Assessment of the safety of genetically modified DP4114xMON810xMIR604xNK603 Maize and sub-combinations for food and feed uses under assimilated Regulation (EC) No. 1829/2003

Reference number RP1506

Regulated Products Risk Assessment Unit

Science, Evidence and Research Division, FSA

Risk Assessment Team

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Regulated Product Dossier Assessment

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Abbreviations

Acronym	Definition
ACNFP	Advisory Committee on Novel Foods and Processes
ACRE	Advisory Committee on Releases to the Environment
ADF	Acid Detergent Fiber
BLAST	Basic Local Alignment Search Tool
bp	Base pair
BW	Body weight
CaMV	Cauliflower mosaic virus
C12:0	Dodecanoic acid / Lauric acid
C14:0	Tetradecanoic acid/ Myristic acid.
C17:0	Heptadecanoic acid/ Margaric acid
C17:1	Heptadecenoic / (cis-10)

Acronym	Definition
C20:2	Eicosadienoic acid / (all cis-11, 14)
C22:0	Docosanoic acid /Behenic acid
CHT	Conventional Herbicide Treated
CRY	Crystal proteins from *Bacillus thuringiensis*
DNA	Deoxyribonucleic acid
EC	European Commission
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate
EU	European Union
FSA	Food Standards Agency
FSS	Food Standard Scotland

Acronym	Definition
GM	Genetically modified
GMO	Genetically modified organism
HECT- E3	Homologous to E6AP C-terminus - ubiquitin ligase
HLA-DQ2/ HLA-DQ8	Celiac disease genes
IgE	Immunoglobulin E
IHT	Intended herbicide-treated
LLOQ	Lower limit of quantification
NDF	Neutral Detergent Fibre
NOS	Transcription terminator
ORFs	Open reading frames
PAT	Phosphinothricin-acetyl-transferase
PCR	Polymerase chain reaction

Acronym	Definition
PMEM	Post-market environmental monitoring
PMI	Phosphomannose-isomerase
T-DNA	Transfer-deoxyribonucleic acid

Summary

Following the submission of application RP1506 from Corteva Agrisciences LLC Represented by Corteva Agriscience UK Limited to the Food Standards Agency (FSA) under assimilated Regulation (EC) No. 1829/2003, FSA/FSS (Food Standards Scotland) have undertaken a safety assessment on genetically modified DP4114xMON810xMIR604xNK603 maize. To support the safety assessment by FSA/FSS, the Advisory Committee on Novel Foods and Processes (ACNFP) provided advice to FSA/FSS on the data submitted for the authorisation of genetically modified DP4114xMON810xMIR604xNK603 maize, as outlined in this document. The advice of the ACNFP has been taken into account in this safety assessment which represents the opinion of FSA/FSS on the safety of genetically modified DP4114xMON810xMIR604xNK603 maize.

DP4114xMON810xMIR604xNK603 maize (*Zea mays L.*) has been obtained by traditional crossing of genetically modified DP4114, MON810, MIR604 and NK603 maize. No additional genetic modification was used to produce this maize hybrid. Therefore, these maize plants produce the transgenic proteins inherited from the single GM maize events. Each single event has been previously assessed and authorised in the EU, during which time the UK was a member state (EFSA, 2018b; EFSA, 2009a; EFSA, 2009b; EFSA, 2009c). The individual events that comprise the stack have therefore not been re-assessed.

In providing its scientific advice, the ACNFP considered data on the composition and agronomic characteristics of the stack, the potential for interactions between the individual events, DNA sequencing and updated bioinformatics analyses, and additional toxicological studies provided by the applicant as part of application RP1791. As the single events have been previously safety assessed and authorised, this safety assessment focused on stability of the transformation events, expression of the transformation events, and potential interactions resulting from

the combination of the transformation events as required by Implementing Regulation (EU) No 503/2013 (EC, 2013).

The introduced genes in DP4114xMON810xMIR604xNK603 maize are *cry1F*, *cry34Ab1*, *cry35Ab1*, *pat*, *cry1Ab*, *cry3A*, *pmi*, and *CP4 epsps*. The correspondent proteins produced are Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1Ab, mCry3A, PMI and CP4 EPSPS. These proteins confer the following traits:

- Herbicide tolerance to glyphosate and glufosinate-ammonium herbicides due to the presence of the CP4 EPSPS and PAT proteins, respectively.
- Protection against lepidopteran target pests based on the presence of the Cry1F and Cry1Ab proteins, conferring independent modes of action for insect protection
- Protection against coleopteran target pests based on the presence of the Cry34Ab1, Cry35Ab1 and mCry3A proteins, conferring independent modes of action for insect protection

Maize is one of the most important crops worldwide and it is grown over a wide range of climatic conditions, well-suited for warm, temperate climates. Maize, grown on 15 million hectares in the EU (14% of the EU's arable land, and 8% of worldwide maize acreage), is the leading cereal in terms of global production volumes. Its principal use is animal feed (83%), followed by starch manufacturing (15%) and cornmeal (2%). The methods of production and manufacturing are well known and have a long history of safe use. Silage maize is cultivated for feed and is mainly used on-farm. Grain maize is used for feed (poultry, corn-cob-mix for pigs), food (maize-meal-products, snacks, cornflakes, oil) or for industrial purposes and non-food products (starch, paper, industrial alcohol). The genetic modification in DP4114xMON810xMIR604xNK603 maize does not impact the production or manufacturing processes currently used for maize.

The scope of the application is for the authorisation for import, processing, and food and feed use of DP4114xMON810xMIR604xNK603 maize. The application does not cover cultivation and therefore no DP4114xMON810xMIR604xNK603 maize will be grown in the UK.

Molecular characterisation confirmed that the genetic insertions in DP4114xMON810xMIR604xNK603 maize were equivalent to those present in the single event GM lines, and the conclusions reached for the single events remain valid for DP4114xMON810xMIR604xNK603 maize and its sub-combinations, irrespective of its origin.

Updated bioinformatics analysis on the open reading frames (ORFs) and newly expressed proteins in maize DP4114xMON810xMIR604xNK603 supported the previous conclusions on the safety of the single maize events reached by the EFSA GMO Panel. Bioinformatic analysis of the sequence regions flanking the insertion sites did not reveal unintended changes or interactions that would need further evaluation. No biologically relevant changes in protein expression values were observed between DP4114xMON810xMIR604xNK603 maize and in the single event maize lines and there are no mechanisms known that could specifically impact on expression levels of any of the sub-combinations. The field trials (including locations and management practices) for the production of test materials for the comparative analysis were considered appropriate, and no differences between DP4114xMON810xMIR604xNK603 maize and the conventional counterpart or the non-GM reference varieties that would raise safety concerns were observed.

