TECHNICAL REPORT

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Assessment of the outcomes of the project "Risk Assessment of Genetically Engineered Organisms in the EU and Switzerland" (RAGES)

European Food Safety Authority (EFSA)
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Abstract

The "*Risk Assessment of genetically engineered organisms in the EU and Switzerland*" (RAGES) project (2016-2019) evaluated the risk assessment of genetically modified plants (GMPs) as performed by the European Food Safety Authority (EFSA) and its Swiss counterpart. RAGES claims shortcomings in several areas of the European risk assessment of GMPs, in particular: (1) the risk posed by herbicide tolerant GMPs on animal and human health; (2) the risk assessment approach of GMPs producing insecticidal proteins; (3) the risk assessment of nutritionally altered GMPs; (4) the assessment of GMPs combining different traits; (5) the environmental risk assessment of GMPs that can persist and spontaneously propagate in the environment; and (6) genetically modified organisms (GMOs) produced using new genetic engineering technologies. Following a request of the European Commission, EFSA reviewed the main scientific arguments raised by RAGES, and assessed whether the reports published by RAGES contain elements that could lead the GMO Panel to reconsider the outcome of its previous scientific opinions on GMPs. EFSA confirms that its risk assessment approach and data requirements are adequate and sufficient for the risk assessment of current GMPs, and that they are consistent with the applicable EU legislation and internationally agreed standards. Moreover, EFSA has gained more than 20 years of experience in the risk assessment of GMPs, and conducts a continued scientific review of all relevant evidence by following the scientific and technical developments in the GMO area. Overall, EFSA concludes that the final RAGES reports do not contain elements that would lead the GMO Panel to reconsider the outcome of its previous scientific opinions on GMPs. Therefore, EFSA considers that the previous GMO Panel risk assessment conclusions remain valid.

Keywords: Combinatorial effects, herbicide tolerant, insect resistant, new breeding techniques, next generation effects, spatio-temporal controllability

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Summary

The 3-year project (2016-2019) "*Risk Assessment of genetically engineered organisms in the EU and Switzerland*" (RAGES) evaluated the risk assessment of genetically modified plants (GMPs) as performed by the European Food Safety Authority (EFSA) and its Swiss counterpart. RAGES claims shortcomings in several areas of the European risk assessment of GMPs, in particular: (1) the risk posed by herbicide tolerant (HT) GMPs on animal and human health; (2) the risk assessment approach of GMPs producing insecticidal proteins; (3) the risk assessment of nutritionally altered GMPs; (4) the assessment of GMPs combining different traits; (5) the environmental risk assessment of GMPs that can persist and spontaneously propagate in the environment; and (6) genetically modified organisms (GMOs) produced using new genetic engineering technologies.

Following a request of the European Commission (EC), EFSA reviewed the main scientific arguments raised by RAGES, and assessed whether the reports published by RAGES contain elements that could lead the GMO Panel to reconsider the outcome of its previous scientific opinions on GMPs. The reports of the RAGES project have been analysed by EFSA. The main scientific arguments put forward in the RAGES reports are addressed in this Technical Report.

EFSA notes that:

(1) Data for the agronomic, phenotypic and compositional characterisation of HT GMPs and derived food and feed products are gathered in field trials where GMPs are exposed to conventional and to the intended herbicide(s) in line with good agricultural practices, and thus they provide a robust basis for the comparative analysis of HT GMPs.

(2) All potential routes of environmental exposure of non-target organisms (NTOs) to GMPs for cultivation are typically considered as part of the risk assessment of GMPs, including those producing insecticidal proteins. EFSA appraises and analyses NTO data following a weight of evidence approach and applies the principles and methods available at the time of the risk assessment.

(3) Its guidance documents provide the necessary principles, strategy and data requirements for a comprehensive and adequate risk assessment of nutritionally altered GMPs for food and feed uses.

(4) The current risk assessment approach for stacked events in GMPs is sufficient to address the identification of risks for animals and consumers. EFSA does not consider the use of long-term animal studies with whole food and feed appropriate to explore the safety of GMPs in the absence of specific hypotheses to test.

(5) The approach for the environmental risk assessment of GMPs that can persist and spontaneously propagate in the environment is fit for purpose and consistent with EU requirements and internationally agreed approaches.

(6) It is currently assessing the adequacy and sufficiency of its current guidelines for the risk assessment and post-market environmental monitoring of GMOs produced through new genetic engineering technologies. The risk assessment approaches applied to GMPs are generally considered suitable for GMPs that induce silencing through RNA interference, without the necessity to develop specific guidance.

Overall, issues raised by RAGES reveal a different perspective on protection goals and the types of evidence and efforts needed to inform the risk assessment of GMPs and derived food and feed products than that of EFSA.

EFSA conducts the risk assessment in an independent and transparent way and provides scientific advice to risk managers on the risks that the use of GMPs may pose to human health, animal health and the environment. EFSA confirms that its risk assessment approach and data requirements are adequate and sufficient for the risk assessment of current GMPs, and that they are consistent with the applicable EU legislation and internationally agreed standards. Moreover, EFSA has gained more than 20 years of experience in the risk assessment of GMPs and conducts a continued scientific review of all relevant evidence by following the scientific and technical developments in the GMO area. Such developments

inform the update of EFSA's risk assessment approaches and guidelines, a process in which stakeholders play an important role.

EFSA concludes that the final RAGES reports do not contain elements that could lead the GMO Panel to reconsider the outcome of its previous scientific opinions on GMPs. Therefore, EFSA considers that the previous GMO Panel risk assessment conclusions remain valid.

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1. Introduction

The 3-year project (2016-2019) "*Risk Assessment of genetically engineered organisms in the EU and Switzerland*" (RAGES) evaluated the risk assessment of genetically modified (GM) plants as performed by the European Food Safety Authority (EFSA) and its Swiss counterpart. The RAGES consortium was composed of the non-governmental organisations Testbiotech e.V., European Network of Scientists for Social and Environmental Responsibility (ENSSER), Critical Scientists Switzerland and GeneWatch UK. The RAGES consortium reported the results and conclusions of the project in six separate reports, an overview report and a summary report (see Section 2.1), which were all published online on 15 January 2020.[1]

Since 2018, the European Commission (DG SANTE) and EFSA's GMO Unit followed the RAGES activities by participating to the two workshops organised by RAGES and the final workshop held at EU Parliament where the final results were reported.

1.1. Terms of Reference as provided by the requestor

On 17 February 2020, EFSA received a request from the European Commission to "*analyse the scientific elements included in the published reports (6 sub-reports, overview and summary reports) and to provide the Commission with a response indicating whether or not these reports contain elements that could lead the GMO Panel to reconsider the outcome of its previous opinions on genetically modified crops*".

2. Data and Methodologies

2.1. Data

In delivering this technical report, EFSA considered the scientific arguments put forward by RAGES in the six reports entitled:

1. Serious shortcomings in the European risk assessment of herbicide tolerant GE plants for human health;[2]
2. Insecticidal *Bt* plants. EFSA's risk assessment approach for GM *Bt* plants fails by design;[3]
3. Health risk assessment of genetically engineered nutritionally-altered GM plants;[4]
4. Assessment of health risks associated with the consumption of products derived from genetically engineered plants with a combination of traits;[5]
5. Environmental risk assessment of genetically engineered plants that can persist and spontaneously propagate in the environment;[6]
6. New genetic engineering technologies.[7]

Along with the six reports, RAGES published the following complementary documents which were also considered by EFSA:

- Overview: The RAGES project;[8]

[1] Available at https://www.testbiotech.org/en/content/research-project-rages
[2] Available at https://www.testbiotech.org/en/content/rages-subreport-risk-assessment-herbicide-tolerant-ge-plants
[3] Available at https://www.testbiotech.org/en/content/rages-subreport-insecticidal-bt-crops
[4] Available at https://www.testbiotech.org/en/content/rages-subreport-risk-assessment-nutritionally-altered-gm-crops
[5] Available at https://www.testbiotech.org/en/content/rages-subreport-assessment-combinatorial-effects
[6] Available at https://www.testbiotech.org/en/content/rages-subreport-risk-assessment-next-generation-effects
[7] Available at https://www.testbiotech.org/en/content/rages-subreport-new-genetic-engineering-technologies
[8] Available at https://www.testbiotech.org/en/content/overview-rages-project

- Summary report of the results from the RAGES project 2016-2019[9] with a summary table.[10,11]

Whenever relevant, EFSA took into account for assessing the case studies used by RAGES data reported in applications for GMO authorisation on the EU market, or in the scientific literature.

