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 genetically engineered stacked Soybean MON87751 x MON87701 x MON87708 x MON89788 and subcombinations

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Content

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 soybean plants (hereinafter referred to as the Soybean) 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 which sufficiently represent the regions in which they will be cultivated. Consequently, expression data and assessment of plant composition are not sufficiently reliable to inform the next steps in risk assessment. This is especially relevant in this case, since (i) the EPSPS enzymes are known to show unintended effects under stress conditions and (ii) the Bt content is known to be influenced by environmental factors.

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

[1] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32021D0066&qid=1612781461058

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 Soybean.

4. Risks indicating toxicological health impacts, potentially enhanced by combinatorial effects caused by the stacking, were not assessed against relevant, reasoned and plausible hypotheses. For example, there was no examination determining 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 here since it is known that enzymes (protease inhibitors) produced by the plants can multiply the toxicity of the Bt toxins and 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.

5. Risks indicating immunological health impacts, potentially enhanced by combinatorial effects caused by the stacking, were not assessed against relevant, reasoned and plausible hypotheses. For example, there was no examination to determine to which extent changes in the microbiome caused by the consumption of the Soybean will impact its immunogenic properties. This is especially relevant in this case, since it is known that enzymes (protease inhibitors) produced in the plants can prolong exposure to the toxins in the gut after ingestion.

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 Soybean 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 Soybean (‘event-specific’) which only is functional with the Soybean, 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 Soybean MON87751 x MON87701 x MON87708 x MON89788 and subcombinations.

The following designations appear in this document:

The Soybean: Genetically engineered stacked Soybean MON87751 x MON87701 x MON87708 x MON89788 and its segregating subcombinations;
The Parental Plants: Single events of genetically engineered Soybean MON87751, MON87701, MON87708, MON89788;
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.

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 2001/189[2] 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.[3]

In order to gain an authorisation, an application must be made to the competent authority of a Member State.[4] 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

[2] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC.
[3] Articles 4(2) and 16(2) of the GM Regulation.
[4] Articles 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).[5] 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).[6] In preparing its opinion, the Authority must consult the national competent authorities of the Member States.[7] 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.[8]

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.[9] 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.[10] 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";[11]

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."[12]

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."

[5] Articles 5(3)(e), 5(3)(f) 17(3)(e), and 17(3)(f) of the GM Regulation.
[6] Articles 6(3)(a) and 18(3)(a) of the GM Regulation.
[7] Articles 6(4) and 18(4) of the GM Regulation.
[8] Articles 7(1) and 19(1) of the GM Regulation.
[9] Articles 7(3), 19(3) and 35(2) of the GM Regulation.
[10] Article 5(3) and 5(4) of Decision 1999/468.
[11] Article 5 of the Food Safety Regulation (also reflected in Recital (3)).
[12] Emphasis added.

1.3 Particular Provisions of Directive 2001/18

Directive 2001/18[13] 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"[14], 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."

[13] Directive 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").

[14] The 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 health[15].

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)"[16].

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:

[15] Article 191(1) TFEU: " *Union policy on the environment shall contribute to the pursuit of the following objectives:... – protecting human health...*"

[16] Directive 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"[17], 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"[18]and, as a catch-all formula "other potential interactions with the environment"[19]. 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"[20]; "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"[21]; and "Potential interactions with the abiotic environment"[22].

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"[23]. 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

[17] Directive 2001/18, Annex III A, section II, C.2(i)
[18] Directive 2001/18, Annex IIIA, section IV B12.
[19] Directive 2001/18, Annex IIIA, section IV B.16.
[20] Directive 2001/18, Annex IIIB, section D no.7.
[21] Directive 2001/18, annex IIIB, section D no.8.
[22] Directive 2001/18, annex IIIB, section D no11.
[23] Decision 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 hand 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 2001/187EC..." (Article 5(5)(b)).[24]

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)).[25] 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

[24] For such cases, Articles 13 to 24 of Directive 2001/18 are declared inapplicable.
[25] 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 products[26].

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 1928/2003, 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 that[27]:

> "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

[26] See 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.
[27] Court 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 counties, 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 Soybean:

> "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 GMO panel assessed the four-stacked soybean event MON87751 x MON87701 x MON 87708 x MON 89788 derived from crossing genetically engineered soybean events. The parental soybeans had undergone previous assessment (EFSA, 2019a). The Soybean contains genes conferring resistance to two herbicides:
MON 87751 expressing the insecticidal proteins Cry1A.105 and Cry2Ab2, and also Cry1A.105 which is synthetic, without a natural template;
MON 87701 expressing the insecticidal protein Cry1Ac;
MON 89788 expressing CP4 EPSPS protein for tolerance to glyphosate-containing herbicides;
MON 87708 expressing dicamba mono-oxygenase (DMO), for tolerance to the herbicide, dicamba.

Consequently, the stacked GE soybean is resistant to two groups of complementary herbicides (glyphosate and dicamba) and produces three insecticidal proteins. The herbicides can be applied in combination or individually. No experimental data were provided for potentially segregated subcombinations of the Soybean.

Implementing Regulation 503/2013 was applied in this case.

The stacked GE soybean plants (hereinafter referred to as the Soybean) was authorised on 22 January 2021, published in the Official Journal of the EU on 26 January 2021.[28]

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)

"*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.*

[28] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32021D0066&qid=1612781461058

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 soybean 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. Furthermore, other gene products, such as ncsRNA from additional open reading frames, were not assessed. Thus, uncertainties remain about other biologically active substances arising from the method of genetic engineering and the newly introduced gene constructs.

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

2.1.2 Impact of environmental factors, agricultural practice and genetic backgrounds

There are several reasons why the data presented (Monsanto, 2016a) do not meet the requirements of Implementing Regulation 503/2013: (1) the field trials were not conducted in all relevant regions where the Soybean will 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.

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

Environmental stress can cause unexpected patterns of expression of the newly introduced DNA (see, for example, Trtikova et al., 2015). There is strong evidence that climate conditions 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 in various environmental conditions.

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 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 describing 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 Soybean in higher concentrations compared to the parental plants. Therefore, the Soybean 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.

The acronyms (ARNE, ILTH, KSLA, NCBD, PAGR) used in the report (Monsanto, 2014) show that the data were taken from some of the field trials carried out for compositional analysis (Monsanto, 2016b). Only 4 samples (each for grain and forage) from 5 field sites located in Arizona, Illinois, Kansas, North Carolina and Pennsylvania were used in this case. The field trials were carried out for one year only (2013). No extreme weather conditions (except frost) were reported during the field trials carried out by Monsanto (Monsanto, 2014).

In summary, the available publications strongly indicate that GE plants inheriting a combination of EPSPS and Bt toxin expression are likely to be influenced by environmental stress factors. However, no specific data on more extreme climate conditions in other countries, such as Brazil or Argentina, were requested or used for detailed comparison to assess the genome x environment interactions.

The gaps in risks assessment are further underlined by meteorological data:

Weather data from the US in 2013[29], show that in all field trials assessed for gene expression, there were no exceptional weather conditions that might have induced specific stress. The plants were therefore not exposed to stress conditions that might have led to responses in gene expression.

The trial sites for gene expression located in Arkansas, Pennsylvania, Illinois, Kansas and North Carolina are only partly representative of the climatic conditions in other soybean growing regions in the US, or conditions in other relevant soybean producing countries, such as Brazil.

For example, the selected field trial sites in the US only represent a limited range of climatic and environmental conditions in major soybean growing regions. USDA data show additional soybean production areas in Minnesota, Wisconsin, North Dakota, South Dakota and other states.[30]

According to climate data, precipitation in soybean growing regions, e.g. Illinois or Kansas is significantly different to that of other soybean growing regions, such as North or South Dakota.[31] The same is true for lower average temperatures in states, such as North Dakota, in comparison to other soybean growing regions in the US.[32]

Even more striking is the difference between the climatic conditions in the major US soybean growing regions and the climate in soybean growing regions in Brazil, another major producer of GE soybean. Data show much more precipitation in soybean growing states, e.g. Paraná or Mato

[29]Weather data accessed via https://www.usclimatedata.com
[30]https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?fattributeid=1&cropid=2222000&sel_year=2021&startrow=1&ftypeid=47&regionid=us&cntryid=USA&nationalGraph=False
[31]https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=2222000&regionid=us&nationalGraph=False&cntryid=USA&sel_year=2021&startRow=1&fctypeid=23&fcattributeid=1
[32]https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=2222000&regionid=us&nationalGraph=False&cntryid=USA&sel_year=2021&startRow=1&fctypeid=24&fcattributeid=1

Grosso, compared to the US.[33] There is also a much higher average and maximum temperature in Brazilian soybean growing regions, such as Mato Grosso, compared to US soybean growing regions.[34]

The Soybean plants tested in field trials do not therefore sufficiently represent the Soybean as imported. 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.

