

Safety Assessment on Genetically Modified Stacked GA21 X T25 Maize for Food and Feed Uses Under Assimilated Regulation (EC) No. 1829/2003 (RP2023)

Food Standards Agency¹, Food Standards Scotland²

¹ Regulated Products Risk Assessment, Food Standards Agency, UK, ² Risk Assessment, Food Standards Scotland, UK

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FSA Research and Evidence

The Food Standards Agency (FSA) and Food Standards Scotland (FSS) received an application from Syngenta Limited ("the applicant") under assimilated Regulation (EC) No. 1829/2003 for GA21 x T25 maize.

GA21 x T25 maize is modified to introduce the *mEPSPS* and *pat* transgenes. The corresponding proteins produced confer tolerance to glyphosate herbicide (mEPSPS) and tolerance to glufosinate ammonium herbicide (PAT). The scope of the application is for the authorisation for import, processing, and food and feed use of GA21 x T25 maize. The application does not cover cultivation and no GA21 x T25 maize will be grown in the UK.

FSA and FSS reviewed the technical dossier and supplementary information provided by the applicant. The safety assessment included molecular characterisation, a comparative assessment, a food/feed safety assessment encompassing toxicity and allergenicity assessments, and a nutritional assessment.

Advice on the environmental risk assessment from the Advisory Committee on Release to the Environment (ACRE) was also taken into account by FSA/FSS who concluded that genetically modified GA21 x T25 maize is as safe as the conventional counterpart.

This safety assessment represents the opinion of the FSA and FSS.

This is a joint FSA and FSS publication.



1. Introduction

1.1. Background

On April 25th 2023, the Food Standards Agency (FSA) received application RP2023 (EFSA-Q-2016-00775) for the authorisation of genetically modified GA21 x T25 maize (unique identifier: MON-ØØØ21-9 x ACS-ZMØØ3-2), submitted by Syngenta Crop Protection NV/SA, Brussels, Belgium, represented by Syngenta Limited, (Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY United Kingdom) (hereafter referred to as "the applicant") according to Regulation (EC) No. 1829/2003, as assimilated in UK law.

The 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 4th October 2024, declared the application valid.

In carrying out the safety assessment, FSA/FSS assessed the data submitted for the authorisation of genetically modified GA21 x T25 maize, as outlined in this document. This assessment represents the opinion of FSA/FSS on the safety of genetically modified GA21 x T25 maize.

GA21 x T25 maize (*Zea mays* L.) was obtained by a conventional breeding cross of two GM single event maize lines: GA21 and T25. No additional genetic modification was used to produce this maize hybrid. Therefore, these maize plants produce only the transgenic proteins inherited from both single GM maize events. Each single event was previously assessed by EFSA and authorised for use in the EU, while the UK was a Member State (EFSA, 2013, EFSA, 2013; EFSA, 2011), as shown in [Table 1](#). The stacked GA21 x T25 maize has also been assessed and authorised in the EU, with a positive EFSA opinion (EFSA-GMO-DE-2016-137) published in 2023 (EFSA, 2023). The individual events that comprise the stack have therefore not been re-assessed. The assessment of sub-combinations from the GA21 x T25 maize stack is not applicable as there are no sub-combinations produced from this stack.

Table 1. Single maize events assessed by the European Food Safety Authority (EFSA) when the UK was a Member State of the European Union.

Event	Scope	Application number or Mandate	EFSA opinion date
T25	Import	EFSA-GMO-NL-2007-46	EFSA (2013)
GA21	Import	EFSA-GMO-UK-2005-19	EFSA (2007)
GA21	Import Renewal	EFSA-GMO-RX-GA21	EFSA (2007)
GA21	Cultivation	EFSA-GMO-UK-2008-60	EFSA (2011a)

FSA/FSS 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 RP2023. As the single events were previously safety assessed and authorised, this safety assessment focusses on the combined transformation events including stability and expression of the transformation events, and potential interactions resulting from the combination of the transformation events as required by Regulation (EU) No 503/2013 (EC, Commission Implementing Regulation (EU) No. 503/2013 of 3 April 2013 on Applications for Authorisation of Genetically Modified Food and Feed in Accordance with Regulation (EC) No. 1829/2003 of the European Parliament and of the Council and Amending Commission Regulations (EC) No. 641/2004 and (EC) No. 1981/2006, 2013).

