Technical background for a request for internal review of administrative acts under Article 10 of Regulation (EC) No. 1367/2006 against the decision of the EU Commission to give market authorisation to genetically engineered stacked maize MON 87427 x MON 89034 x MIR162 x MON 87411 and subcombinations

Testbiotech e. V.
Institute for Independent Impact Assessment in Biotechnology

Prepared by Testbiotech, March 2021, 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 2019). Implementing Regulation 503/2013 was applied in the risk assessment. Therefore, the Regulation must be complied with, setting new standards compared to previous assessments of single or stacked events.

The stacked GE maize (hereinafter referred to as the Maize) was authorised on 22 January 2021, published in the Official Journal of the EU on 26 January 2021.[1]

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

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

[1] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32021D0065&qid=1612781010137

A) The EFSA risk assessment should have been rejected for following reasons:
The EFSA risk assessment is contrary to EU Implementing Regulation 503/2013 which requests that *"for silencing approaches by RNAi expression, potential 'off target' genes should be searched by in silico analysis to assess if the genetic modification could affect the expression of other genes which raise safety concerns."*

The interaction between the ncRNAs (non coding RNAs)[2] produced in the GE plants and the microbiome of humans or animals was not assessed, despite new findings showing that these interactions are likely to occur, and adverse impacts on human and animal health are a plausible consequence.

That a combination of DvSnf7 dsRNA, Bt toxins and residues from spraying can trigger effects on the immune system or other adverse health effects, either directly or via the microbiome, even if these were absent in the parental plants, has to be considered a plausible hypothesis. Therefore, the stacked Maize needs to be tested for synergistic effects due to other plant constituents or additional factors, which may enhance stability or uptake of DvSnf7 dsRNA from the gut.

In conclusion, EFSA and the applicant failed to ensure that the final risk characterisation clearly demonstrates that the genetically modified food and feed derived from the stacked Maize has no adverse effects on human and animal health or the environment.

B) The decision of the EU Commission fails to fulfill the requirements for the following reason:
Since EFSA and the applicant did not ensure that the final risk characterisation clearly demonstrates that the genetically modified food and feed has no adverse effects on human and animal health, the EU Commission decision to allow the import was not in accordance with the EU regulations.

C) The Request
Art. 10 of EU Regulation 1367/2006 allows NGOs active in the field of environmental protection to request re-examination of Commission decisions. Based upon this regulation, we request the re-examination of the risk analysis by EFSA and the EU Commission as well as immediate withdrawal of market authorisation for maize MON 87427 x MON 89034 x MIR162 x MON 87411.

The following designations appear in this document:

The Maize: Genetically engineered stacked maize MON 87427 x MON 89034 x MIR162 x MON 87411 and its segregating subcombinations;
The Parental Plants: Single events of genetically engineered maize MON 87427, MON 89034, MIR162, MON 87411;
The Applicants: Monsanto (owned by Bayer);
GMO or GE: This abbreviation is used for genetically engineered organisms which are subjected to the approval process under EU Directive 2001/18;
GM Regulation: This abbreviation is used for the regulatory framework for genetically engineered organisms that are subject to the approval process under EU Directive 2001/18.

[2] In this version, 'ncRNA' is replacing 'ncsRNA' which was used in previous version.

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

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

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

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

In order to gain an authorisation, an application must be made to the competent authority of a Member State.[5] That application should include, among other things a copy of the studies available to show whether the food or feed complies with Articles 4(1) or 16(1), and an analysis, supported by data of whether the characteristics of the genetically modified organism are not different from their comparators (their conventional counterparts).[6] Article 5(5) also provides that the application must be accompanied by a technical dossier meeting the requirements of Directive 2001/18/EC.

The application is then considered by EFSA, which will provide an opinion, among other matters, on whether the food/feed complies with the criteria referred to in Articles 4(1) / 16(1).[7] In preparing its opinion, the Authority must consult the national competent authorities of the Member States.[8] On

[3]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.
[4]Articles 4(2) and 16(2) of the GM Regulation.
[5]Articles 5(2) and 17(2) of the GM Regulation.
[6]Articles 5(3)(e), 5(3)(f) 17(3)(e), and 17(3)(f) of the GM Regulation.
[7]Articles 6(3)(a) and 18(3)(a) of the GM Regulation.
[8]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.[9]

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

Regulation 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety ("the Food Safety Regulation") outlines the "General Principles of Food Law" upon which European measures, such as the GM Regulation, should be based. These include:

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

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

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

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

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

1.3 Particular Provisions of Directive 2001/18

Directive 2001/18[14] requires that the placing on the market of a genetically modified organism (GMO) as or in a product may only take place after written consent by the competent authority has been given (Article 19). The application for such consent (notification, Article 13) must be accompanied by an environmental risk assessment, by other information, and by a monitoring plan (Article 13(2.b, 2.a, and 2.e)).

