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Assessment of genetically modified maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA GMO-NL-2020-171)

EFSA Panel on Genetically Modified Organisms (GMO),
Ewen Mullins, Jean-Louis Bresson, Tamas Dalmay, Ian Crawford Dewhurst,
Michelle M Epstein, Leslie George Firbank, Philippe Guerche, Jan Hejatko,
Francisco Javier Moreno, Hanspeter Naegeli, Fabien Nogué, Nils Rostoks,
Jose Juan Sánchez Serrano, Giovanni Savoini, Eve Veromann, Fabio Veronesi,
Michele Ardizzone, Ana Martin Camargo, Giacomo De Sanctis, Antonio Fernandez Dumont,
Andrea Gennaro, Jose Ángel Gómez Ruiz, Tilemachos Goumperis, Dafni Maria Kagkli,
Franco Maria Neri, Nikoletta Papadopoulou, Tommaso Raffaello and Franz Streissl

Abstract

Genetically modified maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 was developed by crossing to combine four single events: DP4114, MON 89034, MON 87411 and DAS-40278-9. The GMO Panel previously assessed the four single maize events and two of the subcombinations and did not identify safety concerns. No new data on the single maize events or the assessed subcombinations were identified that could lead to modification of the original conclusions on their safety. The molecular characterisation, comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment indicate that the combination of the single maize events and of the newly expressed proteins in the four-event stack maize does not give rise to food and feed safety and nutritional concerns. Therefore, no post-market monitoring of food/feed is considered necessary. In the case of accidental release of viable four-event stack maize grains into the environment, this would not raise environmental safety concerns. The GMO Panel assessed the likelihood of interactions among the single events in eight of the maize subcombinations not previously assessed and concludes that these are expected to be as safe as the single events, the previously assessed subcombinations and the four-event stack maize. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9. Post-market monitoring of food/feed is not considered necessary. The GMO Panel concludes that the four-event stack maize and its subcombinations are as safe as its non-GM comparator and the tested non-GM maize varieties with respect to potential effects on human and animal health and the environment.

Keywords: genetic engineering, herbicide tolerant, insect resistant, maize (*Zea mays*), import and processing

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Correspondence: nif@efsa.europa.eu

Panel members: Ewen Mullins, Jean-Louis Bresson, Tamas Dalmay, Ian Crawford Dewhurst, Michelle M Epstein, Leslie George Firbank, Philippe Guerche, Jan Hejatko, Francisco Javier Moreno, Hanspeter Naegeli, Fabien Nogué, Nils Rostoks, Jose Juan Sánchez Serrano, Giovanni Savoini, Eve Veromann and Fabio Veronesi.

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Summary

Following the submission of application EFSA-GMO-NL-2020-171 under Regulation (EC) No 1829/2003 from Pioneer Overseas Corporation (referred to hereafter as 'the applicant'), the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') was asked to deliver a Scientific Opinion on the safety of genetically modified (GM) herbicide-tolerant and insect-resistant maize (*Zea mays* L.) DP4114 × MON 89034 × MON 87411 × DAS-40278-9 (referred to hereafter as 'four-event stack maize') and its subcombinations independently of their origin, according to Regulation (EU) No 503/2013 (referred to hereafter as 'subcombinations'). The scope of application EFSA-GMO-NL-2020-171 is for import, processing, and food and feed uses within the European Union (EU) of maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9, and does not include cultivation in the EU. The term 'subcombination' refers to any combination of up to three of the events present in the four-event stack maize. The safety of subcombinations occurring as segregating progeny in the harvested grains of maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 is evaluated in the context of the assessment of the four-event stack maize. The safety of subcombinations that have either been or could be produced by crossing through targeted breeding approaches, and which can be bred, produced and marketed independently of the four-event stack, are risk assessed separately in the present scientific opinion.

The four-event stack maize was produced by crossing to combine four single maize events: DP4114 expressing Cry1F protein to confer protection against certain lepidopteran pests, Cry34Ab1 and Cry35Ab1 proteins to confer protection against certain coleopteran pests and PAT protein to confer tolerance to glufosinate-ammonium-containing herbicides; MON 89034 expressing Cry1A.105 and Cry2Ab2 to confer protection against certain lepidopteran pests; MON 87411 expressing the Cry3Bb1 protein to confer protection against certain coleopteran larvae and the DvSnf7 dsRNA to confer protection against western corn rootworm and the CP4 EPSPS protein for tolerance to glyphosate containing herbicides; and DAS-40278-9 expressing AAD-1 to catalyse the degradation of the general class of herbicides known as aryloxyphenoxypropionates (AOPP) and to confer tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) herbicides.

The GMO Panel evaluated the four-event stack maize and its subcombinations with reference to the scope and appropriate principles described in its applicable guidelines for the risk assessment of GM plants and the post-market environmental monitoring. The GMO Panel considered the information submitted in application EFSA-GMO-NL-2020-171, additional information provided by the applicant during the risk assessment, the scientific comments submitted by the Member States and the relevant scientific literature. For application EFSA-GMO-NL-2020-171, previous assessments of the four single events (DP4114, MON 89034, MON 87411 and DAS-40278-9), and two of the subcombinations provided a basis for the assessment of the four-event stack maize and all its subcombinations. No safety concerns were identified by the GMO Panel in the previous assessments. No safety issue concerning the four single maize events was identified by the updated bioinformatic analyses, nor reported by the applicant since the publication of the previous GMO Panel scientific opinions. Therefore, the GMO Panel considers that its previous conclusions on the safety of the single maize events remain valid.

For the four-event stack maize, the risk assessment included the molecular characterisation of the inserted DNA and analysis of protein expression. An evaluation of the comparative analysis of agronomic, phenotypic and compositional characteristics was carried out, and the safety of the newly expressed proteins and the whole food and feed were evaluated with respect to potential toxicity, allergenicity and nutritional characteristics. Environmental impacts and post-market environmental monitoring (PMEM) plan were also evaluated. The molecular characterisation data establish that the events DP4114, MON 89034, MON 87411 and DAS-40278-9 combined in the four-event stack maize have retained their integrity. Protein expression analysis showed that the levels of the newly expressed proteins are similar in the four-event stack maize and in the single events.

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic-phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials were appropriate to support the comparative analysis. The comparative analysis of agronomic and phenotypic characteristics and grain and forage composition identified no differences between maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 and the non-GM comparator (referred to hereafter as comparator) that required further assessment except for the changes for the levels in forage of phosphorus, and in grain of linoleic acid (C18:2), oleic acid (C18:1), α-linolenic acid (C18:3) and lignoceric acid (C24:0). These changes were

further assessed for food/feed safety and environmental impact and raised no concern. The molecular characterisation, the comparative analysis and the outcome of the toxicological, allergenicity and nutritional assessment indicate that the combination of the single maize events and of the newly expressed proteins in the four-event stack maize does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9, is as safe as the comparator and the selected commercial non-GM maize reference varieties (referred to hereafter as non-GM reference varieties). Considering the combined events and their potential interactions, the outcome of the comparative analysis, and the routes and levels of exposure, the GMO Panel concludes that maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 would not raise safety concerns in the case of accidental release of viable GM maize grains into the environment.

Since no new safety concerns were identified for the previously assessed subcombinations, and no new data leading to the modification of the original conclusions on safety were identified, the GMO Panel considers that its previous conclusions on these maize subcombinations remain valid.

For the remaining subcombinations included in the scope of application EFSA-GMO-NL-2020-171, no experimental data were provided. The GMO Panel assessed the possibility of interactions between the events in these subcombinations and concludes that these subcombinations would not raise safety concerns. These subcombinations are therefore expected to be as safe as the single events, the previously assessed subcombinations and the four-event stack maize.

Given the absence of safety concerns for foods and feeds from maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 and its subcombinations, the GMO Panel considers that post-market monitoring of these products is not necessary. The PMEM plan and reporting intervals are in line with the intended uses of the four-event stack maize and its subcombinations. Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issues pertaining to the intended uses of maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 and its subcombinations.

The GMO Panel concludes that maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 and its subcombinations, as described in this application, are as safe as the comparator and the selected non-GM reference varieties with respect to potential effects on human and animal health and the environment.

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1. Introduction

The scope of application EFSA-GMO-NL-2020-171 is for food and feed uses, import and processing of the genetically modified (GM) herbicide-tolerant and insect-resistant maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 and all its subcombinations independently of their origin and does not include cultivation in the European Union (EU).

1.1. Background

On 11 December 2020, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands application EFSA-GMO-NL-2020-171 for authorisation of maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 (Unique Identifier DP-ØØ4114-3 × MON-89Ø34-3 × MON-87411-9 × DAS-4Ø278-9), submitted by Pioneer Overseas Corporation according to Regulation (EC) No 1829/2003[1]. Following receipt of application EFSA-GMO-NL-2020-171, EFSA informed EU Member States (MS) and the European Commission (EC), and made the application available to them. Simultaneously, EFSA published a summary of the application.[2]

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003 and Regulation (EU) No 503/2013[3], with the EFSA guidance documents, and, when needed, asked the applicant to supplement the initial application. On 26 April 2021, EFSA declared the application valid.

From validity date, EFSA and its Panel on Genetically Modified Organisms (hereafter to as 'GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application EFSA-GMO-NL-2020-171. Such time limit was extended whenever EFSA and/or its GMO Panel requested supplementary information to the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and EC (for further details, see the section 'Documentation', below). In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of the EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[4]. The EU Member States had 3 months to make their opinion known on application EFSA-GMO-NL-2020-171 as of date of validity.

1.2. Terms of Reference as provided by the requestor

According to Articles 6 and 18 of Regulation (EC) No 1829/2003, EFSA and its GMO Panel were requested to carry out a scientific risk assessment of maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 in the context of its scope as defined in application EFSA-GMO- NL-2020-171.

According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation including the opinions of the nominated risk assessment bodies of the EU Member States.[5] In addition to the present scientific opinion, EFSA was also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No 1829/2003, but not to give an opinion on them because they pertain to risk management.[2]

2. Data and methodologies

2.1. Data

The GMO Panel based its scientific risk assessment of four-event stack maize on the valid application EFSA-GMO-NL-2020-171, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by EU MS and relevant peer-reviewed scientific publications. As part of this comprehensive information package, the GMO Panel received additional

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on Genetically Modified Food and Feed. OJ L 268, 18.10.2003, pp. 1–23.
[2] Available online: https://open.efsa.europa.eu/study-inventory/EFSA-Q-2020-00833.
[3] 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 L157, 8.6.2013, pp. 1–48.
[4] 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. OJ L 106, 12.3.2001, pp. 1–38.
[5] Opinions of the nominated risk assessment bodies of EU Member States can be found at the Open EFSA Portal https://open.efsa.europa.eu/questions, querying the assigned Question Number.

unpublished studies submitted by the applicant in order to comply with the specific provisions of Regulation (EU) No 503/2013. A list of these additional unpublished studies is provided in Appendix A.

2.2. Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 503/2013, its applicable guidelines (EFSA GMO Panel, 2010a, 2011a,b, 2015, 2017; EFSA Scientific Committee, 2011) and explanatory notes and statements (EFSA, 2010, 2014, 2017a,b, 2018a, 2019a, b; EFSA GMO Panel, 2010b, 2018a, 2021a) for the risk assessment of GM plants.

