ANNEX

Assessment of the allegations included in the request for internal review of Commission Implementing Decision on GM maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and sub-combinations

1. Preliminary considerations:

The Commission would like to provide clarification in relation to certain statements made in Sections 1 and 3 of your request.

1.1. EU law applicable to the authorisation of the GM stack maize

You claim that the Commission Implementing Decision (EU) 2018/2046[1] authorising the placing on the market of the GM maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and sub-combinations (hereinafter, "the GM stack maize") is not in accordance with EU law; in particular, you make references to Directive 2001/18/EC[2], Regulation (EC) No 1829/2003[3] and Commission Implementing Regulation (EU) No 503/2013.[4]

In point 1.3 of your request you refer to the application of the procedural requirements of Directive 2001/18/EC[5] to the authorisation of the GM stack maize, and in particular to Article 13 of that Directive, which regulates the notification procedure applicable to the authorisation of deliberate releases of GMOs. The Commission would like to clarify that the authorisation of the GM stack maize was adopted pursuant to Regulation (EC) No 1829/2003 and not under Directive 2001/18/EC. Therefore, all references to procedural requirements of Directive 2001/18/EC in your request for internal review (including the references to the notification procedure under Article 13 of the Directive) are irrelevant for the assessment of the compliance of the authorisation decision with EU law.

Furthermore, in point 1.5 of your request you indicate that Commission Implementing Regulation (EU) No 503/2013 was applied to the risk assessment of the GM stack maize. The Commission would like to clarify that Implementing Regulation (EU) No 503/2013 is not applicable to the authorisation of the GM stack maize since that Regulation only applied with general character to applications submitted after 8 December 2013. Although the Regulation entered into force of 28 June 2013, at the moment when the application for the GM stack

[1] Commission Implementing Decision (EU) 2018/2046 of 19 December 2018 authorising the placing on the market of products containing, consisting of or produced from genetically modified maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122, and genetically modified maize combining two, three or four of the single events MON 87427, MON 89034, 1507, MON 88017 and 59122 and repealing Decision 2011/366/EU (OJ L 327, 21.12.2018, p. 70–76).

[2] 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).

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

[4] 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).

[5] You refer in particular to the application of Article 13 (which refers to the notification procedure) and of Article 19 of the Directive (which refers to the written consent for the placing on the market).

maize was submitted (26 November 2013) a transitional regime[6] which allowed applicants to choose between the application of Implementing Regulation (EU) No 503/2013 or Regulation (EC) No 641/2004[7] was in force. In the present case, the applicant requested, at the time of submission if its application for authorisation of the GM stack maize, the application of Regulation (EC) No 641/2004. Therefore, the assessment of the GM maize was carried out under Regulation (EC) No 1829/2003 and Regulation (EC) No 641/2004.

1.2. Scope of the environmental risk assessment (ERA)

1.2.1 Assessment of the health effects of GM food and feed

In point 1.3 of your request, you indicate that "*the environmental risk assessment has to include all effects which the placing of a GMO on the market may have on human health, including any possible cumulative effects*".

This statement is incorrect.

The environmental risk assessment and the food and feed safety assessment to be carried out under Regulation (EC) No 1829/2003 serve different objectives:

- According to Articles 5(3) and (4) and 17(3) and (4) of Regulation (EC) 1829/2003, applicants are required to submit data demonstrating the human and animal health safety of the GM food and feed, including data on molecular characterisation, comparative assessment, toxicity, allergenicity, and nutrition. The objective is to assess all possible effects on human and animal health due to the consumption of GM food and feed.
- In addition, in case of food and feed containing or consisting of GMOs (living GMOs), Articles 5(5)(a) and 17(5)(a) of Regulation (EC) No 1829/2003 impose an environmental risk assessment, with the objective to assess the environmental and health impacts due to the "*release"* of GMOs in the environment within the meaning of the relevant legislation[8]. This assessment must be carried out in accordance with the principles of Annex II of Directive 2001/18/EC.

For this reason, the Commission cannot agree with your claim that the environmental risk assessment must cover all effects that placing a genetically modified product on the market may have on human health or animal health. The effects on human and animal health assessed as part of the safety assessment are different from those assessed under the environmental risk assessment.

The objective of the environmental risk assessment is to assess the effects on health and the environment of the release of the GMO into the environment. This assessment, which focuses on the protection of workers and persons in contact or in the vicinity of a GMO release, is

[6] Article 9(1) of Implementing Regulation (EU) No 503/2013.
[7] Commission Regulation (EC) No 641/2004 of 6 April 2004 on detailed rules for the implementation of Regulation (EC) No 1829/2003 of the European Parliament and of the Council as regards the application for the authorisation of new genetically modified food and feed, the notification of existing products and adventitious or technically unavoidable presence of genetically modified material which has benefited from a favourable risk evaluation (OJ L 102, 7.4.2004, p. 14–25).
[8] Article 2(3) of Directive 2001/18/EC.

distinct from the safety assessment carried out under Regulation (EC) 1829/2003 for the assessment of the effects on health due to the consumption of the GM food and feed.

Therefore, the protection of human health mentioned in Annex II of Directive 2001/18/EC is to be interpreted as covering health issues related to the release of GMOs in the environment, and not as covering the health effects of the consumption of the GM food and feed, which is regulated by Regulation (EC) 1829/2003.

1.2.2 Assessment of pesticide residues

In points 1.3, 1.6 and 1.7 of your request you indicate that the environmental risk assessment also includes the assessment of the potential effects of the use of herbicides or pesticides on the GMO plant grown in the EU or GM products imported from third countries.

The Commission would like to clarify that the assessment of the potential effects of the use of herbicides on human health, including possible cumulative effects, is not a condition for the authorisation of GM food and feed under Regulation (EC) No 1829/2003.

This has been confirmed by the General Court of Justice of the European Union in case T-177/13[9], where it considered 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 on maximum residue levels ("MRLs") of pesticides in or on food and feed of plant and animal origin.[10] The safety of GM food and feed products with a possible presence of pesticide residues is, therefore, guaranteed by the combined application of Regulation (EC) No 1829/2003 and Regulation (EC) No 396/2005.

In any event, the Commission would like to clarify that Regulation No 396/2005 is applicable to pesticide residues on food and feed not only grown in the EU but also imported from third countries, as well as for both conventional and GM products. As any other food and feed, GM products placed on the Union market have to comply with the corresponding MRLs under Regulation No 396/2005. If a herbicide is used on a tolerant GM crop cultivated in a third country, and the resulting residue levels are not covered by the MRLs already set in the EU (for which it has previously been established that they are protective for consumers), an applicant would first have to submit relevant data based on the good agricultural practice (GAP) pertinent to the GM crop. If considered necessary on the basis of such assessment, MRLs for the commodity in question would be set at a level that is the lowest achievable level with that GAP and where the residues of the pesticide substance have no harmful effects on human health. Then, the import of such crop within the EU would only be possible if it complies with the set MRLs set under Regulation No 396/2005. For GM crops where not only the magnitude but also the nature of residues is different compared to its conventional counterpart, additional data are required to address the occurrence of specific metabolites (see section 3.1.3.2 for further explanation).

[9] Case T-177/13, *TestBioTech eV v European Commission*, Judgement of the General Court of 15 December 2016 (ECLI:EU:T:2016:736), paragraphs 228 and 233.
[10] Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC, OJ L 70, 16.3.2005, p. 1–16.

In point 1.6 of your request, you also indicate that it is worrying that EFSA, in its assessment of residues from spraying with glyphosate[11], stated that existing data are not sufficient to conclude on health risks derived from the consumption of products derived from herbicide-resistant maize.

The Commission would like to clarify that EFSA´s reasoned opinion on the review of the existing MRLs for glyphosate indicated that the available data were insufficient to derive MRLs and risk assessment values specific to EPSPS maize. However, EFSA evaluated a GAP for conventional maize that leads to higher residue levels than the GAP underlying the existing MRL for maize. For that GAP, EFSA derived an MRL and risk assessment values. 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.

1.3. Analysis of publications

In the introductory paragraph of Section 3 of your request you claim that some recent publications presented in your background document (Trtikova *et al*., 2017[12]; Santos-Vigil *et al*., 2018[13]; Fang *et al*., 2018[14]) were not available when the application was submitted and when EFSA performed its risk assessment of the GM stack maize.

