Assessment of genetically modified maize MON 89034 3 1507 3 NK603 3 DAS-40278-9 and subcombinations independently of their origin for food and feed uses, import and processing, under Regulation (EC) No 1829-2003 (application EFSA-GMO-NL-2013-112)

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Abstract

Maize MON 89034 × 1507 × NK603 × DAS-40278-9 (four-event stack maize) was produced by conventional crossing to combine four single events: MON 89034, 1507, NK603 and DAS-40278-9. The GMO Panel previously assessed the four single events and four of their subcombinations and did not identify safety concerns. No new data on the maize single events or their four subcombinations that could lead to modification of the original conclusions on their safety have been identified. The molecular characterisation, comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment indicates 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/feed safety and nutritional concerns. The GMO Panel concludes that the four-event stack maize, as described in this application, is as safe as and nutritionally equivalent to its non-GM comparator and the non-GM reference varieties tested. In the case of accidental release of viable grains of the four-event stack maize into the environment, this would not raise environmental safety concerns. The GMO Panel assessed the likelihood of interactions among the single events in the six maize subcombinations for which no experimental data were provided, and concludes that these are expected to be as safe as and nutritionally equivalent to 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 the four-event stack maize. No post-market monitoring for food/feed is 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 reference varieties with respect to potential effects on human and animal health and the environment.

Keywords: GMO, maize (*Zea mays*), MON 89034, 1507, NK603, DAS-40278-9, insect resistance, herbicide tolerance, Regulation (EC) No 1829/2003

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Question number: EFSA-Q-2013-00079

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Summary

Following the submission of application EFSA-GMO-NL-2013-112 under Regulation (EC) No 1829/2003 from Dow AgroSciences, the Panel on Genetically Modified Organisms of the European Food Safety Authority (hereafter referred to as the 'GMO Panel') was asked to deliver a scientific opinion on genetically modified (GM) maize MON 89034 × 1507 × NK603 × DAS-40278-9 and its subcombinations independently of their origin (referred to hereafter as the 'subcombinations'). The scope of application EFSA-GMO-NL-2013-112 is for the placing on the market of maize MON 89034 × 1507 × NK603 × DAS-40278-9 and all its subcombinations independently of their origin for food and feed uses, import and processing.

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 MON 89034 × 1507 × NK603 × DAS-40278-9 is evaluated in the context of the assessment of the four-event stack maize in Section 3.3 of the present GMO Panel scientific opinion. The safety of subcombinations that have either been, or could be produced by conventional crossing through targeted breeding approaches, and which can be bred, produced and marketed independently of the four-event stack, are risk assessed in Section 3.4 of the present scientific opinion.

In delivering its scientific opinion, the GMO Panel considered the data available on the single events, the four-event stack maize, three two-event stack and one three-event stack subcombinations, the scientific comments submitted by the Member States and the relevant scientific literature. The four-event stack maize was produced by conventional crossing to combine four single maize events: MON 89034 expressing the Cry1A.105 and Cry2Ab2 proteins which confer resistance to specific lepidopteran pests; 1507 expressing the Cry1F protein which confers protection against specific lepidopteran pests and phosphinothricin acetyltransferase protein for tolerance to glufosinate-containing herbicides; NK603 expressing the CP4 EPSPS protein for tolerance to glyphosate-containing herbicides; and DAS-40278-9 expressing the aryloxyalkanoate dioxygenase-1 protein for tolerance to 2,4-dichlorophenoxyacetic acid- and aryloxyphenoxypropionate-containing herbicides.

The GMO Panel evaluated the four-event stack maize and its subcombinations with reference to the scope and appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed, the environmental risk assessment of GM plants and the post-market environmental monitoring (PMEM) of GM plants.

For application EFSA-GMO-NL-2013-112, previous assessments of the four single maize events MON 89034, 1507, NK603 and DAS-40278-9, the three-event stack maize MON 89034 × 1507 × NK603 and the two-event stack maize MON 89034 × NK603, MON 89034 × 1507 and 1507 × NK603 provided a basis to evaluate the four-event stack maize and all its subcombinations. No concerns on their safety 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 analyses of agronomic/phenotypic and compositional characteristics was undertaken, 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. An evaluation of environmental impacts and the PMEM plan was also undertaken.

The molecular characterisation data establish that the events stacked in maize MON 89034 × 1507 × NK603 × DAS-40278-9 have retained their integrity. Protein expression analyses show that the levels of the newly expressed proteins in the four-event stack maize are similar to those of either the single event DAS-40278-9 or the already assessed three-event stack maize MON 89034 × 1507 × NK603. No indications of interactions that may affect the integrity of the events and the levels of the newly expressed proteins in this four-event stack maize are identified.

The comparative analysis of forage and grain composition and agronomic/phenotypic characteristics identified no differences between maize MON 89034 × 1507 × NK603 × DAS-40278-9 and the non-GM comparator that required further assessment for food and feed safety or environmental impact, except for levels of cystine, isoleucine, phenylalanine, raffinose, manganese and β-carotene in grain and levels of total fat in forage, and plant height, insect damage and pollen shape which were further assessed and not found to have a safety impact.

The molecular characterisation, comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment indicates 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 the maize MON 89034 × 1507 × NK603 × DAS-40278-9, as described in this application, is as safe as and nutritionally equivalent to its non-GM comparator and the non-GM reference varieties tested.

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 MON 89034 × 1507 × NK603 × 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 two-event stack maize MON 89034 × 1507, MON 89034 × NK603 and 1507 × NK603 as well as the three-event stack maize MON 89034 × 1507 × NK603, 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 six subcombinations included in the scope of EFSA-GMO-NL-2013-112 for which no experimental data have been provided, the GMO Panel assessed the possibility of interactions between the events, and concluded that different combinations of the events MON 89034, 1507, NK603 and DAS-40278-9 would not raise safety concerns. These subcombinations are therefore expected to be as safe as and nutritionally equivalent to the single events, the previously assessed subcombinations and the four-stack maize MON 89034 × 1507 × NK603 × DAS-40278-9.

Given the absence of safety concerns for foods and feeds from maize MON 89034 × 1507 × NK603 × DAS-40278-9 and all 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.

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1. Introduction

The scope of application EFSA-GMO-NL-2013-112 is for food and feed uses, import and processing in the European Union (EU) of the genetically modified (GM) herbicide-tolerant insect-resistant maize MON 89034 × 1507 × NK603 × DAS-40278-9 and all its subcombinations independently of their origin.

1.1. Background

On 11 January 2013, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands application EFSA-GMO-NL-2013-112 for authorisation of maize MON 89034 × 1507 × NK603 × DAS-40278-9 (hereafter referred to as 'the four-event stack maize') (Unique Identifier MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-ØØ6Ø3-6 × DAS-4Ø278-9), submitted by Dow AgroSciences (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003.[1]

Following receipt of application EFSA-GMO-NL-2013-112, EFSA informed EU Member States and the European Commission, and made the application available to them. Simultaneously, EFSA published the summary of the application.[2]

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003 and, when needed, asked the applicant to supplement the initial application. On 29 August 2014, EFSA declared the application valid.

From the validity date, EFSA and its scientific Panel on Genetically Modified Organisms (hereafter referred to as 'the GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application EFSA-GMO-NL-2013-112. 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 European Commission (for further details, see the Section 'Documentation', below).

In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC.[3] The EU Member States had 3 months to make their opinion known on application EFSA-GMO-NL-2013-112 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 the four-event stack maize and all its subcombinations independently of their origin, for food and feed uses, import and processing.

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 and thus will be part of the EFSA overall opinion in accordance with Articles 6(5) and 18(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.[4]

2. Data and methodologies

2.1. Data

The GMO Panel based its scientific assessment of the four-event stack maize on the valid application EFSA-GMO-NL-2013-112, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by EU Member States and relevant peer-reviewed scientific publications.

[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, p. 1–23.
[2] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2013-00079.
[3] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.
[4] These particulars can be found in the technical report by EFSA on the application EFSA-GMO-NL-2013-112, made available in the EFSA Register of Questions.

