

Technical background for a request for internal review of administrative acts under Article 10 of Regulation (EC) No. 1367/2006 against the decision of the EU Commission to give market authorisation to stacked maize MON 87427 x MON87460 x MON 89034 x MIR162 x NK603 and subcombinations

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Summary

Basic principles of the GMO regulation are:

- First, before any GMO is authorised, the risk and safety assessment must show that the genetically modified organism is safe. GMOs must not: “*have adverse effects on human health, animal health or the environment*” (Articles 4(1)(a) and 16(1)(a) of the GM Regulation).
- Second, when assessing the safety of GMOs, the authority should err on the side of caution and apply the precautionary principle. In cases of doubt or where “*the possibility of harmful effects on health is identified but scientific uncertainty persists*” provisional measures may be taken to protect against any such risk eventuating as harm.

Further,

- Regulation 1829/2003 states that genetically engineered organisms “*should only be authorised for placing on the Community market after a scientific evaluation of the highest possible standard.*” (Recital 9 of Regulation 1829/2003).
- Annex II of Directive 2001/18 requires the examination of the direct and indirect as well as the immediate and delayed effects of the GMO on human health and the environment.
- Directive 2001/18 requires post-marketing monitoring “*in order to trace and identify any direct or indirect, immediate, delayed or unforeseen effects on human health or the environment of GMOs as or in products after they have been placed on the market.*”

The application was declared to be valid by EFSA (EFSA, 2019a). Implementing Regulation 503/2013 was applied in the risk assessment. Therefore, the Regulation must be complied with, setting new standards compared to previous assessments of single or stacked events.

The stacked GE Maize (hereinafter referred to as the Maize) was authorised on 22 January 2021, published in the Official Journal of the EU on 26 January 2021.¹

Testbiotech examined EFSA’s opinion and the decision of the EU Commission. In this technical background, which is based upon the analysis of the risk analysis that was carried out, we show that EFSA’s opinion and the decision of the Commission do not fulfil the requirements of EU Regulations.

This technical background is additionally underpinned by a legal analysis and is based upon scientific findings. It is upon this basis that we elucidate the grounds for the complaint. The grounds for the request for internal review are:

A) EFSA’s risk assessment should have been rejected for following reasons:

1. The plants were not exposed to bioclimatic conditions that sufficiently represent the regions in which these plants will be cultivated. Consequently, expression data and the assessment of plant composition as well as agronomic and phenotypic characteristics are not sufficiently reliable to inform the next steps in risk assessment. This is especially relevant since (i) the plants are intended for cultivation in drought conditions, (ii) the EPSPS enzymes are known to show unintended effects under stress conditions (such as drought) and (iii) the Bt content is known to be influenced by environmental factors (such as drought).
2. The plants were not exposed to agricultural practices which sufficiently represent the conditions under which these plants will be cultivated. Consequently, expression data and the assessment of

¹<https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32021D0061&qid=1612780708978>

plant composition as well as agronomic and phenotypic characteristics are not sufficiently reliable to inform the next steps in risk assessment.

3. No more detailed examinations were requested on gene expression, plant composition, agronomic and phenotypic characteristics despite data from other events and previous applications indicating that environmental stress factors, herbicide applications rates, genetic backgrounds and stacking are likely to impact gene expression and plant metabolism in the stacked Maize.

4. Risks which indicate toxicological health impacts, potentially enhanced by combinatorial effects caused by the stacking, were not assessed against relevant, reasoned and plausible hypotheses. For example, it was not examined to which extent the toxicity of the selectivity of the Bt toxins are changed by the mixed toxicity of whole food and feed. This is especially relevant in this case since it is known that enzymes (protease inhibitors) produced in the plants can multiply the toxicity of the Bt toxins and prolong exposure to the toxins in the gut after ingestion.

5. Risks which indicate immunological health impacts, potentially enhanced by the combinatorial effects of stacking, were not assessed against relevant, reasoned and plausible hypotheses. For example, it was not examined to which extent changes in the microbiome caused by the consumption of the Maize will impact its immunogenic properties. This is especially relevant since it is known that enzymes (protease inhibitors) produced in the plants can prolong exposure to the toxins in the gut after ingestion. Furthermore, residues from spraying with glyphosate (Roundup) are known to impact the composition of the microbiome.

6. The environmental risk assessment and the biological characteristics of the hybrid offspring of teosinte and the Maize were not assessed against relevant, reasoned and plausible hypotheses. This is especially relevant since the offspring may show invasive characteristics due to the unintended effects caused by the EPSPS enzymes, higher resistance to damage by insects and potentially higher tolerance to drought conditions.

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

B) The decision of the EU Commission fails to fulfill the requirements for the following reason:

Since 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, the EU Commission decision to allow the import was not in accordance with the EU regulations.

The EU Commission should have requested method(s) for post market monitoring specific to the Maize ('event-specific') which only is functional with the Maize, not being functional if applied to other transformation events already authorised.

C) The Request

Art. 10 of EU Regulation 1367/2006 allows NGOs active in the field of environmental protection to request re-examination of Commission decisions. Based upon this regulation, we request the re-examination of the risk analysis by EFSA and the EU Commission as well as immediate withdrawal of market authorisation for maize MON 87427 x MON87460 x MON 89034 x MIR162 x NK603.

The following designations appear in this document:

The Maize: Genetically engineered stacked maize MON 87427 x MON87460 x MON 89034 x MIR162 x NK603 and its segregating subcombinations;²

The Parental Plants: Single events of genetically engineered maize MON 87427, MON87460 x MON89034, MIR162 and NK603;

The Applicants: Monsanto (owned by Bayer);

GMO or GE: This abbreviation is used for genetically engineered organisms which are subjected to the approval process under EU Directive 2001/18;

GM Regulation: This abbreviation is used for the regulatory framework for genetically engineered organisms that are subject to the approval process under EU Directive 2001/18.

1. Legal Framework

1.1 The Aarhus Regulation

The Aarhus Regulation is intended to implement the Aarhus Convention. The cornerstone of the Aarhus Convention is the principle that environmental NGOs are deemed to have a legal interest of their own in bringing certain judicial proceedings “on behalf of” the environment. This principle is enshrined in Article 2(5) read with Article 9 of the Convention.

The preamble to the Aarhus Convention provides as follows:

“... Recognizing that adequate protection of the environment is essential to human well-being and the enjoyment of basic human rights, including the right to life itself,

Recognizing also that every person has the right to live in an environment adequate to his or her health and well-being, and the duty, both individually and in association with others, to protect and improve the environment for the benefit of present and future generations,

Considering that, to be able to assert this right and observe this duty, citizens must have access to information, be entitled to participate in decision-making and have access to justice in environmental matters, and acknowledging in this regard that citizens may need assistance in order to exercise their rights...

Recognizing further the importance of the respective roles that individual citizens, non-governmental organizations and the private sector can play in environmental protection...”

Recitals (18), (19) and (21) of the Aarhus Regulation in turn provide that:

“(18) Article 9(3) of the Aarhus Convention provides for access to judicial or other review procedures for challenging acts and omissions by private persons and public authorities which contravene provisions of law relating to the environment. Provisions on access to justice should be consistent with the Treaty. It is appropriate in this context that this Regulation address only acts and omissions by public authorities.

(19) To ensure adequate and effective remedies, including those available before the Court of Justice of the European Communities under the relevant provisions of the Treaty, it is appropriate that the Community institution or body which issued the act to be challenged or which, in the case of an alleged administrative omission, omitted to act, be given the opportunity to reconsider its former decision, or, in the case of an omission, to act.

² Corrected detail: In this paragraph, ‘MON87460’ was missing in the original technical dossier.

Where previous requests for internal review have been unsuccessful, the non-governmental organisation concerned should be able to institute proceedings before the Court of Justice in accordance with the relevant provisions of the Treaty.”

Articles 10 and 12 of the Aarhus Regulation are designed to achieve within the Union the Aarhus Convention’s goal of allowing access to justice in environmental matters. Accordingly, Articles 10 and 12 establish administrative and judicial review procedures which enable NGOs meeting the requirements of Article 11 of the Aarhus Regulation to challenge the acts and omissions of the Community institutions which contravene provisions of European environmental law.

1.2 The GM Regulation and other key provisions on food safety

The GM Regulation provides that, in order to protect human and animal health, food and feed that consists of, contains, or is produced from genetically modified organisms should undergo a risk and safety assessment before it is placed on the market in the European Union.

Recitals (2), (3) and (9) make clear that: (a) “A high level of protection of human life and health should be ensured in the pursuit of [Union] policies”; (b) “In order to protect human and animal health, food and feed consisting of, containing or produced from genetically modified organisms...should undergo a safety assessment through a [Union] procedure before being placed on the market within the [Union]”; and (c) “...genetically modified food and feed should only be authorised for placing on the Community market after a scientific evaluation of the highest possible standard, to be undertaken under the responsibility of [EFSA], of any risks which they present for human and animal health and, as the case may be, for the environment...”

“Genetically modified organism” is defined in Article 2(2) of Directive 2001/189³ as “an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination”, where an “organism” is defined in Article 2(1) as “any biological entity capable of replication or of transferring genetic material”. Food and/or feed that consists of, contains, or is produced from, genetically modified organisms must not:

“have adverse effects on human health, animal health or the environment” (Articles 4(1)(a) and 16(1)(a) of the GM Regulation); or

“differ from the food which it is intended to replace to such an extent that its normal consumption would be nutritionally disadvantageous for the consumer” and/or “differ from feed which it is intended to replace to such an extent that its normal consumption would be nutritionally disadvantageous for animals or humans” (Articles 4(1)(c) and 16(1)(d) of the GM Regulation) respectively;

Be placed on the market “unless it is covered by an authorisation granted in accordance with” the GM Regulation.⁴

In order to gain an authorisation, an application must be made to the competent authority of a Member State.⁵ That application should include, among other things a copy of the studies available to show whether the food or feed complies with Articles 4(1) or 16(1), and an analysis, supported

³Directive 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.

⁴Articles 4(2) and 16(2) of the GM Regulation.

⁵Articles 5(2) and 17(2) of the GM Regulation.

by data of whether the characteristics of the genetically modified organism are not different from their comparators (their conventional counterparts).⁶ Article 5(5) also provides that the application must be accompanied by a technical dossier meeting the requirements of Directive 2001/18/EC.

The application is then considered by EFSA, which will provide an opinion, among other matters, on whether the food/feed complies with the criteria referred to in Articles 4(1) / 16(1).⁷ In preparing its opinion, the Authority must consult the national competent authorities of the Member States.⁸ On the basis of the opinion of EFSA, any relevant provisions of Union law and other legitimate factors relevant to the application under consideration, the Commission produces a draft decision.⁹

The Commission's draft decision is submitted to the Standing Committee on the Food Chain and Animal Health. This Standing Committee assists the Commission in accordance with the procedure outlined in Article 5 of Decision 1999/468 laying down the procedures for the exercise of implementing powers conferred on the Commission.¹⁰ This provides for the Standing Committee to issue an opinion on the application. If the opinion is in accordance with the Commission's draft decision the Commission adopts the decision. If it is not, the Commission has to submit a proposal to the Council.¹¹ If the Council neither adopts nor opposes the proposal within the relevant period, the Commission adopts the decision.

Regulation 178/2002 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 ("the Food Safety Regulation") outlines the "General Principles of Food Law" upon which European measures, such as the GM Regulation, should be based. These include:

The "General Objective" of "a high level of protection of human life and health and the protection of consumers' interests";¹²

The principle of "Risk Analysis". According to Article 6 of the Food Safety Regulation:

"(1) In order to achieve the general objective of a high level of protection of human health and life, food law shall be based on risk analysis except where this is not appropriate to the circumstances or the nature of the measure.

(2) Risk assessment shall be based on the available scientific evidence and undertaken in an independent, objective and transparent manner."¹³

The "Precautionary Principle". According to Article 7(1) of the Food Safety Regulation: "In specific circumstances where, following an assessment of available information, the possibility of harmful effects on health is identified but scientific uncertainty persists, provisional risk management measures necessary to ensure the high level of health protection chosen in the [Union] may be adopted, pending further scientific information for a more comprehensive risk assessment."

⁶Articles 5(3)(e), 5(3)(f) 17(3)(e), and 17(3)(f) of the GM Regulation.

⁷Articles 6(3)(a) and 18(3)(a) of the GM Regulation.

⁸Articles 6(4) and 18(4) of the GM Regulation.

⁹Articles 7(1) and 19(1) of the GM Regulation.

¹⁰Articles 7(3), 19(3) and 35(2) of the GM Regulation.

¹¹Article 5(3) and 5(4) of Decision 1999/468.

¹²Article 5 of the Food Safety Regulation (also reflected in Recital (3)).

¹³Emphasis added.

1.3 Particular Provisions of Directive 2001/18

Directive 2001/18¹⁴ requires that the placing on the market of a genetically modified organism (GMO) as or in a product may only take place after written consent by the competent authority has been given (Article 19). The application for such consent (notification, Article 13) must be accompanied by an environmental risk assessment, by other information, and by a monitoring plan (Article 13(2.b, 2.a, and 2.e)).

The environmental risk assessment

Recital (19) of Directive provides that “[a] case-by-case environmental risk assessment should always be carried out prior to a release. It should also take due account of potential cumulative long-term effects associated with the interaction with other GMOs in the environment.” Moreover, “[n]o GMOs, as or in products, intended for deliberate release are to be considered for placing on the market without first having been subjected to satisfactory field testing at the research and development stage in ecosystems which could be affected by their use.”

Recital 33 of the Directive indicates that the environmental risk assessment submitted as part of the notification procedure has to be “full”. Recital 55 stresses the importance of following “closely” the development and use of GMOs.

Article 13 (2.b) provides that the notification shall be accompanied by “the” environmental risk assessment and the conclusions required in Annex II, section D. Annex II section D provides that information on the points listed in sections D1 or D2 should be included, as appropriate, in notifications with a view to assisting in drawing conclusions on the potential impact from the release or the placing on the market of GMOs. This information is to be based on the environmental risk assessment carried out in accordance with the principles laid down by sections B and C of Annex II to the Directive.

Accordingly, the principles with which environmental risk assessments should comply are laid down in Annex II to the Directive. Annex II indicates that the environmental impact assessment is not limited to an examination of the effects of genetically modified products containing GMO on the natural environment, it must also examine the effects on human health. This follows from the general objective of Directive 2001/18 as laid down in Article 1 – “[i]n accordance with the precautionary principle, the objective of this Directive is...to protect human health and the environment”¹⁵, in Recital 5 of the Directive, and the reference to “human health or the environment” in Annex II itself, where this reference appears five times in the introductory remarks and in each of the four parts A to D of that Annex. Further, section A of Annex II states that:

“The objective of an [environmental risk assessment] is, on a case by case basis, to identify and evaluate potential adverse effects of the GMP, either direct, indirect, immediate or delayed, on human health and the environment which the deliberate release or the placing on the market of GMOs may have. The [environmental risk assessment] should be conducted with a view to identifying if there is a need for risk management and if so, the most appropriate methods to be used.”

¹⁴Directive 2001/18/EC of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC, OJ 2001, L 106 p.1. (“the Directive”).