The environmental risk assessment

[9]Articles 7(1) and 19(1) of the GM Regulation.
[10]Articles 7(3), 19(3) and 35(2) of the GM Regulation.
[11]Article 5(3) and 5(4) of Decision 1999/468.
[12]Article 5 of the Food Safety Regulation (also reflected in Recital (3)).
[13]Emphasis added.
[14]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").

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

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

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

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

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

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[16].

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

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

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

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

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

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

Other information

"Other information" which has to accompany every notification under Article 13 of Directive 2001/18, shall include "considerations for human health and animal health, as well as plant health: (i) toxic or allergenic effects of the GMO and/or their metabolic products[18], furthermore "identification and description of non-target organisms which may be adversely affected by the release of the GMO, and the anticipated mechanisms of any identified adverse interaction"[19]and, as a catch-all formula "other potential interactions with the environment"[20]. For genetically modified higher plants (GMHP), Annex IIIB applies, this requires the notifier to supply, with his notification, the following information: "Information on any toxic, allergenic, or other harmful effects on human health arising from the genetic modification"[21]; "Information on the safety of the GMHP to animal health, particularly regarding any toxic, allergenic or other harmful effects arising from the genetic

[17] 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.
[18]Directive 2001/18, Annex III A, section II, C.2(i)
[19]Directive 2001/18, Annex IIIA, section IV B12.
[20]Directive 2001/18, Annex IIIA, section IV B.16.
[21]Directive 2001/18, Annex IIIB, section D no.7.

modification, where the GMHP is intended to be used in animal feedstuffs"[22]; and "Potential interactions with the abiotic environment"[23].

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

The monitoring plan

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

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

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

In 2002, the Council adopted, by way of a Decision, guidance notes "supplementing Annex VII"[24]. The guidance notes "shall be used as a supplement to Annex VII of Directive 2001/18/EC" (Article 1). The guidance notes repeat in the introduction that the purpose of the monitoring plans is to "trace and identify any direct or indirect, immediate, delayed or unforeseen effects on human health or the environment of GMOs as or in products after they have been placed on the market".

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

With regard to unforeseen effects, the guidance notes indicate: "it is very difficult if not impossible to predict the appearance of potential, unforeseen or unanticipated effects that were not highlighted

[22]Directive 2001/18, annex IIIB, section D no.8.
[23]Directive 2001/18, annex IIIB, section D no11.
[24]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.

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)).[25]

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

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

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

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

It follows from these provisions that for genetically modified food or feed, information and conclusions about the risk assessment must be given. This risk assessment must have been carried out in accordance with the principles set out in Annex II to Directive 2001/18 (Article 5(5.a) and Article 17(5.a) see section 2 above). Also a monitoring plan has to be submitted with the application for authorisation (Article 5(5.b) and Article 17 (5.b)). Where EFSA expresses an opinion in favour of the authorisation, it has to address the monitoring plan (Article 6(5.g) and Article 18(5.g)) and indicate “post-market monitoring requirement based on the outcome of the risk assessment” (Article 6(5.e) and Article 18(5.e)).

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

[25]For such cases, Articles 13 to 24 of Directive 2001/18 are declared inapplicable.
[26]Further, under Article 7(1) the Commission has to provide an explanation for the difference, where its decision is not in accordance with EFSA’s opinion.

occasionally did add supplementary conditions on the placing on the market of genetically modified food products[27].

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[28]:

> "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.

[27]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.

[28]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.

Implementing Regulation 503/2013 requests:

> "*Toxicological assessment shall be performed in order to:*
> *(a) demonstrate that the intended effect(s) of the genetic modification has no adverse effects on human and animal health;*
> *(b) demonstrate that unintended effect(s) of the genetic modification(s) identified or assumed to have occurred based on the preceding comparative molecular, compositional or phenotypic analyses, have no adverse effects on human and animal health;*"
>
> "*In accordance with the requirements of Articles 4 and 16 of Regulation (EC) No 1829/2003, the applicant shall ensure that the final risk characterisation clearly demonstrates that:*
> *(a) the genetically modified food and feed has no adverse effects on human and animal health;*"

More specifically, Implementing Regulation 503/2013 requests:

> *"For silencing approaches by RNAi expression, potential 'off target' genes should be searched by in silico analysis to assess if the genetic modification could affect the expression of other genes which raise safety concerns."*

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.

