Technical background for a request for internal review of administrative acts under Article 10 of Regulation (EC) No. 1367/2006 against the decision of the EU Commission to give market authorisation to stacked maize MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 and subcombinations

Prepared by Testbiotech, January 2019, www.testbiotech.org

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 2017a) and forwarded to EU Member States in March 2014; 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.

In 19 December 2018, based upon an EFSA opinion, the EU Commission issued market authorisation[1] for the genetically engineered maize MON 87427 x MON89034 × 1507 × MON88017 × 59122 (hereinafter designated the Maize or “SmartStax +”) and its segregating progeny (sub-combinations). 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. EFSA did not request the applicant to provide data to verify whether the source of the transgene is allergenic.
2. EFSA did not request the applicant to assess the possible role of Bt toxins as adjuvants despite strong indications of this being relevant for hazard identification.
3. No data were requested on potential synergistic or antagonistic effects resulting from the combination of the transformation events in regard to toxicology and allergenicity.
4. EFSA did not request data necessary to assess whether the expected agricultural practices influence the expression of the studied endpoints.

[1] http://europa.eu/rapid/press-release_MEX-18-6883_en.htm

5. EFSA did not request data on field trials to show the different meteorological and agronomic conditions under which the crop is to be grown
6. EFSA did not request sufficiently reliable protein expression data from the newly introduced proteins.
7. EFSA did not request data on any subcombinations.
8. EFSA did not request the applicant to assess gene flow to other cultivated or wild plant species.
9. In conclusion, EFSA and the applicant did not ensure that the final risk characterisation clearly demonstrates that the genetically modified food and feed has no adverse effects on human and animal health or the environment.

B) The decision of the EU Commission fails to fulfill the requirements for the following reason:
(1) 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.

(2) Furthermore, the EU Commission did not request "method(s) (…) 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".

(3) The Commission should also have requested the publication of validated measures allowing the Bt concentration of the maize to be measured by independent scientists as is the case with other plant protection compounds used in food and feed production. This is necessary to make sure that the environment as well as human and animals coming into contact with the material (via dust or consumption) are not exposed to higher quantities of Bt toxins than described in the application.

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 MON89034 × 1507 × MON88017 × 59122 (SmartStax).

The following designations appear in this document:

The Maize: Genetically engineered stacked maize MON87427 x MON89034 × 1507 × MON88017 × 59122 ("SmartStax +") and its segregating subcombinations.
The Parental Plants: Single events of genetically engineered maize MON87427, MON89034, 1507, MON88017, 59122**.**
The Applicants: Monsanto (owned by Bayer)
GMO: 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 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

---

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.

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.

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."

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)).

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.
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").

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."

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

---

[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.

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

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: (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

[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.

[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.

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 "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".

---

[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.

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 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

[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.

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 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.

---

[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.

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.”

- 1.3.6: When appropriate, depending on the nature of the newly expressed protein(s), it shall be necessary to assess the extent to which the processing steps lead to the concentration or to the elimination, denaturation and/or degradation of these protein(s) in the final product.

- 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 2018a), in its assessment of residues from spraying with glyphosate, explicitly states that existing data are not sufficient to conclude on health risks of consuming relevant products derived from herbicide resistant maize:

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

1.7. The interface between pesticide and GMO regulation

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

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

Since the application of the complementary herbicide is a regular part of agricultural practice in the cultivation of herbicide resistant plants, it can be expected that residues from spraying are always present in the harvest. Thus, in regard to herbicide resistant plants, specific assessment of residues

from spraying with complementary herbicides has to be considered to be a prerequisite before any authorisation for genetically engineered plants can be granted.

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

2. Overview EFSA risk assessment of the Maize

Introduction
The GMO Panel assessed the five-event stacked maize MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 which is derived from crossing five genetically engineered maize events. (EFSA, 2017a). The maize contains genes for glyphosate and glufosinate resistance and produces several proteins which confer resistance to specific lepidopteran pests.

- MON 87427 expressing CP4 EPSPS protein for tolerance to glyphosate-containing herbicides (not expressed in the pollen);
- MON 89034 expressing Cry1A.105 and Cry2Ab2 insecticidal proteins;
- 1507 expressing the Cry1F insecticidal protein and phosphinothricin acetyl transferase (PAT) protein for tolerance to glufosinate-containing herbicides;
- MON 88017 expressing the Cry3Bb1 and CP4 EPSPS protein for tolerance to glyphosate-containing herbicides; and
- 59122 expressing the Cry34Ab1 and Cry35Ab1 insecticidal proteins and the PAT protein for tolerance to glufosinate-containing herbicides.

Consequently, the stacked maize produces six insecticidal toxins (Cry1A.105, Cry2Ab2 and Cry1F that target lepidoptera insects and Cry3Bb1, Cry34Ab1 and Cry35Ab1 that target coleoptera). Further, resistance to each of the complementary herbicides is based on a pair of enzymes. The pairwise enzymes are likely to confer high tolerance to applications of these weed killers onto the maize.

No experimental data were provided for 14 maize subcombinations.

Molecular characterisation
The process of genetic engineering used here led to several deletions and insertions in the maize plants. In order to assess the sequences encoding the newly expressed proteins or any other open reading frames (ORFs) present within the insert and spanning the junction sites, it was simply assumed that the proteins that might emerge from these DNA sequences would raise no safety issues; no detailed investigations were carried out in this regard.

Furthermore, other gene products, such as miRNA 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.

Environmental stress can also cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015). However, the expression of the additional enzymes was only measured under field conditions in the US for one year. It is unclear, to which extent specific environmental conditions will influence the overall concentration of the enzymes in the plants. The plants should have been subjected to a much broader range of defined environmental conditions and stressors to gather reliable data on gene expression and functional genetic stability.

The levels of Cry1A.105 in maize MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 are higher for grain and pollen than in the parental line MON 89034. The same observations were made regarding SmartStax maize MON 89034 x 1507 x MON 88017 x 59122 (see Testbiotech, 2011). In addition, most of the other Bt toxins as produced in the plant show a higher concentration compared to the single events. It is surprising that these effects were not investigated further.

Much more surprisingly, EFSA and the applicant omitted to assess the stacked event in regard to its new characteristics. By crossing MON 87427 with MON 88017 (or the previously authorized SmartStax maize MON 89034 x 1507 x MON 88017 x 59122), the content of EPSPS enzymes that confer resistance to glyphosate is increased in vegetative and female reproductive tissues, conferring tolerance to glyphosate in the leaves, stalk, and root tissues and tissues that develop into grain or grain and silks. In consequence, it has to be expected that these plants can and will be exposed to higher and also repeated dosages of glyphosate. Higher applications of glyphosate will not only lead to a higher burden of residues in the harvest, but may also influence the expression of the transgenes or other genome activities in the plants. The same aspect is relevant in regard to the resistance to glufosinate which is also based on two enzyms. This aspect, which highly relevant in regard to this specific stacked event, was completely ignored in the risk assessment as performed.

EFSA should have requested that Monsanto submit data from field trials with the highest dosage of the complementary herbicides that can be tolerated by the plants, also including repeated spraying. The material derived from those plants should have been assessed by using Omics techniques to investigate changes in the activity of the transgene, as well as the natural genome of the plants.

Comparative analysis (for compositional analysis and agronomic traits and GM phenotype)
Field trials for compositional and agronomic assessment of maize MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 were conducted in the US only during one year (2010) and not in other relevant maize production areas, such as Brazil or Argentina.

Regarding agronomic parameters, the following statistically significant differences were found:

(i) between the five-event stack maize <u>not treated with the intended herbicides</u> and the non-GM comparator differences were identified for: early stand count, ear height, plant height, stalk lodged plants, grain moisture and test weight;

(ii) between the five-event stack maize <u>treated with the intended herbicides</u> and the non-GM comparator differences were in: days to 50% pollen shed, days to 50% silking, ear height, plant height, stalk lodged plants, grain moisture and test weight.

All these endpoints fell under equivalence category I.

Compositional data revealed many statistically significant differences:

- Statistically significant differences between the five-event stack maize (not treated) and the non-GM comparator were identified for 47 endpoints. All the endpoints fell under equivalence category I or II.

- Statistically significant differences between the five-event stack maize (treated) and the non-GM comparator were identified for 50 endpoints.

Here, endpoints fell under equivalence category I except for thiamine levels which fell under category III.

Several experts from EU Member States (EFSA, 2017b) took note of the very high number of significant differences (~ 75% of the analytes evaluated) and came to the conclusion that it is highly likely that the genetic modification resulted in unintended effects. However, requests for further tests (toxicological data, sprayings under practical conditions, exposure to a wider range of environmental conditions) were not followed by EFSA.

It has to be assumed that this event is essentially different from its comparator in regard to many compositions and biological characteristics. Even if changes taken as isolated data might not directly raise safety concerns, the overall number of effects and their strong significance has to be taken as a starting point for much more detailed investigations. It is not acceptable that EFSA failed to require further studies e.g.

- No data from Omics (proteomics, transcriptomics, metabolomics) were used to assist the compositional analysis and the assessment of the phenotypical changes.
- More powerful statistical analysis, such as multidimensional analysis, was not applied to the data.
- No field trials were conducted that lasted more than one season. Thus, based on current data, site-specific effects can hardly be assessed.
- Further, no data were generated representing more extreme environmental conditions, such as those caused by climate change. Although no application has been filed for cultivation, data on the interaction between the plants and the environment have to be considered as one of the starting points in risk assessment of the plant, and must be made available and assessed in detail. However, EFSA (2017a) stated that: *"Considering the scope of application EFSA-GMO-BE-2013-118, interactions with the biotic and abiotic environment are not considered to be relevant issues."*
- In addition, more varieties carrying the transgenes should have been included in the field trials to see how the gene constructs interact with the genetic background of the plants.

As mentioned, EFSA and the applicant omitted to assess the stacked event in regard to its new characteristics. By crossing MON 87427 with MON 88017 (or the previously authorised SmartStax maize MON 89034 x 1507 x MON 88017 x 59122), the content of EPSPS enzymes that confer resistance to glyphosate is increased in in vegetative and female reproductive tissues, conferring tolerance to glyphosate in the leaves, stalk, and root tissues and tissues that develop into grain or grain and silks. In consequence, it has to be expected that these plants can and will be exposed to higher and also repeated dosages of glyphosate. The specific pattern of applications of the complementary herbicides will not only lead to a higher burden of residues in the harvest, but may also influence the composition of the plants and agronomic characteristics. This aspect was ignored by the risk assessment as performed.