¹⁵The importance of the protection of human health is reinforced by the multiple references to it in the Directive – see: Article 13(6), in Recital 5 of the Directive, and the reference to “human health or the environment” in Annex II itself, where this reference appears five times in the introductory remarks and in each of the four parts A to D of that Annex.

Finally, it is to be noted that it follows from Article 191(1) TFEU (The Treaty of the Functioning of the European Union) that in EU law, the “protection of the environment” includes the protection of human health¹⁶.

The introductory remarks to Annex II of the Directive state: “A general principle of environmental risk assessment is also that an analysis of the ‘cumulative long-term effects’ relevant to the release and the placing on the market is to be carried out. ‘Cumulative long-term effects’ refers to the accumulated effects of consents on human health and the environment”. Thus, the continued consumption of genetically modified plants, where herbicide residues might be present, should be submitted to risk assessment as a matter of course.

Section B sets out the general principles governing the performance of an environmental risk assessment, which include “identified characteristics of the GMP and its use which have the potential to cause adverse effects should be compared to those presented by the non-modified organism from which it is derived and its use under corresponding situations.”

Section C.2 of Annex II describes the “Steps in the environmental risk assessment”. As a first step, that part requires the identification of characteristics that may cause adverse effects, and gives a general indication of what has to be done, noting that “it is important not to discount any potential adverse effect on the basis that it is unlikely to occur”. Section C.2 then alerts to “Potential adverse effects of GMOs will vary from case to case and may include: - disease to humans including allergenic or toxic effects...” Finally, Section C.2 outlines the steps involved in reaching an overall assessment of the risk posed by a genetically modified plant. These include the evaluation of the potential consequences of the adverse effects (for which the evaluation should assume that such an effect will occur), the evaluation of the likelihood of and the risk posed the occurrence of each potential adverse effect, and the identification of risk management strategies.

The conclusions of the risk assessment shall be part of the notification, in order to allow the competent authority to draw its own conclusions (Annex II, part D). The conclusions on the risk assessment shall include “Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMOs [GMHP] and persons working with, coming into contact with or in the vicinity of the GMO [GMHP] release(s)”¹⁷.

It follows from these provisions 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 also includes the potential effects of the use of herbicides or pesticides on the GMO plant or product. Of particular importance is the fact that the assessment of a particular potential adverse effect may not be excluded from the overall assessment on the basis that it is considered it is unlikely to occur. Although the likelihood of a potential adverse effect is one factor of the evaluation, the magnitude of its potential consequences and the risks it would pose to the environment and human health must still be assessed, and both of these elements should be taken into account in the overall risk assessment.

Other information

“Other information” which has to accompany every notification under Article 13 of Directive 2001/18, shall include “considerations for human health and animal health, as well as plant health:

¹⁶Article 191(1) TFEU: “ *Union policy on the environment shall contribute to the pursuit of the following objectives:...* – *protecting human health...*”

¹⁷Directive 2001/18, Annex II, part D1 no.6 and part D2 no.6. Part D1 refers to GMOs other than higher plants, part D2 to genetically modified higher plants (GMHP). For reasons of simplification the two sections D1 no. 6 and D2 no. 6 were assembled in one text.

(i) toxic or allergenic effects of the GMO and/or their metabolic products”¹⁸, furthermore “identification and description of non-target organisms which may be adversely affected by the release of the GMO, and the anticipated mechanisms of any identified adverse interaction”¹⁹ and, as a catch-all formula “other potential interactions with the environment”²⁰. For genetically modified higher plants (GMHP), Annex IIIB applies, this requires the notifier to supply, with his notification, the following information: “Information on any toxic, allergenic, or other harmful effects on human health arising from the genetic modification”²¹; “Information on the safety of the GMHP to animal health, particularly regarding any toxic, allergenic or other harmful effects arising from the genetic modification, where the GMHP is intended to be used in animal feedstuffs”²²; and “Potential interactions with the abiotic environment”²³.

This wording with regard to the “other information” is thus again very broad and tries to cover all effects that the GMO product might have on human health or animal health. The choice of the terms “arising from the genetic modification” clarifies that information is to be supplied not only on the effects caused directly by the GMO, but also on all other harmful effects on human or animal health and which are, in one way or another, related to the genetically modified plant.

The monitoring plan

According to Article 13(2)(e), a monitoring plan has to accompany the notification; the plan shall be established in accordance with Annex VII to the Directive. Its objectives are underlined by recital 43 of Directive 2001/18 which states: “it is necessary to introduce into this Directive an obligation to implement a monitoring plan in order to trace and identify any direct or indirect, immediate, delayed or unforeseen effects on human health or the environment of GMOS as or in products after they have been placed on the market”. The use of the word “any” both in the Recital 43 and in Annex VII itself demonstrates that the purpose of the monitoring plan is to discover all possible impacts of adverse effects of GMOs, including those effects not foreseen in the environmental risk assessment (“unforeseen”).

This interpretation is confirmed by the provisions in Annex VII on the design of the monitoring plan: the plan has to

1. be detailed on a case by case basis (Annex VII, C.1);
2. take into account the relevant environmental conditions where the GMO is expected to be released (C.2);
3. incorporate general surveillance for unanticipated adverse effects (C.3);
4. provide for case-specific monitoring, though routine surveillance practices that “were already established” are allowed in appropriate cases (C.3.1 and C.3.2);
5. facilitate the observation “in a systematic manner” of the release of the GMO in the receiving environment and the interpretation of these observations “with respect to human health or the environment” (C.4).

In 2002, the Council adopted, by way of a Decision, guidance notes “supplementing Annex VII”²⁴. The guidance notes “shall be used as a supplement to Annex VII of Directive 2001/18/EC” (Article 1). The guidance notes repeat in the introduction that the purpose of the monitoring plans is to

¹⁸Directive 2001/18, Annex III A, section II, C.2(i)

¹⁹Directive 2001/18, Annex IIIA, section IV B12.

²⁰Directive 2001/18, Annex IIIA, section IV B.16.

²¹Directive 2001/18, Annex IIIB, section D no.7.

²²Directive 2001/18, annex IIIB, section D no.8.

²³Directive 2001/18, annex IIIB, section D no11.

²⁴Decision 2002/811/EC of 3 October 2002 establishing guidance notes supplementing Annex VII to Directive 2001/18/EC, OJ 2002, L 280 p.27.

“trace and identify any direct or indirect, immediate, delayed or unforeseen effects on human health or the environment of GMOs as or in products after they have been placed on the market”.

The guidance notes first repeat the objective and general principle of the monitoring plan of Annex VII to Directive 2001/18 and then add: “In addition, monitoring of potential adverse cumulative long-term effects should be considered as a compulsory part of the monitoring plan”(part B). They clarify what is to be understood by the terms “direct effects”, “indirect effects”, “immediate effects” and “delayed effects”.

With regard to unforeseen effects, the guidance notes indicate: “it is very difficult if not impossible to predict the appearance of potential, unforeseen or unanticipated effects that were not highlighted in the risk assessment. General surveillance for potential unforeseen or unanticipated effects should, therefore, be considered as a part of the monitoring strategy” (part C). This statement indicates that the notifier may not limit his monitoring plan to those risks identified in the environmental risk assessment which had to be made according to Article 13(2.b) and Annex II section D to Directive 2001/18.

The guidance notes also expressly state that the time-period for monitoring would depend on the circumstances, but could extend to a number of years (part C- 1.5). This is another indication that potential cumulative effects of genetically modified plants and herbicide residues are to be controlled.

Case-specific monitoring (part C-1.3.1) should focus on “all the potential effects on human health and the environment identified in the risk assessment”. It should begin with determining the case-specific objectives of the monitoring strategy, which “include” the identification of the occurrence and impact of potential adverse effects of the GMO or its use that were made in the environmental risk assessment. The strategy should indicate that these assumptions are to be confirmed by the case-specific monitoring. With regard to potential effects on human health, the guidance notes specify that such effects will depend on the inherent nature of a GMO and its specific genetic modification.

For unforeseen adverse effects that were not predicted in the risk assessment, the guidance notes make provision for a “general surveillance” (part C- 1.3.2) which consists of “routine observation (“look – see”) approach”. Such surveillance should be carried out over a longer period of time and possibly a wider area than the case-specific monitoring, though the type of general surveillance would depend on the type of unforeseen adverse effects. The notes indicate that the general surveillance could make use of established routine surveillance practices “where compatible”; then the established routine surveillance practice should be described in the plan, including any necessary alignment to the general surveillance. “Food surveys“ are expressly mentioned (part C - 1.7) as one example of existing systems.

The guidance notes contain a number of other indications, such as the monitoring methodology (part C- 2) and analysis, reporting and review (part C-3) which will not be set out here.

Overall, the main purpose of the monitoring plan is to confirm the assumptions that were made in the environmental risk assessment on (the absence of) potential adverse effects. However, the guidance notes expressly indicate that the monitoring strategy should also include a strategy with regard to unforeseen events not assessed in the environmental risk assessment.

1.4 Particular provisions of Regulation 1829/2003

Regulation 1829/2003 applies to genetically modified food and feed. Articles 3 to 14 apply to genetically modified food, Articles 15 to 23 to genetically modified feed. The placing on the market of genetically modified food or feed requires an authorisation (Article 4 for food, Article 16 for feed).

Article 5(5) of Regulation 1829/2003 provides that an application for GMOs or food containing or consisting of GMOs must be accompanied by, amongst others, “information and conclusions about the risk assessment carried out in accordance with the principles set out in Annex II to Directive 2001/18/EC or, where the placing on the market of the GMO has been authorised under part C of Directive 2001/18/EC, a copy of the authorisation decision”. Furthermore, such an application shall be accompanied by “a monitoring plan for environmental effects conforming with Annex VII to Directive 2001/18/EC...” (Article 5(5)(b)).²⁵

Article 6(4) provides: “In the case of GMOs or food containing or consisting of GMOs, the environmental safety requirements referred to in Directive 2001/18/EC shall apply to the evaluation to ensure that all appropriate measures are taken to prevent the adverse effects on human and animal health and the environment which might arise from the deliberate release of GMOs...”

Under, Articles 5(3)(k) and 17(3)(k) of the GM Regulation an application for marketing authorisation has to contain a proposal for post-marketing monitoring regarding the use of the food for human consumption and feed for animal consumption “where appropriate”. Similarly, in giving a positive opinion in relation to an application EFSA has to include such post-marketing monitoring requirements “where applicable” (Articles 6(5)(e) and 18(5)(e) of the GM Regulation).

The authorisation of a genetically modified food is granted by the Commission by way of the so-called comitology procedure (Article 7 and Article 35). The authorisation has to include the particulars referred to in Article 6(5), which includes where appropriate a monitoring plan. In its decision, the Commission is not bound by the opinion of EFSA. Instead, the Commission has to take the EFSA opinion into account, as well as “any relevant provision of Community law and other legitimate factors relevant to the matter under consideration” (Article 7(1)).²⁶ In other words, the Commission has to determine, whether the monitoring plan has to include the control of potential adverse effects of the genetically modified plant during the use and consumption stage. Even when the EFSA, in any of its opinions, does not comment on the need for such a control, the Commission was obliged to decide on that issue.

The provisions on feed containing or consisting of GMOs mirror the provisions on genetically modified food: A provision corresponding to Article 5(5) of Regulation 1829/2003 is laid down in Article 17(5), a provision corresponding to Article 6(4) is found in Article 18(4). In addition, where appropriate EFSA also has to give the particulars of the relevant monitoring plan (Article 18(5.g)). The Commission, when authorising the genetically modified feed, also has to also refer to the monitoring plan (Article 19(2)).

It follows from these provisions that for genetically modified food or feed, information and conclusions about the risk assessment must be given. This risk assessment must have been carried out in accordance with the principles set out in Annex II to Directive 2001/18 (Article 5(5.a) and Article 17(5.a) see section 2 above). Also a monitoring plan has to be submitted with the application

²⁵ For such cases, Articles 13 to 24 of Directive 2001/18 are declared inapplicable.

²⁶ Further, under Article 7(1) the Commission has to provide an explanation for the difference, where its decision is not in accordance with EFSA's opinion.

for authorisation (Article 5(5.b) and Article 17 (5.b)). Where EFSA expresses an opinion in favour of the authorisation, it has to address the monitoring plan (Article 6(5.g) and Article 18(5.g)) and indicate “post-market monitoring requirement based on the outcome of the risk assessment” (Article 6(5.e) and Article 18(5.e)).

The European Commission has the responsibility for authorising the placing on the market of genetically modified food or feed. Accordingly, it has an obligation to attach the necessary conditions to the authorisation in order to ensure that the food or feed has no adverse effects on human health, animal health or the environment (Article 4(1)). It has its own responsibility in this regard and may not rely on the – non-binding – opinion of EFSA; in the past, the Commission occasionally did add supplementary conditions on the placing on the market of genetically modified food products²⁷.

Under Regulation 1829/2003, genetically modified food or feed placed on the market, must be monitored according to the principles laid down in Directive 2001/18 (see section 2 above). The monitoring plan must attach greater importance to potential adverse effects and to the unforeseen effects of the genetically modified food or feed on human or animal health than in the application of Directive 2001/18 alone, as it is the very purpose of Regulation 1829/2003, expressed in Recitals 2 and 3 and its Articles 1, 4 and 16, to protect human health. Further, the information and conclusions concerning the risk assessment must take into consideration this need to protect human and animal health.

The Court of Justice confirmed this interpretation and stated that²⁸:

“Regulation 1829/2003 applies to the specific field of food and feed. As regards food, its first objective, referred to in article 4(1), is also to avoid adverse effects on human health and the environment. However, Directive.. 2001/18 [was] drafted primarily from the angle of the concept of ‘deliberate release’ which is defined in article 2(3).. as an intentional introduction of a GMO into the environment, without specific containment measures designed to limit their ‘contact’ with the ‘general population and the environment’. That approach thus appears to be more general, including with regard to the placing on the market of a GMO as a product. In this respect, ... recitals 25, 28 and 32 in the preamble to Directive 2001/18 link the need to introduce an assessment and authorisation procedure to the situation in which the placing on the market includes a deliberate release into the environment. Although Regulation 1829/2003 also includes, in particular in Articles 5(5) and 6(4), aspects of environmental risk assessment of food, it is, as regards food, based overwhelmingly on an appraisal emphasizing protection of human health which is linked to the specific fact that that food is, by definition, intended for human consumption. Thus, in accordance with recital 3 in the preamble, in order to protect human health, foods containing, consisting or produced from GMOs must undergo a ‘safety’ assessment. Regulation 1829/2003 thus introduces an additional level of control. That regulation would be rendered nugatory, if the view were to be taken that an assessment carried out and an authorisation issued pursuant to Directive ... 2001/18 covered all subsequent potential risks to human health and the environment”.

The least which one can conclude from these remarks by the European Court of Justice is that the safety assessment – in other words the environmental risk assessment and the post-marketing

²⁷See for example Commission decision 2010/135/EU, OJ 2010, L 53 p.11, Recital 18 and Article 4(e), where additional monitoring measures were requested.