While it is correct that these publications were not available in 2013 when the application was submitted, those publications have been already analysed by EFSA, which concluded that they do not bring new elements that would change the conclusions of its previous risk assessments. More information in this regard is provided in the following sections of this annex.

1.4 Methodology of the risk assessment carried out by EFSA

The submitted application concerns a GM stack maize, i.e a GM maize combining several (five in this case) single events, and covers also its sub-combinations, i.e the stack maize combining two, three or four of the single events present in the GM stack maize. It has to be recalled that the EFSA GMO Panel evaluated the GM stack maize and its sub-combinations in accordance with the relevant principles described in its guidelines for the risk assessment of GM plants and derived food and feed[15] (hereinafter, "the EFSA GMO Panel Guidance 2011"), for the ERA of GM plants[16], for the post-market environmental monitoring (PMEM) of GM

[11] EFSA, Reasoned Opinion on the review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2018;16(5):5263.

[12] Trtikova M, et al., Teosinte in Europe – Searching for the origin of a novel weed. Scientific Reports, 2017, 71: 1560.

[13] Santos-Vigil K.I., *et al.,* Study of the allergenic potential of Bacillus thuringiensis Cry1Ac toxin following intra-gastric administration in a murine model of food-allergy. International immunopharmacology, 2018, 61: 185.

[14] Fang J, *et al.,* Overexpressing exogenous 5 enolpyruvylshikimate-3-phosphate synthase (EPSPS) genes increases fecundity and auxin content of transgenic Arabidopsis plants. Frontiers in Plant Sciences, 2018, 9: 233.

[15] EFSA GMO Panel, Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011;9(5):2150.

[16] EFSA GMO Panel, Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 2010;8(11):1879.

plants[17] and for statistical considerations for the safety evaluation of GMOs[18], and after the risk assessments of the single events had been finalised[19,20,21,22,23]. For the GM stack maize, as mentioned in the EFSA GMO Panel scientific opinion on the GM stack maize[24] (hereinafter, "the EFSA scientific opinion"), and as for any GM stack crop, the assessment focused on issues related to (a) stability of the inserts, (b) expression of the introduced genes and their products and (c) potential synergistic or antagonistic effects resulting from the combination of the events.

When assessing a GM stack crop, the EFSA GMO Panel considers all datasets provided by the applicant (including additional information provided during the risk assessment phase), which include the data available on the single events, the stack itself and, in case of segregating crops such as maize, its sub-combinations, if available. Moreover, the scientific comments submitted by the Member States and the relevant scientific literature are taken into account.

2. Assessment of the allegations in Section 2 of your request:

In Section 2 of your request, you provide an overview of the EFSA risk assessment of the GM stack maize. Most of your claims in this Section are further detailed in the Section 3 of your request and are addressed in the following section of this annex. This section addresses only the claims which are not repeated in Section 3 of your request.

Regarding molecular characterisation aspects, you claim that other gene products, such as microRNA ("miRNA") from additional Open Reading Frames (ORFs) were not assessed and that material derived from the GM stack maize should have been assessed using "omics" techniques. As regards the comparative analysis, you claim that a more powerful statistical

[17] EFSA GMO Panel, Guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. EFSA Journal 2011;9(8):2316.
[18] EFSA GMO Panel, Statistical considerations for the safety evaluation of GMOs. EFSA Journal 2010;8(1):1250.
[19] EFSA GMO Panel, Scientific Opinion on application (EFSAGMO-BE-2012-110) for the placing on the market of tissue-selective herbicide-tolerant genetically modified maize MON 87427 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2015;13(6):4130.
[20] EFSA, Scientific Opinion of the Panel on Genetically Modified Organisms on application (Reference EFSA-GMO-NL-2007-37) for the placing on the market of the insect-resistant genetically modified maize MON89034, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2008;6(12):909.
[21] EFSA GMO Panel, Scientific opinion on an application for renewal of authorisation for continued marketing of maize 1507 and derived food and feed submitted under Articles 11 and 23 of Regulation (EC) No 1829/2003 by Pioneer Overseas Corporation and Dow AgroSciences LLC. EFSA Journal 2017;15(1):4659.
[22] EFSA GMO Panel, Application (Reference EFSA-GMO-CZ-2005-27) for the placing on the market of the insect-resistant and herbicide-tolerant genetically modified maize MON88017, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2009;7(5):1075.
[23] EFSA GMO Panel, Scientific opinion on an application for renewal of authorisation for continued marketing of maize 59122 and derived food and feed submitted under articles 11 and 23 of Regulation (EC) No 1829/2003 by Pioneer Overseas Corporation and Dow AgroSciences LLC. EFSA Journal 2017;15(6):4861.
[24] EFSA GMO Panel, Scientific Opinion on application EFSA-GMO-BE-2013-118 for authorisation of genetically modified maize MON 87427 X MON 89034 X 1507 X MON 88017 X 59122 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):4921.

analysis, such as multidimensional analysis, should have been applied to investigate the high number of significant effects.

These claims must be rejected.

In relation to molecular characterisation, the EFSA GMO Panel Guidance 2011 does not require the checking for unintended gene silencing coming from the newly created ORFs. EFSA has recognised that there is evidence in the peer-reviewed scientific literature for small RNA molecules to be produced from different sequences of the inserted T-DNA[25] and that these small RNA molecules would be unintended and linked to the genetic transformations. Possible unintended effects in the GM stack maize resulting from the potential production of such small RNAs are covered by the comprehensive comparative analysis, consisting of compositional and agronomic/phenotypic characterisation of the GM plant.

No relevant differences were identified between the GM stack maize and the non-GM comparator, in grain and forage composition and in the tested agronomic and phenotypic characteristics, which would have required further assessment regarding food and feed safety and environmental impact, except for a decrease of thiamin in the GM maize. The decrease in thiamin was considered not to give rise to a substantial modification in the composition of the food and feed derived from the GM stack maize and the nutritional assessment identified no concerns related to this decrease. As referred to in the EFSA scientific opinion, the GMO Panel did not identify indication for potential occurrence of unintended effects based on the molecular, compositional or phenotypic analyses.

Regarding "omics" techniques, the EFSA GMO Panel Guidance 2011 does not require such analyses. Last year in 2018, an EFSA Scientific Colloquium was organised and addressed the state of the art of "omics" technologies and next steps for its integration in the risk assessments performed by EFSA. As indicated in the event report[26], *"a consistent reporting framework for data collection, processing, interpretation, storage and curation should be further drawn up together with national and international organisations before 'omics' technologies can be routinely used in risk assessment"*.

In relation with the comparative assessment, the Commission considers that the statistical approach used for the identification of possible unintended effects of the genetic modifications was sufficient and in accordance with the approach described in the EFSA GMO Panel Guidance 2011. Further clarifications regarding the observed significant differences, which can be linked to correlations existing among several endpoints, are given in the section 3 of this annex.

Based on the molecular, agronomic, phenotypic or compositional characteristics, the combination of maize events MON 87427, MON 89034, 1507, MON 88017 and 59122 in the GM stack maize did not give rise to issues regarding food and feed safety and nutrition.

[25] 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 2015/687 to authorise genetically modified oilseed rape MON88302. EFSA supporting publication 2015:EN-864.

[26] EFSA, EFSA Scientific Colloquium 24 – 'omics in risk assessment: state of the art and next steps'. EFSA supporting publication 2018:EN-1512.

Therefore, your allegations on these points must be rejected.

3. Assessment of the allegations in Section 3 of your request – Grounds of your request:

3.1. Inadequate food and feed safety assessment

Your specific allegations on the inadequacy of the food and feed risk assessment are assessed below.

3.1.1 Allergenicity

In point 3.1 of your request, you claim that EFSA has not requested the applicant to provide data to verify whether the source of the transgene is allergenic.

You refer to a recent study published by Santos-Vigil *et al.*[27], not available at the time of EFSA risk assessment, according to which the Cry1Ac protein can act as allergen if ingested. You disagree with EFSA's assessment of that publication[28], stating that it was biased because EFSA (i) did not identify relevant findings that would need further investigation, (ii) criticised the relatively high dosage of Cry1Ac used in this study, and (iii) referred to other studies supporting its previous opinions without checking their scientific robustness and reliability. You therefore conclude that EFSA assessment is not valid. You also indicate that the Cry1A.105 protein expressed by the GM maize is synthetically derived from Cry1Ac, therefore you claim that the GM maize protein has structural similarity with Cry1Ac, and consequently the source of Cry1A.105 has to be investigated in detail.