EFSA should have requested that Monsanto submit data from field trials with the highest dosage of the complementary herbicides that can be tolerated by the plants, also including repeated spraying. The material derived from those plants should have been assessed by using Omics techniques to investigate changes in plant composition or agronomic characteristics

Based on the available data, no final conclusions can be drawn on the safety of the plants.

Toxicology
No toxicological tests were conducted with maize MON 87427 x MON 89034 x 1507 x MON 88017 x 59122. This is unacceptable for several seasons:

1. The stacked maize differs from the parental lines with regard to the overall amount of toxin produced which is greater than in the parental lines.

2. Despite many significant changes in the composition of the plants and agronomic characteristics, no testing of the whole plant (feeding study) was requested. It has to be assumed that this event is essentially different from its comparator in regard to many compositions and biological characteristics. Even if changes taken as isolated data might not directly raise safety concerns, the overall number of effects and their great significance has to be taken as a starting point for much more detailed investigation of their potential health impacts.

3. Beyond that, the residues from spraying were considered to be not within the remit of the GMO panel. However, without detailed assessment of these residues no conclusion can be drawn on the safety of the imported products: due to specific agricultural practices established in 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.

The assessment of herbicide and metabolite data was also requested by Member State experts (EFSA, 2017b): *"The amount of residues of the herbicide treatment should be assessed including amounts of herbicide metabolites present in the produced material. For this analysis the notifier should take into consideration that CP4 EPSPS transgenes are expressed at a higher level in GM maize MON87427 x MON89034 x 1507 x MON88017 x 59122 which might affect the maximum level of glyphosate herbicides that could be used in the crop."*

The publication by Kleter et al. (2011) also shows that using herbicides to spray genetically engineered herbicide-resistant plants does indeed lead to patterns of residues and exposure that need to be assessed in detail.

Moreover, more detailed assessment would be in accordance with pesticide regulation, which requires specific risk assessment of imported plants if pesticide usage is different in the exporting countries than in the EU. In this regard, it should be taken into account that EFSA (2015a) explicitly stated that no conclusion can be drawn on the safety of residues from spraying with glyphosate occurring in genetically engineered plants resistant to this herbicide.

There is a common understanding that commercially traded formulations of glyphosate, such as Roundup, can be more toxic than glyphosate itself. Therefore, the EU has already taken measures to remove problematic additives known as POE tallowmine 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. (…)." (www.testbiotech.org/node/1637)*

The European Food Safety Authority (EFSA) agrees that further investigations and data are needed (EFSA, 2015).

In any case, both the EU pesticide regulation and the GMO regulation require a high level of protection for health and the environment. Thus, in regard to herbicide-resistant plants, specific assessment of residues from spraying with complementary herbicides must be considered to be a prerequisite for granting authorisation. In addition, cumulative effects have to be investigated if a plant contains or produces other compounds with potential toxicity.

By crossing MON 87427 with MON 88017 (or the previously authorised SmartStax maize MON 89034 x 1507 x MON 88017 x 59122), the content of EPSPS enzymes that confer resistance to glyphosate is increased in vegetative and female reproductive tissues, conferring tolerance to glyphosate in the leaves, stalk, and root tissues and tissues that develop into grain or grain and silks. In consequence, it has to be expected that these plants can and will be exposed to higher and also repeated dosages of glyphosate. These applications of glyphosate will not only lead to a higher burden of residues in the harvest, but may also influence the composition of the plants and agronomic characteristics. EFSA should have requested that Monsanto submit data from field trials with the highest dosage of glyphosate that can be tolerated by the plants, also including repeated spraying. The material derived from those plants should have been assessed in regard to organ toxicity, immune responses and reproductive toxicity, including taking combinatorial effects with other plants components and the Bt toxins into account.

In the context of risk assessment of this stacked event, the residues from spraying with the complementary residues must also be considered to be a potent co-stressor. The impact on cells and organisms exposed to several stressors in parallel can be of great importance for the efficacy of Bt toxins. As, for example, Kramarz et al. (2007 and 2009) show, parallel exposure to chemical toxins can lead to Bt toxins having an effect on organisms that are not normally susceptible. In addition, Bøhn et al. (2016) show additive effects of several Cry toxins. Cry toxins interact with Roundup / glyphosate when co-exposed to *Daphnia magna*. These cumulative effects also have to be assessed in regard to food and feed usages (see also Bøhn, 2018).

In regard to immunogenicity (non-IgE-mediated immune adverse reactions), it is generally acknowledged that Bt toxins are immunogenic (Santos 2018-Vigil et al., 2018; Rubio-Infante & Moreno-Fierros, 2016; Adel-Patient et.al., 2011; Andreassen et.al., 2015a,b; Andreassen et.al., 2016; see also Then & Bauer-Panskus, 2017). These observed effects are likely to be dose-dependent. Stacked events have a much higher concentration of Bt toxins than other plants, such as the single plants which were tested in feeding studies. Further, the concentration of Bt toxins in the plants varies substantially (see Testbiotech 2011).

Moreover, it is evident that Bt toxins can survive digestion to a much higher degree than has been assumed by EFSA: Chowdhury et al., (2003) as well as Walsh et al. (2011) have found that Cry1A proteins can frequently and successfully still be found in the colon of pigs at the end of digestion when they were fed with Bt maize. Thus, the Cry1A proteins can show much higher stability, at least in monogastric species, than predicted by current in vitro digestion experiments. Thus, Bt toxins are not degraded quickly in the gut and can persist in larger amounts until digestion is completed, and there is enough time for interaction between various food compounds. Consequently, there is substantiated concern that especially the stacked event can trigger immune responses and have adverse health effects.

There are further relevant issues: for example, the potential impact on the intestinal microbiome also needs to be considered. Such effects might be caused by the residues from spraying since glyphosate has been shown to have negative effects on the composition of the intestinal flora of cattle (Reuter et al., 2007) and poultry (Shehata et al., 2013). Further, Bremmer and Leist (1997) examined the possible conversion of NAG to glufosinate in rats. Up to 10% deacetylation occurred at a low dose of 3 mg/kg bw as shown by the occurrence of glufosinate in the faeces. The authors concluded, however, that most of the conversion was caused by bacteria in the colon and rectum, although toxicity findings indicate partial bioavailability (Bremmer & Leist, 1997, see also EFSA 2017b). In general, antibiotic effects and other adverse health effects might occur from exposure to a diet containing these plants that were not assessed under pesticide regulation. These adverse effects on health might be triggered by the residues from spraying with the complementary herbicide (see also van Bruggen et al., 2017). Further attention should be paid to the specific toxicity of the metabolites of the active pesticide ingredients that might occur specifically in the stacked event. For example, glufosinate is classified in the EU as showing reproductive toxicity.[28] However, there seems to be no detailed assessment of EFSA of the metabolites arising from spraying glufosinate onto these plants.

Both the EU pesticide regulation and the GMO regulation require a high level of protection for health and the environment. Thus, in regard to herbicide-resistant plants, specific assessment of residues from spraying with complementary herbicides must be considered to be a prerequisite for granting authorisation. In addition, cumulative effects have to be investigated if a plant contains or produces other compounds of potential toxicity.

Moreover, cumulative effects must be investigated if a plant contains or produces other compounds of potential toxicity. It should be acknowledged that no new methodology is needed to assess the health risks emerging from the combinatorial application of the herbicides and their potential interaction with the other plant constituents. Suitable methodology to assess combinatorial effects that emerge from *simultaneous exposure* to a *fixed combination* of potential stressors via a *defined route of exposure* (as it is the case with food and feed products derived from genetically engineered plants that are made resistant to several herbicides) is available and widely used. For example, chronic feeding or multigenerational studies are a well-established method of generating the relevant data.

As a result, the toxicological assessment carried out by EFSA is not acceptable.

Allergenicity
No data were presented to show that plant composition is unchanged in regard to allergenic potential.

There might be various reasons why allergenic potential in the stacked event is increased: higher applications of glyphosate will not only cause a higher burden of residues in the harvest, but may also change the composition of the plants in regard to naturally occurring allergens. Higher concentration of Bt toxins might trigger adjuvant effects in regard to other components in the diet. No data were presented to assess such potential effects.

Consequently, in regard to allergenicity the assessment cannot be regarded as conclusive.

[28] http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN

Others
For 14 subcombinations of the five-event stacked maize, no experimental data were provided at all. There is, therefore, a high level of uncertainty in regard to all levels of risk assessment as mentioned above.

Environmental risk assessment
Any spillage from the kernels has to be monitored closely. EFSA completely overlooked that populations of teosinte are abundant in Spain and France; these have to be considered to be wild relatives that enable gene flow and potential spread of the transgenes throughout the fields and the environment (Trtikova et al., 2017).

In this regard, the opinion of EFSA (2017a) is extensively flawed since the authority refers to completely outdated literature on the occurrence of wild relatives in Europe: "*Populations of sexually compatible indigenous wild relatives of maize are not known in Europe (Eastham and Sweet, 2002; OECD, 2003), therefore vertical gene transfer is not considered to be an environmental issue in the EU.*" However, since 2009, teosinte, a wild relative of maize, is known to occur in Spain. There are further reports from France about its occurrence that might encompass further regions in the EU (Trtikova et al., 2017).

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

Further, as shown by Pascher (2016), EFSA is also underestimating the risks posed by occurrence of volunteers from maize plants.

Consequently, environmental risk assessment carried out by EFSA is not acceptable.

Conclusions
The EFSA risk assessment should not be accepted. EFSA did not request any empirical data regarding toxicity and impact on the immune system, and did not name the knowledge gaps or uncertainties. Combinatorial effects were ignored as well as the consequences of spraying higher dosages of the complementary herbicides . The environmental risk assessment is based on incorrect assumptions. The monitoring plan has to be rejected because no evaluated method was made available that would allow case specific identification. Further, no system is foreseen to perform case specific monitoring of spillage and potential health effects.

3. Grounds for the request to reviewing the decision

The grounds for the request for reviewing the decision to allow the Maize for import are based on the violation of EFSA' s obligation to perform risk assessment in accordance with EU regulations (grounds 1-10). Further, the EU Commission failed to comply with its obligation as risk manager (grounds 11-13). In assessing the request, it should be taken into account that some of the crucial publications presented in the request (Trtikova et al., 2017 Santos-Vigil et al., 2018; Fang et al., 2018) were not available at the time when EFSA performed its risk assessment of the Maize and Monsanto filed its application.

