TECHNICAL REPORT

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Scientific advice on the Testbiotech's request for internal review of Commission Implementing Decision (EU) No 2018/2046 on maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and subcombinations (application EFSA-GMO-BE-2013-118)

European Food Safety Authority (EFSA)

Abstract

Following a request of the European Commission, the European Food Safety Authority (EFSA) reviewed the scientific arguments raised by Testbiotech against the Commission Implementing Decision (EU) 2018/2046 authorising the placing on the market of products containing, consisting of, or produced from maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and its subcombinations (application EFSA-GMO-BE-2013-118). Testbiotech argued that the risk assessment of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and its subcombinations performed by the EFSA GMO Panel is inadequate as regards molecular characterisation, comparative analyses, toxicological and allergenicity assessment, and environmental assessment. EFSA analysed each of the scientific arguments put forward in the technical background of Testbiotech's complaint on maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and its subcombinations and concludes that none reveals new information that would invalidate the previous risk assessment conclusions and risk management recommendations made by the EFSA GMO Panel. Therefore, EFSA considers that the previous EFSA GMO Panel risk assessment conclusions and risk management recommendations on maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and its subcombinations remain valid and applicable.

Key words: maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122, molecular characterisation, comparative analyses, food-feed safety

Requestor: European Commission

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Summary

Following a request of the European Commission (EC), the European Food Safety Authority (EFSA) assessed the scientific content of the technical background of Testbiotech's request for an internal review of the Commission Implementing Decision (EU) No 2018/2046 under Regulation (EC) No 1367/2006, authorising the placing on the market of genetically modified maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and its subcombinations (application EFSA-GMO-BE-2013-118).

In this request for internal review, Testbiotech criticises the risk assessment conducted by the EFSA GMO Panel on this five-event stack maize and its subcombinations. The arguments brought forward by Testbiotech are related to: the reliability of protein expression data; representativeness of the test material used in the comparative analyses from the point of view of agricultural practices, meteorological and agronomic conditions; potential synergistic or antagonistic effects resulting from the combination of the transformation events in regard to toxicology and allergenicity; allergenicity of the sources of the transgenes; adjuvanticity of Cry toxins; gene flow to other cultivated or wild plant species; and assessment of the subcombinations within the scope of the risk assessment.

EFSA analysed each of the scientific arguments put forward in the technical background of Testbiotech's request and concludes that none reveals new information that would invalidate the previous risk assessment conclusions and risk management recommendations on maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and its subcombinations made by the EFSA GMO Panel. Therefore, EFSA considers that the previous EFSA GMO Panel risk assessment conclusions and risk management recommendations on this five-event stack maize and its subcombinations remain valid and applicable.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

The EFSA scientific Panel on Genetically Modified organisms (EFSA GMO Panel) performed a pre-market risk assessment of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and its subcombinations, in the context of application EFSA-GMO-BE-2013-118; the scientific opinion of the EFSA GMO Panel was published on 1 August 2017 (EFSA GMO Panel, 2017a). On 19 December 2018, the European Commission (EC) issued the Commission Implementing Decision (EU) 2018/2046[1], authorising the placing on the market of products containing, consisting of or produced from maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and its subcombinations. On 28 January 2019, the EC received from a non-governmental organisation, Testbiotech[2], a request for internal review of this Commission Implementing Decision, under Article 10 of Regulation (EC) No. 1367/2006.

On 5 February 2019, EFSA received a request from EC for an in-depth analysis of all scientific elements present in the technical background of the request by Testbiotech for internal review[3].

2. Data and Methodologies

2.1. Data

In delivering this report, EFSA considered the scientific arguments put forward in the technical background of Testbiotech's request for internal review, as well as relevant data supplied in the frame of application EFSA-GMO-BE-2013-118 or published in the scientific literature.

2.2. Methodologies

EFSA applied the appropriate principles described in the applicable guidelines for risk assessment of GM plants and derived food and feed products and other relevant documents (EFSA GMO Panel, 2010a, b, 2011a, b; Annex I of the minutes of the 115th GMO Panel plenary meeting, describing the strategy for assessing subcombinations[4]).

3. Assessment

The EFSA assessment below focuses on two sections of the technical background, prepared by Testbiotech, in which the risk assessment of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 performed by the EFSA GMO Panel (EFSA GMO Panel, 2017a) is criticised: Sections 2 "Overview EFSA risk assessment of the Maize"; and Section 3 "Grounds for the request to review the decision". Whereas Section 1 of the technical background has not been considered, as it refers to the legal framework, which is out of EFSA's remit.

The correspondence between the sections of Testbiotech's technical background and the present EFSA technical report is illustrated in the table below:

[1] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018D2046&from=EN
[2] Registered under identification number 151554816791-61 in the Transparency Register http://ec.europa.eu/transparencyregister/public/homePage.do
[3] Mandate number M-2019-0030, Question number EFSA-Q-2019-00080 on the EFSA Register of Questions registerofquestions.efsa.europa.eu/roqFrontend/login
[4] https://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf

Testbiotech's technical background	EFSA technical report
Section 2 presents an overview of the EFSA risk assessment and contains: **A**: scientific arguments that are addressed only in this section; **B**: scientific arguments addressed in more detail also in Section 3, where different grounds for the request are described.	**Section 3.1** discusses all scientific arguments type **A**
Section 3 presents 12 grounds for the request to review the decision: 9 are related to the risk assessment performed by EFSA 3 are related to the EC decision	**Section 3.2** discusses each of the nine grounds separately. The assessment of these grounds covers also the scientific arguments type **B** of Section 2 of Testbiotech's technical background.