These claims must be rejected.

First of all, the Commission considers that the allergenicity of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and its sub-combinations was duly assessed by EFSA, in line with its guidelines. It has also to be noted that the Cry1Ac protein is not present in the GM stack maize. For allergenicity, the main aspects considered in case of a GM stack crop application are those related to possible interactions between proteins, and potential concerns arising from new information not previously assessed in the context of the single event applications.

In the context of the respective single event applications, the GMO Panel had comprehensively assessed the safety of each newly expressed protein, including Cry1A.105, and no concerns on allergenicity for any of these proteins were identified. During that assessment, information on the transgene source of the newly expressed protein was the initial piece of evidence considered in the weight-of-evidence approach. This information is used to calibrate whether or not additional information is needed on a case-by-case basis. According to the applicable EFSA GMO Panel Guidance 2011, and in line with international

[27] See note 13.
[28] EFSA, 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.

guidelines[29], the assessment of newly expressed proteins requires the use of specific serum screening whenever their transgenes originate from sources considered to be allergenic. Foods recognised to be common allergenic food and of public health importance are listed in Annex II of the European Regulation on food information to consumers.[30,31] Whenever a newly expressed protein derived from a transgene of any of these sources is produced in a GM plant, it would trigger the request for specific serum screening from EFSA.

For the GM stack maize, because the sources of the transgenes are not considered common allergenic food, the EFSA GMO Panel did not consider necessary to request specific serum screening on any of the newly expressed proteins.

In addition, no new information on allergenicity of the newly expressed proteins, which might change the previous conclusions of the GMO Panel on the single events, became available during the risk assessment of the GM stack maize. Therefore, the GMO Panel did not identify reasons for concerns regarding the simultaneous presence of these proteins in the GM stack maize affecting their allergenicity, nor regarding the overall allergenicity of the GM stack maize.

The study by Santos-Vigil *et al.* on the allergenic potential of Cry1Ac protein in a murine model of food-allergy, which is related to the risk assessment of GM crops with Cry1Ac protein, has been carefully analysed by EFSA upon a request from the Commission. In reply to this Commission mandate, EFSA published a technical report[32], addressing the relevance of the study. It concluded that it does not bring new elements that would lead the EFSA GMO Panel to reconsider the outcome of its previous opinions on GM crops with Cry1Ac protein, which therefore remain valid and applicable, because shortcomings in the study design and data interpretation limit the possibility to unambiguously attribute findings to the intrinsic properties of Cry1Ac protein and to draw relevant conclusions for the risk assessment. EFSA also highlighted the contrasting evidence available on the topic from studies performed with different Cry proteins, i.e. Cry1Ac vs Cry1Ab, even though the identity in amino acid sequences between these two proteins can be higher than 90%.

The Commission does not share your views that EFSA's assessment is biased in the above-mentioned technical report. As underlined in this technical report, EFSA has previously assessed Cry1Ac protein, taking into account internationally agreed principles, the EU GM legislation and its own guidance documents. Based on this, EFSA had concluded on the safety of Cry1Ac protein in different GM crops. More generally, the Commission would like to stress that EFSA is a European agency that operates independently of any undue external influence from the European institutions, Member States and stakeholders in the public or private sectors.

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[29] Codex Alimentarius, 2003-2009. Foods derived from modern biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Rome

[30] Directive 2003/89/EC of the European Parliament and of the Council of 10 November 2003 amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs.

[31] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004

[32] See note 28.

Therefore, your allegations on this point must be rejected.

3.1.2 Adjuvanticity

In point 3.2 of your request you claim that EFSA has not requested the applicant to assess the possible role of Bt toxins as adjuvants despite the fact that several scientific publications indicate that Cry (Bt) proteins can trigger immune responses such as adjuvanticity in mammals, in particular Cry1Ac protein.

You also refer to the structural similarity between Cry1Ac and Cry1A.105, expressed in the GM stack maize, to infer that Cry1A.105 is likely to be immunogenic.[33] You state that according to the scientific literature, adjuvant effects are likely to be dependent on the dosage to which the mammals are exposed. You also state that, in the GM stack maize, the concentrations of Cry proteins will sum up and therefore the GM stack maize needs to be more carefully assessed than a GM maize producing a single Cry protein.

You also claim that EFSA failed to consider that Cry proteins will not be quickly degraded in the gut but are likely to be present in substantial concentrations in the large intestine.

Finally, you indicate that so far only two Bt toxins have been investigated for their potential impact of the immune system, namely Cry1Ac and Cry1Ab, and you claim that the Cry proteins present in the GM stack maize have not been investigated for this aspect, nor the effects caused by the combination of several Cry proteins. In this regard, you stress the need for more detailed investigation of potential immunogenic effects of Cry proteins, referring to a minority opinion expressed by one GMO Panel member in the context of the EFSA opinion on another GM stack maize application[34].

These claims must be rejected.

First, the Commission would like to recall that the EFSA scientific opinion on the GM stack maize does not contain a minority opinion. Moreover, the Commission considers that the potential adjuvant activity of proteins produced by maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and its sub-combinations was comprehensively assessed by EFSA, in line with the applicable guidance document, as referred to in the EFSA scientific opinion. According to the EFSA GMO Panel Guidance 2011, when known functional aspects of the newly expressed proteins or structural similarity to known adjuvants may indicate an adjuvant activity, the possible role of these proteins as adjuvants is considered. If newly expressed proteins with a potential adjuvant activity are expressed together, possible interactions increasing adjuvanticity and impacting the allergenicity of the GM crop are assessed.

No concerns on adjuvanticity were identified in the context of the single event applications. The EFSA GMO Panel has duly taken into account the levels of expression of the Cry proteins in the GM stack maize. It has to be emphasised that the levels of individual Bt proteins in the GM stack maize are similar to those in the respective single maize events (see Appendix A of the EFSA scientific opinion). Following an analysis of the available experimental evidence, the GMO Panel did not find indications that the presence of the Bt

[33] This claim has been addressed in the previous sub-section.
[34] Your claims related to this minority opinion are addressed in section 3.3.2 of this annex.

proteins, at the levels expressed in the GM stack maize, might act as adjuvants with the potential to enhance a specific immunoglobulin E (IgE) response and to favour the development of an allergic reaction. Therefore, the EFSA GMO Panel did not consider that there was a safety concern for the health of humans or animals that consume food/feed derived from the GM stack maize containing Cry proteins.

As mentioned in the EFSA scientific opinion, an adjuvant effect of Cry proteins has been suggested based on animal studies with Cry1Ac. However, the studies were performed using relatively high doses and routes of administration that are different from those occurring during intake of GM maize containing Cry proteins by human consumers. In addition, the adjuvant effect observed enhanced the immune response to co-administered proteins, but it was not shown to induce an allergic reaction or an IgE response.

Regarding protein degradation studies, such *in vitro* studies are considered by the GMO Panel as additional information for the safety assessment of the newly expressed proteins, in line with international guidelines[35] and the EFSA GMO Panel Guidance 2011.

The EFSA GMO Panel recently provided supplementary guidance on specific topics for the allergenicity risk assessment of GM plants[36], and in particular for *in vitro* protein digestibility tests. A refined *in vitro* digestion test is proposed, which extends the conditions currently used in the classical pepsin resistance test, in order to better reflect the range of conditions found *in vivo*. However, and as stated in the guidance, the EFSA GMO Panel considers that additional investigations are needed on the proposed *in vitro* protein digestibility tests, before any additional recommendation for applicants can be provided. To this end, an EFSA procurement[37] is ongoing, to evaluate the proposed revisions to the *in vitro* digestion test. After the completion of this procurement, EFSA will assess whether the proposed revised test adds value to the allergenicity risk assessment and, if so, what further steps are needed for its final implementation.