For this application, in the context of the contracts OC/EFSA/GMO/2018/04 and OC/EFSA/GMO/2020/01 the contractors performed preparatory work for the evaluation of the applicant's literature search and for the completeness and quality of DNA sequencing information on maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9, respectively.

3. Assessment

3.1. Introduction

Application EFSA-GMO-NL-2020-171 covers the four-event stack maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 and all its 10 subcombinations independently of their origin (Table 1).

Table 1: Stacked maize events covered by the scope of application EFSA-GMO-NL-2020-171

Degree of stacking	Event	Unique identifiers
Four-event stack	DP4114 × MON 89034 × MON 87411 × DAS-40278-9	DP-ØØ4114-3 × MON-89Ø34-3 × MON-87411-9 × DAS-4Ø278-9
Three-event stack	DAS-40278-9 × DP4114 × MON 87411	DAS-4Ø278-9 × DP-ØØ4114-3 × MON-87411-9
	MON 89034 × DP4114 × MON 87411	MON-89Ø34-3 × DP-ØØ4114-3 × MON-87411-9
	MON 89034 × DAS-40278-9 × MON 87411	MON-89Ø34-3 × DAS-4Ø278-9 × MON-87411-9
	MON 89034 × DAS-40278-9 × DP4114	MON-89Ø34-3 × DAS-4Ø278-9 × DP-ØØ4114-3
Two-event stack	DP4114 × MON 87411	DP-ØØ4114-3 × MON-87411-9
	DAS-40278-9 × MON 87411	DAS-4Ø278-9 × MON-87411-9
	DAS-40278-9 × DP4114	DAS-4Ø278-9 × DP-ØØ4114-3
	MON 89034 × MON 87411	MON-89Ø34-3 × MON-87411-9
	MON 89034 × DP4114	MON-89Ø34-3 × DP-ØØ4114-3
	MON 89034 × DAS-40278-9	MON-89Ø34-3 × DAS-4Ø278-9

The term 'subcombination' refers to any combination of up to three of the maize events DP4114, MON 89034, MON 87411 and DAS-40278-9.

'Subcombination' also covers combinations that have either been or could be produced in the future by crossing through targeted breeding approaches (EFSA GMO Panel, 2011a). These are maize stacks that can be bred, produced and marketed independently of the four-event stack maize. These subcombinations are assessed in Section 3.5 of this scientific opinion.

The safety of subcombinations occurring as segregating progeny in harvested grains of maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 is evaluated in the context of the assessment of the four-event stack maize in Section 3.5 of the present scientific opinion.

The four-event stack maize was produced by crossing to combine four single maize events: DP4114 expressing Cry1F protein to confer protection against certain lepidopteran pests, Cry34Ab1 and Cry35Ab1 proteins to confer protection against certain coleopteran pests and PAT protein to confer tolerance to glufosinate-ammonium-containing herbicides; MON 89034 expressing Cry1A.105 and Cry2Ab2 to confer protection against certain lepidopteran pests; MON 87411 expressing the Cry3Bb1 protein to confer protection against certain coleopteran larvae and the DvSnf7 dsRNA to confer protection against western corn rootworm and the CP4 EPSPS protein for tolerance to glyphosate containing herbicides; and DAS-40278-9 expressing AAD-1 to catalyse the degradation of the general class of herbicides known as aryloxyphenoxypropionates (AOPP) and to confer tolerance to

2,4-dichlorophenoxyacetic acid (2,4-D) herbicides. It should be noted that the assessment of herbicide residues in maize herbicide-tolerant crops relevant for this application has been investigated by the EFSA Pesticides Unit (EFSA, 2018b).

All four single maize events and the two-event stacks MON 89034 × MON 87411 and MON 89034 × DAS-40278-9 have been previously assessed by the GMO Panel (see Table 2) and no safety concerns for human and animal health or environmental safety were identified.

Table 2: Single maize events and subcombinations of maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 previously assessed by the GMO Panel

Event	Application or mandate	EFSA Scientific Opinion
DP4114	EFSA-GMO-NL-2014-123	EFSA GMO Panel (2018b)
MON 89034	EFSA-GMO-NL-2007-37; EFSA-GMO-RX-015	EFSA (2008); EFSA GMO Panel (2019a)
MON 87411	EFSA-GMO-NL-2015-124	EFSA GMO Panel (2018c)
DAS-40278-9	EFSA-GMO-NL-2010-89	EFSA GMO Panel (2016)
MON 89034 × MON 87411	EFSA-GMO-NL-2017-139 EFSA-GMO-NL-2017-144	EFSA GMO Panel (2021b) EFSA GMO Panel (2019b)
MON 89034 × DAS-40278-9	EFSA-GMO-NL-2013-112; EFSA-GMO-NL-2013-113	EFSA GMO Panel (2019c); EFSA GMO Panel (2019d)

3.2. Updated information on single events

Since publication of the scientific opinions on the single maize events by the GMO Panel (see Table 2), no safety issue concerning the four single events has been reported by the applicant.

Updated bioinformatic analyses of the junction regions for maize events DP4114, MON 89034, MON 87411 and DAS-40278-9, using up-to-date sequence databases and methodology specified in EFSA guidance (EFSA GMO Panel, 2011a), confirmed that no known endogenous genes were disrupted by any of the inserts.

Updated bioinformatic analyses of the amino acid sequence of the newly expressed Cry1F, Cry1A.105, Cry2Ab2, Cry34Ab1, Cry35Ab1, Cry3Bb1, PAT, CP4 EPSPS and AAD-1 proteins confirmed previous results indicating no significant similarities to known toxins and allergens. Updated bioinformatic analyses of the newly created open reading frames (ORFs) within the inserts or spanning the junctions between the insert and the flanking regions for events DP4114, MON 89034, MON 87411 and DAS-40278-9 indicate that the production of a new peptide showing significant similarities to toxins or allergens for any of the events in maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 is highly unlikely, which confirmed previous analyses (Table 2).

According to Regulation (EU) No 503/2013, when silencing approaches by RNAi have been used in GM plant applications, a bioinformatic analysis to identify potential off-target genes is required. The applicant has performed an updated RNAi off-target search in the available maize transcriptome following the recommendations by the GMO Panel.[6] This updated bioinformatics analysis confirms previous results that do not indicate an off-target effect of the DvSnf7 dsRNA expression that would need further assessment.

In order to update the bioinformatics analyses to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis with microbial DNA for maize events DP4114, MON 89034, MON 87411 and DAS-40278-9. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.4.4.2.

Based on the above information, the GMO Panel considers that its previous conclusions on the safety of the single maize events remain valid.

3.3. Systematic literature review

The GMO Panel assessed the applicant's literature searches on maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9, which include a scoping review, according to the guidelines given in EFSA (2010, 2019b).

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

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application EFSA-GMO-NL-2020-171. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 at present. The GMO Panel considered the overall quality of the performed literature searches acceptable.

The literature searches did not identify any relevant publications on maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9.

3.4. Risk assessment of the four-event stack maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9

3.4.1. Molecular characterisation[7]

In line with the requirements laid down by Regulation (EU) 503/2013, the possible impact of the combination of the events on the integrity of the events, the expression levels of the newly expressed proteins or the biological functions conferred by the individual inserts are considered below.

3.4.1.1. Genetic elements and their biological function

Maize events DP4114, MON 89034, MON 87411 and DAS-40278-9, were combined by crossing to produce the four-event stack maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9. The structure of the inserts introduced into the four-event stack maize is described in detail in the respective EFSA scientific opinions (Table 2) and no new genetic modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 3.

Intended effects of the inserts in maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 are summarised in Table 4. Based on the known biological function of the newly expressed proteins and the DvSnf7 dsRNA (Table 4) no foreseen interactions at the biological level are identified.

Table 3: Genetic elements in the expression cassettes of the events stacked in maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
DP4114	*ubi*ZM1 region (*Zea mays*)	–	–	*cry1F** (*Bacillus thurigiensis*)	ORF25 (*Agrobacterium tumefaciens*)
	*ubi*ZM1 region (*Zea mays*)	–	–	*cry34Ab1** (*Bacillus thurigiensis*)	*pinII* (*Solanum tuberosum*)
	Peroxidase (*Triticum aestivum*)	–	–	*cry35Ab1** (*Bacillus thurigiensis*)	*pinII* (*Solanum tuberosum*)
	35 S (cauliflower mosaic virus)	–	–	*pat** (*Streptomyces viridochromogenes*)	35 S (cauliflower mosaic virus)
MON 89034	e35S (cauliflower mosaic virus)	*cab* (*Triticum* sp.)	–	*cry1A.105** (*Bacillus thurigiensis*)	*hsp17* (*Triticum* sp.)
	35 S (figwort mosaic virus)	–	CTP (Zea mays)	*cry2Ab2** (*Bacillus thurigiensis*)	*nos* (*Agrobacterium tumefaciens*)
MON 87411	e35S (cauliflower mosaic virus)	–	–	*An inverted repeat for partial coding sequence of DvSnf7* (*Diabrotica virgifera virgifera*)	E9 (*Pisum sativum*)
	pIIG (Zea mays)	*cab* (*Triticum* sp.)	–	*cry3Bb1** (*Bacillus thurigiensis*)	*hsp17* (*Triticum aestivum*)

[7] Dossier: Part II – Section 1.2 and additional information 12/07/2021, 04/11/2021, 10/02/2022 and 15/06/2022.

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
	TubA (*Oryza sativa*)	TubA (*Oryza sativa*)	CTP2 (*Arabidopsis thaliana*)	*cp4 epsps** (*Agrobacterium* sp.)	TubA (*Oryza sativa*)
DAS-40278-9	ZmUbi1 (*Zea mays*)	–	–	*aad-1** (*Sphingobium herbicidovorans*)	*ZmPer5* 3′UTR (*Zea mays*)

CaMV: cauliflower mosaic virus; CTP: chloroplast transit peptide.
–: When no element was specifically introduced to optimise expression.
*: Codon optimised for expression in plants.