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

Due to increased weed pressure, it has to be expected that these plants will be exposed to much higher and also repeated dosages of glyphosate alone and / or in combination with dicamba. Higher applications of the complementary herbicides 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 in substantial amounts of data showing changes in the composition of GE herbicide-resistant soybeans (Miyazaki et al., 2019; see also Testbiotech, 2021).

As stated by experts of Member States, higher application rates of the complementary herbicides can cause stress responses in the plants and impact gene expression (EFSA, 2019b). However, this aspect was ignored in the EFSA risk assessment.

Glyphosate was applied at a rate of 0.87 kg a.e./ha (Monsanto, 2016b) only. While currently, ‘on top’ glyphosate applications use an average rate of 3 to 4 kg/ha, the amount expected in the US would be an average overall rate of 6 to 7 kg/ha (USDA, 2019; Miyazaki et al., 2019). Even higher amounts can be expected in South America (see, for example, Bombardi, 2016; Miyazaki et al., 2019). In contrast, the amount of glyphosate used in the field trials was just 0,87 kg a.e./ha, which is close to the lowest limit recommended by the company.

The same flaw in the design of the field trials was seen for dicamba. Only 0,56 kg a.e./ha of dicamba was sprayed, while in agricultural management practice the double amount can be expected (see also Miyazaki et al, 2019).

In a statement, EFSA (2019b) indicated that the design of the field trials should avoid major differences in the application of the herbicides:
“The complementary herbicides are kept at a similar application rate across sites: indeed, for the experimental treatments to be comparable between different locations, the application rate should not differ too strongly between them.”

This statement is in direct contradiction to the requirements of Implementing Regulation (EU) No 503/2013. It is evident that EFSA, in making this statement, is violating the provisions of

[33] https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=2222000&regionid=br&nationalGraph=False&cntryid=BRA&sel_year=2021&startRow=1&fctypeid=23&fcattributeid=1
[34] https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=2222000&regionid=br&nationalGraph=False&cntryid=BRA&sel_year=2021&startRow=1&fctypeid=24&fcattributeid=1
https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=2222000&regionid=br&nationalGraph=False&cntryid=BRA&sel_year=2021&startRow=1&fctypeid=24&fcattributeid=5

Implementing Regulation 503/2013 since, under anticipated agricultural conditions, there will be strong differences between the herbicide regimes for herbicide-resistant soybeans in comparison to conventional agriculture (see also Testbiotech, 2021).

The effects of glyphosate applications on plant metabolism in herbicide-resistant plants is not only shown in the overview provided by Miyazaki et al. (2019) but has also been confirmed by de Campos et al. (2020) and Zanatta et al. (2020). This underlines the need to test the Soybean in agronomic conditions specific to herbicide-resistant plants, and not to keep to similar application rates across all the field trial sites.

EFSA should have requested the applicant to submit data from field trials with the highest dosage of the complementary herbicides that can be expected in practice, including repeated spraying and the application of each of the relevant herbicides alone and in combination.

However, no such data were presented by the applicant or requested by EFSA. At the same time, the data presented by the applicant are purposefully flawed: expression data from the stacked Soybean were only generated from crops treated with the complementary herbicide. These data were then compared to data from the parental plants which were not treated with the complementary herbicide (Monsanto, 2014). This is not in accordance with scientific and regulatory standards, which request that GE plants used for comparison should be generated under equal conditions. Thus, no comparison can be made as requested in Implementing Regulation 503/2013 (especially requested in the case of herbicide tolerant GE plants) to assess whether anticipated agricultural practices influence the expression of the studied endpoints.

Furthermore, no data were made available to show herbicide application practices in important soybean producing areas, such as Argentina, Brasil, Paraguay and Uruguay. As shown by Miyazaki et al. (2019), these herbicide regimes may be significantly different compared to those in the US. Neither were any such data available for the parental plants or subcombinations.

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

2.1.2.3 Impact of stacking and influence of genetic backgrounds on gene expression

The data presented show that the expression of biologically active compounds, such as “Gly m 4” protein, is lower in the stacked event compared to its conventional comparator (Monsanto, 2016c). Further significant differences are natural phyto-estrogens (daidzein and genistein) and the concentration of the newly expressed Cry1A.105, DMO and EPSPS enzymes (Monsanto, 2014). This indicates influence from the process of stacking and the resulting overall genomic background of the stacked event.

As shown above, the EPSPS enzymes cause unintended effects, which can interfere with the activity of the other gene constructs, e.g. via the auxin hormone. Even if no such effects were observed in the parental plants, these enzymes are now produced in the stacked Soybean at higher concentrations. Therefore, the likelihood of interaction between the genetic constructs and gene expression on plant composition - as well as impacts on agronomic and phenotypic characteristics - is higher in the stacked event compared to the parental plants. With exposure to a broader range of

environmental conditions, it is not unlikely that the differences between the stacked Soybean and its parental plants will be substantially increased.

In addition, it is known that the genomic background of the variety can influence the expression of the inserted genes and plant metabolism (see for example Barbosa et al., 2012; Zanatta et al., 2020; de Campos et al., 2020).

Therefore, EFSA should have requested additional data from several stacked varieties, including those cultivated in South America.

However, these issues have not yet been taken into account in EFSA risk assessment. Nor do they use more sensitive methods, such transcriptomics, proteomics and metabolomics, to explore and assesses unintended changes in the stacked Soybean.

Consequently, the Soybean plants tested in field trials do not sufficiently represent the Soybean 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 and the biological characteristics of the plant as requested in EU Regulation 503/2013.

2.1.3 Conclusion - molecular characterisation and gene expression

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

Therefore, to gather reliable data on gene expression and functional genetic stability, the plants should have been subjected to a much broader range of defined environmental conditions and stressors. Whatever the case, they should have been tested in the soybean 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 application rates of complementary herbicides being sprayed on the plants, including repeated spraying.

However, only 4 samples (each for grain and forage) from 5 field sites, all located in the US, were used for generating the data on gene expression. Furthermore, only one variety of the stacked Soybean was used in the trials. The impact of environmental factors and agricultural practices was assessed without taking into account more extreme climate conditions as might be expected from climate change. Herbicide applications in the field trials did not represent current agricultural practices since lower application rates were used. Data to compare sprayed and unsprayed GE Soybean plants are completely missing.

In summary, the Soybean plants tested in field trials do not sufficiently represent the Soybean as imported. Consequently, the data presented by the applicant (Monsanto, 2016a) and accepted by EFSA are insufficient to conclude on the impact of the combination of traits and gene constructs (stacking), of environmental factors, of herbicide applications and of different genetic backgrounds on gene expression and plant metabolism.

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

The data provided by Monsanto (Monsanto, 2016a) do not reflect anticipated agricultural management practices or the different meteorological and agronomic conditions under which the crop is to be grown. There are three reasons: (2.2.1) the field trials were not conducted in all relevant regions where the Soybean 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 transgenic stacked variety was included in the field trials.

2.2.1 Data on environmental factors and stress conditions - and their impact on plant composition and phenotype

Field trials for the assessment of plant composition and agronomic and phenotypic characteristics of the stacked Soybean were only conducted in the US, and not in other relevant soybean production countries, e.g. Brazil, Argentina, Paraguay or Uruguay. As stated in the EFSA opinion (2019a), "*No exceptional weather conditions were reported at any of the selected field trial sites.*"

It is unacceptable that EFSA failed to require further field trials to take current agricultural management practices into account, including more than one growing season, all relevant regions, a broader range of varietal backgrounds and more extreme environmental conditions, such as those caused by climate change.

The gaps in risks assessment are further underlined by meteorological data:

Weather data from the US in 2013[35], show that in all field trials assessed for gene expression, there were no exceptional weather conditions that might have induced specific stress. The plants were therefore not exposed to stress conditions that might have led to responses in gene expression.

[35]Weather data accessed via https://www.usclimatedata.com

The trial sites for gene expression located in Arkansas, Pennsylvania, Illinois, Kansas and North Carolina are only partly representative of the climatic conditions in other soybean growing regions in the US, or conditions in other relevant soybean producing countries, such as Brazil.

For example, the selected field trial sites in the US only represent a limited range of climatic and environmental conditions in major soybean growing regions. USDA data show additional soybean production areas in Minnesota, Wisconsin, North Dakota, South Dakota and other states.[36]

According to climate data, precipitation in soybean growing regions, e.g. Illinois or Kansas is significantly different to that of other soybean growing regions, such as North or South Dakota.[37] The same is true for lower average temperatures in states, such as North Dakota, in comparison to other soybean growing regions in the US.[38]

Even more striking is the difference between the climatic conditions in the major US soybean growing regions and the climate in soybean growing regions in Brazil, another major producer of GE soybean. Data show much more precipitation in soybean growing states, e.g. Paraná or Mato Grosso, compared to the US.[39] There is also a much higher average and maximum temperature in Brazilian soybean growing regions, such as Mato Grosso, compared to US soybean growing regions.[40]

There are other environmental factors relevant for plant composition: for example, Petineli et al (2020) show that transgenic, glyphosate-resistant soybeans display physiological and nutritional differences if exposed to sulfur fertiliser compared to conventional soybeans.