Finally, the Commission would like to note that no adjuvant effect has been observed when Cry proteins are expressed at the levels observed in GM plants so far assessed by the EFSA GMO Panel. Adjuvanticity of Cry proteins is a matter of scientific debate where most of the scientific groups agreed on the limited and contrasting evidence available for such an activity, including as regards dose-response relationships. Consequently, on the basis of current knowledge, EFSA and other risk assessment bodies concluded that there are currently no indications of safety concern regarding Cry proteins in the context of the GM plants assessed, as stated in EFSA technical report on your request for internal review ("EFSA technical report").[38]

[35] Codex Alimentarius, 2003-2009. Foods derived from modern biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Rome

[36] EFSA GMO Panel, Guidance on allergenicity assessment of genetically modified plants. EFSA Journal 2017;15 (5):4862. This guidance document was not applicable to the assessment of the GM stack maize.

[37] https://ted.europa.eu/udl?uri=TED:NOTICE:289049-2017:TEXT:EN:HTML

[38] EFSA, 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.

In any case, EFSA is committed to incorporating latest scientific developments regarding adjuvant and allergenic potential of proteins in its risk assessment process, when appropriate, as illustrated by the development of EFSA supplementary guidance on allergenicity[39].

Therefore, your allegations on this point must be rejected.

3.1.3 Potential synergistic or antagonistic effects of the stacked events, affecting toxicity and allergenicity of the GM maize

In point 3.3 of your request you claim that EFSA did not request data on potential synergistic or antagonistic effects resulting from the combination of the transformation events regarding toxicology and allergenicity. We address your claim in the sub-sections below.

3.1.3.1 Interactions affecting allergenicity

Regarding allergenicity of the combination of the transformation events, you refer to the potential of Bt toxins to trigger immune responses and show adjuvant effects in mammals, and to the presence of the proteins in the intestine due to slower *in vivo* degradation rate (compared to *in vitro* studies), and possible interactions with other feed components impacting their degradation rate.

These claims must be rejected.

In relation to interactions between the events potentially affecting allergenicity, two aspects are considered by EFSA, (i) the assessment of the newly expressed proteins simultaneously present in the GM plant, (ii) and the assessment of endogenous allergenicity.

As mentioned above, the EFSA GMO Panel has comprehensively taken into account the levels of expression of the Cry proteins in the GM stack maize, and the levels of individual Cry proteins in the GM stack maize are similar to those in the respective single maize events. As none of the proteins expressed by the GM stack maize showed allergenicity, and considering current knowledge, EFSA did not identify reasons for concerns regarding the simultaneous presence of these newly expressed proteins in the GM stack maize.

With regard to the assessment of endogenous allergenicity, the EFSA GMO Panel requests experimental data to analyse the allergen repertoire of a GM plant on a case-by-case basis (EFSA GMO Panel Guidance 2011). Taking into account that maize is currently not considered a common allergenic food[40,41] and that no indications of concern were identified from the molecular characterisation and compositional analysis, the EFSA GMO Panel did

[39] See note 36.

[40] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

[41] OECD (Organisation for Economic Co-operation and Development), Consensus Document on compositional considerations for new varieties of maize (Zea mays): key food and feed nutrients, anti-nutrients and secondary plant metabolites. Series on the Safety of Novel Food and Feeds (ENV/JM/MONO(2002)25), 6, 1–42

not considered necessary to request experimental data on the allergen repertoire of the GM stack maize.

Your other claims regarding the allergenicity of Bt proteins have been addressed in sections 3.1.1 and 3.1.2 of this annex.

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

3.1.3.2 Interactions affecting toxicity

Regarding toxicity, you claim that the combination of the transformation events can result in effects due to interactions at protein level, but also in relation with pesticides residues and that organ and reproductive toxicity should have been investigated.

More specifically, you claim that the applicant did not provide sufficient data on potential interactions between the newly expressed proteins and that negative impacts caused by Bt toxins on human and animal health cannot be excluded a priori. You also refer to several mechanisms of action of Bt proteins, of which not all of them are dependent of the specific mechanisms occurring in the target insect species. You also refer to various publications showing effects of Bt toxins in mammals, possibly after high concentration exposure, and with stronger effects in longer term.

You refer to the need to assess the potential effects of pesticides residues, including their possible combinatorial effects. You recall that the GM stack maize is tolerant to glyphosate and glufosinate-ammonium based herbicides, which should be seen, according to you, as potential co-stressors. You also highlight that in the GM stack maize the content of EPSPS protein is increased, due to the combination of MON 87427 and MON 88017 events (compared to the single events GM maize) and you make a link with a higher, and repeated, exposition to glyphosate.[42] You claim that the applicant only presented data on potential interactions between the different newly expressed proteins (Cry proteins and the enzymes conferring tolerance to the herbicides) while the potential interactions between the Cry proteins and residues from spraying with herbicides and their metabolites are more relevant for toxicology.

You also refer to potential effects of Bt toxins and/or herbicides residues on the intestinal microbiome, which could trigger adverse health effects. You indicate that plant material from the GM stack maize should have been assessed regarding organ toxicity, immune responses and reproductive toxicity, taking also into account possible combinatorial effects caused by Bt toxins and other plant components.

These claims must be rejected.

Contrary to your claim, the EFSA GMO Panel has assessed the various combinations of the six newly expressed proteins in the GM stack maize for potential toxicological effects.

For GM crops with stacked events, the toxicological assessment starts from the safety profile of the individual newly expressed proteins (previously assessed in the context of the single

[42] Your claims regarding the field trials design are addressed in section 3.2 of this annex.

events applications), corroborated by up-to-date scientific data and updated bioinformatics. The potential for adverse effects relevant for humans and animals of new protein combinations is then evaluated. To this aim, the EFSA GMO Panel uses generally accepted concepts of mixture toxicology and applies three different models to predict toxicity[43].

First, the individual proteins (Bt proteins, PAT and CP4 EPSPS) were considered safe for humans and animals and no new data impacting the protein toxicological assessment performed in the context of the single event applications were identified (EFSA scientific opinion).

For the newly expressed Bt proteins (Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1 and Cry35Ab1), as mentioned in the EFSA scientific opinion, the EFSA GMO Panel considered up-to-date available information on their biological function, mode of action, and available toxicological information.

Based on the scientific literature and as elaborated by EFSA in its technical report, the mode of action of Cry proteins is a multistep process, starting from proteolytic processing in the insect gut releasing the activated toxin (in case of paracrystallin forms) to the binding to different insect protein molecules/receptors in gut cells leading to oligomerization, membrane insertion, and pore formation. Binding interactions (protein-protein and protein-carbohydrate) have been proposed between Cry toxin domains and receptors, and recognition of unique carbohydrate structures present in invertebrates has been proposed to explain specificity of Cry toxins to insects and nematodes but not to vertebrates. Such cellular receptors have not been identified in animals and humans, this representing a major limitation for a possible effect of these proteins on the gastrointestinal tract after oral ingestion.

According to EFSA, *in vitro* evidences regarding potential cytotoxic effects of Bt proteins in mammalian cells do not offer, for the time being, sufficient evidence to change current conclusions of the GMO Panel[44]. Other mode of actions for Cry proteins have been hypothesized (e.g. detergent mode[45]). However these derive from *in vitro* models and their translatability to *in vivo* conditions is not established.

Regarding the enzymes (PAT and CP4 EPSPS) conferring herbicide tolerance to the GM stack maize, as stated in the EFSA scientific opinion, an interaction among these enzymes is unlikely since they are known to catalyse distinct biochemical reactions and to act on unrelated substrates in the plant, with high substrate specificity.

Overall, there are no indications that the combination of the Bt proteins Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1 and Cry35Ab1 or of PAT and CP4 EPSPS enzymes in the GM stack maize and its sub-combinations would raise toxicological concerns for food and feed due to the absence of a mechanism of toxicity relevant for humans and animals. Given the current knowledge of their biological functions, the EFSA GMO Panel considers that possible

[43] More details can be found in EFSA technical report.
[44] Such as, for example, EFSA, Statement on a request from the European Commission related to an emergency measure notified by France under Article 34 of Regulation (EC) 1829/2003 to prohibit the cultivation of genetically modified maize MON 810. EFSA Journal 2014;12(8):3809;
[45] COGEM Report 14/001: https://www.cogem.net/showdownload.cfm?objectId=39FB5B0E-B9A0-BB5C-5B4F558545968DB1&objectType=mark.hive.contentobjects.download.pdf

interactions between Bt proteins and enzymes newly expressed in the GM stack maize are not plausible.