2.2. Methodologies

EFSA considered the risk assessment principles described in its applicable guidelines (EFSA GMO Panel, 2010a, 2011a), other relevant EFSA documents (EFSA GMO Panel, 2015a, 2017a), Regulation (EU) No 503/2013[12] and Commission Directive (EU) 2018/350.[13]

At the two workshops organised by RAGES (30 October 2018 in Brussels, Belgium, and 29 October 2019 in Neuchâtel, Switzerland), representatives of EFSA's GMO Unit and the EC commented verbally on earlier versions of the RAGES reports, focusing on scientific and legal requirements respectively. The aim was to provide RAGES with detailed comments on their draft reports, for consideration during preparation of the final reports.

EFSA's assessment focuses on scientific elements and does not cover legal aspects, as they are not in EFSA's remit and not in the frame of the mandate received from the EC.

3. Assessment

EFSA's assessment described below is structured according to the six reports and complementary documents published by the RAGES consortium. For each of these documents, the main outcomes reported by RAGES are briefly summarised and followed by EFSA's assessment and conclusions.

3.1. "Serious shortcomings in the European risk assessment of herbicide tolerant GE plants for human health" report

3.1.1. Main issues reported by RAGES

- Field trials for the agronomic/phenotypic and compositional characterisation of herbicide tolerant (HT) GMPs systematically underestimate risks, as non-representative herbicide regimes are applied in such trials;
- Herbicides applied on HT GMPs (and associated metabolites) interact with plant constituents leading to cumulative and/or combinatorial effects that are not sufficiently taken into account. These changes can have toxicological, hormonal or immunological effects on animals and humans following dietary intake;
- Current set of regulatory HT GMP studies are insufficient to assess animal and human health safety, especially for stacked events or GMPs with complex traits.

[9] Available at https://www.testbiotech.org/en/content/summary-report-results-rages-project-2016-2019
[10] Available at https://www.testbiotech.org/en/content/tabled-overview-rages
[11] The summary table organises some of the main issues identified of the RAGES project by scientific areas i.e. molecular characterisation, plant composition and agronomic and phenotypic characteristics, toxicology and allergenicity end ERA. Each issue is followed by its relevance and by the concrete measures to be put in place.
[12] Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L 157, 8.6.2013, p. 1-48. http://data.europa.eu/eli/reg_impl/2013/503/oj
[13] Commission Directive (EU) 2018/350 of 8 March 2018 amending Directive 2001/18/EC of the European Parliament and of the Council as regards the environmental risk assessment of genetically modified organisms. OJ L 67, 9.3.2018, p. 30–45. http://data.europa.eu/eli/dir/2018/350/oj

3.1.2. EFSA's assessment

3.1.2.1. Non-representative herbicide regimes in pre-market risk assessments

Similar criticisms on the non-representative herbicide regime applied on HT GMPs have been previously addressed by EFSA (EFSA, 2015a, 2019, 2020).

Saying that EFSA does not require the sprayings of field trials in accordance with current agricultural practices is incorrect. The herbicide management of field trials for HT GMPs is challenging because it needs to produce relevant and interpretable information for the comparative assessment and, simultaneously, to expose the GMPs to all the intended herbicides to which they are tolerant. Considering that in such trials only the GMPs - but not the non-GM comparator(s) nor reference varieties - are tolerant to the intended herbicides, the experimental design allows the experimental materials to be managed with different herbicide regimes.

More specifically, the requirements for HT GMPs stipulate to perform field trials for the agronomic/phenotypic and compositional analysis with and without the application of intended herbicide(s) as well as with the application of the conventional herbicide(s). This mandatory requirement was first introduced in the EFSA guidance for risk assessment of food and feed (EFSA GMO Panel, 2011a), and retained in Regulation (EU) No 503/2013 later on. To further clarify and standardise the type of information the applicants should report with regards to the application of herbicides (e.g. timing, dose, volumes, coadjuvants), the GMO Panel self-tasked the development of the EFSA guidance on the agronomic and phenotypic characterisation of GMPs (EFSA GMO Panel, 2015a), that includes these elements.

In the field trials for comparative analysis of HT GMPs, the intended herbicides are kept at a similar application rate across sites, to ensure comparability between locations, while the combinations of conventional herbicides applied at the selected sites reflect different weed management practices, chosen to maintain the weed pressure under control. EFSA verifies that the timing and rate of the applied intended herbicides are in line with the recommendations of the manufacturers. This information is routinely verified by the GMO Panel and specifically discussed in the section of the scientific opinion on management practices. EFSA highlights that the production of the material used for the comparative studies is conducted in field trials managed in line with good agricultural practices (GAPs). The current agronomic, phenotypic and compositional comparative analysis is considered comprehensive and conservative, taking into account that intended herbicides are applied on top of conventional herbicide management and that a range of different herbicide management regimes have to be applied.

3.1.2.2. Cumulative and/or combinatorial effects in HT GMPs that can have health effects on animals and humans and current set of regulatory HT GMP studies insufficient to assess animal and human health safety, especially for stacked events or GMPs with complex traits

Similar criticisms on the toxicological methodology and assessment of HT GMPs and complex traits have been previously addressed by EFSA (EFSA, 2019).

Since these criticisms largely overlap with those raised in the report on "Assessment of health risks associated with the consumption of products derived from genetically engineered plants with a combination of traits", these are addressed in the respective Section 3.4.

RAGES also provided a review on the toxicity of the intended herbicides applied to HT GMPs. RAGES acknowledged this was not in the scope of its report and EFSA is not commenting further on this topic.

3.1.3. Conclusion

EFSA does not support the statement that the EU assessment of HT GMPs excludes systematically many of the potential risks from the evaluation. The production of the material used for the comparative analysis studies is conducted in field trials managed in line with GAPs and the current compositional analysis is considered comprehensive. Issues related to EFSA's safety assessment of HT GMPs regarding potential health effects for humans and animals largely overlap with those discussed in Section 3.4, and are addressed there.

3.2. "Insecticidal *Bt* plants. EFSA's risk assessment approach for GM *Bt* plants fails by design" report

3.2.1. Main issues reported by RAGES

- EFSA's non-target organisms (NTOs) risk assessments of insect resistant GMPs are reduced to the assessment of their newly expressed proteins (NEPs);
- EFSA assumes that insecticidal proteins from *Bacillus thuringiensis* (*Bt* proteins) have a single target and specific mode-of-action against target organisms;
- EFSA omits specific environmental routes of exposure through which NTOs can be exposed to *Bt* proteins;
- EFSA ignores or dismisses relevant evidence by denying documented negative effects on several groups of NTOs;
- Expression of *Bt* proteins in teosinte through vertical gene flow from GM *Bt*-maize is an emerging hazard that EFSA failed to assess properly.

3.2.2. EFSA's assessment

3.2.2.1. Assessment of potential intended and unintended effects

It is incorrect to claim that EFSA's NTO risk assessments of GMPs for cultivation only address the NEPs. EFSA's NTO risk assessments are designed to evaluate whether the deployment of GMPs can have direct or indirect adverse effects on NTOs and the ecosystem services they provide. This evaluation covers potential adverse environmental effects on NTOs due to intended and unintended changes in the GMP. In line with internationally agreed risk assessment practices, potential harmful effects on NTOs associated with the newly expressed proteins are evaluated within different tiers that progress from laboratory studies representing highly controlled, worst-case expected environmental concentrations using microbial-derived (purified) proteins (Tier 1a), to laboratory bioassays with more realistic exposure to the newly expressed protein (Tier 1b), semi-field (Tier 2) and field (Tier 3) studies carried out under less controlled conditions. In lower tiers, representative NTOs are exposed to concentrations of the novel protein in excess of predicted worst-case exposure levels in the field. The aim of these high-dose laboratory studies is to maximise the likelihood of detecting adverse effects on NTOs. Moving to a higher tier is only considered relevant by EFSA if adverse effects are detected at a lower tier, or if unacceptable scientific uncertainty remains (EFSA GMO Panel, 2010a), thereby iteratively addressing risk in a manner consistent with the level of concern and the uncertainty in the assessment. The tiered approach has proven adequate to categorise the newly expressed proteins in GMPs that pose low risk to NTOs and require little or no further testing, from those that pose high risk and require further evaluation. Overall, this approach is considered sufficiently accurate and conservative to detect harmful effects that might be manifested in the field. Whether the safety assessment of the newly expressed proteins requires testing is evaluated on a case-by-case basis, depending on the experience and existing knowledge on the source and properties of the protein (and derived constituents), their function/activity, whether they are known to be toxic to NTOs, and whether NTOs will be exposed to the proteins.

In addition of the NTO risk assessment of the NEPs of GMPs, the GMO Panel requires laboratory bioassays with plant material for all GMPs for which authorisation for cultivation is in the scope of the application, to capture potential unintended effects, unknown complexities and variability in GMPs, irrespective of the intended traits. In this respect, EFSA requested applicants to submit *in planta* data for cultivation applications of insect resistant GMPs (e.g. EFSA GMO Panel 2011b, 2012a) and HT GMPs (e.g. EFSA GMO Panel 2011c, 2012b).