Toxicological testing of newly expressed proteins were conducted as part of the previous EU applications, showing no adverse effects. In addition, the 90-day feeding study performed on DP4114xMON810xMIR604xNK603 maize as part of this application raised no safety concerns. No relevant similarity between the inserted protein sequences and known protein toxins or allergens was identified through updated bioinformatic studies.

The ACNFP concludes that considering the nature of the introduced traits, the lack of differences in the agronomic and compositional analyses, and the proposed levels of exposure, there is no evidence that the import, processing, and food and feed use of DP4114xMON810xMIR604xNK603 maize would raise any safety concerns. The ACNFP concludes that DP4114xMON810xMIR604xNK603 maize is as safe as its conventional counterpart.

1. Introduction

1.1 Background

On April 7th 2022, the Food Standards Agency (FSA) received application RP1506 (EFSA-GMO-NL-2018-150) for the authorisation of genetically modified DP4114xMON810xMIR604xNK603 maize (unique identifier: DP-ØØ4114-3xMON-ØØ81Ø-6xSYN-IR6Ø4-5xMON-ØØ6Ø3-6), submitted by Corteva Agrisciences LLC Represented by Corteva Agriscience UK Limited (European Development Centre 3B Park Square) (hereafter referred to as "the applicant") according to Regulation (EC) No. 1829/2003, as assimilated in UK law.

FSA and FSS checked the application for compliance with the relevant requirements of Regulation (EC) No. 1829/2003, and assimilated Regulation (EU) No. 503/2013, and on 8th April 2022, declared the application valid.

FSA and FSS would like to thank the following members of the ACNFP who participated in the assessment: Dr Camilla Alexander White, Dr Andy Greenfield, Dr Anton Alldrick, Alison Austin, Prof George Bassel, Dr Mark Berry, Prof Dimitris Charalampopoulos, Dr Cathrina Edwards, Prof Susan Fairweather-Tait, Prof Paul Fraser, Dr Hamid Ghouddusi, Prof Wendy Harwood, Prof Huw D. Jones, Dr Ray Kemp, Dr Elizabeth Lund, Emeritus Professor Harry J. McArdle, Rebecca McKenzie, Prof Clare Mills, Dr Lesley Stanley, Prof Hans Verhagen, Dr Maureen Wakefield, Prof Bruce Whitelaw, Dr Christine Bosch, Dr Antonio Peña-Fernández and Dr Kimon

Andreas Karatzas (associate members); and Prof Pete Lund and Prof Alastair Macrae (co-opted members of ACNFP-PGT Subcommittee).

1.2 Terms of Reference

According to Articles 6 and 18 of assimilated Regulation (EC) No. 1829/2003, the FSA/FSS were requested to carry out a scientific safety assessment of genetically modified DP4114xMON810xMIR604xNK603 maize for authorisation in the scope of the application, namely the import, processing, and food and feed use of DP4114xMON810xMIR604xNK603 maize.

FSA/FSS sought safety advice from the Advisory Committee on Novel Foods and Processes (ACNFP) on DP4114xMON810xMIR604xNK603 maize, which will inform the FSA/FSS safety assessment. The FSA/FSS safety assessment is to be seen as the opinion requested under Articles 6(6) and 18(6) of assimilated Regulation (EC) No. 1829/2003.

In addition to the present advice on the safety of genetically modified DP4114xMON810xMIR604xNK603 maize, the ACNFP were also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No. 1829/2003. These articles concern details that must be included in positive opinions/outcomes of assessment of GMO foods and feeds, including labelling details, any relevant conditions or restrictions, and monitoring plans.

2. Applicant details

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3. Data and methodologies

3.1 Data

The data for application RP1506 submitted according to legal requirements contained in Regulation (EC) 1829/2003 and provided by the applicant at the time of submission are specified below. To inform the FSA/FSS safety assessment of the application for renewal of the authorisation of genetically modified DP4114xMON810xMIR604xNK603 maize for food and feed uses in accordance with Articles 11 and 23 of Regulation (EC) No. 1829/2003, the ACNFP was asked to provide safety advice. It considered the requirements described in applicable guidance for the safety assessment of GM food and feed applications under assimilated Regulation (EC) No. 1829/2003, and based its scientific safety assessment on the data within application RP1506, additional information provided by the applicant, and any relevant peer-reviewed scientific publications.

3.2 Methodologies

The ACNFP conducted its assessment in accordance with the principles described in assimilated Regulation (EU) No. 503/2013, applicable guidance, explanatory notes, and statements (EFSA GMO Panel 2010; EFSA GMO Panel 2011; EFSA GMO Panel, 2015a; EFSA GMO Panel, 2017). Independent contractors performed preparatory work and delivered reports on the methods applied by the applicant in performing sequencing and bioinformatics analyses.

4. Assessment

4.1 Molecular characterisation

The molecular characterisation section of this safety assessment considers the sequence and structure of the newly expressed proteins, and the sequences at the insertion locus. Bioinformatics analyses performed on the transgenic sequences are also assessed to ensure the newly expressed proteins do not raise any safety concerns. Additionally, the expression of the new proteins is assessed. Finally, bioinformatics analyses performed on the flanking regions either side of the inserted material (and the junctions between them) are assessed to ensure no sequences occur that could raise safety concerns.

4.1.1 Transformation process and vector constructs

DP4114xMON810xMIR604xNK603 maize was obtained by traditional crossing between genetically modified DP4114 (also referred to as 4114), MON810, MIR604, and NK603 maize, respectively. No vector has been used to produce this maize hybrid. Therefore, these maize plants produce the transgenic proteins inherited from these single GM maize events. The single events bred together to create DP4114xMON810xMIR604xNK603 maize behave as independent genetic loci and the DNA fragments used to transform the previously assessed single events are summarised in Table 1. Since maize grain is the product of genetic segregation and recombination of genetic components according to Mendelian law, F2 grain

imported into the EU, produced from selfed DP4114xMON810xMIR604xNK603 hemizygous hybrid (F1) seeds, will include a mixture of DP4114xMON810xMIR604xNK603 sub-combinations (with fewer of these independently segregating events based on the Mendelian segregation ratios). No new genetic modification has been introduced to obtain DP4114xMON810xMIR604xNK603 maize. Similarly, no further genetic modification has taken place in any of the sub-combinations.

Table 1. Genetic elements in the expression cassettes of the single events stacked in DP4114xMON810xMIR604xNK603 maize and References of their previous assessments.

