.I., Ilhuicatzi-Alvarado, D., García-Hernández, A.L., Herrera-García, J.S., Moreno-Fierros, L. (2018) Study of the allergenic potential of Bacillus thuringiensis Cry1Ac toxin following intra-gastric administration in a murine model of food-allergy. Int Immunopharmacol, 61: 185-196. https://doi.org/10.1016/j.intimp.018.05.029.

64 EFSA, Dumont AF, Lanzoni A, Waigmann E and Paoletti C, 2018b. Relevance of new scientific information (Santos-Vigil et al., 2018) in relation to the risk assessment of genetically modified crops with Cry1Ac. EFSA supporting publication 2018:EN-1504. 13 pp. doi:10.2903/sp.efsa.2018.EN-1504.

65 Parenti, M.D., Santoro, A., Del Rio, A., Franceschi, C. (2019) Literature review in support of adjuvanticity/immunogenicity assessment of proteins. EFSA Supporting Publications, 16(1): 1551E

66 Section 4, point vi.

GM stack maize assessed when compared to their non-GM comparators and non-GM reference varieties tested[67].

In view of the above considerations, your claims on this point must be rejected.

1.5 Environmental risk assessment

In section 2.5 of your request, you claim that the conclusion by EFSA that the potential environmental effects from the spread of genes from occasional feral GM maize plants will not differ from that of conventional maize varieties is incorrect, and conclude that the GM stack maize needs to be examined in detail for next generation effects, volunteer potential (persistence) and gene flow.

1.5.1 Likelihood of gene flow

In section 2.5.1 of your request, you claim that, according to publications by Diaz *et al*. (2019)[68] and by Le Corre *et al*. (2020)[69], teosinte has changed its characteristics to facilitate gene flow with maize, and that the risk of crop-wild introgression should not be underestimated. Based on those publications, you claim that without more data on the teosinte species growing in the EU, the likelihood of gene flow from maize to teosinte cannot be assessed.

The Commission would like to recall that the scope of the authorisation decision is for placing on the market of the GM stack maize for food and feed uses. Since that GM stack maize is not authorised for cultivation in the EU, the likelihood that harm will occur via the pathway you depicted is negligible under import conditions. As stated in the EFSA Scientific opinion on the GM stack maize, the potential of maize grains (be it GM or not) being spilled during import to establish, grow and produce pollen was extremely low and transient. Therefore, the likelihood/frequency of cross- pollination and gene flow between occasional feral, GM maize plants resulting from grain spillage, and weedy or cultivated *Zea* plants was considered extremely low[70].

In view of the above considerations, your claims on this point must be rejected.

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67 Section 3.4.3.4 of EFSA scientific opinion on the GM stack maize (see *supra* footnote 8).

68 Díaz, A., Taberner, A., Vilaplana, L. (2020) The emergence of a new weed in maize plantations: characterization and genetic structure using microsatellite markers. Genet Resour Crop Evol, 67: 225-239. https://doi.org/10.1007/s10722-019-00828-z.

69 Le Corre, V., Siol, M., Vigouroux, Y., Tenaillon, M.I., Délye, C. (2020) Adaptive introgression from maize has facilitated the establishment of teosinte as a noxious weed in Europe. PNAS USA, 117(41): 25618-25627. https://doi.org/10.1073/pnas.2006633117.

70 Section 3.4.4.2, point *Plant-to-plant gene transfer* of the EFSA scientific opinion of the GM stack maize (see *supra* footnote 8).

1.5.2 Enhanced fitness and next generation effects

In sections 2.5.2 and 2.5.3, of your request, you state that if the characteristics of the GM stack maize were transferred to teosinte, this would render the latter herbicide-resistant, insect-tolerant and drought-tolerant. In addition, you claim that according to a number of publications, additional EPSPS enzymes result in enhanced overall fitness of hybrid offspring, which in your view can enhance the spread of teosinte. Finally, you contend that because teosinte can overwinter in the fields and transfer genetic information to the next generation, it has the potential to become a super-weed. On that basis you conclude that potential hybrid and next generation effects cannot be predicted from the data of the original event and should have been further investigated.

First of all, the Commission would like to recall that the scope of the authorisation decision is for placing on the market of the GM stack maize in question for food and feed uses. Since that GM stack maize is not authorised for cultivation in the EU, the likelihood that harm will occur via the pathway to harm you depicted is negligible under import conditions, as explained in section 1.6.1 of this Annex.

Even if cross- pollination would occur, EFSA was of the opinion that environmental effects resulting from the spread of genes from occasional feral GM stack maize plants in Europe would not differ from that of conventional maize varieties. As stated in the EFSA opinion on the GM stack maize[71], the fitness advantage provided by the intended traits and the observed differences (namely, in days to 50% silking and final stand count) will not allow the GM stack maize to overcome other biological and abiotic factors limiting the plant's persistence and invasiveness. Therefore, those traits and differences will not affect the persistence and invasiveness of the GM stack maize. Thus, it is very unlikely that the GM stack maize will differ from conventional maize hybrid varieties in its ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable GM stack maize grains.