As part of its assessments of GMPs, EFSA has regularly evaluated relevant NTO studies reported in the peer-reviewed scientific literature. Examples include: (1) the risk assessment of GMP applications for cultivation; (2) updates of the risk assessment conclusions and risk management recommendations of the maize events MON 810, Bt11 and 1507; (3) annual post-market environment monitoring reports on the cultivation of maize MON 810; (4) requests from the European Commission to assess relevant scientific publications; and (5) emergency measures and safeguard clauses on maize MON 810 invoked by several EU Member States.

3.2.2.2. Activity spectrum/mode of action of *Bt* proteins

It is incorrect to claim that EFSA does not consider the spectrum of activity of insecticidal *Bt* proteins when assessing their environmental risk on NTOs. For all GMP applications for cultivation, earlier-tier studies have been requested from applicants on a range of NTOs belonging to different taxonomical groups, going beyond that of the target pests.

For the selection of focal species for toxicity testing, EFSA GMO Panel (2010a) has proposed the following steps: (1) identification of relevant functional groups; (2) categorisation of NTO species from identified functional groups; (3) species prioritisation based on ecological criteria; and (4) final selection of focal species taking into consideration practical criteria (availability and amenability for testing). At least one relevant focal NTO from each relevant functional group should be selected for testing. For species selection, EFSA's arthropod databases on bio-ecological information on non-target arthropod species are available (Meissle et al., 2012; Riedel et al., 2015).

3.2.2.3. Exposure assessment

It is incorrect to claim that EFSA does not consider all potential routes through which NTOs from the different environmental compartments can be exposed to the newly expressed proteins, e.g. exposure to *Bt* pollen. EFSA's risk assessments of GMPs for cultivation cover all relevant direct and indirect exposure pathways to which terrestrial, soil and aquatic NTOs can be exposed to different matrixes (plant, soil, aquatic environments), as well as the level of exposure to such proteins.

EFSA notes that, in the context of GMP applications for import and processing for food/feed uses, excluding cultivation in the EU, interactions of insect resistant GMPs with NTOs are not considered a relevant issue by the GMO Panel. Due to the limited environmental exposure of NTOs to spilled GM seeds or occasional feral GMPs arising from spilled seed, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GMP, no consequential harm to NTOs is identified.

EFSA has quantified the risk to non-target Lepidoptera associated with the ingestion of maize MON 810, Bt11 and 1507 (referred to hereafter as *Bt*-maize) pollen deposited on their host plants through estimates of larval mortality based on mathematical models developed by Perry et al. (2010, 2011, 2012, 2013). Since 2009, EFSA and its GMO Panel have published seven scientific outputs on this topic, either on their own initiative or on request of the European Commission, applying and further refining the model in a stepwise approach, whilst taking into account new relevant scientific publications, like those cited by RAGES (Hofmann et al., 2014). In these scientific outputs, EFSA and its GMO Panel acknowledge that: (1) uncertainties pertaining to the structure of the Perry et al. (2010, 2012) models, mostly caused by the lack of data from bioassays estimating the sensitivity of a wider range of 'real' NT Lepidoptera for most of the assessed *Bt*-maize events; and (2) uncertainties contributing to the variability in exposure of NT Lepidoptera to *Bt*-maize pollen.

In April 2018, EFSA launched an open call to develop a model to quantify risks to NT Lepidoptera associated with the ingestion of *Bt*-maize pollen deposited on their host plants.[14] The model is expected to be publicly available at the end of 2020, together with a detailed user guide/manual that explains how to use it.

3.2.2.4. Weight of evidence for NTO risk assessment

EFSA rejects the claim of bias in its appraisal of NTO studies supporting the risk assessment of GMPs for cultivation. In all its GMP assessments, EFSA considers all scientific evidence available following a weight-of-evidence approach. This information comprises: data submitted by applicants in the initial dossier, additional data supplied by applicants upon EFSA's request, scientific comments provided by EU Member States, and relevant peer-reviewed publications.

The careful evaluation of the quality of NTO studies used in support of the NTO risk assessment of insect resistant GMPs is a critical step in the environmental risk assessment (ERA) of GMPs. EFSA concurs that studies supporting the ERA of GMPs should comply with defined quality standards and strict procedures, as this increases confidence in the results and adds certainty to the conclusions drawn. EFSA also recognises the added value of critical appraisal tools to promote a systematic, predictable

[14] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2017-00702

and transparent evaluation of the quality of studies. Any study should be reproducible and performed minimising the probability of erroneous or inconclusive results. According to the weight of evidence approach, EFSA evaluates the relevance and reliability (methodological quality) of NTO studies. When assessing the relevance of NTO studies for the risk assessment of GMPs for cultivation, it is important that results seen under worst case exposure conditions in laboratory settings are brought in the context of expected environmental exposure levels. To characterise the risk of GMPs to NTOs, EFSA assembles, weighs and integrates all available evidence.

EFSA notices that some relevant publications dealing with the interactions of insecticidal *Bt* proteins on NTOs, including several meta-analyses (e.g. Marvier et al., 2007; Duan et al., 2008; Wolfenbarger et al., 2008; Naranjo, 2009; Comas et al., 2014; Romeis et al., 2014; Dang et al., 2017; Pellegrino et al., 2018) have not been considered/cited by RAGES. Overall, these studies have collectively concluded that non-target effects of *Bt* plants are minimal or negligible, especially in comparison to the negative effects of the use of insecticides for controlling of target organisms (Romeis et al., 2019).

3.2.2.5. Teosinte as an emerging hazard

Following a mandate from the European Commission in 2016, EFSA assessed whether the scientifically relevant information on teosinte contained new evidence that would change or invalidate its previous ERA conclusions and risk management recommendations on the cultivation of maize MON 810, Bt11, 1507 and GA21 (EFSA, 2016). In its assessment, EFSA explored whether plausible pathways to harm from the cultivation of GM maize could be hypothesised for situations where GM maize and teosinte would grow sympatrically, focusing on four specific areas of risk that are typically considered in ERAs of GMPs: (1) altered persistence and invasiveness of GM maize × teosinte hybrids; (2) cross-pollination of maize by GM maize × teosinte hybrids; (3) interactions of GM maize × teosinte hybrids with other organisms; and (4) interactions of GM maize × teosinte hybrids with abiotic environment and biogeochemical processes. For each of these pathways, EFSA considered unlikely that environmental harm would be realised. EFSA therefore concluded that there were no data in the scientific information provided by the European Commission on teosinte, or in any other scientific report, indicating the necessity to revise the previous ERA conclusions and risk management recommendations for the GM maize events made by the GMO Panel (EFSA, 2016). EFSA is not aware of new evidence that would invalidate the conclusions of EFSA (2016). EFSA notes that, in the context of risk assessed GMP applications for import and processing for food/feed uses, excluding cultivation in the EU, the likelihood/frequency of cross-pollination between occasional feral GM maize plants resulting from grain spillage and teosinte is considered extremely low.

3.2.3. Conclusion

EFSA disagrees with the claims made by RAGES with regards to the ERA of insect resistant GMPs. EFSA considers all potential routes of environmental exposure of NTOs to GMP material and their associated newly expressed proteins, and all available scientific evidence. EFSA appraises and analyses the data following a weight of evidence approach and applies the principles and methods available at the time for the risk assessment. EFSA strives to maintain scientific rigour in the assessment of the risk that insect resistant GMPs could pose to NTOs, and consistently provides risk managers with the most up to date scientific advice accounting for new relevant scientific publications and developments in the field.

3.3. "Health risk assessment of genetically engineered nutritionally -altered GM plants" report

3.3.1. Main issues reported by RAGES

- Lack of specific EFSA guidance for the risk assessment of nutritionally altered GM plants.
- Lack of comprehensive nutritional (safety) assessment: inappropriate compositional analysis, poor understanding of the overall health effect of nutritional changes, vulnerable groups not considered, impact of plant processing not assessed, changes in food from animals fed with nutritionally altered GMPs not assessed.
- Post-market monitoring (PMM) plans proposed for nutritionally altered GMPs are inadequate. The report from RAGES also covered labelling which is not in the EFSA's remit and thus was not discussed during EFSA's assessment.

3.3.2. EFSA's assessment

3.3.2.1. Lack of specific guidance for the risk assessment of nutritionally altered GM plants

Similar criticisms on the lack of specific guidance for assessing GMPs with altered nutrient composition have been previously addressed by EFSA (EFSA, 2015b).