Table 4: Characteristics and intended effects of the events stacked in maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9

Event	Protein	Donor organism and biological function	Intended effects in GM plant
DP4114	Cry1F	Based on genes from *Bacillus thuringiensis* subsp. aizawai. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (cry) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event DP4114 expresses Cry1F which is a protein toxic to certain lepidopteran larvae feeding on maize.
	Cry34Ab1	Based on genes from *Bacillus thuringiensis* strain PS149B1. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (cry) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event DP4114 expresses the Cry34Ab1 and Cry35Ab1; in complex these proteins are toxic to certain coleopteran larvae feeding on maize
	Cry35Ab1	Based on genes from *Bacillus thuringiensis* strain PS149B1. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (cry) genes (Schnepf et al., 1998; Ellis et al., 2002)	
	PAT	Based on a gene from *Streptomyces viridochromogenes* Tü494. Phosphinothricin-acetyl-transferase (PAT) enzyme acetylates L-glufosinate-ammonium (Thompson et al., 1987; Wohlleben et al., 1988; Eckes et al., 1989).	Event DP4114 expresses the PAT protein, which confers tolerance to glufosinate ammonium-based herbicides.
MON 89034	Cry1A.105	Based on genes *from B. thuringiensis* subsp. kurstaki and subsp. aizawai. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (cry) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event MON 89034 expresses a modified version of the Cry1A-type protein. Cry1A.105 is a protein toxic to certain lepidopteran larvae feeding on maize
	Cry2Ab2	Based on a gene from *Bacillus thuringiensis* subsp. kurstaki. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (cry) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event MON 89034 expresses the Cry2Ab2, a protein toxic to certain lepidopteran larvae feeding on maize
MON 87411	DvSnf7 dsRNA	Based on genes from western corn rootworm (WCR) (*Diabrotica virgifera virgifera* LeConte). The full-length Snf7 protein is part of the intracellular protein trafficking pathway (ESCRT) which is important for the maintenance of a	Event MON 87411 expresses DvSnf7 dsRNA which is a small RNA toxic to western corn rootworm feeding on maize

Event	Protein	Donor organism and biological function	Intended effects in GM plant
		functional intracellular transport of transmembrane proteins (Baum et al., 2007; Ramaseshadri et al., 2013)	
	Cry3Bb1	Based on genes from *Bacillus thuringiensis*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (cry) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event MON 87411 expresses the Cry3Bb1, a protein toxic to certain coleopteran larvae feeding on maize
	CP4 EPSPS	Based on a gene from *Agrobacterium* strain CP4 (Barry et al., 2001). 5-Enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	Event MON 87411 expresses the bacterial CP4 EPSPS protein which confers tolerance to glyphosate containing herbicides as it has lower affinity towards glyphosate than the plant endogenous enzyme
DAS-40278-9	AAD-1	Based on a gene from *Sphingobium herbicidovorans*. Aryloxyalkanoate dioxygenase (AAD-1) facilitates the breakdown of phenoxy auxin and aryloxyphenoxypropionate herbicides into carbon sources for the bacterium (Wright et al., 2009)	Event DAS-40278-9 expresses AAD-1 protein which degrades the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and thus confers tolerance to this herbicide.

3.4.1.2. Integrity of the events in the four-event stack maize

The genetic stability of the inserted DNA over multiple generations in the single maize events DP4114, MON 89034, MON 87411 and DAS-40278-9 has been previously demonstrated (Table 2). Integrity of these events in maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 was shown by Southern analyses. In addition, the sequence of the events (inserts and their flanking regions) was determined in the four-event stack maize and compared to the sequences originally reported for the four single events. The sequences of the events in the stack and in the single events were found to be identical, thus confirming that the integrity of these events was maintained in the four-event stack maize. The quality of the methodology and datasets was assessed by the EFSA GMO Panel and is in compliance to the requirements listed in the EFSA Technical Note (EFSA GMO Panel, 2018a).

3.4.1.3. Information on the expression of the inserts

Cry1F, Cry1A.105, Cry2Ab2, Cry34Ab1, Cry35Ab1, Cry3Bb1, PAT, CP4 EPSPS and AAD-1 protein levels were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from a field trial at six locations: five in the USA and one in Canada in 2019. Samples analysed included leaves (V9 and R1), roots (V9 and R1), pollen (R1), forage (R4) and grain (R6), in each case treated and not treated with intended herbicides.

In order to assess the possible changes in protein expression levels which may result from potential interactions between the events, protein levels were determined for the four-event stack and the corresponding single events in different parts of the plant.

The levels of all the newly expressed proteins in the four-event stack and the corresponding singles were comparable in all tissues (Appendix B). There is no indication of an interaction between the events, including a potential effect of the DvSnf7 dsRNA, that may affect the levels of the newly expressed proteins in this stack.

3.4.1.4. Conclusions of the molecular characterisation

The molecular data establish that the events stacked in maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the four-event stack maize and in the single events. Therefore, there is no indication of an interaction that may affect the integrity of the events or the levels of the newly expressed proteins in this stack. Based on the known biological function (Table 4)

of the newly expressed proteins, the only potential functional interactions are among the Cry proteins in susceptible insects, which will be dealt with in Section 3.4.4.

3.4.2. Comparative analysis[8]

3.4.2.1. Overview of studies conducted for the comparative analysis

Application EFSA-GMO-NL-2020-171 presents data on agronomic and phenotypic characteristics as well as on forage and grain composition of maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 (Table 5 and Appendix A).

Table 5: Main comparative analysis studies to characterise maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 provided in the application EFSA-GMO-NL-2020-171

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic and phenotypic characteristics	Field trials, 2019, US and Canada, 11 sites[(a)]	PH1KTF/3AAXI2080	16[(b)]
Compositional analysis	Field trials, 2019, US and Canada, eight sites[(a)]		
Seed germination	F_1 seed germination study – controlled conditions		–

(a): Eight field trials conducted in 2019 were used for both the agronomic/phenotypic and the compositional analysis: in the US, two field trials in Iowa and South Dakota and one field trial in Indiana, Nebraska and Pennsylvania; in Canada, one field trial in Ontario. Three field trials were used only for the agronomic/phenotypic analysis and were conducted in 2019 in the US in Illinois, Minnesota and Wisconsin.

(b): The non-GM maize hybrids with their corresponding comparative relative maturity indicated in brackets were: Pioneer P9537 (98), Pioneer 36Y03 (100), Pioneer 37Y12 (99), Pioneer P0312 (100), Pioneer P9903 (99), Prairie Brand 1,032 (103), Becks 5,234 (102), Becks 5,337 (103), Becks 5,385 (103), Mycogen MY06R30 (103), Pioneer P0157 (101), Pioneer P0164 (101), Pioneer P0216 (102), Pioneer P0506 (105), Pioneer P0589 (103) and Pioneer P0574 (105).

3.4.2.2. Experimental field trial design and statistical analysis

At each field trial site, the following materials were grown in a randomised complete block design with four replicates: the four-event stack maize not exposed to the intended herbicides, the four-event stack maize exposed to the intended herbicides, the comparator PH1KTF/3AAXI2080 and four non-GM reference varieties.

The agronomic, phenotypic and compositional data were analysed as specified by EFSA GMO Panel (2010b, 2011a). This includes, for each of the two treatments of the four-event stack maize, the application of a difference test (between the four-event stack maize and the comparator) and an equivalence test (between the four-event stack maize and the set of non-GM commercial reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[9]

3.4.2.3. Suitability of selected test materials

Selection of the test materials

To obtain the four-event stack maize, the single events DP4114 and MON 87411 and the single events MON 89034 and DAS-40278-9 were transferred in the genetic background of the two different non-GM maize inbred lines, PH1KTF and 3AAXI2080, respectively. In subsequent subsections, maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 refers to hybrid (F_1 generation) obtained crossing GM inbred line PH1KTF (carrying events DP4114 and MON 87411) with GM inbred line 3AAXI2080 (carrying events MON 89034 and DAS-40278-9).

The comparator used in the field trials is the non-GM hybrid maize PH1KTF/3AAXI2080, which has a similar genetic background as DP4114 × MON 89034 × MON 87411 × DAS-40278-9 (as documented by the pedigree and by the additional information), and is therefore considered to be the suitable comparator.

[8] Dossier: Part II – Section 1.3; additional information: 12/07/2021.

[9] In detail, the four outcomes are: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence).

The four-event stack and the comparator both have a relative maturity (CRM) of 101, which is considered appropriate for growing in environments across the US and Canada, where the comparative field trials were conducted.

Commercial non-GM reference varieties with a CRM ranging from 98 to 105 were selected by the applicant and, at each selected site, four reference varieties were tested (see Table 5). On the basis of the provided information on relative maturity classes and year of commercialization, the GMO Panel considers the selected non-GM reference varieties appropriate for the comparative assessment.

Seed production and quality

Seeds of the four-event stack maize and the comparator used in the 2019 field trials were produced from plants free of diseases, harvested and stored under similar conditions, before being sown in the field trial sites. The seed lots were verified for their identity via event specific quantitative polymerase chain reaction analysis.

The grains were tested for their germination capacity under warm and cold temperature conditions.[10] Germination capacity of maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 was compared with the one of its comparator and the results[11] of these studies indicate that the seed germination of four-event stack maize was not different than that of its comparator.

Conclusion on suitability

The GMO Panel is of the opinion that the four-stack maize, the comparator and the non-GM maize reference varieties were properly selected and are of adequate quality. Therefore, the test materials are considered suitable for the comparative analysis.

3.4.2.4. Representativeness of the receiving environments

Selection of field trial sites

The selected field trial sites were located in commercial maize-growing regions of the US and Canada. The soil and climatic characteristics of the selected fields were diverse,[12] corresponding to optimal, near-optimal and sub-optimal conditions for maize cultivation (Sys et al., 1993). The GMO Panel considers that the selected sites, including the subset chosen for the compositional analysis, reflect commercial maize-growing regions in which the test materials are likely to be grown.

Meteorological conditions

Some exceptional weather conditions were reported at four of the selected sites.[13] However, due to the lack of major impacts on plant growth at these sites, the GMO Panel considers that the exceptional weather conditions did not invalidate the selection of the field trial sites for the comparative analysis.

Management practices

The field trials included plots containing four-stack maize, plots with the comparator and plots with non-GM maize reference varieties, all managed according to local agricultural practices. In addition, the field trials included plots containing four-event stack maize managed following the same agricultural practices, but conventional herbicide was replaced with one application of quizalofop-containing herbicide that was applied at the BBCH 12 growth stage,[14] and at BBCH 14 growth stage a mixture of 2,4-D and glyphosate-containing herbicides and one application of glufosinate were applied. The GMO Panel considers that the management practices including sowing, harvesting and application of plant protection products were appropriate for the selected receiving environments.

[10] Warm temperature condition corresponds to 25°C for 7 days and cold temperature to 10°C for 7 days followed by 5 days at 25°C.

[11] The GM maize DP4114 × MON89034 × MON87411 × DAS40278 showed a mean germination of 99% and 97%, while the comparator showed a mean of 96% and 97% under warm and cold temperature conditions, respectively.

[12] Soil types of the field trials were clay loam, sandy clay loam, silty clay loam, loam, silt loam and sandy loam; soil organic carbon ranged from 1.1% to 2.7%; pH ranged from 5.5 to 7.1; historically, average temperatures and sum of precipitations during the usual crop growing season ranged, respectively, from 12.9 to 17.0°C and from 487 to 908 mm.

[13] Frost events were registered at one field trial in Illinois and South Dakota, windstorm at one field trial in Minnesota and heavy rainfall at one field trial in Pennsylvania.

[14] BBCH scale describes phenological stages (Meier, 2001).

Conclusion on representativeness

The GMO Panel concludes that the geographical locations, soil and climatic characteristics, meteorological conditions and management practices of the field trials are typical of the receiving environments where the test materials could be grown.

3.4.2.5. Agronomic and phenotypic analysis

Eleven agronomic and phenotypic endpoints plus information on abiotic stressors, disease incidence and arthropod damage were evaluated in the field trials (see Table 5).[15] Three endpoints (ear count, dropped ears and lodging) were not analysed with formal statistical methods because of lack of variability in the data.

The statistical analysis (Section 3.4.2.2) was applied to eight endpoints, with the following results:

- For the four-event stack maize (not treated with the intended herbicides), the test of difference identified statistically significant differences with the comparator for early stand count, days to flowering, plant height, final stand count and harvest grain moisture. All these endpoints fell under equivalence category I.
- For the four-event stack maize (treated with the intended herbicides), the test of difference identified statistically significant differences with the comparator for early stand count, days to flowering, plant height, days to maturity, final stand count and harvest grain moisture. All these endpoints fell under equivalence category I.