In relation to plant protection products and their residues, it should be emphasised that the risk assessment of the GM stack maize was conducted in accordance with the applicable EFSA GMO Panel Guidance 2011 and therefore on materials treated, during the field trials, with the intended herbicides, sprayed in combination and in accordance with GAP (see details in section 3.2 of this annex). The likelihood of compositional and/or agronomic/phenotypic unintended effects resulting from the possible herbicide-caused stress is covered by analysing the compositional and agronomic/phenotypic characteristics of the treated and untreated GM plants. As specified in the EFSA scientific opinion, these analyses did not reveal statistically significant differences/lack of equivalences (unintended effects) for the GM stack maize versus its comparator and reference commercial varieties that could raise safety concerns for humans and animals health. Thus, a potential combined effect of the newly expressed proteins and the herbicide treatment was considered.

Regarding the possible "combinatorial" effects of pesticide residues, EFSA's Scientific Committee recently developed, in the context of the MIXTOX project, a guidance on new approaches and tools for harmonising how to assess risks to humans and the environment from combined exposure to multiple chemicals. This document[46] is intended to support all relevant areas within EFSA's remit, including human health and environmental aspects. In addition, the EFSA Pesticides Panel is working on two pilot assessments on the risks posed to humans by residues of multiple pesticides in food.[47]

However, the Commission would like to emphasise that such ongoing EFSA work is independent from the GMO assessment since, as explained in section 1 of this annex, the assessment of herbicide residues present on food and feed is not a condition for the authorisation of GM food and feed under Regulation (EC) No 1829/2003.

EFSA has already assessed several requests for setting MRL import tolerances for GM crops cultivated in third countries. In such respect, EFSA requires metabolism studies in the GM crops and evaluates if GM-crop specific metabolites are observed and should be added to the residue definition. If this is the case, EFSA requests toxicological studies on those metabolites. In the case of GM maize, metabolism studies of glyphosate[48] and glufosinate[49] have been explored and supported residue definitions.

As stated in the EFSA scientific opinion, based on the outcome of compositional analysis for the GM stack maize, which did not show statistically significant differences/lack of equivalences versus the non-GM comparator and the reference varieties that could raise safety concerns and impact the nutritional profile of derived food and feed, the GMO Panel was of the opinion that there was no need to investigate further organ toxicity, immune responses and reproductive toxicity.

[46] EFSA Scientific Committee, Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA Journal 2019;17(3):5634
[47] https://www.efsa.europa.eu/en/press/news/181205
[48] See note 11.
[49] EFSA, Reasoned opinion on the review of the existing maximum residue levels (MRLs) for glufosinate according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2015;13(1):3950.

Regarding the use of animal feeding studies to address potential uncertainties on stacked events, EFSA underlines that the sensitivity of such studies to indicate the presence of adverse effects related to the test material is limited, due to various hurdles, such as dose level, unless a clear test-hypothesis is identified, which was not the case for the GM stack maize[50].

Therefore, the Commission cannot agree with your claim that more data were needed to conclude on the safety of the GM stack maize and your allegations on this point must be rejected.

3.2 Inadequate field trials design/experiments

Your specific allegations on the inadequacy of the field trials design/experiments are assessed below.

3.2.1 Inadequate agricultural practices during field trials

In point 3.4 of your request you claim that EFSA did not request data from the field trials necessary to assess whether expected agricultural practices influence the expression of the studied endpoints.

You stress the importance of the relevance of the material used in the comparative assessment. In the case of herbicide-tolerant GM crops, such as the GM stack maize, you emphasise that the field trials have to be designed so as to assess the potential impact of the agricultural practices. In this respect, you claim that the overall dosage, the timing of the spraying and the number of spraying of the complementary herbicides (those to which the GM stack maize is tolerant) was not reflecting the regional agricultural practices in the receiving environments of the GM stack maize. You also claim that different patterns in the herbicides application (formulation of the herbicides, use of them in combination or not, dose..) were not considered in the field trials design. You therefore claim that there was no assessment of whether the expected agricultural practices influence the composition and agronomic/phenotypic characteristics of the GM maize and that the findings of the comparative assessment cannot be accepted.

You claim that scientific publications show that the application of complementary herbicides can change plant composition, in particular Zobiole *et al*., 2012[51].

You also highlight that the GM stack maize combines two events producing the CP4 EPSPS protein, and therefore the content of CP4 EPSPS protein is increased, as compared to the GM maize with the single events. You make a link with a higher, and repeated, exposition to glyphosate of the GM stack maize.

You also refer to a publication by Fang *et al*., 2018[52] showing effects of EPSPS enzyme on plant growth hormone metabolism and you claim that a higher expression of EPSPS,

[50] EFSA Scientific Committee, EFSA guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. EFSA Journal 2011;9(12):2438; EFSA, Explanatory statement for the applicability of the Guidance of the EFSA Scientific Committee on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed for GMO risk assessment. EFSA Journal 2014;12(10):3871.

[51] Zobiole L.H.S., *et al.*, Glyphosate effects on photosynthesis, nutrient accumulation, and nodulation in glyphosate-resistant soybean. J. Plant Nutr. Soil Sci., 2012, 175: 319.

potentially triggered by high dose of glyphosate, can lead to changes in plant composition and phenotypic characteristics.

You also claim that data presented show a high number of significant differences (~75% of the analytes evaluated for plants composition), and that apart from the relevance of each significant result, the overall number of significant effects should have called for more detailed investigations.

Your claims cannot be accepted.

First, as mentioned above, in case of herbicide tolerant crops, the experimental design of the field trials recommended by the EFSA GMO Panel (EFSA GMO Panel Guidance 2011 and EFSA GMO Panel Guidance on the ERA of GM plants) includes the GM crop, its non-GM comparator and non-GM reference varieties, all treated with conventional herbicides according to local requirements; and the GM crop treated with the intended (complementary) herbicide(s), in addition to the same conventional herbicides.

The EFSA GMO Panel considered the applied management practices and the environmental conditions under which the plants were cultivated as representative of the regions where the GM stack maize can be grown. The outcome of the comparative analysis allowed the EFSA GMO Panel to conclude that the GM stack maize would not raise safety concerns.

As regards the applied herbicides, as described in the EFSA scientific opinion, the material used for the comparative analysis has been obtained from eight sites, reflecting diverse agronomic practices and environmental conditions. At the eight selected sites, conventional herbicides at the planting/pre-emergence and/or post-emergence phase were applied. In addition, the glyphosate- and glufosinate ammonium-containing herbicides were applied on the GM stack maize in two sequential applications.

According to EFSA, it is not surprising that the complementary herbicides are kept at a similar application rate across sites: indeed, for the experimental treatments to be comparable between different locations, the application rate should not differ too strongly between them. However, the different combinations of conventional/complementary herbicides applied at the selected sites represent different possible weed management practices, and the chosen weed control schemes are likely to maintain the weed pressure under reasonable control.

The timing and rate of the applied intended herbicides are in line with the recommendations of the manufacturers and also, more generally, with the literature recommending the use in pre-emergence of a residual herbicide followed by an in-crop glyphosate application[53]. They are also in line with the official recommendations from the Ministry of Agriculture, Food and Rural Affairs of Ontario (2016/2017), to which you refer to in your background document, which advises four possible strategies for both glyphosate- and glufosinate-tolerant maize, the pre-emergence application of a residual herbicide followed by an in-crop application of glyphosate and glufosinate being one of these strategies. It is the one, which was applied in most of the cases by the applicant.

[52] See note 14.

[53] Nurse RE, *et al.*, Weed control and yield are improved when glyphosate is preceded by a residual herbicide in glyphosate-tolerant maize (Zea mays), Crop Protection, 2006, 25(11): 1174.

EFSA recognises that different weed control strategies might be selected by farmers in different countries and in accordance with specific local management practices, the specific weed population, crop rotation, and profitability linked to the herbicide selection. However, it is considered unfeasible in practice to assess herbicide tolerant GM plants under all possible scenarios. The EFSA GMO Panel considered that the different strategies selected by the applicant allowed to assess whether the agricultural practices, representative of the regions where the GM plant can be grown, influence the expression of relevant agronomic, phenotypic and compositional endpoints of the GM stack maize.

Regarding the publication with glyphosate-tolerant soybean that you mention (Zobiole *et al*., 2012), the authors identify that in soybean a higher and later glyphosate application correlates significantly and negatively with several plant physiological parameters; however, no data were collected on bean composition. The authors infer that the observed glyphosate impact on plant physiology might have an impact also on soybean yield. Specific data on the influence of glyphosate application on maize grain yield are available, where the authors conclude that, under certain conditions, maize yield is reduced in case of late glyphosate application[54].