The body of EFSA guidance documents provides principles, strategy and data requirements for the risk assessment of GMPs for food and feed uses, including nutritionally altered GMPs. An external scientific report published by EFSA in 2013 "Review of the strategies for the comprehensive food and feed safety and nutritional assessment of GM plant per se" concluded that the comparative assessment is the most reliable method, with possible adjustments, to also assess nutritionally altered plants (ADAS, 2013). In the case of nutritionally altered GMPs, dietary intake estimations became a key element in the nutritional assessment, where different consumption scenarios (replacement of conventional foods, replacement of similar foods, etc.) are considered. This information is typically provided as part of application dossiers and subsequently complemented following GMO Panel requests, when needed. EFSA does not support the claim that the risk assessment for nutritionally altered GM plant needs the development of specific guidance, and considers the current GMO Panel guidance documents (EFSA GMO Panel, 2010a, 2011a, 2015a) adequate to conduct the RA of these GM plants. In addition, on a case-by-case basis, EFSA collects and analyses additional data to identify and characterise any possible risk that could emerge from particular plants.

3.3.2.2. Lack of comprehensive nutritional (safety) assessment

EFSA agrees with RAGES on the importance of a regular reviewing of the scientific literature regarding the effect of the nutrients on human health (positive and/or negative), considering the vast number of studies that continuously becomes available, often with contradictory outcomes. At the same time, EFSA wants to point out that the EU risk assessment of GMOs focuses on their safety and not on potential human and animal health benefits (irrelevant for the authorisation). The need for systematic review of scientific studies was introduced with Regulation (EU) 503/2013, according to which applicants must submit all available studies to demonstrate that the GM food and feed complies with the requirements (e.g. must not have adverse effects on human health, animal health or the environment) as described in articles 5 and 17 of Regulation (EC) 1829/2003 and detailed in the Explanatory note on literature search recently published by EFSA (EFSA et al., 2019).

RAGES claims lack of specific requirements for the compositional data of nutritionally altered plants to take into account the importance of G × E interactions and herbicide applications. EFSA points out that the applicable GMO Panel guidance documents (EFSA GMO Panel, 2011a, 2015a) cover the analysis of possible G × E interactions for all GMPs, i.e. also for nutritionally altered GMPs. In addition, EFSA wishes to stress that the nutritional assessment always considers the composition analysis of the GMPs treated with the intended herbicide/s or, alternatively, the processed foods derived from the treated GMPs.

The nutritional assessment carries out by EFSA follows a step-wise approach considering different pieces of information, including the most relevant and recent scientific evidence available at the time of the

assessment. The most recent information on Dietary Reference Values (DRVs) and/or Tolerable upper intake levels (UL) for the different nutrients are used during the assessment. For those components where significant differences in the GM plant are identified in comparison to its conventional counterpart and when these differences are not explained by natural variability, EFSA conducts a specific nutritional assessment to identify or discard any safety concern. The biological relevance of the identified nutrients, the role of the GMP and its products as contributors to total intake of the respective nutrient, and the magnitude and direction of the observed changes are considered during the nutritional assessment. When needed, nutrient intakes are estimated using consumption data of conventional foods likely to be replaced by those derived from the GMPs, and/or taking into account target foods where the GM derived food (e.g. oil) will be incorporated (Codex Alimentarius, 2009). Typically, overly conservative scenarios replacing not only the corresponding conventional foods but also similar ones (e.g. replacement of all vegetable oils by GM derived oil) are also considered. Nutritional intakes are compared to DRV and UL or, when reference values are not available for particular nutrients, to maximum doses without adverse effects derived from human intervention studies. For new compounds not present in the conventional plant (e.g. specific fatty acids), and even in the absence of any type of reference value, scientific evidence must be provided to support their safety (e.g. information on levels in commonly consumed foods, scientific literature on the absence of safety concerns, etc.). The uncertainties linked to the overly conservative intake scenarios typically used (e.g. 100% replacement) are considered when interpreting the outcome of the assessment and concluding on the absence/presence of any nutritional concern in the population.

EFSA acknowledges that, in certain cases, consumption data on particular vulnerable populations are needed for the assessment of nutritionally altered plants as well as representative data across European countries. In this respect, the most recent assessments have progressed on both aspects making use of the EFSA Comprehensive Consumption database,[15] where consumption data in more than 20 European countries cover different age classes (infants, toddlers, children, adolescents, adults, elderly) and vulnerable groups (pregnant and lactating women). The appropriate labelling of food products containing, consisting of or produced from the GMP as required in the authorizations, allows incorporating or excluding these products from the diet depending on individual health needs.

EFSA does not support the RAGES claim that the effect of processing and storage on the nutritionally altered GMPs is not considered in the nutritional assessment. Composition data of both raw agricultural commodities (i.e. seeds) and processed products (i.e. oil) are compared and considered during the risk assessment of GM plants with modified fatty acid profile. As it occurs during the processing of conventional seeds, small amounts of trans-fatty acids might be generated; these fatty acids are nutritionally assessed to identify any concern linked to their intake. Oxidative stability studies are requested when the genetic modification implies a fatty acid composition that could make the oil more susceptible to oxidation reactions (higher levels of polyunsaturated fatty acids) as compared to oils from the conventional counterpart. Food commodities other than oil are also considered during the nutritional assessment.

RAGES claims that EFSA does not cover in the risk assessment the safety of food derived from food producing animals fed with nutritionally altered plants. Reg. (EC) 1829/2003 provides the basis for ensuring a high level of protection of human life and health, animal health and welfare, environment and consumer interests in relation to genetically modified food and feed produced from a GMO. However, the assessment of the safety of food products obtained from animals fed with genetically modified feed is not in the scope of this Regulation.

3.3.2.3. Post-market monitoring plans

EFSA disagrees with RAGES regarding the inadequacy of post-market monitoring (PMM) plans and labelling proposals for nutritionally altered GMPs.

As set in Regulation (EU) No 503/2013, once the safety of the GMP is demonstrated, PMM is appropriate to confirm the predicted consumption, the application of conditions of uses, or identified effects. PMM

[15] https://www.efsa.europa.eu/en/food-consumption/comprehensive-database

requirements are set out in the Commission Implementing Decisions[16] authorising nutritionally altered GM plants.

3.3.3. Conclusion

EFSA considers that the body of EFSA guidance documents provides principles, strategy and data requirements for the risk assessment of GMPs for food and feed uses, including GM food and feed with nutritional traits. EFSA carries out a comprehensive nutritional assessment of all GMPs, in particular of those with modified nutrient profile, using a stepwise approach that contemplates the use of different dietary intake scenarios as needed. PMM plans as described in the Commission Implementing Decisions are adequate tools to monitor changes in the information considered during the pre-market risk assessment that might indicate the need of revisiting that initial assessment.

3.4. "Assessment of health risks associated with the consumption of products derived from genetically engineered plants with a combination of traits" report

3.4.1. Main issues reported by RAGES

- The risk assessment of GMPs expressing a combination of traits should take into consideration combinatorial effects and toxicity of mixtures;
- The assessment should be based also on the use of long -term feeding studies with materials derived from plants exposed to dosage of intended herbicide(s) reflecting the current agricultural practices and therefore being representative of the consumed products to investigate toxicological, reproductive, hormonal, immunological effects on consumers;

3.4.2. EFSA's assessment

EFSA has previously comprehensively addressed similar criticisms on the methodology applied by EFSA for the risk assessment of genetically engineered plants with a combination of traits with respect to the assessment of combinatorial effects and mixed toxicity and on allergenicity (EFSA, 2019). Key points are summarised below.

3.4.2.1. General principles for the EFSA assessment of GMPs with stacked events

In accordance with the principles described in GMO Panel guidelines (2010a, 2010b and 2011a), the assessment of GMPs with stacked events relies on the outcome of the risk assessment of the single events and, in addition, evaluates potential synergistic or antagonistic effects resulting from the combination of the events. To conduct this assessment, EFSA considers all datasets provided by the applicant, which include the data on the single events, on the stack itself and, in case of segregating plants like maize, on its subcombinations, when available. The GMO Panel approach for the risk assessment of subcombinations is described in detail in the Annex 1 of the notes of the 118th Plenary meeting of the GMO Panel. [17]

[16] Commission Implementing Decision (EU) 2015/686 of 24 April 2015 authorising the placing on the market of products containing, consisting of, or produced from genetically modified soybean MON 87769 (MON-87769-7) pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council (notified under document C(2015) 2757) OJ L 112, 30.4.2015, p. 16–21 http://data.europa.eu/eli/dec_impl/2015/686/oj
Commission Implementing Decision (EU) 2015/698 of 24 April 2015 authorising the placing on the market of products containing, consisting of, or produced from genetically modified soybean 305423 (DP-3Ø5423-1) pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council (notified under document C(2015) 2773) OJ L 112, 30.4.2015, p. 71–76 http://data.europa.eu/eli/dec_impl/2015/698/oj
Commission Implementing Decision (EU) 2015/696 of 24 April 2015 authorising the placing on the market of products containing, consisting of, or produced from genetically modified soybean MON87705 (MON-877Ø5-6) pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council (notified under document C(2015) 2770) OJ L 112, 30.4.2015, p. 60–65 http://data.europa.eu/eli/dec_impl/2015/696/oj