2. Grounds for the request to reviewing the decision

Introduction

- The EFSA GMO panel assessed the four-stacked maize MON 87427 x MON 89034 x MIR162 x MON 87411, which is derived from crossing genetically engineered maize events (EFSA, 2019). The parental plants were assessed by EFSA in previous opinions. The maize contains genes conferring double resistance to glyphosate and producing three insecticides.
- MON 87427 expressing CP4 EPSPS protein for tolerance to glyphosate-containing herbicides;
- MON 89034 expressing the insecticidal proteins Cry1A.105 (artificially synthesized) and Cry2Ab2;
- MIR162 expressing the insecticidal protein Vip3Aa20 and phosphomannose isomerase (PMI) which is a selectable marker;
- MON 87411 produces an insecticidal dsRNA (DvSnf7 dsRNA) as well as the EPSPS protein for tolerance to glyphosate-containing herbicides. In addition it also produces the insecticidal protein Cry3Bb1 (artificially synthesized).

Consequently, the stacked GE maize has double resistance to glyphosate, making it tolerant to high dosages and repeated sprayings as applied in fields with herbicide-resistant weeds. Further, it produces three toxins against the larvae of *Lepidoptera* (butterflies) that feed on the plants (‘pest insects’) and one toxin (Cry3Bb1) against the larvae of Coleoptera (beetles) that feed below the ground. In addition, it produces a biologically active molecule (dsRNA) that can negatively impact metabolism in larvae of *Coleoptera* (beetle). The maize produces the PMI protein (phosphomannose isomerase) derived from *Escherichia coli*; expression of PMI enables transformed maize cells to utilise mannose and, therefore, survive on specific media used for selecting the maize plants after the process of genetic engineering (so-called marker genes).

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

The stacked GE maize (hereinafter referred to as the Maize) was authorised on 22 January 2021, published in the Official Journal of the EU on 26 January 2021.[29]

[29]https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32021D0065&qid=1612781010137

This request focuses in particular on risk assessment of the dsRNA (ncRNA) DvSnf7 produced in the stacked Maize.

Regulation 1829/2003 requests "*genetically modified food and feed should only be authorized for placing on the Community market after a scientific evaluation of the highest possible standard, to be undertaken under the responsibility of the European Food Safety Authority (Authority), of any risks which they present for human and animal health and, as the case may be, for the environment.*" (Recital 9).

In addition, Implementing Regulation 503/2013 requests:
"*Toxicological assessment shall be performed in order to:*
(a) demonstrate that the intended effect(s) of the genetic modification has no adverse effects on human and animal health;
(b) demonstrate that unintended effect(s) of the genetic modification(s) identified or assumed to have occurred based on the preceding comparative molecular, compositional or phenotypic analyses, have no adverse effects on human and animal health;"

"*In accordance with the requirements of Articles 4 and 16 of Regulation (EC) No 1829/2003, the applicant shall ensure that the final risk characterisation clearly demonstrates that:*
(a) the genetically modified food and feed has no adverse effects on human and animal health;"

More specifically, Implementing Regulation 503/2013 requests:
"For silencing approaches by RNAi expression, potential 'off target' genes should be searched by in silico analysis to assess if the genetic modification could affect the expression of other genes which raise safety concerns."

2.1.2 Background - biology and technology

The production of artificial double stranded RNA (dsRNA), i.e. 'non coding RNAs' (ncRNAs), in genetically engineered plants developed to kill pest insects via RNAi (RNA interference) is a new technology. For the following overview also see RAGES (2020).

Very generally, RNAi processes are based on the silencing of gene functions. The effects can be enacted by uptake of ncRNAs across species' borders, the plant and animal kingdoms and the domains of life (bacteria, archaea and eukaryotes). In nature, plants use ncRNA to interact with their microbiome, e.g. soil organisms. Based on these signalling molecules, there is a two-way communication between the associated microbial fauna and the plants that is directly related to plant health and soil fertility.

For example, plants can induce gene silencing in some eukaryotic pathogens, pests, parasites or symbiotic microorganisms as a defence strategy. However, pathogens also developed similar mechanisms, proving the existence of a two-way ncRNAs traffic between pathogens and their plant hosts. Similar mechanisms and pathways are also known to occur in plant-insect interaction. Therefore, genetically engineered plants were developed to produce artificial miRNA which, after uptake by pest insects such as the corn rootworm, will downregulate gene activity in the insect and thus kill it. When ingested, the biologically active molecules produced in plants may also actively interfere with gene regulation in humans (animals) or their intestinal microbiome. Potential effects are dependent on several factors such as stability, further splicing and regions within DNA where it can interact.

RNAi is a mechanism of gene silencing that occurs naturally in the cells of fungi, plants and animals, but RNAi-based GM crops artificially induce this gene silencing through the production of novel RNAi in the GMO. Although the effects of gene silencing have been observed for a considerable time, the RNAi mechanism has only been explained in the past 15 years (Couzin, 2002; Roberts et al., 2015). Naturally occurring RNAi is a gene-silencing mechanism and can also confer resistance to invading nucleic acids. Initially it was found that RNAi is exogenously triggered as an immune response by the infiltration of foreign RNA (in most cases double stranded RNA (dsRNA)) into an organism's cell (for example by an invading viral pathogen) (Mello & Conte, 2004). Shortly after, endogenous genomic sources of RNAi (centromeric regions, transposons and other repetitive sequences) were also found (Lippman & Martienssen, 2004).