3.1. Issues mentioned under Section 2, not detailed further in Section 3 of the technical background

As regards molecular characterisation, Testbiotech indicates that other gene products, such as miRNA from newly created open reading frames, were not assessed. EFSA points out that EFSA GMO Panel guidelines (EFSA GMO Panel, 2011a) do not require the checking for unintended gene silencing coming from the newly created ORFs. EFSA already addressed this issue in the context of another request from Testbiotech (Section 3.1 of EFSA, 2015a). Testbiotech also indicates that omics techniques should have been used during the risk assessment. Omics analyses are not required by EFSA GMO Panel guidelines. In addition, EFSA underlines the outcome of its Scientific colloquium 'Omics in risk assessment: state of the art and next steps', indicating that "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" (EFSA, 2018a). Other issues mentioned by Testbiotech, related to the variability of protein expression, comparison of protein levels between the five-event stack and the single events, representativity of field trials, and expected herbicide treatment are discussed in detail under specific headings of Section 3.2 of this report.

As regards comparative analyses, Testbiotech indicates that a more powerful statistical approach, such as multidimensional analysis, should have been used to investigate the high number of significant effects. The statistical approach used for the identification of unintended effects is described in EFSA guidance (EFSA GMO Panel, 2011). EFSA underlines that a large portion of the significant differences can be linked to the correlations existing among several endpoints (e.g. levels of crude protein and all amino acids) and such correlations can be easily identified. Other issues mentioned by Testbiotech, related to representativeness of the field trials and the test material used, are discussed in detail under specific headings of Section 3.2 of this report.

As regards toxicology and allergenicity, Testbiotech indicates that the EFSA assessment is not acceptable since it did not properly take into account: 1) the overall higher amount of toxins present in the five-stack as compared to single parental events; 2) the number of statistically significant changes in comparative analysis studies, which would have required animal feeding studies on material from the stacked event; and 3) the presence of residual levels of pesticides, their possible metabolites and the occurrence of combinatorial and cumulative effects related to these. Additionally, Testbiotech refers to possible effects on the immune system and on the intestinal microbiome by Bt proteins and/or pesticide residues, quoting some papers previously assessed by the EFSA GMO Panel. In summary, Testbiotech indicates the necessity of a specific assessment of complementary herbicides for herbicide-resistant plants, and investigations of cumulative/combinatorial effects of new proteins/pesticides, for example using of animal feeding studies, considering the lack of alternative methodology so far. Furthermore, it is also stated by Testbiotech that "Bt toxins can survive digestion to a much higher degree than has been assumed by EFSA". In addition, Testbiotech raises the point that no data were presented to show that the plant composition is unchanged with regard to its allergenic potential. All these aspects are discussed in detail in Section 3.2 of this report.

The other issues mentioned by Testbiotech, related to the environmental risk assessment and to the assessment of subcombinations, are discussed in detail under specific headings of this report.

3.2. Issues mentioned under Section 3 of the technical background

Section 3 of the Testbiotech technical background contains 12 grounds for the request to review the decision, of which nine are referring to the risk assessment performed by EFSA, and three to the EC decision. For the nine grounds related to risk assessment, EFSA assessed whether any of the scientific arguments put forward by Testbiotech would invalidate the previous EFSA GMO Panel conclusions on the safety of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122.

3.2.1. "EFSA did not request the applicant to provide data to verify whether the source of the transgene is allergenic"

The EFSA GMO Panel performed an allergenicity assessment of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 in line with its guidance document (EFSA GMO Panel, 2011a). The GM plant under discussion in this report is a plant where single transformation events have been stacked together. The main aspects considered in a stack application are those related to possible interactions and concerns arising from new information not previously assessed in the single event applications. In the context of the respective single event applications relevant for this maize stack, the assessment of the newly expressed proteins was comprehensively performed considering data of different nature. In such regard, information on the transgene source of the newly expressed protein under assessment 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 guidance document (EFSA GMO Panel, 2011a) and in line with international guidelines (Codex Alimentarius, 2003-2009), 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.[5,6] 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. In the case of the five-event stack maize discussed in this report, 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.

Testbiotech mainly refers to a publication dealing with the Cry1Ac protein where its potential allergenicity and adjuvanticity are discussed (Santos-Vigil et al 2018). EFSA published a technical report addressing the relevance of such study and checking whether or not the new scientific information contained elements that could lead the EFSA GMO Panel to reconsider the outcome of its previous opinions on genetically modified crops with Cry1Ac (EFSA, 2018b). In such report, EFSA concluded that the publication by Santos-Vigil et al (2018) does not bring new elements that would lead the EFSA GMO Panel to reconsider the outcome of its previous opinions on genetically modified crops with Cry1Ac. For more information on these aspects, please see also Section 3.2.2.

EFSA conclusions

The arguments put forward in the technical background of Testbiotech's complaint do not reveal new information that would invalidate the previous risk assessment conclusions made by the EFSA GMO Panel.

[5] EC, 2003. Directive 2003/89/EC of the European Parliament and of the Council of 10 November 2003 amending Directive2000/13/EC as regards indication of the ingredients present in foodstuffs.

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

3.2.2. "EFSA did not request the applicant to assess the possible role of Bt toxins as adjuvants despite strong indication of relevance for hazard identification"

According to the applicable EFSA guidance (EFSA GMO Panel, 2011a), when known functional aspects of the newly expressed protein or structural similarity to known adjuvants may indicate an adjuvant activity, the possible role of these proteins as adjuvants is considered. In the case that 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. This aspect has been transparently described in the EFSA GMO Panel's scientific opinion on maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 (EFSA GMO Panel, 2017a). Following an analysis of the available experimental evidence, the EFSA GMO Panel did not find indications that the presence of the Bt proteins at the levels expressed in this five-event 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. EFSA recognises that such available evidence is limited and underlines its involvement in actively following/contributing to the debate on the topic.