[17] http://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf

3.4.2.2. Assessment of potential synergistic or antagonistic effects resulting from the combination of the transformation events in regard to toxicology and allergenicity

The assessment of potential interactions (synergistic or antagonistic effects) resulting from the combination of events in stacks and of relevance for humans, animals and the environment is based on molecular characterisation data (e.g. integrity of the events and expression levels of the newly expressed proteins), on the outcome of the comparative analysis studies (including results on HT GMPs treated with the intended herbicide/s) and on the safety assessment of interactions among NEPs (see EFSA, 2019 for details). In particular the assessment of combination of compounds (e.g. NEPs, constituents) is built on the current knowledge on the biological characteristics of the compounds. Additional studies are requested in case interactions of relevance for safety are expected on the basis of the mode of action on NEPs and the information on their toxicological assessment. EFSA is actively working at developing new methodologies for the assessment of mixed toxicity that can be horizontally relevant for food and feed risk assessment. A data model for probabilistic cumulative dietary exposure assessment of pesticides has been published (EFSA PPR Panel, 2012), and procedures for establishing cumulative assessment groups of pesticides on the basis of their common toxicological effects are under development. EFSA also strives for continuously improving the assessment of combined exposure to multiple substances, horizontally applicable to risk assessment areas (EFSA et al., 2020a, 2020b). Dedicated risk assessment methodologies were developed and applied in two pilot cumulative risk assessments (CRA) of pesticide residues, one considering chronic effects on the thyroid system (EFSA et al., 2020c) and the other considering acute effects on the nervous system (EFSA et al., 2020d). EFSA and the EC are currently defining an implementation plan to assess in the coming years the effects of pesticides on other organs and body functions.

In relation to allergenicity and the immune system in general, EFSA performs its risk assessment according to relevant guidelines (EFSA GMO Panel, 2011a; Regulation 503/2013), the principles of which are aligned with international documents (Codex Alimentarius, 2009). According to RAGES, the potential impacts on the immune system (such as adjuvant effects) have not been appropriately investigated. In particular, RAGES claims that the potential immune adverse effects of *Bt* proteins were not addressed because their additive or combinatorial effects were not sufficiently assessed and because they are not degraded in the gut upon oral consumption. EFSA previously published comprehensive scientific reports addressing similar questions on the EFSA assessment of GMPs and the potential effects of *Bt* proteins on the immune system (e.g. EFSA GMO Panel, 2017b; EFSA et al., 2018a; EFSA 2019). Briefly, the GMO Panel does not find indications that *Bt* proteins in GM maize assessed by the Panel might act as adjuvants with the potential to enhance a specific immunoglobulin E (IgE) response and to favour the development of an allergic reaction. Furthermore, because none of the newly expressed proteins in the assessed GMPs showed potential for allergenicity, considering current knowledge, no reasons for concerns regarding the simultaneous presence of these newly expressed proteins in the GM maize are expected. In relation to *in vitro* degradation studies with *Bt* proteins, EFSA highlights that the studies provided by applicants are considered as additional information for the safety assessment of the newly expressed proteins in line with international (Codex Alimentarius, 2009) and EFSA guidelines (EFSA GMO Panel, 2011a). It is noted that the GMO Panel published a guidance document in 2017 on allergenicity providing additional considerations and potential refinements to the *in vitro* protein degradation studies (EFSA GMO Panel, 2017a). Subsequently, EFSA launched a procurement call to test the proposed revisions and the outcome of such studies was recently published (Mackie et al, 2019). EFSA is currently considering the added value of such test in risk assessment and recommendations for future developments.

Finally, EFSA underlines its commitment and involvement in actively contributing to the debate on the topic and will continue to invest on the development of more robust and modern approaches (e.g. *in vitro* and *in silico* tools) for allergenicity assessment. Examples of this commitment are: (1) the guidance document on allergenicity assessment previously mentioned (EFSA GMO Panel, 2017a), where new scientific elements were considered in the risk assessment process; (2) the comprehensive EFSA analysis of new publications on the topic and proposals for future development (EFSA et al., 2018a); (3) the proactive collection of information to be used in future discussions (Parenti et al 2019); and (4) production of experimental data (Mackie et al 2019). Furthermore, EFSA was involved in past EU funded projects on the topic (e.g. www.imparas.eu) and is committed to incorporating latest scientific developments in its risk assessment process, when appropriate.

3.4.2.3. Use of animal feeding studies with appropriate stack GM material to investigate toxicological, reproductive, hormonal, immunological effects on consumers

EFSA confirms its opinion that the use of animal studies to investigate possible effects of GMP whole food and feed on consumers should be conducted only when suited to investigate specific hypotheses. EFSA underlines that the sensitivity of animal studies to indicate the presence of adverse effects related to the whole food and feed is in general limited due to various hurdles, such as limitations in dose level selection. In case a clear test-hypothesis is identified, a fit-for purpose design would allow to investigate specific endpoints addressing the risk assessment question (EFSA Scientific Committee, 2011). It is highlighted that, in case studies are deemed necessary, these should be conducted on appropriate test materials, e.g. treated with the intended herbicide in case of HT GMPs. Therefore, EFSA disagrees with RAGES on the use of animal studies on whole GM food and feed to resolve possible gaps in the assessment of long -term, reproductive or immunological adverse effects.

Immunological effects on consumers are addressed in the Section 3.4.2.2, above.

3.4.3. Conclusion

EFSA does not support the statement that the current EU assessment of stacked events is not sufficiently addressing the identification of risks for animals and consumers. EFSA considers not appropriate the use of long-term animal studies with whole food/feed to explore the safety of GMPs in the absence of specific hypotheses to investigate. EFSA is committed to explore new approaches (or New Approach Methodologies [NAMs]) to strengthen GMPs risk assessment.

3.5. "Environmental risk assessment of genetically engineered crops that can persist and spontaneously propagate in the environment" report

3.5.1. Main issues reported by RAGES

- RAGES proposes to expand the current ERA as conducted by EFSA by introducing an assessment of the spatio-temporal controllability of GMPs. If spatio-temporal controllability cannot be ensured, a GMP application for authorisation for cultivation or for import and processing for food/feed uses in the EU should be rejected;
- GMPs that persist and propagate in the environment, and/or that produce viable offspring, raise new ERA challenges;
- EFSA insufficiently considers "next generation" effects caused by offspring resulting from spontaneous propagation (e.g. displacement of native species, weed problems, possible alteration of the plant's interaction with the food web).

3.5.2. EFSA's assessment

3.5.2.1. Spatio-temporal controllability

The RAGES report, and examples given therein, illustrates that RAGES and EFSA have different perspectives on protection goals, and thus on the framing of the ERA. There is no consensus between RAGES and EFSA on what constitutes environmental harm arising from the persistence/invasiveness and vertical gene flow of GMPs. For example, RAGES considers the potential for escape from "spatio-temporal controllability" as a "cut off criterion" based on which GMP applications for authorisation should be rejected. In contrast, for EFSA the fact that GMPs can persist and propagate in the environment and produce viable offspring is not harmful per se – as this will depend on the associated environmental/agronomic impacts, which must be assessed on a case-by-case basis. The use of different protection goals translates into risk assessments being framed differently between RAGES and EFSA, with different perspectives on the types of evidence and efforts necessary to inform the risk assessment of the persistence/invasiveness and vertical gene flow potential of GMPs.

EFSA notes that Directive 2001/18/EC and Commission Directive (EU) 2018/305 on the deliberate release into the environment of GMOs do not mention/refer to "spatio-temporal controllability", so it is not considered a protection goal by law at present. However, the implementation of risk mitigation measures can be recommended/imposed to spatially and/or temporally restrict the use or spread of a GMP, when a risk that needs to be reduced to a level falling within limits of concern is identified. In this respect, applicants should demonstrate that the management and control measures for the GMP are effective. Moreover, applicants should state the post-commercialisation measures they will put in place in order to monitor and verify the efficacy of the risk management measures and to allow changes in risk management strategies if circumstances change or if new data indicating the need for changes to the risk management become available.

3.5.2.2. Novel challenges for risk assessment

According to RAGES, the persistence and propagation of GMPs and the production of viable offspring in the environment raise "new" challenges for risk assessment, suggesting that such aspects are "novel" and have not been addressed in the risk assessment of GMPs so far. EFSA does not support this point of view, as risk assessors typically have considered the persistence and invasiveness potential of GMPs and the potential for vertical gene flow with sexually cross-compatible wild/weedy relatives and their environmental/agronomic impacts over the last 20 years. Consequently, these challenges cannot be considered "new" (EFSA GMO Panel, 2010a).