Overview of reasons for requesting an internal review of the Commission's decision:

Assessment of health risks:

- impact on the immune system (3.1 and 3.2)
- combinatorial effects (3.3)
- design of field trials (3.4 -3.6)
- safety of all subcombinations (3.7)

Assessment of environmental risks:

- gene flow to wild relatives (3.8)

Monitoring:

- traceability and identification of the event (3.11)
- controllability of the characteristics of the relevant products 3.12

The above reasons are further elucidated below. The points raised in Chapter 2 (Overview EFSA risk assessment of the Maize) show that the decision of the EU Commission was not in accordance with EU Regulations and must therefore be revised.

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

According to Santos-Vigil et al (2018), the Bt toxin Cry1Ac can act as an allergen if ingested. This publication which was not available at the time of the risk assessment is nevertheless highly relevant: the Bt toxin Cry1Ac was used as a source for the synthesis of Cry1A.105 as expressed in the Maize.[29] Therefore, the synthetically derived Cry1A.105 toxin produced in the Maize has structural similarity with Cry1Ac. If Cry1Ac is suspected of being an allergen, the source of Cry1A.105 has to be verified as allergenic and therefore investigated in detail.

The EU Commission initially 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. However, EFSA (EFSA, 2018b) came to the conclusion that the Santos-Vigil et al. (2018) publication does not provide any new information and suffers from methodological flaws.

[29] See US patent application Patent 6,326,169

This EFSA opinion cannot, however, solve the problems in the risk assessment of the Maize:

(1) The EFSA opinion is biased
EFSA cites many details that may be seen as calling the Mexican study into question. However, there was never any direct contact with the scientists to exchange viewpoints. Furthermore, EFSA does not identify any relevant findings that need further investigation. This is surprising, since EFSA states that there are as yet not many empirical investigations available. Based on this approach, which is not in accordance with normal scientific standards, EFSA comes to the conclusion that the new publication does not present a challenge to its previous opinions.
On the other hand, studies supporting the previous opinions of EFSA are referred to without checking their scientific robustness and reliability: for example, EFSA several times refers to research from Austria (Reiner et al., 2014) showing that feeding mice with genetically engineered maize (MON810) did not enhance immune responses. However, the findings of Reiner et al. (2014) are highly questionable: in contrast to the research carried out by the Mexican scientists, the Austrian scientists did not measure the amount of Bt toxin taken up by the animals. Therefore, they cannot be sure whether the Bt toxin was present in the feed at all, or if it was only present in very low amounts. For example, the Bt toxin might have degraded after harvest due to storage and processing, or due to the varietal background, or environmental conditions; it might, further, have been produced in the plants at only very low levels (for assessment of such effects see Trtikova et al., 2015). Moreover, the Austrian scientists did not investigate the same protein as the Mexican scientists. Consequently, it cannot be said that Reiner et al. (2014) provide any arguments against the findings of Santos-Vigil et al. (2018).

There might have been a specific reason for EFSA's conclusion: the authority would have called its own previous risk assessments into question if it had come to any other conclusion. Furthermore, EFSA and the EU-Commission are involved in a court case (C-82/17 P) on the potential immune responses caused by Cry1Ac produced in the genetically engineered soybean 'Intacta' (MON 87701 x MON 89788) produced by Monsanto. Both the EFSA and the Commission are involved in this court case and are defending the risk assessment and decision-making process that allowed the soybean to be imported (see also Then & Bauer-Panskus, 2017). This court case might have been an additional and very specific reason for EFSA to take a biased perspective of the research carried out by the Mexican scientists.

(2) The outcome of the EFSA assessment is not valid
Testbiotech asked the Mexican scientists for their comments on the EFSA assessment. In their reply (Moreno-Fierros et al., 2018), showed that EFSA is not correct on crucial details and explain why their own findings are still valid. The Mexican scientists state that their research was not designed to find evidence of detrimental effects from consumption of food derived from genetically engineered plants. They do, however, emphasise that their findings show that these risks should be investigated in more detail: *"We consider our publication contributes to the knowledge of the immunological effects of Cry1Ac toxin and the new information provided should not be negatively judged or disqualified just because it has been considered relevant for the risk assessment of GM plants."*

The Mexican scientists summarise their reply to EFSA as follows: *"We are aware that more studies are required to determine the potential immunological effects derived from the consume of GM plants containing Cry1Ac, we are also aware that the dose of Cry1Ac used in our study was higher than the one reported to be expressed in most GM plants but we*

disagree with the point of view regarding the shortcomings in our study design and data interpretation." As the Mexican scientists state further: *"… there are variations in the expression levels in the distinct plants and tissues. Moreover, there are already stacked events on the market that produce a much higher overall concentration of Bt toxins than plants producing just one Bt toxin."*

EFSA (2018b) criticised the relatively high dosage of the Cry toxin used by Santos-Vigil. However, this is not an argument that can be used to resolve the problems in the risk assessment of the Maize: the Mexican scientists used 50µg of the toxin Cry1Ac to trigger the immune response in mice; this can be considered to be a relatively high dosage for raw products derived from genetically engineered plants producing just one Bt toxin. However, according to the application (EFSA 2017a, 2013, Table 5), the six toxins produced in the kernels of the Maize can add up to a concentration of 70 µg/g (dry weight). Furthermore, according to the application (Monsanto, 2103, Table 34), the concentration of the Bt toxins is much higher in gluten meal produced from the Maize: it can add up to a maximum of 800 µg/g (dry weight), with Cry1A.105 (as single substance) showing a concentration of up to 160µg/g. Therefore, there needs to be a much more careful risk assessment of the products derived from the Maize food & feed production in regard to allergenic reactions compared to those genetically engineered plants producing just one Bt toxin.

So far only very few Bt toxins produced in genetically engineered plants have been investigated in regard to their potential impact upon the immune system which might be allergenic or also Non-IGE. None of the toxins produced in the Maize were investigated in this regard in any empirical research. The effects caused by a combination of these toxins also remained untested.

In conclusion, the EFSA opinion regarding the application for the Maize (EFSA 2017a) cannot be said to fulfil the requirements for assessing allergenicity of the source of the transgene. The Santos-Vigil et al (2018) publication has to be considered to be both valid, and not properly assessed by EFSA (Moreno-Fierros et al., 2018). In awareness of the high concentrations of Bt toxins produced in the Maize and products derived thereof, EFSA should have started with the hypothesis that the consumption of the Maize can trigger allergic reactions – and should therefore have requested empirical investigations.

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

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 et al. 1999; Legorreta-Herrera et al., 2010; Jarillo-Luna et al. 2008; E. González-González et al., 2015; Ibarra-Moreno et al., 2014; Guerrero et al. 2007; Guerrero et al., 2004; Moreno-Fierros et al. 2013; Rubio-Infante et al. 2018) are especially relevant (for review also see Rubio-Infante et al. 2016).

As mentioned, the Bt toxin Cry1Ac was used as a source for the synthesis of Cry1A.105 expressed in the Maize.[30] Therefore, the synthetically derived Cry1A.105 toxin produced in the Maize has

[30] See US patent application Patent 6,326,169

structural similarity with Cry1Ac. If Cry1Ac is immunogenic, Cry1A.105 is also likely to be immunogenic.

All the reactions described in the above publications are likely to be dependent on the dosage to which the mammals are exposed. In this regard and as mentioned above, the investigation of potential immune responses triggered by the Maize is highly relevant: according to the application (EFSA 2017a, Table 5), the six toxins produced in the kernels of the Maize can add up to a concentration of 70 µg/g (dry weight). Furthermore, according to the application (Monsanto, 2013; Table 34), the concentration of the Bt toxins is much higher in gluten meal produced from the Maize: it can add up to a maximum of 800 µg/g (dry weight), with Cry1A.105 (as a single substance) showing a concentration of up to 160µg/g. Therefore, the products derived from the Maize food & feed production need to be much more carefully risk assessed in regard to their impact on the immune system and potential adjuvanticity compared to those genetically engineered plants producing just one Bt toxin.

In its risk assessment, EFSA did not consider that under real conditions and contrary to what is suggested by the findings of in-vitro studies, Bt toxins will not be degraded quickly in the gut but are likely to occur in substantial concentrations in the large intestine and faeces (Chowdhury et al., 2003; Walsh et al., 2011).

In regard to the degradation of the Bt toxins during ingestion, there is specific cause for concern that the Maize or gluten is likely to be fed together with soybeans that naturally produce enzymes that can substantially delay the degradation of Bt toxins in the gut (Pardo-López et al., 2009). In addition, soybeans are known to produce many food allergens. Therefore, the immune responses caused by the allergens in the soybeans might be considerably enhanced by the adjuvant effects of the Bt toxins. Such effects are likely to lead to detrimental effects on health.

Furthermore, it also has to be taken into account that so far only very few Bt toxins produced in genetically engineered plants have been investigated in regard to their potential impact upon the immune system. As yet, only two Bt toxins (Cry1Ac and Cry1Ab) have been tested for their possible effects on the immune system; none of the toxins produced in the Maize were investigated in this regard in any empirical research. The effects caused by a combination of these toxins also remain untested.

The need for more detailed investigations in regard to potential immunogenic effects is also underlined in the minority opinion in another EFSA opinion (Annex II of EFSA, 2018c):
"However, compositional data and reliable information on the actual concentrations of the NEPs[31] are crucial to achieve a sound safety assessment. Indeed, it has been shown that the genetic background of the recipient plant has a major effect on Cry1Ac expression in GM cotton (Adamczyk & Meredith 2004) and maize (Trtikova et al., 2015; Zeljenkova et al., 2014); it may cause an additional variability (not taken into account by the GMO Panel so far) in Bt protein concentrations which might impact on the safety. The risk of increased expression of the newly expressed Bt proteins and of a possible cumulative effect of their combination on the immune system (e.g. resulting in an adjuvant activity) cannot be ruled out although it is difficult to evaluate in the absence of actual experimental data. Indeed, the scope of AP 86[32] is for import and processing which suggests a limited exposure for consumers in the EU. Nevertheless, should those subcombinations (or some of them) be produced and commercialised in the future, the resulting risk

[31] Explanation by Testbiotech: newly expressed proteins
[32] Explanation by Testbiotech: the application concerns another genetically engineered maize, Bt11 x MIR162 x 1507 x GA21

for human health, particularly in workers, might be higher than that of singles or of the fully assessed Bt11 x MIR162 x 1507 x GA21 maize."