Finally, your comments about the unintentionally enhanced fitness due to EPSPS, potential hybrid and next generation effects are not specific to the GM stack maize. Similar comments previously made by you were addressed in EFSA's assessment of the outcomes of the project *Risk Assessment of Genetically Engineered Organisms in the EU and Switzerland*. The Commission refers to the conclusions of the assessment, as reported in section 3.5.2.3 of the EFSA's *Technical Report on the assessment of the outcomes of the project "Risk Assessment of Genetically Engineered Organisms in the EU and Switzerland" (RAGES)*[72].

In view of the above considerations, your claims on this point must be rejected.

[71] Section 3.4.4.1, point *Plant-to-plant gene transfer* of the EFSA scientific opinion of the GM stack maize (see *supra* footnote 8).

[72] See *supra* footnote 31.

2. Post-market monitoring requirement in the Commission Implementing Decision

In section 3.2 of your request, you claim, firstly, that the detection methods provided by the applicant should not have been accepted because they do not allow, under practical conditions, to identify the GM stack maize in question and to distinguish it from other already authorised stacked or single events that inherit the same gene constructs and that can be mixed in the diets. Secondly, you indicate that the post-market monitoring plan should have included some pieces of information, such as import volumes and volumes used in the EU. Finally, you make a number of observations regarding where and how environmental monitoring should be carried out.

Regarding the first claim, in accordance with Articles 5(3)(i) and 17(3)(i) of Regulation (EC) No 1829/2003, the applicant must provide the methods of detection, sampling and identification of the transformation event, in accordance with the requirements set out in Article 8 and Annex III to Regulation (EU) No 503/2013.

For GMOs with stacked events, the Commission's Joint Research Centre ('JRC'), which is the European Union Reference Laboratory for GM food and feed ('EURL GMFF')[73], carries out a verification study to assess the performance of the event-specific methods, previously validated on parental lines, to detect and quantify the transformation event(s) on DNA from the stacked GMO containing several transgenic events. The results of the EURL GMFF verification are available online[74].

The detection methods validated by the EURL GMFF for the purposes of carrying out its tasks pursuant to Regulation (EC) No 1829/2003 are event-specific. Therefore, by applying several of such methods, it is possible to appropriately identify multiple GMO events in a food or feed sample, may the events correspond to different GMOs or to the same GMO. However, distinguishing between the potential presence of an equimolar mixture of single-event GMOs and a stacked-event GMO in a food or feed product usually requires additional information besides the laboratory measurement results.

In any case, in your request, you do not provide any evidence to support your claim that the GM stack maize in question cannot be distinguished from other GM stacked or single events. Therefore, your allegation on this point must be rejected.

As regards the second claim, the Commission notes that, in accordance with Article 5(3)(k) of Regulation (EC) No 1829/2003, the application for authorisation may include, *'where appropriate, a proposal for post-market monitoring regarding the use of the food for human consumption'*. Article 6(5)(e) provides that post-market monitoring requirements may be imposed ('*where applicable'*), *'based on the outcome of the risk assessment'*. In the case at hand, the applicant did not propose a post-market monitoring regarding the use of the GM stack maize in food, and EFSA in its opinion did not identify the need for such monitoring on the basis of the

[73] The JRC/EURL GMFF is in charge of testing and validating the methods of detection and identification proposed by the applicants in accordance with Articles 6(3)(d) and 18(3)(d) of Regulation (EC) No 1829/2003.

[74] Available at: http://gmo-crl.jrc.ec.europa.eu/statusofdossiers.aspx.

risk assessment. Your request does not provide any argumentation or evidence showing that such a monitoring was needed based on the outcome of the risk assessment.

Finally, concerning the monitoring plan for environmental effects, the Commission notes that, as stated in recital 7 of Commission Implementing Decision (EU) 2021/61, EFSA concluded that the plan submitted by the applicant, consisting of a general surveillance plan, was in line with the intended uses of the products. In your request, you simply mention aspects, including where and how environmental monitoring should be carried out, which were not included in the plan, without providing any argumentation or evidence as to the reasons why they should have been included or as to the way in which they were at odds with the requirements of Annex VII to Directive 2001/18/EC[75].

Based on the above considerations, the Commission is of the view that your claims regarding the detection methods and the post-market monitoring requirement in Commission Implementing Decision (EU) 2021/61 are unfounded.

[75] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC - Commission Declaration (OJ L 106, 17.4.2001, p. 1–39).