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

[36] https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?fattributeid=1&cropid=2222000&sel_year=2021&startrow=1&ftypeid=47&regionid=us&cntryid=USA&nationalGraph=False

[37] https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=2222000&regionid=us&nationalGraph=False&cntryid=USA&sel_year=2021&startRow=1&fctypeid=23&fcattributeid=1

[38] https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=2222000&regionid=us&nationalGraph=False&cntryid=USA&sel_year=2021&startRow=1&fctypeid=24&fcattributeid=1

[39] https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=2222000&regionid=br&nationalGraph=False&cntryid=BRA&sel_year=2021&startRow=1&fctypeid=23&fcattributeid=1

[40] https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=2222000&regionid=br&nationalGraph=False&cntryid=BRA&sel_year=2021&startRow=1&fctypeid=24&fcattributeid=1
https://ipad.fas.usda.gov/cropexplorer/cropview/comm_chartview.aspx?cropid=2222000&regionid=br&nationalGraph=False&cntryid=BRA&sel_year=2021&startRow=1&fctypeid=24&fcattributeid=5

The EPSPS enzymes occur in the stacked Soybean in higher concentrations than in the parental plants. Therefore, the Soybean 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.

The stacked Soybean carries a combination of gene constructs (epsps and Bt genes) which are likely to show or to cause major changes in gene expression if exposed to environmental stressors (see above). These genetic elements can synergize and interact with each other. Such effects are not unlikely to impact plant composition and biological characteristics crucial for the assessment of food and feed safety.

However, no experiments were requested to show to which extent specific environmental conditions will influence plant composition and agronomic characteristics. Hence, no data were made available as requested in 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.

Consequently, the Soybean plants tested in the field trials do not sufficiently represent the Soybean as imported. The data presented by the applicant are insufficient to conclude on the impact of environmental factors in the various soybean producing countries, or the impact of stress conditions on gene expression, plant composition, or 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 soybean growing regions, a large number of these plants will be exposed to higher amounts and repeated dosages of the herbicides. It has to be taken into account that the herbicides can be sprayed in combination or individually at high dosages and repeatedly. These agricultural management practices need to be considered to assess whether the expected agricultural practices will influence the expression of the studied endpoints.

However, the herbicides were only sprayed in combination, each just once at an early stage of vegetation and at comparably low dosages: the amount of glyphosate used in the field trials was just 0,87 kg a.e./ha (see Monsanto 2016b), which is close to the lowest limit recommended by the company.

Available publications show (see Miyazaki et al., 2019) that the GE soybeans are repeatedly sprayed with much higher dosages of the complementary herbicides: on its product label Monsanto recommends spraying about 7 kg (a.i.)/ha (Monsanto, 2017), with up to three applications during cultivation. Official figures from the USDA data base show that up to 6-7 kg (a.i.)/ha of glyphosate can be expected in soybean cultivation, including pre- and post-emergence applications (USDA, 2019). Data from South America show that even higher amounts are possible (Avila-Vazquez et al., 2018).

The field trials have the same design flaws for dicamba; only 0,56 kg a.e./ha was sprayed on the plants, while in agricultural management practice, while in agricultural management practice the double amount can be expected (see also Miyazaki et al, 2019).

As stated by the experts of Member States, higher application rates of the complementary herbicides can cause stress responses in the plants and impact gene expression (EFSA, 2019b). 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 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. The findings showing unintended effects in plants inheriting additional EPSPS genes are described in several publications (Beres, 2019; Beres et al., 2018; Wang et al., 2014). 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 Soybean in much higher concentrations than in 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 impact gene expression and plant composition to a greater extent compared to the parental plants.

From the available data, 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 will impact the composition of the plants and agronomic characteristics (see Miyazaki et al., 2019). For example, Zobiole et al. (2012) and also Bøhn et al. (2014) found that glyphosate applications can cause significant changes in soybean plant constituents. More specifically, Zobiole et al. (2012) applied glyphosate at three different dosages (800, 1200 and 2400 g/ha), which resulted in dose-correlated changes in plant agronomic performance and plant composition. The effects of applications of the complementary herbicide on plant metabolism in herbicide-resistant plants was also confirmed by de Campos et al. (2020) and Zanatta et al. (2020).

Therefore, EFSA should have requested the applicant to submit data from field trials with the highest dosage of the complementary herbicides that might be expected in practice, including repeated spraying and the application of each of the relevant herbicides alone and in combination (see also Testbiotech, 2021).

A statement made by EFSA (2019b) says that the design of the field trials should avoid major differences in herbicide applications:
“The complementary herbicides are kept at a similar application rate across sites: indeed, for the experimental treatments to be comparable between different locations, the application rate should not differ too strongly between them.”

This statement is in direct contradiction to the requirements of Implementing Regulation (EU) No 503/2013. Clearly, EFSA is violating the provisions of Implementing Regulation 503/2013 since in anticipated agricultural conditions, there will be substantial differences between the herbicide regimes for herbicide-resistant soybeans in comparison to conventional agriculture (see also Testbiotech, 2021).

As the Miyazaki et al (2019) review and other publications show, the amount of glyphosate application does indeed have a strong impact on plant composition (see above). It is known that soybeans contain many biologically active substances e.g. estrogens, allergens and anti-nutritional compounds. Changes in the composition of these components may not only be triggered by the process of genetic engineering, but also by interactions with the complementary herbicides.

This underlines the need to test the Soybean in the agronomic conditions specific to herbicide-resistant plants, and not to adhere to similar application rates across all the field trial sites.

It should also be taken into account that a mixture of all the complementary herbicides will not always be used in the fields where the soybeans are cultivated; in some cases, just one will be used. This might lead to an increase in dosages of the respective complementary herbicides. The choice of herbicide will depend on the price of the herbicide formulations, the respective weed problems and regional agricultural management practices. For example, in Argentina, Brazil and the US, there will be different prices, different herbicide formulations and varying regimes of herbicide applications in soybean cultivation. None of these specific agronomic practices were considered in the design of the field trials or in EFSA risk assessment.

Consequently, the Soybean plants tested in field trials do not sufficiently represent the Soybean 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 agronomic and phenotypic characteristics of the plant as requested in EU Regulation 503/2013.

2.2.3 Impact of stacking and influence of genetic backgrounds on plant composition and agronomic and phenotypic characteristics

The data as presented show that the expression of biologically active compounds, such as “Gly m 4” protein, is lower in the stacked event compared to its conventional comparator (Monsanto, 2016c). There are further significant differences in natural phyto-estrogens (daidzein and genistein) and the concentration of the newly expressed Cry1A.105, DMO and EPSPS enzymes (Monsanto, 2014). This indicates influence from the stacking process and resulting overall genomic background of the stacked event.

As shown above, the EPSPS enzymes have unintended effects which can interfere with the activity of the other gene constructs, for example, via the auxin hormone. Even if no such effects were observed in the parental plants, the stacked Soybean produces higher concentrations of the enzymes. Therefore, the likelihood of interaction between the gene constructs and gene expression, and the effect on plant composition and agronomic and phenotypic characteristics, is higher in the stacked event compared to the parental plants. If exposed to broader range of environmental conditions, it is not unlikely that the differences between the stacked Soybean and the parental plants will be substantially increased.

In addition, it is known that the genomic background of the variety can influence the expression of the inserted genes and plant metabolism (see, for example, Barbosa et al., 2012; Zanatta et al., 2020; de Campos et al., 2020).

Therefore EFSA should have requested additional data from several stacked varieties, necessarily including those cultivated in South America.

However, these issues have as yet not been taken into account in EFSA risk assessment. Nor do they use more sensitive methods such transcriptomics, proteomics and metabolomics to explore and assesses unintended changes in the stacked Soybean.

In conclusion, the Soybean plants tested in field trials do not represent the Soybean as imported. The data presented by the applicant are insufficient to conclude on the impact of the genetic

background, or the effect of stacking on gene expression, plant composition and the agronomic and phenotypic characteristics. However, reliable data are essential to demonstrate safety as requested in EU Regulation.

2.2.4 Data from compositional analysis show the need for further investigations

Only data from a low number of agronomic parameters (8) were subjected to statistical analysis in accordance with EFSA guidance; 2 of these were found to be significantly different in the stacked plants compared to their conventional comparators. There were certainly significant differences even in this small data set, and EFSA should have requested much more data (see also above).