²⁸Court of Justice, case C-442/09 *Bablok*, Judgment of 6 September 2011, paragraphs 97 – 102.

monitoring evaluation – must be, under Regulation 1829/2003, at least as strict as under Directive 2001/18, if not stricter.

Conclusion

It follows from all these provisions, that under Directive 2001/18, a notifier's documentation must contain a comprehensive environmental risk assessment of the GMO, which includes all potential adverse effects on human and animal health. Unlikely occurrences must also be included in the assessment and evaluated. The monitoring plan must be case specific and also contain a strategy for monitoring events that were not foreseen in the environmental risk assessment.

The purpose of Directive 2001/18 is also to protect human and animal health, and as GMO plants are consumed by humans, the environmental risk assessment and the monitoring plan must, therefore, also contain an assessment of such potential effects (risk assessment) and a strategy to verify whether such adverse effects actually occur. Indeed, the development of allergies or other adverse effects, due to the consumption of genetically modified plants which are herbicide-resistant, and which possibly contain herbicide residues, are not so unlikely that the monitoring of such effects can be omitted.

The competent authority has to give written consent for the placing on the market of a GMO as or in a product (Article 19). The consent has to specify, among other things, the monitoring requirements in accordance with Annex VII to the Directive (Article 19(3.f)). This provision clarifies that the competent authority is not bound, in the monitoring conditions, which it puts on the consent with regard to monitoring, by the monitoring plan of the notifier. Rather, this plan is, legally, a mere proposal. Thus, the competent authority, which gives written consent, has a responsibility of its own to ensure that all direct and indirect, immediate and delayed, cumulative and unforeseen effects of the GMO on human and animal health and the environment are properly monitored.

Under Regulation 1829/2003, the competent authority is required to ensure that a proper safety and risk assessment of the GMO is carried out to ensure that it does not have adverse effects on human health, animal health or the environment. This requires that not only is a thorough and scientifically adequate safety assessment is carried out, but also where appropriate that suitable monitoring is carried out.

1.5 Particular Provisions of Implementing Regulation 503/2013

The application was declared to be valid by EFSA and forwarded to EU Member States in March 2014 and Implementing Regulation 503/2013 was applied in the risk assessment. Therefore the Regulation has to be obeyed, setting new standards compared to previous assessments of single or stacked events. The following provisions of Annex II and Annex III of Regulation 503/2013 are of specific relevance for the request:

Annex II,

I. INTRODUCTION

- 2.2.: “The risk assessment of genetically modified food and feed containing stacked transformation events shall also include an assessment of the following aspects:
 - (a) stability of the transformation events;
 - (b) expression of the transformation events;

(c) potential synergistic or antagonistic effects resulting from the combination of the transformation events shall be subject to an assessment in accordance with Sections 1.4 (Toxicology), 1.5 (Allergenicity) and 1.6 (Nutritional assessment).”

“(…) the application shall include all subcombinations independently of their origin which have not yet been authorised.”

II. SCIENTIFIC REQUIREMENTS:

- 1.1 (e) (ii): “Information relating to the recipient or (where appropriate) parental plants: (….) sexual compatibility with other cultivated or wild plant species;”
- 1.2.2.3: “Protein expression data, including the raw data, obtained from field trials and related to the conditions in which the crop is grown (in regard to the newly expressed proteins).”
- 1.3.1: “In the case of herbicide tolerant genetically modified plants and in order to assess whether the expected agricultural practices influence the expression of the studied endpoints, three test materials shall be compared: the genetically modified plant exposed to the intended herbicide; the conventional counterpart treated with conventional herbicide management regimes; and the genetically modified plant treated with the same conventional herbicide management regimes.”
- 1.3.2.1 (b): “The different sites selected for the field trials shall reflect the different meteorological and agronomic conditions under which the crop is to be grown; the choice shall be explicitly justified. The choice of non-genetically modified reference varieties shall be appropriate for the chosen sites and shall be justified explicitly.”
- 1.4: “Toxicological assessment shall be performed in order to:
(a) demonstrate that the intended effect(s) of the genetic modification has no adverse effects on human and animal health;
(b) demonstrate that unintended effect(s) of the genetic modification(s) identified or assumed to have occurred based on the preceding comparative molecular, compositional or phenotypic analyses, have no adverse effects on human and animal health;”
- 1.4.4.1: “An additional 90-day feeding study with whole food and feed in rodents with the genetically modified plant with the stacked transformation events shall be included where indications of potential adverse effects are identified during the assessment of: (i) the stability of the inserts; (ii) the expression of the inserts; and (iii) the potential synergistic or antagonistic effects resulting from the combination of the transformation events.”
- 1.4.4.2: “When information (…) on the genetically modified food and feed suggest the potential for reproductive, developmental or chronic toxicity or in case of indications of adverse effects from the 90-day feeding study in rodents (such as functional and/or histological modifications of nervous, endocrine, reproductive or immunological tissues/organs), appropriate testing shall be performed.”
- 1.5.1: “The applicant shall verify whether the source of the transgene is allergenic. (….) Where transformation events have been stacked, the applicant shall provide an assessment of any potential for increased allergenicity to humans and animals on a case-by-case

approach. These potential effects may arise from additive, synergistic or antagonistic effects of the gene products.”

- 1.5.3: “In cases when known functional aspects of the newly expressed protein or structural similarity to known strong adjuvants may indicate possible adjuvant activity, the applicant shall assess the possible role of these proteins as adjuvants. As for allergens, interactions with other constituents of the food matrix and/or processing may alter the structure and bioavailability of an adjuvant and thus modify its biological activity.”
- 3.3: In accordance with the requirements of Articles 4 and 16 of Regulation (EC) No 1829/2003, the applicant shall ensure that the final risk characterisation clearly demonstrates that:
 - (a) the genetically modified food and feed has no adverse effects on human and animal health;

Annex III

- 3.1, C: The applicant shall demonstrate that the method(s) fulfils the following requirements:
 1. The method(s) shall be specific to the transformation event (hereafter referred to as ‘event-specific’) and thus shall only be functional with the genetically modified organism or genetically modified based product considered and shall not be functional if applied to other transformation events already authorised; otherwise the method cannot be applied for unequivocal detection/identification/quantification. This shall be demonstrated with a selection of non-target transgenic authorised transformation events and conventional counterparts. This testing shall include closely related transformation events.

Conclusions

It follows from all these provisions, that under Implementing Regulation 503/2013 Directive detailed requests and defined standards onto the risk assessment of EFSA are provided if genetically engineered plants are applied for import in the EU to be used in food and feed production. If these minimum standards are not fulfilled, it can not be concluded that a product derived from a genetically engineered plant was demonstrated to be safe. Beyond that, on a case by case approach, more data have to be requested by EFSA, if it is deemed necessary for risk assessment. Equally to the provisions under Directive 2001/18 and Regulation 1829/2003, products derived from genetically engineered plants have to be shown to be safe for health and the environment. If substantial uncertainties remain after risk assessment as requested under Regulation 503/2013, the overall provisions regarding the protection of health and the environment under Directive 2001/18 and Regulation 1829/2003 still prevail.

1.6. Pesticide regulation

Most relevant for health risk assessment of pesticides is Regulation 1107/2009 for placing on the market of relevant products and Regulation 396/2005 for setting Maximum Residue Levels (MRLs). Both Regulations require a high level of protection for health and the environment (see, for example, Recitals 8 and 24, and Article 1.4. of Regulation 1107/2009 as well as Recital 5 and Article 1 of Regulation 396/2005). In consequence, safety has to be established to make sure that substances or products produced or placed on the market do not have any harmful effect on human or animal health.

More specifically, Article 29 of Regulation 1107/2009 requests that active substances as well as synergists have to be approved and the maximum residue levels for the specific agricultural products have to be determined; Article 4 of Regulation 1107/2009 states that pesticides must not have any harmful effects on human or animal health, taking into account known cumulative and synergistic effects; Recital 5 of Regulation 396/2005 states that residues should not be present at levels presenting an unacceptable risk to humans and, where relevant, to animals; Recital 10 of Regulation 396/2005 requests specific MRLs for each pesticide in food and feed products have to be established. Very relevant in the context of importing products derived from genetically engineered herbicide resistant plants, Recital 26 of Regulation 396/2005 requests that MRLs have to be set for food and feed produced outside the Community if produced by different agricultural practices as regards the use of plant protection products. Article 14 of Regulation 396/2005 adds the presence of pesticide residues arising from sources other than current plant protection uses and their known cumulative and synergistic effects have to be determined, as well as “the results of any evaluations and decisions to modify the uses of plant protection products” (Article 14.2 (d)).

Consequently, even if a particular pesticide is authorised for use on plants grown in the EU or imported from third countries, further investigation of the residues from spraying with the complementary herbicide may be required. Due to the specific agricultural practices that go along with the cultivation of these herbicide resistant plants, there are for example specific patterns of applications, exposure, occurrence of specific metabolites and emergence of combinatorial effects that require special attention. As Kleter et al. (2011) summarise, genetically herbicide resistant crops can change the way that herbicides can be used on these crops, for example (a) post-emergent over-the-top applications (i.e. on the crop itself) instead of directed sprays, avoiding herbicide contact with the crop; or (b) pre-emergent and pre-harvest applications made to the conventional crop and not, or in different quantities, to the genetically engineered crop. Further, the residue profile of the applied pesticide may have been altered on the basis of the nature of the genetic changes introduced and the overall pattern of pesticides applied to the particular crop may have been altered, leading to different exposure to pesticide residues overall.

More specifically, agricultural practice as established in the usage of the herbicides on these plants might result in an increase in the amounts of herbicide that are sprayed and subsequently in the amounts of residues in the harvest. Further, if herbicides are meant to be applied in combination to crops, the residues thereof can lead to a specific pattern of combinatorial exposure of the feed and food chain.

It is worrying that EFSA's pesticide panel (EFSA 2018b), in its assessment of residues from spraying with glyphosate, explicitly states that existing data are not sufficient to conclude on health risks of consuming relevant products derived from herbicide resistant maize:

“For genetically modified crops, data were sufficient to derive MRL for sweet corn (EPSPS modification) and cotton seed (EPSPS modification), noting that MRLs should be tentative pending on the submission of confirmatory methods for enforcement of AMPA and N-acetyl-glyphosate. For sugar beet roots, maize and soybeans (EPSPS modification), soybeans (GAT modification) and rapeseeds (GOX modification), the available data were insufficient to derive MRLs and risk assessment values.”

1.7. The interface between pesticide and GMO regulation

There are several requirements for health risk assessment in the EU GMO and pesticide regulation:

Both require a high level of protection for health and the environment. Both request that the conditions in agricultural production are taken into account. Both request combinatorial effects to be taken into account. Finally, Implementing Regulation 503/2013 explicitly combines the two areas of risk assessment in requesting field trials with and without the application of the complementary herbicide.

Since the application of the complementary herbicide is a regular part of agricultural practice in the cultivation of herbicide resistant plants, it can be expected that residues from spraying are always present in the harvest. Thus, in regard to herbicide resistant plants, specific assessment of residues from spraying with complementary herbicides has to be considered to be a prerequisite before any authorisation for genetically engineered plants can be granted.

It follows that under the EU Regulation, a notifier's documentation must contain a comprehensive safety and environmental risk assessment of the genetically engineered organism, which includes all or potential adverse effects on the environment as well as on human and animal health. This requirement includes long-term potential and accumulative effects and also all other harmful effects on human or animal health which are, in one way or another, related to the genetically modified plant, such as residues from spraying with complementary herbicides. Consequently, authorisation for import and usage in food and feed of genetically engineered plants cannot be granted if the plants contain residues from spraying with complementary herbicides that pose unacceptable risks, or are suspected of causing harm to human and / or animal health (see also Kraemer, 2012).

2. Grounds for the request to reviewing the decision

Introduction

The EFSA GMO panel assessed the five-stacked Maize MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 derived from crossing genetically engineered maize events (EFSA, 2019a). The parental plants were assessed by EFSA in previous opinions. The Maize contains genes conferring triple resistance to glyphosate and produces three insecticides. It was also developed to be drought tolerant:

- MON 87427 expressing CP4 EPSPS protein for tolerance to glyphosate-containing herbicides;
- MON 87460 produces a so-called cold shock protein (CSPB) associated with enhanced abiotic stress tolerance in bacteria; furthermore, it produces neomycin phosphotransferase II (NPTII) which inactivates a range of important antibiotics, including kanamycin and neomycin;
- MON 89034 expressing the insecticidal proteins Cry1A.105 (artificially synthesized) and Cry2Ab2;
- MIR162 expressing the insecticidal protein Vip3Aa20 and phosphomannose isomerase (PMI) which is a selectable marker;
- NK603 expressing two variants of CP4 EPSPS protein for tolerance to glyphosate-containing herbicides.

Consequently, the stacked GE Maize has triple resistance to glyphosate, making it tolerant to high dosages and repeated sprayings as applied in fields with herbicide-resistant weeds. Further, it produces three toxins against the larvae of *Lepidoptera* (butterflies) that feed on the plants ('pest insects'). In addition, the cold shock protein is intended to confer drought tolerance.

Furthermore, it produces the PMI protein (phosphomannose isomerase) derived from *Escherichia coli*. Expression of PMI enables transformed maize cells to utilise mannose, and therefore to survive on specific media used for selecting the maize plants after the process of genetic engineering (so-called marker genes). Finally, neomycin phosphotransferase II (NPTII) is produced in the plants which inactivates a range of important antibiotics, including kanamycin and neomycin.

No experimental data were provided for potentially segregated subcombinations of the Maize.

Implementing Regulation 503/2013 was applied in the EFSA risk assessment.

The stacked GE Maize (hereinafter referred to as the Maize) was authorised on 22 January 2021, published in the Official Journal of the EU on 26 January 2021.²⁹

2.1. Molecular characterisation and gene expression

Annex II of Implementing Regulation 503/2013 requests that

"The risk assessment of genetically modified food and feed containing stacked transformation events shall also include an assessment of the following aspects:

- (a) stability of the transformation events;*
- (b) expression of the transformation events;*
- (c) potential synergistic or antagonistic effects resulting from the combination of the transformation events shall be subject to an assessment in accordance with Sections 1.4 (Toxicology), 1.5 (Allergenicity) and 1.6 (Nutritional assessment)."* (Introduction)

²⁹<https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32021D0061&qid=1612780708978>

“Protein expression data, including the raw data, obtained from field trials and related to the conditions in which the crop is grown (in regard to the newly expressed proteins).” (Scientific requirements 1.2.2.3)

“In the case of herbicide tolerant genetically modified plants and in order to assess whether the expected agricultural practices influence the expression of the studied endpoints, three test materials shall be compared: the genetically modified plant exposed to the intended herbicide; the conventional counterpart treated with conventional herbicide management regimes; and the genetically modified plant treated with the same conventional herbicide management regimes.” (Scientific requirements 1.3.1)

“The different sites selected for the field trials shall reflect the different meteorological and agronomic conditions under which the crop is to be grown; the choice shall be explicitly justified. The choice of non-genetically modified reference varieties shall be appropriate for the chosen sites and shall be justified explicitly.” (Scientific requirements 1.3.2.1)

2.1.1 Assessment of open reading frames

The process of genetic engineering involved several deletions and insertions in the parental GE maize plants. In order to assess the sequences encoding the newly expressed proteins, or any other open reading frames (ORFs) present within the insert and spanning the junction sites, it was assumed that the proteins that might emerge from these DNA sequences would raise no safety concerns; therefore, no detailed investigations were carried out in this regard. Although EFSA identified a putative peptide which might emerge from one of the open reading frames that shows similarity to known allergens, Monsanto did not provide data proving the absence of this additional peptide in the Maize.