The minority opinion also addresses this problem from a more general perspective:
"Indeed, unintended effects on the immune system have never been identified in any application where Bt proteins were expressed; but at the same time, it should also be noted that they could not be observed by the toxicological studies (i.e. 28-day repeated-dose tox studies and/or 90-day feeding trials) currently recommended and performed for the safety assessment of GM plants at EFSA because they do not include appropriate tests for this purpose."

The opinion also addresses the immense importance of risk assessment being conducted thoroughly in this context:
"Allergic reactions in general and particularly food allergy are dramatically increasing in the EU (and worldwide) and have become a most important public health issue. The reasons are unclear, but most specialists involve the changes in environmental conditions, in cultivated plant species and in food habits. Indeed, environmental conditions are known to play a major role in the occurrence and/or severity of the allergic reaction in addition to the genetic background of predisposed individuals and characteristics of the allergen. They include the route and doses of exposure to the protein in question but also the presence in the food/diet of compounds known to modulate (e.g. increase) the immune response to other unrelated proteins present in the food. The potential role of these 'adjuvants' is therefore emphasised and especially in the case of immunoglobulin E (IgE)-mediated allergy.
It is thus a pity that a high-double uncertainty due to both a lack of knowledge and a lack of data, still remains which clarification would improve the assessment, clarify the role/absence of role of GMOs in the increasing allergenic risk and finally allow a solid protection and prevention of at risk consumers."

The EFSA GMO Panel (2018b) also admitted that there is only "*limited experimental evidence available"*. Therefore, in the light of current uncertainties, experimental data on the allergenic potential should have been requested.

In conclusion, the EFSA opinion on the application for the Maize (EFSA 2017a) cannot be said to fulfil the requirements for assessing the possible role of the Bt toxins produced in the plants as adjuvants. The hypothesis that should have been used as a starting point is that the Bt toxins can cause immune responses which are dependent on a dosage-response relationship. Therefore, several dosages of the Bt toxins produced in the Maize should have been tested regarding their impact on the immune system, alone and in combination. Furthermore, combinatorial (adjuvant) effects should have been tested in interaction with known allergens.

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

(1) In regard to the allergenicity of the combination of the transformation events, several studies indicate that Bt toxins (see above) trigger immune responses in mammals. In addition, and as mentioned, Bt toxins under real conditions and contrary to what the findings in-vitro studies suggest, will not be degraded quickly in the gut but are likely to occur in substantial concentrations in the large intestine and faeces (Chowdhury et al., 2003; Walsh et al., 2011)). In regard to the

degradation of the Bt toxins during ingestion, there is specific cause for concern that the Maize or gluten will probably be fed together with soybeans that naturally produce enzymes, which can substantially delay the degradation of Bt toxins in the gut (Pardo-López et al., 2009). In addition, soybeans are known to produce many food allergens. Therefore, the immune responses caused by the allergens in the soybeans might be considerably enhanced by adjuvant effects of the Bt toxins. Such effects are likely to cause detrimental effects on health.

(2) In regard to toxicology and potential synergistic or other combinatorial effects, negative impacts caused by Bt toxins on human and animal health cannot be excluded a priori. Bt toxins have several modes of action (Hilbeck & Otto, 2015). Not all of them are dependent on the specific mechanisms that only occur in the target insect species. Only very few Bt toxins (especially Cry1Ab, for overview see, for example, Then, 2010) were investigated in more detail in regard to their exact mode of action, and there is no data on the Bt toxins produced in the Maize. Further, no data were presented to show that the toxins produced in the plants are only activated and become effective in insects. On the other hand, several publications exist showing 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. (2012) and Bondzio et al. (2013) show that Cry proteins could potentially have an impact on the health of mammals. Two recent publications (de Souza Freire et al., 2014; Mezzomo et al., 2014) confirm hematotoxicity of several Cry toxins, including those being used in genetically engineered plants such as Cry 1Ab and Cry1Ac. These effects seem to occur after high concentrations and tend to become stronger after several days. Such observations call for the study of effects after long-term exposure to lower dosages and / or in combination with relevant herbicides as described above. Therefore, potential synergistic or antagonistic effects must be examined.

In this context it is important that the Maize is also resistant to the herbicides glyphosate and glufosinate, both of which should be seen as potential co-stressors (see Then & Bauer-Panskus, 2017). By crossing MON 87427 with MON 88017 (or the previously authorised SmartStax maize MON 89034 x 1507 x MON 88017 x 59122, see EFSA 2010), the content of EPSPS enzymes that confer resistance to glyphosate is increased in vegetative and female reproductive tissues, conferring tolerance to glyphosate in the leaves, stalk, and root tissues and tissues that develop into grain or grain and silks. Consequently, it has to be expected that these plants can and will be exposed to higher and also repeated dosages of glyphosate. It can be expected that in Argentina, Brazil and the US, there will be different prices, different herbicide formulations and varying regimes of herbicide applications in Maize cultivation. Due to specific agricultural practices in the cultivation of these herbicide-resistant plants, specific patterns of herbicide sprayings and subsequent exposure to specific metabolites, including the emergence of combinatorial effects, require special attention.

The only data presented by the applicant are potential interactions between the Bt toxins and the enzymes which confer resistance to the complementary herbicides. However, these enzymes are not known as potential stressors. Residues from spraying with the complementary herbicides and their metabolites are of much more toxicological relevance. Bøhn et al. (2016) not only show additive effects of several Cry toxins in non-target organisms but also that Cry toxins interact with Roundup / glyphosate when co-exposed to *Daphnia magna*. These cumulative effects also have to be assessed in regard to food and feed usages (see also Bøhn, 2018). In general, it has to be assumed that other toxins present in diets can enhance the effects of Bt toxins. As, for example, Kramarz et al. (2007 and 2009) show, parallel exposure to chemical toxins can lead to Bt toxins having an effect on organisms that are not normally susceptible.

(3) It also has to be taken into account that Bt toxins and/or residues from spraying with the complimentary herbicides can effect health via the intestinal microbiome. For example, glyphosate has been shown to have negative effects on the composition of the intestinal flora of cattle (Reuter et al., 2007) poultry (Shehata et al., 2013) and rats (Mao et al., 2018). Further, Bremmer and Leist (1997) examined the possible conversion of NAG to glufosinate in rats. Up to 10% deacetylation occurred at a low dose of 3 mg/kg bw as shown by the occurrence of glufosinate in the faeces. The authors concluded that most of the conversion was caused by bacteria in the colon and rectum, although toxicity findings indicate partial bioavailability (Bremmer & Leist, 1997). Changes in the gut microbiome are known to be implicated in many diseases. Consequently, potential synergistic or antagonistic effects on gut microorganisms from the consumption of the Maize need to be tested. These tests must take into account combinatorial effects caused by the combination of Bt toxins as well as material derived from the Maize sprayed with the complementary herbicide. However, adverse health effects triggered by exposure of the gut micrombiome to a diet containing the Maize were not considered at all. There are no data at all to show that no effects occur in gut microorganisms after ingestion of products derived from the Maize.

In conclusion, the EFSA opinion on the application for authorisation of the Maize (EFSA 2017a) cannot be said to fulfil the requirements for assessment of potential synergistic or antagonistic effects resulting from the combination of the transformation events in regard to toxicology and allergenicity. The hypothesis which should have been used as a starting point is that there will be synergistic effects between the various Bt toxins and between the various Bt toxins and other stressors, such as residues from spraying. Therefore, the effects of the Bt toxins in regard to mammalian cell systems and intestinal microbiomes should have been tested in combination with other stressors. Furthermore, combinatorial (adjuvant) effects triggered by Bt toxins occurring in high concentration in the Maize and especially in gluten prepared from the Maize, have to be tested in interaction with known allergens, such as the one occurring in soybeans. For this purpose, EFSA should have requested that Monsanto submit data from field trials with the highest dosage of glyphosate that can be tolerated by the plants, also including repeated spraying. The material derived from those plants should have been assessed in regard to organ toxicity, immune responses and reproductive toxicity, also taking combinatorial effects with other plants components and the Bt toxins into account.

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

Under current EFSA risk assessment practice established in the EU, the analysis of the composition of plant material is especially crucial when comparing the genetically modified food and feed with its conventional counterpart. Therefore, the uses of the genetically modified feed and expected agricultural practices need to be taken into account for the selection of the material used in the comparative assessment. The data from the field trials will only represent the material at the point of entry into food and feed production and the processing chain if the field trials are designed in accordance with the practical conditions under which these plants are grown.
As far as herbicide resistant genetically engineered plants are concerned, the field trials need to be designed so that data from the plants treated with the complementary herbicide and untreated plants can be compared in order to assess whether the expected agricultural practices influence the expression of the studied endpoints. This is important since application of the herbicides can influence gene expression, agronomic performance and plant composition. These data are highly

relevant for the assessment of health risks because it is important that the herbicide regime and the material chosen for measuring the endpoints represents those products that will be used for food and feed production.

However, in case of the Maize, the design of the field trials does not allow any assessment of whether the expected agricultural practices will influence the relevant endpoints: according to the applicant (Ref, page 83), only one dosage of each of the complementary herbicides was sprayed on the Maize during the field trials:
"The application was made to all rows when plants were at approximately the V2-V4 growth stage. The glyphosate treatment was applied at approximately 1.1 kg a.i./ha (kilograms of active ingredient per hectare). Following the glyphosate treatment, a sequential treatment of glufosinate was applied (…). The application was made to all rows when plants were at approximately the V3-V6 growth stage. The glufosinate treatment was applied at approximately 0.45 kg a.i./ha (kilograms of active ingredient per hectare)."

EFSA did not any request further data on the expected agricultural practices regarding the application of the complementary herbicides in the various regions where the Maize is to be grown. However, a comparison with existing official recommendations from one of the respective regions (Canada) shows that there are huge differences between what can be applied in practice and what was used in the field trials. A guide for weed control published by the Ministry of Agriculture, Food and Rural Affairs of Ontario (2016/2017) recommends one or two applications of glufosinate and / or glyphosate for herbicide resistant maize. The recommended dosage is much higher than that applied in field trials of the applicant. Further, the latest time for spraying was when the maize had plants had eight leaves (V8), while in the field trials this was between the V2 and V6 stage.