EVENT	PROMOTER	5' UTR	TRANSIT PEPTIDE	CODING REGION	TERMINATOR	REFERENCE
DP4114	*ubi*ZM1 region[1]			*cry1F (Bt)*	ORF25 (*Atum*)	EFSA, 2018
	*ubi*ZM1 region[1]			*cry34Ab1 (Bt)*	pinII (*St*)	
	Pperoxidase			*cry35Ab1 (Bt)*	pinII (*St*)	
	CaMV 35S			*pat (Sv)*	CaMV 35S	
MON810	*e35S* (CaMV)	*Zmhsp70*		*cry1Ab (Bt)*	(deleted during integration)	EFSA, 2009a
MIR604	MLT (*Zm*)			*mcry3A (Bt)*	*Nos (Atum)*	EFSA, 2009b
	ZmUbiInt			*Pmi (E.coli)*	*Nos (Atum)*	EFSA, 2015c
NK603	*ract1 (Os)*	*ract1 (Os)*	*ctp2 (At)*	*CP4 epsps (At)*	NOS *(Atum)*	EFSA, 2003; EFSA, 2007b
	E35S (CaMV)	*Zmhsp70*	*ctp2 (At)*	*CP4 epsps l214p (At)*	NOS *(Atum)*	EFSA, 2009c

At, *Arabidopsis thaliana*; *Atum, Agrobacterium tumefaciens; Bt, Bacillus* thuringiensis; CaMV, cauliflower mosaic virus; ctp, chloroplast transit peptide; *Os, Oryza sativa; Sv, Streptomyces viridochromogenes; St, Solanum tuberosum; Zm, Zea mays*

[1] The ubiZM1 region includes the promoter region with a 5' untranslated region (UTR) and intron from the *Zea mays ubiquitin* gene

4.1.2 Molecular studies performed on DP4114xMON810xMIR604xNK603 maize

Southern blot analysis, using multiple probes on DP4114xMON810xMIR604xNK603 and the single events confirmed the structural stability of the inserts. For all events, it was demonstrated that the insertion was intact and equivalent to that of the single event maize. Sequencing analyses confirmed Southern blot results, with the 5' and 3' genomic flanking regions for the different inserts being re-determined in. DP4114xMON810xMIR604xNK603 maize. All were confirmed to be identical to previously determined sequences for each single event.

The DP4114, MON810, MIR604 and NK603 maize inserts did not show irregular segregation patterns. As the inserts are present at different genetic loci, even in the presence of regulatory elements derived from the same source, the likelihood of molecular interactions between the different inserts is low.

Updated bioinformatic analyses have been performed for each of the events using databases that allow for intraspecies and interspecies similarity searching, these indicate it is unlikely that endogenous genes are interrupted, or their transcriptional or translational activity altered. BLASTn (Basic Local Alignment Search Tool) and BLASTx evaluations for MON810 indicate that a putative HECT E3 ubiquitin ligase sequence is found at the 3' end of the inserted T-DNA sequence. There are no significant phenotypic or metabolic differences between MON810 and non-transgenic, control maize. Moreover, the HECT-ubiquitin gene has no known physiological roles in maize.

No biologically relevant similarity to known proteins, toxins or allergens were identified for the introduced proteins and there were no putative ORFs produced

in DP4114xMON810xMIR604xNK603 maize showing similarity to known proteins, toxins or allergens associated with adverse health effects. Bioinformatics analyses of the insertion site found no sequences likely to contribute to horizontal gene transfer with bacterial species.

4.1.3 Transgenic protein expression

Expression levels of the Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1Ab, mCry3A, PMI and CP4 EPSPS proteins were measured by a quantitative enzyme linked immunosorbent assay (ELISA) on leaf, root, pollen, stalk, whole plant, forage and grain samples from tissues in different growth stages harvested from DP4114xMON810xMIR604xNK603 maize and each single event maize line during the 2015 growing season at four locations in the USA. Expression levels were measured for conventional herbicide-treated (CHT) and intended herbicide-treated (IHT) samples. The inserted genes were expressed in all tissues examined. The analyses of the protein concentrations in grain (Table 2) and forage (Table 3) from DP4114xMON810xMIR604xNK603 maize, the single events and the control maize revealed that the herbicide treatments had no effect on the expression of the newly expressed proteins, and that the stacking of the single events has not substantially changed the protein expression levels in grain of DP4114xMON810xMIR604xNK603 maize compared to the expression of the corresponding proteins in the single events.

Table 2. Comparison of the Expression of Insert-Related Proteins in Grain between DP4114xMON810xMIR604xNK603 Maize and the Single Event Lines, measured by a quantitative enzyme linked immunosorbent assay (ELISA).

Protein ng/mg Tissue Dry Weight	**Reported Statistics**	**IHT DP4114xMON810xMIR604xNK603**	**CHT DP4114xMON810xMIR604xNK603**	**CHT DP4114**	**CHT MON810**	**CHT MIR604**	**CHT NK603**
Cry1F	Mean	2.4	2.1	2	NA	NA	NA
	SD	0.48	0.49	0.45	NA	NA	NA
	Range	1.7 - 3.3	1.4 - 3.3	1.3 - 3.0	NA	NA	NA
Cry34Ab1	Mean	27	23	22	NA	NA	NA
	SD	4.7	5.8	3.2	NA	NA	NA
	Range	20 - 36	11 – 36	18 - 29	NA	NA	NA
Cry35Ab1	Mean	0.5	0.51	0.42	NA	NA	NA
	SD	0.16	0.14	0.15	NA	NA	NA

Protein ng/mg Tissue Dry Weight	Reported Statistics	IHT DP4114xMON810xMIR604xNK603	CHT DP4114xMON810xMIR604xNK603	CHT DP4114	CHT MON810	CHT MIR604	CHT NK603
	Range	0.30 - 0.75	0.28 - 0.72	0.25 - 0.75	NA	NA	NA
PAT	Mean	<0.054	<0.054	<0.054	NA	NA	NA
	SD	ND	ND	ND	NA	NA	NA
	Range	<0.054	<0.054	<0.054	NA	NA	NA
Cry1Ab	Mean	0.28	0.28	NA	0.32	NA	NA
	SD	0.059	0.077	NA	0.072	NA	NA
	Range	0.18 - 0.36	0.14 - 0.48	NA	0.20 - 0.45	NA	NA
mCry3A	Mean	0.31	0.23	NA	NA	0.16 [a]	NA
	SD	0.17	0.082	NA	NA	0.16 [a]	NA
	Range	0.11 - 0.75	0.11 - 0.42	NA	NA	<0.069 - 0.66	NA

Protein ng/mg Tissue Dry Weight	Reported Statistics	IHT DP4114xMON810xMIR604xNK603	CHT DP4114xMON810xMIR604xNK603	CHT DP4114	CHT MON810	CHT MIR604	CHT NK603
PMI	Mean	1.4	1.2 [a]	NA	NA	1.2	NA
	SD	0.31	0.43[a]	NA	NA	0.54	NA
	Range	0.87 - 1.9	<0.27 - 2.1	NA	NA	0.66 - 2.5	NA
CP4 EPSPS	Mean	13	8	NA	NA	NA	8.3
	SD	2.8	2.1	NA	NA	NA	2.7
	Range	8.7 - 18	2.9 – 12	NA	NA	NA	4.5 - 14

SD, Standard deviation; ND, Not determined; all samples were below the LLOQ, NA, Not Applicable

[a] Some, but not all, sample results were below the LLOQ. A value equal to half the LLOQ value was assigned to those samples to calculate the mean and standard deviation.