Monsanto / Bayer declared the relevant DNA sequence to be confidential. This is in contradiction to the EU regulation which requests access to data is given for all risk relevant information. Unless access to the data is given, no independent risk research can be carried out into the specific peptide with allergenic potential.

Furthermore, other biologically active gene products such as non-coding small RNAs (ncsRNAs) from additional open reading frames were not assessed. Thus, uncertainties remain in regard to other biologically active substances arising from the method of genetic engineering and the newly introduced gene constructs.

Consequently, EFSA neither took into account all the relevant data requested by EU regulation nor potential synergistic or antagonistic effects resulting from the combination of the transformation events. This would however be needed to draw reliable conclusions on health safety, including the assessment of toxicity and impact on the immune system.

2.1.3 Impact of environmental factors, agricultural practice and genetic backgrounds

There are several reasons why the data presented do not meet the requirements of Implementing Regulation 503/2013: (1) the field trials were not conducted in all relevant regions where the Maize is to be cultivated, and no extreme weather conditions were taken into account; (2) the field trials did not take current agricultural management practices into account; (3) only one transgenic variety

was included in the field trials; (4) data from previous applications indicate that environmental stress factors, herbicide applications rates, genetic backgrounds and stacking all impact gene expression. This underlines the need for more data to assess the stability and the expression of the stacked event, taking into account potential synergistic or antagonistic effects resulting from the combination in the stacked Maize.

2.1.3.1 Data on environmental factors, stress conditions and their impact on gene expression

Environmental stress can cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015; Lohn et al., 2020). There is plenty of evidence showing that drought or heat can significantly impact the content of Bt in the plant tissue (Adamczyk & Meredith, 2004; Adamczyk et al., 2009; Chen et al., 2005; Dong & Li, 2006; Luo et al., 2008; Then & Lorch, 2008; Trtikova et al., 2015; Jiang et al., 2018; Girón-Calva et al., 2020). Therefore, to assess gene expression, the plants should have been grown under conditions of severe drought, with and without irrigation, with and without application of the complementary herbicide and in comparison to more moderately severe climate conditions. However, no such data were requested or used for detailed comparison to assess the genome x environment interactions.

More specifically, Fang et al. (2018) and Yang et al. (2017) show that stress responses can lead to unintended changes in plant metabolism inheriting additional EPSPS enzymes. In this context, there are strong indications that the EPSPS enzyme, which confers glyphosate tolerance, also interferes with the auxin metabolism in the plants (Fang et al., 2018). This plant hormone plays a key role in growth, fecundity and adaptation to environmental stressors. Thus, changes in the auxin content can also result in changes in plant composition that can raise safety concerns (see also Testbiotech, 2021).

Several publications support these findings showing unintended effects in plants inheriting additional EPSPS genes (Beres, 2019; Beres et al., 2018; Wang et al., 2014). In addition, other authors show the need for further investigations (Vila-Aiub et al., 2009 and Vila-Aiub et al., 2019).

The EPSPS enzymes occur in the stacked Maize in higher concentrations compared to the parental plants. Therefore, the Maize should have been subjected to a broad range of defined environmental conditions and stressors to gather reliable data on gene expression and functional genetic stability. This is especially relevant for the stacked Maize since these plants are the first in which expression of the epsps gene constructs are intended to be exposed to more extreme climate conditions.

Therefore, the Maize should have been tested in all major maize producing countries, such as North and South America. Furthermore, the Maize should have been subjected to defined drought conditions since it must be assumed it will be grown in accordance with its intended purpose.

However, this was not the case: The acronyms (IARL, ILFI, ILMN, ILRD and OHTR) used in the report (Monsanto, 2016a) show the origin of the data from some of the field trials performed for compositional analysis (Monsanto, 2016c). Only five sites, i.e. Iowa (1), Illinois (3), and Ohio (1) were used in this case. They are all relatively close and therefore only represent a very limited region of the maize-producing areas in the US; no other maize-producing country was included.

No extreme weather conditions (except frost) were reported during cultivation in 2014. Monsanto reported some cases of drought and heat (Monsanto, 2016b) as well as cold and flooding, but without providing exact definitions of these environmental factors. Interestingly, at the Kansas site (KSLA), higher temperatures were observed for four months, the plants were irrigated during that

time therefore avoiding drought. Notably, data from KSLA on gene expression were not provided for the risk assessment.

In summary, the available publications strongly indicate that plants inheriting a combination of EPSPS and CSPB are likely to show gene expression responses when grown under stress conditions such as drought. In addition, Bt toxin expression in GE plants is known to be influenced by environmental conditions such as drought.

Thus, in the stacked Maize, there is a combination of a trait which is meant to expose the plant to extreme climate conditions and gene constructs (such as for the EPSPS enzyme and the production of Bt toxins), which are likely to show or cause major changes in gene expression if exposed to environmental stressors, such as drought. In addition, two other proteins are expressed which are absent in conventional maize: NPTII which confers antibiotic resistance (and should have been avoided as requested by EU Directive 2001/18), and PMI which was used for the selecting process during plant production. These traits and genetic elements may synergise and interact with each other. The resulting effects are not unlikely to impact plant composition and biological characteristics crucial for the assessment of food and feed safety. However, no specific data were requested or used for detailed comparison to assess genome x environment interactions.

Some comments made by experts of Member States (EFSA, 2019d) point out this gap in risk assessment. In response, EFSA stated:

“Considering that there is no indication of an interaction between the events (see section 3.4.1.4 of the Scientific Opinion), it was not necessary to request the inclusion of field trials under drought conditions for the five-event stack maize.”

This statement is scientifically problematic. At the very least, the unintended effects of the EPSPS enzymes plausibly interfere with the activity of the other gene constructs, for example, via the auxin hormone (see above). This is especially relevant if the plants are exposed to stress conditions. Even if no such effects were observed in the parental plants (which, to our knowledge, were not tested in field trials under stressful conditions), these enzymes are produced in the stacked Maize at higher concentrations. Therefore, the likelihood of interaction between the gene constructs and gene expression is higher in the stacked compared to the parental plants.

Furthermore, EU regulation does not request that interactions between gene constructs are known before it requests that the data from the plants are *“obtained from field trials and related to the conditions in which the crop is grown (in regard to the newly expressed proteins).”*

It has to be expected that the stacked maize will be grown under drought conditions to an extent that most of the parental GE plants were not tested for in their previous risk assessment. Moreover, it is the first time that the combination of artificial gene constructs will be exposed to more extreme drought conditions. It is obvious that in the absence of adequate data, there can be no assessment of whether interactions will occur under stress conditions. Therefore, there is no scientifically sound way of arguing why much more specific data is not necessary. Consequently, the stacked plants should have been grown under conditions of severe drought, with and without irrigation, with and without application of the complementary herbicide and in comparison to more moderately severe climate conditions.

Consequently, the EFSA response to requests from member states as quoted above is not acceptable. The GMO Panel appears to be abusing its position in order to exert a dominant rhetorical position

that ignores sound scientific arguments and, at the same time, escapes the challenges of a sufficiently robust risk assessment.

In summary, while the plants are expected to show improved performance under more extreme drought conditions, no such data have been reported from the field trials. No data are available as requested by Implementing regulation 503/2013 to assess whether the expected environmental conditions under which the plants are likely to be cultivated will influence the expression of the studied endpoints. Furthermore, no such data are available from the parental plants or any subcombinations.

These gaps in risks assessment are further emphasised in other meteorological data:

- Regarding the US, weather data from 2014 show that there was more precipitation than normal at the trial sites in Illinois. Therefore, a relevant stressor which might influence gene expression, composition or phenotype of the Maize may not be covered by the field trial data.
- Further, the trial sites in Illinois, Iowa and Ohio neither represent the climatic conditions in other maize growing regions of the US nor the conditions in other relevant maize producing countries, such as Brazil.
- For example, in the US, the selected field trial sites (mainly Illinois, Iowa, Kansas, Ohio) only represent a very limited range of climatic and environmental conditions in major maize growing regions. However, USDA data show relevant maize production is also found in North Dakota, Minnesota, Mississippi, Louisiana and other states.
- According to climate data, precipitation in maize production regions such as Iowa, Illinois or Kansas is significantly different to that in other maize production regions, e.g. South Dakota.³⁰ The same is true for lower average temperatures in northern maize growing states, e.g. Minnesota, in comparison to other maize growing regions in the US.³¹
- Even more striking is the difference between the climatic conditions in the major maize growing regions of the US and the conditions in maize growing regions in Brazil, another major producer of GE maize. Data show much more precipitation in maize producing states, such as Paraná or Mato Grosso, compared to the US.³² Furthermore, most Brazilian maize-producing regions have much higher average temperatures compared to US regions, such as Iowa, Illinois or Ohio.³³

Consequently, the Maize plants tested in field trials do not sufficiently represent the imported Maize. The data presented by the applicant are insufficient to conclude on the impact of environmental factors and stress conditions on gene expression, plant composition and the biological characteristics of the plant as requested by the EU Regulation 503/2013.

³⁰https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=0440000®ionid=us&nationalGraph=False&cntryid=USA&sel_year=2021&startRow=1&fctypeid=23&fcattributeid=1

³¹https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=0440000®ionid=us&nationalGraph=False&cntryid=USA&sel_year=2021&startRow=1&fctypeid=24&fcattributeid=1

³²https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=0440000®ionid=br&nationalGraph=False&cntryid=BRA&sel_year=2021&startRow=1&fctypeid=23&fcattributeid=1

³³https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=0440000®ionid=us&nationalGraph=False&cntryid=USA&sel_year=2021&startRow=1&fctypeid=24&fcattributeid=1

2.1.3.2 Data on herbicide application rates and their impact on gene expression

Due to increased weed pressure, it must be assumed that these plants will be exposed to high and also repeated dosages of glyphosate. Higher rates of herbicide application will not only lead to a higher burden of residues in the harvest, but can also influence the expression of the transgenes or other genome activities in the plants. These observations are evidenced by a large amount of data showing substantial changes in the composition of GE herbicide-resistant soybeans (Miyazaki et al., 2019; see also Testbiotech, 2021) while more specific data on GE maize seem to be lacking.

The glyphosate treatment was applied at a rate of 0.87 kg a.e./ha (Monsanto, 2016b) only. At the same time, industry recommendations suggest dosages of up to approx. 3,5 kg a.i./ha glyphosate post-emergence, 7 kg per season, and even higher rates, can be sprayed on herbicide-resistant maize.³⁴

Only 4 samples (each for grain and forage) from 5 closely located field sites (IARL, ILFI, ILMN, ILRD and OHTR, see Monsanto, 2016c), were used for generating the data on gene expression. While some data include samples with and without spraying of the complementary herbicide, some only include data from crops treated with glyphosate. This makes it impossible to assess the impact of applications of the complementary herbicide on gene expression as requested by EU regulation. Therefore, EFSA should not have accepted these data.

Monsanto reasoned that *“there is no known mechanism by which glyphosate application to MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 could affect protein expression levels in this product, and therefore no impact of this treatment is anticipated.”* (Monsanto, 2016) However, this assumption is not sufficiently based on facts: Fang et al. (2018) and Yang et al. (2017) show that stress responses can lead to unexpected changes in plant metabolism inheriting additional EPSPS enzymes. These findings showing unintended effects in plants inheriting additional EPSPS genes are supported in several other publications (Beres, 2019; Beres et al., 2018; Wang et al., 2014). In addition, other authors show the need for further investigations (Vila-Aiub et al., 2009 and Vila-Aiub et al., 2019).

In this context, there are strong indications that the EPSPS enzyme also interferes with the auxin metabolism in the plants (Fang et al., 2018). Auxin has a role in multiple metabolic pathways in plants. Thus, changes in the auxin content can also result in changes in plant composition that can raise safety concerns.

The EPSPS enzymes occur in the stacked Maize in much higher concentrations compared to the parental plants. Therefore, it should be taken into account that unintended effects caused by high concentrations of EPSPS enzymes in combination with higher rates of spraying, may also affect gene expression and plant composition. When the Maize is exposed to higher rates of glyphosate application, the plants may experience stress conditions, impacting gene expression and plant composition as well as the biological characteristics of the Maize.

Similar concerns were also raised by experts of the Member States (EFSA, 2019d): *“The study design is missing the cultivation of MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 under analysis drought conditions. GM plants carrying specific traits require appropriate treatment comparisons to evaluate safety (...). In terms of herbicide resistant plants cultivation with and without the intended stressor (i.e. herbicide) is required. Similar approach would be useful for drought-resistant plants, where plants should be grown both at standard and drought conditions, treated and non-treated with intended herbicides.”*

³⁴www.greenbook.net/monsanto-company/roundup-weathermax; www.greenbook.net/monsanto-company/roundup-ultra

Consequently, the Maize plants tested in field trials do not sufficiently represent the Maize as imported. The data presented by the applicant are insufficient to conclude on the impact of the herbicide applications on gene expression, plant composition or biological characteristics of the plant as requested by the EU Regulation 503/2013.

2.1.2.3 Data on genetic backgrounds and their impact on gene expression

It is very well known that the genomic background of the variety can influence the expression of the inserted genes and / or the concentration of the additional proteins present in the GE plants (see Trtikova et al., 2015). For example, Lohn et al. (2020), in a multigenerational study with Bt maize, show that the Bt content was generally lower in offspring if the maize was crossed with Brazilian varieties, but much higher than expected in offspring from South African plants.

Therefore, EFSA should have requested additional data from several varieties, including those cultivated in South America. However, no such data were provided or requested. Furthermore, no such data are available for the parental plants or any subcombinations.

In conclusion, the Maize plants tested in field trials do not represent the Maize as imported. The data presented by the applicant are insufficient to conclude on the impact of genetic background on gene expression, plant composition and biological characteristics. However, reliable data are needed to demonstrate safety as requested by EU Regulation.

2.1.2.4 Data from other events and previous applications indicate that environmental stress factors, herbicide application rates, genetic backgrounds and stacking all impact gene expression and plant metabolism

The findings on flaws in risk assessment are supported by analysis data from previous applications involving the same parental events. Data presented in Table 1 show widely differing gene expression and content of Vip3Aa20. This might be caused by genetic instability, varietal backgrounds, agricultural practices, environmental factors or stacking.