3.5.2.3. Next generation effects

There is no consensus between RAGES and EFSA regarding which cases should be considered for the assessment of "next generation effects" (see also spatio-temporal controllability, above). In EFSA's view, the need to assess "next generation effects" should be defined on a case-by-case basis.

EFSA notes that the RAGES report does not sufficiently explore the role that mathematical modelling and post-market environmental monitoring can play in the assessment of "next generation effects" when such assessment is needed. The gathering of the data recommended by RAGES through empirical studies may be challenging. Consequently, more weight could be given to mathematical modelling as additional line of evidence to capture some of the spatio-temporal dimensions put forward by RAGES. In addition, post-market environmental monitoring could serve as a complementary tool to ERA to manage uncertainties and risks pertaining to potential "next generation effects".

Possible alteration of the plant's interactions and biological signalling pathways within the food web, with soil organisms or insects such as pollinators and other organisms is addressed in the area of risk dedicated to the assessment of potential adverse effects of GMPs to NTOs.

3.5.2.4. Additional considerations

Proportionality principle

EFSA notes that some of the RAGES demands pertaining to the assessment of persistence, invasiveness and vertical gene flow are disproportionate, and not in tune with the nature of the former and current GMPs for market release in the EU, their intended uses, and the expected level of environmental exposure. At present, the bulk of applications for authorisation of GMPs covers the import/processing for food/feed uses of highly domesticated plants with a low potential to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable grains/seeds. Owing to the nature of most of the former/current novel traits, it is unlikely that such traits will enable GMPs to overcome other biological and abiotic factors limiting their persistence and invasiveness.

Case-by-case approach

In EFSA GMO Panel (2010a), an overarching conceptual frame is provided for the ERA of GMPs and their associated potential adverse environmental effects, including those arising from persistence/invasiveness and vertical gene flow. Hence, EFSA GMO Panel (2010a) does not specify all information requirements for each specific GMP, as it would be impossible to capture the broad array of GMPs, including future ones, for deliberate release into the environment (especially for cultivation in the EU). Consequently, through the problem formulation process, case-specific information

requirements must be defined on a case-by-case basis for each GMP for deliberate release into the environment. ERA will vary dependent on the biology of the GMP under consideration, the introduced traits, the intended uses of the GMP, the scale and frequency of the deliberate release, the receiving environments, and the interactions amongst these variables. Case-specific information from applicants is typically requested by the EFSA GMO Panel, as appropriate.

To cover the full breadth of ERA approaches and information requirements for GMPs in the EU, the analysis of EFSA GMO Panel (2010a) as performed by RAGES should be complemented with a systematic analysis of all EFSA GMO Panel scientific opinions on the assessment of GMP applications for authorisation. In this respect, it should be noted that no application for authorisation of the cultivation of GMPs with enhanced potential for persistence/invasiveness and vertical gene flow has been submitted and thus considered at EU level at the time of writing. This means that some of the higher stages of information required to test formulated hypotheses concerning the persistence and invasiveness of a GMP itself, or any of its introgressed relatives as a result of vertical gene flow, as outlined in EFSA GMO Panel (2010a), remain conceptual and untested in practice.

Agronomic/phenotypic characterisation of GMPs to support the persistence/invasiveness and vertical gene flow assessment

The agronomic/phenotypic characterisation of GMPs, including seed germination, is one of the event-specific datasets that informs the assessment of the persistence/invasiveness potential of GMPs, along with the consideration of the reproductive biology of the parental species, characteristics associated with persistence and invasiveness, factors limiting plant's persistence and invasiveness, and hybridisation and introgression potential with any sympatric compatible relatives. This information frames the following steps of the persistence/invasiveness and vertical gene flow assessment and associated impacts. Irrespective of the nature of a GMP and its intended uses, fitness-related traits, whether intended or unintended, are assessed for each GMP event.

According to the requirements of the Regulation (EU) No 503/2013 and EFSA GMO Panel (2010a, 2015a), applicants are required to perform field trials for the agronomic/phenotypic and compositional characterisation of GMPs, which include fitness-related traits associated with growth habit and vegetative vigour, phenology and reproductive behaviour, and susceptibility to pests, diseases and abiotic stress. If the GMP contains a herbicide tolerance trait(s), agronomic/phenotypic data must be generated with and without the intended herbicide(s). These agronomic/phenotypic data are typically gathered from field trials performed at multiple sites under conditions representative of the receiving environments in which the GMP can be grown, as required by Regulation (EU) No 503/2013. Consequently, fitness-related traits of GM herbicide-tolerant plants treated or untreated with the intended herbicides are typically assessed as part of the mandatory agronomic/phenotypic field trials, representative of the receiving environments in which the GMP can be grown.

For GMPs with more complex traits (e.g. quantitative traits controlled by multiple genes (e.g. photosynthetic capacity and nutrient use efficiency), traits that require lengthy multigene pathways (e.g. to produce new metabolites), or traits that perform new or expanded functions), the GMO Panel (2010a, 2015a) requires: (1) the selection of additional relevant endpoints for the agronomic/phenotypic characterisation; and (2) more emphasis on G×E×M interactions, as actual impacts of such GMPs are likely to be more context-dependent (in terms of genetic background, receiving environments and management practices). The latter may require the testing of the GMP in different genetic backgrounds and receiving environments under different management regimes as part of the pre-market ERA and/or post-market environmental monitoring (e.g. through variety registration trials, studies published in the scientific literature). Such field trials may be carried out across sites over a natural gradient of the stressor or through local manipulation of the environment.

Risk management

Some points raised by RAGES are not in EFSA's remit (e.g. decision to authorise a GMP for deliberate release, acceptability of harm, "cut off criteria" based on which to reject a GMP application for authorisation) and should be addressed by risk managers.

3.5.3. Conclusion

EFSA typically assesses the persistence and invasiveness potential of GMPs and the potential for vertical gene flow with sexually cross-compatible wild/weedy relatives (including possible associated environmental/agronomic impacts) for each GMP application for authorisation in the EU, taking into account the intended of the GMP use and scope of the GMP application, the specificities of the GMP and relevant receiving environments. The approach followed for this assessment is outlined in EFSA GMO Panel (2010a) and is considered fit for purpose and consistent with the requirements given in Directive 2001/18/EC, Commission Directive (EU) 2018/305 and Commission Implementing Regulation (EU) No 503/2013.

EFSA notes that at the time of writing, the bulk of GMP applications for authorisation covers the import and processing for food/feed uses of highly domesticated plants with a low potential to survive until subsequent seasons, or to establish occasional feral plants.

Issues raised by RAGES reveal a different perspective on protection goals and the types of evidence and efforts needed to inform the assessment of the persistence/invasiveness and vertical gene flow potential of GMPs (including possible associated adverse environmental effects) than that of EFSA. Further dialogue between risk assessors, risk managers and stakeholders are recommended to clarify how ERAs can address policy goals and decision-making criteria relevant for the assessment of the persistence/invasiveness and vertical gene flow potential of GMPs.

3.6. "New genetic engineering technologies" report

3.6.1. Main issues reported by RAGES

- New genetic engineering technologies and their potential applications raise new risk assessment challenges, especially RNAi-based GMPs, new plant breeding techniques (NPBTs) and genome editing, synthetic genomics and gene drive modified organisms;
- For several NPBTs, the RAGES project advocates the use and the importance of OMICS as additional tools to assist the RA;
- The report covers also a number of issues like the regulation and detectability of plants derived from NPBTs which are not in the EFSA remit and thus not discussed in EFSA's assessment below.

3.6.2. EFSA's assessment

3.6.2.1. RNAi-based GMPs

EFSA is aware of the particularities that the risk assessment of RNAi-based GMPs can pose. EFSA has taken several actions to determine whether the existing risk assessment approaches for GMPs are appropriate for the risk assessment of RNAi-based GMPs or require complementary or alternative approaches. An overview of EFSA's activities on the risk assessment of RNAi-based GMPs is given in Papadopoulou et al. (2020) and summarised below:

- *International scientific workshop "Risk assessment considerations for RNAi-based GM plants" (4–5 June 2014, Brussels, Belgium)*: At this workshop, experts from academia, risk assessment bodies, non-governmental organisations, the European Commission and the private sector identified scientific uncertainties on the level of exposure of humans, animals and the environment to dsRNA/artificial miRNA and derived small RNAs, as well as limitations of *in silico* methods to unequivocally identify potential off-targets (EFSA, 2014; Ramon et al., 2014);
- *External scientific reports*: EFSA commissioned three external scientific reports in which relevant scientific literature was reviewed systematically to further inform the molecular characterisation, food/feed safety assessment and ERA of RNAi-based GMPs, and address issues identified in the workshop. The report supporting the molecular characterisation addressed dsRNA and miRNA pathways in different species, including mammals, arthropods and plants (Pačes et al., 2017),

while the food/feed safety report focused on the kinetics and possible effects of non-coding (nc) RNAs, including silencing RNAs, upon ingestion by humans and animals (Dávalos et al., 2019). The report in support of the ERA considered environmental RNAi-related aspects in arthropods, nematodes, and annelids and molluscs (Christiaens et al., 2018);