The transgenes present in GA21 x T25 maize are *mepsps* and *pat*. The resultant proteins produced, and the traits conferred, are as follows:

- Double-mutated maize 5-enolpyruvylshikimate-3-phosphate synthase enzyme (mEPSPS), produced by the GA21 event (GA21 maize) conferring glyphosate herbicide tolerance for weed control;
- Phosphinothricin acetyltransferase (PAT) protein, produced by the T25 event (T25 maize), to confer gulfosinate ammonium herbicide tolerance for weed control.

Maize is one of the most important food and feed crops worldwide and is grown over a wide range of climatic conditions, well-suited for warm, temperate climates. Maize is grown over 15 million hectares in the EU (14% of the EU's arable land, and 8% of worldwide maize acreage), and 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 to produce and manufacture maize-derived food and feed 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 GA21 x T25 maize does not impact any 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 GA21 x T25 maize. The application does not cover cultivation and therefore no GA21 x T25 maize will be grown in the UK.

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 GA21 x T25 maize for authorisation in the scope of the application, namely the import, processing, and food and feed use of GA21 x T25 maize.

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 GA21 x T25 maize, FSA/FSS were also required 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

- Name: Syngenta Crop Protection NV/SA
- Address: Syngenta Crop Protection NV/SA
Avenue Louise 489
1050 Brussels
Belgium

(represented by)

- Name: Syngenta Limited
- Address: Jealott's Hill International Research Centre
Bracknell, Berkshire RG42 6EY
United Kingdom

3. Data and methodologies

3.1. Data

The data for application RP2023 submitted according to legal requirements contained in Regulation (EC) 1829/2003 and provided by the applicant at the time of submission are specified below. FSA/FSS 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 RP2023, additional information provided by the applicant, and any relevant peer-reviewed scientific publications.

3.2. Methodologies

The FSA/FSS assessment was conducted 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, 2015, 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 the safety assessment considers the methods used to insert the transgenic material, the sequence and structure of the newly expressed protein, and the sequences at the insertion locus.

The following information is assessed:

- Analyses performed by the applicant to determine insertion locus, copy number, and any deletions that occurred during the insertion of transgenic material;
- Bioinformatics analyses performed on the transgenic sequences to ensure the newly expressed protein does not raise any safety concerns;
- The expression of the new protein;
- Bioinformatics analyses performed on the flanking regions either side of the inserted material (and the junctions between them) to ensure no sequences occur that could raise safety concerns.

4.1.1. Transformation process and vector constructs

GA21 x T25 maize was obtained by conventional crossing of the GM maize single events GA21 and T25. The structure of the inserts introduced into maize GA21 x T25 are described in previous EFSA assessments ([Table 1](#)) and no new genetic modifications were involved. The vectors and methods used to produce the GA21 and T25 single event maize, as shown in Table 2, are as follows:

- A *NotI* restriction fragment from the Plasmid pDPG434 was used to transform GA21 maize via microprojectile bombardment transformation. The plasmid is derived from a pSK- vector which is commonly used in molecular biology and is derived from pUC19;
- The purified plasmid vector pUC/Ac was used for the transformation of T25 maize through protoplast transformation. The plasmid pUC/Ac was constructed by cloning the synthetic *pat* gene between the Cauliflower Mosaic Virus (CaMV)-derived 35S gene promoter and terminator sequences of the pUC derived plasmid pDH51.

Table 2. Genetic elements in the expression cassettes of the single events stacked in GA21 x T25 maize and References of their previous assessments.