Testbiotech refers to several publications where the potential adjuvant capacity of the Cry1Ac protein is discussed (e.g. Vazquez et al 1999). Animal studies on Cry1Ac have suggested that specific proteins derived from *B. thuringiensis* may possess adjuvant activity when applied at relatively high doses (e.g. Vazquez et al., 1999). EFSA has recently provided a detailed analysis of the safety of Cry1Ac protein regarding its potential adjuvant and allergenic effect in the frame of an EC mandate assessing the relevance of the publication by Santos-Vigil et al (2018) mentioned above (Section 3.2.1). Unclarities with regard to the question addressed in the study, shortcomings in the study design and data interpretation limit the possibility to unambiguously attribute findings, raising (or not) safety concerns, to the intrinsic properties of Cry1Ac protein and to draw relevant conclusions for the risk assessment.

Cry1Ac is not present in maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122. In the EFSA technical report mentioned above (EFSA, 2018b), 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%. EFSA also described several explanations hypothesised for such observations, highlighting that the reasons underlying this contrasting evidence remain to be deciphered. The main aspects still to be understood are the following: i) whether or not there is a dose-response relationship for the potential adverse effects reported on specific Cry proteins; ii) what test item should be investigated; and iii) what *in vivo* and/or *in vitro* models should be used.

In addition, EFSA raised the point that studies appropriately designed to provide reliable answers on adjuvant and allergenic potential and, more broadly, on the effects on the immune system of Cry and of any other novel protein in general, are desirable, an aspect also highlighted by Santos-Vigil et al 2018. Future studies addressing allergenicity of proteins should consider limitations of the models currently in use and be performed using relevant routes of administration at appropriate doses, with appropriate positive and negative control proteins, taking into account possible effects of processing and matrices. Moreover, relevant EU funded projects are also contributing to the knowledge (e.g. www.imparas.eu). Approaches for the allergenicity assessment of newly expressed proteins should be revisited accordingly in the light of the latest novelties in this area, once scientific robustness is demonstrated. For instance, a strategy ranking the allergenic potential of known proteins has been suggested as a way forward (FAO/WHO, 2001; Remington et al., 2018; EFSA GMO Panel, 2017b), which might serve as benchmark for the allergenicity assessment of any novel protein in the future.

Testbiotech also refers to *in vitro* degradation studies with the Cry proteins. EFSA highlights that *in vitro* protein degradation studies are considered additional information for the safety assessment of the newly expressed proteins in line with international (Codex Alimentarius, 2003-2009) and EFSA guidelines (EFSA GMO Panel, 2011a). The EFSA GMO Panel published a guidance document in 2017 on allergenicity providing additional considerations on *in vitro* protein degradation studies. In Annex B of such guidance document, a refined *in vitro* digestion test that extends the conditions currently used in the classical pepsin resistance test in order to better reflect the range of conditions found *in vivo*, is proposed. However, the EFSA GMO Panel considers that additional investigation is needed before any additional recommendation in the form of guidance for applicants can be provided on the proposed *in*

vitro protein digestibility tests. To this end, an interim phase period, which is currently ongoing via an EFSA procurement[7], is evaluating the proposed revisions to the *in vitro* gastrointestinal digestion test. After this period, EFSA will assess whether the test adds value to the allergenicity risk assessment and, if so, what further steps are needed for its final implementation in the form of guidance for applicants.

The development of advanced methods for the allergenicity and adjuvanticity assessment of proteins is desirable. EFSA has been involved in past EU funded projects on the topic and is committed to incorporating latest scientific developments in its risk assessment process, when appropriate. EFSA is moving forward the field of allergenicity assessment being proactive in considering new scientific developments in the area (EFSA GMO Panel, 2017b). Future discussions involving the international scientific community could focus on building up new strategies for the allergenicity assessment.

Finally, Testbiotech refers to a minority opinion linked to a maize stack which is not the five-event stack maize under discussion in this report. The EFSA GMO Panel opinion on maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 contains no minority opinion. Further details on the minority opinion cited by Testbiotech are given in Section 3.2.7.

EFSA conclusions

The arguments put forward in the technical background of Testbiotech's complaint do not reveal new information that would invalidate the previous risk assessment conclusions made by the EFSA GMO Panel.

3.2.3. "No data were requested regarding potential synergistic or antagonistic effects resulting from the combination of the transformation events in regard to toxicology and allergenicity"

In accordance with the principles described in EFSA GMO Panel guidelines (EFSA, GMO Panel 2010a, 2011a and 2011b), the EFSA GMO Panel evaluates stack-event crops and (in case of segregating crops like maize) its subcombinations when the assessment of the single events has been finalised, focusing 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 stacks, the EFSA GMO Panel considers all datasets provided by the applicant, which include the data available on the single events, the stack itself and its subcombinations, if available, in case of segregating crops; moreover the scientific comments submitted by the Member States and the relevant scientific literature are taken into account.

- *Potential synergistic or antagonistic effects resulting from the combination of the transformation events – newly expressed proteins*

As regards proteins newly expressed in stack-event crops the toxicological assessment considers in first instance the safety profile of the individual proteins assessed in the single events, 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, i.e. "interaction" (when single components of a mixture can influence the toxicity of one another, leading to a synergistic or antagonistic outcome in combination), "dose addition" (individual components of mixtures may act independently on a common biological target), or "response addition" (the components of mixtures act independently on distinctly different biological targets) (Cassee et al., 2008). The building blocks for this assessment are the current knowledge on the biological characteristics of the newly expressed proteins, their mode of action (MoA) and the outcome of their toxicological assessment. In case combinations relevant for human and animal safety are expected under these models, additional studies can be requested as appropriate.

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

The EFSA GMO Panel used the approach described above for the assessment of the various combinations of the six proteins newly expressed in this five-event stack maize and its subcombinations.