2.2. Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 1829/2003, its applicable guidelines (i.e. EFSA GMO Panel, 2010a,b, 2011a,b) and explanatory notes (i.e. EFSA, 2017a,b) for the risk assessment of GM plants.

In the context of the contracts OC/EFSA/GMO/2013/01 and OC/EFSA/GMO/2014/01, contractors performed preparatory work and delivered reports on the methods applied by the applicant in performing bioinformatic and statistical analyses, respectively.

3. Assessment

3.1. Introduction

Application EFSA-GMO-NL-2013-112 covers the four-event stack maize MON 89034 × 1507 × NK603 × DAS-40278-9 and all its subcombinations independently of their origin (Table 1).

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 harvested grains of the four–event stack maize is evaluated in the context of the assessment of the four-event stack maize in Section 3.3 of the present GMO Panel scientific opinion.

'Subcombination' also covers combinations of up to three of the maize events MON 89034, 1507, NK603 or DAS-40278-9 that have either been or could be produced by conventional 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 3.4 of this scientific opinion.

The four-event stack maize was produced by conventional crossing to combine four single maize events: MON 89034 (expressing the Cry1A.105 and Cry2Ab2 proteins); 1507 (expressing the Cry1F and phosphinothricin acetyl transferase (PAT) proteins); NK603 (expressing the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) and its variant CP4 EPSPS L214P protein); and DAS-40278-9 (expressing the aryloxyalkanoate dioxygenase 1 (AAD-1) protein).

Herbicidal tolerance traits are achieved by the expression of AAD-1 protein from *Sphingobium herbicidovorans*, CP4 EPSPS proteins from *Agrobacterium* sp. strain CP4, and PAT protein from *Streptomyces viridochromogenes*. Insecticidal resistance traits are achieved by the expression of Cry1A.105, Cry2Ab2 and Cry1F proteins from *Bacillus thuringiensis*, which confer protection against specific lepidopteran pests.

Table 1: Stacked maize events covered by the scope of application EFSA-GMO-NL-2013-112

Degree of stacking	Events
Four-event stack maize	MON 89034 × 1507 × NK603 × DAS-40278-9
Three-event stack maize	MON 89034 × 1507 × NK603
	MON 89034 × NK603 × DAS-40278-9
	MON 89034 × 1507 × DAS-40278-9
	1507 × NK603 × DAS-40278-9
Two-event stack maize	MON 89034 × 1507
	MON 89034 × NK603
	MON 89034 × DAS-40278-9
	1507 × NK603
	1507 × DAS-40278-9
	NK603 × DAS-40278-9

All four single maize events and four of the subcombinations (three two-event stacks and one three-event stack) have been previously assessed (see Table 2). No concerns for human and animal health, or environmental safety were identified.

Table 2: Single maize events and subcombinations of maize MON 89034 × 1507 × NK603 × DAS-40278-9 previously assessed by the GMO Panel

Events	Application or mandate	Reference
MON 89034	EFSA-GMO-NL-2007-37	EFSA (2008)
1507	C/NL/00/10	EFSA (2004a)
	C/ES/01/01	EFSA (2005a)
	EFSA-GMO-NL-2004–02	EFSA (2005b)
	EFSA-GMO-RX-1507	EFSA (2009a)
	EFSA-M-2012-0231[(a)]	EFSA GMO Panel (2012)
	EFSA-GMO-RX-001	EFSA GMO Panel (2017a)
NK603	C/ES/00/01	EFSA (2007)
	Article 4 of the Novel Food Regulation (EC) No 258/97	EFSA (2004b)
	EFSA-GMO-NL-2005-22	EFSA (2009b)
	EFSA-GMO-RX-NK603	EFSA (2009b)
DAS–40278–9	EFSA-GMO-NL-2010-89	EFSA GMO Panel (2016)
MON 89034 × 1507	EFSA-GMO-CZ-2008-62	EFSA GMO Panel (2010c)
	EFSA-M-2011-0066[(b)]	EFSA GMO Panel (2011c)
	EFSA-GMO-BE-2013-118	EFSA GMO Panel (2017b)
MON 89034 × NK603	EFSA-GMO-NL-2007-38	EFSA GMO Panel (2009)
	EFSA-M-2011-0066[(b)]	EFSA GMO Panel (2011c)
	EFSA-GMO-BE-2013-117	EFSA GMO Panel (2017c)
1507 × NK603	EFSA-GMO-UK-2004-05	EFSA (2006)
	EFSA-M-2011-0066[(b)]	EFSA GMO Panel (2011c)
	EFSA-GMO-NL-2011-92	EFSA GMO Panel (2017d)
	EFSA-GMO-RX-008	EFSA GMO Panel (2018a)
MON 89034 × 1507 × NK603	EFSA-GMO-NL-2009-65	EFSA GMO Panel (2010d)

(a): Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2012-00712
(b): Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2011-00169

3.2. Updated information on the single events[5]

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

The applicant clarified that the 1507 maize sequence reported for the four-event stack maize contained one silent nucleotide change in the insert sequence compared to the corrected original 1507 maize sequence (EFSA GMO Panel, 2018a,b, 2019). Analysis of the new sequencing data and bioinformatic analyses performed on the new sequence does not identify any need for further safety assessment.

Updated bioinformatic analyses on the junction regions for maize events MON 89034, 1507, NK603 and DAS-40278-9, using the methodology specified in the 2011 GMO Panel Guidance Document (EFSA GMO Panel, 2011a) confirm that no known endogenous genes were disrupted by any of the inserts.

Updated bioinformatic analyses of the amino acid sequence of the newly expressed Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS and AAD-1 proteins reveal no significant similarities to toxins and allergens. Updated bioinformatic analyses of the newly created open reading frames (ORFs) within the insert or spanning the junctions between the insert and genomic DNA indicate that the expression of an ORF showing significant similarities to toxins or allergens is highly unlikely.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination, the applicant performed a sequence identity analysis for maize events MON 89034, 1507, NK603 and DAS-40278-9 with microbial DNA. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.3.4.2.

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

[5] Dossier: Part II – Section 1.2.2.2; Additional information: 13/1/2017, 19/9/2017 and 30/7/2018.

3.3. Risk assessment of the four-event stack maize MON 89034 × 1507 × NK603 × DAS-40278-9

3.3.1. Molecular characterisation[6]

Possible interactions that would affect the integrity of the events, newly expressed proteins levels or the biological function conferred by the individual inserts are considered below.

3.3.1.1. Genetic elements and their biological function

Maize events MON 89034, 1507, NK603 and DAS-40278-9 were combined by conventional crossing to produce the four-event stack maize. The structure of the inserts in the four-event stack maize are 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 MON 89034 × 1507 × NK603 × DAS-40278-9 are summarised in Table 4.

Based on the known biological function of the newly expressed proteins (Table 4), the only foreseen interactions at the biological level are between the three Cry proteins in susceptible insects.

Table 3: Genetic elements in the expression cassettes of the events stacked in maize MON 89034 × 1507 × NK603 × DAS-40278-9

Event	Promoter	5′ UTR	Transit peptide	Coding region*	Terminator
MON 89034	35S (CaMV)	CAB (*Triticum* sp.)	–	cry1A.105 (*Bacillus thuringiensis*)	Hsp17 (*Triticum* sp.)
	35S (FMV)	–	CTP (*Z. mays*)	cry2Ab2 (*B. thuringiensis*)	nos (*Agrobacterium tumefaciens*)
1507[(a)]	*ubi*ZM1 (*Zea mays*)	–	–	cry1F (*B. thuringiensis*)	ORF25PolyA (*A. tumefaciens*)
	35S (CaMV)	–	–	pat (Streptomyces *viridochromogenes*)	35S (CaMV)
NK603[(b)]	ract1 (*O. sativa*)	ract1 (*O. sativa*)	CTP2 (*A. thaliana*)	CP4 epsps (*Agrobacterium* sp.)	nos (*A. tumefaciens*)
	35S (CaMV)	I-Hsp70 (*Z. mays*)	CTP2 (*A. thaliana*)	CP4 *epsps* L214P (*Agrobacterium* sp.)	nos (*A. tumefaciens*)
DAS–40278–9	ZmUbi1 (*Z. mays*)	–	–	aad-1 (*Sphingobium herbicidovorans*)	ZmPer5 3′ UTR (*Z. mays*)

CaMV: cauliflower mosaic virus; FMV: figwort mosaic virus.
*: all gene sequences are codon-optimised for expression in plants.
–: when no element was specifically introduced to optimise expression.
(a): Maize 1507 also contains partial fragments of the *cry1F* and *pat* genes at a single locus in the nuclear genome.
(b): Maize NK603 also includes at the 3′ end an additional 217 bp DNA fragment of the rice actin promoter, lacking sequences needed for promoter activity.