Compositional analysis of 53 endpoints in the grains revealed many (and partly major) statistically significant differences: 25 endpoints were significantly different in plants not sprayed with the complementary herbicides and 16 in the stacked plants that were sprayed, when compared to the conventional comparators. One of them, ("Gly m4") protein, indicated major differences between the transgenic stack and its comparator.

As mentioned above, the data showed a much lower number of significant findings in plant composition and phenotypic characteristics when the plants were sprayed with the complementary herbicides. This indicates that application of the complementary herbicide might have impacted metabolic pathways. This should have been investigated in more detail.

More detailed analysis would have been necessary to investigate changes in plant composition and phenotype caused by the stacking, as well as to investigate 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 Soybean in all relevant major soybean producing countries, or in more extreme drought conditions, such as those occurring due to climate change. The range of differences and their significance are likely to be substantially increased in regional and environmental conditions.

One reason for this are the unintended effects of the EPSPS enzymes, which are known to interfere with gene activity, 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, the enzymes are produced in higher concentrations in the stacked Soybean. Therefore, the likelihood of interaction between the gene constructs and gene expression affecting plant composition as well as agronomic and phenotypic characteristics is higher in the stacked Soybean than in the parental plants.

As explained above, EFSA should have requested further tests to include repeated spraying of the herbicides at higher dosages and exposure to a much broader range of environmental conditions. The plant material should have been used to investigate changes in plant composition or agronomic and phenotypic characteristics in much more detail.

However, instead of assessing the overall patterns of change in plant components, their causes and possible impacts in more detail, EFSA only assessed each of 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, 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 where there are indications

of adverse effects, but is always needed to come to sufficiently robust conclusions to inform the next steps of risk assessment.

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 (EU) No 503/2013.

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

The combination of gene constructs (the EPSPS enzyme and the production of Bt toxins) in the stacked Soybean are likely to cause or lead to major changes in gene expression if exposed to environmental stressors such as drought.

These traits and genetic elements can synergize and interact with each other. Such effects are also likely to impact plant composition and phenotype, especially if exposed to environmental stress conditions, including higher application rates of the complementary herbicides.

Unquestionably, the data provided by the applicant (Monsanto, 2016a) and accepted by EFSA are insufficient to conclude on the impact of the combination of traits and gene constructs, or the effects of environmental factors, herbicide applications and genetic background on gene expression, plant metabolism, plant composition and agronomic and phenotypic characteristics.

Since the field trials did not represent the conditions in which the plants will be grown in practice, the data from Soybean plants tested in field trials do not sufficiently represent the Soybean as imported. Consequently, these data do not fulfill the requirements of Implementing Regulation 503/2013.

Whatever the case, based on the available data, no final conclusions can be drawn on the safety of the plants.

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

The findings showed several significant changes in plant composition and agronomic characteristics; several uncertainties were identified in the feeding studies with the parental plants.

Nevertheless, testing of the whole stacked plant (feeding study) was not requested. Even if 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.

The data presented by the applicant neither took into account cultivation of the stacked Soybean in a sufficiently broad range of climate and regional conditions nor realistic agricultural conditions. This means that in such conditions, the range of differences and their significance are likely to be substantially increased. Thus, without more data, the true range of unintended effects cannot be determined and safety cannot be demonstrated as requested in EU regulation.

It is also shown that a reliable conclusion on the content of insecticidal proteins or the other intended new proteins cannot be drawn 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 from consumption of the Soybean cannot be determined and safety cannot be demonstrated as requested in EU regulation.

Despite these findings and a lack of more specific data and 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, negative impacts of Bt toxins on human and animal health cannot be excluded a priori. Bt toxins have several modes of action. Even though they are produced in the plants, they are altered in their biological characteristics and not identical to their natural templates (Hilbeck & Otto, 2015).

Several publications exist describing the effects of Bt toxins in mammals: some Cry toxins are known to bind to epithelial cells in the intestine of mice (Vázquez Padrón et al., 1999, Vásquez 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 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 that the stacked Soybean is also resistant to glyphosate, and the resulting residues should be seen as potential co-stressors at the stage of consumption (see also Then & Bauer-Panskus, 2017).

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 enhance their toxicity.

Testbiotech is aware of several publications confirming this gap in risk assessment that EFSA has consistently 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 the plants (MacIntosh et al., 1990). 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 (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 described effects 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).

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 interaction with other Bt toxins (Sharma et al., 2004; 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).

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 important 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, 2015). 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 findings shown above, the basic data for toxicity assessment of the stacked Soybean are neither valid nor reliable.

In addition, incorrect assumptions were made concerning the degradation of the Bt toxins at the stage of consumption as well as similarity to known toxins (see below). Therefore, the foundations of the Monsanto (Monsanto, 2016a) risk assessment on toxicity and exposure to Bt toxins are based upon incorrect assumptions.

2.3.3 Immmunogenicity 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; Vázquez-Padrón et al., 2000; Legorreta-Herrera et al., 2010; Jarillo-Luna et al. 2008; 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 in this context (for review also see Rubio-Infante et al. 2016). Since Cry1Ac is also used as an adjuvant in vaccines, risks inherent to food consumption, which can be intensified by synergistic effects, need 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: 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 chronic inflammation and other immune responses (see also Testbiotech, 2021).

In this context, it is relevant that Bt toxins produced in the plants can indeed survive digestion to a much higher degree than has been assumed by EFSA and shown in the data provided by 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. Bt proteins are present in higher concentrations in the stacked Soybean compared to the parental plants. The food and feed products derived from the stacked Soybean need to be much more carefully risk assessed for their impact on the immune system compared to genetically engineered plants producing just one Bt toxin.

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

2.3.3 Effects from residues of spraying with complementary herbicide specific to GE plants and their mixed toxicity

The residues from spraying 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 management 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 requiring specific risk assessment of imported plants if the use of pesticides is different in the exporting countries to use in the EU. In this regard, it should be taken into account that EFSA (2015 and 2018a) explicitly stated that no conclusion can be drawn on the safety of residues from spraying with glyphosate occurring in genetically engineered plants made resistant to this herbicide.

The analysis of the toxicity data for glyphosate and dicamba indicate higher toxicity if the two herbicides are combined (Reuter, 2015). EFSA should have at least requested data on the combined toxicity of the residues from spraying with the complementary herbicides.

Furthermore, there is a general understanding that commercially traded formulations of glyphosate, such as Roundup, can be more toxic than glyphosate itself. The EU has therefore 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. (…)."*[41]

The stacked Soybean 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 using the highest dosage of the complementary herbicides that can be tolerated by the plants, 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 a prerequisite for granting authorisation.

EU legal provisions such as Regulation 1829/2003 (and 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 resulting from combinatorial exposure of various potential stressors need to be tested for mixed toxicity (EFSA 2019c).

Glyphosate is known to cause shifts in the microbial composition and associated microbiomes of plants and animals, thus leading to a specific situation in regard to chronic exposure from food consumption: glyphosate has indeed been shown to cause shifts not only in soil organisms (van Bruggen et al., 2018) but also in the composition of the intestinal flora of cattle (Reuter et al., 2007), poultry (Shehata et al., 2013; Ruuskanen et al, 2020) and rodents (Mao et al., 2018; 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 Soybean, are 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, encompassing 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 benefits both the host and microorganisms. They act at the interphase and communicate between the organisms and their wider environment while at the same time being part of an organism's closer environment. Microbiomes are considered to be vital for the health of higher organisms, i.e. human, animal and plants.

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

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 (2020) 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.*"

A 2019 study commissioned by EFSA on adjuvanticity / immunogenicity assessment of proteins included 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 Soybean. The stacked Soybean combines several EPSPS enzymes enhancing resistance to glyphosate, therefore the plants may be expected to carry a higher burden of the residues from spraying in comparison to the parental plants. These residues may cause a perturbation of the gut microbiome. Further, the stacking of the Soybean results in a combination of several Bt toxins which are likely to show immunogenicity. It has to be considered a plausible hypothesis that the combination of Bt toxins with residues from spraying, can trigger effects on the immune system 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 Soybean. This is in direct contradiction 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).

In conclusion, the EFSA opinion on the application for authorisation of the stacked Soybean (EFSA, 2019a) cannot be said to fulfill assessment requirements of potential synergistic or antagonistic effects resulting from the combination of the transformation events in regard to toxicity.

2.3.4 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 Soybean. This observation is supported in the literature review carried out by the company.