As regards the newly expressed Bt proteins (Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1 and Cry35Ab1), the EFSA GMO Panel considered up-to-date available information on their biological function, MoA, and available toxicological information (EFSA, GMO Panel 2017). The MoA 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 (e.g. Soberón et al, 2016; and Jurat-Fuentes et al. 2017). 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 (Jurat-Fuentes et al, 2017). Such cellular receptors have not been identified in animals and humans (e.g. Koch et al, 2015), this representing a major limitation for a possible effect of these proteins on the gastrointestinal tract after oral ingestion. *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 EFSA GMO Panel (e.g. EFSA, 2014a). Other mode of actions for Cry proteins have been hypothesized (e.g. detergent mode, see COGEM Report 14/001[8]), however these derive from *in vitro* models and their translatability to *in vivo* conditions has not been consolidated, this deserving further investigation. Finally, no new data impacting the toxicological assessment of individual Bt proteins as performed in single events constituting this five-event stack maize have been identified (EFSA, GMO Panel, 2017a).

As regards enzymes PAT and CP4 EPSPS newly expressed in this five-event stack maize, an interaction among these is unlikely since they are known to catalyse distinct biochemical reactions and to act on unrelated substrates in the plant, with high substrate specificity (EFSA GMO Panel, 2017a). Furthermore, no statistically significant differences/lack of equivalences at the compositional level that could raise safety concerns as regards food and feed were observed between the five-event stack maize and comparator. Finally, no new data impacting the toxicological assessment of these individual enzymes as performed in single events constituting this five-event stack maize have been identified (EFSA GMO Panel, 2017a).

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 this five-event stack maize and its subcombinations 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, possible interactions between Bt proteins and enzymes newly expressed in this five-event stack maize (and its subcombinations) are not plausible.

- *Assessment of pesticides residues and of their possible combinatorial effects*

Regarding Testbiotech's consideration that pesticides residues (and their metabolites) and related amounts should be considered in the assessment of GM crops, EFSA recalls that the assessment of residues is not under the remit of the EFSA GMO Panel. However, EFSA remarks that pesticide uses in the EU require a premarketing assessment covering all risks and setting MRL for each food commodity with authorised use. Import of products containing residues of pesticides above the levels described in the EU legislation requires a premarketing assessment setting MRLs for import tolerances. A general default MRL of 0.01 mg/kg applies where a specific assessment has not been conducted, this covering pesticides and pesticides residues in imported food and pesticides not assessed in the EU.

Regarding GM crops, EFSA assessed several requests for setting MRL import tolerances for those cultivated abroad. In such respect, EFSA requires metabolism studies in the GMO crops and evaluates if GMO-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 (EFSA, 2018c) and glufosinate (EFSA, 2015b) have been explored and supported residue definitions.

[8] https://www.cogem.net/showdownload.cfm?objectId=39FB5B0E-B9A0-BB5C-5B4F558545968DB1&objectType=mark.hive.contentobjects.download.pdf

Regarding co-formulants, EFSA reminds that these are general chemicals, such as solvents or surfactants, covered by the general provisions on chemicals under REACH and CLP Regulations handled by the European Chemicals Agency. The pesticides regulation allows banning some substances as co-formulants, as implemented by some MS at the national level. The EC has already banned the use of POE-tallowamine in glyphosate-based products and is finalising a new regulation for setting EU level prohibitions.

Regarding the possible "combinatorial" effects of pesticide residues mentioned by Testbiotech, EFSA considers this not in the remit of the EFSA GMO Panel. Noteworthy, in the context of the MIXTOX project, EFSA is developing a guidance on new approaches and tools for harmonising how to assess risks to humans and the environment from multiple chemicals in the food chain: "chemical mixtures" and their "cocktail effects". This document is intended to support all relevant areas within EFSA's remit, including human health and environmental aspects (2018 draft published for public consultation available[9]). Under this initiative EFSA has also recently published a statement on the genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019).[10]

In the specific case of this five-event stack maize it is important to remind that the risk assessment was conducted in accordance to the applicable guidance (EFSA GMO Panel, 2011a) and therefore on materials (i.e. field trials for comparative analysis and levels of newly expressed protein) treated with the intended herbicides, sprayed in combination and in accordance to good agricultural practices, (see details in paragraph 3.2.4). Compositional analysis did not reveal statistically significant differences/lack of equivalences (unintended effects) for this five-event stack maize versus its comparator and reference commercial varieties, that could raise safety concerns and impact the nutritional profile of derived food and feed (EFSA GMO Panel, 2017a).

- *Investigating organ toxicity, immune responses and reproductive toxicity*

Testbiotech indicates that in view of the overall number of differences observed in the composition of this GM-maize with respect to its comparator (Section 2 - Toxicology), the potential effects of Bt proteins/pesticide residues on the intestinal microbiome and possible Bt combinatorial effects (Section 3.3), organ toxicity, immune responses and reproductive toxicity should be explored on plant material from the five-event stack maize.

The EFSA GMO Panel is of the opinion that based on the outcome of compositional analysis for this five-event stack maize (no statistically significant differences/lack of equivalences versus its comparator and the reference varieties that could raise safety concerns and impact the nutritional profile of derived food and feed), there is no need to investigate further organ toxicity, immune responses and reproductive toxicity (EFSA GMO Panel, 2017a). 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 (EFSA Scientific Committee, 2011; EFSA, 2014b). Considering Directive 2010/63[11] and the 3Rs philosophy, EFSA agrees that tools replacing animal studies should be matter of further investigation. In this context, EFSA has launched procurements on *in silico tools* or *in vitro system* for the assessment of GM newly expressed proteins.[12]

- *Interactions affecting allergenicity*

In relation to interactions between the events affecting allergenicity, two aspects are considered by EFSA, the assessment of the newly expressed proteins simultaneously present in the GM plant and the assessment of endogenous allergenicity. In the case of maize MON 87427 × MON 89034 × 1507 ×