[6] Dossier: Part II – Section A.2; Additional information: 13/1/2017, 19/9/2017, 24/11/2017, 18/1/2018, 12/3/2018 and 30/7/2018.

Table 4: Characteristics and intended effects of the events stacked in maize MON 89034 × 1507 × NK603 × DAS-40278-9

Event	Protein	Donor organism and biological function	Intended effects in the GM plant
MON 89034	Cry1A.105	Based on genes from *Bacillus 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
	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
1507	Cry1F	Based on a gene 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 1507 expresses a truncated version of the Cry1F protein. Cry1F is a protein toxic to certain lepidopteran larvae
	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 1507 expresses the PAT protein which confers tolerance to glufosinate ammonium-based herbicides (Droge-Laser et al., 1994).
NK603	CP4 EPSPS	Based on a gene from *Agrobacterium* strain CP4. 5-Enopyruvyl-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 NK603 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
	CP4 EPSPS L214P	Based on a gene from *Agrobacterium* strain CP4. 5-Enopyruvyl-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 NK603 expresses a modified version of 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

3.3.1.2. Integrity of the events in the four-event stack

The genetic stability of the inserted DNA over multiple generations in the single maize events MON 89034, 1507, NK603 and DAS-402789 was previously demonstrated (see Table 2). Integrity of these events in the four-event stack maize was demonstrated by Southern analyses.

3.3.1.3. Information on the expression of the inserts

Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4-EPSPS and AAD-1 protein levels were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from a field trial across 10 locations in the

US in 2010. Four-event stack maize samples analysed included leaf (V2–V4, V9, R1), pollen (R1), root (R1), forage (R4), whole plant (R6) and grain (R6) treated and not treated with the intended herbicides. The applicant indicated that a small percentage of the non-GM controls showed detectable levels of the proteins, possibly resulting from cross-contamination or sampling error. Additional information requested by the GMO Panel did not allow limiting this observation to particular locations. Considering that the proportion of contaminated controls was very low, and given the high number of samples analysed, the impact on the mean expression values presented in Appendix 1 is considered negligible.

In order to assess changes in protein expression levels which may result from potential interactions between the events, protein levels were determined for the four-event stack maize and one corresponding single event or the previously assessed three-event stack maize MON 89034 × 1507 × NK603 (see Table 2) in different parts of the plant grown without intended herbicide regimes.

The levels of the proteins in the four-event stack maize were comparable in all tissues to those of either the single event DAS-40278-9 or the previously assessed three-event stack MON 89034 × 1507 × NK603 (EFSA GMO Panel, 2010d; Appendix 1). Therefore, there is no indication of interactions that may affect the integrity of the events and the levels of the newly expressed proteins in this stack.

3.3.1.4. Conclusion on molecular characterisation

The molecular data establish that the events stacked in the four-event stack maize have retained their integrity. Protein expression analyses show that the levels of the newly expressed proteins in the four-event stack maize are similar to those of either the single event DAS-40278-9 or the already assessed three-event stack maize MON 89034 × 1507 × NK603. Therefore, there is no indication of an interaction that may affect the integrity of the events and the levels of the newly expressed proteins in this stack.

Based on the known biological function of the newly expressed proteins (Table 4), the only foreseen interactions at the biological level are between the Cry proteins in susceptible insects, which is dealt with in Section 3.3.4.4.

3.3.2. Comparative analysis[7]

3.3.2.1. Choice of comparator and production of material for the comparative analysis

Application EFSA-GMO-NL-2013-112 presents data on agronomic and phenotypic characteristics as well as on forage and grain composition of the four-event stack maize derived from field trials performed at 10 sites in the US during the 2010 growing season (Table 5).

Table 5: Overview of comparative analysis studies with maize MON 89034 × 1507 × NK603 × DAS-40278-9

Study focus	Study details	Comparator	Non-GM commercial reference varieties
Agronomic, phenotypic and compositional analysis	Field study, 2010, US, 10 sites	7SH382 × XHH13	Six

The four-event stack maize was obtained by conventional crossing: events MON 89034, 1507 and NK603 were introgressed in the inbred maize line 7SH382, while event DAS-40278-9 was introgressed in maize XHH13. As documented by the pedigree, the four single events, after backcrossing, were combined in a hybrid maize with a genetic background (F_1) of 7SH382 × XHH13. The same two inbred maize lines (7SH382 and XHH13) were crossed to produce the non-GM hybrid maize used as comparator. On the basis of the provided pedigree, documenting the production of the four-event stack GM maize, the GMO Panel considers that hybrid maize 7SH382 × XHH13 is a suitable comparator.

The field trial sites were located in major maize growing areas of the US,[8] representing regions of diverse agronomic practices and environmental conditions. At each site, the following materials were grown in a randomised complete block design with four replicates: the four-event stack maize treated, a non-GM comparator and three non-GM reference varieties[9] all treated (sprayed) with plant protection

[7] Dossier: Part II – Section A.3; Additional information: 6/3/2017.
[8] The field sites were located in Richland (IA), Lime Springs (IA), Atlantic (IA), Carlyle (IL), Wyoming (IL), Sheridan (IN), Fisk (MO), Brunswick (NE), York (NE) and Germansville (PA).
[9] The reference varieties used were: Dekalb 6170, Golden Harvest 8920, Middlekoop 5513, Middlekoop 6614, Middlekoop C110 and Pioneer 33W82.

products (PPP) according to local requirements, and the four-event stack maize treated with the intended herbicides (2,4-D-, glyphosate-, glufosinate-ammonium- and quizalofop-containing herbicides) in addition to the other PPP.

Statistical analysis of field trials data

The statistical analysis of the agronomic, phenotypic and compositional data from the 2010 field trial study followed the recommendations of the GMO Panel (EFSA GMO Panel, 2010b, 2011a). This included, for each of the two treatments of the four-event stack maize, the application of a difference test (between the GM maize and the non-GM comparator) and an equivalence test (between the GM maize and the set of non-GM maize reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[10]

3.3.2.2. Agronomic and phenotypic analysis

A total of 26 agronomic and phenotypic endpoints, including observations on the biotic and abiotic interactions, were analysed.[11]

Data for 12 endpoints[12] were considered not suitable for a parametric analysis; for these, a Wilcoxon signed-rank (WSR) test was used to check for differences between the GM maize and the non-GM comparator.

The remaining 14 endpoints were analysed as described in Section 3.3.2.1, with the following outcomes:

- For the four-event stack maize not treated with the intended herbicides, statistically significant differences were identified for time to silking, time to pollen shed, ear height, stay green, plant height and insect damage. All endpoints fell under equivalence category I or II, except for plant height (equivalence category III) and insect damage for which the test of equivalence was not applied because the variability among the non-GM reference varieties was estimated to be zero.[13]
- For the four-event stack maize treated with the intended herbicides, statistically significant differences were identified for time to silking, time to pollen shed, ear height, disease incidence, insect damage, stay green, final population, plant height and pollen shape at 30 min. All endpoints fell under category I or II except for insect damage and pollen shape at 30 min, for which the test of equivalence was not applied because the variability among the non-GM reference varieties was estimated to be zero.[14]

Of the endpoints analysed with the WSR test, statistically significant differences were identified for days to maturity (for both GM maize treatments) and pollen shape at 120 min (for GM maize treated with the intended herbicides). However, in both cases, the average values for the GM maize were within the range of the non-GM reference varieties.