Instead, the applicant (Monsanto, 2016a) rejected any need for empirical testing of whole food and feed. In doing so, the applicant referred to the following five arguments:

1) History of safe use of the additional proteins produced in the plants
This claim is not based on science. At a minimum, 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 the additional proteins produced in the plants 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 any impact on the immune system causing non-IGE responses, e.g. increased likelihood of inflammation, is not unlikely. These effects might by exerted via the microbiome. Such risks are especially relevant for the stacked event 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 the additional proteins produced in the plants at the stage of consumption
Since the data on gene expression are inconclusive, this claim lacks the necessary evidence. Furthermore, 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 as currently performed (such as a 90-day feeding study with the parental plants). These risks 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) are more likely.

4) Rapid digestibility of the additionally produced proteins and 5) their deactivation with heat treatment

These claims ignore evidence that degradation and deactivation of the Bt toxins produced in the plants if consumed cannot be seen as equivalent to the toxins tested in isolation.

Furthermore, there are several methods of processing soybeans, such as micronisation, roasting, expanding, extrusion, hydrothermal processing and germination, which all work with different temperatures and durations. The methods used will depend on the product to be placed on the market as well as on the variety of soybean. The degradation of the inhibiting proteins will vary, but they will not be removed completely. In general, each processing company might also prefer to vary the standardised methods, since the goal of the processing is not only to degrade anti-nutritional compounds, such as trypsin-inhibitors, but also to produce a food or feed product with high quality proteins and healthy compounds, such as isoflavones. The structure and function of the Bt toxin could be preserved in food and feed if the method used particularly focusses on the conservation of protein quality in the soybeans. Since the authorisation is not limited to specific purposes, usages such as soybean sprouts or soymilk are examples of where technical treatment is very limited. Such products could reach the market without any further risk assessment (see Then & Bauer-Panskus, 2017).

These problems are especially relevant to the stacked event since the overall concentration of Bt toxins is higher compared to the parental plants, and combinatorial effects may occur which will additionally affect the degradation of the toxins.

6) Lack of a testable hypothesis on potential health impacts

So-called 'weight of evidence' is used by the applicant in this respect to refuse 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 Soybean.

Testbiotech is also aware that feeding studies with similar stacked maize events 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 and 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 Soybean. Safety was not demonstrated in regard to potential toxicity / adverse health effects from the consumption of food and feed derived from the Soybean. Thus, 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)[42]. According to Santos-Vigil et al. (2018), the Bt toxin Cry1Ac can act as an allergen if ingested. This publication is also relevant for Cry1A.105, since the Bt toxin Cry1Ac was used as a source for the synthesis of Cry1A.105 expressed in the stacked Soybean. Therefore, the synthetically derived Cry1A.105 toxin produced in the Soybean has structural similarity with Cry1Ac.

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, 2018b)

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

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). Consequently, the Santos-Vigil et al. (2018) publication has to be considered valid but not properly assessed by EFSA. Clearly, EFSA should have requested testing of the hypothesis that consumption of products derived from the Soybean can trigger allergic responses.

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

2.4.2 Potential adjuvanticity

The synergistic effects between PI and Bt toxins as described above are also relevant to 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-Padron et al. 1999; Vázquez-Padron et al. 2000; 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 in this context (for review also see Rubio-Infante et al. 2016). Since Cry1Ac is also used as adjuvant in vaccines, risks inherent to food consumption, which can be enhanced by synergistic effects, need to be addressed and carefully examined.

In their reply to experts from Member States (EFSA, 2019b), EFSA admits only that “limited experimental evidence” is available to conclude the safety of Bt toxins in regard to immune system responses. Nevertheless, they do acknowledge the need for more detailed testing:
“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 crops events producing Bt toxins have been approved for the EU market, this explanation 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 for the stacked Soybean, the risk assessment is not conclusive and no market authorisation can be granted.

These issues are especially relevant for the stacked event 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, EFSA followed the reasoning put forward by the applicant which referred to the following four arguments to avoid further empirical testing:

1. The additional proteins produced in the plants 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 demonstrated that the additional proteins produced in the plants 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 wrong, 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. 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 in a way to demonstrate that the food and feed from the stacked Soybean has no adverse effects on human and animal health.

In summary, the EFSA assessment of the stacked soybean cannot be said to fulfill the requirements for assessing risks to the immune system as requested in EU regulation.

2.5 Conclusions

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

1. The plants were not exposed to bioclimatic conditions which sufficiently represent the regions in which they will be cultivated. Consequently, expression data and assessment of plant composition are not sufficiently reliable to inform the next steps in risk assessment. This is especially relevant in this case, since (i) the EPSPS enzymes are known to show unintended effects under stress conditions and (ii) the Bt content is known to be influenced by environmental factors.

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 assessment of plant

composition or 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 Soybean.

4. Risks indicating toxicological health impacts, potentially enhanced by combinatorial effects caused by the stacking, were not assessed against relevant, reasoned and plausible hypotheses. For example, there was no examination determining 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 here since it is known that enzymes (protease inhibitors) produced by the plants can multiply the toxicity of the Bt toxins and 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.

5. Risks indicating immunological health impacts, potentially enhanced by combinatorial effects caused by the stacking, were not assessed against relevant, reasoned and plausible hypotheses. For example, there was no examination to determine to which extent changes in the microbiome caused by the consumption of the Soybean will impact its immunogenic properties. This is especially relevant in this case, since it is known that enzymes (protease inhibitors) produced in the plants can prolong exposure to the toxins in the gut after ingestion.

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 Soybean (‘event-specific’) which only is functional with the Soybean, 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 Soybean 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 Soybean. Thus, the monitoring report should at the very least contain detailed information on:
i) actual volumes of the Soybean imported into the EU,
ii) the ports and silos where shipments of the Soybean are unloaded,
iii) the processing plants where the Soybean is transferred to,
iv) the amount of the Soybean used on farms for feed, and
v) transport routes of the Soybean.

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

Furthermore, environmental exposure through organic waste material, by-products, sewage or faeces containing the Soybean 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.

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.

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References

Adamczyk Jr, J.J., & Meredith Jr, W.R. (2004) Genetic basis for variability of Cry1Ac expression among commercial transgenic Bacillus thuringiensis (Bt) cotton cultivars in the United States. Journal of Cotton Science, 8(1): 433-440. http://www.cotton.org/journal/2004-08/1/17.cfm

Adamczyk, J.J., Perera, O., Meredith, W.R. (2009) Production of mRNA from the cry1Ac transgene differs among Bollgard® lines which correlates to the level of subsequent protein. Transgenic Research, 18: 143-149. https://doi.org/10.1007/s11248-008-9198-z

Avila-Vazquez, M., Difilippo, F.S., Lean, B.M., Maturano, E., Etchegoyen, A. (2018) Environmental exposure to glyphosate and reproductive health impacts in agricultural population of Argentina. J Environ Prot, 9: 241-253. https://doi.org/10.4236/jep.2018.93016

Barbosa, H.S., Arruda, S.C., Azevedo, R.A., Arruda, M.A. (2012) New insights on proteomics of transgenic soybean seeds: evaluation of differential expressions of enzymes and proteins. Anal Bioanal Chem, 402: 299-314. https://doi.org/10.1007/s00216-011-5409-1

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

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

Bøhn, T., Cuhra, M., Traavik, T., Sanden, M., Fagan, J., Primicerio, R. (2014) Compositional differences in soybeans on the market: glyphosate accumulates in Roundup Ready GM soybeans. Food Chem, 153: 207-215. https://doi.org/10.1016/j.foodchem.2013.12.054

Bøhn, T. (2018) Criticism of EFSA's scientific opinion on combinatorial effects of ‘stacked’GM plants. Food Chem Toxicol, 111: 268-274. https://doi.org/10.1016/j.fct.2017.11.023

Bøhn, T., Rover, C.M., Semenchuk, P.R. (2016) Daphnia magna negatively affected by chronic exposure to purified Cry-toxins. Food Chem Toxicol, 91: 130-140. https://doi.org/10.1016/j.fct.2016.03.009

Bombardi, L.M. (2016) Pequeno ensaio cartográfico sobre o uso de agrotóxicos no Brasil. Laboratório de Geografia Agrária-USP, Blurb, São Paulo. OpenEdition. https://journals.openedition.org/confins/12594

Bondzio, A., Lodemann, U., Weise, C., Einspanier, R. (2013) Cry1Ab treatment has no effects on viability of cultured porcine intestinal cells, but triggers hsp70 expression. PloS One, 8: e67079. https://doi.org/10.1371/journal.pone.0067079

Broderick, N.A., Robinson, C.J., McMahon, M.D., Holt, J., Handelsman, J., Raffa, K.F. (2009) Contributions of gut bacteria to Bacillus thuringiensis-induced mortality vary across a range of Lepidoptera. BMC Biol, 7: 11. https://doi.org/10.1186/1741-7007-7-11

de Campos, B.K., Galazzi, R.M., dos Santos, B.M., Balbuena, T.S., dos Santos, F.N., Mokochinski, J.B., Eberlin, M.N., Arruda, M.A.Z. (2020) Comparison of generational effect on proteins and metabolites in non-transgenic and transgenic soybean seeds through the insertion of the cp4-EPSPS gene assessed by omics-based platforms, Ecotoxicol Environ Saf, 202: 110918. https://doi.org/10.1016/j.ecoenv.2020.110918