[9] See 2018 draft published for public consultation https://www.efsa.europa.eu/en/consultations/call/180626-0

[10] EFSA Scientific Committee, More et al, 2019. Statement on the genotoxicity assessment of chemical mixtures. EFSA Journal 2019;17(1):5519, 11 pp. https://doi.org/10.2903/j.efsa.2019.5519

[11] Directive 2010/63/EU of The European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes

[12] NP/EFSA/GMO/2018/01 Exploring in silico protein toxicity prediction methods to support the food and feed risk assessment; OC/EFSA/GMO/2017/01 Refined Protocol for in vitro digestion of proteins for allergenicity assessment

MON 88017 × 59122, as none of the newly expressed proteins showed allergenicity and considering current knowledge, no reasons for concerns regarding the simultaneous presence of these newly expressed proteins in this five-event stack maize are expected. Testbiotech also refers to *in vitro* studies on protein degradation during ingestion. This aspect is addressed above in section 3.2.2.

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, 2011). In 2017, the EFSA GMO Panel published a guidance document providing additional considerations relevant for the assessment of endogenous allergenicity. Although this guidance document is not applicable to the five-event stack maize under discussion here, it provides a comprehensive description of the aspects considered for the assessment of endogenous allergenicity. The main focus of the document is on: i) relevant crops subjected to such analysis; ii) relevant allergens to be quantified; iii) methodology to be used for the quantification; and iv) principles to be followed for data interpretation and risk assessment considerations. Taking into account that maize is currently not considered a common allergenic food[13] (OECD, 2002) and that no indications of concern were identified from the molecular characterisation and compositional analysis, the EFSA GMO Panel did not considered necessary to request experimental data on the allergen repertoire of this five-event stack maize. The EFSA GMO Panel identified no indications of a potentially increased allergenicity of food and feed derived from the five-event stack maize with respect to that derived from the non-GM comparator.

EFSA has previously highlighted that the types of GM crops requiring an analysis of the allergen repertoire may be revised in the future. For such revisions, risk assessors, risk managers, health professionals and stakeholders can provide valuable feedback (EFSA, 2017).

EFSA conclusions

The arguments put forward in the technical background of Testbiotech's complaint do not reveal new information that would invalidate the previous risk assessment conclusions and risk management recommendations made by the EFSA GMO Panel.

3.2.4. "EFSA did not request data from the field trials which are necessary to assess whether expected agricultural practices influence the expression of the studied endpoints"

Maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 is tolerant to glyphosate- and glufosinate ammonium-containing herbicides. Compared to conventional maize varieties, this five-event stack maize provides more flexibility to farmers as they can control weeds in post-emergence applying two non-selective complementary herbicides. In case of herbicide tolerant crops, the experimental design recommended by the EFSA GMO Panel (EFSA GMO Panel, 2010a; EFSA GMO Panel, 2011a) includes materials from the GM crop treated with plant protection products (PPP) according to local requirements (including conventional herbicides), and from the GM crop treated with the complementary herbicides, in addition to PPP.

- *Lack of representativeness of the applied complementary herbicides*

In order to produce grain and forage used for the compositional analysis of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122, the applicant selected 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. Independently of the differences in application of PPP, the glyphosate- and glufosinate ammonium-containing herbicides were applied

[13] 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.

in two sequential applications, between the V2 and V6 stages and at a rate of 1.1 kg a.e./ha and 0.5 kg a.i/ha, respectively. 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 as well as with those of the Ministry of Agriculture, Food and Rural Affairs of Ontario (2016/2017) to which Testbiotech refers in its document. For both glyphosate-and glufosinate-tolerant maize, the Ministry of Agriculture, Food and Rural Affairs of Ontario (2016/2017) recommends four possible strategies[14]. The strategy applied at the selected eight sites corresponds in most of the cases to a pre-emergence application of a residual herbicide followed by an in-crop application of glyphosate and glufosinate. 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 different strategies selected by the applicant were considered able to assess whether the agricultural practices, representative of the regions where the plant can be grown, influence the expression of relevant agronomic, phenotypic and compositional endpoints in forage and grains of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122.

- *Possible changes in plant composition due to glyphosate application*

Regarding the Zobiole et al. (2012) publication, 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. Maize specific data on the influence of glyphosate application on grain yield are available in Soltani et al. (2016), where the authors conclude that under certain conditions maize yield is reduced in case of late glyphosate application. It is also important to notice that in Zobiole et al. (2012), to reduce possible undesirable effects of glyphosate, it is recommended to use pre-emergence herbicides and to anticipate the glyphosate treatment rather than to increase its rate of application.

In application EFSA-GMO-BE-2013-118, in accordance with the applicable guidance documents (EFSA GMO Panel, 2011a), the agronomic/phenotypic characterisation and the compositional analysis were conducted on endpoints and materials collected from maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 plants exposed to the complementary herbicides or to the conventional ones. The applied management practices and the environmental conditions under which the plants were cultivated were considered representative of the regions where the plant can be grown. The outcome of the comparative analysis allowed the EFSA GMO Panel to conclude that maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 would not raise safety concerns.

- *Possible exposure to higher dosage and repetitive application of glyphosate on maize MON 87427 x MON 89034 x 1507 x MON 88017 x 59122*

Concerning the additive effect of herbicide tolerance traits, in the specific case of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 there are two genes coding for the CP4 EPSPS and two for the PAT proteins. EFSA does not share the Testbiotech expectation that maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 will be exposed to higher and repeated dosage of glyphosate beyond official recommendations. There is evidence that the use of glyphosate in early stage guarantees a more effective weed control and prevents yield loss (Soltani et al., 2016). In addition,

[14] (1) A single application of the complementary herbicide; (2) the complementary herbicide tank-mixed with a residual herbicide; (3) two in-crop applications of the complementary herbicide and (4) a pre-emergence application with a residual herbicide followed with an in-crop application of the complementary herbicide.

the presence of two distinct herbicide tolerant traits in the five-event stack maize makes it more likely that, in case of glyphosate tolerant weeds, the farmers will opt for a more effective herbicide. EFSA believes that there is no reason to expect that this plant will be exposed to higher doses of a single complementary herbicide.