3.3.2.3. Compositional analysis

Forage and grain harvested from the field trial study in the US in 2010 (Table 5) were analysed for 82 constituents (9 in forage and 73 in grain), including the key constituents recommended by the OECD (OECD, 2002). For 17 grain constituents,[15] more than 50% of the observations were below the

[10] The results of the equivalence test are categorised into four possible 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).

[11] Early population, final population, time to silking, time to pollen shed, plant height, ear height, yield, pollen colour (measured at 0, 30, 60 and 120 minutes), pollen shape (measured at 0, 30, 60 and 120 minutes), stay green, herbicide injury (after each of the four herbicide applications), disease incidence, insect damage, days to maturity, root lodging, stalk lodging and seedling vigour.

[12] Days to maturity, root lodging, stalk lodging, seedling vigour, pollen colour and shape (at 0 and 120 min) and herbicide injury (at 4 time points).

[13] Estimated mean values for plant height (cm) were 274.9 (non-GM comparator) and 284.6 (untreated GM maize); the equivalence interval was (256.9–282.5). Estimated mean values for insect damage (% plant tissue/leaf area damaged) were 14.25 (non-GM comparator), 11.44 (untreated GM maize) and 13.95 (non-GM commercial reference varieties).

[14] Estimated mean values for insect damage (% plant tissue/leaf area damaged) were as follows 14.25 (non-GM comparator), 11.78 (treated GM maize) and 13.95 (non-GM commercial reference varieties). Estimated mean values for pollen shape at 30 min were 58.88% (non-GM comparator), 63.5% (treated GM maize) and 59.76% (non-GM commercial reference varieties).

[15] Sodium, furfural, ascorbic acid and the fatty acids caprylic (8:0), capric (10:0), lauric (12:0), myristic (14:0), myristoleic (14:1), pentadecanoic (15:0), pentadecenoic (15:1), palmitoleic (16:1), heptadecanoic (17:0), heptadecenoic (17:1), γ-linolenic (18:3), eicosadienoic (20:2), eicosatrienoic (20:3) and arachidonic (20:4).

limit of quantification. The statistical analysis was applied to the remaining 65 constituents (9 in forage[16] and 56 in grain[17]). 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, 23 grain endpoints showed statistically significant differences with the non-GM comparator. All these endpoints fell under equivalence category I or II, except for levels of isoleucine, raffinose, manganese and β-carotene which fell under equivalence category III or IV. Levels of cystine, phenylalanine, iron, pyridoxine and folic acid in grain fell under equivalence category III or IV, although no statistically significant differences were identified with the non-GM comparator.
- For the four-event stack maize treated with the intended herbicides, 27 grain endpoints and three forage endpoints showed statistically significant differences with the non-GM comparator. All these endpoints fell under equivalence category I or II, except for levels of cystine, isoleucine, phenylalanine, raffinose, manganese and β-carotene in grain, which fell under equivalence category III or IV; and for total fat in forage, for which the test of equivalence was not applied (because the variability associated to the non-GM reference varieties was estimated to be zero). Levels of iron, pyridoxine and folic acid in grain fell under equivalence category III or IV, although no statistically significant differences were identified with the non-GM comparator.

Table 6: Outcome of the comparative compositional analysis in grains and forage of maize MON 89034 × 1507 × NK603 × 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	36	19[(d)]	32	23[(d)]
	Category III/IV	5[(e)]	4[(f)]	3[(e)]	6[(f)]
	Not categorised	1[(g)]	–	–	1[(g)]
Total endpoints		65		65	

(a): Comparison between maize MON 89034 × 1507 × NK603 × DAS-40278-9 and its non-GM 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 (see Section 3.3.2.1).
(d): Endpoints with significant differences between maize MON 89034 × 1507 × NK603 × DAS-40278-9 and its non-GM comparator falling in equivalence category I-II (treated and not treated).
For grain, both treated and not treated: glutamic acid, glycine, leucine, threonine, tryptophan, 18:1 oleic, 18:2 linoleic, 20:1 eicosenoic, protein, copper and zinc. For not treated only: aspartic acid, ferulic acid, phytic acid, 20:0 arachidic, ash, moisture, magnesium and riboflavin (B2). For treated only: arginine, histidine, inositol, 18:3 linolenic, ADF, carbohydrates, total fat, calcium, phosphorus and niacin (B3).
For forage: calcium and phosphorus (treated only).
(e): The following endpoints in grain fell under equivalence category III or IV, although no statistically significant differences were identified with respect to the conventional counterpart: iron, pyridoxine (B6) and folic acid (B9) (both treated and not treated), cystine and phenylalanine (non-treated only).
(f): Endpoints with significant differences between maize MON 89034 × 1507 × NK603 × DAS-40278-9 and its non-GM comparator and falling in equivalence category III-IV. Quantitative results for these endpoints are reported in Table 7.
(g): The endpoint total fat in forage was not categorised for equivalence. Quantitative results are reported in Table 7.

[16] Protein, fat, ash, moisture, carbohydrates, acid detergent fibre (ADF), neutral detergent fibre (NDF), calcium and phosphorus.
[17] Proximates (protein, fat ash, moisture and carbohydrates by calculation), fibre fractions (acid detergent fibre (ADF), neutral detergent fibre (NDF) and total detergent fibre (TDF)), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, selenium and zinc), 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 (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), arachidic acid (20:0), eicosenoic acid (20:1) and behenic acid (22:0)), vitamins (β-carotene, thiamine, riboflavin, niacin, pyridoxine, folic acid and α-tocopherol) and other compounds (inositol, *p*-coumaric acid, ferulic acid, phytic acid, raffinose and trypsin inhibitor).

The GMO Panel assessed all significant differences between the four-event stack maize and the non-GM comparator, taking into account potential impact on plant metabolism and the natural variability observed for the set of non-GM commercial reference varieties. Quantitative results for the endpoints showing significant differences between the four-event stack maize and the non-GM comparator and not falling under category I/II are given in Table 7.

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

Endpoint	Maize MON 89034 × 1507 × NK603 × DAS-40278-9		Non-GM comparator	Non-GM reference varieties	
	Not treated	Treated[(a)]		Mean	Equivalence limits
Forage					
Total fat (% dw)	2.024	1.966*	2.172	2.001	–
Grain					
Cystine (% AA)	1.902	1.866*	1.927[(b)]	2.085	(1.928, 2.254)
Isoleucine (% AA)	3.780*	3.784*	3.746	3.685	(3.600, 3.769)
Phenylalanine (% AA)	5.305	5.352*	5.276[(b)]	5.087	(4.925, 5.257)
Raffinose (% dw)	0.115*	0.108*	0.102[(b)]	0.200	(0.121, 0.330)
Manganese (mg/100 g dw)	0.489*	0.496*	0.518	0.596	(0.503, 0.690)
β-Carotene (mg/kg dw)	3.612*	3.476*	3.360[(b)]	1.260	(0.735, 1.787)

dw: dry weight; % AA: percentage total amino acid.
For the GM maize, significantly different entries are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by the greyscale background: white (the test of equivalence was not performed), light grey (equivalence category III) and dark grey (equivalence category IV).
(a): Treated with the intended herbicides as described in Section 3.3.2.1.
(b): Mean values for cystine, phenylalanine, raffinose and β-carotene in the non-GM comparator were out of the equivalence limits derived from the selected non-GM reference varieties.

3.3.2.4. Conclusion on comparative analysis

The GMO Panel concludes that the differences in the agronomic and phenotypic characteristics identified between the four-event stack maize and its non-GM comparator do not require further assessment, except for plant height, insect damage and pollen shape at 30 min. These differences are further assessed for their environmental impact in Section 3.3.4.1.

The GMO Panel concludes that none of the differences identified in forage and grain composition between the four-event stack maize, its non-GM comparator and the non-GM commercial reference varieties needs further assessment regarding food and feed safety, except for the changes in levels of cystine, isoleucine, phenylalanine, raffinose, manganese and β-carotene in grain and in levels of total fat in forage, which are further assessed in Section 3.3.3.