Chen, D., Ye, G., Yang, C., Chen Y., Wu, Y. (2005) The effect of high temperature on the insecticidal properties of Bt Cotton. Environ Exp Bot, 53: 333-342. https://doi.org/10.1016/j.envexpbot.2004.04.004

Chowdhury, E.H., Kuribara, H., Hino, A., Sultana, P., Mikami, O., Shimada, N., Guruge, K.S., Saito, S., Nakajima, Y. (2003) Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11. J Anim Sci, 81(10): 2546-2551. https://doi.org/10.2527/2003.81102546x

Dong, H.Z., & Li, W.J. (2006) Variability of endotoxin expression in Bt transgenic cotton. J Agron Crop Sci, 193: 21-29. https://doi.org/10.1111/j.1439-037X.2006.00240.x

EFSA (2015) Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. EFSA J, 13 (11): 4302. http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2015.4302/full

EFSA (2018a) Review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005. EFSA J, 16(5): e05263. https://doi.org/10.2903/j.efsa.2018.5263

EFSA (2018b) Relevance of new scientific information (Santos-Vigil et al., 2018) in relation to the risk assessment of genetically modified crops with Cry1Ac. EFSA supporting publication, 15(11): EN 1504. https://doi.org/10.2903/sp.efsa.2019.EN-1504

EFSA (2019a) Scientific Opinion on the assessment of genetically modified soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2016 128). EFSA J, 17(11): 5847. https://doi.org/10.2903/j.efsa.2019.5847

EFSA (2019b) Application EFSA-GMO-NL-2016 128; Comments and opinions submitted by Member States during the three-month consultation period. EFSA Register of Questions, http://registerofquestions.efsa.europa.eu/roqFrontend/ListOfQuestionsNoLogin?0&panel=ALL

EFSA (2019c) Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA J, 17(3): 5634. https://doi.org/10.2903/j.efsa.2019.5634

EFSA (2020) Editorial: Exploring the need to include microbiomes into EFSA's scientific assessments. EFSA J, 18(6): e18061. https://doi.org/10.2903/j.efsa.2020.e18061

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

Girón-Calva, P.S., Twyman, R. M., Albajes, R., Gatehouse, A. M., Christou, P. (2020) The impact of environmental stress on Bt crop performance. Trends Plant Sci, 25(3), 264-278. https://doi.org/10.1016/j.tplants.2019.12.019

González-González, E., García-Hernández ,A.L., Flores-Mejía, R., López-Santiago, R., Moreno-Fierros, L. (2015) The protoxin Cry1Ac of Bacillus thuringiensis improves the protection conferred by intranasal immunization with Brucella abortus RB51 in a mouse model. Veterinary Microbiology, 175(2-4): 382-388. https://doi.org/10.1016/j.vetmic.2014.11.021

Guerrero, G.G. & Moreno-Fierros, L. (2007) Carrier potential properties of Bacillus thuringiensis Cry1A toxins for a diphtheria toxin epitope. Scandinavian Journal of Immunology, 66(6): 610-618. https://doi.org/10.1111/j.1365-3083.2007.01992.x

Guerrero, G.G., Dean, D.H., Moreno-Fierros, L. (2004) Structural implication of the induced immune response by Bacillus thuringiensis cry proteins: role of the N-terminal region. Mol Immunol, 41(12): 1177-1183. https://doi.org/10.1016/j.molimm.2004.06.026

Gujar, T., Kalia, V., Kumari, A., Prasad, T.V. (2004) Potentiation of insecticidal activity of Bacillus thuringiensis subsp. kurstaki HD-1 by proteinase inhibitors in the American bollworm, Helicoverpa armigera (Hübner). Indian J Exp Biol, 42: 157-163. http://hdl.handle.net/123456789/23352

Hilbeck, A. & Otto, M. (2015) Specificity and combinatorial effects of Bacillus thuringiensis Cry toxins in the context of GMO risk assessment. Front Environ Sci, 3: 71. https://doi.org/10.3389/fenvs.2015.00071

Huffmann, D.L., Abrami, L., Sasik, R., Corbeil, J., van der Goot, G., Aroian, R.V. (2004) Mitogenactivated protein kinase pathways defend against bacterial pore-forming toxins. PNAS USA, 101: 10995-11000. https://doi.org/10.1073/pnas.0404073101

Ibarra-Moreno, S., García-Hernández, A.L., Moreno-Fierros L. (2014) Coadministration of protoxin Cry1Ac from Bacillus thuringiensis with metacestode extract confers protective immunity to murine cysticercosis. Parasite Immunol, 36(6): 266-270. https://doi.org/10.1111/pim.12103

Ito, A., Sasaguri, Y., Kitada, S., Kusaka, Y., Kuwano, K., Masutomi, K., Mizuki, E., Akao, T., Ohba, M. (2004) Bacillus thuringiensis crystal protein with selective cytocidal action on human cells. J Biol Chem, 279: 21282-21286. https://doi.org/10.1074/jbc.M401881200

Jarillo-Luna, A., Moreno-Fierros L., Campos-Rodríguez R., Rodríguez-Monroy, M.A., Lara-Padilla, E., Rojas-Hernández, S. (2008) Intranasal immunization with Naegleria fowleri lysates and Cry1Ac induces metaplasia in the olfactory epithelium and increases IgA secretion. Parasite Immunology, 30(1): 31-38. https://doi.org/10.1111/j.1365-3024.2007.00999.x

Jiang, Y., Ling, L., Zhng, L., Wang, K., Li, X., Cai, M., ... & Cao, C. (2018) Comparison of transgenic Bt rice and their non-Bt counterpart in yield and physiological response to drought stress. Field Crops Res, 217: 45-52. https://doi.org/10.1016/j.fcr.2017.12.007

Khalique, F. & Ahmed, K. (2005) Compatibility of bio-insecticide with chemical insecticide for management of Helicoverpa armigera Huebner. Pak J Biol Sci, 8: 475-478. https://dx.doi.org/10.3923/pjbs.2005.475.478

Kleter, G.A., Unsworth, J.B., Harris, C.A. (2011) The impact of altered herbicide residues in transgenic herbicide-resistant crops on standard setting for herbicide residues. Pest Manag Sci, 67(10):1193-1210. https://doi.org/10.1002/ps.2128

Kramarz, P., de Vaufleury, A., Gimbert, F., Cortet, J., Tabone, E., Andersen, M.N., Krogh, P.H. (2009) Effects of Bt-maize material on the life cycle of the land snail Cantareus aspersus. Appl Soil Ecol, 42: 236-242. https://doi.org/10.1016/j.apsoil.2009.04.007

Kramarz, P.E., de Vaufleury, A., Zygmunt, P.M.S., Verdun, C. (2007) Increased response to cadmium and Bacillus thuringiensis maize toxicity in the snail Helix aspersa infected by the nematode Phasmarhabditis hermaphrodita. Environ Toxicol Chem, 26: 73-79. https://doi.org/10.1897/06-095R.1

Legorreta-Herrera, M., Oviedo Meza, R., Moreno-Fierros L. (2010) Pretreatment with Cry1Ac protoxin modulates the immune response, and increases the survival of plasmodium -infected CBA/ Ca mice. BioMed Research International: 198921. https://doi.org/10.1155/2010/198921

Luo, Z., Dong, H., Li, W., Ming, Z., Zhu, Y. (2008) Individual and combined effects of salinity and waterlogging on Cry1Ac expression and insecticidal efficacy of Bt cotton. Crop Protection, 27(12): 1485-1490. https://doi.org/10.1016/j.cropro.2008.06.006

Ma, Y., Zhang, Y., Chen, R.-R., Ren, X.-L., Wan, P.-J., Mu, L.-L., Li, G.-Q. (2013) Combined effects of three crystalline toxins from Bacillus thuringiensis with seven proteinase inhibitors on beet armyworm, Spodoptera exigua Hübner (Lepidoptera: Noctuidae). Pestic Biochem Physiol, 105: 169-176. https://doi.org/10.1016/j.pestbp.2013.01.007

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

Mao, Q., Manservisi, F., Panzacchi, S., Mandrioli, D., Menghetti, I., Vornoli, A., Bua, L., Falcioni, L., Lesseur, C., Chen, J., Belpoggi, F., Hu, J. (2018) The Ramazzini Institute 13-week pilot study on glyphosate and Roundup administered at human-equivalent dose to Sprague Dawley rats: effects on the microbiome. Environmental Health, 17: 50. https://doi.org/10.1186/s12940-018-0394-x