3.4.2.6. Compositional analysis

Forage and grain of the DP4114 × MON 89034 × MON 87411 × DAS-40278-9 maize harvested from the field trials (Table 5) were analysed for 80 constituents (10 in forage and 70 in grain), including those recommended by OECD (2002). The statistical analysis as described in Section 3.4.2.2 was not applied to 11 grain constituents[16] because their concentration in more than half of the samples were below the limit of quantification.

The statistical analysis was applied to a total of 69 constituents (10 in forage[17] and 59 in grain[18]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 6:

- For the four-event stack maize not treated with the intended herbicides, statistically significant differences with the non-GM comparator were found for 35 endpoints (5 in forage and 30 in grains). All these endpoints fell under equivalence category I or II except for phosphorus in forage which fell under equivalence category III, oleic acid (C18:1), and α-linolenic acid (C18:3) in grain which fell under equivalence category III and lignoceric acid (C24:0) in grain which fell under equivalence category IV, both in grain.
- For the four-event stack maize treated with the intended herbicides, statistically significant differences with the non-GM comparator were found for 21 endpoints (4 in forage and 17 in grains). All these endpoints fell under equivalence category I or II except for oleic acid (C18:1), linoleic acid (C18:2) and α-linolenic acid (C18:3) which fell under equivalence category III, and lignoceric acid (C24:0) which fell under equivalence category IV (all in grain).

[15] Early stand count, days to flowering, plant height, days to maturity, lodging, final stand count, ear count, dropped ears, yield, harvest grain moisture and 100-kernel weight.

[16] Lauric acid (C12:0), myristic acid (C14:0), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), eicosadienoic acid (C20:2), copper, riboflavin, β-tocopherol, δ-tocopherol, furfural and raffinose.

[17] Moisture, crude protein, crude fat, ash, carbohydrates, crude fibre, acid detergent fibre (ADF), neutral detergent fibre (NDF), calcium and phosphorus.

[18] Proximates and fibre content (ash, carbohydrates, crude fat, crude fibre, crude protein, moisture, acid detergent fibre (ADF), neutral detergent fibre (NDF) and total dietary fibre (TDF)), minerals (calcium, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc), vitamins (α-tocopherol, β-carotene, γ-tocopherol, total tocopherols, thiamine, niacin, pantothenic acid, pyridoxine, folic acid), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine), fatty acids (palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), α-linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), behenic acid (C22:0), lignoceric acid (C24:0)) and other compounds (ferulic acid, inositol, p-coumaric acid, phytic acid, trypsin inhibitor).

Table 6: Outcome of the comparative compositional analysis of forage and grain of maize DP4114x MON 89034 × MON 87411 × DAS-40278-9. The table shows the number of endpoints in each category

		Test of difference[a]			
		Not treated[c]		Treated[c]	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence[b]	Category I/II	33	31[d]	48	16[d]
	Category III/IV	–	4[e]	–	4[e]
	Not categorised	1[f]	–	1[f]	–
	Total endpoints	69		69	

(a): Comparison between the four-event stack maize and its comparator.
(b): Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.
(c): Treated/not treated with the intended herbicides.
(d): Endpoints with significant differences between the four-event stack maize and its comparator and falling under equivalence category I–II. For forage, not treated only: NDF. Treated only: crude protein. Both treated and not treated: moisture and crude fat. For grains, not treated only: crude protein, carbohydrates, palmitic acid (C16:0), linoleic acid (C18:2), alanine, glutamic acid, histidine, isoleucine, leucine, phenylalanine, proline, serine, threonine, tyrosine, valine, phosphorous, potassium and β-carotene. Treated only: total dietary fibre, zinc and *p*-coumaric acid. Both treated and not treated: moisture, ash, palmitoleic acid (C16:1), stearic acid (C18:0), calcium, pyridoxine, γ-tocopherol, total tocopherols, phytic acid and ferulic acid.
(e): Endpoints with significant differences between the four-event stack maize and its comparator and falling in equivalence category III/IV. In forage, not treated only: phosphorus. In grain, not treated only: none. Treated only: linoleic acid (C18:2). Both not treated and treated: oleic acid (C18:1), α-linolenic acid (C18:3) and lignoceric acid (C24:0). Quantitative results for these endpoints are reported in Table 7.
(f): Endpoints that were not categorised for equivalence and for which no significant differences were identified between the four-event stack maize and its comparator: folic acid in grain (both treated and not treated).

The GMO Panel assessed all the significant differences between the four-event stack maize and its comparator, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. Quantitative results for the endpoints showing significant differences between the four-event stack maize and its comparator and falling under equivalence category III/IV are given in Table 7.

Table 7: Quantitative results (estimated means and equivalence limits) for compositional endpoints in maize that are further assessed based on the results of the statistical analysis

	Endpoint	Maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9		Comparator	Non-GM reference varieties	
		Not treated[a]	Treated[a]		Mean	Equivalence limits
Forage	Phosphorus (% dw)	0.289*	0.270	0.256	0.250	0.214–0.286
Grain	Oleic acid (C18:1) (% FA)	23.2*	23.3*	24.2	28.2	23.8–32.6
Grain	Linoleic acid (C18:2) (% FA)	59.7*	59.8*	59.3	54.9	50.1–59.8
Grain	α-Linolenic acid (C18:3) (% FA)	1.82*	1.84*	1.76	1.53	1.25–1.81
Grain	Lignoceric acid (C24:0) (% FA)	0.345*	0.349*	0.338	0.264	0.197–0.33

dw: dry weight; % FA: percentage total fatty acids.
(a): Treated with the intended herbicides quizalofop and a mixture of glufosinate, glyphosate and 2,4-dichlorophenoxyacetic acid (2,4-D).
For the four-event stack maize, significantly different values are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds: white (equivalence category I or II), light grey (equivalence category III) and dark grey (equivalence category IV).

3.4.2.7. Conclusions on the comparative analysis

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic-phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials were appropriate to support the comparative analysis.

Taking into account the natural variability observed for the set of non-GM reference varieties, the GMO Panel concludes that:

- None of the differences identified in the agronomic and phenotypic characteristics between the four-event stack maize and the comparator needs further assessment for environmental safety.
- None of the differences identified in forage and grain composition between the four-event stack maize and the comparator needs further assessment regarding food and feed safety except for the levels of phosphorus in forage (not treated), linoleic acid (C18:2) in grain (treated), and oleic acid (C18:1), α-linolenic acid (C18:3) and lignoceric acid (C24:0) in grain (both not treated and treated), which are further assessed in Section 3.4.3.

3.4.3. Food/Feed safety assessment[19]

3.4.3.1. Effects of processing

The four-stack event maize will undergo existing production processes used for conventional maize. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the four-event stack maize into food and feed products is not expected to result in products being different from those of conventional non-GM maize varieties.

3.4.3.2. Stability of newly expressed proteins

Protein stability is one of several relevant parameters to consider in the weight-of-evidence approach in protein safety assessment (EFSA GMO Panel, 2010c, 2011a, 2017, 2021a). The term protein stability encompasses several properties such as thermal stability, pH-dependent stability, proteolytic stability and physical stability (e.g. tendency to aggregate), among others (Li et al., 2019). It has been shown, for example, that when characteristics of known food allergens are examined, one prominent trait attributed to food allergens is protein stability (Helm, 2001; Breiteneder and Mills, 2005; Foo and Mueller, 2021; Costa et al., 2022).

Effect of temperature and pH on newly expressed proteins

The effects of temperature and pH on the Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1A.105, Cry2Ab2, Cry3Bb1, CP4 EPSPS and AAD-1 proteins have been previously evaluated by the GMO Panel (Table 2). No new information has been provided in the context of this application.

In vitro *protein degradation by proteolytic enzymes*

The resistance to degradation by pepsin of the newly expressed Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1A.105, Cry2Ab2, Cry3Bb1, CP4 EPSPS and AAD-1 proteins have been previously evaluated by the GMO Panel (Table 2). No new information has been provided in the context of this application.

3.4.3.3. Toxicology

Testing of newly expressed proteins

Nine proteins (Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1A.105, Cry2Ab2, Cry3Bb1, CP4 EPSPS and AAD-1) are newly expressed in the four-event stack maize (Section 3.4.1). The GMO Panel has previously assessed these proteins in the context of the single maize events (Table 2), and no safety concerns were identified for humans and animals (i.e. farmed and companion animals). The GMO Panel is not aware of any new information that would change its previous conclusions on the safety of these proteins. The potential for a functional interaction among the proteins newly expressed in four-event stack maize has been assessed with regard to human and animal health.

The six insecticidal proteins Cry1F, Cry34Ab1, Cry35Ab1, Cry1A.105, Cry2Ab2, Cry3Bb1 are delta-endotoxins acting through cellular receptors found in target insect species. It is reported that the gastrointestinal tract of mammals, including humans, lacks receptors with high specific affinity to Cry proteins (Hammond et al., 2013; Koch et al., 2015; Jurat-Fuentes and Crickmore, 2017).

[19] Dossier: Part II – Sections 1.4, 1.5, 1.6, 2, 3, 4; additional information: 12/7/2021, 31/3/2022, 15/6/2022.

The three enzymatic proteins (CP4 EPSPS, PAT and AAD-1) catalyse distinct biochemical reactions, acting on unrelated substrates and are not expected to interact. The CP4 EPSPS act on the shikimic acid pathway for the biosynthesis of aromatic amino acids in plants, showing high substrate specificity. The PAT enzyme acts on the glufosinate-ammonium-based herbicides and the AAD-1 enzyme degrades 2,4-D and AOPP class of herbicides. On the basis of the known biological function of the individual newly expressed proteins (Table 4), there is currently no expectation for possible interactions relevant to the food and feed safety of the four-event stack maize.

The GMO Panel concludes that there are no safety concerns for human and animal health related to the newly expressed proteins Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1A.105, Cry2Ab2, Cry3Bb1, CP4 EPSPS and AAD-1 in the four-event stack maize.

Testing of new constituents other than proteins

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no new constituents other than the newly expressed proteins have been identified in seed and forage from the four-event stack maize, with the exception of the intended expression of DvSnf7 dsRNA and derived siRNAs, designed to control coleopteran pests via RNAi. The GMO Panel has previously assessed these compounds in the context of the single maize event MON 87411 (EFSA GMO Panel, 2018b) and concluded that no safety concerns are associated with their presence. The GMO Panel is not aware of any new information that would change its previous conclusions on the safety of these compounds. On the basis of the known biological function of these constituents (Table 4), there is currently no expectation for possible interactions with other new compounds (newly expressed proteins) or other constituents relevant to the food and feed safety of this four-event stack maize.

Information on altered levels of food and feed constituents

No altered levels of food/feed constituents have been identified in seed and forage from the four-event stack maize, except for phosphorus in forage, and linoleic acid (C18:2), oleic acid (C18:1), α-linolenic acid (C18:3) and lignoceric acid (C24:0) in grain. These changes are considered not to represent a toxicological concern, considering the biological role of the affected constituent and the magnitude of the changes. Therefore, no further toxicological studies are needed. Further information on the relevance of these findings is provided in Section 3.4.3.6.