Thus, compared to the official recommendations from the Ministry of Agriculture, Food and Rural Affairs of Ontario (2016/2017), which is representative for an important region of maize production, three substantial differences can be identified compared to the herbicide regime applied in the field trials (Monsanto, 2013): time of spraying, number of sprayings and overall dosage of herbicide applied (see also Table 1). Further, a distinction is made whether applications are made in combination with other herbicides, while in the field trials (Monsanto, 2013) the complementary herbicides were only applied in combination. In addition, it should be noted that there are various mixtures and combinations that can be sprayed on the Maize when it is grown in regions with high weed pressure – this will also imply higher dosages.

Table 1: Comparison of herbicide regimes recommended for herbicide resistant corn by the Ministry of Agriculture, Food and Rural Affairs of Ontario (2016/2017) and applied by Monsanto (2013) in the field trials with the Maize

Source	Ministry of Agriculture, Ontario	Ministry of Agriculture, Ontario	Monsanto (2013)
Herbicide (applied postemergence)	one pass (1-8 leaf stage of corn)	two pass (up to 8 leave stage of corn)	field trials (one pass, 2-6 leaf stage of corn)
Glyphosate	1,8 kg/h[33]	1,8 kg/h[34]	1,1 kg/h[35]
Glufosinate	0,5 kg/h[36]	0,5 + 0,4 kg/h[37]	0,45 g/h[38]

[33] Ministry of Agriculture, Food and Rural Affairs of Ontario (2016/2017), table 9-9, page 162
[34] Ministry of Agriculture, Food and Rural Affairs of Ontario (2016/2017), table 9-9, page 166
[35] Monsanto (2013), page 83
[36] Ministry of Agriculture, Food and Rural Affairs of Ontario (2016/2017), table 9-12, page 171
[37] Ministry of Agriculture, Food and Rural Affairs of Ontario (2016/2017), table 9-12, page 172
[38] Monsanto (2013), page 83

There are many findings in existing publications showing that the application of the complementary herbicides can change plant composition. However, only very few investigations have systematically assessed such effects in regard to all relevant plant species, including maize. For example, Zobiole et al (2012) tested whether spraying 0.8, 1.2 and 2.4 kg/ha of glyphosate affected the nutritional status of genetically engineered soybeans. This study showed that increasing glyphosate applications and spraying plants in later stages of growth decreased nutrient accumulation, nodulation, leaf area, and shoot biomass production in the plants.

Crossing MON 87427 with MON 88017 (or the previously authorised SmartStax maize MON 89034 x 1507 x MON 88017 x 59122, see EFSA 2010) increases the content of EPSPS enzymes that confer resistance to glyphosate in vegetative and female reproductive tissues, and therefore confers tolerance to glyphosate in the leaves, stalk, and root tissues and tissues that develop into grain or grain and silks. Consequently, there is an expectation that these plants can and will be exposed to higher and repeated dosages of glyphosate beyond official recommendations. These applications of glyphosate will not only lead to a higher burden of residues in the harvest but may also influence the composition of the plants and agronomic characteristics.

As recent research shows, there are previously undetected mechanisms that can cause these changes in genetically engineered plants resistant to glyphosate: Fang et al. (2018) found interactions between the additional enzyme (EPSPS) inserted to confer resistance to glyphosate and the plants own growth hormone (auxin) metabolism. This plant hormone plays a key role in growth, fecundity and adaptation to environmental stressors. Thus, higher expression of the EPSPS enzyme, potentially triggered by high dosage spraying with glyphosate, can lead to changes on several levels in plant composition and phenotypical characteristics. Therefore, the dosage and number of sprayings with the complementary herbicides have to be taken into account particularly for this stacked Maize which inherits a double resistance to glyphosate.

It should further be taken into account that the mixture of the complementary herbicide (glyphosate and glufosinate) will not always be used in the fields where the Maize is cultivated; in some cases just one of them 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 problem and regional agricultural practices. For example, it can be expected that in Argentina, Brazil and the US, there will be different prices, different herbicide formulations and varying regimes of herbicide applications under which the Maize is cultivated. None of these specific agronomic practices were considered in the design of the field trials or in EFSA risk assessment.

There was, therefore, no assessment of whether the expected agricultural practices influence the expression of the studied endpoints. The tested plant materials are not representative for the material that will enter the food chain. The data as presented show a high number of significant differences (~ 75% of the analytes evaluated for plants composition). Even if changes taken as isolated data might not directly raise safety concerns, the overall number of effects and their clear significance has to be taken as a starting point for much more detailed investigations. If the field trials had been performed under practical conditions and with the respective herbicide regimes, a much broader range of significant differences could have been expected. Based on these data, the field trials should have been designed to assess whether the expected agricultural practices influence the expression of the studied endpoints, and to make sure that the plant materials used in the assessment of comparative plants composition and agronomical characteristics is representative of those designed for import. Consequently, the findings of comparative assessment of plant composition and agronomic characteristics cannot be accepted.

The hypothesis that should have been used as a starting point is that the plant composition can be changed by the herbicide regime (dosage, number of sprayings, time of sprayings, mix of active ingredients). EFSA should have requested representative data on herbicide regimes as established in all the regions where the Maize will be cultivated. In addition, EFSA should have requested that Monsanto submit data from field trials with the highest dosage of glyphosate that can be tolerated by the plants, also including repeated spraying. The material derived from those plants should have been assessed in regard to gene expression, plant composition and agronomic characteristics. Further, the material should have been used to investigate organ toxicity, immune responses and reproductive toxicity, also taking combinatorial effects with other plant components and the Bt toxins into account.

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

Variation in gene expression, plant composition and phenotypical characteristics of genetically engineered plants may have several sources: variation due to environmental factors or genetic factors, or a combination of both genetic and environmental factors. Environmental stress can cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015). Genome x environment interactions can also cause dysfunctional reactions or other unexpected effects in transgenic plants (Zeller et al., 2010; Matthews et al., 2005; Meyer et al., 1992; Then & Lorch, 2008; Zhu et al., 2018. Therefore, the different sites selected for the field trials need to reflect the different meteorological and agronomic conditions under which the crop is to be grown.

Especially relevant in this context are the findings of Fang et al. (2018), which show that stress conditions can affect the characteristics of genetically engineered plants inheriting additional EPSPS enzymes on several levels. With this Maize, it is important to keep in mind that compared to previous stacked events an additional EPSPS enzyme was added by further crossing with MON87427.

However, in this case, the design of the field trials only represents a very narrow range of different meteorological and agronomic conditions under which the Maize will be grown:

(1) In regard to meteorological conditions, the field trials carried out by Monsanto (2013) only represent one year and one maize growing region. In addition, data from the field trials affected by more extreme weather conditions were excluded from the assessment:
"MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 treated (T) and untreated (NT) and the conventional counterpart (EXP262), were initially planted at twelve field trial sites during the 2010 growing season in order to collect compositional and agronomic/phenotypic data. From the twelve sites, two sites were adversely affected by weather conditions, one could not be used for compositional analyses due to excessively dirty samples (…). It is common practice to plant an excess number of field trial sites to reduce the risk of having an insufficient number of field trials to analyze because of the potential for uncontrollable weather conditions such as frost, flooding, disease etc." (Monsanto, page 82)

As a result, and in respect to ongoing climate change and the various regions where the Maize will be cultivated, including Argentina and Brazil, the range of the respective data from the field trials

used in risk assessment is too narrow to reflect the real conditions in cultivation. These data do not represent the different meteorological conditions under which these plants are cultivated in other regions and in other years.

In regard to agronomic conditions, the data from field trials also fail to reflect the different conditions under which the crop is to be grown. As shown above, further data would be needed on established herbicide regimes in all the regions where the Maize will be cultivated (see above). In addition, reactions to defined biotic stressors, such as bacterial and fungal diseases, were not tested.

In summary, the data used for assessing the expression of the additional enzymes, the comparison of the plants composition and the agronomic characteristics were only collected from field conditions in the US for one year and exclude data from more extreme weather conditions - so that the plants were not exposed to a sufficiently broad range of agronomic practices and relevant stress factors (biotic and abiotic).

Consequently, the outcome of comparative assessment of plant composition and agronomic characteristics cannot be accepted. The tested plant materials are not representative for the material that will enter the food chain. The data presented show a high number of significant differences (~ 75% of the analytes evaluated for plants composition). Even if changes taken as isolated data do not necessarily raise direct safety concerns, the overall number of effects and their strong significance has to be taken as a starting point for much more detailed investigations. If the field trials had been conducted under more realistic conditions, a much broader range of significant differences could have been expected to emerge.

A much broader range of significant differences could also have been expected to emerge if the field trials had been conducted under practical conditions with the respective herbicide regimes. The field trials should have been designed to assess whether different meteorological and agronomic conditions under which the crop is expected to be grown would influence the expression of the studied endpoints; and to ensure that the plant materials used in the assessment of comparative plant composition and agronomic characteristics is representative of those designated for import.

Based on the scientific evidence available, EFSA should have started from the hypothesis that differences in the meteorological and agronomic conditions can affect the expression rate of the additionally inserted DNA sequences, plant composition and agronomic characteristics. Thus, EFSA should have requested further studies e.g.

- field trials that lasted more than one season,
- field trials in other maize growing regions,
- field trials with the highest dosage of glyphosate that can be tolerated by the plants, including repeated spraying.
- data from climate chamber or green house experiments where the Maize was subjected to a broad range of defined stressors,
- in addition, more varieties carrying the transgenes should have been included in the field trials to see how the gene constructs interact with the genetic background of the plants.

Since the data used in the risk assessment do not sufficiently reflect the different meteorological and agronomic conditions under which the crop is to be grown, the outcome regarding the expression data assessment, as well as that for the comparative assessment of plant composition and agronomic characteristics, cannot be accepted. Under the comparative approach conducted by EFSA, the outcome of the assessment of the molecular data, plant composition and agronomic conditions are also decisive for follow-on steps in risk assessment, such as the toxicological investigations. Both

the incorrectly designed field trials and lack of reliable data had a serious impact on the outcome of the overall risk assessment and this cannot, therefore, be regarded as conclusive.

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

As shown above, different meteorological and agronomic conditions can influence the expression of the additional DNA sequences inserted in the plants (see, for example, Trtikova et al., 2015). This is especially relevant in this case since Fang et al. (2018) showed that stress conditions can affect the characteristics of genetically engineered plants inheriting additional EPSPS enzymes. This issue is especially relevant for the Maize in which – compared to previous stacked events – an additional EPSPS enzyme was added by further crossing with MON87427, causing an higher overall expression rate compared to the parental plants. As EFSA (2017) states:
"(…) CP4 EPSPS and PAT showed the expected higher levels in the stack resulting from the combination of MON 87427 and MON 88017 (producing CP4 EPSPS) and 1507 and 59122 (producing PAT) events." (EFSA 2017a, page 13).