- *Note on the strategy for the prediction and risk assessment of off-targets*: In 2017, EFSA's GMO Panel published an internal note[18] on the strategy to identify/predict off-targets and risk assess their potential impact in RNAi-based GMPs. It built on the available scientific knowledge and is expected to evolve with the progress of the knowledge in the field;
- EFSA GMO Panel scientific opinions of RNAi-based GMPs: EFSA's GMO Panel assessed applications for GMO authorisation for EU market for food and feed uses of potato EH92-527-1 (including cultivation in the EU) and soybeans MON87705, 305423, MON87705 × MON89788, and 305423 × 40-3-2 (excluding cultivation) designed to downregulate plant endogenous transcripts that modulate amylose and starch content in potato tubers or fatty acid profile in soybeans (EFSA GMO Panel, 2006; EFSA GMO Panel, 2012c, 2013a, 2015b, 2016, respectively). More recently, the GMO Panel also assessed (for food and feed uses excluding cultivation) the maize events MON87411 and MON87427×MON89034×MIR162×MON87411 that constitute cases of GMPs expressing RNAi for plant pest control (EFSA GMO Panel, 2018a, 2019b, respectively).

To our knowledge, at the international level no dedicated guidelines have been developed for the risk assessment and regulation of RNAi-based GMPs, confirming that existing science-based risk assessment approaches for GMPs are generally considered suitable for RNAi-based GMPs. However, as reported above, some specificities have been identified for the risk assessment of RNAi-based GMPs.

3.6.2.2. Synthetic genomics

As synthetic biology (SynBio) is a fast-evolving field and as pointed out by RAGES, the European Commission has asked EFSA to evaluate the adequacy of its existing guidelines for the molecular characterisation and ERA of GMPs and GM microorganisms obtained by SynBio.[19,20] The mandate has been addressed via a case study approach, focusing on SynBio products that could reach the EU market within the next decade.

EFSA commissioned two horizon scanning exercises to identify the most realistic and forthcoming SynBio cases of relevance to this remit (Unkel et al., 2019; van der Vlugt, 2019).

Between 31 March and 4 June 2020, interested persons were invited to submit online their public comments on the draft scientific opinions on GMPs and GM microorganisms separately through an online EUSurvey.[21] The outcome of the online public consultations will be reported in technical reports published in the EFSA journal. These reports will contain the comments received and explain how they have been considered for finalisation of the GMO Panel scientific opinions.

The final GMO Panel scientific opinions should be issued by the end of 2020.

3.6.2.3. New plant breeding techniques and genome editing

Upon request of the European Commission, EFSA has been mandated to assess whether its previous GMO Panel scientific opinion on SDN-3 (EFSA GMO Panel, 2012d) is still valid and applicable to plants developed through Site-Directed Nucleases type 1 and type 2 (SDN-1 and SDN-2) and oligonucleotide

[18] http://www.efsa.europa.eu/sites/default/files/event/171025-m.pdf

[19] The mandate is available at http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2018-01000

[20] The mandate is available at http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2018-00921

[21] The draft GMO Panel scientific opinion on GMPs has been published at http://www.efsa.europa.eu/en/consultations/call/public-consultation-gmo-panel-draft-scientific-opinion-evaluation, and the one on microorganisms at http://www.efsa.europa.eu/en/consultations/call/public-consultation-draft-efsa-scientific-committee-opinion

directed mutagenesis (ODM).[22] While SDN-1, SDN-2, and ODM are genome editing approaches that can be used to alter plant endogenous genomic sequences by introducing site-directed mutations to the plant genome, SDN- 3 facilitate the insertion of exogenous DNA at a precise genomic locus. Between 15 April and 4 June 2020, interested persons were invited to submit online their public comments on the draft GMO Panel scientific opinion through an online EUSurvey.[23] The outcome of the online public consultation will be reported in a technical report in the EFSA journal. This report will contain the comments received and explain how they have been considered for finalisation of the GMO Panel scientific opinion. The final output is scheduled for adoption by EFSA's GMO Panel by the end of October 2020.

On 10 January 2020, EFSA has received an additional mandate from the European Commission[24], asking to provide an overview on the risk assessment of plants developed through new genomic techniques (NGTs). By October 2020, EFSA should review its previous opinions on cisgenesis and intragenesis (EFSA GMO Panel, 2012e) and on SDN-3 (EFSA GMO Panel, 2012d) and current work on this matter (i.e. SDN-1, SDN-2, and ODM), as well as that carried out at national level by Competent Authorities and national institutions since 2012.

3.6.2.4. Gene drive modified organisms

EFSA has been requested by the European Commission to assess, through a problem formulation exercise, whether: (1) the deliberate release of gene drive modified organisms could pose potential new hazards and risks to human/animal health and the environment, considering relevant comparators; (2) the scientific considerations/requirements given in its existing guidelines for the risk assessment of genetically modified animals (EFSA GMO and AHAW Panels, 2012; EFSA GMO panel, 2013b) are adequate for the molecular characterisation and ERA of gene drive modified organisms; and (3) there is a need for updated guidance in relation to previous documents EFSA GMO and AHAW Panels, 2012; EFSA GMO panel, 2013b).[25]

Considering the current societal debate on the potential applications of gene drive, given the need for greater dialogue, and in line with its policy on openness and transparency, EFSA has organised two consultations at different development stages of the GMO Panel scientific opinion to collect input from its stakeholders (including EU Member States) and other interested parties:

- One was organised in the shape of a stakeholder workshop "Problem formulation for the environmental risk assessment of gene drive modified insects" (15 May 2019, Brussels), that took place early in the development process of this GMO Panel scientific opinion. Points raised by the workshop participants, on defining protection goals, formulating specific pathways to harm and on structuring risks have been considered by the GMO Panel during its deliberations, and are reported in EFSA et al. (2020e);
- The other was organised in the shape of an online public consultation. Between 17 February and 24 April 2020, interested persons were invited to submit online their public comments on the draft GMO Panel scientific opinion through an online EUSurvey.[26] Comments received will be analysed and taken into account by the GMO Panel during the revision of its draft scientific opinion. The outcome of the online public consultation will be reported in a technical report for publication in the EFSA journal. This report will contain the comments received and explain how they have been considered for finalisation of the GMO Panel scientific opinion.

EFSA notes that representatives of RAGES participated to the stakeholder workshop. Some issues addressed by RAGES were also voiced as concerns at the stakeholder workshop.

The final GMO Panel scientific opinion should be issued by the end of 2020.

[22] The mandate is available at http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2019-00297
[23] Published at http://www.efsa.europa.eu/en/consultations/call/public-consultation-applicability-efsa-opinion-site-directed
[24] The mandate is available at http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2020-00103
[25] The mandate is available at http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2018-00619
[26] Published at http://www.efsa.europa.eu/en/consultations/call/public-consultation-gmo-panel-scientific-opinion-evaluation

3.6.2.5. The use of OMICS for the characterisation of GMOs

In April 2018 EFSA held a dedicated Colloquium on the potential use of different OMICS approaches in risk assessment, such as the use of metabolomics in support of the compositional characterisation of GMPs or the use of OMICS for ERA (EFSA et al., 2018b). The Colloquium concluded that the use of OMICS approaches in the risk assessment of GMPs is potentially useful, but that there are still significant steps to be made in terms of standardisation and validation of the current experimental methodologies, data analysis (statistics) and data interpretation. Thus, EFSA considers that OMICS techniques in support of GMP risk assessment are not sufficiently standardised for routine use yet. However, for some specific purposes, OMICS data can already support the GMO risk assessment on a regular basis. In this respect, EFSA notes that applicants regularly provide genomics data (i.e next generation sequencing data) for the molecular characterisation of transformation events. The GMO Panel has also published a technical note on the quality of DNA sequencing for the molecular characterisation of GMPs that includes specific data submission and quality recommendations for the submission of next generation sequencing (e.g. targeted and whole genome sequencing) data (EFSA GMO Panel, 2018c). In addition, the GMO Panel has on a case-by-case basis requested transcriptomics data (RNA-Seq) to measure and compare global mRNA transcript levels to support the risk assessment of specific events (EFSA GMO Panel, 2018b).

3.6.3. Conclusion

The applied risk assessment approaches for GMPs are generally considered suitable for RNAi-based GMPs, without the necessity of developing specific guidance. Regarding the NGT, since 2019, EFSA is addressing several mandates from the European Commission that may have implications for future applications for GMO authorisation on the EU market and their risk assessment: those mandates address genome editing, synthetic biology, gene drive and NGTs. In addition, EFSA is continuously monitoring the development in the OMICs area; while several types of OMICS are not yet sufficiently standardized to be used as a routine data set in risk assessment, some types of OMICS (e.g. genomics data in the form of next generation sequencing data) are already part of the data routinely received in the GMO risk assessment, or can be requested on a case-by-case basis.