- *Interaction between EPSPS and plant metabolism*

With regards to the publication by Fang et al. (2018), the authors report on increased fitness of Arabidopsis plants overexpressing EPSPS genes conferring tolerance to glyphosate. 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 increase in fitness. Whether these findings can be generalised requires further research. Event specific data has been generated to assess the composition of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 following the application of complementary herbicides and the EFSA GMO Panel concluded that the five-event stack maize is as safe as the non-GM comparator and the non GM reference varieties tested.

- *The data presented show a high number of significant differences (~75% of the analytes evaluated for plants composition)*

Testbiotech remarks that, apart from the relevance of each significant result, the overall number of significant differences should have called for more detailed investigations. The EFSA GMO Panel indeed considered further the whole set of statistical outcomes during the risk assessment and found that the effective number of significant results is much lower, 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 maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and the 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 this five-event stack maize is similar to that expected from the non-GM comparator and non-GM commercial reference varieties.

EFSA conclusions

The arguments put forward in the technical background of Testbiotech's complaint do not reveal new information that would invalidate the previous risk assessment conclusions made by the EFSA GMO Panel.

3.2.5. "EFSA did not request data on field trials that reflect the different meteorological and agronomic conditions under which the crop is to be grown"

Considering the scope of application EFSA-GMO-BE-2013-118 (which excludes cultivation), maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and its subcombinations are likely to be introduced under receiving environments (REs) different from those tested to support the application. Therefore, it is important that the REs selected for the comparative assessment allow drawing conclusions on the safety of the GM plants produced in other possible REs (EFSA GMO Panel, 2015). Since the REs are highly diverse and dynamic over time, it is considered unfeasible, in practice, to assess such GM events under all possible REs (EFSA GMO Panel, 2010a). 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.

For the compositional and agronomic/phenotypic characterisation of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122, the applicant selected respectively eight and nine sites located in major maize producing areas of the US[15], 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 the following meteorological and agronomic conditions:

- Field history at the selected sites;
- Soil information including soil texture, organic matter and soil pH;
- Planting information including planting date, planting depth, soil moisture and temperature at planting depth, air temperature, plot size and number of rows per plot;
- Information on the maintenance pesticides including the day and rate of application and the active ingredient of the different insecticides, fungicides and herbicides at the nine sites;
- Information on the fertilisers applied at the nine sites including day of the fertilisation, formulation (% N-P-K) and rate;
- Information on the application of the complementary herbicides including day, crop stage and rate;
- Irrigation information including the day, the applied amount and the irrigation method;
- Historical and in-season meteorological data, including minimum and maximum temperatures and precipitation.

In light of the information provided, the EFSA GMO Panel considered that the meteorological and agronomic variability at the sites selected for the compositional and agronomic/phenotypic characterisation of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 is able to ensure a sufficient range of environmental conditions reflecting those under which the five-event maize might be cultivated in practice (EFSA GMO Panel, 2017a).

More specifically, replies to each of the scientific elements reported in section 3.5 of Testbiotech's technical background are given below:

- *field trials that lasted more than one season*

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 where the plant is to be grown. The field trials may be conducted in a single year or spread over multiple years. In particular, in case the sites cover a restricted range of growing conditions, it is recommended to replicate the field trials over more than one year (EFSA GMO Panel, 2010b). The EFSA GMO Panel considers that the sites selected for the compositional and agronomic/phenotypic characterisation of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 ensure a sufficient range of environmental conditions; hence, there was no need to request additional field trials conducted over more than one season.

- *field trials in other maize growing regions*

Since the REs are highly diverse and dynamic over time, it is considered unfeasible, in practice, to assess GM events under all possible REs (EFSA GMO Panel, 2010a). 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. The EFSA GMO Panel considers that

[15] The map of yield per harvested surface by County in north America is available at https://www.nass.usda.gov/Charts_and_Maps/Crops_County/cr-pr.php

the sites selected for the compositional and agronomic/phenotypic characterisation of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 ensure a sufficient range of environmental conditions; hence, there was no need to request additional field trials conducted in other maize growing regions.

- *field trials with the highest dosage of glyphosate that can be tolerated by the plants, including repeated spraying*

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 sufficient control.

The timing and rate of the applied intended herbicides are in line with the recommendations of the manufacturers and more in general with the literature recommending the use in pre-emergence of a residual herbicide followed by an in-crop glyphosate (Nurse et al. 2006). More details are reported above (Section 3.2.4).

- *data from climate chamber or green house experiments where the maize was subjected to broad range of defined stressors*

For 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 GM plant's agronomic/phenotypic characteristics across sites over a natural gradient of the stressor, or through local manipulation of the environment (EFSA GMO Panel, 2015). However, given that the traits present in maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 are not designed to target abiotic stressors, the EFSA GMO Panel considered sufficient to perform the agronomic/phenotypic characterisation of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 under typical environmental conditions.

Under these conditions, a variety of abiotic and biotic stressors including pests, fungal and bacterial diseases are naturally present. Different responses to these stressors between the five-event stack maize and the non-GM comparator were collected and reported by the applicant. The data on environmental interactions with biotic and abiotic stressors were considered appropriate for the scope of the application. No differences were identified in the comparative analysis for biotic and abiotic interactions between maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and its non-GM comparator.