3.3.3. Food and feed safety assessment[18]

3.3.3.1. Effects of processing

The four-event stack maize will undergo existing production processes used for conventional maize. 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 non-GM maize varieties.

[18] Dossier: Part II – Sections A.4, A.5 and A.6; Additional information: 19/9/2017 and 30/7/2018.

3.3.3.2. Influence of temperature and pH on newly expressed proteins

Effects of temperature and pH on Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS and AAD-1 proteins have been previously evaluated by the GMO Panel (Table 2). In the context of this application, no new studies addressing these aspects were provided by the applicant.

3.3.3.3. Toxicology

Testing of newly expressed proteins

Six proteins (Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS[19] and AAD-1) are newly expressed in the four-event stack maize (see Section 3.1). The GMO Panel has previously assessed these proteins in the context of the single maize events (see Table 2), and no safety concerns were identified for humans and animals. The GMO Panel is not aware of any new information that would change these conclusions.

The potential for a functional interaction between the proteins newly expressed in the four-event stack maize has been assessed with regard to human and animal health. The CP4 EPSPS, PAT and AAD-1 proteins are enzymes that catalyse distinct biochemical reactions and act on unrelated substrates with high substrate specificity. The Cry1A.105, Cry2Ab2 and Cry1F proteins are delta endotoxins with high specific insecticidal properties 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). On the basis of the known biological functions of the individual newly expressed proteins (see Table 4), there is currently no expectation for possible interactions relevant for the food and feed safety of the four-event stack maize.

In vitro protein degradation studies on Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS and AAD–1 proteins have been previously evaluated by the GMO Panel (see Table 2). In the context of this application, no new studies addressing *in vitro* protein degradation of these newly expressed proteins were provided by the applicant.

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

Testing of new constituents other than newly expressed proteins

No new constituents other than newly expressed proteins have been identified in the four-event stack maize. Therefore, no further food and feed safety assessment of components other than the newly expressed proteins is required.

Information on altered levels of food and feed constituents

Cystine, isoleucine, phenylalanine, raffinose, manganese and β-carotene in grain and total fat in forage were significantly different in the four-event stack maize when compared to its comparator and showed lack of equivalence with the set of non-GM reference varieties (see Section 3.3.2.3). Taking into account the known biological role of these compounds, these differences are considered of no toxicological concern by the GMO Panel. Further information on the safety of these maize constituents is provided in Section 3.3.3.5.

Testing of the whole genetically modified food and feed

Based on the outcome of the studies considered in the molecular characterisation and comparative analysis, no substantial modifications of toxicological relevance in the composition of the four-event stack maize, and no indication of possible unintended effects relevant to food and feed safety have been identified (see Sections 3.3.1. and 3.3.2.3). Therefore, animal studies on food and feed from the four-event stack maize are not necessary (EFSA GMO Panel, 2011a).

3.3.3.4. Allergenicity

For the allergenicity assessment, a weight-of-evidence approach was followed, taking into account all of the information obtained on the newly expressed proteins, as no single piece of information or experimental method yields sufficient evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a). In addition, when known functional aspects of the newly expressed protein or

[19] Including its variant CP4 EPSPS L214P.

structural similarity to known adjuvants may indicate an adjuvant activity, the possible role of these proteins as adjuvants is considered. When newly expressed proteins with a potential adjuvant activity are expressed together, possible interactions increasing adjuvanticity and impacting the allergenicity of the GM crop are assessed.

Assessment of allergenicity of the newly expressed protein

For allergenicity, the GMO Panel has previously evaluated the safety of Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS and AAD-1 proteins individually, and no concerns on allergenicity were identified in the context of the applications assessed (see Table 2). No new information on allergenicity of these proteins that might change the previous conclusions of the GMO Panel has become available. Based on the current knowledge, and as none of the newly expressed proteins showed allergenicity, no reasons for concerns regarding the simultaneous presence of these newly expressed proteins in the four-event stack maize affecting their allergenicity are expected.

For adjuvanticity, Cry1Ac protein has been suggested to possess adjuvant activity based on animal studies when applied at relatively high doses (e.g. Vazquez et al., 1999). The Panel has previously evaluated the safety of Cry1A.105, Cry2Ab2 and Cry1F proteins, and no concerns on adjuvanticity were identified in the context of the applications assessed (see Table 2). The levels of the individual Cry proteins in the four-event stack maize are similar to those of the three-event stack MON 89034 × 1507 × NK603 (see Appendix 1). From the limited evidence available, the GMO Panel does not find indications that the presence of the Cry proteins at the levels expressed in the four-event stack maize might act as adjuvants with the potential to enhance a specific immunoglobulin E (IgE) response and to favour the development of an allergic reaction.

Assessment of allergenicity of the whole GM plant

The GMO Panel regularly reviews the available publications on food allergy to maize. However, to date, maize has not been considered to be 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 3.3.1, 3.3.2 and 3.3.3), the GMO Panel identifies no indications of a potentially increased allergenicity of foods and feeds from the four-event stack maize with respect to those from its non-GM comparator.

3.3.3.5. Nutritional assessment of GM food and feed

The intended trait of the four-event stack maize is insect resistance and herbicide tolerance, with no intention to alter the nutritional parameters. However, levels of cystine, isoleucine, phenylalanine, raffinose, manganese and β-carotene in grain were significantly different from its non-GM comparator and showed a lack of equivalence with the set of non-GM reference varieties (see Section 3.3.2.3). In addition, for total fat in forage from the GM-maize the levels were significantly different from its non-GM comparator and the test of equivalence could not be applied because of the lack of variation among the non-GM reference varieties. The biological role of these compounds, the contribution of maize to their total intake and the magnitude and direction of the observed changes are considered in the nutritional assessment.

Human nutrition

Among the three amino acids showing significant differences as compared to the non-GM comparator, two of them are essential amino acids: isoleucine and phenylalanine. For these two amino acids an increase of approximately 1% (percentage of total amino acids) is observed in the GM maize as compared to the non-GM comparator. For cystine, the oxidised dimer form of the non-essential amino acid cysteine, a decrease of approximately 3% was observed as compared to the non-GM comparator. Overall, these changes are not considered relevant from a nutritional point of view.

Raffinose is a non-digestible trisaccharide composed of galactose, fructose and glucose, considered as an antinutrient since cannot be broken down by gastrointestinal enzymes; as a consequence,

[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.

raffinose passes into the large intestine where is fermented by the intestinal microflora causing flatulence (OECD, 2002). An increase of approximately 13% in the levels of raffinose in the GM-maize is observed as compared to the non-GM comparator. Maize contains very small amounts of raffinose as compared to other foods of plant origin such as soybean, garlic and onions among many others. Based on this, and considering that a daily dose of 15 g of raffinose is considered safe (Voragen, 1998), the increase observed in raffinose is not considered relevant from a nutritional point of view.

β-Carotene is a precursor of vitamin A and is found in plant derived foods; together with preformed vitamin A (mainly retinol and retinyl esters) present in foods of animal origin, it contributes to the total dietary intake of vitamin A. Milk, meat, vegetables and derived products are the main sources of vitamin A in the diet (EFSA NDA Panel, 2012). An increase up to approximately 8% is observed as compared to the non-GM comparator (from 560 μg RE[21]/kg dw to 602 μg RE/kg dw). Considering the magnitude of the change, the consumption of maize and the Tolerable Upper Intake Levels (UL) for vitamin A (800–3,000 μg RE/day), the increase observed in β-carotene is not considered relevant from a nutritional point of view.

Manganese is an essential dietary mineral which is a component of a number of metalloenzymes involved in amino acid, lipid and carbohydrate metabolism. Nuts, dried fruits and chocolate are the main dietary sources of manganese; cereal-based products also contribute to its intake mainly due to the levels found in wheat grain (25 mg/kg, Bawiec et al., 2014). A decrease of up to approximately 6% is observed in the GM maize as compared to the non-GM comparator. Considering the relatively low levels in maize, the magnitude of the change and the relatively low contribution of maize to the dietary intake of manganese, the observed decrease is not considered relevant from a nutritional point of view.