Table 3. Comparison of the Expression of Insert-Related Proteins in forage between DP4114xMON810xMIR604xNK603 Maize and the Single Event Lines, measured by a quantitative enzyme linked immunosorbent assay (ELISA).

Protein ng/mg Tissue Dry Weight	**Reported Statistics**	**IHT DP4114x MON810 xMIR604 xNK603**	**CHT DP4114x MON810 xMIR604 xNK603**	**CHT DP4114**	**CHT MON810**	**CHT MIR604**	**CHT NK603**
Cry1F	Mean	8.2	7.6	7.1	NA	NA	NA
	SD	1.4	1.2	1.1	NA	NA	NA
	Range	5.2 - 11	5.4 – 10	6.0 - 11	NA	NA	NA
Cry34Ab1	Mean	77	60	69	NA	NA	NA
	SD	23	19	21	NA	NA	NA
	Range	36 - 110	30 – 96	48 - 130	NA	NA	NA
Cry35Ab1	Mean	24	20	22	NA	NA	NA
	SD	4.8	4.4	5.7	NA	NA	NA
	Range	15 - 30	11 – 28	14 - 30	NA	NA	NA
PAT	Mean	2	2	1.9	NA	NA	NA

Protein ng/mg Tissue Dry Weight	Reported Statistics	IHT DP4114x MON810 xMIR604 xNK603	CHT DP4114x MON810 xMIR604 xNK603	CHT DP4114	CHT MON810	CHT MIR604	CHT NK603
	SD	0.76	0.83	0.61	NA	NA	NA
	Range	0.86 - 3.2	0.90 - 3.4	1.2 - 3.0	NA	NA	NA
Cry1Ab	Mean	11	9.6	NA	9.6	NA	NA
	SD	2.2	2.7	NA	2.7	NA	NA
	Range	7.0 - 14	6.2 – 14	NA	6.0 - 16	NA	NA
mCry3A	Mean	11	9.3	NA	NA	9.5	NA
	SD	2.8	1.9	NA	NA	3.4	NA
	Range	8.2 - 17	5.4 – 13	NA	NA	5.4 - 18	NA
PMI	Mean	7.7	6.3	NA	NA	6.1	NA
	SD	1.3	1.3	NA	NA	2.1	NA
	Range	5.0 - 9.6	4.0 - 8.6	NA	NA	3.0 - 9.6	NA
	Mean	110	98	NA	NA	NA	93

Protein ng/mg Tissue Dry Weight	Reported Statistics	IHT DP4114x MON810 xMIR604 xNK603	CHT DP4114x MON810 xMIR604 xNK603	CHT DP4114	CHT MON810	CHT MIR604	CHT NK603
CP4 EPSPS	SD	24	25	NA	NA	NA	23
	Range	68 - 160	60 - 140	NA	NA	NA	54 - 130

NA = Not Applicable

4.1.4 Genetic stability

Southern blot analyses carried out in this application on DP4114xMON810xMIR604xNK603 maize confirmed the integrity of the inserted sequences of DP4114, MON810, MIR604 and NK603, as discussed above in the section on molecular studies.

Molecular analyses, agronomic characterisation and protein expression analysis confirmed the phenotypic stability of DP4114xMON810xMIR604xNK603 maize, showing stable inheritance and expression of the *cry1F*, *cry34Ab1*, *cry35Ab1*, *pat*, *cry1Ab*, *mCry3A*, *pmi* and *CP4 epsps* genes following traditional crossing between DP4114, MON810, MIR604 and NK603 maize. There is no evidence that any of the sub-combinations segregating from DP4114xMON810xMIR604xNK603 maize or otherwise combined through breeding would be less genetically or phenotypically stable. This was confirmed for NK603xMON810 maize that has been previously risk assessed (EFSA, 2005b).

4.1.5 Conclusion on the molecular characterisation

On the basis of the molecular characterisation data provided, it was demonstrated that the insertion was intact and equivalent to that of the single event maize. Sequencing analyses confirmed Southern blot results, with the 5' and 3' genomic flanking regions for the different inserts being re-determined in DP4114xMON810xMIR604xNK603 maize. All were confirmed to be identical to previously determined sequences for each single event. The molecular characterisation data presented confirmed equivalence and structural stability of the inserts with respect to the individual event containing maize lines, as well as genetic stability. Updated bioinformatics analyses performed for each of the events and the flanking sequences, raised no safety concerns. The expression levels of the transgenic proteins were determined using suitable methodologies, and do not cause a safety concern.

4.2 Comparative analysis

The purpose of the comparative analysis is to compare the GM plant with its conventional counterpart, a non-GM plant with a similar genetic background. This comparison takes two forms; firstly, a comparison of the agronomic characteristics of the plant as it grows in the field which looks at the yields derived from the plants, as well as their observable characteristics such as height and colour, and a comparison of the composition of the plant after harvest which considers the nutritional value and safety of the genetically modified plant.

All individual events in the stacked DP4114xMON810xMIR604xNK603 maize were previously assessed, whereby equivalence was demonstrated for all single events.

In addition to the information already available, the applicant provided a comparative assessment of the stacked DP4114xMON810xMIR604xNK603 maize.

The GM maize was equivalent to the conventional counterpart and to reference varieties for its agronomic characteristics and composition.

4.2.1 Experimental field trial design

Test material DP4114xMON810xMIR604xNK603 maize, along with the control material (conventional counterpart, consisting of non-GM near-isoline hybrid maize seed), and 20 non-GM reference Pioneer® brand commercial maize lines (35F38, 36B08, 35P12, 35K02, 34Y02, P0965, 34B39, 34F06, 34H31, 33W82, P1184, P1319, 3335, P1395, XL5246, XL5354, XL5475, XL5435, XL6077, and XL6272) were tested during 2015 at 10 sites for agronomic, and 8 sites for compositional analysis in North-America.