Mason, K.L., Stepien, T.A., Blum, J.E., Holt, J.F., Labbe, N.H., Rush, J.S., Raffa, K.F., Handelsman, J. (2011) From commensal to pathogen: translocation of Enterococcus faecalis from the midgut to the hemocoel of Manduca sexta. mBio 2: e00065-00011. https://doi.org/10.1128/mBio.00065-11

Mesén-Porras, E., Dahdouh-Cabia, S., Jimenez-Quiros, C., Mora-Castro, R., Rodríguez, C., Pinto-Tomás, A. (2020) Soybean protease inhibitors increase Bacillus thuringiensis subs. israelensis toxicity against Hypothenemus hampei. Agronomía Mesoamericana, 31: 461-478. https://doi.org/10.15517/am.v31i2.36573

Mesnage, R., Clair, E., Gress, S., Then, C., Székács, A., Séralini, G.-E. (2013) Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. J Appl Toxicol, 33: 695-699. https://doi.org/10.1002/jat.2712

Mesnage, R., Teixeira, M., Mandrioli, D., Falcioni, L., Ducarmon, Q.R., Zwittink, R.D., Mazzacuva, F., Caldwell, A., Halket, J., Amiel, C., Panoff, J.-M., Belpoggi, F. Antoniou, M.N. (2021) Use of shotgun metagenomics and metabolomics to evaluate the impact of glyphosate or Roundup MON 52276 on the gut microbiota and serum metabolome of Sprague-Dawley rats. Environ Health Perspect, 129(1): 017005. https://doi.org/10.1289/EHP6990

Mezzomo, B.P. (2013) Hematotoxicity of Bacillus thuringiensis as spore-crystal strains Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa in Swiss albino mice. J Hematol Thromb Dis, 1(1): 1-9. http://repositorio.unb.br/handle/10482/18532

Miyazaki, J., Bauer-Panskus, A., Bøhn, T., Reichenbecher, W., Then, C. (2019) Insufficient risk assessment of herbicide-tolerant genetically engineered soybeans intended for import into the EU. Environ Sci Eur, 31(1): 92. https://doi.org/10.1186/s12302-019-0274-1

Monsanto (2014) Assessment of Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS protein levels in soybean tissues collected from MON 87751 × MON 87701 × MON87708 × MON 89788 produced in United States field trials during 2013. Monsanto, Technical Report, MSL0026209, accessed via EFSA

Monsanto (2015) Acute dietary risk assessment of CP4 EPSPS, Cry1Ac, Cry1A.105, Cry2Ab2, and DMO proteins from consumption of MON 87751x MON 87701 x MON 87708 x MON 89788 soybean in the EU. Monsanto Technical Report, MSL0026316, accessed via EFSA

Monsanto (2016a) Application for authorization to place on the market MON 87751 x MON 87701 x MON 87708 x MON 89788 soybean in the European Union according to Regulation (EC) No 1829/2003 on genetically modified food and feed, EFSA-GMO-NL-2016-128 / EFSA-Q-2016-00009, accessed via EFSA

Monsanto (2016b) Compositional Analyses of Soybean Seed and Forage from MON 87751 x MON87701 x MON 87708 x MON 89788 Grown in the United States During the 2013 Season, Monsanto Techical Report, MSL0027823, accessed via EFSA

Monsanto (2016c). Comparison of Endogenous Allergens from MON87751 x MON 87701 x MON 87708 x MON 89788 and Conventional Soybeans Grown in the United States During the 2013 Season. Monsanto Techical Report, MSL0027890, accessed via EFSA.

Monsanto (2017) Roundup PowerMaxII product label. Greenbook. https://assets.greenbook.net/16-50-19-15-03-2018-63045R2-9_PMAXII_SpecimenLabel_2017.pdf

Moreno-Fierros, L., García, N., Gutiérrez, R., López-Revilla, R., Vázquez-Padrón, R.I. (2000) Intranasal, rectal and intraperitoneal immunization with protoxin Cry1Ac from Bacillus thuringiensis induces compartmentalized serum, intestinal, vaginal and pulmonary immune responses in Balb/c mice. Microbes and Infection, 2(8): 885-890. https://doi.org/10.1016/S1286-4579(00)00398-1

Moreno-Fierros, L., García-Hernández, A.L., Ilhuicatzi-Alvarado, D., Rivera-Santiago, L., Torres-Martínez, M., Rubio-Infante, N., Legorreta-Herrera, M. (2013) Cry1Ac protoxin from Bacillus thuringiensis promotes macrophage activation by upregulating CD80 and CD86 and by inducing IL-6, MCP-1 and TNF-α cytokines. International Immunopharmacology, 17(4): 1051-1066. https://doi.org/10.1016/j.intimp.2013.10.005

Moreno-Fierros, L., Santos-Vigil, K., Ilhicatzi-Alvarado, D. (2018) Response to assessment of the relevance of new scientific information (Santos-Vigil et al., 2018) in relation to the risk assessment of genetically modified crops with Cry1Ac by European Food Safety Authority (EFSA). https://www.testbiotech.org/sites/default/files/Response%202018-EFSA_Supporting_Publications_Santos_Vigill_1.pdf

Motta, E.V., Mak, M., De Jong, T.K., Powell, J.E., O'Donnell, A., Suhr, K.J., Riddington, I.M., Moran, N.A. (2020) Oral and topical exposure to glyphosate in herbicide formulation impact the gut microbiota and survival rates of honey bees. Appl Environ Microbiol, 6: e01150-20. https://doi.org/10.1128/AEM.01150-20

Pardo-López, L., Muñoz-Garay, C., Porta, H., Rodríguez-Almazán, C., Soberón, M., Bravo, A. (2009) Strategies to improve the insecticidal activity of Cry toxins from Bacillus thuringiensis. Peptides, 30(3): 589-595. https://www.sciencedirect.com/science/article/pii/S0196978108003264

Parenti, M.D., Santoro, A., Del Rio, A., Franceschi, C. (2019) Literature review in support of adjuvanticity/immunogenicity assessment of proteins. EFSA Supporting Publications, 16(1): 1551E. https://doi.org/10.2903/sp.efsa.2019.EN-1551

Petineli, R., Moraes, L.A., Heinrichs, R., Moretti, L.G., Moreira, A. (2020) Conventional and transgenic soybeans: physiological and nutritional differences in productivity under sulfur fertilization. Commun Soil Sci Plant Anal, 51(15): 2045-2053. https://doi.org/10.1080/00103624.2020.1822387

Reardon, B.J., Hellmich, R.L., Sumerford, D.V., Lewis, L.C. (2004) Growth, Development, and Survival of Nosema pyrausta -Infected European Corn Borers (Lepidoptera: Crambidae) Reared on Meridic Diet and Cry1Ab. J Econ Entomol, 97: 1198-1201. https://doi.org/10.1093/jee/97.4.1198

Reuter, W. (2015) Toxicology of glyphosate, isoxaflutole, dicamba and possible combination effects. Available via Testbiotech. https://www.testbiotech.org/content/toxicology-glyphosate-isoxaflutole-dicamba-and-possible-combination-effects

Reuter, T., Alexander, T.W., Martínez, T.F., McAllister, T.A. (2007) The effect of glyphosate on digestion and horizontal gene transfer during in vitro ruminal fermentation of genetically modified canola. J Sci Food Agric, 87(15): 2837–2843. https://doi.org/10.1002/jsfa.3038

Rubio-Infante, N. & Moreno-Fierros, L. (2016) An overview of the safety and biological effects of Bacillus thuringiensis Cry toxins in mammals. J Appl Toxicol, 36(5): 630-648. https://doi.org/10.1002/jat.3252

Rubio-Infante, N., Ilhuicatzi-Alvarado, D., Torres-Martínez, M., Reyes-Grajeda, J.P., Nava-Acosta, R., González-González, E., Moreno-Fierros, L. (2018) The macrophage activation induced by Bacillus thuringiensis Cry1Ac protoxin involves ERK1/2 and p38 pathways and the interaction with cell-Surface-HSP70. J Cell Biochem, 119(1): 580-598. https://doi.org/10.1002/jcb.26216

Ruuskanen, S., Rainio, M.J., Gomez-Gallego, C., Selenius, O., Salminen, S., Collado, M.C., Saikkonena, K., Saloniemia, I., Helander, M. (2020) Glyphosate-based herbicides influence antioxidants, reproductive hormones and gut microbiome but not reproduction: A long-term experiment in an avian model. Environ Pollut, 266(1): 115108. https://doi.org/10.1016/j.envpol.2020.115108