However, in the case of the GM stack maize (treated and not treated with the intended herbicides), the comparative analysis carried out did not show statistically significant differences/lack of equivalences versus its non-GM comparator and the reference varieties that could raise safety concerns and impact the nutritional profile of derived food and feed.

Concerning the combination of herbicide tolerance traits, the Commission does not agree with your view that the GM stack maize will be exposed to higher and repeated dosage of a single complementary herbicide. As stated in EFSA technical report, there is evidence that the use of glyphosate in early stage guarantees a more effective weed control and prevents yield loss[55]. In addition, the presence of two distinct herbicide tolerant traits in the GM stack maize makes it more likely that, in case of glyphosate tolerant weeds, the farmers will opt for a more effective herbicide.

It is also interesting to note that in the Zobiole *et al.* publication[56] that you mention, it is recommended to use pre-emergence herbicides and to anticipate the glyphosate treatment rather than to increase its rate of application, to reduce possible undesirable effects of glyphosate.

With regard to the effects of EPSPS enzyme, which confers tolerance to glyphosate in GM crops, the publication by Fang *et al.*, 2018, reports on increased fitness of Arabidopsis plants overexpressing *epsps* genes. This increase in fitness is observed in the absence of glyphosate treatment. Thus, the authors conclude that it is the surplus of EPSPS that may affect plant growth and confer the increased fitness. Whether these findings can be generalised to a broader range of species, transgenic EPSPS-expressing events and environmental conditions requires further research.

[54] Soltani N, *et al*., Biologically effective dose of glyphosate as influenced by weed size in corn. Canadian Journal of Plant Science, 2016, 96(3): 455.

[55] See note 54.

[56] See note 51.

EFSA considered that the levels of CP4 EPSPS showed the expected higher levels in the GM stack maize, resulting from the combination of MON 87427 and MON 88017 events, both producing CP4 EPSPS protein. However, they were well within the range of values determined for other GM maize plants producing CP4 EPSPS proteins previously assessed by the EFSA GMO Panel.

It has to be noted that fitness-related traits are assessed for each GM crop, treated or untreated with the intended herbicides in case of herbicide-tolerant GM plants. For the GM stack maize, the GMO Panel considered, given the magnitude of the observed differences, the outcome of the equivalence test and the nature of the endpoints, that none of the agronomic and phenotypic differences between the GM stack maize and the non-GM comparator were relevant for further assessment.

Regarding the significant differences observed in compositional analysis, the EFSA GMO Panel considered further the whole set of statistical outcomes during the risk assessment and found that the effective number of significant results is lower than what you claim, as the changes observed in the level of all 18 amino acids (30% of the analytes) are highly correlated with the change in the level of a single analyte, crude protein in grain. Hence, the number of significant differences *per se* was not considered a reason for concern.

Considering the outcome of the comparative analysis, the EFSA GMO Panel concluded that the difference between the GM stack maize and its non-GM comparator in thiamin levels in grain was the only endpoint needing further assessment regarding food and feed safety. The EFSA GMO Panel assessed the reduced thiamin levels and concluded that the nutritional impact of the foods and feeds derived from the GM stack maize is similar to that expected from the non-GM comparator and non-GM commercial reference varieties.

In sum, the Commission considers that the field trials were carried out under appropriate conditions that allow drawing conclusions as regards the safety of the GM stack maize and therefore rejects this allegation.

3.2.2 Narrow range of meteorological and agronomic conditions

In point 3.5 of your request you claim that EFSA did not request data on field trials that reflect the different meteorological and agronomic conditions under which the crop is to be grown, and you claim that the design of the field trials represents only a narrow range of different meteorological and agronomic conditions.

Regarding to meteorological conditions, you indicate that the field trials were conducted only over one season, and in one country (United States), and that data from two sites "adversely affected by weather conditions" were excluded from the assessment.

For agronomic conditions, you claim that the field trials fail to represent the different conditions under which the crop is to be cultivated, in particular concerning possible different herbicides regimes. Moreover, you claim that reactions to biotic stressors were not tested.

You also claim that the plant materials used for the comparative analysis is not representative and that more varieties carrying the transgenes should have been included in the field trials to see how the gene constructs interact with the genetic background of the plants.

Finally, you refer to some publications concerning the unexpected effects of environmental stress on patterns of gene expression in GM plants and genotype X environment interactions.

Your claims regarding the higher content of CP4 EPSPS protein in the GM stack maize, the findings of Fang et al., the herbicides regime, and the number of endpoints with significant differences have been addressed in the above section.

Your claims cannot be accepted.

As stated above, EFSA approach requires that each field trial is replicated at a minimum of eight sites, chosen to be representative of the range of likely receiving environments (REs) where the GM plant is to be grown, thereby reflecting relevant meteorological, soil and agronomic conditions. The field trials may be conducted in a single year or spread over multiple years. Since the REs are highly diverse and dynamic over time, it is considered unfeasible, in practice, to assess GM crops under all possible REs[57]. Therefore, applicants should select localities sufficiently different to capture the environmental variability within the set of possible receiving environments in which the test materials may be grown.

As described in EFSA scientific opinion, the material used for the comparative analysis was grown in eight (for the compositional analysis) and nine (for the agronomic/phenotypic characterisation) 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. This was documented in the field production data provided by the applicant, including information on meteorological and agronomic conditions.

In light of the information provided by the applicant, the EFSA GMO Panel considered that the meteorological and agronomic variability at the sites selected for the compositional and agronomic/phenotypic characterisation of the GM stack maize ensure a sufficient range of environmental conditions, reflecting those under which the GM stack maize might be cultivated in practice. Therefore, the EFSA GMO Panel considered that there was no need to request additional field trials conducted in other maize growing regions or conducted over more than one season.

Regarding the sites affected by weather conditions that have been excluded from the assessment, this was indicated by the applicant in the application dossier, and supported by justifications, which were accepted by the EFSA GMO Panel in the light of its applicable guidance. In general, the EFSA GMO Panel acknowledges that some sites and/or replicates may not produce data of an appropriate quality for a range of reasons outside the control of the applicant, such as weather conditions[58].

As regards, the potential impacts of biotic and abiotic stressors, the Commission disagrees with your view that they have not been assessed for the GM stack maize. In field trials conditions, a variety of abiotic and biotic stressors including pests, fungal and bacterial diseases are naturally present. Different responses to these stressors between the GM stack maize and its non-GM comparator were collected and reported by the applicant. The data on

[57] EFSA GMO Panel, Guidance on the environmental risk assessment of GM plants. EFSA Journal 2010;8(11):1879.

[58] EFSA GMO Panel, Guidance on the agronomic and phenotypic characterisation of genetically modified plants. EFSA Journal 2015;13(6):4128.

environmental interactions with biotic and abiotic stressors were considered appropriate by the EFSA GMO Panel for the scope of the application (which excluded cultivation). No differences were identified in the comparative analysis for biotic and abiotic interactions between the GM stack maize and its non-GM comparator.

In addition, in the case of GM plants having traits specifically intended to reduce their susceptibility to abiotic stressors such as drought, the EFSA GMO Panel considers relevant to characterise the agronomic/phenotypic characteristics of the GM plant across sites over a natural gradient of the stressor, or through local manipulation of the environment[59]. As the GM stack maize does not contain such type of traits, the EFSA GMO Panel considered sufficient to perform its agronomic/phenotypic characterisation under typical environmental conditions.

Considering the appropriateness of the genetic background selected by the applicant and the sufficient variability of the REs where the field trials were conducted, the EFSA GMO Panel did not see the need to request additional varieties carrying the five transformation events to be included in the field trials.

Finally, regarding the cited publications on the effects of environmental stress on patterns of gene expression (e.g Trtikova *et al*., 2015[60]) or about genotype X environment interactions, the Commission would like to stress that the findings reported by Trtikova *et al*., 2015, were already assessed by EFSA[61] and present no new scientific information that would invalidate the EFSA GMO Panel previous risk assessment conclusions for GM maize with Cry1Ab protein.