In gene expression, genes (encoded by DNA) are transcribed into an intermediate product, messenger RNA (mRNA), which is subsequently translated into a protein. RNAi acts at the RNA level by, for example, cleaving an invasive viral RNA or mRNA, preventing it from being translated into a protein. There are two kinds of small RNA molecules (dsRNA or ncRNAs) that act in the RNAi pathway: miRNAs (micro RNAs) and siRNAs (small interfering RNAs).

miRNAs are derived from short stem-loop RNA molecules and typically silence genes by repression of translation. siRNAs are derived by longer regions of dsRNAs and typically work by cleaving the mRNA before translation (Wilson & Doudna, 2013). The structure and length of siRNAs are very similar to miRNAs.

In both the exogenously and endogenously induced RNAi pathway, dsRNA is incorporated into the nucleus and is enzymatically cleaved into smaller pieces of RNA (Carthew & Sontheimer, 2009; Pačes et al., 2017). These small RNA molecules are directed to mRNA molecules or invasive RNAs that have a complementary sequence. This binding event can either lead to the cutting of the mRNA or prevent the mRNA from being translated into the corresponding protein, both of which can lead to an overall reduction in the level of the protein (see Pačes et al., 2017).

Until now, commercially-orientated RNAi-based GM organisms (i.e. those intended for uncontained cultivation, food or feed) are restricted to plants. In RNAi-based GM crops, a single functional gene or, more typically, a suite of novel genes is inserted into the organism (usually a plant) using first generation genetic engineering techniques. These novel genes induce the production of dsRNA. The dsRNA is then processed into small interfering RNAs, which then mediate the interference with mRNA in a sequence-specific manner. The processing of dsRNA into siRNAs can be directed to occur either within the GM organism itself, or within an organism that ingests the GM organism. For more details, see Pačes et al. (2017).

Genetic modification of RNAi is not intended to produce a novel protein as most commercial GMOs do (e.g. production of the Bt protein in GM Bt crops and production of the EPSPS protein in GM Roundup Ready crops). However, the production of a novel protein is not the only risk that is considered in the risk assessment of GMOs. The main concerns regarding RNAi-based GM crops relate to the lack of specificity of siRNAs and the potential adverse impacts this may cause on the GM plant, ecosystems and human health. Whilst all GM crops are subject to concerns regarding safety for the environment and inclusion as food for humans and animals, there are specific concerns regarding the GM plants that contain RNAi constructs, as described below.

2.1.3 Unintended effects in RNAi-based GM crops

One major concern regarding unintended effects within RNAi-based GM plants is the lack of specificity of siRNAs. siRNAs may be generated that are sufficiently complementary to mRNAs in the GM plant other than the mRNA intended to be silenced. This could enact gene silencing of an unintended gene (an off-target effect) within the GM plant. Many siRNAs are produced during cleavage of dsRNA. Each siRNA has the potential to recognize putative off-target sites due to a lack of specificity (Ramon et al., 2014).

Off-target effects could result in unexpected effects in the resulting GM plant, e.g. by altering a biochemical pathway. Off-target effects would need to be assessed robustly as plant chemistry is complex.

Further research is needed on how transgenic siRNAs can affect the expression levels of other non-coding RNAs and other RNAs, such as mRNAs in RNAi-based GM plants. This could lead to changes in protein and enzymatic content, putatively altering the nutritional value of the GM plant.

One of the principal ecological concerns regarding RNAi-based GM crops are unintended adverse effects on non-target organisms in the environment, i.e. the dsRNA produced by the GM plant may affect organisms in the environment other than the intended pest, e.g. beneficial insects (Zhang et al., 2017).

There are also concerns for human and animal health, but these are poorly delineated as there is a considerable lack of knowledge regarding the unintended transmission of dsRNA and siRNAs following ingestion. Processing dsRNA into siRNAs following ingestion is the key mechanism for the pesticide action of RNAi-based GM crops. The concern is whether dsRNA, or the siRNAs derived from them, might also be active when the GM plant is ingested as a food.