However, contrary to EFSA statements, it is important to note that not only the expression of the two enzymes conferring resistance to herbicides, but also the Bt toxins show a clear tendency towards higher expression rates in the stacked Maize (in grain and forage) compared to the expression of the Bt toxins in parental plants grown in comparison (see fig 1).

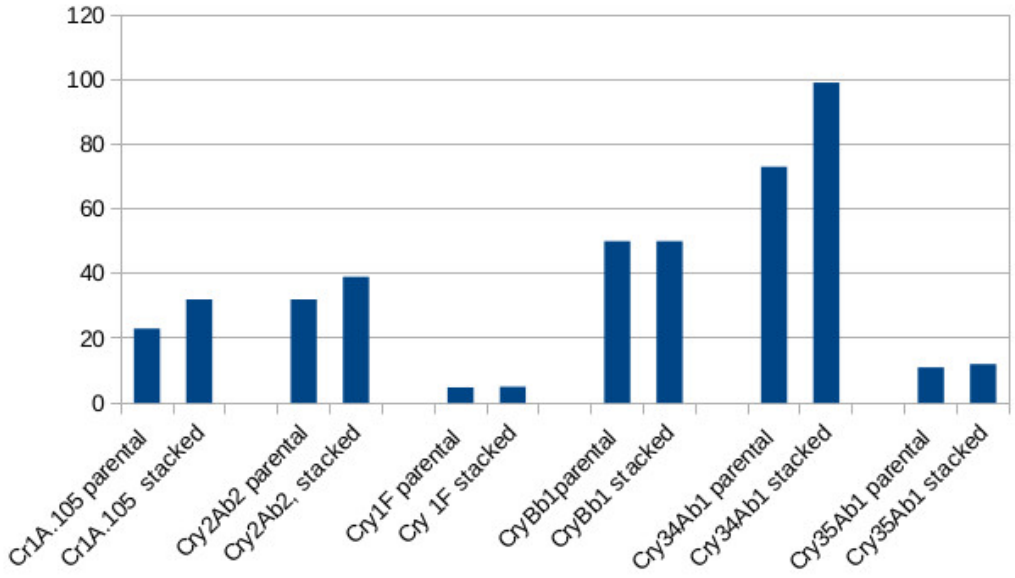

Fig 1: Expression of Bt toxins in the stacked Maize in comparison to the parental plants (forage, dry weight, µg/g) (source: Monsanto, 2013)

This effect might be caused by the stacking and the interference of the EPSPS enzyme with the plant's growth hormone (Fang et al., 2018), and might be enhanced by agricultural practices and conditions e.g. spraying with the complementary herbicide, meteorological conditions, impact of stressor and varietal backgrounds. Whatever the case, the expression of the newly expressed proteins is different from that of the parental plants.

Based on the scientific evidence available, EFSA should have tested the hypothesis that differences in the meteorological and agronomic conditions as well as different herbicide applications and

varietal backgrounds can affect the expression rate of the additional inserted DNA sequences. Furthermore, the subcombination should have been grown in parallel in the field trials with the Maize to allow for direct comparison.

Thus, EFSA should have requested further studies e.g.

- field trials that lasted more than one season,
- field trials in other maize growing regions,
- field trials with the highest dosage of glyphosate that can be tolerated by the plants, including repeated spraying.
- field trials including the subcombinations grown in parallel,
- experiments in climate chambers or green house experiments where the Maize was subjected to a broad range of defined stressors,
- in addition, more varieties carrying the transgenes should have been included in the field trials to see how the gene constructs interact with the genetic background of the plants.

Since the data used in the risk assessment do not sufficiently represent protein expression data, including the raw data, obtained from field trials and related to the conditions in which the crop is grown, the molecular risk assessment performed by EFSA cannot be accepted.

3.7 EFSA did not request data on all subcombinations.

The expression of the Bt toxins in the Maize shows a notably different pattern of expression compared to the stacked maize MON89034 x 1507 x MON88017 x 59122, which was assessed previously and produces the same Bt toxins (EFSA 2010): most of the toxins show higher expression in the 4-stacked (EFSA 2010) compared to the 5-stacked (EFSA 2017a) without any explanation being given (see Table 2).

Table 2: Comparison of the gene expression of Bt toxins (kernels, dry weight, µg/g) **in the Maize (EFSA 2017a) and one of its subcombination (EFSA 2010)**

	Stacked Maize (MON87427 x MON89034 x 1507 x MON88017 x 59122)	Subcombination (MON89034 x 1507 x MON88017 x 59122)
Cry1A.105	6-14	1,7-4,9
Cry1F	1,6-2,8	2,12-7,43
Cry2Ab2	1,2-2,8	2,7-7,5
Cry3Bb1	6,1-9,6	10-38
Cry34Ab1	29-42	43,6-102
Cry35Ab2	0,39-0,80	1,24-2,65

Despite these huge differences compared to previous applications, Monsanto did not present any data on subcombinations for the Maize. This is unacceptable since the subcombinations might not only show effects from stacking, but also other circumstances have to be considered: for example, some of the subcombinations might only be resistant to one of the complementary herbicides or produce toxins only for specific target pest organisms and will therefore be cultivated under different agronomic conditions. Consequently, there are several reasons why the subcombinations might in response show different expressions of the inserted DNA as well as different plant composition in comparison to the Maize and / or conventional varieties. Therefore,

without specific risk assessment data for each of the subcombinations, these cannot be approved for import.

The need to investigate the risks of the subcombinations in more detail is also highlighted in a statement made by an expert on the EFSA GMO panel in a minority opinion in 2018 (EFSA 2018c). The statement explains why subcombinations with stacked Bt toxins should be investigated more thoroughly. These arguments are also highly relevant for the risk assessment of the Maize:

"It is not acceptable that the same weight and reliability are given to the assessment of a GM crop for which a complete data set is available and can be comprehensively evaluated and to GM crops for which no specific data are provided, particularly when there is a health concern resulting from a possible over expression of the NEPs.[39]

In the case of AP 86[40]*, the safety assessment of the three subcombinations only relies on the data from the four-event stack, from the single events and from subcombinations previously assessed in other applications. Despite missing data on comparative analysis, the GMO Panel concludes that it expects no adverse effect on human health based on an extrapolation. The criteria and procedure used are not detailed and remain mostly implicit; the level of confidence that should be required for this extrapolation is not given and there is no critical appraisal of its limitations. No evaluation of the resulting uncertainty has been performed. These weaknesses may invalidate the conclusion which anticipates the absence of safety concerns and does not require that additional specific data shall be provided to EFSA to guarantee the safety of these three subcombinations should they be produced and imported to the EU market in the future.*

This MO[41] *is not only a question of principle since a risk for the human health may result from a possible over expression of NEPs due to the different factors of variability already mentioned if those subcombinations were to be produced. Hopefully the risk might be low, but the uncertainty could be much decreased if sufficient specific data were provided by the Applicant before all 'sub-combinations independently of their origin' are approved.*

Allergic reactions in general and particularly food allergy are dramatically increasing in the EU (and worldwide) and have become a most important public health issue. The reasons are unclear, but most specialists involve the changes in environmental conditions, in cultivated plant species and in food habits. Indeed, environmental conditions are known to play a major role in the occurrence and/or severity of the allergic reaction in addition to the genetic background of predisposed individuals and characteristics of the allergen. They include the route and doses of exposure to the protein in question but also the presence in the food/diet of compounds known to modulate (e.g. increase) the immune response to other unrelated proteins present in the food. The potential role of these 'adjuvants' is therefore emphasised and especially in the case of immunoglobulin E (IgE)-mediated allergy.

It is thus a pity that a high-double uncertainty due to both a lack of knowledge and a lack of data, still remains which clarification would improve the assessment, clarify the role/absence of role of GMOs in the increasing allergenic risk and finally allow a solid protection and prevention of at risk consumers.

One solution is at least to reduce the uncertainty on the levels of expression of NEPs by measuring

[39] newly expressed proteins
[40] Bt11 x MIR162 x 1507 x GA21 and three subcombinations, application EFSA-GMO-DE-2010-86
[41] minority opinion

and collecting actual reliable data for each subcombination through well-designed field trials and taking into account all the factors of variability including the genetic background of varieties used for producing the subcombinations/stacks." (EFSA 2018c, page 34)

In conclusion, the EFSA risk assessment is unacceptable. Based on the evidence available, EFSA should have tested the hypothesis that effects can emerge from the specific subcombinations of inserted DNAs that might be dependent on varietal backgrounds. In addition, the different subcombinations might be grown under different agronomic conditions compared to the Maize. Therefore, gene expression of the newly introduced DNA and plant composition can be substantially different compared to the Maize. Therefore, the subcombinations should have been grown in parallel in the field trials with the Maize in order to allow for direct comparison.

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

Monsanto (2013) gave incorrect information on the potential gene flow from the Maize to wild relatives in its application:
"There are no compatible wild relatives of maize in Europe." (page 12)

"It has been established that Z. mays can grow and potentially hybridize only with itself in the EU receiving environment since no wild relatives are found on the European continent. Z. mays volunteer potential is practically non-existent (see Section E.2.1; (OECD 2003). In addition, although maize grain from the previous crop year can over-winter in mild winter conditions and germinate the following year, it cannot persist as a weed (Hallauer, 1995). These qualities prevent Z. mays populations from persisting both in in-field and off-field." (page 180)

This information is incorrect or at least insufficient for several reasons:

- Monsanto completely ignored the appearance of teosinte in Spain and France (see Testbiotech, 2016; Trtikova et al, 2017). Thus, the statement that no wild relatives of maize would occur in Europe is simply wrong.
- In its assessment of the volunteer potential, the information provided by Monsanto is largely outdated. As Pascher et al (2016) shows, the volunteer potential of maize is higher than assumed by Monsanto.
- Further, in awareness of the findings of Fang et al. (2018), the Maize, with a double resistance to glyphosate needs to be examined in detail regarding next generation effects, volunteer potential (persistence) and gene flow. There are substantial reasons for following a hypothesis that the Maize can show higher fitness compared to conventional maize or genetically engineered maize with just one set of EPSPS enzymes.