EFSA acknowledges that, in general, environmental or genetic factors or a combination of both can affect gene expression, plant composition and phenotypical characteristics of GM plants, and that this is well documented in peer-reviewed literature. Therefore, it is considered key that the sites selected for the field trials reflect the different meteorological and agronomic conditions under which the crop is to be grown. As already mentioned, the EFSA GMO Panel considered that the site locations selected for the agronomic/phenotypic and compositional field trials were sufficiently diverse to capture variability in agrometeorological conditions.

In addition, interactions between the environmental factors and the different test materials were analysed, described and commented during the assessment of the GM stack maize. The EFSA GMO Panel considered that the information provided by the applicant was adequate for the comparative analysis.

The Commission considers that, in accordance with EFSA Guidance, the production of material for the comparative assessment was adequate, and that EFSA assessed the comparative analysis performed by the applicant in a comprehensive manner.

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[59] See note 57.

[60] Trtikova M *et al*., Transgene expression and Bt protein content in transgenic Bt maize (MON810) under optimal and stressful environmental conditions. PloS one, 2015, 10(4): e0123011.

[61] EFSA, 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:EN878.

The Commission therefore rejects these claims.

3.3 Inadequate protein expression dataset

3.3.1 Missing reliable protein expression data from the newly expressed proteins

In point 3.6 of your request you claim that EFSA did not request sufficiently reliable protein expression data from the newly expressed proteins and that consequently the molecular risk assessment by EFSA cannot be accepted. In particular, you claim that, in the GM stack maize, not only the two enzymes conferring herbicide tolerance have an higher overall expression rate compared to the GM single event plants, but also the Bt toxins in grain and forage. You state that this might be caused by several factors (such as interference of EPSPS enzyme, agricultural practices, impact of stressors or varietal background) affecting the expression of the events.

You also claim that sub-combinations of the GM stack maize should have been grown in parallel in the field trials to allow a direct comparison with the GM stack maize[62].

Your claims cannot be accepted.

EFSA acknowledges that in general protein expression can be affected by several factors, e.g. genetic background, environmental conditions and agricultural practices, as it is well documented in peer-reviewed literature. Protein expression variability was considered by the EFSA GMO Panel during its assessment of the GM stack maize application.

As referred to in the EFSA technical report, EFSA considers that certain agricultural practices (including herbicide treatments) or environmental conditions could impose stress on plants (conventional or GM) which may in some cases influence protein expression. However, such conditions can be temporary and the possible consequences on the protein levels are unpredictable, i.e. may result in either higher or reduced protein levels.

As mentioned in section 3.2.2, the GM stack maize was not designed to target abiotic stressors and therefore the EFSA GMO Panel considered protein expression measurements of GM plants grown under typical field conditions adequate (EFSA GMO Panel Guidance 2011). In addition, the EFSA GMO Panel considered that the protein expression data produced from field trials across five locations in representative maize-growing areas sufficiently accounted for protein expression variability possibly arising from diverse environmental conditions.

In order to assess the changes in protein expression levels that may result from potential interactions between the events, protein levels were determined for the GM stack maize and the corresponding single events in different parts of the plant. The methodology applied to quantify the newly expressed proteins levels was validated and considered acceptable by the EFSA GMO Panel. Contrary to your allegations, the results showed that the levels of the Cry proteins were similar in the GM stack maize and the parental single events maize (<1.4-fold difference for grain and forage tissues). The CP4 EPSPS and PAT protein levels showed

[62] The design of the field trials is addressed in section 3.2.1 of this annex.

expected differences resulting from the combination, respectively, of events MON 87427 and MON 88017, and events 1507 and 59122 (Appendix A of the EFSA scientific opinion).

Therefore, the EFSA GMO Panel concluded that there was no indication of interactions that may affect the levels of the newly expressed proteins in the GM stack maize.

In addition, it should be noted that the CP4 EPSPS and PAT protein levels in the GM stack maize are well within the range of values determined for other GM maize plants producing PAT or CP4 EPSPS proteins previously assessed by the EFSA GMO Panel.

Therefore, your allegation in this respect is not pertinent and should be rejected.

3.3.2 Absence of data for all maize sub-combinations

In point 3.7 of your request, you claim that EFSA did not request data on all sub-combinations.

You claim that the applicant should have submitted data on the sub-combinations of the GM stack maize, as those could be cultivated with different agricultural practices than the GM stack maize, and that the sub-combinations should have been grown in parallel with the GM stack maize[63]. In this regard, you compare the GM stack maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 with the four-event stack maize MON 89034 x 1507 x MON 88017 x 59122, previously assessed by EFSA[64] and producing the same Cry proteins, and claim that the Cry proteins have a higher expression in the four-event stack maize than in the GM stack maize.

You call for a specific risk assessment of each of the sub-combinations, and you refer to a minority opinion expressed by one GMO Panel member in the context of the EFSA opinion on another maize application.

Your claims cannot be accepted.

Regarding the safety assessment of sub-combinations of GM crops with stacked events, the EFSA GMO Panel established principles[65] and those were followed to assess the safety of the sub-combinations of the GM stack maize.

For sub-combinations for which no specific data were provided, a weight of evidence approach that takes as a starting point the results of the assessment of (i) the five single events MON 87427, MON 89034, 1507, MON 88017 and 59122, (ii) the comprehensive dataset

[63] The design of the field trials is addressed in section 3.2.1 of this annex.
[64] EFSA Panel on Genetically Modified Organisms, Scientific Opinion on application (EFSA-GMO-CZ-2008-62) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Dow AgroSciences and Monsanto. EFSA Journal 2010; 8(9):1781.
[65] GMO Panel approach for the risk assessment of sub-combinations as required by Implementing Regulation (EU) No 503/2013, Annex I of Minutes of the 115th Plenary meeting of the Scientific Panel on GMO.

generated for the GM stack maize, and (iii) the data available on eleven maize sub-combinations previously assessed by the GMO Panel[66], was followed.

For those sub-combinations, as stated in the EFSA scientific opinion, the EFSA GMO Panel assessed genetic stability, expression of the events and potential functional interactions between them. Based on the outcome of the risk assessments of the single events, of the GM stack maize, and previously assessed sub-combinations, there was no expected effect linked to specific sub-combinations and the EFSA GMO Panel concluded that these sub-combinations do not raise safety concern. Therefore, there was no trigger to request additional data for the sub-combinations not previously assessed.

As regards your claim on the alleged different protein expression levels in the GM stack maize, as compared to a four-event stack maize, it has to be noted that the protein expression levels for these two stack maize were collected from different field trials, performed in the context of independent applications. As stated in EFSA technical report, the direct comparison of protein expression levels of plants grown in different trials is scientifically not appropriate, because protein expression can be influenced by many factors (e.g. environmental factors). In addition, for both stack maize, the assessment of the newly expressed proteins raised no concerns relevant for food and feed safety.

Finally, in relation with the minority opinion expressed by one GMO Panel member in the context of another stack maize application (maize Bt11 x MIR162 x 1507 x GA21) regarding the sub-combinations, in particular on the relevance of protein levels and potential adjuvanticity of Cry proteins in the sub-combinations, in addition to the aspects described in the EFSA opinion on that GM maize[67], additional considerations on the topic can be found in the minutes of the GMO Panel plenary meeting[68] where that application was discussed. The following aspects were underlined and used by all the other GMO Panel members to conclude positively on the sub-combinations: (i) no indications of interactions between the events raising safety issues were identified; (ii) the comparison of levels of newly expressed proteins between that stack maize and each of the single events did not reveal an interaction manifesting at protein expression level; (iii) data on genetic stability and protein expression for seven sub-combinations previously assessed did not show evidence for interactions; (iv) the potential adjuvanticity of Cry proteins was extensively discussed considering all available information (see also section 3.1.2).

The same considerations are also valid for the GM stack maize at stake. Therefore, the Commission considers that the EFSA GMO Panel has adequately assessed the maize sub-combinations and your claim in this regard must be rejected.

3.4. Inadequate environmental risk assessment (gene flow)

[66] Maize 1507 x 59122 ; maize MON 89034 x MON 88017 ; and maize MON 89034 x 1507 x MON 88017 x 59122 and its sub-combinations.
[67] EFSA GMO Panel, Scientific Opinion on the assessment of genetically modified maizeBt11 x MIR162 x 1507 x GA21 and three subcombinations independently of their origin, for food and feed uses under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2010-86). EFSA Journal2018;16(7):5309.
[68] Minutes of the 123rd GMO Panel plenary meeting:
https://www.efsa.europa.eu/sites/default/files/event/180530-m.pdf

In point 3.8 of your request, you claim that EFSA did not request the applicant to assess gene flow to other cultivated or wild plant species.