There are at least two ways in which the dsRNA expressed in GMO plants and its subsequent processing into siRNA can impact mammalian health via ingestion:
(1) Uptake from the gut into the bloodstream in the same way as other plant miRNAs as described (see, for example, Yang et al., 2015; Liang et al., 2015; Hirschi et al, 2015; Beatty et al., 2014). If the bioactive molecules produced in the plants interfere with mammalian gene regulation, adverse effects could be the result (see Testbiotech, 2018). Based on current knowledge, this interference scenario cannot be excluded. The need for further investigation is supported by the outcome of a meeting of a USA governmental scientific advisory panel in 2014, which maintained that risks arising from RNAi-based GM crops to immune-compromised individuals should be tested (US Environment Protection Agency, 2014): "*The stability of dsRNA should be tested in individuals that manifest specific diseases (e.g., Crohn's, colitis, irritable bowel syndrome, etc.), the immune compromised, elderly, as well as children. These individuals may have compromised digestion or increased sensitivity to dsRNA exposure.*"

(2) It is well known that endogenous miRNA plays a key role in gene regulation in the gut microbiome, as well as in the communication between the mammalian host and its gut microbiome (see, for example, Williams et al., 2017). It is plausible that the dsRNA produced in GM maize MON 87411 can interact with the gut microbiome directly without direct uptake from the gut. For example, the Snf7 gene which is targeted by the dsRNA produced in maize MON 87441, is involved in crucial biological processes in insects and in yeast (Testbiotech, 2018).

2.1.4 Methodological problems in risk assessment

The risk assessment of RNAi-based GM crops is compromised by large, significant knowledge gaps regarding RNAi mechanisms and pathways for adverse effects within the environment and on human and animal health. More research is needed in this area before any meaningful risk assessment of food and feed can take place (Pačes et al., 2017; Roberts et al., 2015).

In order to begin identifying these gaps and issues unique to the risk assessment of RNAi-based crops, EFSA convened an international scientific workshop in June 2014 (EFSA, 2014; Ramon et al., 2014). Following the workshop, two literature reviews were commissioned by EFSA, one on baseline data to inform the risk assessment of RNAi-based GM plants in general (Pačes et al., 2017) and one (Christiaens et al., 2018) to inform the risk assessment.

From Pačes et al. (2017) and other published literature, EFSA currently considers that bioinformatics searches for off-target effects of siRNAs can usefully (in terms of risk assessment) be conducted for plants, but give insufficiently reliable predictions for animals or humans (EFSA, 2017). Thus, EFSA has only issued a protocol for off-target bioinformatics searches in plants, not for animals or humans who might ingest the plant (EFSA, 2017). This means that it may be possible to look for off-target effects within the GM plant that might give rise to unexpected effects. However, the risks arising from the possibility and potential effects of uptake of dsRNA from GM plants by humans and animals from RNAi-based GM plants cannot be assessed at the present time (EFSA, 2017; Christiaens et al., 2018).

This affects both the environmental risk assessment (for non-target organisms) and also food and feed safety risk assessment (for consumers). In 2014 an EFSA workshop (ESFA, 2014) identified the following issues as relevant for risk assessment of effects on health:
"*Throughout the different discussion topics, the following issues were identified as knowledge gaps, where more research could be warranted:*
- The RNAi and metabolic profiling in RNAi-based plants could be further explored and corroborated to support risk assessment. In this context, 'omics' techniques should be further investigated as supporting tools.
- The use of bioinformatics to predict potential off-target effects in consumers should be further explored.
- Possible changes in microbiota, residing in human or animal guts, following consumption of food and feed products derived from RNAi-based plants could be a research topic."

None of these research topics have yet been progressed to the point where they can assist the food and feed risk assessment of RNAi-based GM crops (see, for example, the discussion on -omics techniques in risk assessment related to the genome editing process).

As the BSE crisis showed, the risk of bioactive compounds being transmitted to humans via the food and feed chain poses a high risk to farm animals and humans (see Pačes et al., 2017). There are clearly knowledge gaps in how RNAi-based GM crops could have environmental effects (via negative effects on non-target wild animals) and affect human health (via the uptake of dsRNA in food).

2.2 Risk assessment of the parental plant MON 87411

In 2018, EFSA published its opinion on the parental maize which produces insecticidal dsRNA (DvSnf7). Several uncertainties were stated in the EFSA opinion on the risk assessment of DvSnf7 (EFSA, 2018a).

To assess potential off-target effects, the structure of the dsRNA can be compared to genomic regions in organisms that might come into contact with the molecules. Implementing Regulation (EU) No 503/2013 states that when silencing approaches with RNAi are used in genetically engineered plants, a bioinformatics analysis is required in order to identify potential 'off-target' genes. An important starting point is the collection of relevant data to make comparisons with the RNA networks of non- target organisms, including mammals and humans that are exposed to the plants via food and feed.

However, in the assessment of the parental plant MON 87411 (EFSA, 2018a), the additional dsRNA produced in the plants was only compared with RNA expressed in plants. EFSA concluded that there was similarity that would raise concerns. However, no comparison was made in regard to mammals and microorganisms.