Table 1: Gene expression and content of Vip3Aa20 present in maize MIR162 in grain ($\mu\text{g/g}$ dry weight, mean values)

Application (EFSA opinion)	Details from field trials	Content of Vip3Aa20
MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 (EFSA 2019a)	Field trials at five locations in the USA in 2014 (sprayed with glyphosate)	38
MON 87427 x MON 89034 x MIR162 x NK603 (EFSA 2019b)	Field trials at five locations in the USA in 2013 (sprayed with glyphosate)	59
Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 (EFSA 2019c)	Field trials at three locations in the US in 2012 (not sprayed with complementary herbicides)	100
Bt11 x MIR162 x 1507 x GA21 (EFSA 2018a)	Field trials at one single location in the US 2008 (sprayed?)	28
Bt11 x MIR162 x MIR604 x GA21 (EFSA 2015a)	Single location in the US in 2006 (sprayed?)	140
MIR162 (EFSA 2012)	Bloomington, Illinois 2005, Hybrid A	46
	York, Nebraska, 2005, Hybrid B	41
	Bloomington, Illinois, 2006, Hybrid A	124
	Bloomington, Illinois, 2006, Hybrid B	84
	Brazil, Ituiutaba, 2007	62
	Brazil, Uberlandia, 2007	59

These data show a range of mean values between 28 $\mu\text{g/g}$ and 140 $\mu\text{g/g}$ for Vip3Aa20 in the grain, while in other cases 166 $\mu\text{g/g}$ and more were measured as maximum range in the grain (EFSA, 2012); this is evidence of highly variable gene expression, with the actual content of the additional protein being unpredictable.

The factors influencing the content might seem variable. As EFSA (2012) stated in previous opinions, “*a year-to-year and site-to-site variation is evident*”. In addition, genetic backgrounds of different varieties and effects from stacking seem to be relevant as well. There is no justification for not requesting additional data on the impact of drought conditions on Vip3Aa20 gene expression.

There is already plenty of evidence in other cases that, for example, drought or heat can significantly impact the content of Bt in the plant tissue (Adamczyk & Meredith, 2004; Adamczyk et al., 2009; Chen et al., 2005; Dong & Li, 2006; Luo et al., 2008; Then & Lorch, 2008; Trtikova et al., 2015; Girón-Calva et al., 2020)

However, EFSA and the applicant fail to give a full overview of existing data from previous applications and findings in more detail, which is needed for conclusive and sufficiently robust risk assessment, taking into account realistic conditions under which the Maize is likely to be grown.

It also should have been taken into account that previous research indicates the expression of Cry1A.105, Cry2Ab2 and EPSPS proteins in genetically engineered maize can induce changes in the overall proteome of the respective GM maize line, with impacts on associated endogenous metabolic pathways. Several publications point out the unintended changes in the genome, the transcriptome, the proteome or the metabolome of transgenic maize. (see Agapito-Tenfen et al., 2013; Agapito-Tenfen et al., 2014; Benevenuto et al., 2017; Mesnage et al., 2016; Ben Ali et al., 2020).

Several of these transgenes are also present in the stacked Maize. In addition, four other proteins are expressed which are absent in conventional maize: NPTII which confers antibiotic resistance (which should have been avoided as requested by EU Directive 2001/18), PMI which was used for the selecting process during plant production, Vip3Aa20 (insecticidal toxicity) and CSPB (for drought tolerance). These traits and genetic elements can synergise and interact with each other. These effects are also likely to impact plant composition and biological characteristics crucial for the assessment of food and feed safety. However, no specific data for a detailed comparison to assess the genome x environment interactions were presented by the applicant.

Robust data should have been requested to assess whether metabolic changes with relevance to food and feed safety occur in the stacked maize, and EFSA should have requested a much more detailed investigation into potential biologically active gene products and changes in metabolic pathways under more extreme climate conditions.

However, EFSA risk assessment has not yet taken these issues into account. Nor does EFSA use more sensitive methods, such as transcriptomics, proteomics and metabolomics, to explore and assess unintended changes in the stacked Maize.

In summary, EFSA did not take into account all relevant data as requested by EU regulation to come to reliable conclusions regarding health safety, including the assessment of toxicity and impact on the immune system.

Consequently, the Maize plants tested in the field trials do not sufficiently represent the Maize as imported. The data presented by the applicant are insufficient to conclude on the impact of environmental factors, stress conditions, herbicide application rates, genetic backgrounds and stacking on gene expression, plant composition or the biological characteristics of the plant as requested by the EU Regulation 503/2013.

2.1.3 Conclusion on molecular characterisation and gene expression

We conclude that the available data strongly indicate gene expression of several of the additional genes is likely to depend on, or be influenced by, stacking, varietal background, herbicide spraying or environmental factors, such as stress conditions.

Therefore, the plants should have been exposed to a much broader range of defined environmental conditions and stressors to gather reliable data on gene expression and functional genetic stability. This is especially relevant in this case since the stacked Maize is meant to be cultivated under more extreme drought conditions, such as those caused by ongoing climate change. Whatever the case, it should have been tested in the maize producing countries in South America. Furthermore, EFSA should have requested the applicant to submit data from field trials which represent current agricultural practices with much higher rates of complementary herbicide applications on the plants, including repeated spraying. In addition, EFSA should have requested data for several varieties, including those cultivated in South America.

However, only 4 samples (each for grain and forage) from 5 closely located field trials were used to generate the data on gene expression. Furthermore, only one variety of the stacked Maize was used in the trials. The impact of environmental factors and agricultural practices was assessed without taking more extreme climate conditions into account, although cultivation in drought conditions is

one of the intended traits in the stacked Maize. For example, according to weather data, in 2014 there was more precipitation in most field trial sites than normal. Herbicide applications in the field trials did not represent current agricultural practices, the rates were much lower. Relevant data on comparison of sprayed and unsprayed GE Maize plants are completely missing.

In summary, the Maize plants tested in field trials do not sufficiently represent the imported Maize. Consequently, the data presented by the applicant and accepted by EFSA are insufficient to conclude on the impact of the combination of traits and gene constructs (stacking), or the impact of environmental factors, herbicide applications and of genetic background on gene expression and plant metabolism. However, reliable data are needed to demonstrate safety as requested by EU Regulation.

Based on the available data, no final conclusions can be drawn on the safety of the plants. Consequently, the data do not fulfill the requirements of Implementing Regulation 503/2013 and Regulation 1829/2003.

2.2. Comparative assessment of plant composition and agronomic and phenotypic characteristics

Implementing Regulation 503/2013 requests:

“In the case of herbicide tolerant genetically modified plants and in order to assess whether the expected agricultural practices influence the expression of the studied endpoints, three test materials shall be compared: the genetically modified plant exposed to the intended herbicide; the conventional counterpart treated with conventional herbicide management regimes; and the genetically modified plant treated with the same conventional herbicide management regimes.”

“The different sites selected for the field trials shall reflect the different meteorological and agronomic conditions under which the crop is to be grown; the choice shall be explicitly justified. The choice of non-genetically modified reference varieties shall be appropriate for the chosen sites and shall be justified explicitly.”

However, the data provided by Monsanto neither represent expected agricultural practices nor the different meteorological and agronomic conditions under which the crop is to be grown. There are three reasons for this: (2.2.1) the field trials were not conducted in all relevant regions where the Maize will be cultivated and no extreme weather conditions were taken into account; (2.2.2) the field trials did not take current agricultural management practices into account; (2.2.3) only one stacked transgenic variety was included in the field trials.

2.2.1 Data on environmental factors and stress conditions - and their impact on plant composition and agronomic and phenotypic characteristics

Field trials for the assessment of plant composition and agronomic and phenotypic characteristics of the stacked Maize were conducted in the US for one year only, but not in other relevant maize production areas such as Brazil and Argentina. As shown in the EFSA opinion (2019a), “no exceptional weather conditions were reported at any of the selected field trial sites”. In addition, and contrary to expected agricultural practices and different meteorological and agronomic conditions under which the crop is to be grown, EFSA states it “considers that the selected sites reflect commercial maize-growing regions in which the test materials are likely to be grown.”

No extreme weather conditions (except frost) were observed during cultivation in 2014. Monsanto reported some cases of drought and heat as well as (Monsanto, 2016b) cold and flooding, but without providing exact definitions of these environmental factors. Interestingly, higher temperatures were observed during four months at the site in Kansas (KLSA), and the plants were irrigated to avoid drought conditions. While the plants are expected to show improved performance under more extreme drought conditions, no such data were reported from the field trials.

Taking into account the purpose of the genetic engineering in this case, it is not acceptable that EFSA failed to require further studies:

- No field trials were conducted that lasted more than one season. Thus, based on current data, it is hardly possible to assess site-specific effects. However, as our analysis on gene expression shows, specific site-by-site and year-on-year effects have to be expected.
- No data were generated representing more extreme environmental conditions, such as those caused by climate change resulting in more extreme droughts.
- No data were generated to represent the growing conditions in other relevant maize growing regions outside the US.

These gaps in risks assessment are also evident in other meteorological data:

- US weather data from 2014 show that there was more precipitation than normal at the trial sites in Illinois. This means that a relevant stressor that might influence gene expression, composition or phenotype of the Maize may not be covered by the field trial data.
- The trial sites in Illinois, Iowa and Ohio neither represent the climatic conditions in other maize growing regions of the US nor the conditions in other relevant maize producing countries, such as Brazil.
- As far as the US is concerned, only a very limited range of climatic and environmental conditions in major maize growing regions is represented in the selected field trial sites (mainly Illinois, Iowa, Kansas, Ohio). However, USDA data show relevant maize production in North Dakota, Minnesota, Mississippi, Louisiana and other states.
- According to climate data, precipitation in maize production regions, such as Iowa, Illinois or Kansas, is significantly different to that of other maize-producing regions, such as South Dakota.³⁵ The same is true for lower average temperatures in northern maize growing states, e.g. Minnesota, in comparison to other maize growing regions in the US.³⁶
- Even more striking is the difference between the climatic conditions in the major maize growing regions of the US and the conditions in maize growing regions in Brazil, another major producer of GE maize. Data show much more precipitation in maize producing states like Paraná or Mato Grosso compared to the US.³⁷ Furthermore, the average temperature in most Brazilian maize production regions is much higher compared to US regions, e.g. Iowa, Illinois or Ohio.³⁸

³⁵https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=0440000®ionid=us&nationalGraph=False&cntryid=USA&sel_year=2021&startRow=1&fctypeid=23&fcattr_ibuteid=1

³⁶https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=0440000®ionid=us&nationalGraph=False&cntryid=USA&sel_year=2021&startRow=1&fctypeid=24&fcattr_ibuteid=1

³⁷https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=0440000®ionid=br&nationalGraph=False&cntryid=BRA&sel_year=2021&startRow=1&fctypeid=23&fcattr_ibuteid=1

³⁸https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=0440000®ionid=us&nationalGraph=False&cntryid=USA&sel_year=2021&startRow=1&fctypeid=24&fcattr_ibuteid=1

Fang et al. (2018) as well as Yang et al. (2017) show that stress responses can lead to unintended changes in plant metabolism inheriting additional EPSPS enzymes. In this context, there are strong indications that the EPSPS enzyme, which confers glyphosate tolerance, also interferes with the auxin metabolism in the plants (Fang et al., 2018). This plant hormone plays a key role in growth, fecundity and adaptation to environmental stressors. Thus, changes in the auxin content can also result in changes in plant composition that can raise safety concerns.

Several publications support these findings showing unintended effects in plants inheriting additional EPSPS genes (Beres, 2019; Beres et al., 2018; Wang et al., 2014). Other authors also show the need for further investigations (Vila-Aiub et al., 2009 and Vila-Aiub et al., 2019).

The EPSPS enzymes occur in the stacked Maize in higher concentrations compared to the parental plants. Therefore, the Maize should have been subjected to a broad range of defined environmental conditions and stressors to gather reliable data on gene expression and functional genetic stability. This is especially relevant to the stacked Maize since EPSPS gene constructs will, for the first time, be expressed in these plants in more extreme climate conditions.

The stacked Maize carries a combination of a trait making the plants more tolerant to extreme climate conditions and gene constructs likely to show or cause major changes in gene expression if exposed to environmental stressors, such as drought. In addition, two other proteins are expressed which are absent in conventional maize: NPTII which confers antibiotic resistance - and should have been avoided as requested by EU Directive 2001/18 - and PMI which was used in the selecting process during plant production. These traits and genetic elements can synergise and interact. The effects are not unlikely to impact plant composition and biological characteristics crucial for the assessment of food and feed safety. However, no specific data needed for detailed comparison to assess the genome x environment interactions were presented by the applicant.

Whatever the case, it should have been tested in all the major maize producing countries such as North and South America. Furthermore, the Maize should have been subjected to defined drought conditions as there is an expectation that it will be grown in accordance with its intended purpose.

However, no experiments were requested to show to which extent specific environmental conditions will influence plant composition or agronomic and phenotypic characteristics. No data were made available as requested by Implementing regulation 503/2013 to assess whether the expected environmental conditions under which the plants are likely to be cultivated will influence the expression of the studied endpoints. Furthermore, no such data are available for the parental plants or any subcombinations. Some comments made by experts of Member States (EFSA, 2019d) point out this gap in risk assessment. In response, EFSA stated:

“Considering that there is no indication of an interaction between the events (see section 3.4.1.4 of the Scientific Opinion), it was not necessary to request the inclusion of field trials under drought conditions for the five-event stack maize.”

This statement is scientifically problematic. It has to be assumed that the stacked maize will be grown under drought conditions to an extent that most of the parental GE plants were not tested for in their previous risk assessment. Moreover, it is the first time that the artificial gene constructs in combination will be exposed to more extreme drought conditions. It is obvious that in the absence of adequate data, it cannot be assessed whether interactions will occur under stress conditions. Therefore, there is no scientifically sound way of arguing why much more specific data is not necessary. For this reason, the stacked plants should have been grown under conditions of severe

drought, with and without irrigation, with and without application of the complementary herbicide and in comparison to more moderately severe climate conditions.

In conclusion, the EFSA response to the requests from Member States quoted above is not acceptable. The GMO Panel appears to be abusing its position in order to exert a dominant rhetorical position that ignores sound scientific arguments and escapes the challenges of a sufficiently robust risk assessment.

Consequently, the Maize plants tested in field trials do not sufficiently represent the imported Maize. The data presented by the applicant are insufficient to conclude on the impact of environmental factors and stress conditions in the various maize producing countries on gene expression, plant composition and the agronomic and phenotypic characteristics of the plant, as requested by the EU Regulation 503/2013.

2.2.2 Data on herbicide application rates and their impact on plant composition and agronomic and phenotypic characteristics

Due to high weed pressure in many maize growing regions, there is an expectation that these plants will be exposed to higher amounts and repeated dosages of glyphosate. It also has to be taken into account that the herbicides can be sprayed repeatedly and at high dosages. Such agricultural practices have to be taken into account to assess whether the expected agricultural practices will influence the expression of the studied endpoints.

However, this requirement was mostly ignored by EFSA and the applicant: glyphosate was only sprayed at an early stage of vegetation and at comparably low dosages (0.87 kg a.e./ha, see Monsanto, 2016b). Industry recommendations suggest dosages to be sprayed on herbicide resistant maize of up to approx. 3,5 kg a.i./ha glyphosate post-emergence, 7 kg per season, and even higher rates.³⁹ (see also Testbiotech, 2021).