Event	Promoter	5' UTR	Transit Peptide	Coding Region	Terminator	Reference
GA21	Actin 1 (<i>Oryza sativa</i>)	Actin 1 (<i>Oryza sativa</i>)	OTP (<i>Helianthus annuus</i>)	mEPSPS (<i>Zea mays</i>)	nos (<i>Agrobacterium tumefaciens</i>)	EFSA, 2007, 2011a
T25	35 S (CaMV)	-	-	<i>pat</i> (<i>Streptomyces viridochromogenes</i>)	35 S (CaMV)	EFSA, 2013

CaMV: cauliflower mosaic virus; UTR: untranslated region.

-: when no additional genetic element was specifically introduced to optimise expression or to target proteins to subcellular compartments.

4.1.2. Molecular studies performed on stacked GA21 x T25 maize

A comparative Southern blot analysis of GA21 x T25 maize, using two gene-specific probes, confirmed the presence of the GA21 and T25 inserts and demonstrated that the stacked GA21 x T25 maize has maintained the integrity, number, structure and organisation of the GA21 and T25 maize inserts as seen in the corresponding single event lines. Sequence comparisons of the GA21 and T25 inserts in GA21 x T25 maize showed that no nucleotide changes were identified in the stacked maize when compared to the corresponding sequences in the single event maize lines.

BLASTn (Basic Local Alignment Search Tool) and BLASTx evaluations of GA21 maize suggest that the GA21 insert disrupted an identified open reading frame (ORF) with similarity to a hypothetical chloroplast cytochrome c gene. However, it was considered that the presence of a functional cytochrome c biosynthesis gene in the chloroplast genome of GA21 x T25 maize is sufficient to compensate for any possible disruption of a gene in the nuclear genome. The analyses also showed that the sequence flanking the 3' region of the GA21 insert is a highly repetitive region of the maize genome. None of the alignments indicated that an essential nuclear maize gene was interrupted.

T25 BLAST analysis demonstrated sequence homology of the 5' and 3' flanking sequences with several putative retrotransposon genes and which suggest interruption upon transformation. Due to the fact the maize sequences flanking the T25 insert are highly repetitive in the maize genome, corresponding with a likely retrotransposon region which are common in the maize genome, a loss of function due to the integration of the T25 T-DNA is highly unlikely.

Overall, BLASTN and BLASTX analyses performed on maize genomic sequence flanking the 5' and 3' region of the GA21 and T25 maize inserts showed that no known essential maize genes were interrupted and that no unintended modifications occurred at either insertion site.

No biologically relevant similarity to known proteins, toxins or allergens were identified for the introduced proteins and there were no putative ORFs produced in GA21 x T25 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. An independent, outside contractor verified the outcomes and methodologies of all bioinformatic analyses. The FSA/FSS was satisfied that the methods and results were satisfactory.

4.1.3. Transgenic protein expression

Expression levels of the mEPSPS and PAT proteins were measured by a quantitative enzyme linked immunosorbent assay (ELISA) on samples from leaves, roots, whole plants, pollen, and kernel tissues in different growth stages. Samples were harvested from GA21 x T25 maize and each single event maize line during the 2012 growing season at three locations in the USA. Expression levels were measured for plants that had not been treated with the intended herbicide. The analyses of both protein concentrations (Table 3, 4) in the GA21 x T25 maize and the single events revealed that the stacking of the single events did not substantially change the protein expression levels in GA21 x T25 maize compared to the expression of the corresponding proteins in the single events. Significant differences were

observed for two out of thirteen endpoints: (1) mEPSPS concentrations in growth stage V6 whole plant, 2) PAT concentration in growth stage V6 leaves for which statistical comparisons were performed; however, these differences were not consistently observed across tissue types or developmental stages and would not raise safety concerns.