3.7. Overview and Summary report of the results from the RAGES project 2016-2019

RAGES published two additional documents entitled *Overview: The RAGES project*[8] and *Summary report of the results from the RAGES project 2016-2019*,[9] the latter supported by a summary table.[10]

3.7.1. Main issues reported by RAGES

The complementary documents, in addition to the summary of findings reported in the six reports discussed above (Section 3), underline several crosscutting issues such as:

- EFSA's risk assessment frame is too narrow and reductionistic and fails to account for the complexity of biological systems, new combinations of interactions or interactions with microbiome;
- Any type of genetic engineering method needs to be regulated because the resulting characteristics cannot be compared with the ones that might be obtained applying conventional breeding methods;
- The precautionary principles should be applied more consistently and comprehensively and as long as the issues identified by RAGES are not solved the market approvals for GMOs should not be granted;
- Transparency and access to data is not adequate.

3.7.2. EFSA's assessment

3.7.2.1. Legal frame, principles of and continuous development in risk assessment of GMPs

EFSA would like to remind the frame and the principles of risk assessment that govern EFSA's activities.

The risk assessment approach followed by EFSA is consistent with the applicable EU legislation and internationally agreed standards (such as Codex Alimentarius or OECD documents). EFSA acts in line with principles, strategies and requirements outlined in the above-mentioned documents, centred around the case-by-case principle; the comparative approach; the problem formulation process; an iterative, stepwise/staged/tiered-based testing approach; the combination of different lines of evidence in a weight of evidence approach and the consideration of the previously gained knowledge, experience and familiarity. In addition, EFSA conducts a continued scientific review of all relevant evidence, including studies with adverse findings.

EFSA does not support the RAGES statement in the summary report of the project on the narrow framing of the RA conducted by the GMO Panel. One of the EFSA's aims is to ensure the highest level of protection of the human and animal health, and the environment. This has been achieved by constantly integrating scientific and technical developments into the GMO risk assessment as, evidenced by publication of guidelines, scientific opinions and technical reports; the organisation of open workshops and conferences; and procurements launched and funded by EFSA.[27] In some cases, these activities were performed upon request of the EC, in other cases these were EFSA self-tasks triggered by challenges raised from MS, NGOs, applicants and/or by new challenges encountered during the RA process of GMO applications. The breadths of topics addressed and the consistent undertaking of such activities cannot be considered narrow or reductionistic, on the contrary it is evident from the number of documents published by EFSA and reported in the sections above, that the majority of the issues discussed in the RAGES reports were already subject to an EFSA development, are currently under discussion or have been just taken onboard for future projects.

In the overview of the RAGES project as well as in the other individual reports (see Section 3.5.2), it becomes clear that the RAGES project consortium has different views on the types of evidence and efforts necessary to inform the risk assessment of GMOs, from those outlined in the relevant Regulation and the GMO Panel guidance documents. In EFSA's view, this underlines the need for further dialogue between risk assessors, risk managers and stakeholders to define clear protection goals and decision-making criteria for the risk assessment of GMPs, as this will frame the risk assessment; the dialogue with stakeholders is a very important part for the constantly ongoing evolution of the risk assessment. Science and knowledge evolve, and the risk assessment takes into account the state of the art to also evolve. In this process stakeholders play an important role be it by participating to workshops, public consultations, preparing reports, and publishing scientific papers. EFSA acknowledges the contribution of RAGES project and of its partners in this process.

3.7.2.2. Genetic modification methods subject to regulation under the GMO legal framework, precautionary principle and market authorization

EFSA notes that some issues raised by RAGES are not in EFSA's remit as risk assessor, and thus should be addressed to risk managers.

Examples include remarks on the applicable regulatory frame for (novel) genetic modification methods that the RAGES consortium considers as producing GMOs in the legal sense. It is not in the EFSA remit to decide on the methods that should be covered by the regulation; however, it is within the EFSA remit and appropriate to scrutinise the adequacy of existing guidelines for the RA of GMOs created by different methods/approaches if so needed. Examples for such tasks are the currently ongoing work on the EC mandates addressing gene drives, synthetic biology and SDN-1, 2 and ODM.

Another example are remarks on the interplay between the GMO and pesticide regulations in the risk assessment of HT GMPs and their respective intended herbicides. EFSA underlines that in case of HT

[27] For a complete list see https://www.efsa.europa.eu/en/calls/procurement

GMPs the GMO and pesticide regulations work together in a complimentary mode and EFSA's responsibility is to perform its risk assessment in line with the applicable regulatory frame.

Furthermore, RAGES underlines that the precautionary principle should be applied more consistently and comprehensively and that market approvals for GMOs should not be granted as long as the issues identified by RAGES are not solved. EFSA notes that both the application of the precautionary principle and the granting of market authorisations fall under the responsibility of the risk managers. EFSA's role is to provide a science based- risk assessment that risk managers take into consideration when reaching an authorisation decision.

3.7.2.3. Transparency in the RA

In some comments of the summary table, the RAGES advocates for more accessibility to the data. In this respect EFSA recalls that Regulation (EU) 2019/1381[28] on the transparency and sustainability of the EU risk assessment in the food chain shall come into force on 27 March 2021. This Regulation has several objectives, among which: (1) ensure better transparency of the EU decision-making cycle; (2) enhance sustainability through stronger involvement of MS in EFSA risk assessment work. The requirements of the Transparency Regulation will introduce numerous innovations for which EFSA is currently preparing, among others:

- All scientific studies/information supporting an EU application procedure will be made public proactively and automatically, early in the risk assessment process, with the exception of duly justified confidential information.
- MS will be more involved in supporting the production of scientific opinions by EFSA.
- Public consultations will be organised during several phases of the risk assessment

3.7.3. Conclusion

EFSA conducts the risk assessment in an independent and transparent way and provides advice to risk managers on the risks the use of GMO may pose to human and animal health and the environment. The risk assessment approach followed by EFSA is consistent with the applicable EU regulation and internationally agreed standards. EFSA conducts a continued scientific review of all relevant evidence and follows the scientific and technical developments in the GMO area as demonstrated with the continued development of new guidance, numerous publications and events covering the different aspects of the RA of GMO. In this process, stakeholders play an important role; thus, EFSA acknowledges the contribution of the RAGES project and of its partners in this process.

4. Conclusions

Overall, EFSA concludes that the RAGES reports do not contain elements that could lead the GMO Panel to reconsider the outcome of its previous opinions on GMPs. Therefore, EFSA considers that the previous GMO Panel risk assessment conclusions remain valid. Some of the concerns expressed in the RAGES reports are not in the EFSA's remit and are not further assessed in the current report.

[28] Regulation (EU) 2019/1381 of the European Parliament and of the Council of 20 June 2019 on the transparency and sustainability of the EU risk assessment in the food chain and amending Regulations (EC) No 178/2002, (EC) No 1829/2003, (EC) No 1831/2003, (EC) No 2065/2003, (EC) No 1935/2004, (EC) No 1331/2008, (EC) No 1107/2009, (EU) 2015/2283 and Directive 2001/18/EC. OJ L 231, 6.9.2019, p. 1–28. http://data.europa.eu/eli/reg/2019/1381/oj

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1. Letter from the European Commission to the EFSA Executive Director, dated 17 February 2020, requesting technical and scientific assistance on the final reports from the RAGES project concerning the risk assessment of GM plants.

2. RAGES reports

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Abbreviations

AHAW	Animal health and animal welfare
Bt	*Bacillus thuringiensis*
CRA	cumulative risk assessment
DRVs	dietary reference values
dsRNA	double strand RNA
EC	European Commission
ENSSER	European network of scientists for social and environmental responsibility
EFSA	European food safety Authority
ERA	environmental risk assessment
EU	European Union
G × E	genotype × environment
GAPs	good agricultural practices
GE	genetically engineered
GM	genetically modified
GMO	genetically modified organism
GMP	genetically modified plant
HT	herbicide tolerant
IgE	immunoglobulin E
miRNA	micro RNA
MS	member States
NAMs	new approach methodologies
NEPs	newly expressed proteins
nc	non-coding
NGOs	non-governmental organisations
NGTs	new genomic techniques
NTO	non target organism
NPBTs	new plant breeding techniques
ODM	oligonucleotide directed mutagenesis
OECD	organisation for economic co-operation and development
PMM	post market monitoring
RA	risk assessment
RAGES	risk assessment of genetically engineered organisms in the EU and Switzerland
RNA	ribonucleic acid
RNA-Seq	RNA sequencing
RNAi	RNA interference
SDN	site directed nucleases
SynBio	synthetic biology
UL	tolerable upper intake levels