Each site utilised a randomised complete block design and contained four blocks, each containing:

- Conventional herbicide-treated (CHT) DP4114xMON810xMIR604xNK603 maize (CHT refers to treatment with a mixture of nicosulfuron, dicamba, and diflufenzopyr)
- Intended herbicide-treated (IHT) DP4114xMON810xMIR604xNK603 maize (IHT refers to treatment with a mixture of glyphosate and glufosinate)
- Non-GM near-isoline CHT control maize (conventional counterpart)
- Four of the non-GM CHT commercial maize lines selected among 20 maize lines

The agronomic/phenotypic data and compositional data from these field trials were analysed as specified previously in guidance provided by EFSA (EFSA GMO Panel 2010; EFSA GMO Panel 2011; EFSA GMO Panel, 2015). This includes the application of a test of difference between DP4114xMON810xMIR604xNK603 maize

and the conventional counterpart, and a test of equivalence between DP4114xMON810xMIR604xNK603 maize and the non-GM reference varieties.

4.2.2 Suitability of field trials and test materials

The field trial sites represent a wide diversity of environmental conditions (e.g. temperature, precipitation, soil type, biotic factors) and crop management practices (e.g. planting data, fertilisation, pest management) for the geographic distribution in commercial maize-growing regions.

The production of the test and control substances was performed under comparable environmental conditions (Puerto Rico, 2012 growing season) following good agricultural practices and with quality control mechanisms in place to ensure genetic identity, purity, and health. The GM line and the conventional counterpart were tested for the presence or absence of the intended GM event(s) using polymerase chain reaction (PCR) methodology. The seed lots were stored under similar conditions and seed treatments were the same for the test and control substances. Seed for the reference substances were grown and processed under commercial quality conditions. Germination of DP4114xMON810xMIR604xNK603 maize under warm, cold, and diurnal growing conditions were comparable to that of the control maize under corresponding growing conditions.

The compositional assessment of DP4114xMON810xMIR604xNK603 maize has been based on the analysis of nutrient composition of forage (R4 growth stage) and grain (R6 growth stage).

The ACNFP is satisfied that the field trials, and the materials used in the field trials are appropriate for the comparative assessment. The geographical locations, soil conditions, meteorological conditions, and the management practices used were

all considered typical of the receiving environments where DP4114xMON810xMIR604xNK603 maize could be grown.

4.2.3 Comparative analysis (agronomic characteristics)

Difference and equivalence tests were performed for 8 out of 10 agronomic characteristics (early population, first flowering, plant height, days to maturity, final population, 100-kernel weight, grain moisture, and yield).

Results in Table 4 show that statistically significant differences were observed for CHT test material compared to the conventional counterpart for plant height, grain moisture, and yield but equivalence test results confirmed equivalence to the reference maize in all cases.

For IHT DP4114xMON810xMIR604xNK603 maize, statistically significant differences from the control maize were observed for days to maturity, plant height, grain moisture, and yield but the equivalence test results concluded equivalence to the reference maize in all cases.

For both CHT and IHT test material, a significant difference at the 0.10 significance level was observed compared to the conventional counterpart for early population and final population. Data values were however within the corresponding reference maize data range and no statistically significant genotype-environment interaction was observed. While early population was consistently lower when compared to the control maize, this observation is not a concern from a weediness or persistence perspective. The differences observed between DP4114xMON810xMIR604xNK603 maize and the control maize for early population and final population were likely affected by the germination percentage of the seed lots prior to planting.

Table 4. Comparative analysis results for 8 agronomic characteristics in DP4114xMON810xMIR604xNK603 maize. The table shows the number of endpoints in each category.

		Test of difference[a]			
		CHT [c] stack maize		**IHT [c] stack maize**	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence[b]	**Category I**	3	3[d]	2	4[e]
	Category II	0	0	0	0
	Category III	0	0	0	2
	Category IV	2	0	0	0
	No category	0	0	0	0
	Total endpoints	8		8	

[a]Comparison between both DP4114xMON810xMIR604xNK603 maize entries and the conventional counterpart

[b]The test of equivalence with the reference varieties is categorised into four different outcomes; category I (equivalence with the reference varieties is demonstrated), category II (equivalence is more likely than not), category III (equivalence is less likely than not), and category IV (non-equivalence is demonstrated). No category means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.

[c] CHT: conventional herbicide-treated DP4114xMON810xMIR604xNK603 maize with nicosulfuron, dicamba and diflufenzopyr;

IHT : Intentional herbicide-treated DP4114xMON810xMIR604xNK603 maize with glyphosate and glufonsinate

[d] Plant height, Grain moisture, Yield

[e] Days to maturity, Plant height, Grain moisture, Yield

Lodging and ear count were not included in the comparative analysis because 50% or more of the data values were at a uniform value across all entries and sites, and no biologically relevant differences in terms of magnitude or trends were observed for the associated individual site mean values and overall data ranges.

4.2.4 Compositional analysis

Maize forage and grains harvested from the field trial study in the USA in 2015 were analysed for 80 constituents (10 in forage and 70 in grain, see Appendix 1 at the end of the document on page 50), including the key constituents recommended by OECD (2002). The statistical analysis was not applied to 10 grain constituents because their concentration in more than half of the observations were below the limit of quantification.

The statistical analysis was applied to the remaining 70 constituents (see Appendix 1 at the end of the document on page 50) (10 in forage and 60 in grain). Results of the test of difference and the test of equivalence are summarised in Table 5, as follows:

In the CHT stack maize (treated with the conventional herbicides) significant differences with the comparator were identified for 29 endpoints (1 in forage and 28 in grain, see Appendix 1 at the end of the document on page 50, letters [d,f,h]); of those, phosphorus in forage fell under equivalence category IV, while the other endpoints fell under category I/II (see Appendix 1 at the end of the document on page 50).

- In the IHT stack maize (treated with the intended herbicides), significant differences with the comparator were identified for 51 endpoints (5 in forage and 46 in grain, see Appendix 1 at the end of the document on page 50, letters [e,g,i]); three forage endpoints fell under equivalence category III/IV, while the other endpoints fell under category I/II (see Appendix 1 at the end of the document on page 50).

Table 5. Comparative compositional analysis results of grains and forage from maize DP4114 X MON 810 X MIR604 X NK603. The table shows the number of endpoints in each category.

		Test of difference[a]			
		CHT [c] stack maize		**IHT [c] stack maize**	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence[b]	**Category I**	31	24[d]	16	44[e]
	Category II	9	4 [f]	2	4[g]
	Category III	0	0	0	2
	Category IV	0	1[h]	0	1[i]
	No category	1	0	1	0
	Total endpoints	70 Appendix 1		70 Appendix 1	

[a]Comparison between both DP4114xMON810xMIR604xNK603 maize entries and the conventional counterpart

[b]The test of equivalence with the reference varieties is categorised into four different outcomes; category I (equivalence with the reference varieties is demonstrated), category II (equivalence is more likely than not), category III (equivalence is less likely than not), and category IV (non-equivalence is demonstrated). No category means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.