From the data that is available, it has to be assumed that the specific patterns of complementary herbicide applications will not only lead to a higher burden of residues in the harvest, but may also influence the composition of the plants, agronomic and phenotypic characteristics, for example, due to stress conditions caused by high amounts of complementary herbicides and unintended stress responses as described by Fang et al. (2018) and Yang et al., (2017).

Several publications report unintended effects in plants inheriting additional EPSPS genes (Beres, 2019; Beres et al., 2018; Wang et al., 2014). In addition, other authors show the need for further investigations (Vila-Aiub et al., 2009 and Vila-Aiub et al., 2019).

In this context, there are strong indications that the EPSPS enzyme, which confers glyphosate tolerance, also interferes with auxin metabolism in the plants (Fang et al., 2018). Auxin plays a role in multiple metabolic pathways in plants. Changes in the auxin content can result in changes in plant composition which can raise concerns about safety.

The EPSPS enzymes occur in the stacked Maize in much higher concentrations compared to the parental plants. Therefore, it should be taken into account that unintended effects caused by high concentrations of EPSPS enzymes in combination with higher rates of spraying, may also affect gene expression and plant composition. If the Maize is exposed to higher rates of glyphosate

³⁹www.greenbook.net/monsanto-company/roundup-weathermax; www.greenbook.net/monsanto-company/roundup-ultra

application, this may cause stress conditions, which can in turn impact gene expression and plant composition as well as the biological characteristics of the Maize.

EFSA should have requested the applicant to submit data from field trials; at a minimum, data on complementary herbicide application rates as recommended by industry, including repeated applications. However, no such data were provided or requested. Furthermore, no such data are available for the parental plants or any subcombinations.

Consequently, the Maize plants tested in the field trials do not sufficiently represent the imported Maize. The data presented by the applicant are insufficient to conclude on the impact of the herbicide applications on gene expression, plant composition and the agronomic and phenotypic characteristics of the plant as requested by the EU Regulation 503/2013.

2.2.3 Data on genetic backgrounds and their impact on plant composition and agronomic and phenotypic characteristics

It is very well known that the genomic background of the variety can influence the expression of the inserted genes and / or the concentration of the additional proteins present in the GE plants (see Trtikova et al., 2015). For example, Lohn et al. (2020), in a multigenerational study with Bt maize, show that the Bt content was generally lower in offspring if the maize was crossed with Brazilian varieties, but much higher than expected in offspring from South African plants.

Therefore, EFSA should have requested additional data from several varieties, including those cultivated in South America, to examine how the gene constructs interact with the genetic background of the plants. This approach is supported by the analysis of gene expression given above, but was ignored in the EFSA risk assessment. However, no such data were provided or requested. Furthermore, no such data are available for the parental plants or any subcombinations.

In conclusion, the Maize plants tested in field trials do not represent the imported Maize. The data presented by the applicant are insufficient to conclude on the impact of genetic background on gene expression, plant composition and the agronomic and phenotypic characteristics. However, reliable data are needed to demonstrate safety as requested by EU Regulation.

2.2.4 Data from compositional analysis show the need for further investigation

Only data from a low number of agronomic parameters (13) were subjected to statistical analysis in accordance with EFSA guidance; of these, 6 (no spraying) and 8 (spraying of the complementary herbicide) were found to be statistically and significantly different.

Compositional analysis of 54 endpoints in the grains revealed many (and partly major) statistically significant differences: 39 endpoints were statistically significantly different in plants sprayed with the complementary herbicides, 47 in plants not sprayed with glyphosate (but other conventional herbicides).

Even if changes taken as isolated data might not directly raise safety concerns, the overall number of significant effects has to be taken as a starting point for much more detailed investigations: more than half of the parameters measured for agronomic characteristics as well as for plant composition were significantly different.

Looking at these data, EFSA should have concluded that the plants are likely to be substantially different compared to their conventional counterparts. Therefore, more detailed analysis would have been necessary to investigate changes in the plant composition and phenotype caused by the stacking, including investigation into potential unintended changes in metabolic pathways and the emergence of unintended biologically active gene products.

Furthermore, the data presented did not take into account cultivation of the stacked maize under more extreme drought conditions, even though these are the most relevant environmental conditions in which the plants will be cultivated. Under such environmental conditions, the range of differences and their significance are likely to be substantially increased.

As explained above, EFSA should have requested further tests, with repeated herbicide applications using higher dosages and with exposure to a much wider range of environmental conditions, taking more extreme drought conditions into account. Furthermore, the plant material should have been assessed in regard to changes in plant composition or agronomic and phenotypic characteristics in much more detail.

However, instead of assessing the overall pattern of changes in plant components, their causes and possible impacts in more detail, EFSA only assessed the observed changes in isolation for evidence of potential harm. This approach turns the comparative approach into a trivial concept of assessing bits and pieces, and it ignores questions on the overall safety of the whole food and feed. A more in-depth investigation of unintended changes is not only necessary if there are findings indicating adverse effects, it is always needed to come to sufficiently robust conclusions to inform the next steps in risk assessment.

Previous research indicates that expression of Cry1A.105, Cry2Ab2 and EPSPS proteins in genetically engineered maize can induce changes in the overall proteome of the respective GM maize line, with impacts on associated endogenous metabolic pathways. This research should have been taken into account. Several publications also report unintended changes in the genome, the transcriptome, the proteome or the metabolome of transgenic maize (see Agapito-Tenfen et al., 2013; Agapito-Tenfen et al., 2014; Benevenuto et al., 2017; Mesnage et al., 2016; Ben Ali et al., 2020).

Several of these transgenes are also present in the stacked Maize. In addition, four other proteins are expressed that are absent in conventional maize: NPTII which confers antibiotic resistance - and should have been avoided as requested by EU Directive 2001/18 - PMI which was used for selecting process during plant production, Vip3Aa20 (insecticidal toxicity) and CSPB (for drought tolerance). These traits and genetic elements can synergise and interact. These effects are also likely to impact plant composition and biological characteristics crucial to the assessment of food and feed safety. However, no specific data needed for detailed comparison to assess the genome x environment interactions were presented by the applicant.

At a minimum, the unintended effects of the EPSPS enzymes will very plausibly interfere with the activity of the other gene constructs, for example, via the auxin hormone (see above). This is especially relevant if the plants are exposed to stress conditions. Even if no such effects were observed in the parental plants (which, to our knowledge, were not tested in field trials under stress conditions), these enzymes are now produced in the stacked Maize at higher concentrations. Therefore, the likelihood of interaction between the gene constructs and gene expression, plant

composition as well as agronomic and phenotypic characteristics is higher in the stacked Maize compared to the parental plants.

Thus, robust data should have been presented to assess whether metabolic changes with relevance to food and feed safety occur in the stacked maize. EFSA should have requested a much more detailed investigation into potential biologically active gene products and changes in metabolic pathways under more extreme climate conditions.

However, these issues have not yet been taken into account in EFSA risk assessment. Neither do they use more sensitive methods, such as transcriptomics, proteomics and metabolomics to explore and assesses unintended changes in the stacked Maize.

Based on the available data, no final conclusions can be drawn on the safety of the plants. The data do not fulfill the requirements of Implementing Regulation 503/2013.

2.2.5 Conclusion on comparative assessment of plant composition and phenotypic and agronomic characteristics

The stacked Maize has a trait enabling cultivation of the plants under more extreme climate conditions. The trait is combined with gene constructs (the EPSPS enzyme and the production of Bt toxins) which are likely to cause or to show major changes in gene expression if exposed to environmental stressors, such as drought. In addition, three other proteins are expressed in the stacked Maize which are absent in conventional maize: NPTII which confers antibiotic resistance - which should have been avoided as requested by EU Directive 2001/18 - PMI which was used in the selecting process during plant production and Vip3Aa20 (insecticidal toxicity). These traits and genetic elements can synergise and interact with each other. These effects are also likely to impact plant composition phenotype, especially if exposed to environmental stress conditions.

However, the data provided by the applicant and accepted by EFSA are insufficient to conclude on the impact of the combination of traits and gene constructs, of environmental factors, of herbicide applications and the genetic background on gene expression, plant metabolism, plant composition and the agronomic and phenotypic characteristics. Since the field trials did not represent the conditions under which the plants are to be grown in practice, the data from GE plants tested in field trials do not sufficiently represent the imported GE plants. Consequently, these data do not fulfill the requirements of Implementing Regulation 503/2013.

In summary, the Maize plants tested in field trials do not sufficiently represent the Maize as imported. Based on the available data, no final conclusions can be drawn on the safety of the plants. Consequently, the data do not fulfill the requirements of Implementing Regulation 503/2013 and Regulation 1829/2003.

2.3 Toxicity

Implementing Regulation 503/2013 requests:

“Toxicological assessment shall be performed in order to:

(a) demonstrate that the intended effect(s) of the genetic modification has no adverse effects on human and animal health;

(b) demonstrate that unintended effect(s) of the genetic modification(s) identified or assumed to have occurred based on the preceding comparative molecular, compositional or phenotypic analyses, have no adverse effects on human and animal health;”

“In accordance with the requirements of Articles 4 and 16 of Regulation (EC) No 1829/2003, the applicant shall ensure that the final risk characterisation clearly demonstrates that:

(a) the genetically modified food and feed has no adverse effects on human and animal health;”

2.3.1 Findings from molecular characterisation and comparative approach

As explained above, many significant changes were identified: more than half of the parameters measured for agronomic characteristics and plant composition were significantly different. Even if the changes taken as isolated data might not directly raise safety concerns, the overall number of effects should have been considered as a starting point for much more detailed investigation into their potential health impacts.

However, the data presented by the applicant did not take into account cultivation of the stacked maize under more extreme drought conditions, i.e. neither under realistic agricultural conditions nor considering all relevant countries of cultivation. Drought would have been especially relevant since this the Maize was developed to be grown in such conditions. The range of differences and their significance are likely to be substantially increased in these conditions. Thus, without more data, the true range of unintended effects cannot be determined and safety cannot be demonstrated as requested by EU regulation.

It is also shown that no reliable conclusions can be drawn on the content of insecticidal proteins and the other intended new proteins from the available data. For example, the overall concentration of the three insecticidal proteins is relevant for the assessment of overall toxicology as well as for the immune system. Thus, without more data, the true impact of intended effects on health at the stage of consumption cannot be determined and safety cannot be demonstrated as requested by EU regulation.

Despite these findings, and in awareness of the lack of more specific data and the resulting major uncertainties, no testing of the whole stacked plant (feeding study) was requested.

2.3.2 Toxicity of the Bt toxins

In regard to toxicology and potential synergistic or other combinatorial effects, the negative impacts of Bt toxins on human and animal health cannot be excluded a priori. Bt toxins have several modes of action. They are produced in the plants but their biological characteristics are altered and not identical to their natural templates (Hilbeck & Otto, 2015).

Several publications describe the effects of Bt toxins in mammals: some Cry toxins are known to bind to epithelial cells in the intestines of mice (Vázquez-Padrón et al., 1999, Vázquez-Padrón et al., 2000). As far as potential effects on health are concerned, Thomas and Ellar (1983), Shimada et al. (2003) Huffmann et al. (2004), Ito et al. (2004), Mesnage et al. (2013) and Bondzio et al. (2013) show that Cry proteins could potentially have an impact on the health of mammals. Further publications (de Souza Freire et al., 2014; Mezzomo et al., 2014) confirm hematotoxicity of several Cry toxins, including those being used in genetically engineered plants, such as Cry 1Ab and Cry1Ac. These effects seem to occur after high concentrations and tend to become stronger after several days. Such observations call for the study of effects after long-term exposure to various dosages, including in combination with material sprayed with the complementary herbicides. In this context, it is important to consider that the stacked maize is also resistant to the herbicide glyphosate, and the resulting residues should be seen as potential co-stressors at the stage of consumption (see also Then & Bauer-Panskus, 2017).

It has to be considered that the concentration of the insecticidal proteins is much higher in gluten meal produced from the maize, it can reach much higher concentrations than in the kernels. Therefore, the food and feed products derived from the stacked Maize need to be much more carefully risk assessed in regard to their toxicity compared to genetically engineered plants producing just one Bt toxin.

Relevant findings show that the selectivity and efficacy of Bt toxins produced in GE plants can be influenced by many co-factors (see, for example, Then, 2010; Hilbeck & Otto, 2015). Higher toxicity can also cause lower selectivity (Then, 2010): if synergistic or additive effects occur that increase efficacy of the Bt toxin, its selectivity may be decreased and a wider range of non-target organisms may become susceptible.

One crucial impact factor in this context are protease inhibitors (PI), which show synergistic effects with Bt toxins, strongly enhancing their toxicity. It is likely that PI delay the degradation of Bt proteins and thereby also enhance their toxicity. In many of its comments on EFSA opinions, Testbiotech has highlighted these effects by referring, for example, to Pardo-López et al. (2009). However, EFSA has never provided a detailed response.

Testbiotech is aware of several publications confirming this gap in risk assessment that EFSA has constantly ignored or denied: as Monsanto already showed in the 1990s, maize, cotton and soybeans produce protease inhibitors (PI), which considerably enhance the toxicity of Bt proteins in plants. In the presence of PIs, Bt toxin will degrade much more slowly than in isolation. This results in a much higher toxicity of the Bt toxin (if it is taken up together with the plant tissue) compared to the isolated toxin (MacIntosh et al., 1990; Zhao et al., 1999; Zhang et al., 2000; Gujar et al., 2004; Zhu et al., 2007; Pardo-López et al., 2009; Ma et al., 2013; Mesén-Porras et al., 2020). The effects described indicate, for example, a 20-fold higher toxicity of Bt proteins if produced in the plants and taken up with PIs (MacIntosh et al., 1990). Differences in toxicity between toxins produced in isolation compared to those produced by the plants are also described for Vip3A efficacy in transgenic plants (Khan et al., 2020).

It also should be taken into account that the toxicity of Bt toxins can not only be enhanced through interaction with plant enzymes such as PI, but also by Bt toxins (Sharma et al., 2004; Sharma et al., 2010; Tabashnik et al., 2013; Bøhn et al. 2016; Bøhn, 2018), gut bacteria (Broderick et al., 2009), residues from spraying with herbicides (Bøhn et al. 2016; Bøhn, 2018) and other co-stressors (Kramarz et al., 2007; Kramarz et al., 2009; Khalique and Ahmed, 2005; Singh et al., 2007; Zhu et al., 2005; Mason et al., 2011; Reardon et al., 2004).

Therefore, any risk assessment that does not take synergistic effects caused by the combination of plant material or other stressors with the Bt toxin into account is not reliable and systematically underestimates the risks (see also Testbiotech, 2021).

These issues are especially relevant for the stacked events since the overall concentration of Bt toxins is higher and combinatorial effects with other stressors (such as residues from spraying) more likely.

In summary, the evidence for enhanced toxicity of Bt proteins produced in maize, cotton and soybeans was published by Monsanto 30 years ago (MacIntosh et al., 1990) and has since then been confirmed in multiple studies. Crucially, EFSA has never assessed this aspect in any of its opinions.