Testing of the whole genetically modified food and feed

Based on the outcome of the molecular characterisation, comparative analysis and toxicological assessment, no indication of findings relevant to food/feed safety related to the stability and expression of the inserts or to interaction between the transformation events, and no modifications of toxicological concern in the composition of the four-event stack maize have been identified (see Sections 3.4.1, 3.4.2 and 3.4.3.3). Therefore, animal studies on food/feed derived from the four-event stack maize are not necessary (EFSA GMO Panel, 2011a). In accordance to Regulation (EU) No 503/2013, the applicant provided a 90-day oral repeated-dose toxicity study in rats on whole food and feed from each of the maize single event composing the four-event stack maize.

The GMO Panel had previously concluded that these studies are in line with Regulation (EU) No 503/2013 and do not show adverse effects related to diets incorporating the single-event maize DP4114 (EFSA GMO Panel, 2018b), MON 89034 (EFSA GMO Panel, 2019e), MON 87411 (EFSA GMO Panel, 2018c) and DAS-40278-9 (EFSA GMO Panel, 2021c).

3.4.3.4. Allergenicity

For the allergenicity assessment, a weight-of-evidence approach was followed, taking into account all the information obtained on the newly expressed proteins, as no single piece of information or experimental method yields sufficient evidence to predict allergenicity and adjuvanticity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a; Commission Regulation (EU) No 503/2013). Furthermore, an assessment of specific newly expressed proteins in relation to their potential to cause celiac disease was performed (EFSA GMO Panel, 2017).

Assessment of allergenicity of the newly expressed proteins

The GMO Panel has previously evaluated the safety of Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1A.105, Cry2Ab2, Cry3Bb1, CP4 EPSPS and AAD-1 proteins individually, and no evidence of allergenicity was identified in the context of the applications assessed (Table 2). No new information on allergenicity of

the proteins newly expressed in this four-event stack maize that might change the previous conclusions of the GMO Panel has become available. Based on the current knowledge, and as there is no evidence of allergenicity of the newly expressed proteins, there are no expected concerns of allergenicity as a consequence of their presence in this four-event stack maize.

The GMO Panel has previously evaluated the safety of the newly expressed proteins, and no evidence of adjuvanticity were identified in the context of the applications assessed (Table 2). This aspect has been discussed in detail by EFSA (2018b; Parenti et al., 2019). To date, there is no evidence for adjuvanticity in the GMOs assessed by the Panel. This four-event stack maize has similar levels of the individual Bt proteins as those in the respective single maize events (see Section 3.4.1). The GMO Panel did not find indications that the Bt proteins at the levels expressed in this four-event stack maize might be adjuvants able to enhance an allergic reaction.

The applicant also provided information on the safety of the Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1A.105, Cry2Ab2, Cry3Bb1, CP4 EPSPS and AAD-1 proteins regarding their potential to cause a celiac disease response. For such assessment, the applicant followed the principles described in the EFSA GMO Panel guidance document (EFSA GMO Panel, 2017). The assessment of the Cry34Ab1, Cry2Ab2 and CP4 EPSPS proteins identified no perfect or relevant partial matches with known celiac disease peptide sequences. The assessment of the Cry1F, Cry1A.105, Cry35Ab1, Cry3Bb1, PAT and PMI proteins revealed partial matches containing the Q/E-X1-P-X2 motif and required further investigations. Several of these partial matches have been previously assessed by the EFSA GMO Panel (2019f, 2021b,d, 2022a,b). Based on additional considerations on the position and nature of amino acids flanking the motifs, such as the presence of two consecutive prolines and the charge and size of adjacent amino acids (EFSA GMO Panel, 2017), the relevant peptides containing the motif do not raise concern as they fail to mimic gluten sequences. Therefore, no indications of safety concern were identified by the GMO Panel.

Assessment of allergenicity of the whole GM plant or crop

The GMO Panel regularly reviews the available publications on food allergy to maize. However, maize is not considered a common allergenic food[20] (OECD, 2002). Therefore, the GMO Panel does not request experimental data to analyse the allergen repertoire of GM maize. In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (see Sections 3.4.1, 3.4.2 and 3.4.3), the GMO Panel identifies no indications of a potentially increased allergenicity of food and feed derived from this four-event stack maize with respect to that derived from the non-GM comparator and the non-GM reference varieties tested.

3.4.3.5. Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013 the applicant provided dietary exposure estimates to Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1A.105, Cry2Ab2, Cry3Bb1, CP4 EPSPS and AAD-1 proteins newly expressed in the four-event stack maize. Dietary exposure was estimated based on protein expression levels reported in this application for the four-event stack maize treated with the intended herbicides, the currently available consumption data and feed practices, the foods and feeds currently available on the market and the described processing conditions.

For the purpose of estimating dietary exposure, the levels of newly expressed proteins in the four-event stack maize grains, forage and pollen were derived from replicated field trials (four replicates from six locations, n = 24) in 2019 in the US and Canada. Table 8 describes the protein expression levels used to estimate both human and animal dietary exposure.

[20] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

Table 8: Mean values (n = 24, μg/g dry weight and μg/g fresh weight) for newly expressed proteins in grains, forage and pollen from maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 treated with the intended herbicides[(a)]

Protein	Tissue/developmental stage		
	Grains/R6 (μg/g dry weight/μg/g fresh weight)	Pollen/R1 (μg/g dry weight)[(b)]	Forage/R4 (μg/g dry weight)
Cry1F	1.7/1.4	34	5.4
Cry34Ab1	19/16	19	78
Cry35Ab1	0.78/0.66	< 0.22[(c)]	21
Cry1A.105	1.8/1.5	4.2	9.4
Cry2Ab2	3.0/2.5	0.58	53
Cry3Bb1	4.5/3.8	36	55
CP4 EPSPS	2.9/2.4	26	16
PAT	0.030/0.025[(c)]	< 0.22[(c)]	1.8
AAD-1	5.0/4.2	130	11

(a): Intended herbicides: glufosinate, glyphosate, 2,4-dichlorophenoxyacetic acid (2,4-D) herbicide and aryloxyphenoxypropionate (AOPP) herbicides.
(b): Concentrations values in pollen were adjusted to 6% moisture content before using them to estimate dietary exposure to the different newly expressed protein via the consumption of pollen supplements.
(c): All pollen samples analysed for Cry35Ab1 and PAT proteins were below LOQ (0.22 μg/g dry weight). A total of 23 out of 24 grain samples analysed for PAT protein were reported below LOQ (0.054 μg/g dry weight/0.047 μg/g fresh weight). Half of the LOQ value was assigned to the left-censored data to calculate the mean.

Human and animal dietary exposure assessment to DvSnf7 dsRNA and its derived siRNAs was not conducted because these molecules are generally rapidly denaturated, depurinated and degraded shortly after ingestion, and therefore, they are considered generally not to exert any biological effects once ingested by humans and animals (EFSA GMO Panel, 2018b).

Human dietary exposure

Chronic and acute dietary exposure to Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1A.105, Cry2Ab2, Cry3Bb1, CP4 EPSPS and AAD-1 proteins newly expressed in the four-event stack maize were provided. The applicant followed the methodology described in the EFSA Statement 'Human dietary exposure assessment to newly expressed protein in GM foods' (EFSA, 2019a) to estimate human dietary exposure in average and high consumers making use of summary statistics of consumption.

Human dietary exposure was estimated across different European countries on different population groups: young population (infants, toddlers, 'other children'), adolescents, adult population (adults, elderly and very elderly) and special populations (pregnant and lactating women). Since no specific consumption data were available on commodities containing, consisting of or obtained from the four-event stack maize grains, a conservative scenario with 100% replacement of conventional maize by the GM maize was considered. Consumption figures for all relevant commodities (e.g. corn flakes, sweet corn, popcorn, etc.) were retrieved from the EFSA Comprehensive European Food Consumption Database (EFSA consumption database).[21] Corn oil, corn starch and corn syrup were excluded from the assessment since no proteins are expected to be present in these commodities.

Mean protein expression values on fresh weight basis are considered as the most adequate to estimate human dietary exposure (both acute and chronic) when working with raw primary commodities that are commonly consumed as processed blended commodities (EFSA, 2019a). Different recipes and factors were considered to estimate the amount of maize in the consumed commodities before assigning newly expressed protein levels to the relevant commodities.[22] No losses in the newly expressed proteins during processing were considered, except for certain commodities excluded from the exposure estimations (corn oil, corn starch, corn syrup).

The highest acute dietary exposure (high consumers) was estimated in the age class 'Other children' with exposure estimates that ranged between 0.380 μg/kg body weight (bw) per day for PAT

[21] https://www.efsa.europa.eu/en/applications/gmo/tools. Data accessed: June 2020.
[22] Example: 100 g of maize bread are made with approximately 74 g of maize flour, and a reverse yield factor of 1.22 from the conversion of maize grains into flour is used. This results in ~14.6 μg of Cry34Ab1 per gram of maize bread as compared to the 16 μg/g reported as mean concentration in the maize grains.

protein and 243 μg/kg bw per day for Cry34Ab1 protein. The main contributor to the exposure in the dietary survey with the highest estimates was corn grains.

The highest chronic dietary exposure (high consumers) was estimated in the age class 'Infants', with exposure estimates that ranged between 0.14 μg/kg bw per day for PAT protein and 90 μg/kg bw per day for Cry34Ab1 protein. The main contributor to the exposure in the dietary survey with the highest estimates was sweet corn.

An ad hoc dietary exposure scenario was provided for consumers of pollen supplements under the assumption that these supplements might be made of pollen from DP4114 × MON 89034 × MON 87411 × DAS-40278-9 maize. Consumption data on pollen supplements are available for few consumers across eight different European countries.[23] The low number of consumers available adds uncertainty to the exposure estimations which should be carefully interpreted, and it prevents from estimating exposure for high consumers of pollen supplements. In average consumers of pollen supplements, the highest acute dietary exposure would range from 0.156 μg/kg bw per day for Cry35Ab1 and PAT proteins to 90.4 μg/kg bw per day for AAD-1, in the elderly population. Similarly, the highest chronic dietary exposure in average consumers would range from 0.104 μg/kg bw per day for Cry35Ab1 and PAT proteins to 60.3 μg/kg bw per day for AAD-1, also in the elderly population.

Animal dietary exposure

Dietary exposure to Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1A.105, Cry2Ab2, Cry3Bb1, CP4 EPSPS and AAD-1 proteins in the four-event stack maize was estimated across different animal species as below described, assuming the consumption of maize products commonly entering the feed supply chain (i.e. maize grains and forage). A conservative scenario with 100% replacement of conventional maize products by the four-event stack maize products was considered.

Mean levels (dry weight) of the newly expressed proteins in grains and forage from four-stack event maize treated with the intended herbicide used for animal dietary exposure are listed in Table 8.

The applicant estimated dietary exposure to Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1A.105, Cry2Ab2, Cry3Bb1, CP4 EPSPS and AAD-1 proteins in livestock (i.e. poultry, swine, cattle and sheep), based on estimates for body weights, daily feed intakes and inclusion rates (percentage) of maize grains and forage in diets/rations (OECD, 2013). Estimated dietary exposure in livestock animals was calculated based on the consumption of maize grain and forage alone or in combination, as reported in Appendix C.