This gap in risk assessment was also acknowledged in comments made by experts of Member States (EFSA, 2018b), such as the BVL (Germany):
"The applicant has not provided data on potential RNAi-targets of DvSnf7 dsRNA in non-target organisms, including humans. (…) Thus, additional data like bioinformatic evaluations should be considered. As demonstrated by a history of safe consumption of dsRNAs with high homology in conventional food and feed, the identification of sequence similarities between the dsRNA produced by MON 87411 and transcripts of exposed species would not directly indicate an increased risk of adverse effects. Nevertheless, a bioinformatic search for potential targets in transcripts of human and likely exposed non target species (farm animals) would back the weight of evidence approach if no matching targets where identified. The German Competent Authority therefore recommends a bioinformatic evaluation, comparable to study no.: RAR-2015-0373, to identify potential target genes in human and other relevant non target species. Additional information might be recommended according to the outcome of the bioinformatics evaluation."

However, no such data were requested by EFSA for the parental plants or the stacked events. Instead, EFSA seems to be of the opinion that such data would not allow reliable prediction of the potential effects of the molecules. The protocol of the EFSA panel meeting (EFSA, 2017) states:
"*In plants a set of parameters allows for a reasonable prediction of RNAi off-target genes while in human and animals the extent of complementarity between the small RNA and the target is more limited and therefore these prediction tools do not allow for sufficiently reliable predictions (Pinzón et al., 2017). Therefore the GMO Panel considers that only the search for small RNA off-targets in the GM plant could have value for the risk assessment of GM plants."*

This is an interesting statement since it exposes some limitations in current knowledge. Pinzón et al. (2017) show that further research is needed to make reliable predictions in regard to dsRNA effects in mammals. This publication cannot be used as justification not to assess health risks of MON 87411. At the same time, EFSA neither tried to overcome these limitations in current knowledge nor did it consider that risk assessment cannot be concluded without sufficient data and meaningful analysis.

Instead, EFSA (2018a) simply accepted these limitations by restricting its considerations and risk assessment to potential off-target effects in the plants, leaving aside effects in humans and livestock and their gut microbiomes that are exposed to the maize via the food and feed chain. This is akin to someone who has lost something in the dark and then only searches where street lamps shed light because that is where the light is available.

A similar approach was taken by EFSA in assessing the concentration of dsRNA and its downstream metabolic products in the plants. EFSA (2018a) states:
"The applicant provided a measure of the levels of DvSnf7 dsRNA in different tissues including grain and forage. However, the dsRNA is an intermediate molecule which is processed by dicer to siRNA molecules and the levels of dsRNA are not a good proxy for the levels of the active siRNAs in the plant (Paces et al., 2017). Therefore, the levels of the DvSnf7 dsRNA were not considered relevant for the risk assessment of maize MON 87411."

As a result, the data on the concentration of the biologically active molecules in the plants were not assessed. However, such data are necessary to assess the risks for the food chain and the fate of these molecules in the environment (see below).

Instead of performing detailed risk assessment, EFSA (2018a), contrary to scientific publications, (see below) simply assumed that:
"the amount of RNAs taken up and absorbed after oral ingestion is considered negligible in humans and animals (mammals, birds and fish)."

EFSA risk assessment of the dsRNA expressed in the plants on a molecular level might be described as the perfect example of a 'don´t look – don´t find' strategy, incompatible with existing regulation.

Moreover, greater uncertainties were observed in the MON 87411 feeding study, indicating unintended effects via the consumption of grains: the company conducted a 90-day feeding trial with maize MON 87411 in rats. In this feeding trial only one dosage of maize (33 %) was included as part of the diet, instead of different dosages as requested by existing guidance. Nevertheless, EFSA still accepted the data.

The stability of the test and control materials was not tested; therefore, it remains unclear if the diet is comparable to diets fed under practical conditions if, for example, the maize is fed to animals closer to the date of harvest.

The most relevant finding was weight depression in the rats fed with the maize. As EFSA (2018a) summarises : "*Statistically significant lower mean feed consumption (as g/cage per day only) were observed in males fed test diet (~ 9% in study week intervals 5–6, 9–10, 10–11, 11–12). This was associated with a statistically significant decrease in mean body weights, compared to the concurrent control (~ 7% in weeks 11 and 12) and in mean cumulative body weight (~ 12% in study week intervals 0–10, 0–11 and 0–12). Moreover, statistically significant lower mean weekly body weight change was also observed in males (study week intervals 0–1, 3–4, and 6–7) and in females (study week interval 7–8) fed the test diet, compared to the concurrent controls.*"

However, in the absence of test diet-related clinical signs and histopathological changes in the digestive tract, the GMO panel considered the changes to be non-adverse. Further, EFSA (2018a), without citing specific references, very generally questions whether the uptake of the dsRNA can be expected at all: "*Dietary ncRNAs [non coding RNAs] are generally rapidly denaturated, depurinated and degraded shortly after ingestion due to enzymes and conditions (e.g. pH) in the gastrointestinal tract lumen; in addition, the presence of barriers (e.g. mucus, cellular membranes) limits the cellular uptake of ncRNAs by gastrointestinal cells, and a rapid intracellular degradation of possible uptaken ncRNA occurs. Due to the above, the amount of RNAs taken up and absorbed after oral ingestion is considered negligible in humans and animals (mammals, birds and fish).*"

This assessment of toxicology has to be rejected for several reasons. As shown by Dávalos et al. (2019) and Nawaz et al. (2019) (see below), the uptake of ncRNA from plants via ingestion in sufficient amounts to exert effects on gene regulation in mammalian cells must be seen as a certainty. Further, the impact on the host via its microbiome is another way in which human or animal health could be affected.