In its opinion, EFSA (2017) was aware of the occurrence of teosinte in the EU and tried to assess the risks of gene flow. However, EFSA (2017) is wrong for several reasons:

- Without more data on the teosinte species growing in the EU, the likelihood of gene flow from the Maize to teosinte cannot be assessed (Trtikova et al, 2017). The same is true for gene flow from teosinte to genetically engineered plants.
- Furthermore, the characteristics of potential hybrids and next generations have to be investigated and cannot be predicted simply from the data of the original event. It is well known that there can be next generation effects and interference from genetic background that cannot be predicted from the assessment of the original event (Kawata et al., 2009; Cao et al., 2009; Yang et al., 2017; Bollinedi et al., 2017; Lu and Yang, 2009; Vacher et al., 2004;

Adamczyk & Meredith, 2004; Adamczyk et al., 2009). This issue is relevant for gene flow from maize to as well from teosinte to maize.

- Finally, it is well established under EU regulation that it is the applicant who has to present data sufficient to show that the respective event is safe before the application can be considered to be valid (see Kraemer, 2016). Thus, an application with incorrect or missing information on crucial aspects of environmental risk assessment cannot be accepted as a starting point for EFSA risk assessment.

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

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

As shown, the EFSA risk assessment suffers from substantial flaws:
1. EFSA did not request the applicant to provide data to verify whether the source of the transgene is allergenic.
2. EFSA did not request the applicant to assess the possible role of Bt toxins as adjuvants despite strong indications of this being relevant for hazard identification.
3. No data were requested regarding potential synergistic or antagonistic effects resulting from the combination of the transformation events in regard to toxicology and allergenicity.
4. EFSA did not request data which are necessary to assess whether the expected agricultural practices influence the expression of the studied endpoints.
5. EFSA did not request data on field trials to reflect the different meteorological and agronomic conditions under which the crop is to be grown
6. EFSA did not request sufficiently reliable protein expression data from the newly introduced proteins.
7. EFSA did not request data on all subcombinations.
8. EFSA did not request the applicant to assess gene flow to other wild plant species or from wild plants species to volunteers of the Maize.

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

3.10 The EU Commission decision to allow import was not in accordance with the EU regulations.

For the reasons outlined above (point 3.1.-3.9 and, in addition, the arguments presented in Chapter 2.), the Commission should not have accepted the opinion of EFSA (2017a) 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.

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

The EU Commission is responsible for the post-market monitoring plan. In general, methods for detection should be event-specific to allow effective monitoring based on identification at all stages of import and processing. However, based on the methods provided, under practical conditions it is not possible to identify the Maize and distinguish it from other already authorised stacked or single events that inherit the same gene constructs and can be mixed in the diets. Therefore, the monitoring plan provided by the applicant cannot be accepted.

Post-market monitoring must be established in a way that allows the collection of reliable information on the detection of indications showing whether any (adverse) effects on health and the / or the environment may be related to the Maize. Thus, the monitoring report should at the very least contain detailed information on:
i) actual volumes of the Maize imported into the EU,
ii) the ports and silos where shipments of the Maize are unloaded,
iii) the processing plants where the Maize is transferred to,
iv) the amount of the Maize used on farms for feed, and
v) transport routes of the Maize.

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

Furthermore, environmental exposure through organic waste material, by-products, sewage or faeces containing the Maize during or after the production process, and during or after human or animal consumption should be part of the monitoring procedure (see also EFSA, 2017b).

3.12 The Commission should also have requested the publication of fully validated measures that would allow independent scientists to measure Bt concentrations in the Maize

Besides the methods of detection, other methods for quantifying exposure to Bt toxins need to be made publicly available in order to facilitate monitoring. Food and feed producers, farmers as well as experts dealing with environmental exposure (for example via which waste material, spillage and manure) have to be able to gather independent information on their exposure to the toxins via independent laboratories. As yet, these methods are regarded as confidential business information and are not made available upon request by EFSA. Thus, the Commission should ensure that the relevant data are both publicly available and also reliable.

As existing evidence shows (Székács et al., 2011; Shu et al., 2018), the methods need to be carefully evaluated to ensure that the results are reliable, comparable and reproducible. Therefore, fully evaluated methods have to be published that allow the Bt concentration in the maize to be measured by independent scientists as is the case for other plant protection compounds used in food

and feed production. This is necessary to make sure that the environment as well as human and animals coming into contact with the material (for example, via dust, consumption or manure) are not exposed to higher quantities of Bt toxins than described in the application.

4. Final conclusion

The Commission should not have accepted the EFSA (2017) 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.

References

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

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

Adel-Patient, K., Guimaraes, V.D., Paris, A., Drumare, M.F., Ah-Leung, S., Lamourette, P., ... & Créminon, C. (2011) Immunological and metabolomic impacts of administration of Cry1Ab protein and MON 810 maize in mouse. PloS one, 6(1): e16346. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0016346

Andreassen, M., Rocca, E., Bøhn, T., Wikmark, O.G., van den Berg, J., Løvik, M., ... & Nygaard, U. C. (2015a) Humoral and cellular immune responses in mice after airway administration of Bacillus thuringiensis Cry1Ab and MON810 cry1Ab-transgenic maize. Food and Agricultural Immunology, 26(4): 521-537. http://www.tandfonline.com/doi/abs/10.1080/09540105.2014.988128

Andreassen, M., Bøhn, T., Wikmark, O.G., Van den Berg, J., Løvik, M., Traavik, T., & Nygaard, U. C. (2015b) Cry1Ab Protein from Bacillus thuringiensis and MON810 cry1Ab transgenic Maize Exerts No Adjuvant Effect After Airway Exposure. Scandinavian Journal of Immunology, 81(3): 192-200. http://onlinelibrary.wiley.com/doi/10.1111/sji.12269/full

Andreassen, M., Bøhn, T., Wikmark, O. G., Bodin, J., Traavik, T., Løvik, M., & Nygaard, U. C. (2016) Investigations of immunogenic, allergenic and adjuvant properties of Cry1Ab protein after intragastric exposure in a food allergy model in mice. BMC Immunology, 17(1): 10. https://bmcimmunol.biomedcentral.com/articles/10.1186/s12865-016-0148-x

Bøhn, T., Rover, C.M., Semenchuk, P.R. (2016) Daphnia magna negatively affected by chronic exposure to purified Cry-toxins. Food and Chemical Toxicology, 91: 130-140. https://www.sciencedirect.com/science/article/pii/S0278691516300722

Bøhn, T. (2018) Criticism of EFSA's scientific opinion on combinatorial effects of ‘stacked’ GM plants. Food and Chemical Toxicology, 111: 268-274. https://www.sciencedirect.com/science/article/pii/S0278691517306907

Bollinedi, H., S. G.K, Prabhu, K.V., Singh, N.K., Mishra, S., Khurana, J.P., Singh, A.K. (2017) Molecular and Functional Characterization of GR2-R1 Event Based Backcross Derived Lines of Golden Rice in the Genetic Background of a Mega Rice Variety Swarna. PLoS ONE 12(1): e0169600. https://doi.org/10.1371/journal.pone.0169600

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(7): e67079. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0067079

Bremmer, J.N. and Leist, K.-H. (1997) Disodium-N-acetyl-L-glufosinate; AE F099730 – Hazard evaluation of Lglufosinate produced intestinally from N-acetyl-L-glufosinate. Hoechst Schering AgrEvo GmbH, Safety Evaluation Frankfurt. TOX97/014. A58659. Unpublished.

Cao, Q.-J., Xia, H., Yang, X., Lu, B.-R. (2009) Performance of Hybrids between Weedy Rice and Insect-resistant Transgenic Rice under Field Experiments: Implication for Environmental Biosafety Assessment. J. Integr. Plant Biol. 51, 1138-1148. https://doi.org/10.1111/j.1744-7909.2009.00877.x

Chowdhury, E. H., Kuribara, H., Hino, A., Sultana, P., Mikami, O., Shimada, N., ... & 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. Journal of Animal Science, 81(10): 2546-2551. https://academic.oup.com/jas/article-abstract/81/10/2546/4789819

de Souza Freire I., Miranda-Vilela A.l., Pereira Barbosa L. C., Soares Martins E., Gomes Monnerat R., Koppe Grisolia C. (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://www.mdpi.com/2072-6651/6/10/2872/htm

EFSA (2010) Scientific Opinion on application (EFSA-GMO-CZ-2008-62) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON 89034 x 1507 x MON 88017 x 59122 and all subcombinations of the individual events as present in its segregating progeny, for food and feed uses, import and processing under Regulation (EC), No 1829/2003 from DowAgroSciences and Monsanto, EFSA Panel on Genetically Modified Organisms (GMO), www.efsa.europa.eu/en/efsajournal/pub/1781.htm

EFSA (2015) Request for the evaluation of the toxicological assessment of the co-formulant POE-tallowamine. EFSA Journal 2015; 13(11): 4303. https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/j.efsa.2015.4303

EFSA (2017a) Scientific Opinion on application EFSA-GMO-BE-2013-118 for authorisation of genetically modified maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and subcombinations independently of their origin, for food and feed uses, import and processing submitted under Regulation (EC) No 1829/2003 by Monsanto Company. EFSA Journal 2017;15(8):4921, 32 pp. https://doi.org/10.2903/j.efsa.2017.4921

EFSA (2017b) Application EFSA-GMO-BE-2013-118, Comments and opinions submitted by Member States during the three-month consultation period, Register of Questions, http://registerofquestions.efsa.europa.eu/roqFrontend/ListOfQuestionsNoLogin?0&panel=ALL

EFSA (2018a) Reasoned Opinion on the review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2018;16(5):5263, 230 pp. 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 2018:EN-1504. 13 pp. doi:10.2903/sp.efsa.2018.EN-1504. https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2019.EN-1504

EFSA GMO Panel (2018c) Scientific opinion on the assessment of genetically modified maize Bt11 x MIR162 x 1507 x GA21 and three subcombinations independently of their origin, for food and feed uses under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2010-86). EFSA Journal 2018;16(7):5309, 35 pp. https://doi.org/10.2903/j.efsa.2018.5309

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. Frontiers in Plant Sciences, 9: 233. https://doi.org/10.3389/fpls.2018.00233

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. Vet. Microbiol. 175: 382–388, http://dx.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: 610–618. http://dx.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, Molecular Immunology, 41: 1177-1183. http://dx.doi.org/10.1016/j.molimm.2004.06.026

Hallauer, A.R. (1995) Potential for outcrossing and weediness of genetically modified insect protected corn. Department of Agronomy, Iawa State University, USA.