3.4.3.6. Nutritional assessment of the GM food/feed

The intended traits of the four-event stack maize are herbicide tolerance and insect resistance, with no intention to alter nutritional parameters. However, levels of phosphorus in forage (not treated), linoleic acid (C18:2) in grain (treated), and oleic acid (C18:1), α-linolenic acid (C18:3) and lignoceric acid (C24:0) in grain (both not treated and treated) were significantly different from its comparator and showed a lack of equivalence with the set of non-GM reference varieties (Section 3.4.2.6). The biological relevance of these compounds, the role of maize as contributor to their total intake and the magnitude and direction of the observed changes were considered during the nutritional assessment.

Human nutritional assessment

A small decrease of 3% and 4% of oleic acid in treated and not treated, respectively, was reported compared to its comparator. No dietary reference values (DRVs) for *cis*-monounsaturated fatty acids are proposed by EFSA since they are synthesised by the body (EFSA NDA Panel, 2010). Considering this information and the fact that many other foods are also source of oleic acid in the diet, the GMO Panel concludes that the observed decrease does not raise nutritional concerns.

As regards to linoleic acid, a slight increase of 1% was observed for both treated and not treated four-event stack maize compared to its comparator. An adequate intake (AI) for linoleic acid of 4 E% has been proposed (EFSA NDA Panel, 2010). Although linoleic acid is the most abundant fatty acid in maize, considering that no tolerable upper intake level (UL) is set and the magnitude of the increase, the GMO Panel identified no nutritional concern.

α-Linolenic acid counts for < 2% of total fatty acid content in maize. A small increase of 5% and 3% in treated and not treated four-event stack maize respectively was reported compared to its comparator EFSA NDA Panel proposed an AI of 0.5 E% (EFSA NDA Panel, 2010). Considering that no

[23] https://www.efsa.europa.eu/en/food-consumption/comprehensive-database. Data accessed: 14 February 2022.

UL has been proposed for α-linolenic acid, the GMO Panel concludes that the observed increase does not raise nutritional concerns.

A small increase of 4% and 2% of lignoceric acid in treated and not treated four-event stack maize respectively was reported compared to its comparator. Current nutritional recommendations for saturated fatty acids indicate that their intake should be as low as is possible within the context of a nutritionally adequate diet (EFSA NDA Panel, 2010). Considering the very low levels of lignoceric acid (< 1% total FA) in maize, the GMO Panel concludes that the levels of lignoceric acid in the four-event stack maize do not represent a nutritional concern in humans.

Animal nutritional assessment

Diets for animals are usually balanced for the content of major minerals, including phosphorus, and eventually supplemented when the amount provided by feed is not enough to satisfy nutritional requirements. Phosphorus in cereals is mainly bound to phytic acid, largely reducing its bioavailability especially in non-ruminant animals. The observed increase of phosphorus in not treated four-event stack maize forage compared to its comparator does not pose an issue for animals.

Oleic acid (C18:1) is a non-essential fatty acid. The decrease in GM maize grains does not represent a problem for animal nutrition.

Linoleic (C18:2 n-6) is the most dominant fatty acid in maize grain, while α-linolenic acid (C18:3 n-3) is scarcely present. These fatty acids are called essential, because they cannot be synthetized in the body, and they must be supplied in diets. The observed increased content of both fatty acids in four-event stack maize grains is not an issue for animal nutrition.

Lignoceric acid (C24:0) is a very long saturated fatty acid present in several feed at low concentration. It can be synthesised in the body (van den Ingh et al., 2019) and the observed increase in four-event stack maize grains does not pose an issue for animal nutrition.

3.4.3.7. Conclusion on the food/feed safety assessment

The newly expressed proteins Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1A.105, Cry2Ab2, Cry3Bb1, CP4 EPSPS and AAD-1 and the DvSnf7 dsRNA and derived siRNAs in the four-event stack maize do not raise safety concerns for human and animal health. No interactions between the newly expressed proteins relevant for food and feed safety were identified, and no overall toxicological concerns on the four-event stack maize were identified. Similarly, the GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity related to the presence of the newly expressed proteins in maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9, or regarding the overall allergenicity of this four-event stack maize. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 does not represent any nutritional concern, in the context of the scope of this application.

3.4.4. Environmental risk assessment[24]

Considering the scope of application EFSA-GMO-NL-2020-171, which excludes cultivation, the environmental risk assessment (ERA) of four-event stack maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA (i.e. in the gastrointestinal tract of animals and humans ingesting GM material) and of microorganisms present in environments exposed to faecal material (manure and faeces) of these animals; and (2) the accidental release into the environment of viable four-event stack maize grains during transportation and/or processing (EFSA GMO Panel, 2010a).

3.4.4.1. Persistence and invasiveness of the GM plant

Maize is highly domesticated, not winter hardy in colder regions of Europe, and generally unable to survive in the environment without appropriate management. Survival is limited mainly by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens, herbivores and cold climate conditions (OECD, 2003), even though occasional feral GM maize plants may occur outside cultivation areas in the EU (e.g. Pascher, 2016). Field observations indicate that maize grains may survive and overwinter in some EU regions, resulting in volunteers in subsequent crops (e.g. Gruber et al., 2008; Palaudelmàs et al., 2009; Pascher, 2016). However, maize

[24] Dossier: Part II – Section 5; additional information: 15/6/2022.

volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). Thus, the establishment and survival of feral and volunteer maize in the EU is currently limited and transient.

It is unlikely that the intended traits of the four-event stack maize will provide a selective advantage to maize plants except when they are exposed to quizalofop- and/or 2,4-D- and/or glyphosate- and/or glufosinate-ammonium-containing herbicides or infested by insect pests that are susceptible to the Cry1A.105 and/or Cry3Bb1 and/or Cry34Ab1 and Cry35Ab1 proteins and to the *DvSnf7* dsRNA. However, if this was to occur this fitness advantage will not allow the GM plant to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers that four-event stack maize will be equivalent to conventional maize hybrid varieties in their ability 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 four-event stack maize grains.

3.4.4.2. Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer (HGT) of DNA, or through vertical gene flow via cross-pollination from feral plants originating from spilled grains.

Plant to microorganism gene transfer

The probability and potential adverse effects of HGT of the recombinant DNA have been assessed in previous GMO Panel Scientific Opinions for the single events (see Table 1). This assessment included consideration of homology-based recombination processes, as well as non-homologous end joining and microhomology-mediated end joining. Possible fitness advantages that the bacteria in the receiving environments would gain from acquiring recombinant DNA were considered. No concern as a result of an unlikely, but theoretically possible, HGT of the recombinant genes to bacteria in the gut of domesticated animals and humans fed GM material or other receiving environments was identified.

The applicant submitted an updated bioinformatic analysis for each of the single events to assess the possibility for HGT by homologous recombination.

The updated bioinformatic analyses for maize events DP4114, MON 89034, MON 87411 and DAS-40278-9 confirm the assessments provided in the context of previous Scientific Opinions (EFSA GMO Panel, 2021b, 2022a,b).

Synergistic effects of the recombinant genes, for instance due to combinations of recombinogenic sequences, which would cause an increase in the likelihood for HGT or a selective advantage were not identified.

Therefore, the GMO Panel concludes that the unlikely, but theoretically possible, horizontal transfer of recombinant genes from this four-event stack maize to bacteria does not raise any environmental safety concern.

Plant-to-plant gene transfer

The potential for occasional feral four-event stack maize plants originating from grain import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer were considered.

For plant-to-plant gene transfer to occur, imported GM maize grains need to germinate and develop into plants in areas containing sympatric wild relatives and/or cultivated maize with synchronous flowering and environmental conditions favouring cross-pollination.

Maize is an annual predominantly cross-pollinating crop. Cross-fertilisation occurs mainly by wind (OECD, 2003). Vertical gene transfer from maize is limited to *Zea* species. Wild relatives of maize outside cultivation are not known/reported in Europe (Eastham and Sweet, 2002; OECD, 2003; EFSA, 2016, 2022; Trtikova et al., 2017). Therefore, potential vertical gene transfer is restricted to maize and weedy *Zea* species, such as teosintes, and/or maize-teosinte hybrids, occurring in cultivated areas (EFSA, 2016, 2022; Trtikova et al., 2017; Le Corre et al., 2020).

The potential of spilled maize grains to establish, grow and produce pollen is extremely low and transient (see Section 3.4.4.1). Therefore, the likelihood/frequency of cross-pollination between occasional feral GM maize plants resulting from grain spillage, and weedy or cultivated *Zea* plants is considered extremely low (EFSA, 2016, 2022). Even if cross-pollination would occur, the GMO Panel is

of the opinion that environmental effects as a consequence of the spread of genes from occasional feral GM maize plants in Europe will not differ from that of conventional maize varieties for the reasons given in Section 3.4.4.2, even if exposed to the intended herbicides.

3.4.4.3. Interactions of the GM plant with target organisms

Taking the scope of application EFSA-GMO-NL-2020-171 into account (no cultivation), potential interactions of occasional feral four-event stack maize plants arising from grain import spills with the target organisms are not considered a relevant issue.

3.4.4.4. Interactions of the GM plant with non-target organisms

Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled four-event stack maize grains is limited, and because ingested dsRNA and proteins are degraded before entering the environment through faecal material of animals fed GM maize, the GMO Panel considers that potential interactions of the four-event stack maize with non-target organisms do not raise any environmental safety concern. Interactions that may occur between the insecticidal proteins will not alter this conclusion.

3.4.4.5. Interactions with the abiotic environment and biogeochemical cycles

Given that environmental exposure to spilled grains or occasional feral four-event stack maize plants arising from grain import spills is limited, and because ingested dsRNA and proteins are degraded before entering the environment through faecal material of animals fed GM maize, the GMO Panel considers that potential interactions with the abiotic environment and biogeochemical cycles do not raise any environmental safety concern.

3.4.4.6. Conclusion of the environmental risk assessment

The GMO Panel concludes that it is unlikely that four-event stack maize would differ from conventional maize varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-NL-2020-171, interactions of occasional feral four-event stack maize plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from four-event stack maize to bacteria does not indicate a safety concern. Therefore, considering the combined traits and their interactions, the outcome of the agronomic and phenotypic analysis, and the routes and levels of exposure, the GMO Panel concludes that four-event stack maize would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

3.5. Risk assessment of the subcombinations[25]

Subcombinations previously assessed in the frame of other applications are discussed in Section 3.5.1. The subcombinations that have not been previously assessed are discussed in Section 3.5.2.

3.5.1. Subcombinations previously assessed

The GMO Panel has previously assessed two subcombinations (see Table 2) and no safety concerns were identified. Literature searches covering the 10 years before submission of the application and the period since the time of validity of the application revealed no new scientific information relevant to the risk assessment of these maize stacks. Consequently, the GMO Panel considers that its previous conclusions on these subcombinations remain valid.

3.5.2. Subcombinations not previously assessed

Eight of the 10 subcombinations included in the scope of this application have not been previously assessed by the GMO Panel, and no experimental data were provided for these maize stacks (Table 9). In this case, following the strategy defined by the GMO Panel,[26] the risk assessment takes as its starting point the assessment of the single maize events, and uses the data generated for the four-

[25] Dossier: Part II – Section 1.2.
[26] 115th GMO Panel meeting (Annex 1 of the minutes: http://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf).

event stack as well as all the additional data available on subcombinations- previously assessed by the GMO Panel (Table 2).