[c] CHT: conventional herbicide-treated DP4114xMON810xMIR604xNK603 maize with nicosulfuron, dicamba and diflufenzopyr;

IHT : Intentional herbicide-treated DP4114xMON810xMIR604xNK603 maize with glyphosate and glufonsinate

[d] see Appendix 1 at the end of the document on page 50

[e] see Appendix 1 at the end of the document on page 50

[f] In grains: Glycine, Phenylalanine, Threonine, Ferulic Acid

[g] In grains: ADF, Potassium, Zinc, Ferulic Acid

[h] In forage: Phosphorus

[i] In forage: Phosphorus

Fisher's exact test performed on the remaining 10 analytes (C12:0, C14:0, C17:0, C17:1, C20:2, C22:0, Vit B2, *beta*-tocopherol, *delta*-tocopherol, furfural) with 50% or more sample values below the LLOQ in one or more entries, confirmed that no statistically significant differences in proportions were identified at the significance level of 0.10 with the exception of delta-tocopherol. Thirty-one sample values from CHT stack maize and all sample values for IHT stack maize were above the reference maize data range for delta-tocopherol. Three sample values for both stack maize entries were above the tolerance interval for delta-tocopherol. An assessment of the biological relevance of delta-tocopherol was therefore performed. The applicants concluded that no biological relevance is attributed to apparent differences in the delta-tocopherol level, as delta-tocopherol is not considered the most biologically active form of vitamin E and is of little concern as vitamin E is routinely included in animal diet formulation vitamin premixes.

4.2.5 Conclusion on the comparative analysis

The ACNFP assessed the field trials used to generate material for the comparative analyses and considered the locations selected were representative of commercial maize production, and that the meteorological conditions and management practices used during the field trails were appropriate.

The ACNFP also assessed the results from the comparative analysis, including all the significant differences between DP4114xMON810xMIR604xNK603 maize and its

conventional counterpart, and the information provided did not raise safety concerns.

4.3 Food/feed safety assessment

The food/feed safety assessment covers the likelihood that the newly expressed proteins, or the whole genetically modified food or feed, will cause safety concerns when consumed by humans and/or animals. This includes looking at the concentrations of newly expressed protein in the final products that will be consumed, as well as the anticipated rates of consumption by humans and animals to understand the anticipated magnitude of exposure to any transgenic proteins. Any toxicological or allergenic risks that can be identified and any effects on nutritional quality are also assessed.

4.3.1 Effects of processing

In the EU, most of the maize is used for animal feed, and only about 8% is processed into food products (highly refined starch, maize flour). The majority of the starch is used for sweeteners and fermentation including high fructose maize syrup and ethanol. The maize germ can be processed to obtain maize oil, which can be further processed into margarine, cooking oil, and baking and frying fats. Wet and dry milling processes are used to separate grain into components for food, feed, and fuel processing.

The effects of processing have previously been assessed for all individual GM events within the stacked DP4114xMON810xMIR604xNK603 maize. Considering the genetic modifications in DP4114xMON810xMIR604xNK603 maize, none of the processing outcomes are likely to be affected by the traits introduced in DP4114xMON810xMIR604xNK603 maize. The processed products will therefore be comparable to those produced from the corresponding single event GM maize lines and conventional maize.

4.3.2 Activity and stability of the newly expressed protein

The DP4114xMON810xMIR604xNK603 maize expresses the Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1Ab, mCry3A, PMI and CP4 EPSPS proteins. All newly expressed proteins have previously been assessed (EFSA, 2003; EFSA, 2004; EFSA, 2005a; EFSA, 2005c; EFSA, 2007a; EFSA, 2009a; EFSA, 2009b; EFSA, 2009c; EFSA, 2009d; EFSA, 2009e; EFSA, 2017a; EFSA, 2017b, EFSA, 2018b), including analysis of their modes of action, specificity of their biological activity, thermal stability and resistance to proteolysis.

The potential for interactions between two or more of the Cry proteins from *B. thuringiensis* has been evaluated by the EFSA GMO Panel as part of submissions containing the same or similar proteins. Cry1F, Cry1Ab, Cry34Ab1, Cry35Ab1 and mCry3A are insecticidal proteins that bind to cell surface receptors in the insect midgut. The gastrointestinal tract of mammals, including humans, lacks receptors with specific affinity to Cry proteins. The EFSA GMO panel (EFSA, 2016b) concluded that on the basis of the known biological function of the newly expressed proteins, there is currently no expectation for possible interactions relevant to the food and feed safety assessment of these proteins.

4.3.3 Toxicological testing of the newly expressed proteins

The proteins expressed from the transgenes in DP4114xMON810xMIR604xNK603 maize have been previously assessed and have a history of safe consumption as part of approved single and stacked GM events, including analyses of relatedness to other proteins with a history of safe use, absence of toxicity to mammals, absence of adverse effects on fast growing species, lack of homology to known toxins, lack of resistance to proteolysis, and degradation upon heating. Furthermore, for each of the introduced proteins in DP4114xMON810xMIR604xNK603 maize, 90-day feeding studies found no safety concerns.

Previous assessments on the individual events did not identify any potential for reproductive, developmental or chronic toxicity and no adverse effects were identified. Furthermore, no hazard was identified due to combining the four events, or unintended or intended compositional modifications in DP4114xMON810xMIR604xNK603 maize. In addition, a previous assessment of one of the sub-combinations, NK603xMON810 maize (EFSA, 2005b), no potential for adverse effects due to stacking were identified.

To further support previous conclusions, bioinformatics analyses were performed, in which consistent with previous data, no biologically relevant sequence similarities to known protein toxins that could be harmful to human or animal health were identified.

4.3.4 Toxicological testing of new constituents other than the newly expressed proteins

The genetic modifications in DP4114xMON810xMIR604xNK603 maize do not aim to change the composition of the crop or processed products produced from it, with no compositional differences between DP4114xMON810xMIR604xNK603 maize, the conventional counterpart, and the reference varieties identified that raise safety concerns.

4.3.5 Toxicological testing of the whole genetically modified food or feed

In accordance with assimilated Regulation (EU) No. 503/2013, the applicant provided a 90-day feeding study with DP4114xMON810xMIR604xNK603 maize. No diet-related differences were observed in Crl:CD(SD) rats fed a diet containing either untreated or intended herbicide-treated maize grain, at a high or low dietary concentration, compared with rats fed diets containing non-transgenic, near-isogenic maize grain or non-transgenic commercial maize grain.

4.3.6 Assessment of allergenicity

In accordance with assimilated Regulation (EU) No. 503/2013, the applicant used a weight-of-evidence approach to assess the allergenicity potential of Cry1F, Cry34Ab1, Cry35Ab1, Cry1Ab, mCry3A, PMI PAT, and CP4 EPSPS proteins as no single method is sufficient to predict allergenicity (Codex Alimentarius, 2009).