Whatever the case, the EFSA risk assessment is contrary to EU Implementing Regulation 503/2013 which requests that *"for silencing approaches by RNAi expression, potential 'off target' genes should be searched by in silico analysis to assess if the genetic modification could affect the expression of other genes which raise safety concerns."*

2.3 New findings

A report commissioned and published by EFSA in 2019 (Dávalos et al., 2019) considers the role of ncRNA in the risk assessment of GE plants. Dávalos et al. summarise current findings on ncRNAs produced by plants; they discuss to which extent they can be taken up via food or feed consumption and show cross kingdom activity due to unintentional interaction with human or animal gene regulation. The findings of Dávalos et al. (2019) and Nawaz et al. (2019) were not mentioned by EFSA either in 2018 or 2019. Therefore, these publications have to be regarded as new information and also as new evidence since they go beyond the EFSA assessment.

2.3.1 Potential off-target genes in mammals

As Dávalos et al. (2019) show, there are many matches between the ncRNA produced in food and medical plants and regulatory pathways in human and animals. There is no doubt that in cases where relevant plant molecules are transmitted into the cells of humans and animals, RNAi effects, such as gene silencing, can occur and, for example, genes in animals can be downregulated by plant nscRNA.

Therapeutic effects from the uptake of ncRNA from the gut have been evidenced in several publications. Some of the research shows that biological effects can be achieved with very low dosages (for references see Dávalos et al., 2019).

2.3.2 Stability of ncRNA

It appears that some findings depend on the specific type of ncRNA. For example, naked synthetic ncRNA used by some researchers, is degraded very quickly compared to ncRNA produced by plants (for references see Dávalos et al., 2019).

The Dávalos et al. (2019) study found strong indications that plant miRNAs are more stable than previously anticipated. This is due to structural properties influencing their stability and turnover (EFSA, 2019a): *"However, when assessing the stability of plant ncRNAs outside the plant, compelling evidence exists that plant miRNAs are highly stable under different conditions including food storage, processing, cooking, or simulated digestion. Moreover, they seem to survive after long incubation in serum, or are detected in the gastric content of mice, suggesting that plant miRNAs are more resistant to degradation than synthetic or animal miRNAs."*

2.3.3 ncRNA uptake from the gut

Contrary to assumptions made in the assessment of the parental plants (EFSA, 2018a) and for stacked events (EFSA, 2019), research by Dávalos et al. (2019) shows that the uptake of ncRNA

from plants and microorganisms via the gut into the cells of humans and animals is an established fact.

It is known that there are many barriers between the intestine, the blood stream, the cells and the cell nuclei, which lower the likelihood of such RNAi effects occurring. However, according to Dávalos et al. (2019), there are mechanisms that can allow the molecules to pass through these barriers: plant ncRNA is protected against degradation by methylation, it can be excreted and taken up in vesicles (such as exosomes); nano-particles are also produced by plants which can serve as transport elements.

The ncRNA molecules originating from plants can reportedly be found in many bodily fluids of humans and animals, including blood and milk. Similar findings have been reported by Nawaz et al. (2019): "*Strong evidence suggested that plant-food-miRNAs can survive digestion, enter the body and affect gene expression patterns.*"

In this context, Dávalos et al. (2019) see the need for further research to explore the uptake and biological effects of ncRNA: *"Exogenous plant-derived ncRNAs have been found in exosomes or macrovesicles. How they reach these types of structures in biological fluids is unknown. In summary, supporting and contradicting evidence concerning the existence of systemic effects of dietary plant-derived exogenous ncRNAs is heavily debated. Important aspects such as the precise mechanism/s of transport of plant ncRNAs from food into the systemic circulation, the amount of exogenous ncRNAs reaching tissues or the molecular mechanisms of cellular uptake need to be determined."*

2.3.4 Interactions in the microbiome

There is strong evidence that ncRNAs originating in the host (e.g. produced by intestinal epithelial cells) are taken up by the gut microbiota and can manipulate its gene regulation. The same evidence is available for ncRNA produced in the gut microbiome: it can be taken up by the host and enact RNAi in its cells, demonstrating the existence of bidirectional ncRNAs based host-microbial interactions (for details see Dávalos et al., 2019).

In this context, Dávalos et al. (2019) show that plant-derived ncRNA does not necessarily have to be taken up from the intestine to exert its effects. Instead, interaction with the intestinal microbiome can emerge which, in a next step, may impact the health of the animal or human host.