Table 9: Maize stacks not previously assessed and covered by the scope of application EFSA-GMO-NL-2020-171

Degree of stacking	Event
Three-event stack	DAS-40278-9 × DP4114 × MON 87411
	MON 89034 × DP4114 × MON 87411
	MON 89034 × DAS-40278-9 × MON 87411
	MON 89034 × DAS-40278-9 × DP4114
Two-event stack	DP4114 × MON 87411
	DAS-40278-9 × MON 87411
	DAS-40278-9 × DP4114
	MON 89034 × DP4114

3.5.2.1. Stability of the events

The genetic stability of the inserted DNA over multiple generations in the four single maize events was demonstrated previously (see Table 2). Integrity of the events was demonstrated in the four-event stack maize (Section 3.4.1.2) and the previously assessed maize subcombinations (see Table 2). The GMO Panel finds no reasons to expect the loss of integrity of the events in the maize subcombinations not previously assessed (see Table 9).

3.5.2.2. Expression of the events

The GMO Panel assessed whether any combination of the four events by crossing could result in significant changes in expression levels of the newly expressed proteins, as this could indicate an unexpected interaction between the events. Based on current knowledge of the molecular elements introduced, there is no reason to expect interactions that would affect the levels of the newly expressed proteins in the eight subcombinations compared with those in the single maize events. This assumption was confirmed by comparing the levels of the newly expressed proteins of each single maize event with those of the four-event stack maize. The levels were comparable in the four-event stack maize and in the single events (Section 3.4.1.3 and Appendix B). In addition, the potential impact of the DvSnf7 dsRNA on the levels of the newly expressed proteins was assessed by comparing the protein expression levels in the four-event stack and the respective singles. The data indicate that there is no impact of the DvSnf7 dsRNA on the expression levels of the newly expressed proteins. This supports the conclusion that interactions affecting the expression levels of the newly expressed proteins are not expected in the eight subcombinations not previously assessed and included in the scope of application EFSA-GMO-NL-2020-171.

3.5.2.3. Potential functional interactions between the events

The GMO Panel assessed the potential for interactions between maize events in the eight subcombinations not previously assessed (Table 9), taking into consideration intended traits and unintended effects.

Based on the known biological functions of the individual newly expressed proteins and dsRNA (Table 4), there is currently no expectation for possible interactions relevant for the food and feed or environmental safety between these proteins in those subcombinations. The GMO Panel took into account all the intended and potential unintended effects considered in the assessment of the four single events, the previously assessed subcombinations (Table 2) and the four-event stack maize. It is concluded that none of these events would raise safety concerns when combined in any of these maize subcombinations. The GMO Panel considers that no further data are needed to complete the assessment of subcombinations from the four-event stack maize.

3.5.3. Conclusions

Since no new safety concerns were identified for the previously assessed subcombinations, the GMO Panel considers that its previous conclusions on these maize subcombinations remain valid. For the remaining eight subcombinations included in the scope of application EFSA-GMO-NL-2020-171, the

GMO Panel assessed the possibility of interactions among the events and concluded that these combinations would not raise safety concerns. These subcombinations are therefore expected to be as safe as and nutritionally equivalent to the single maize events, the previously assessed subcombinations and the four-event stack maize.

3.6. Post-market monitoring[27]

3.6.1. Post-market monitoring of GM food/feed

The GMO Panel concluded that the four-event stack maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9, as described in this application, does not raise any nutritional concern and is as safe as the comparator and the non-GM reference varieties tested (Section 3.4.3). Two of the subcombinations have been previously assessed and no safety concerns were identified. The subcombinations not previously assessed and included in the scope of this application (eight) are expected to be as safe as the single maize events, the previously assessed maize subcombinations and the four-event stack maize (Section 3.5.2). Therefore, the GMO Panel considers that post-market monitoring of food and feed from the four-event stack maize and its subcombinations, as described in this application, is not necessary.

3.6.2. Post-market environmental monitoring

The objectives of a post-market environmental monitoring (PMEM) plan, according to Annex VII of Directive 2001/18/EC, are: (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the ERA are correct; and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the ERA.

Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific rationale of the PMEM plan provided by the applicant (EFSA GMO Panel, 2011b).

As the ERA did not identify potential adverse environmental effects from maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 includes: (1) the description of a monitoring approach involving operators (federations involved in import and processing), reporting to the applicant, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by Crop Life Europe for the collection of information recorded by the various operators; and (3) the review of relevant scientific publications retrieved from literature searches (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis for the duration of the authorisation period.

The GMO Panel considers that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan. The PMEM plan and reporting intervals are in line with the intended uses of the four-event stack maize and its subcombinations.

3.6.3. Conclusion on post-market monitoring

No post market monitoring of food and feed is necessary. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9.

4. Overall conclusions

The GMO Panel was asked to carry out a scientific assessment of maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 and subcombinations for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

No new information was identified on the four single maize events (DP4114, MON 89034, MON 87411 and DAS-40278-9) that would lead to a modification of the original conclusions on their safety.

[27] Dossier: Part II – Sections 4 and 6.

The molecular characterisation, the comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment indicate that the combination of the single maize events, the newly expressed proteins and the dsRNA in the four-event stack maize do not give rise to food/feed safety and nutritional concerns. The GMO Panel concludes that the four-event stack maize, as described in this application, does not raise any nutritional concern and is as safe as its comparator and the selected non-GM reference varieties.

The GMO Panel concludes that there is a very low likelihood of environmental effects resulting from the accidental release of viable grains from the four-event stack maize into the environment. Since no new data were identified on the previously assessed subcombinations that would lead to a modification of the original conclusions on their safety, the GMO Panel considers that its previous conclusions on these maize stacks remain valid. For the remaining subcombinations included in the scope of application EFSA-GMO-NL-2020-171, no information has been provided. The GMO Panel assessed the possible interactions between the events in these subcombinations and concludes that these combinations of events DP4114, MON 89034, MON 87411 and DAS-40278-9 would not raise safety concerns. These subcombinations are therefore expected to be as safe as the maize single events, the previously assessed subcombinations and the four-event stack maize.

Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issues pertaining to the intended uses of maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 and its subcombinations.

In addition, the GMO Panel considered the additional unpublished studies listed in Appendix A. This new information does not raise any concern for human and animal health and the environment regarding the four-event stack maize and its subcombinations. Given the absence of safety and nutritional concerns for foods and feeds from the four-event stack maize and all its subcombinations, the GMO Panel considers that PMM of these products is not necessary. The PMEM plan and reporting intervals are in line with the intended uses of the four-event stack maize and its subcombinations. In conclusion, the GMO Panel considers that maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 and its subcombinations, as described in this application, are as safe as the comparator and the selected non-GM reference varieties with respect to potential effects on human and animal health and the environment.

5. Documentation as provided to EFSA

- Application submitted for the authorisation of genetically modified maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 by Pioneer Overseas Corporation on behalf of Pioneer Hi-Bred International, Inc. on 11 December 2020 (EFSA Ref. EFSA-GMO-NL-2020-171; EFSA-Q-2020-00833).
- The application was made valid on 26 April 2021.
- Additional Information (1) was requested on 11 May 2021.
- Additional Information (1) was received on 12 July 2021.
- Additional Information (2) was requested on 9 August 2021.
- Additional Information (2) was received on 4 November 2021.
- Additional Information (3) was requested on 10 December 2021.
- Additional Information (3) was received on 10 February 2022.
- Additional Information (4) was requested on 7 February 2022.
- Additional Information (4) was received on 31 March 2022.
- Additional Information (5) was requested on 24 March 2022.
- Additional information (5) was received on 15 June 2022.
- Supplementary information was provided on voluntary basis on 31 March 2022 and on 23 September 2022.

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Abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid
AAD-1	aryloxyalkanoate dioxygenase
ADF	acid detergent fibre
AI	adequate intake
AOPP	aryloxyphenoxypropionates
bw	body weight
CRM	comparative relative maturity
DRV	dietary reference value
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphat synthase
ERA	environmental risk assessment
GM	genetically modified
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
HR	homologous recombination
LOQ	limit of quantification
MS	Member States
NDF	neutral detergent fibre
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin-acetyl-transferase
PMEM	post-market environmental monitoring
UL	tolerable upper intake level
WCR	western corn rootworm

Appendix A – Additional studies

List of additional studies performed by or on behalf of the applicant with regard to the evaluation of the safety of maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 for humans, animal or the environment.

Study identification	Title
PHI-2019-021/760	(2020) DvSnf7 double-stranded RNA Concentration of a Maize Line Containing the Combined Trait Product DP-ØØ4114-3 × MON-89Ø34-3 × MON-87411-9 × DAS-4Ø278-9: U.S. and Canada Test Sites (EU Study Format)
PHI-2019-147	(2020) Evaluation of Germination and Viability of a Maize Line Containing the Combined Trait Product DP-ØØ4114-3 × MON-89Ø34-3 × MON-87411-9 × DAS-4Ø278-9

Appendix B – Protein expression data

Mean, standard deviation and range of protein levels (ng/mg dry weight) from maize DP4114 × MON 89034 × MON 87411 × DAS-40278-9 (not treated) and DP4114, MON 89034, MON 87411, DAS-40278-9 (not treated), from field trials performed across six locations in the USA and Canada in 2019 (n = 24)[(a)].

Protein	Event(s)	Leaf (V9)	Leaf (R1)	Root (V9)	Root (R1)	Pollen (R1)	Forage (R4)	Grain (R6)
Cry1F	**DP4114 × MON 89034 × MON 87411 × DAS-40278-9**	11[(b)] ± 3.8[(c)] (7.0–22)[(d)]	13 ± 3.7 (5.2–22)	7.8 ± 2.5 (2.2–11)	5.3 ± 0.96 (3.3–6.7)	33 ± 2.4 (28–38)	5.8 ± 1.1 (4.1–7.9)	1.6 ± 0.41 (1.0–2.3)
	DP4114	11 ± 3.5 (7.0–22)	9.9 ± 2.2 (5.7–15)	5.3 ± 1.3 (1.8–8.7)	4.6 ± 1.7 (1.0–7.9)	34 ± 6.4 (17–54)	5.9 ± 1.4 (3.8–9.5)	1.8 ± 0.60 (0.76–3.6)
Cry1A.105	**DP4114 × MON 89034 × MON 87411 × DAS-40278-9**	82 ± 8.2 (64–95)	58 ± 16 (14–82)	19 ± 5.9 (6.7–29)	13 ± 3.2 (8.0–17)	3.8 ± 0.62 (2.8–5.5)	9.0 ± 2.0 (5.7–14)	1.8 ± 0.79 (0.84–4.1)
	MON 89034	78 ± 14 (53–110)	56 ± 10 (31–78)	13 ± 5.0 (4.6–23)	11 ± 3.9 (4.2–19)	2.9 ± 0.59 (1.3–4.2)	10 ± 1.5 (8.1–14)	2.5 ± 0.73 (1.3–4.1)
Cry2Ab2	**DP4114 × MON 89034 × MON 87411 × DAS-40278-9**	130 ± 37 (73–200)	200 ± 34 (140–270)	41 ± 16 (7.4–64)	33 ± 7.7 (19–45)	0.54 ± 0.097 (0.33–0.74)	52 ± 14 (34–80)	3.4 ± 2.1 (0.75–8.9)
	MON 89034	110 ± 25 (80–160)	140 ± 30 (88–210)	32 ± 14 (8.0–48)	27 ± 13 (9.0–53)	0.66 ± 0.12 (0.43–0.82)	55 ± 15 (26–93)	3.6 ± 1.5 (1.9–6.7)
Cry34Ab1	**DP4114 × MON 89034 × MON 87411 × DAS-40278-9**	39 ± 9.2 (27–59)	66 ± 13 (33–92)	22 ± 8.1 (8.1–36)	22 ± 5.4 (11–33)	16 ± 2.8 (11–22)	86 ± 35 (41–230)	19 ± 6.4 (10–37)
	DP4114	40 ± 13 (27–71)	61 ± 8.8 (47–85)	26 ± 11 (11–52)	25 ± 8.9 (4.1–44)	15 ± 2.1 (10–21)	82 ± 27 (38–140)	21 ± 7.7 (7.6–40)
Cry35Ab1	**DP4114 × MON 89034 × MON 87411 × DAS-40278-9**	33 ± 7.4 (24–48)	55 ± 12 (20–75)	11 ± 4.1 (4.0–18)	8.5 ± 3.3 (3.5–15)	< LLOQ[(e),(f)]	23 ± 5.1 (14–37)	0.62 ± 0.3 (0.3–1.8)
	DP4114	44 ± 10 (29–69)	50 ± 8.4 (34–71)	11 ± 5.2 (2.6–18)	8.2 ± 3.7 (1.3–17)	< LLOQ[(e),(f)]	21 ± 7.2 (11–39)	0.73 ± 0.39 (0.18–2.2)
Cry3Bb1	**DP4114 × MON 89034 × MON 87411 × DAS-40278-9**	200 ± 31 (130–250)	180 ± 61 (25–250)	160 ± 38 (74–210)	110 ± 20 (71–140)	36 ± 4.1 (28–43)	38 ± 16 (12–83)	3.1 ± 1.1 (1.8–5.6)
	MON 87411	220 ± 89 (1.9–400)	150 ± 88 (0.87–270)	140 ± 57 (25–230)	96 ± 40 (25–190)	38 ± 3.8 (32–47)	39 ± 17 (8.2–71)	4.1 ± 2.1 (1.8–8.9)