You claim that the potential for plant to plant gene transfer has been incorrectly assessed by the applicant in the application dossier, because it did not take into account recent publications (i.e. Pascher 2016[69]; Trtikova *et al*., 2017[70]; Fang *et al*., 2018[71]) related to the appearance of maize wild relative (teosinte) in Spain and France, higher volunteer potential of maize and possibly enhanced fitness of GM maize. You claim in this regard that the applicant should have provided additional evidence showing that no adverse effects can occur through gene flow from maize to teosinte and/or from teosinte to maize volunteers.

Your claim cannot be accepted.

First, it has to be noted that the scientific publications to which you refer to were published after the submission of the GM stack maize application and therefore could not have been considered by the applicant in its assessment of vertical gene flow. EFSA is of the opinion that the applicant considered all scientific evidence available at the time of submission of the application. In addition, an updated literature search performed by applicant (covering the period from the time of validity of the application until November 2016) did not identify scientific information that could change the conclusions on the GM stack maize.

EFSA notes that the assessment of the potential plant-to-plant gene transfer in the GM stack maize application followed the step-wise approach described in its guidance on the ERA of GM plants and that all relevant scientific evidence was considered, including that on the presence of teosinte plants.

Considering the scope of the GM stack maize application, which excludes cultivation, the potential for occasional feral GM stack maize plants originating from grain import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer were taken into account. As stated in the EFSA scientific opinion, for plant-to-plant gene transfer to occur, imported GM maize grains need to germinate and develop into plants in areas containing sympatric wild relatives and/or cultivated maize with synchronous flowering and environmental conditions favouring cross-pollination.

Maize is an annual predominantly cross-pollinating crop. Cross-fertilisation occurs mainly by wind[72]. Vertical gene transfer from maize is limited to *Zea* species. Wild relatives of maize outside cultivation are not known/reported in Europe (Eastham and Sweet, 2002[73]; OECD, 2003[69]; EFSA, 2016[74]; Trtikova *et al*., 2017[75]). Therefore, potential vertical gene transfer is

[69] Pascher K, Spread of volunteer and feral maize plants in Central Europe: recent data from Austria. Environmental Sciences Europe, 2016, 28: 30.
[70] See note 12.
[71] See note 14.
[72] OECD (Organisation for Economic Co-operation and Development), Consensus Document on the biology of Zea mays subsp. mays (Maize). Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO(2003)11), 27: 1–49.
[73] Eastham K and Sweet J, Genetically modified organisms (GMOs): The significance of gene flow through pollen transfer. European Environment Agency, Environmental issue report, 2002, 28: 1–75.
[74] EFSA, Relevance of new scientific evidence on the occurrence of teosinte in maize fields in Spain and France for previous environmental risk assessment conclusions and risk management recommendations on the cultivation of maize events MON810, Bt11, 1507 and GA21. EFSA supporting publication 2016:EN-1094.

restricted to maize and weedy *Zea* species, such as teosintes, and/or maize-teosinte hybrids, occurring in cultivated areas (EFSA, 2016, Trtikova *et al*., 2017).

The potential of spilled maize grains during import and/or processing to establish, grow and produce pollen is extremely low and transient. Therefore, likelihood/frequency of cross-pollination between occasional feral GM maize plants resulting from grain spillage, and weedy or cultivated *Zea* plants is considered extremely low (EFSA, 2016[76]). Even if cross-pollination would occur, EFSA considered that environmental effects because of the spread of genes from occasional feral GM maize plants in Europe will not differ from that of conventional maize varieties.

Therefore, the EFSA GMO Panel did not consider necessary to request additional information from the applicant to conclude on the potential for vertical gene flow.

Therefore, your claim in this regard must be rejected.

3.5. Lawfulness of the adoption of the authorisation decision

In point 3.10 of your request and based on all your previous claims in points 3.1 to 3.9 of your request, you claim that the Commission should have not relied on EFSA's scientific opinion on the GM stack maize to authorise the placing on the market of the product.

As explained above, the Commission considers that all your claims concerning EFSA's risk assessment of the GM stack maize are unfounded and must be rejected. Consequently, the Commission considers that the decision to authorise the placing on the market of the GM stack maize through the adoption of the Commission Implementing Decision was correct.

Therefore, your allegation on this point must be rejected.

3.6 Detection method for the GM stack maize

In point 3.11 of your request you claim that no specific detection method for the stacked event was made available, and therefore it is difficult to distinguish the event from the parental lines for monitoring purposes.

In accordance with Article 3(e) of Regulation (EC) No 641/2004 the applicant should provide a description of a method(s) of detection, sampling and event-specific identification of the transformation event, as provided for in Articles 5(3)(i) and 17(3)(i) of Regulation (EC) 1829/2003 and in accordance with Annex I to this Regulation.

For GMOs with stacked events, the Joint Research Centre (JRC), as European Union Reference Laboratory (EURL) for GM food and feed, 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 EURL verification are available online[77]. Authorisation decisions for GMOs with stacked events always include the detection methods

[75] See note 12.
[76] See note 74.
[77] Available at: http://gmo-crl.jrc.ec.europa.eu/statusofdossiers.aspx

for the events present in the GMO at stake, the conditions in which validation of the methods is taking place and the reference material used, as it is the case for the present GM stack maize. The detection methods are always tested on material produced from the GMO with the stacked events as part of validation process.

Therefore, your allegation on this point must be rejected.

3.7. Public access to the methods for quantifying protein (Bt toxins) expression levels

In point 1.7 of your request you claim that public access to the methods for quantifying protein expression levels should be ensured by the Commission so as to allow independent scientists to quantify exposure to Bt toxins and, in particular, you claim that these methods are not made available upon request by EFSA.

First, the Commission would like to note that the public access version of the application dossier of the GM stack maize contained essential information regarding methods for quantifying protein expression levels, which was used by EFSA for its risk assessment.

Second, the Commission understands that you submitted a request to EFSA under Regulation (EC) No 1049/2001, regarding public access to documents[78], asking for access to "all data" submitted in relation to several GMO applications. In reply to your request, EFSA provided you access to the GM stack maize application dossier submitted by the applicant, with exclusion of certain information including on methods for quantifying protein expression levels.

The Commission would like to remind you that Regulation (EC) No 1049/2001 applies independently to each institution, body or agency with regard to the documents held by each one of them[79].

Pursuant to Article 4(2), 1st indent, of Regulation (EC) No 1049/2001, "[t]he institutions shall refuse access to a document where disclosure would undermine the protection of commercial interests of a natural or legal person, including intellectual property". Article 4(4) of the same Regulation establishes that "[a]s regards third-party documents, the institution shall consult the third party with a view to assessing whether an exception in paragraph 1 or 2 is applicable, unless it is clear that the document shall or shall not be disclosed". Further to this, in the case of the GM stack maize application dossier, EFSA, after consulting the third party, considered in its reply to your request that some of the information in the application dossier could not be disclosed.

Pursuant to Article 7(2) of Regulation (EC) No 1049/2001, and as you were informed in the EFSA's reply to your access to documents request, in the event of a total or partial refusal, the applicant can make a confirmatory application asking the institution or body to reconsider its

[78] Regulation (EC) No 1049/2001 of the European Parliament and of the Council of 30 May 2001 regarding public access to European Parliament, Council and Commission documents (OJ L 145, 31.5.2001, p. 43–48).
[79] With regard to EFSA, see Article 41 of Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety (OJ L 31, 1.2.2002, p. 1–24).

position. The decision on the confirmatory application contains the final position of the institution or body on the disclosure of the documents in question.

The Commission understands that you have not made use of the possibility provided by Article 7(2) to submit a confirmatory application against EFSA's decision on your access to documents request and that, therefore, you did not challenge EFSA's decision not to disclose the information on methods for quantifying protein expression levels.

In any case, the question of the public access to the methods for quantifying protein expression is not connected to the legality of Implementing Decision (EU) 2018/2046 by which the Commission granted authorisation to the GM stack maize, which is the subject of your present request for administrative review.

Therefore, your claim must be rejected.