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

Schnepf, E., Crickmore, N.V., Van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R., Dean, D. (1998). Bacillus thuringiensis and its pesticidal crystal proteins. Microbiol Mol Biol Rev , 62(3), 775-806. https://doi.org/10.1128/MMBR.62.3.775-806.1998

Sharma, H.C., Sharma, K.K., Crouch, J.H. (2004) Genetic transformation of crops for insect resistance: potential and limitations. Crit Rev Plant Sci, 23(1): 47-72. https://doi.org/10.1080/07352680490273400

Shehata, A.A., Schrödl, W., Aldin, A.A., Hafez, H.M., Krüger., M. (2013) The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro. Curr Microbiol, 66(4): 350-358. https://doi.org/10.1007/s00284-012-0277-2

Shimada, N., Kim, Y.S., Miyamoto, K., Yoshioka, M., Murata, H. (2003) Effects of Bacillus thuringiensis Cry1Ab toxin on mammalian cells. J Veterinary Sci, 65: 187-191. https://doi.org/10.1292/jvms.65.187

Singh, G., Rup, P.J., Koul, O. (2007) Acute, sublethal and combination effects of azadirachtin and Bacillus thuringiensis toxins on Helicoverpa armigera (Lepidoptera: Noctuidae) larvae. Bull Entomol Res, 97: 351-357. https://doi.org/10.1017/S0007485307005019

de Souza Freire, I., Miranda-Vilela, A.L., Barbosa, L.C.P., Martins, E.S., Monnerat, R.G., Grisolia, C.K. (2014) Evaluation of cytotoxicity, genotoxicity and hematotoxicity of the recombinant spore-crystal complexes Cry1Ia, Cry10Aa and Cry1Ba6 from Bacillus thuringiensis in Swiss mice. Toxins, 6: 2872-2885. https://doi.org/10.3390/toxins6102872

Suppa, A., Kvist, J., Li, X., Dhandapani, V., Almulla, H., Tian, A.Y., Kissane, S., Zhou, J., Perotti, A., Mangelson, H., Langford, K., Rossi, V., BrownJ.B., Orsini, L. (2020) Roundup causes embryonic development failure and alters metabolic pathways and gut microbiota functionality in non-target species. Microbiome, 8(1): 1-15. https://doi.org/10.1186/s40168-020-00943-5

Tabashnik, B.E., Fabrick, J.A., Unnithan, G.C., Yelich, A.J., Masson, L., Zhang, J., Bravo, A., Soberón, M. (2013) Efficacy of genetically modified Bt toxins alone and in combinations against pink bollworm resistant to Cry1Ac and Cry2Ab. PLOS ONE, 8(11): e80496. https://doi.org/10.1371/journal.pone.0080496

Tang, Q., Tang, J., Ren, X., Li, C. (2020) Glyphosate exposure induces inflammatory responses in the small intestine and alters gut microbial composition in rats. Environ Poll, 261: 114129. https://doi.org/10.1016/j.envpol.2020.114129

Testbiotech (2021) Risk assessment of GE plants in the EU: Taking a look at the ‘dark side of the moon’, www.testbiotech.org/node/2692

Then, C. (2010) Risk assessment of toxins derived from Bacillus thuringiensis: synergism, efficacy, and selectivity. Environ Sci Pollut Res Int, 17: 791-797. https://doi.org/10.1007/s11356-009-0208-3

Then, C. & Lorch, A. (2008) A simple question in a complex environment: How much Bt toxin do genetically engineered MON810 maize plants actually produce? In: Breckling, B., Reuter, H. & Verhoeven, R. (2008) Implications of GM-Crop Cultivation at Large Spatial Scales. Theorie in der Ökologie 14. Frankfurt, Peter Lang: 17-21. http://www.mapserver.uni-vechta.de/generisk/gmls2008/beitraege/Then.pdf

Then, C., & Bauer-Panskus, A. (2017) Possible health impacts of Bt toxins and residues from spraying with complementary herbicides in genetically engineered soybeans and risk assessment as performed by the European Food Safety Authority EFSA. Environ Sci Eur, 29(1):1. https://doi.org/10.1186/s12302-016-0099-0

Thomas, W.E., Ellar, D.J. (1983) Bacillus thuringiensis var israelensis crystal delta-endotoxin: effects on insect and mammalian cells in vitro and in vivo. J Cell Sci, 60(1): 181-197. https://jcs.biologists.org/content/60/1/181.short

Trtikova, M., Wikmark, O.G., Zemp, N., Widmer, A., Hilbeck, A. (2015) Transgene expression and Bt protein content in transgenic Bt maize (MON810) under optimal and stressful environmental conditions. PLoS One, 10(4): e0123011. https://doi.org/10.1371/journal.pone.0123011

United States Department of Agriculture (USDA), National Agricultural Statistics Service (NASS) (2019) Agricultural chemical use program. http://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Chemical_Use/index

Van Bruggen, A.H.C., He, M.M., Shin, K., Mai, V., Jeong, K.C., Finckh, M.R., Morris, J.G. (2018) Environmental and health effects of the herbicide glyphosate. Sci Total Environ 616–617:255–268. https://doi.org/10.1016/j.scitotenv.2017.10.309

Vázquez-Padrón, R.I., Gonzáles-Cabrera, J., García-Tovar, C., Neri-Bazan, L., Lopéz-Revillac, L., Hernández, M., Moreno-Fierro, L., de la Riva, G.A. (2000) Cry1Ac protoxin from Bacillus thuringiensis sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. Biochem Biophys Res Commun, 271(1): 54-58. https://doi.org/10.1006/bbrc.2000.2584

Vázquez-Padrón, R.I., Moreno-Fierros, L., Neri-Bazán, L., de la Riva, G.A., López-Revilla, R. (1999) Intragastric and intraperitoneal administration of Cry1Ac protoxin from Bacillus thuringiensis induces systemic and mucosal antibody responses in mice. Life Sciences, 64: 1897-1912. https://doi.org/10.1016/S0024-3205(99)00136-8

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

Vila-Aiub, M.M., Neve, P., Powles, S.B. (2009) Fitness costs associated with evolved herbicide resistance alleles in plants. New Phytol, 184(4): 751-767. https://doi.org/10.1111/j.1469-8137.2009.03055.x

Walsh, M.C., Buzoianu, S.G., Gardiner, G.E., Rea, M.C., Gelencsér, E., Jánosi, A., Jánosi, A., Epstein, M.M., Lawlor, P.G. (2011) Fate of transgenic DNA from orally administered Bt MON810 maize and effects on immune response and growth in pigs. PLoS ONE, 6(11): e27177. https://doi.org/10.1371/journal.pone.0027177

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

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

Zanatta, C.B., Benevenuto, R.F., Nodari, R.O., Agapito-Tenfen, S.Z. (2020) Stacked genetically modified soybean harboring herbicide resistance and insecticide rCry1Ac shows strong defense and redox homeostasis disturbance after glyphosate-based herbicide application. Environ Sci Eur, 32: 104. https://doi.org/10.1186/s12302-020-00379-6

Zdziarski, I.M., Carman, J.A., Edwards, J.W. (2018) Histopathological Investigation of the Stomach of Rats Fed a 60% Genetically Modified Corn Diet. Food Sci Nutr, 9: 763-796. https://doi.org/10.4236/fns.2018.96058

Zhang, J., Wang, C., Qin, J. (2000) The interactions between soybean trypsin inhibitor and δ-endotoxin of Bacillus thuringiensis in Helicoverpa armigera larva. J Invertebr Pathol, 74(5): 259-266. https://doi.org/10.1006/jipa.2000.4936

Zhao, J.Z., Fan, Y.L., Fan, X.L., Shi, X.P., Lu, M.G. (1999) Evaluation of transgenic tobacco expressing two insecticidal genes to delay resistance development of Helicoverpa armigera. Chin Sci Bull, 44: 1871-1874. https://doi.org/10.1007/BF02886343

Zhu, Y.C., Abel, C.A., Chen, M.S. (2007) Interaction of Cry1Ac toxin (Bacillus thuringiensis) and proteinase inhibitors on the growth, development, and midgut proteinase activities of the bollworm, Helicoverpa zea. Pestic Biochem Physiol, 87(1): 39-46. https://doi.org/10.1016/j.pestbp.2006.05.004

Zhu, Y.C., Adamczyk, J.J., West, S. (2005) Avidin, a potential biopesticide and synergist to Bacillus thuringiensis toxins against field crop insects. J Econ Entomol, 98: 1566-1571. https://doi.org/10.1093/jee/98.5.1566

Zobiole, L.H.S., Kremer, R.J., de Oliveira Jr, R.S., Constantin, J. (2012) Glyphosate effects on photosynthesis, nutrient accumulation, and nodulation in glyphosate resistant soybean. J Plant Nutr Soil Sci, 175(2): 319-330. https://doi.org/10.1002/jpln.201000434