All newly expressed proteins have already been assessed in the EU while the UK was a member state, and they (or in any sub-combination of these events) were not identified as potential allergens for humans or animals. Updated bioinformatics analyses were performed, comprising in silico searches against up-to-date allergen databases. No matches were identified to known allergenic proteins apart from mCry3A which confirmed a previously identified sequence match of mCry3A with the allergenic alpha-parvalbumin from frog. This is considered a false positive as no IgE cross-reactivity has been confirmed using serum from an individual with food-induced anaphylaxis who was sensitised to parvalbumin.

Assessment of the non-IgE-mediated adverse immune reactions to the eight proteins expressed in DP4114xMON810xMIR604xNK603 maize were completed using in silico approaches in line with the EFSA guidance, 2017. The proteins do not contain HLA-DQ2 or HLA-DQ8 restricted epitopes or the motifs of HLA-DQ2 restricted epitopes implicated as potential hazards for celiac disease induction.

The ACNFP considered the bioinformatics analyses and found no allergenicity-related concerns for the newly expressed protein.

4.3.7 Anticipated intake/extent of use

In accordance with assimilated Regulation (EU) No. 503/2013, the applicant estimated potential exposure in the European Union (EU) to Cry1F, Cry34Ab1,

Cry35Ab1, PAT, Cry1Ab, mCry3A, PMI and CP4 EPSPS proteins from consumption of maize grain containing DP4114xMON810xMIR604xNK603 maize.

Estimated acute exposures to the introduced proteins ranged from 0.000654 (PAT) to 0.410439 (Cry34Ab1) mg/kg body weight/day for high consumers and from 0.000053 (PAT) to 0.033528 (Cry34Ab1) mg/kg body weight for average consumers. Estimated chronic exposures to the introduced proteins ranged from 0.000286 (PAT) to 0.152375 (Cry34Ab1) mg/kg body weight/day for high consumers and from 0.000053 (PAT) to 0.033509 (Cty34Abl) mg/kg body weight for average consumers. The anticipated human dietary intake of DP4114xMON810xMIR604xNK603 maize is considered to be negligible, based on data available on the consumption of maize and derived products in the EU. Therefore, no nutritional impact is expected and the risk to consumers is considered negligible.

The estimated potential dietary exposures to Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1Ab, mCry3A, PMI, and CP4 EPSPS proteins in DP4114xMON810xMIR604xNK603 maize grain for livestock animals were calculated and ranged from 0.0054 to 1.73 mg/kg BW/day, and exposures as a percent of total crude protein exposure in maize grain ranged from 0.0000517% to 0.0350%. The consumption of DP4114xMON810x MIR604xNK603 maize is not expected to pose a risk to livestock animals.

4.3.8 Nutritional assessment

DP4114xMON810xMIR604xNK603 maize, containing the introduced traits for agronomic purposes only, is not nutritionally disadvantageous, and is as safe as conventional maize varieties, as confirmed by bioinformatics and compositional analysis.

4.3.9 Conclusion of the food/feed safety assessment

The ACNFP assessed the food/feed safety of the genetically modified DP4114xMON810xMIR604xNK603 maize in terms of their toxicological potential, allergenic potential, and nutritional quality. It concluded that the genetically modified maize shared no identity with known toxins and allergens, and the overall allergenicity of DP4114xMON810xMIR604xNK603 maize was not different to conventional maize. The ACNFP concluded that based on the comparative and compositional analysis, DP4114xMON810xMIR604xNK603 maize is not nutritionally disadvantageous, and is as safe as conventional maize varieties. These conclusions are confirmed by bioinformatics analyses.

4.4 Environmental risk assessment and monitoring plan

4.4.1 Environmental risk assessment

The environmental risk assessment of DP4114xMON810xMIR604xNK603 maize is within the remit of Advisory Committee on Releases to the Environment (ACRE), and their assessment will form part of the final scientific assessment published by FSA/FSS.

The scope of the application only covers the import, processing, and food and feed use of DP4114xMON810xMIR604xNK603 maize, and no deliberate release of viable plant material or derived products is expected. Therefore, only accidental release of viable GM seeds or propagating material during import, transportation, storage, handling, and processing will be considered.

ACRE considered the ability of DP4114xMON810xMIR604xNK603 maize to persist under GB environmental conditions, interactions of feral DP4114xMON810xMIR604xNK603 maize with the environment, and the potential for horizontal gene transfer (HGT) to the environment. ACRE concluded that DP4114xMON810xMIR604xNK603 maize would not raise safety concerns in the

event of accidental release of viable seeds or propagating material into the environment.

ACRE's advice is available at the following link:

ACRE advice: applications to market GM soybeans and maize - GOV.UK (www.gov.uk)

4.4.2 Post-market environmental monitoring (PMEM) plan

Assessing any proposals for the PMEM plan is within the remit of ACRE, and its assessment will form part of the final safety assessment published by FSA/FSS.

Briefly, general surveillance will be used to identify the occurrence of unanticipated adverse effects due to the unintended release of DP4114xMON810xMIR604xNK603 maize. Exposure (via accidental release) can be controlled by clean-up measures, and the application of current practices used for the control of any adventitious maize plants, such as manual or mechanical removal, and the application of herbicides.

General surveillance will be predominantly based on collaboration with third parties, such as operators involved in the import, handling, and processing of DP4114xMON810xMIR604xNK603 maize. These third parties will report any potential unanticipated adverse effects to the authorisation holder, who will investigate.

The authorisation holder will submit an annual report including results of the general surveillance and any unanticipated adverse effects. If information that confirms an adverse effect becomes available, the authorisation holder will investigate, and based on a scientific evaluation, define, and implement management measures to protect human and animal health, or the environment, as necessary.

5. Analytical methods

Analytical methods

The FSA and FSS have decided, where appropriate, to make use of the European Union Reference Laboratory (EURL) laboratory reports completed prior to the end of the transition period for a GMO for which an application has also now been made to GB.

The FSA and FSS accepted the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF) report, showing that the detection methods for the stacked events DP4114xMON810xMIR604xNK603 were validated.

The methods and validation report are available via the following links:

DP4114 x MON 810 x MIR604 x NK603 documents | European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF) (europa.eu)

6. Overall conclusions and recommendations

To support the safety assessment by FSA/FSS, the ACNFP was asked to provide advice on the data submitted for the authorisation for import, processing, and food and feed use of genetically modified DP4114xMON810xMIR604xNK603 maize in accordance with assimilated Regulation (EU) No. 1829/2003.