Table 3. mEPSPS protein concentrations (µg/g) in tissues of GA21 and GA21 x T25 maize hybrids of four growth stages grown at three different locations in the USA during the 2012 growing season, on a dry weight basis (DW), measured by a quantitative enzyme linked immunosorbent assay (ELISA).

Tissue/ Stage	Hybrid	Mean µg/g dw	Range µg/g dw	SEM	SD	P	
V6							
Leaves	GA21	111	80.5–132	6.64	25.7	0.534	
	GA21 × T25	116	83.5–149				
Roots	GA21	55.5	36.6–72.9	3.85	14.9	0.783	
	GA21 × T25	57	35.4–75.4				
Whole plants	GA21	130	85.2–174	6.16	23.8	0.016	
	GA21 × T25	113	74.3–153				
R1							
Leaves	GA21	100	58.6–142	12.9	50.1	0.316	
	GA21 × T25	105	66.0–136				
Roots	GA21	—	<LOQ a _{-38.9}	—	—	—	
	GA21 × T25	—	<LOQ a _{-42.2}	—	—	—	
Pollen	GA21	—	<LOD b ₋₂₄₀	—	—	—	
GA21 × T25	—	<LOD b ₋₁₀₃	—	—	—		
Whole plants	GA21	70.3	26.6–116	16.44	63.67	0.724	
	GA21 × T25	73.3	39.0–117				
R6							
Kernels	GA21	—	<LOQ c _{-12.4}	—	—	—	
	GA21 × T25	—	—	<LOQ c _{-9.09}	—	—	—
Senescence							
Kernels	GA21	—	<LOD d _{-<LOQ} c	—	—	—	
	GA21 × T25	—	<LOD d _{-<LOQ} c	—	—	—	

LOD = Limit of detection

LOQ = Limit of quantification

SEM = Standard error of the mean

SD = Standard deviation

— = not applicable (not analysed)

Protein concentration units are µg/g dw (dry weight)

N = 15 for both the single event hybrid and stacked-trait hybrid unless noted otherwise

Results significantly different at $P < 0.05$ are shown in bold italic type

^a LOQ for roots = 12.8 µg/g

^b LOD for pollen = 37.50 µg/g

^c LOQ for kernels = 4.0 µg/g

^d LOD for kernels = 2.00 µg/g

Table 4. PAT protein concentrations (µg/g) in tissues of T25 and GA21 x T25 maize hybrids of four growth stages grown at three different locations in the USA during the 2012 growing season, on a dry weight basis (dw), measured by a quantitative enzyme linked immunosorbent assay (ELISA).

Tissue/ Stage	Hybrid	Mean µg/g dw	Range µg/g dw	SEM	SD	P
V6						
Leaves	T25	63.9	47.1–79.0	3.24	12.53	0.047
	GA21 × T25	68.3	58.5–79.4			
Roots	T25	23.4	14.0–29.9	1.64	6.37	0.811
	GA21 × T25	23	16.5–29.3			
Whole plants	T25	53.2	37.8–66.6	4.3	16.67	0.208
	GA21 × T25	49.3	20.2–66.2			
R1						
Leaves	T25	35.8	23.9–50.0	2.24	8.38	0.074
	GA21 × T25	41.5	32.9–61.8	2.15	8.34	
Roots	T25	10.5	2.70–22.3	4	14.95	0.903
	GA21 × T25	10.1	0.630–20.9	3.99	15.46	
Pollen	T25	—	<LOD ^a	—	—	—
	GA21 × T25	—	<LOD ^a			
Whole plants	T25	23.2	12.2–36.6	2.28	8.53	0.454
	GA21 × T25	21.9	15.6–36.0	2.25	8.72	
R6						
Kernels	T25 ^b	0.209	0.113–0.390	0.0534	0.2	0.153
	GA21 × T25	0.241	0.0915–0.439	0.0533	0.2064	
Senescence						
Kernels	T25 ^c	0.123	0.0522–0.211	0.0242	0.0871	0.194
	GA21 × T25	0.105	0.0514–0.246	0.0239	0.0924	