Animal nutrition

Cystine is a conditionally essential amino acid. The magnitude of the decrease observed in the GM maize as compared to the non-GM comparator does not constitute an issue for animal nutrition. Isoleucine and phenylalanine are essential amino acids and the increase observed in the GM-maize as compared to the non-GM comparator is not an issue for animal nutrition.

Raffinose is fermented in the forestomachs (ruminants) and in the hind gut (rabbit, horse) and provides energy. High level of raffinose in monogastric diet is detrimental to digestion and production performance, but maize contains very low level of raffinose as compared to other common feed used in animal diets, such as soybean. Raffinose, in specific conditions, has been demonstrated to be a prebiotic compound that can improve intestinal health in poultry and fishes. Taking into account the magnitude and the biological role of these changes, these do not to pose an issue for animal nutrition.

Manganese is an important trace element in animal nutrition; the decreased level observed does not constitute an issue, since complete diets are balanced with mineral premixes. Moreover, maize grains are also considered a poor source of manganese (McDonald et al., 2011).

β-Carotene is converted in the liver into retinol and an increase does not represent an issue for animal health.

Forage is not provided to animals as source of fat; a decrease in total fat content of forage does not affect animal nutrition since complete diets are balanced to energy content.

Conclusion on human and animal nutrition

Based on the current knowledge on the biological role of the compounds assessed, the magnitude and direction of the changes identified, and the relevance of maize as contributor to the intake of these compounds, the GMO Panel concludes that the nutritional impact of foods and feeds from the four-event stack maize is expected to be the same as those from the comparator and non-GM reference varieties.

3.3.3.6. Conclusion on food and feed safety assessment

The individual proteins Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS and AAD-1 newly expressed in the four-event stack maize do not raise safety concerns for human and animal health. Interactions between these newly expressed proteins raising food and feed safety concerns (toxicological, allergenicity and adjuvanticity) are not expected. The nutritional impact of the four-event stack maize foods and feeds is expected to be the same as those from the comparator and non-GM reference

[21] RE: retinol equivalent. One μg RE equals 6 μg of β-carotene.

varieties. The GMO Panel concludes that the four-event stack maize, as described in this application, is as safe as and nutritionally equivalent to the comparator and the non-GM reference varieties tested.

3.3.4. Environmental risk assessment[22]

Considering the scope of application EFSA-GMO-NL-2013-112, which excludes cultivation, the environmental risk assessment (ERA) of the four-event stack maize mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable four-event stack maize grains during transportation and/or processing (EFSA GMO Panel, 2010a).

3.3.4.1. 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. Occasional feral GM maize plants may occur outside cultivation areas in the EU (e.g. Pascher, 2016), but survival is limited mainly by a combination of low competitiveness, the absence of a dormancy phase and susceptibility to plant pathogens, herbivores and cold climate conditions (OECD, 2003). 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 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 and the observed differences in plant height, insect damage and pollen shape at 30 min (see Section 3.3.2.2) will provide a selective advantage to maize plants, except when they are exposed to glufosinate-ammonium-, glyphosate-, 2,4-D and/or AOPP-containing herbicides, or infested by insect pests that are susceptible to the Cry1A.105, Cry2Ab2 and/or Cry1F proteins. However, this fitness advantage will not allow the four-event stack maize to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits and the observed differences in plant height, insect damage and pollen shape at 30 min do not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers it very unlikely that the four-event stack maize will differ from conventional maize hybrid varieties in its 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.

3.3.4.2. Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through 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 2). 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 in order to assess the possibility for HGT by homologous recombination.

The updated bioinformatic analysis for maize event 1507 revealed sufficient length and sequence identity for homologous recombination for two copies of the ORF25 terminator with the same *A. tumefaciens* genomic sequence. Because of its length (~700 bp) and the opposite orientation of the two ORF25 copies in maize event 1507, a potential for a facilitated HGT by double homologous recombination (DHR) is unlikely. The occurrence of a DHR would result in the insertion of the *pat* gene cassette which is expected to be less efficiently translated in potential bacterial recipients because of the plant-codon optimisation of the *pat* gene and because the *pat* gene is under the control of plant virus element.

[22] Dossier: Part II – Section E; Additional information: 30/7/2018.

The updated bioinformatic analysis for maize events MON 89034 and NK603 do not reveal any new DNA sequence that could provide sufficient length and identity which could facilitate HGT by DHR, confirming the previous conclusions (EFSA GMO Panel, 2017b,c,d).

Bioinformatic analysis for maize event DAS-40278-9 does not reveal sufficient length and sequence identity with known sequences from bacteria which would facilitate homologous recombination.

Synergistic effects of the recombinant genes, for instance due to combinations of recombinogenic sequences, which would cause an increase in the likelihood for horizontal gene transfer or a selective advantage are 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; 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, Trtikova et al., 2017).

The potential of spilled maize grains to establish, grow and produce pollen is extremely low and transient (see Section 3.3.4.1). 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). 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 would not differ from that of conventional maize varieties for the reasons given in Section 3.3.4.1, even if exposed to the intended herbicides.

3.3.4.3. Interactions of the GM plant with target organisms

Taking the scope of application EFSA-GMO-NL-2013-112 into account (no cultivation), potential interactions of occasional feral four-event stack maize plants arising from grain import spills with target organisms are not considered a relevant issue.

3.3.4.4. 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 proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the four-event stack maize with non-target organisms are not considered to raise any environmental safety concern. Interactions that may occur between the Cry proteins would not alter this conclusion.

3.3.4.5. 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 proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions with the abiotic environment and biogeochemical cycles are not considered to raise any environmental safety concern.

3.3.4.6. Conclusions on environmental risk assessment

The GMO Panel concludes that it is unlikely that the four-event stack maize would differ from conventional maize varieties in its ability to persist under EU environmental conditions. Considering the scope of application EFSA-GMO-NL-2013-112, 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 the four-event stack maize to bacteria does not indicate a safety concern. Therefore, considering the combined traits and their interactions, the outcome of the comparative analysis, and the routes and levels of exposure, the GMO Panel concludes that the four-event stack maize would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

3.3.5. Conclusion on the four-event stack maize MON 89034 × 1507 × NK603 × DAS–40278–9

No new data on the single maize events MON 89034, 1507, NK603 and DAS-40278-9 leading to a modification of the original conclusions on their safety are identified.

The molecular characterisation, comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment indicates 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 the four-event stack, as described in this application, is as safe as and nutritionally equivalent to its non-GM comparator and the non-GM reference varieties tested.

Considering the combined traits and their interactions, the outcome of the comparative analysis, and the routes and levels of exposure, the GMO Panel concludes that the four-event stack maize would not raise safety concerns in the case of accidental release of viable GM maize grains into the environment.

No scientific information that could change the conclusions on this four-event stack was retrieved in a literature search covering the period since the time of validity of the application.[23]

In conclusion, the GMO Panel considers that the four-event stack maize is as safe as its non-GM comparator and the non-GM maize reference varieties tested with respect to potential effects on human and animal health and the environment.

3.4. Risk assessment of the subcombinations[24]

Subcombinations previously assessed in the frame of other applications are discussed in Section 3.4.1.

The strategy followed for the assessment of those subcombinations for which no specific data have been submitted and which have not been previously assessed by the GMO Panel (see Table 8), has been described by the GMO Panel.[25] In this case, the risk assessment takes as its starting point the assessment of the single maize events, and uses the data generated for the four-event stack, as well as all the additional data available on subcombinations previously assessed by the GMO Panel (see Table 2).

3.4.1. Subcombinations previously assessed

The GMO Panel has previously assessed the two-event maize stacks MON 89034 × 1507, MON 89034 × NK603 and 1507 × NK603, and the three-event maize stack MON 89034 × 1507 × NK603 (see Table 2) and did not identify any safety concerns. No scientific information relevant to the risk assessment of these maize stacks became available since the validation of application EFSA-GMO-NL-2013-112. Consequently, the GMO Panel considers that its previous conclusions on these subcombinations remain valid.