Instead, the toxicity of the Bt toxins was assessed on the basis of feeding studies, using only isolated Bt proteins produced by bacteria for gavage experiments in mice (Monsanto 2016d). The data from these experiments were then used to calculate NOAEL (No-Observed-Adverse-Effect Level) and to assess the impact of exposure at the stage of consumption. Therefore, considering the above findings, the basic data for toxicity assessment of the stacked Maize are neither valid nor reliable. In addition, incorrect assumptions were made on the degradation of the Bt toxins at the stage of consumption and similarity to known toxins (see below). Therefore, the Monsanto risk assessment depends entirely on incorrect assumptions in regard to toxicity and exposure.

2.3.3 Immunogenicity of the Bt toxins

There are several studies indicating that immune responses in mammals can be triggered by Bt toxins and have to be considered in this context. Studies with the Cry1Ac toxin (Moreno-Fierros et al., 2000; Vázquez-Padrón et al. 1999; Legorreta-Herrera et al., 2010; Jarillo-Luna et al. 2008; E. González-González et al., 2015; Ibarra-Moreno et al., 2014; Guerrero et al. 2007; Guerrero et al., 2004; Moreno-Fierros et al. 2013; Rubio-Infante et al. 2018) are especially relevant (for review also see Rubio-Infante et al. 2016). Since Cry1Ac is also used as an adjuvant in vaccines, the risks to food consumption can be promoted through synergistic effects, this needs to be addressed and carefully examined.

The synergistic effects described by MacIntosh et al. (1990), Zhao et al. (1999), Zhang et al. (2000) Gujar et al. (2004), Zhu et al. (2007), Pardo-López et al. (2009), Ma et al. (2013), Mesén-Porras et al. (2020) causing higher toxicity of the Bt toxins are also relevant in risk assessment in regard to the immune system: combination with protease inhibitors is likely to be associated with a delay in the degradation of the Bt toxins after consumption. This delay in degradation extends the exposure of the intestinal immune system to Bt toxins and may trigger or enhance chronic inflammation and other immune responses (see also Testbiotech, 2021). Certainly, a study testing corn with a combination of Bt toxins (Cry1Ab and Cry34Ab1) indicates inflammation in rats (Zdziarski et al., 2018).

In this context, it is relevant that Bt toxins produced by plants can survive digestion to a much higher degree than has been assumed by EFSA and shown by the data of the applicant. Chowdhury et al. (2003) and Walsh et al. (2011) showed that when pigs were fed with Bt maize, Cry1A proteins could frequently and successfully still be found in the colon of pigs at the end of the digestion process. This means that Bt toxins are not degraded quickly in the gut and can persist in larger

amounts until digestion is completed; therefore, there is enough time for interaction between various food compounds.

It has to be considered that the concentration of the insecticidal proteins is much higher in gluten meal produced from the maize, and that it can reach a much higher concentrations compared to the kernels.

These issues are especially relevant for the stacked events since the overall concentration of Bt toxins is higher compared to the parental plants.

Not only is the concentration of Bt toxins higher in the stacked Maize, there is also a higher likelihood of combinatorial effects with other stressors (such as residues from spraying). However, neither EFSA nor the applicant considered the potential enhancement of toxic or immunogenic effects caused by interaction with plant components such as PI. In this context, potential impacts on the microbiome also have to be taken into account (see below).

2.3.4 Effects of complementary herbicide residues specific to GE plants and their mixed toxicity

The residues from spraying with complementary herbicides were considered to be outside the remit of the GMO panel. However, without detailed assessment of these residues, no conclusion can be drawn on the safety of the imported products: due to specific agricultural practices in the cultivation of these herbicide-resistant plants, there are, for example, specific patterns of spraying, exposure, occurrence of specific metabolites and emergence of combinatorial effects that require special attention (see also Kleter et al., 2011).

More detailed assessment is also in accordance with pesticide regulation that requires specific risk assessment of imported plants if the usage of pesticides is different in the exporting countries compared to the usage in the EU. In this regard, it should be taken into account that EFSA (2015b; 2015c and 2018b) explicitly stated that no conclusions can be drawn on the safety of residues from spraying with glyphosate in genetically engineered plants resistant to this herbicide.

Further, there is a common understanding that commercially traded formulations of glyphosate, such as Roundup, can be more toxic than glyphosate itself. Therefore, the EU has already taken measures to remove problematic additives known as POE tallowamine from the market.

Problematic additives are still allowed in those countries where the genetically engineered plants are cultivated. The EU Commission has confirmed the respective gaps in risk assessment:

“A significant amount of food and feed is imported into the EU from third countries. This includes food and feed produced from glyphosate-tolerant crops. Uses of glyphosate-based plant protection products in third countries are evaluated by the competent authorities in those countries against the locally prevailing regulatory framework, but not against the criteria of Regulation (EC) No. 1107/2009. (...).”⁴⁰

The stacked Maize combines several EPSPS enzymes conferring enhanced resistance to glyphosate; therefore, a higher burden of the residues from spraying might be expected compared to the parental plants. Consequently, EFSA should have requested the company to submit data from field trials with the highest dosage of the complementary herbicides that can be expected under current

⁴⁰www.testbiotech.org/content/eu-commission-request-consider-impact-glyphosate-residues-feed-animal-health-february-2016

agricultural practises, including repeated spraying. The material derived from those plants should have been assessed in regard to organ toxicity, immune system responses and reproductive toxicity, also taking combinatorial effects with other plant components into account.

Whatever the case, both the EU pesticide regulation and the GMO regulation require a high level of protection for health and the environment. Thus, in regard to herbicide-resistant plants, specific assessment of residues from spraying with complementary herbicides must be considered to be a prerequisite for granting authorisation.

EU legal provisions such as Regulation 1829/2003 (as well as Implementing Regulation 503/2013) state that “*any risks which they present for human and animal health and, as the case may be, for the environment*” have to be avoided. Therefore, potential adverse effects that result from combinatorial exposure of various potential stressors need to be tested in regard to mixed toxicity (EFSA 2019e).

Glyphosate is particularly problematic in regard to chronic exposure when it is consumed in food. This is because glyphosate is known to cause shifts in the microbial composition and associated microbiomes of plants and animals: glyphosate (Roundup) has been shown to cause shifts not only in soil organisms (van Bruggen et al., 2018) but also in the composition of the intestinal flora in cattle (Reuter et al., 2007), poultry (Shehata et al., 2013; Ruuskanen et al., 2020) and rodents (Mao et al., 2018; Mesnage et al., 2019; Mesnage et al., 2021; Tang et al., 2020) as well as honey bees (Motta et al., 2020) and Daphnia (Suppa et al., 2020). Therefore, antibiotic effects caused by chronic exposure to food and feed derived from glyphosate-resistant GE plants, including the stacked Maize, is not unlikely to trigger significant changes in intestinal bacteria (see also Testbiotech, 2021).

In general, the microbiome can be seen as a common network of life, circumventing and closely interacting with plants, animals and humans. Microbial networks are thought to have co-evolved with their hosts and have developed a mutualistic relationship that benefit both the host and microorganisms. They act at the interphase and communicate between an organism and its wider environment while at the same time being part of the closer environment of an organism. Microbiomes are considered key for the health of higher organisms, i.e. humans, animal and plants.

Just recently, a document published by EFSA (EFSA, 2020), called attention to the role of the microbiome in environmental risk assessment and food and feed safety. In regard to food and feed safety, EFSA (2020d) considers microbiomes to be highly relevant to the health status of their hosts. Therefore, it is desirable to understand the importance of their role in risk assessment. EFSA expects that gut microbiome research (not only in the case of GE plants) will play a relevant role in regulatory science with potential implications for future risk assessments and predictive risk models. As EFSA states: “*considering that the gut microbiome is a biological component directly and indirectly involved in the metabolism of food/feed components and chemicals and in the protection of the host against adverse environmental exposure, it would be useful to establish criteria on how to evaluate the potential adverse impacts of perturbators on this defensive barrier, and consequently, on human/animal health.*”

In 2019, EFSA published a study on the adjuvanticity / immunogenicity assessment of proteins, including the role of the microbiome. Parenti et al. (2019) state that “*one of the most important drivers of immune response is the gut microbiota and other microbial constituent of the human body which are able to regulate host-pathogen balance and to produce systemic pro-inflammatory stimuli. The lifelong antigenic load represented by foods and bacteria/bacterial products leads to a*

profound remodeling of the gut microbiota and these changes are emerging as a driving force of the functional homeostasis of the immune system. As a matter of fact, a perturbation of the gut microbiota homeostasis due to irregular lifestyles, stress and age may lead to gut microbiota dysbiosis. This condition may predispose the host to metabolic disorders and inflammation.”

This finding is highly relevant for the risk assessment of the stacked Maize. Several EPSPS enzymes are combined in the stacked Maize for enhanced resistance to glyphosate, therefore a higher burden of residues from spraying can be expected compared to the parental plants. These residues may cause a perturbation of the gut microbiome. At the same time, stacking in the Maize results in a combination of several Bt toxins that are likely to show immunogenicity. It is plausible that this combination of Bt toxins, together with residues from spraying, can trigger effects on the immune system either directly or via the microbiome. This hypothesis needs to be tested before any conclusion can be drawn on the health safety of food and feed.

However, no attempts have been made to integrate the microbiome into the risk assessment of food and feed derived from the stacked Maize. This is contrary to Regulation 1829/2003 which requests “*genetically modified food and feed should only be authorised for placing on the Community market after a scientific evaluation of the highest possible standard, to be undertaken under the responsibility of the European Food Safety Authority (Authority), of any risks which they present for human and animal health and, as the case may be, for the environment.*” (Recital 9).

2.3.5 Conclusions on toxicity

Despite all these open questions regarding potential health impacts, we are not aware of a single sub-chronic or chronic feeding study performed with whole food and feed derived from the stacked Maize. This observation is supported by the literature review carried out by the company. In this context, it is relevant to consider that the outcome of the feeding studies with the parental plants raised several questions concerning their results, methodology and reliability (see comments from the experts of Member States, EFSA, 2019d)

Testbiotech is also aware that feeding studies with similarly stacked maize events indicated potential health impacts, such as inflammatory responses in the stomach (Zdziarski et al., 2018). Inflammatory responses are an alarm signal typical of many chronic diseases and therefore require close attention. While the applicant provided some data in regard to celiac disease, other diseases associated with symptoms of chronic inflammation were not considered at all.

EFSA should have requested the applicant to submit data from field trials which represent the environmental and agricultural conditions under which the Maize will be grown. Drought and high rates of complementary herbicide applications should have been taken into account. The material derived from the plants should have been assessed, especially in regard to long-term organ toxicity, immune responses and impact on the gut microbiome, also taking combinatorial effects and mixed toxicity into account.

Instead, the applicant referred to the so-called ‘weight of evidence’ (Monsanto, 2016a) to justify why safety should be assumed, rejecting the need for empirical testing of whole food and feed. In doing so, the applicant combines 6 elements which are not sufficient to demonstrate safety (for additional references see above):

1) History of safe use of the CP4 EPSPS, CspB, NptII, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI proteins and their source organism

This claim is not based on science. At the very least, the Cry and VIP toxins produced in the plants do not occur in nature and history of safe use cannot be claimed.

2) Lack of structural or functional relationship of CP4 EPSPS, CspB, NptII, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI to proteins that adversely affect human or animal health

This claim should be treated with some caution since the Cry toxins share some similarity with several other bacterial protein toxins, including colicin A and diphtheria toxin (Schnepf et al. 1998). Furthermore, the mode of action of Cry toxins shows there is more than one way they can exert toxicity. Changes in the structure of the toxins as well as synergistic effects with plant constituents (PI) or other stressors (residues from spraying) can lower selectivity and enhance toxicity. Finally, some Bt toxins are known to trigger immune responses, therefore, it is not unlikely that they will impact the immune system causing non-IGE responses, such as the increased possibility of inflammation. These effects might be exerted via the microbiome. Such risks are especially relevant for stacked events since the overall concentration of Bt toxins is higher and combinatorial effects with other stressors (such as residues from spraying) are more likely.

3) Negligible human exposure to CP4 EPSPS, CspB, NptII, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI proteins from maize consumption

Since the data on gene expression are inconclusive, this claim lacks the necessary evidence. Long-term consumption may cause a permanent pattern of exposure to some of the plant components or their mixtures, which is unique to the human or animal body and may cause changes in health (due to direct effects or effects triggered via the microbiome) that escape the tests (such as a 90-day feeding study with the parental plants). These risks are especially relevant for stacked events since the overall concentration of Bt toxins is higher and combinatorial effects with other stressors (such as residues from spraying) are more likely.

4) Quick digestibility of CP4 EPSPS, CspB, NptII, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI and 5) its deactivation upon heat treatment

These claims ignore evidence that degradation and deactivation of the Bt toxins produced in the plants and consumed with the plant constituents cannot be seen as being equivalent to the toxins tested in isolation. Further, heat treatment is (by far) not always necessary when maize products are used in food and feed.

These problems are especially relevant for stacked events since the overall concentration of Bt toxins is higher compared to the parental plants and combinatorial effects additionally affecting the degradation of the toxins.

6) Lack of testable hypothesis on potential health impacts

This so-called ‘weight of evidence’ is used by the applicant to deny any testable hypothesis which could be subjected to further investigations. This claim has to be rejected. Testable hypotheses are for example: (i) higher toxicity of Bt toxins if combined with residues from spraying; (ii) higher toxicity and immunogenicity of Bt toxins if combined with protease inhibitors; (iii) changes in the intestinal flora after long-term consumption of food and feed derived from the stacked Maize.

Testbiotech is also aware that feeding studies with similar stacked maize varieties indicated potential health impacts, such as inflammation in the stomach (Zdziarski et al., 2018). Inflammatory

responses are an alarm signal typical of many chronic diseases which therefore require close attention.

In conclusion, the EFSA opinion (EFSA, 2019a) cannot be said to fulfill the requirements for assessment of potential synergistic or antagonistic effects resulting from the combination of the transformation events in the stacked Maize. Safety in regard to potential toxicity / adverse health effects caused by consumption of food and feed derived from the stacked Maize was not demonstrated. Therefore, the requirements of EU Regulation (1829/2003 and Implementing Regulation 503/2013) are not fulfilled.

2.4. Allergenicity

Implementing Regulation 503/2013 requests:

“In cases when known functional aspects of the newly expressed protein or structural similarity to known strong adjuvants may indicate possible adjuvant activity, the applicant shall assess the possible role of these proteins as adjuvants. As for allergens, interactions with other constituents of the food matrix and/or processing may alter the structure and bioavailability of an adjuvant and thus modify its biological activity.”

“In accordance with the requirements of Articles 4 and 16 of Regulation (EC) No 1829/2003, the applicant shall ensure that the final risk characterisation clearly demonstrates that:

(a) the genetically modified food and feed has no adverse effects on human and animal health;”

2.4.1 Potential allergenicity

EFSA does not mention that Cry1Ac is thought to be allergenic (Santos-Vigil et al., 2018)⁴¹. According to Santos-Vigil et al. (2018), the Bt toxin Cry1Ac can act as an allergen if ingested. This publication is relevant: the Bt toxin Cry1Ac was used as a source for the synthesis of Cry1A.105 expressed in the stacked Maize. Therefore, the synthetically derived Cry1A.105 toxin produced in the Maize has structural similarity with Cry1Ac. If Cry1Ac is thought to be an allergen, the source of Cry1A.105 has to be verified as allergenic and therefore investigated in detail.