- *The data presented show a high number of significant differences (~75% of the analytes evaluated for plants composition)*

Testbiotech remarks that, apart from the relevance of each significant result, the overall number of significant effects should have called for more detailed investigations. The EFSA GMO Panel indeed considered further the whole set of statistical outcomes during the risk assessment and found that the effective number of significant results is much lower, 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 maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and the 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 this five-event stack maize is similar to that expected from the non-GM comparator and non-GM commercial reference varieties.

- *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*

The selections of test materials and sites are closely related and interdependent, because a given GM line is not generally appropriate for cultivation in all the possible REs in which lines with the GM event

may be introduced. Particular attention should therefore be paid to the selection of the line used in the transformation or to the variety in which the GM event is introgressed. A variety with high adaptability will guarantee the potential for testing the materials in a wider range of REs, whereas a variety with lower adaptability would be tested in a narrower range of REs, in which case additional genetic backgrounds carrying the transgenes might be requested. 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.

- *Data from the field trials affected by more extreme weather conditions were excluded from the assessment*

In designing the field trials, applicants should consider the likelihood 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 (EFSA GMO Panel, 2015). In Table 4 of Study Number SCR-2016-0200, the applicant indicated the data missing or excluded from analysis. The applicant provided justifications for data exclusion, also reported in the additional information received on 2/10/2015. The justifications were considered acceptable by the GMO Panel in the light of the applicable EFSA guidance (EFSA GMO Panel, 2011a).

- *Findings reported by Trtikova et al. (2015)*

The findings reported by Trtikova et al. (2015) were already assessed by EFSA (2015c) and present no new scientific information that would invalidate the EFSA GMO Panel's previous risk assessment conclusions, or the recommendations on risk management, for Bt-maize events for which it has issued a scientific opinion.

- *Genotype X environment interactions*

EFSA acknowledges that, in general, environmental or genetic factors or a combination of the two can affect gene expression, plant composition and phenotypical characteristics of genetically modified 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. 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 in Study MSL0024063 and Report SCR-2011-0585. The EFSA GMO Panel considered that the information provided by the applicant was adequate for the comparative analysis, since the applicant provided per-site summary statistics to aid the interpretation of the results, as required by EFSA guidance documents (EFSA GMO Panel, 2011a).

- *Findings reported by Fang et al. (2018) and additional EPSPS enzyme added by further crossing with MON87427*

With regards to the publication by Fang et al. (2018), the authors report on increased fitness of Arabidopsis plants overexpressing EPSPS genes conferring tolerance to glyphosate. 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 increase in fitness. Whether these findings can be generalised to a broader range of varieties transgenic EPSPS-expressing events and environmental conditions requires further research.

EFSA considered that the levels of CP4 EPSPS showed the expected higher levels in the stack resulting from the combination of the 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.

EFSA conclusions

The arguments put forward in the technical background of Testbiotech's complaint do not reveal new information that would invalidate the previous risk assessment conclusions made by the EFSA GMO Panel.

3.2.6. "EFSA did not request sufficiently reliable protein expression data from the newly introduced proteins"

EFSA considers that certain agricultural practices (including herbicide treatments) or environmental conditions could impose stress on plants which may in some cases influence protein expression (e.g. Ahmad et al., 2016). 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 (e.g. Hendawey, 2009; Merewitz et al., 2011). 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 is also observed in GM plant applications submitted to EFSA and it was considered by the EFSA GMO Panel during its assessment of application EFSA-GMO-BE-2013-118.

Maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and the respective singles were 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, 2011a). In addition, the EFSA GMO Panel considered that the 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.

The ELISA methods used to quantify the levels of the newly expressed proteins produced in the five-event stack and the respective singles were validated and considered acceptable. The results showed that the levels of the Cry proteins were similar in the five-event stack and the single events (<1.4-fold difference for grain and forage tissues). The CP4 EPSPS and PAT protein levels showed expected differences resulting from the combination of maize MON 87427 and MON 88017 events both producing CP4 EPSPS protein, and maize 1507 and 59122 events, both producing PAT protein in the five-event stack maize. Therefore, there is no indication of an interaction that may affect the levels of the newly expressed proteins in this stack. In addition, the determined CP4 EPSPS and PAT protein levels in the five-event stack 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.

EFSA conclusions

The arguments put forward in the technical background of Testbiotech's complaint do not reveal new information that would invalidate the previous risk assessment conclusions and risk management recommendations made by the EFSA GMO Panel.

3.2.7. "EFSA did not request data on all subcombinations"

Testbiotech refers to differences in expression levels of Cry proteins between maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and maize MON 89034 × 1507 × MON 88017 × 59122 (EFSA GMO Panel, 2010c). This argumentation is used to claim the necessity for data on expression levels for all subcombinations of the five-event stack maize. In addition, Testbiotech linked this aspect to a minority opinion in the frame of another application on maize Bt11 × MIR162 × 1507 × GA21 (EFSA GMO Panel, 2018). As already mentioned, the EFSA GMO Panel opinion on maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 contains no minority opinion. Nevertheless, EFSA takes the opportunity to comment and to clarify the general issues raised by Testbiotech.

First, EFSA underlines that the protein expression levels for the two above mentioned stacks were collected from different field trials, performed in the context of independent applications. 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). Second, neither in maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and

maize MON 89034 × 1507 × MON 88017 × 59122 the assessment of the newly expressed proteins raised concerns relevant for food and feed safety.

Regarding the comparison proposed by Testbiotech in table 2 (page 36): as explained above, so comparing values collected in different field trials, and possibly in different genetic backgrounds, is subject to many confounding effects.

At the 115th plenary meeting, the EFSA GMO Panel endorsed the strategy to follow for the safety assessment of subcombinations[16]. The principles described in Annex 1 of this document are followed to assess the safety of subcombinations of GM stacked crops, including those of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 (EFSA GMO Panel, 2017a) and maize Bt11 × MIR162 × 1507 × GA21 (EFSA GMO Panel, 2018).