3.4.2. Subcombinations not previously assessed

Six out of the ten 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 (see Table 8).

[23] Additional information: 22/12/2017.
[24] Additional information: 7/5/2018 and 27/7/2018.
[25] 115th GMO Panel meeting (Annex 1 of the minutes: https://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf)

Table 8: Subcombinations of maize MON 89034 × 1507 × NK603 × DAS-40278-9 not previously assessed and covered by the scope of application EFSA-GMO-NL-2013-112

Degree of stacking	Events
Three-event stack maize	MON 89034 × NK603 × DAS-40278-9
	MON 89034 × 1507 × DAS-40278-9[(a)]
	1507 × NK603 × DAS-40278-9
Two-event stack maize	MON 89034 × DAS-40278-9[(a)]
	1507 × DAS-40278-9[(a)]
	NK603 × DAS-40278-9

(a): Subcombinations assessed in parallel in the context of application EFSA-GMO-NL-2013-113 (EFSA GMO Panel, 2019).

3.4.2.1. Stability of the events

The genetic stability of the inserted DNA over multiple generations in the four single maize events has been demonstrated previously (see Table 2). Integrity of the events has been demonstrated in the four-event stack maize (Section 3.3.1.2) and the previously assessed maize subcombinations (see Table 2). The GMO Panel finds no reasons to expect loss of integrity of the events in the maize subcombinations not previously assessed (see Table 8).

3.4.2.2. Expression of the events

The GMO Panel assessed whether any combination of the four events by conventional 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 subcombinations compared with those in the single maize events. This assumption is confirmed by comparing the levels of the newly expressed AAD-1 protein of the single event maize DAS-40278-9 and CP4 EPSPS, Cry1A.105, Cry2Ab2, Cry1F and PAT proteins of the three-event maize stack maize MON 89034 × 1507 × NK603 with those of the four-event stack maize. The levels were similar in the four-event stack maize and in maize DAS-40278-9 and MON 89034 × 1507 × NK603. Therefore, there is no indication of an interaction manifesting at protein expression level. This confirms that interactions affecting expression levels of the newly expressed proteins are not expected in the six maize subcombinations not previously assessed and included in the scope of application EFSA-GMO-NL-2013-113.

3.4.2.3. Potential functional interactions between the events

The GMO Panel assessed the potential for interactions between maize events in the subcombinations not previously assessed (Table 8), taking into consideration the intended traits and unintended effects.

Based on the known biological functions of the individual newly expressed proteins (Table 4), there is currently no expectation for possible interactions relevant for the food and feed or environmental safety between these proteins in the six subcombinations not previously assessed. 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 effects would raise safety concerns when combined in any of these maize subcombinations. Therefore, the GMO Panel is of the opinion that no additional data are needed to complete the assessment of subcombinations from the four-event stack maize.

3.4.3. Conclusion

Since no new safety concerns are identified for the previously assessed two-event stack maize MON 89034 × 1507, MON 89034 × NK603 and 1507 × NK603 as well as for the three-event stack maize MON 89034 × 1507 × NK603, the GMO Panel considers that its previous conclusions on these maize subcombinations remain valid. For the remaining six subcombinations included in the scope of application EFSA-GMO-NL-2013-112 for which no experimental data have been provided, the GMO Panel has assessed the possibility of interactions between the events and concludes 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 two-event

maize stacks MON 89034 × 1507, MON 89034 × NK603 and 1507 × NK603 and the three-event stack maize MON 89034 × 1507 × NK603 as well as the four-event stack maize.

3.5. Post-market monitoring[26]

3.5.1. Post-market monitoring of GM food and feed

The GMO Panel concludes that four-event stack maize, as described in this application, is nutritionally equivalent to and as safe as the non-GM comparator and the non-GM reference varieties tested, and no post-market monitoring (EFSA GMO Panel, 2011a) of food and feed is considered necessary.

The two-event stack maize MON 89034 × 1507, MON 89034 × NK603 and 1507 × NK603 and the three-event stack maize MON 89034 × 1507 × NK603 have been previously assessed and no safety concerns were identified. The six subcombinations not previously assessed and included in the scope of application EFSA-GMO-NL-2013-112 are expected to be as safe as and nutritionally equivalent to the single maize events, the previously assessed maize subcombinations and the four-event stack maize MON 89034 × NK603 and 1507 × NK603. Therefore, the GMO Panel considers that post-market monitoring of the four-event stack maize and its subcombinations, as described in this application, is not necessary.

3.5.2. 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 does not identify potential adverse environmental effects from the four-event stack maize, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for the four-event stack maize and its subcombinations 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 EuropaBio 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 and a final report at the end 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 the four-event stack maize. 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.

3.5.3. Conclusion on post-market monitoring

No post market monitoring for 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 the four-event stack maize and its subcombinations.

4. Overall conclusions and recommendations

No new data on the four single maize events MON 89034, 1507, NK603 and DAS-40278-9 that would lead to a modification of the original conclusions on their safety are identified.

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

[26] Dossier: Part II – Sections D and E4.

stack maize does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that the four-event stack maize, as described in this application, is as safe as and nutritionally equivalent to its non-GM comparator and the non-GM reference varieties tested, and no post-market monitoring of food and feed is considered necessary.

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 on the previously assessed two-event stack maize MON 89034 × 1507, MON 89034 × NK603 and 1507 × NK603 and the three-event stack maize MON 89034 × 1507 × NK603 that would lead to a modification of the original conclusions on their safety are identified, the GMO Panel considers that its previous conclusions on these maize stacks remain valid.

For the additional six maize subcombinations included in the scope of application EFSA-GMO-NL-2013-112 for which no experimental data have been provided, the GMO Panel assessed possible interactions between the events, and concludes that combinations of maize events MON 89034, 1507, NK603 and DAS-40278-9 would not raise safety concerns in these maize subcombinations. These subcombinations are therefore expected to be as safe as and nutritionally equivalent to the single maize events, all the previously assessed subcombinations and the four-event stack maize.

Given the absence of safety concerns for foods and feeds from the four-event stack maize and all its subcombinations, the GMO Panel considers that post-market monitoring of these products is not necessary.

The PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of the four-event stack maize and its subcombinations.

Documentation provided to EFSA

1) Application EFSA-GMO-NL-2013-112 received from the Competent Authority of Netherlands in support to Dow AgroSciences request for placing maize MON 89034 × 1507 × NK603 × DAS-40278-9 on the EU market according to Regulation (EC) No 1829/2003, 11 January 2013, 6 February 2013.
2) Receipt of application EFSA-GMO-NL-2013-112 acknowledged by EFSA, 21 February 2013.
3) Application EFSA-GMO-NL-2013-112 validated by EFSA, 29 August 2014.
4) Request for supplementary information to the applicant, 29 August 2014.
5) Receipt of supplementary information from the applicant, 15 September 2014.
6) Request for supplementary information to the applicant, 23 November 2016.
7) Request for supplementary information to the applicant, 20 December 2016.
8) Receipt of supplementary information from the applicant, 13 January 2017.
9) Receipt of supplementary information from the applicant, 6 March 2017.
10) Request for supplementary information to the applicant, 16 March 2017.
11) Request for supplementary information to the applicant, 20 April 2017.
12) Request for supplementary information to the applicant, 18 May 2017.
13) Receipt of supplementary information from the applicant, 9 June 2017.
14) Receipt of supplementary information from the applicant, 19 September 2017.
15) Request for supplementary information to the applicant, 25 September 2017.
16) Request for supplementary information to the applicant, 7 November 2017.
17) Receipt of supplementary information from the applicant, 24 November 2017.
18) Receipt of supplementary information from the applicant, 22 December 2017.
19) Request for supplementary information to the applicant, 17 January 2018.
20) Receipt of supplementary information from the applicant, 18 January 2018.
21) Receipt of supplementary information from the applicant, 12 March 2018.
22) Request for supplementary information to the applicant, 21 March 2018.
23) Request for supplementary information to the applicant, 4 May 2018.
24) Receipt of supplementary information from the applicant, 7 May 2018.
25) Request for supplementary information to the applicant, 18 June 2018.
26) Receipt of supplementary information from the applicant, 26 July 2018.
27) Receipt of supplementary information from the applicant, 30 July 2018.
28) Request for supplementary information to the applicant, 29 August 2018.
29) Receipt of supplementary information from the applicant, 10 October 2018.
30) Receipt of supplementary information from the applicant, 1 October 2018.