The EU Commission also noted that the Santos-Vigil et al. (2018) publication was relevant for the risk assessment of genetically engineered plants producing Bt toxins, and therefore requested the European Food Safety Authority (EFSA) for an assessment. In response, EFSA (EFSA, 2018c) came to the conclusion that the Santos-Vigil et al. (2018) publication does not provide any new information and suffers from methodological deficiencies. However, this EFSA opinion is based on a rather biased interpretation of existing publications, and it does not provide any evidence that the Santos-Vigil (2018) findings are invalid or irrelevant (for more details see Moreno-Fierros et al., 2018)⁴². Consequently, the Santos-Vigil et al. (2018) publication has to be considered valid but not properly assessed by EFSA. Certainly, EFSA should have requested testing of the hypothesis that the consumption of products derived from the maize can trigger allergic reactions.

In conclusion, the EFSA assessment of the stacked maize cannot be said to fulfil the requirements for assessing allergenicity of the source of the transgene.

⁴¹see also: www.testbiotech.org/en/press-release/can-bt-toxins-cause-allergies

⁴²www.testbiotech.org/sites/default/files/Response%202018-EFSA_Supporting_Publications_Santos_Vigil_1.pdf

2.4.2 Potential adjuvanticity

The synergistic effects between PI and Bt toxins as described above are also relevant for risk assessment in regard to adjuvanticity: the combination with protease inhibitors is likely to be associated with a delay in the degradation of the Bt toxins after consumption. This delay in degradation extends the exposure of the intestinal immune system to Bt toxins and may trigger or enhance relevant effects.

There are several studies indicating that immune responses, such as adjuvanticity in mammals, are triggered by Bt toxins and have to be considered in this context. Studies with the Cry1Ac toxin (Moreno-Fierros et al., 2000; Vázquez-Padrón et al. 1999; Legorreta-Herrera et al., 2010; Jarillo-Luna et al., 2008; E. González-González et al., 2015; Ibarra-Moreno et al., 2014; Guerrero et al., 2007; Guerrero et al., 2004; Moreno-Fierros et al. 2013; Rubio-Infante et al., 2018) are especially relevant (for review also see Rubio-Infante et al., 2016). Since Cry1Ac is also used as an adjuvant in vaccines, the risks to food consumption can be promoted through synergistic effects, this needs to be addressed and carefully examined. The need for more detailed investigations in regard to potential immunogenic effects is also pointed out in the minority opinion in another EFSA opinion (Annex II of EFSA, 2018a).

In its answers to experts from Member States (EFSA, 2019d), EFSA admits only that “limited experimental evidence” is available to conclude on the safety of Bt toxins in regard to immune system responses. However, the need for more detailed testing is acknowledged:

“EFSA has previously highlighted that the testing of adjuvant and allergenic potential of proteins requires stronger and fit-for-purpose standardised study design, and that future studies should consider limitations of current models, using relevant routes and methods of administration, doses, appropriate control proteins, and realistic exposure regimes. These aspects will require a broader discussion with the involvement of the international scientific community and its stakeholders to define a consensus on a fit-for-purpose study design for this assessment.

Given the fact that potential effects of Bt toxins on the immune system have meanwhile been discussed for many years (for overview see, for example, Then & Bauer-Panskus, 2017), and already 45 GE crop events producing Bt toxins have been approved for the EU market, this explanatory excuse cannot be accepted. In accordance with EU Regulation 1829/2003, safety of whole food and feed has to be demonstrated before approval for import can be issued. Since this is not the case with the stacked Maize, the risk assessment is not conclusive and no market authorisation can be granted.

These issues are especially relevant for stacked events since the overall concentration of Bt toxins is higher and combinatorial effects with other stressors (such as residues from spraying) are more likely. This is also relevant for immune responses exerted via the microbiome (see above). However, neither EFSA nor the applicant considered the potential enhancement of toxic or immunogenic effects caused by interaction with plant components.

2.4.3 Conclusion on allergenicity and adjuvanticity

Considering these uncertainties, EFSA should have requested empirical testing of allergenic or adjuvant effects. Instead, the applicant refers to the so-called ‘weight of evidence’ (Monsanto, 2016a) as an explanation of why safety should be assumed without experimental testing. In doing so, the applicant combines four elements which are not sufficient to demonstrate safety (for references see above):

1. The CP4 EPSPS, CspB, NptII, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI proteins expressed in MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 are encoded by genes from organisms that are not a source of known allergens

This statement is misleading. Cry1A.105 is derived from Cry1Ac which is thought to be an allergen.

2. Bioinformatics analyses the additional proteins produced in the plants demonstrated that they do not share immunologically relevant amino acid sequence similarities with known allergens.

This statement is misleading for the same reason as above. Cry1A.105 is derived from Cry1Ac which is thought to be an allergen.

3. Digestive fate experiments conducted with those proteins demonstrate that they are rapidly digested in simulated digestive fluids

This claim is incorrect, it ignores evidence that degradation and deactivation of the Bt toxins produced in the plants and consumed with the plant constituents cannot be seen as equivalent to the toxins tested in isolation.

4. Also, no structural similarity has been seen between the newly expressed proteins and known strong adjuvants.

This statement is also misleading. Cry1A.105 is derived from Cry1Ac which is used as a ‘strong’ adjuvant in vaccines.

In conclusion, allergenicity and adjuvanticity were not assessed to sufficiently demonstrate that the food and feed from the stacked Maize has no adverse effects on human and animal health.

2.5 Environmental risk assessment

The appearance of teosinte in Spain and France (see Testbiotech, 2016; Trtikova et al., 2017) has to be considered in more detail. In its assessment of the volunteer potential, the information provided by Monsanto is largely outdated. As Pascher et al. (2016) show, the volunteer potential of maize is higher than assumed by Monsanto. Further, in awareness of the biological characteristics of maize MON87460 and the findings of Fang et al. (2018), the stacked maize needs to be examined in detail for next generation effects, volunteer potential (persistence) and gene flow. In doing so, the hypothesis that the maize and its offspring will show a higher fitness compared to conventional maize, is evident. This might also concern adaptation to a colder climate due to the biological characteristics of the cold shock protein (CSPB). Under these circumstances, even a rare single outcrossing event that goes unnoticed can have a huge long-term impact on agro-ecosystems.

Therefore, the conclusion of EFSA is simply wrong, stating that:

“Even if cross-pollination would occur, the GMO Panel is of the opinion that environmental effects as a consequence of the spread of genes from occasional feral GM maize plants in Europe will not differ from that of conventional maize varieties.”

2.5.1 Likelihood of gene flow

Without more data on the teosinte species growing in the EU, the likelihood of gene flow from the maize to teosinte cannot be assessed (Trtikova et al., 2017). The same is true for gene flow from teosinte to genetically engineered plants.

There are new publications confirming that the risks from crossings of GE maize and teosinte cannot be predicted from the data assessed by EFSA (2019a): as already shown by Trtikova et al. (2017), another publication, Le Corre et al. (2020), confirms that European teosinte plants from Spain and France have, in fact, integrated larger genomic parts from European maize varieties.

As Le Corre et al. (2020) show, teosinte has changed its biological characteristics in ways that will facilitate further genetic exchange with maize plants. Similarly, Diaz et al. (2019) show that these new weeds seem to have a complex origin. Therefore, the likelihood of hybridisation with the GE maize has strongly increased. For example, teosinte has now altered its flowering time. Furthermore, teosinte has already acquired herbicide-resistance from conventional European maize varieties. In awareness of their findings, the authors emphasise that their results show that risks of crop-wild introgression should not be underestimated in forecasting the risk of invasiveness. They show that crop-wild introgression can be a two-way street, enhancing gene flow to both partners, maize and teosinte. The scientists have therefore explicitly warned that the risk of the plants becoming invasive should not be underestimated (see also Testbiotech, 2021).

2.5.2 Enhanced fitness

If the known characteristics of the stacked Maize were to be transferred to teosinte, this would render them herbicide-resistant, toxic for pest insects and drought tolerant.

In addition, a higher overall fitness of hybrid offspring can result from the additional EPSPS enzymes. Fang et al. (2018) showed that higher fitness does occur in GE glyphosate-resistant *Arabidopsis thaliana* in a glyphosate-free environment as an unintended effect. According to this research, the expressed enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) not only makes the plants resistant to glyphosate, it also interferes with metabolic processes associated with their growth and fecundity. As a consequence, plant offspring can produce more seeds and be more resistant (tolerant) to environmental stressors such as drought and heat. The authors stated that the observed effects are likely to be caused by increased production of the hormone auxin in the transgenic plants. This plant hormone plays a key role in growth, fecundity and adaptation to environmental stressors. The general findings regarding enhanced fitness of plants inheriting additional EPSPS genes are supported in several other publications (Beres, 2019; Beres et al., 2018; Wang et al., 2014; Yang et al., 2017). In addition, other authors show the need for further investigations (Vila-Aiub et al., 2009 and Vila-Aiub et al., 2019). Thus, if gene flow occurs, this effect, which was completely ignored by EFSA and the applicant, can strongly enhance the spread of teosinte in the fields and possibly beyond the fields.

2.5.3 Next generation effects

Unlike maize, teosinte can overwinter in the fields and pass new genetic information to offspring - from where it has the potential to spread and become a new European super-weed. These risks are not only a concern for farmers, they could also seriously damage the environment and protected species.

It is well known that there can be next generation effects and interference from genetic background that cannot be predicted from the assessment of the original event (Kawata et al., 2009; Cao et al., 2009; Yang et al., 2017; Bollinedi et al., 2017; Lu and Yang, 2009; Vacher et al., 2004; Adamczyk & Meredith, 2004; Adamczyk et al., 2009; for overview see Bauer-Panskus et al., 2020). This issue is relevant for gene flow from maize to teosinte as well from teosinte to maize.

Therefore, the characteristics of potential hybrids and next generations have to be investigated and cannot be predicted simply from the data of the original event, as suggested by EFSA.

2.5.4 Conclusion on environmental risk assessment

Without detailed consideration and examination of the hazards associated with the potential gene flow from maize to teosinte and from teosinte to maize, no conclusion can be drawn on the environmental risks of spillage from the stacked maize.

This is especially relevant for the stacked Maize which combines several traits which are likely to result in higher fitness of hybrid offspring.

EFSA should have requested data from the applicant to show that no adverse effects can occur through gene flow from the maize to teosinte and / or from teosinte to the maize volunteers. In the absence of such data, the risk assessment and the authorisation have to be regarded as not valid.

The risk assessment as performed does not fulfill EU regulations, which require the examination of the direct and indirect as well as the immediate and delayed effects of the GMO on human health and the environment.

2.6. Conclusions

The EFSA risk assessment cannot be accepted. Given the findings, there are multiple violations of EU Regulations requesting that the safety of the GE plants is demonstrated:

1. The plants were not exposed to bioclimatic conditions that sufficiently represent the regions in which these plants will be cultivated. Consequently, expression data and the assessment of plant composition as well as agronomic and phenotypic characteristics are not sufficiently reliable to inform the next steps in risk assessment. This is especially relevant since (i) the plants are intended for cultivation in drought conditions, (ii) the EPSPS enzymes are known to show unintended effects under stress conditions (such as drought) and (iii) the Bt content is known to be influenced by environmental factors (such as drought).
2. The plants were not exposed to agricultural practices which sufficiently represent the conditions under which these plants will be cultivated. Consequently, expression data and the assessment of plant composition as well as agronomic and phenotypic characteristics are not sufficiently reliable to inform the next steps in risk assessment.

3. No more detailed examinations were requested on gene expression, plant composition, agronomic and phenotypic characteristics despite data from other events and previous applications indicating that environmental stress factors, herbicide applications rates, genetic backgrounds and stacking are likely to impact gene expression and plant metabolism in the stacked Maize.
4. Risks which indicate toxicological health impacts, potentially enhanced by combinatorial effects caused by the stacking, were not assessed against relevant, reasoned and plausible hypotheses. For example, it was not examined to which extent the toxicity of the selectivity of the Bt toxins are changed by the mixed toxicity of whole food and feed. This is especially relevant in this case since it is known that enzymes (protease inhibitors) produced in the plants can multiply the toxicity of the Bt toxins and prolong exposure to the toxins in the gut after ingestion.
5. Risks which indicate immunological health impacts, potentially enhanced by the combinatorial effects of stacking, were not assessed against relevant, reasoned and plausible hypotheses. For example, it was not examined to which extent changes in the microbiome caused by the consumption of the Maize will impact its immunogenic properties. This is especially relevant since it is known that enzymes (protease inhibitors) produced in the plants can prolong exposure to the toxins in the gut after ingestion. Furthermore, residues from spraying with glyphosate (Roundup) are known to impact the composition of the microbiome.
6. The environmental risk assessment and the biological characteristics of the hybrid offspring of teosinte and the Maize were not assessed against relevant, reasoned and plausible hypotheses. This is especially relevant since the offspring may show invasive characteristics due to the unintended effects caused by the EPSPS enzymes, higher resistance to damage by insects and potentially higher tolerance to drought conditions.

3. The EU Commission decision was not in accordance with the EU regulations.

3.1. The above reasons and points raised in Chapter 2 show that the decision of the EU Commission was not in accordance with EU Regulations and must therefore be revised.

The Commission should not have accepted the opinion of EFSA (2019a) and the Member states should not have voted on it. The flaws described above cannot be corrected at this stage. Instead, authorisation must be revoked and the process of risk assessment re-started.

3.2 The EU Commission should have requested method(s) for post market monitoring specific to the Maize ('event-specific') which only is functional with the Maize, not being functional if applied to other transformation events already authorised.

The EU Commission is responsible for the post-market monitoring plan. In general, methods for detection should be event-specific to allow effective monitoring based on identification at all stages of import and processing. However, based on the methods provided, under practical conditions it is not possible to identify the Maize and distinguish it from other already authorised stacked or single

events that inherit the same gene constructs and can be mixed in the diets. Therefore, the monitoring plan provided by the applicant cannot be accepted.

Post-market monitoring must be established in a way that allows the collection of reliable information on the detection of indications showing whether any (adverse) effects on health and the / or the environment may be related to the Maize. Thus, the monitoring report should at the very least contain detailed information on:

- i) actual volumes of the Maize imported into the EU,
- ii) the ports and silos where shipments of the Maize are unloaded,
- iii) the processing plants where the Maize is transferred to,
- iv) the amount of the Maize used on farms for feed, and
- v) transport routes of the Maize.

Environmental monitoring should be carried out in regions where viable kernels of the Maize are transported, stored, packaged, processed or used for food/feed. In case of losses and spread of the Maize, all receiving environments need to be monitored.

Furthermore, environmental exposure through organic waste material, by-products, sewage or faeces containing the Maize during or after the production process, and during or after human or animal consumption should be part of the monitoring procedure.

Final conclusion

The Commission should not have accepted the EFSA (2019a) opinion and the Member States should not have voted on it. The flaws described above cannot be corrected at this stage.

Instead, authorisation has to be revoked and the process of risk assessment re-started.

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