This is of specific interest in the case of DvSnf7: the Snf7 gene which is targeted by the dsRNA produced in maize MON 87441, is involved in important biological processes in insects as well as in yeast. The essential role of the Snf7 as part of the ESCRT pathway is well described (see www.yeastgenome.org/locus/S000004015).

Thus, there is a plausible hypothesis on how the additional dsRNA might affect the gut microbiome community after ingestion, and further research is needed to understand the impact of exogenous dsRNA in mammalian host microbiota composition and identify microbial targets along with their effect on physiological conditions.

There is broad consensus on the role of the gut microbiome in human and animal health. For example, in 2019, in a study commissioned by EFSA, Parenti et al. (2019) states that "*one of the most important drivers of immune response is the gut microbiota and other microbial constituent of the human body which are able to regulate host-pathogen balance and to produce systemic pro-*

inflammatory stimuli. The lifelong antigenic load represented by foods and bacteria/bacterial products leads to a profound remodeling of the gut microbiota and these changes are emerging as a driving force of the functional homeostasis of the immune system. As a matter of fact, a perturbation of the gut microbiota homeostasis due to irregular lifestyles, stress and age may lead to gut microbiota dysbiosis. This condition may predispose the host to metabolic disorders and inflammation."

2.3.5 Conclusions

Therefore, the interaction between the ncRNAs produced by GE plants and the microbiome of humans or animals has to be considered in food and feed safety assessment. In this context, the barrier for ncRNA to pass from plants to gut microorganisms seems to be much lower compared to those identified in the human or animal body.

In summary, it is clear that interference with gene regulation following the absorption and processing of dsRNAs to siRNA within humans and animals after ingestion of RNAi-based GM crops is both feasible and plausible. As Nawaz et al. (2019) conclude: *"Based on the currently available evidence, off-target effects from the ingestion of novel siRNA present in foods derived from either GM crops or foliar insecticidal or anti-viral spray application, cannot be ignored and thus should form an integral part of the risk assessment of these products."*

2.4 Consequences for market authorisation of stacked maize MON 87427 x MON 89034 x MIR162 x MON 87411 and subcombinations

EFSA food and feed risk assessment of maize MON 87411, which produces an insecticidal ncRNA (EFSA, 2018b), did not consider effects on the microbiome and no empirical data were made available on the uptake of the molecules from the gut (Testbiotech, 2018). Also EFSA, in assessing the stacked Maize, did not consider the Dávalos et al. (2019) and Nawaz et al. (2019) findings.

The Implementing Directive 503/2013 request that "*potential 'off target' genes should be searched by in silico analysis to assess if the genetic modification could affect the expression of other genes which raise safety concerns"* was not fulfilled.

As shown by Dávalos et al. (2019) and Nawaz et al. (2019), the uptake of ncRNA from plants via ingestion in sufficient amounts to exert effects on gene regulation in mammalian cells must be seen as a certainty. Further, the impact on the host via its microbiome is another way in which human or animal health could be affected.

Therefore, further risk assessment has to be performed

- to trace the fate of the artificial ncRNA after ingestion
- to identify the potential target site in the microbial community in the gut and mammalian cells
- to assess the magnitude of potential effects if identified.

Additional questions have arisen from risk assessment in respect to the mixed toxicity of the stacked Maize. These questions are highly relevant for demonstrating the safety of the plants because other newly expressed proteins, residues from spraying or plant constituents, can influence the impact on the microbiome in the gut or the uptake from the gut.

It is known from several studies that uptake of miRNA from the mammalian gut and its detection is dependent on specific factors. For example, Liang et al. (2015) describe mechanisms for uptake and measurement that need to be taken into account to successfully quantify the uptake, Yang et al. (2015) and Wang et al. (2012) show that the health status of the recipient can be decisive; Baier et al. (2014) show that packaging in liposomes enhances uptake; Yang et al. (2015) show that dosage and also prolonged duration of exposure is important.

It has to be considered a plausible hypothesis that a combination of DvSnf7 dsRNA, Bt toxins and residues from spraying, can trigger effects on the immune system or other adverse health effects, either directly or via the microbiome, even if these were absent in the parental plants (see Testbiotech, 2021). Therefore, the stacked Maize needs to be tested, including taking into account synergistic effects due to other plant constituents or additional factors which may enhance stability or uptake of DvSnf7 dsRNA from the gut.

It is evident that in order to demonstrate safety of the stacked Maize, these factors, effects and mechanisms have to be taken into account for the health risk assessment of DvSnf7 dsRNA.

The Implementing Directive 503/2013 request that "*potential 'off target' genes should be searched by in silico analysis to assess if the genetic modification could affect the expression of other genes which raise safety concerns"* has to be fulfilled.

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

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

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

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

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

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

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

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

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

Final conclusion

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

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

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