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Abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid
AAD-1	aryloxyalkanoate dioxygenase 1
ADF	acid detergent fibre
AOPP	aryloxyphenoxypropionate
cry	crystal protein
DHR	double homologous recombination
Dw	dry weight
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	environmental risk assessment
GM	genetically modified
GMO Panel	EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
IgE	immunoglobulin E
NDF	neutral detergent fibre
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin acetyltransferase
PPP	plant protection products
PMEM	post-market environmental monitoring
RE	retinol equivalent
TDF	total detergent fibre
UL	tolerable upper intake levels
UTR	untranslated region
WSR	Wilcoxon signed-rank

Appendix A – Protein expression data

Mean, standard deviation and range of protein levels (μg/g dry weight) from maize MON 89034 × 1507 × NK603 × DAS-40278-9, MON 89034 × 1507 × NK603, and DAS-402789 unsprayed tissues from field trials performed across 10 locations in the US in 2010 (n = 40)

	DAS-40278-9	MON 89034 × 1507 × NK603	MON 89034 × 1507 × NK603 × DAS-40278-9
AAD-1			
Leaf (V2-V4)	11.81 ± 6.28 (2.59–24.32)		11.83 ± 6.13 (2.40–24.39)
Leaf (V9)	4.43 ± 2.53 (0.41–12.07)		4.23 ± 1.48 (0.65–7.44)
Pollen (R1)	97.28 ± 14.23 (61.52–132.26)		100.95 ± 12.97 (78.60–131.70)
Root (R1)	4.16 ± 2.33 (0.71–11.24)		3.90 ± 1.86 (0.92–10.22)
Leaf (R1)	6.05 ± 2.41 (2.10–11.32)		6.40 ± 2.95 (1.73–14.22)
Forage (R4)	4.79 ± 1.41 (ND–8.26)		4.43 ± 1.39 (1.83–7.46)
Whole plant (R6)	4.95 ± 2.28 (ND–11.61)		5.37 ± 2.57 (1.52–12.47)
Grain R6	3.04 ± 1.02 (1.27–6.71)		3.03 ± 0.74 (1.37–5.47)
CP4-EPSPS			
Leaf (V2–V4)		158.50 ± 40.11 (94.00–252.99)	162.19 ± 43.39 (103.00–279.04)
Leaf (V9)		107.37 ± 15.23 (67.00–139.75)	108.66 ± 25.44 (64.00–191.95)
Pollen (R1)		192.88 ± 42.25 (124.00–287.37)	186.91 ± 41.96 (86.35–265.53)
Root (R1)		42.21 ± 8.85 (21.73–66.59)	40.26 ± 10.29 (13.07–94.18)
Leaf (R1)		86.88 ± 16.65 (47.50–134.89)	97.54 ± 15.25 (60.00–138.90)
Forage (R4)		59.46 ± 11.38 (22.23–97.08)	57.85 ± 16.56 (23.05–106.00)
Whole plant (R6)		30.69 ± 16.24 (11.41–73.34)	32.83 ± 15.72 (7.23–67.87)
Grain (R6)		7.57 ± 2.34 (3.89–13.32)	7.94 ± 1.97 (3.44–15.28)
Cry1A.105			
Leaf (V2–V4)		148.44 ± 39.15 (76.57–223.79)	155.51 ± 57.17 (67.60–281.33)
Leaf (V9)		51.73 ± 29.14 (19.99–130.71)	53.48 ± 24.39 (16.86–147.51)
Pollen (R1)		15.50 ± 2.24 (ND–19.10)	15.15 ± 1.61 (12.25–18.15)
Root (R1)		22.27 ± 7.50 (10.30–39.84)	21.05 ± 9.54 (7.56–42.72)
Leaf (R1)		30.60 ± 16.57 (7.02–80.80)	32.95 ± 16.23 (10.04–84.70)
Forage (R4)		27.04 ± 5.84 (16.19–41.63)	26.71 ± 5.54 (16.06–41.27)

	DAS-40278-9	MON 89034 × 1507 × NK603	MON 89034 × 1507 × NK603 × DAS-40278-9
Whole plant (R6)		10.55 ± 5.57 (4.09–32.95)	9.94 ± 3.96 (4.33–26.35)
Grain (R6)		4.81 ± 1.08 (2.28–10.00)	4.91 ± 0.64 (3.09–6.96)
Cry2Ab2			
Leaf (V2–V4)		142.80 ± 59.34 (52.20–273.00)	146.21 ± 59.52 (66.90–281.70)
Leaf (V9)		60.23 ± 18.70 (25.80–106.23)	64.00 ± 20.83 (30.00–118.09)
Pollen (R1)		1.30 ± 0.61 (0.50–2.88)	1.27 ± 0.58 (0.43–2.91)
Root (R1)		31.99 ± 9.79 (14.10–49.74)	28.29 ± 7.89 (ND–50.85)
Leaf (R1)		48.85 ± 18.44 (14.10–88.74)	52.85 ± 15.31 (23.10–83.85)
Forage (R4)		51.25 ± 10.44 (15.75–72.45)	49.53 ± 11.79 (27.60–82.50)
Whole plant (R6)		34.04 ± 24.58 (8.83–99.46)	31.96 ± 17.82 (10.93–88.61)
Grain (R6)		3.35 ± 0.78 (2.17–6.00)	3.34 ± 0.89 (1.38–6.25)
Cry1F			
Leaf (V2–V4)		14.81 ± 3.59 (8.45–24.55)	16.29 ± 4.54 (9.50–27.26)
Leaf (V9)		9.06 ± 3.69 (2.48–18.81)	8.82 ± 3.94 (1.57–21.32)
Pollen (R1)		19.15 ± 3.43 (13.02–25.74)	18.86 ± 4.21 (11.31–26.60)
Root (R1)		4.71 ± 1.03 (2.42–6.96)	4.56 ± 1.34 (2.22–7.58)
Leaf (R1)		7.12 ± 3.77 (0.51–14.64)	7.61 ± 3.28 (0.48–12.90)
Forage (R4)		8.23 ± 1.89 (1.94–11.22)	7.99 ± 2.18 (1.77–13.24)
Whole plant (R6)		4.26 ± 1.15 (2.31–6.81)	4.59 ± 1.39 (2.22–8.28)
Grain (R6)		2.21 ± 0.58 (1.32–3.85)	2.50 ± 0.44 (1.32–3.79)
PAT			
Leaf (V2–V4)		8.11 ± 5.66 (ND–23.28)	8.23 ± 5.13 (2.40–20.92)
Leaf (V9)		6.29 ± 6.91 (1.86–29.22)	5.00 ± 2.99 (1.23–12.64)
Pollen (R1)		ND ± N/A (ND–ND)	ND ± N/A (ND–ND)
Root (R1)		0.16 ± 0.06 (0.06–0.35)	0.14 ± 0.06 (ND–0.24)
Leaf (R1)		3.66 ± 1.35 (1.81–7.12)	4.00 ± 1.35 (1.21–7.12)
Forage (R4)		0.34 ± 0.10 (0.13–0.60)	0.35 ± 0.13 (0.06–0.75)

	DAS-40278-9	MON 89034 × 1507 × NK603	MON 89034 × 1507 × NK603 × DAS-40278-9
Whole plant (R6)		ND ± N/A (ND–0.06)	ND ± N/A (ND–0.09)
Grain (R6)		ND ± N/A (ND–ND)	ND ± N/A (ND–ND)