Hilbeck, A. & Otto, M. (2015) Specificity and combinatorial effects of Bacillus thuringiensis Cry toxins in the context of GMO risk assessment. Frontiers Environmental Science, 3: 71.

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. Proceedings of the National Academy of Sciences, 101(30): 10995-11000. http://www.pnas.org/content/101/30/10995.short

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: 266–270. http://dx.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. Journal of Biological Chemistry, 279: 21282-21286. http://www.jbc.org/content/279/20/21282.short

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 Immunol., 30: 31–38, http://dx.doi.org/10.1111/j.1365-3024.2007.00999.x

Kawata, M., Murakami, K., Ishikawa, T. (2009) Dispersal and persistence of genetically modified oilseed rape around Japanese harbors. Environmental Science and Pollution Research, 16(2): 120-126. https://link.springer.com/article/10.1007/s11356-008-0074-4

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 Managment Science, 67(10): 1193-1210. https://onlinelibrary.wiley.com/doi/abs/10.1002/ps.2128

Kraemer, L. (2012) The consumption of genetically modified plants and the potential presence of herbicide residues. Legal dossier compiled on behalf of Testbiotech, http://www.testbiotech.de/sites/default/files/Legal_Dossier_Kraemer_Pesticide_RA_PMP.pdf

Kraemer, L. (2016) Teosinte plants in the European environment and its implication for market authorisation of genetically engineered maize Legal analysis commissioned by Testbiotech, http://www.testbiotech.org/node/1773

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. Applied Soil Ecology, 42(3): 236-242. https://www.sciencedirect.com/science/article/pii/S0929139309000808

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. Environmental Toxicology and Chemistry, 26(1): 73-79. https://onlinelibrary.wiley.com/doi/abs/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, J Biomed Biotechnol, http://dx.doi.org/10.1155/2010/198921

Lu, B.-R., Yang, C., 2009. Gene flow from genetically modified rice to its wild relatives: Assessing potential ecological consequences. Biotechnol. Adv., Biotechnology for the Sustainability of Human SocietyInvited Papers from IBS 2008 27, 1083–1091. https://doi.org/10.1016/j.biotechadv.2009.05.018

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

Matthews, D., Jones, H., Gans, P., Coates, S., Smith, L.M.J. (2005) Toxic Secondary Metabolite Production in Genetically Modified Potatoes in Response to Stress. Journal of Agricultural and Food Chemistry, 53(20): 7766-7776. https://doi.org/10.1021/jf050589r

Mesnage, R., Clair, E., Gress, S., Then, C., Székács, A., Séralini, G.-E. (2012) Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. Journal of Applied Toxicology, 33(7): 695–699. https://onlinelibrary.wiley.com/doi/abs/10.1002/jat.2712

Meyer, P., Linn, F., Heidmann, I., Meyer, H., Niedenhof, I., Saedler, H., 1992. Endogenous and environmental factors influence 35S promoter methylation of a maize A1 gene construct in transgenic petunia and its colour phenotype. Mol. Gen. Genet. MGG 231, 345–352. https://doi.org/10.1007/BF00292701

Mezzomo, B.P., Miranda-Vilela, A.L., de Souza Freire, I., Pereira Barbosa, L.C., Portilho, F.A., Marques Lacava, Z.G., Koppe Grisolia, C. (2013) Hematotoxicity of Bacillus thuringiensis as Spore-crystal Strains Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa in Swiss Albino Mice, J Hematol Thromb Dis, 1:1.

Ministry of Agriculture, Food and Rural Affairs of Ontario (2016/2017) Guide to weed control, 2016–2017, Publication 75, http://www.omafra.gov.on.ca/english/crops/pub75/pub75toc.htm

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 Infect., 2: 885–890. http://dx.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, Int. Immunopharmacol. 17 (2013) 1051–1066, http://dx.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 of European Food Safety Authority (EFSA). www.testbiotech.org/node/2304

Monsanto (2013) Application for authorization to place on the market MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 maize in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed EFSA-GMO-BE-2013-118, EFSA-Q-2013-00926, Part II Scientific information. Made available upon request by EFSA.

OECD (2003) Consensus Document on the Biology of Zea Mays Subsp. Mays (Maize). http://www.oecd.org/ (referenced from Monsanto, 2013)

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

Pascher, K. (2016) Spread of volunteer and feral maize plants in Central Europe: recent data from Austria. Environmental Sciences Europe, 28(1): 30. https://link.springer.com/article/10.1186/s12302-016-0098-1

Reuter, T., Alexander, T.W., Martinez, 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: 2837-2843. https://onlinelibrary.wiley.com/doi/abs/10.1002/jsfa.3038

Reiner, D., Lee, R.Y., Dekan, G., Epstein, M.M. (2014) No adjuvant effect of Bacillus thuringiensis-maize on allergic responses in mice. PLoS ONE, 9(8): e103979. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0103979

Rubio Infante, N., & Moreno-Fierros, L. (2016) An overview of the safety and biological effects of Bacillus thuringiensis Cry toxins in mammals. Journal of Applied Toxicology, 36(5): 630-648. http://onlinelibrary.wiley.com/doi/10.1002/jat.3252/full

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. International immunopharmacology, 61: 185–196. https://www.sciencedirect.com/science/article/pii/S1567576918302467

Shehata, A.A., Schrödl, W., Aldin, A.A., Hafez, H.M., Krüger, M. (2012) The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro. Curr Microbiol 6(4): 350-358. https://link.springer.com/article/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 Vet Med Sci, 65: 187-191. https://www.jstage.jst.go.jp/article/jvms/65/2/65_2_187/_article/-char/ja/

Shu, Y., Romeis, J., Meissle, M. (2018) No interactions of stacked Bt maize with the non-target aphid Rhopalosiphum padi and the spider mite Tetranychus urticae. Frontiers in Plant Science, 9: 39. https://www.frontiersin.org/articles/10.3389/fpls.2018.00039

Székács, A., Weiss, G., Quist, D., Takács, E., Darvas, B., Meier, M., Swain, T., Hilbeck, A. (2011) Interlaboratory comparison of Cry1Ab toxin quantification in MON 810 maize by ezyme-immunoassay. Food and Agricultural Immunology, 23(2): 99-121. www.tandfonline.com/doi/abs/10.1080/09540105.2011.604773

Testbiotech (2016) Cultivation of genetically engineered maize: Risks not under control - Overview: Why the EU should not allow the cultivation of transgenic maize engineered to produce insecticidal toxins. Testbiotech Background, 24 - 11 – 2016. https://www.testbiotech.org/node/1759

Testbiotech (2011) Expression of Bt toxins in 'SmartStax'. https://www.testbiotech.org/sites/default/files/SmartStax_Expression_data_Testbiotech.pdf

Then, C. (2010) Risk assessment of toxins derived from Bacillus thuringiensis: synergism, efficacy, and selectivity. Environmental Science and Pollution Research, 17(3): 791-797. https://link.springer.com/article/10.1007/s11356-009-0208-3

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. Environmental Sciences Europe, 29(1): 1. https://enveurope.springeropen.com/articles/10.1186/s12302-016-0099-0

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: Implications of GM-Crop Cultivation at Large Spatial Scales., Theorie in Der Ökologie. Peter Lang, Frankfurt, pp. 17–21.

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. Journal of Cell Science, 60(1): 181–197. http://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. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0123011

Trtikova, M., Lohn, A., Binimelis, R., Chapela, I., Oehen, B., Zemp, N., Widmer, A., Hilbeck, A. (2017) Teosinte in Europe – Searching for the Origin of a Novel Weed. Scientific Reports, 7: 1560. https://www.nature.com/articles/s41598-017-01478-w

Vacher, C., Weis, A.E., Hermann, D., Kossler, T., Young, C., Hochberg, M.E. (2004) Impact of ecological factors on the initial invasion of Bt transgenes into wild populations of birdseed rape (Brassica rapa). Theor. Appl. Genet. 109, 806–814. https://doi.org/10.1007/s00122-004-1696-7

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. Science of The Total Environment, 616: 255-268. https://www.sciencedirect.com/science/article/pii/S0048969717330279

Vázquez, R.I., Moreno-Fierros L., Neri-Bazán L., De La Riva, G.A., López-Revilla, R. (1999) Bacillus thuringiensis Cry1Ac protoxin is a potent systemic and mucosal adjuvant, Scand. J. Immunol. 49, 578–584, http://dx.doi.org/10.1046/j.1365-3083.1999.00534.x

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(21): 1897 1912. http://www.academia.edu/download/43177637/Intragastric_and_intraperitoneal_adminis20160228-1573-204m62.pdf

Vásquez-Padrón. R.I., Gonzáles-Cabrera. J., Garcia-Tovar. C., Neri-Bazan. L., Lopéz-Revilla. R., Hernández. M., Morena-Fierros. 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 and Biophys Research Comm, 271: 54-58. https://www.sciencedirect.com/science/article/pii/S0006291X00925841

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, 6834. https://doi.org/10.1038/s41598-017-07089-9

Walsh, M. C., Buzoianu, S. G., Gardiner, G. E., Rea, M. C., Gelencsér, E., Jánosi, A., ... & 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. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0027177

Zeljenkova, D., Ambrusova, K., Bartusova, M., Kebis, A., Kovriznych, J., et al. (2014) Ninety-day oral toxicity studies on two genetically modified maize MON810 varieties in Wistar Han RCC rats (EU 7th Framework Programme project GRACE). Archives of Toxicology, 88: 2289–314. https://link.springer.com/article/10.1007/s00204-014-1374-8

Zeller, S.L., Kalininal, O., Brunner, S., Keller, B., Schmid, B. (2010) Transgene x Environment Interactions in Genetically Modified Wheat. 5(7): e11405. http://www.plosone.org/article/info:doi/10.1371/journal.pone.0011405

Zhu, X., Sun, L., Kuppu, S., Hu, R., Mishra, N., Smith, J., Esmaeili, N., Herath, M., Gore, M.A., Payton, P., Shen, G., Zhang, H., 2018. The yield difference between wild-type cotton and transgenic cotton that expresses IPT depends on when water-deficit stress is applied. Sci. Rep. 8: 2538. https://doi.org/10.1038/s41598-018-20944-7

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: 319–330. https://doi.org/10.1002/jpln.201000434