LOD = Limit of detection

SEM = Standard error of the mean

SD = Standard deviation

— = not applicable (not analysed)

Protein concentration units are µg/g dw (dry weight)

N = 15 for both the single event hybrid and stacked-trait hybrid unless noted otherwise

Results significantly different at $P < 0.05$ are shown in bold italic type

^a LOD for pollen = 0.025 µg/g

^b N = 14 for single event hybrid (N = 4 for samples collected at Bagley, IA)

^c N = 13 for single event hybrid (N = 3 for samples collected at Bagley, IA)

4.1.4. Genetic stability

Southern blot analyses carried out in this application on GA21 x T25 maize confirmed the integrity of the inserted sequences of GA21 and T25, as discussed above in the section on molecular studies (section 4.1.2).

Molecular analyses, agronomic characterisation and protein expression analysis confirmed the phenotypic stability of GA21 x T25 maize, showing stable inheritance and expression of the *mepsps* and *pat* genes following traditional crossing between GA21 and T25 maize lines. The phenotypic stability was confirmed and demonstrated that expression of the transgenic proteins in GA21 x T25 maize is not substantially different from the expression in the GA21 and T25 single maize events.

4.1.5. Conclusion on the molecular characterisation

The molecular characterisation data provided demonstrated that the genetic insertions in GA21 x T25 maize were intact and equivalent to that of the individual events present in the single event GM lines. Updated bioinformatics analyses on the open reading frames (ORFs) and newly expressed proteins in maize GA21 x T25 raised no safety concerns.

The expression levels of the transgenic proteins were determined using suitable methodologies and no biologically relevant changes in protein expression were observed between GA21 x T25 maize and the single event maize lines.

An independent, outside contractor assessed the outcomes and methodologies of all bioinformatic analyses and was satisfied that the methods and results were satisfactory.

The FSA/FSS have reviewed the data provided by the applicant to support the molecular characterisation of GA21 x T25 maize and are satisfied that the events within GA21 x T25 maize are equivalent to those already assessed by EFSA and given a positive opinion while the UK was an EU Member State.

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 secondly 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 GA21 x T25 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 GA21 x T25 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 for GA21 x T25 maize, along with the control material (conventional counterpart, consisting of non-GM near-isoline hybrid maize seed), and 6 commercially available non-GM maize reference lines (H-6044, NK Symba, NK Thermo, X36344, H-7191 and H-7540), were tested during 2012 and 2016 at 13 sites for agronomic, and during 2012 at 8 sites for compositional analysis in North America.

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

- Conventional herbicide – treated (CHT) GA21 x T25 maize (referring to treatment with conventional herbicide only, not with intended herbicide);
- Intended herbicide-treated (IHT) GA21 x T25 maize (referring to treatment with trait-specific herbicides glyphosate and glufosinate);
- Non-GM near-isoline CHT control maize (conventional counterpart);
- Six non-GM reference varieties (H-6044, NK Symba, NK Thermo, X36344, H-7191 and H-7540).

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 GA21 x T25 maize and the conventional counterpart, and a test of equivalence between GA21 x T25 maize and the non-GM reference varieties.

4.2.2. Suitability of field trials and test materials

The field trial sites represent sufficient 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 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 GA21 x T25 maize under six temperature regimes were comparable to that of the control maize under corresponding growing conditions.

The compositional assessment of GA21 x T25 maize was based on the analysis of nutrient composition of forage (R4 growth stage) and grain (R6 growth stage).

The FSA/FSS 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 GA21 x T25 maize could be grown.

4.2.3. Comparative analysis (agronomic characteristics)

Difference and equivalence tests were performed for 9 agronomic characteristics (early stand count, days to 50% pollen shed, days to 50% silking, plant height, total lodged plants, final stand count, grain moisture, test weight and yield).

The endpoint “total lodged plants” was not analysed with formal statistical methods because of a lack of variability in the data. In addition, data on abiotic stressors, disease incidence and insect damage were collected from the field trials.