DP4114xMON810xMIR604xNK603 maize contains *cry1F*, *cry34Ab1*, *cry35Ab1*, *pat*, *cry1Ab*, *cry3A*, *pmi*, *CP4 epsps* transgenic genes. The corresponding proteins produced confer 1) herbicide tolerance to glyphosate (CP4 EPSPS) and glufosinate-ammonium (PAT) herbicides 2) protection against lepidopteran target pests (Cry1F and Cry1Ab) 3) protection against coleopteran target pests (Cry34Ab1, Cry35Ab1

and mCry3A). The molecular characterisation data established that DP4114xMON810xMIR604xNK603 maize contains 4 transgenic inserts and bioinformatics analyses of these inserts, and the flanking sequences, raised no safety concerns. The stability of the inserts was confirmed in previous assessments of each single event authorised in the EU and it was demonstrated that the insertions in the stack were intact and equivalent to that of the corresponding single event maize. The expression levels of the transgenic protein in maize grain and forage were determined using suitable methodologies, and do not cause a safety concern.

The field trials used to generate material for the comparative analyses were deemed appropriate, and the locations selected were considered representative of commercial maize production. The meteorological conditions and management practices used during the field trails were appropriate. The ACNFP also assessed the results from the comparative analysis, including all the significant differences between DP4114xMON810xMIR604xNK603 maize and its conventional counterpart, and found no safety concerns when compared to reference varieties.

The food/feed safety of the newly expressed protein was assessed, and no safety concerns were raised in terms of their toxicological potential, allergenic potential, and nutritional quality. Based on the comparative analysis and the nutritional assessment, DP4114xMON810xMIR604xNK603 maize does not cause any nutritional concerns.

FSA/FSS concluded, based on ACNFP advice, that DP4114xMON810xMIR604xNK603 maize is as safe as its conventional counterpart with respect to its potential effects on human and animal health.

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8. Appendices

Appendix 1

1) List of 80 tested analytes for the comparative compositional analysis:

10 Analytes in forage:

- Proximates and fiber content: ash, carbohydrates, crude fat, crude fibre, crude protein, moisture, acid detergent fibre (ADF), neutral detergent fibre (NDF).
- Minerals: calcium, phosphorus

70 Analytes in grain:

- 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, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc
- Vitamins: *a*-tocopherol, ß-carotene, c-tocopherol, total tocopherols, thiamine, niacin,pantothenic acid, pyridoxine, folic acid),), *b*-tocopherol, *d*-tocopherol.
- 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), a-linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), lignoceric acid (C24:0), lauric acid (C12:0), myristic acid (C14:0), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), eicosadienoic acid (C20:2), behenic acid (C22:0), erucic acid (C22:1)

- Secondary Metabolite and Anti-Nutrient: ferulic acid, inositol, *p*-coumaric acid, phytic acid, raffinose, trypsin inhibitor, furfural.

2) Categories reported in Table 5 for CHT [(c)] DP4114xMON810xMIR604xNK603 maize

Category I (equivalence demonstrated)

i. Not different

In forage: Moisture,Crude Protein, Crude Fat, Crude Fiber, ADF , NDF, Ash, Carbohydrates, Calcium.

In grains: Moisture, Total Dietary Fiber, Crude Fat, ADF, Carbohydrates, Palmitoleic Acid (C16:1), Alpha-Linolenic Acid (C18:3), Arachidic Acid (C20:0), Lignoceric Acid (C24:0), Tryptophan, Calcium, Manganese, Zinc, Vitamin B1 (Thiamine), Vitamin B3 (Niacin), Vitamin B5 (Pantothenic Acid), Vitamin B9 (Folic Acid), α-Tocopherol, Total Tocopherols, Inositol, Phytic Acid

ii. Significantly different [d]

In grains: NDF, Ash, Palmitic Acid (C16:0), Oleic Acid (C18:1), Linoleic Acid (C18:2), Eicosenoic Acid (C20:1), Arginine, Aspartic Acid, Histidine, Lysine, Methionine, Proline, Tyrosine, Copper, Iron, Magnesium, Phosphorus, Potassium, Beta Carotene, Vitamin B6 (Pyridoxine), γ-Tocopherol, p-Coumaric Acid, Raffinose, Trypsin Inhibitor

Category II (equivalence more likely than not)

i. Not different

In grains: Crude Protein, Stearic Acid (C18:0), Alanine, Cystine, Glutamic Acid, Isoleucine, Leucine, Serine, Valine

ii. Significantly different [f]

In grains: Glycine, Phenylalanine, Threonine, Ferulic Acid

Category III (equivalence less likely than not)

i. Not different

None

ii. Significantly different

None

Category IV (non-equivalence demonstrated

i. Not different

None

ii. Significantly different [h]

In forage: Phosphorus

Not categorised

i. Not different

In grains: Sodium

ii. Significantly different

None

3) Categories reported in Table 5 for IHT (c) DP4114xMON810xMIR604xNK603 maize

Category I (equivalence demonstrated)

i. Not different

In forage: Crude Fat, Crude Fiber, ADF, Ash

In grains: Moisture, Crude Fat, Arachidic Acid (C20:0), Lignoceric Acid (C24:0), Methionine, Calcium, Vitamin B3 (Niacin), Vitamin B5 (Pantothenic Acid), Vitamin B9 (Folic Acid), α-Tocopherol, Inositol, Raffinose

ii. Significantly different [e]

In forage: Moisture, NDF

In grains: Total Dietary Fiber, Crude Protein, Crude Fiber, NDF, Ash, Carbohydrates, Palmitic Acid (C16:0), Palmitoleic Acid (C16:1), Oleic Acid (C18:1), Linoleic Acid (C18:2), Alpha-Linolenic Acid (C18:3), Eicosenoic Acid (C20:1), Alanine, Arginine, Aspartic Acid, Cystine, Glutamic Acid, Glycine, Histidine, Isoleucine, Leucine, Lysine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, Valine, Copper, Iron, Magnesium, Manganese, Phosphorus, Beta Carotene, Vitamin B1 (Thiamine), Vitamin B6 (Pyridoxine), γ-Tocopherol, Total Tocopherols, p-Coumaric Acid, Phytic Acid, Trypsin Inhibitor

Category II (equivalence more likely than not)

i. Not different

In forage: Calcium

In grains: Stearic Acid (C18:0)

ii. Significantly different [g]

In grains: ADF, Potassium, Zinc, Ferulic Acid

Category III (equivalence less likely than not)

i. Not different

None

ii. significantly different [i]

In forage: Crude Protein, Carbohydrates

Category IV (non-equivalence demonstrated)

i. not different

None

ii. Significantly different [i]

In forage: Phosphorus

Not categorised

i. Not different

In grains: Sodium

ii. Significantly different

None

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