Protein	Event(s)	Leaf (V9)	Leaf (R1)	Root (V9)	Root (R1)	Pollen (R1)	Forage (R4)	Grain (R6)
PAT	**DP4114 × MON 89034 × MON 87411 × DAS-40278-9**	19 ± 4.8 (9.1–27)	20 ± 4.7 (7.7–26)	0.69 ± 0.31 (< LLOQ-1.1)[f]	0.49 ± 0.21 (0.15–0.83)	< LLOQ[e],[f]	1.8 ± 0.45 (0.93–2.8)	< LLOQ[e],[f]
	DP4114	16 ± 3.3 (10–24)	17 ± 3.5 (11–23)	0.55 ± 0.29 (< LLOQ-1.0)[f]	0.53 ± 0.26 (< LLOQ-1.0)[f]	< LLOQ[e],[f]	1.9 ± 0.49 (0.98–2.8)	< LLOQ[e],[f]
CP4 EPSPS	**DP4114 × MON 89034 × MON 87411 × DAS-40278-9**	26 ± 5.9 (15–35)	34 ± 5.8 (22–44)	27 ± 9.0 (8.2–43)	24 ± 5.0 (14–31)	26 ± 3.4 (18–33)	17 ± 2.8 (11–21)	2.4 ± 0.75 (1.3–4.2)
	MON 87411	27 ± 9.7 (2.2–44)	23 ± 7.2 (9.8–36)	24 ± 11 (0.71–43)	20 ± 5.4 (11–31)	23 ± 3.3 (15–32)	13 ± 2.4 (9.3–18)	2.1 ± 1.2 (0.56–7.1)
AAD-1	**DP4114 × MON 89034 × MON 87411 × DAS-40278-9**	14 ± 3.2 (8.4–18)	15 ± 4.0 (9.7–25)	8.8 ± 2.8 (3.4–14)	6.5 ± 2.0 (3.1–12)	120 ± 12 (100–140)	11 ± 2.5 (6.3–18)	4.6 ± 1.6 (2.1–8.9)
	DAS-40278-9	7.8 ± 2.3 (3.3–12)	6.9 ± 1.6 (4.3–12)	7.1 ± 3.3 (1.6–13)	6.0 ± 3.3 (1.6–13)	130 ± 19 (50–140)	12 ± 2.0 (7.1–15)	4.7 ± 1.4 (2.4–7.0)

(a): Number of samples is n = 24 except for: n = 21 for Cry2Ab2 in leaf (V9) of DP4114 × MON 89034 × MON 87411 × DAS-40278-9, n = 23 for Cry3Bb1 in leaf (V9) and leaf (R1) of DP4114 × MON 89034 × MON 87411 × DAS-40278-9 and for AAD-1 in grain (R6) of DAS-40278-9.
(b): Mean.
(c): Standard deviation.
(d): Range.
(e): < LLOQ: All values were below LLOQ.
(f): LLOQ for Cry35Ab1 and PAT in pollen is 0.22 ng/mg dw; 0.054 ng/mg dw in root and grain. A value equal to half of the limit of quantification was used to estimate the mean values when samples were reported as below LOQ.

Appendix C – Animal dietary exposure

Table C.1: Dietary exposure to Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1A.105, Cry2Ab2, Cry3Bb1, CP4 EPSPS and AAD-1 proteins (mg/kg bw per day) in selected animals, based on the consumption of maize grains and forage

Cry1F	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
Beef cattle[(a)]	500	12	80	80	0.033	0.10	0.14
Dairy cattle	650	25	30	60	0.02	0.12	0.14
Ram/ewe	75	2.5	30	NA	0.017	NA	NA
Lamb	40	1.7	30	30	0.022	0.069	0.091
Breeding pigs	260	6	70	20	0.027	0.025	0.052
Finishing pigs	100	3	70	NA	0.036	NA	NA
Broiler	1.7	0.12	70	NA	0.084	NA	NA
Layer	1.9	0.13	70	10	0.081	0.037	0.12
Turkey	7	0.50	50	NA	0.061	NA	NA

Cry34Ab1	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
Beef cattle[(a)]	500	12	80	80	0.36	1.50	1.9
Dairy cattle	650	25	30	60	0.22	1.80	2.0
Ram/ewe	75	2.5	30	NA	0.19	NA	NA
Lamb	40	1.7	30	30	0.24	0.99	1.2
Breeding pigs	260	6	70	20	0.31	0.36	0.67
Finishing pigs	100	3	70	NA	0.40	NA	NA
Broiler	1.7	0.12	70	NA	0.94	NA	NA
Layer	1.9	0.13	70	10	0.91	0.53	1.4
Turkey	7	0.50	50	NA	0.68	NA	NA

Cry35Ab1	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
Beef cattle[(a)]	500	12	80	80	0.015	0.40	0.42
Dairy cattle	650	25	30	60	0.009	0.48	0.49
Ram/ewe	75	2.5	30	NA	0.0078	NA	NA
Lamb	40	1.7	30	30	0.010	0.27	0.28
Breeding pigs	260	6	70	20	0.013	0.10	0.11
Finishing pigs	100	3	70	NA	0.016	NA	NA
Broiler	1.7	0.12	70	NA	0.039	NA	NA
Layer	1.9	0.13	70	10	0.037	0.14	0.18
Turkey	7	0.50	50	NA	0.028	NA	NA

PAT	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
Beef cattle[(a)]	500	12	80	80	0.00058	0.035	0.035
Dairy cattle	650	25	30	60	0.00035	0.042	0.042
Ram/ewe	75	2.5	30	NA	0.00030	NA	NA
Lamb	40	1.7	30	30	0.00038	0.023	0.023
Breeding pigs	260	6	70	20	0.00048	0.0083	0.0088
Finishing pigs	100	3	70	NA	0.00063	NA	NA
Broiler	1.7	0.12	70	NA	0.0015	NA	NA

PAT	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
Layer	1.9	0.13	70	10	0.0014	0.012	0.014
Turkey	7	0.50	50	NA	0.0011	NA	NA

Cry1A.105	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
Beef cattle[(a)]	500	12	80	80	0.035	0.18	0.22
Dairy cattle	650	25	30	60	0.021	0.22	0.24
Ram/ewe	75	2.5	30	NA	0.018	NA	NA
Lamb	40	1.7	30	30	0.023	0.12	0.14
Breeding pigs	260	6	70	20	0.029	0.043	0.072
Finishing pigs	100	3	70	NA	0.038	NA	NA
Broiler	1.7	0.12	70	NA	0.089	NA	NA
Layer	1.9	0.13	70	10	0.086	0.064	0.15
Turkey	7	0.50	50	NA	0.064	NA	NA

Cry2Ab2	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
Beef cattle[(a)]	500	12	80	80	0.06	1.02	1.1
Dairy cattle	650	25	30	60	0.035	1.2	1.3
Ram/ewe	75	2.5	30	NA	0.030	NA	NA
Lamb	40	1.7	30	30	0.038	0.68	0.71
Breeding pigs	260	6	70	20	0.05	0.24	0.29
Finishing pigs	100	3	70	NA	0.06	NA	NA
Broiler	1.7	0.12	70	NA	0.15	NA	NA
Layer	1.9	0.13	70	10	0.14	0.36	0.51
Turkey	7	0.50	50	NA	0.11	NA	NA

Cry3Bb1	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
Beef cattle[(a)]	500	12	80	80	0.086	1.06	1.1
Dairy cattle	650	25	30	60	0.052	1.27	1.3
Ram/ewe	75	2.5	30	NA	0.045	NA	NA
Lamb	40	1.7	30	30	0.057	0.70	0.76
Breeding pigs	260	6	70	20	0.073	0.25	0.33
Finishing pigs	100	3	70	NA	0.095	NA	NA
Broiler	1.7	0.12	70	NA	0.22	NA	NA
Layer	1.9	0.13	70	10	0.22	0.38	0.59
Turkey	7	0.50	50	NA	0.16	NA	NA

CP4 EPSPS	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
Beef cattle[(a)]	500	12	80	80	0.056	0.31	0.36
Dairy cattle	650	25	30	60	0.033	0.37	0.40
Ram/ewe	75	2.5	30	NA	0.029	NA	NA
Lamb	40	1.7	30	30	0.037	0.20	0.24
Breeding pigs	260	6	70	20	0.047	0.07	0.12
Finishing pigs	100	3	70	NA	0.061	NA	NA

CP4 EPSPS	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
Broiler	1.7	0.12	70	NA	0.14	NA	NA
Layer	1.9	0.13	70	10	0.14	0.11	0.25
Turkey	7	0.50	50	NA	0.10	NA	NA

AAD-1	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
Beef cattle[(a)]	500	12	80	80	0.096	0.21	0.31
Dairy cattle	650	25	30	60	0.058	0.25	0.31
Ram/ewe	75	2.5	30	NA	0.05	NA	NA
Lamb	40	1.7	30	30	0.064	0.14	0.20
Breeding pigs	260	6	70	20	0.081	0.05	0.13
Finishing pigs	100	3	70	NA	0.11	NA	NA
Broiler	1.7	0.12	70	NA	0.25	NA	NA
Layer	1.9	0.13	70	10	0.24	0.075	0.31
Turkey	7	0.50	50	NA	0.18	NA	NA

(a): The inclusion rate for beef cattle would be 160% of the diet, resulting the DDE to each protein an overestimation.
NA indicates that a forage inclusion rate was not provided in the reference and therefore no exposure calculations were done.