For subcombinations for which no specific experimental data were provided, the EFSA GMO Panel assessed the possibility of interactions between the events and concluded that the subcombinations do not raise safety concern. In the case of subcombinations of maize Bt11 × MIR162 × 1507 × GA21 a minority opinion was raised on the relevance of protein levels and potential adjuvanticity of Cry proteins in subcombinations.

In addition to the aspects described in the relevant EFSA opinion (EFSA GMO Panel, 2018), additional considerations on the topic can be found in the minutes of the 123rd plenary meeting[17] where the application was discussed, and the following aspects underlined:

i) No indications of interactions between the events raising safety issues;
ii) Comparison of levels of newly expressed proteins between the four-event stack 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 subcombinations previously assessed did not show evidence for interactions.
iv) Potential adjuvanticity of Cry proteins was extensively discussed considering all available information; the topic is a matter of scientific debate where limited and contrasting evidence is available for such an activity, including scarce information regarding dose-response relationships;
v) 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.
vi) EFSA and other risk assessment bodies have previously commented on the topic (EFSA, 2009; VKM, 2012). Consequently, on the basis of available knowledge, EFSA and other risk assessment bodies conclude that there are currently no indications of safety concern regarding Cry proteins in the context of the GM plants assessed.

For additional information, please see also Section 3.2.2 and the EFSA technical report (EFSA, 2018b).

In the case of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122, based on the outcome of the risk assessment of the single events and of the five-event stack, there was no expected effect linked to specific subcombinations. Therefore, there was no trigger to request additional data for the subcombinations not previously assessed.

3.2.8. "EFSA did not request the applicant to assess geneflow to other cultivated or wild plant species"

Testbiotech claims that in application EFSA-GMO-BE-2013-118 the applicant did not consider some recent scientific publications - i.e. Pascher (2016); Trtikova et al. (2017), Fang et al. (2018) - to assess the potential for plant to plant gene transfer and should have provided additional evidence

[16] https://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf
[17] Minutes of the 123rd GMO Panel plenary meeting – link https://www.efsa.europa.eu/sites/default/files/event/180530-m.pdf

showing that no adverse effects can occur through gene flow from maize to teosinte and/or from teosinte to maize volunteers.

EFSA notes that the scientific publications referred to by Testbiotech were published after the submission of application EFSA-GMO-BE-2013-118 to the Belgian Competent Authority on 15 November 2013 and therefore could not have been considered by the applicant for the assessment of vertical gene flow. Therefore, the applicant considered all scientific evidence available at the time of submission of the application. In addition, the applicant performed a literature search to find and assess relevant scientific publications from the time of validity of the application until November 2016. The three papers mentioned above have been published after such date. The applicant conducted the search in line with the scope of the application, which excludes cultivation, and following the principles of systematic review described in EFSA (2010). No scientific information that could change the conclusions on maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 was found in that search.

Testbiotech does not agree with EFSA's assessment on the potential plant to plant gene flow for application EFSA-GMO-BE-2013-118 (EFSA GMO Panel, 2017a).

EFSA notes that the assessment of the potential plant-to-plant gene transfer in application EFSA-GMO-BE-2013-118 followed the step-wise approach described in its guidance on the environmental risk assessment of GM plants (EFSA GMO Panel, 2010a) and that all relevant scientific evidence was considered, including that on the presence of teosinte plants.

Considering the scope of the application, which excludes cultivation, the potential for occasional feral maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 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. 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 (OECD, 2003). 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; OECD, 2003; EFSA, 2016; Trtikova et al., 2017). Therefore, potential vertical gene transfer is 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). 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.

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

EFSA conclusions

The scientific arguments put forward in the technical background of Testbiotech's complaint reveal no new information that would invalidate the previous risk assessment conclusions and risk management recommendations made by the EFSA GMO Panel.

3.2.9. "In conclusion, EFSA and the applicant did not ensure that the final risk characterisation clearly demonstrates that the genetically modified food and feed has no adverse effects on human and animal health or the environment"

The EFSA GMO Panel conducted the risk assessment of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 in line with its guidance documents (EFSA GMO Panel, 2010a, 2011a and b).

During the assessment of this five-event stack maize, when aspects of hazard identification and characterisation were found to be lacking, EFSA and its GMO Panel requested additional information from the applicant. Based on the information provided in the valid application EFSA-GMO-BE-2013-118, the additional information submitted during the risk assessment, and the available scientific literature relevant for this application, the EFSA GMO Panel concluded on the safety five-event stack maize and its subcombinations.

4. Conclusions

The arguments raised by Testbiotech did not put forward new information that would invalidate the previous risk assessment conclusions and risk management recommendations made by the EFSA GMO Panel for GM maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and its subcombinations. Therefore, EFSA considers that the previous risk assessment conclusions on this five-event stack maize remain valid.

Documentation provided to EFSA

1. Letter from the European Commission to the EFSA Executive Director, dated 5 February 2019, requesting technical and scientific assistance 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

2. Acknowledgement letter, dated 22 February 2019, from the EFSA Executive Director to the European Commission

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Abbreviations

3R	Replacement, reduction, and refinement
a.e.	Acid equivalent
a.i.	Active ingredient
Bt	*Bacillus thuringiensis*
CP4 EPSPS	5-enolpyruvylshikimate-3-phosphate from *Agrobacterium tumefaciens* strain CP4
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
GMO	Genetically modified organisms
IgE	Immunoglobulin E
MoA	Mode of action
MRL	Maximum residue levels
MS	Member State(s)
OECD	Organisation for Economic Co-operation and Development
PAT	Phosphinothricin acetyltransferase
PPP	Plant protection products
POE	Polyethoxylated
RE	Receiving environment(s)