Statistically significant differences were observed for CHT-GA21 x T25 (not treated with intended herbicides) test material compared to the conventional counterpart for “early stand count”, which fell under

equivalence category III (equivalence less likely than not), and “final stand count”, which fell under category I (equivalence demonstrated). However, equivalence test results confirmed equivalence to the reference maize.

Early and final stand counts were significantly lower in the CHT-GA21 x T25 than in the control which reflected variability in seedling emergence but did not affect grain yield. Since “early stand count” was recorded prior to application of the trait-specific herbicide, and both entries of GA21 x T25 maize were from the same seed lot, the difference in outcomes is likely due to random variation and has no biological relevance.

For IHT-GA21 x T25 maize (treated with intended herbicides), a statistically significant difference was shown for days to 50% pollen shed, however it fell under equivalence category I (equivalence demonstrated).

The mean values for all agronomic characteristics measured were within the observed ranges.

4.2.4. Compositional analysis

Maize forage and grains harvested from the field trial study in the USA in 2012 were analysed for 81 constituents (9 in forage and 72 in grains, see Appendix 1), including the key constituents recommended by OECD (2002). The statistical analysis was not applied to the following 16 grain constituents because of the large number of values below the limit of detection: selenium, sodium, 13 fatty acids (caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), myristoleic acid (14:1), pentadecanoic acid (15:0); pentadecanoic acid (15:1); heptadecanoic acid (17:0); heptadecenoic acid (17:1), gamma linolenic acid (18:3), eicosadienoic acid (20:2), eicosatrienoic acid (20:3), and arachidonic acid (20:4)), and furfural. In addition, moisture levels in grains were not compared since ears were dried in the field or mechanically after being harvested.

The statistical analysis was applied to the remaining 65 constituents (see Appendix 1 - 9 in forage and 72 in grains). Results of the test of difference and the test of equivalence are summarised in [Table 5](#).

For maize CHT-GA21 x T25 (not treated with the intended herbicides), statistically significant differences with its conventional counterpart were found for 12 components in grain; however, all of them fell under equivalence category I or II, confirming their equivalence to the conventional counterpart and references.

For maize IHT-GA21 x T25 (treated with the intended herbicides), statistically significant differences with its conventional counterpart were found for 14 components in grain; all of them fell under equivalence category I or II.

Table 5. Comparative compositional analysis results of grains and forage from maize GA21 x T25. The table shows the number of endpoints in each category.

a) CHT ^c GA21 x T25 maize			
		Test of difference ^a	Test of difference ^a
		Not different	Significantly different
Test of equivalence ^(b)	Category I	48 ^(d)	11 ^(e)
	Category II	4 ^(f)	1 ^(g)
	Category III	0	0
	Category IV	0	0
	No category	1 ^(h)	0
	Total endpoints	53 Appendix 1	12 Appendix 1
b) IHT ^c GA21 x T25 maize			
		Test of difference ^a	Test of difference ^a
		Not different	Significantly different
Test of equivalence ^b	Category I	48 ⁱ	12 ^l
	Category II	2 ^m	2 ⁿ
	Category III	0	0
	Category IV	0	0
	No category	1 ^o	0
	Total endpoints	51 Appendix 1	14 Appendix 1

^a Comparison between both GA21 x T25 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 GA21 x T25 maize with not intended herbicides;

IHT : Intentional herbicide-treated GA21 x T25 maize with glyphosate and glufosinate

d,e,f,g,h,i,l,m,n,o see Appendix 1

The “not categorised” outcome for trypsin inhibitor in both entries CHT-GA21 x T25 and IHT-GA21 x T25 maize was due to a zero estimate of variance among the reference lines and therefore it was not possible to draw any conclusions regarding equivalence to the reference lines; however, the levels did not differ from those in the control grain.

No statistically significant differences were observed in levels of trypsin inhibitor in the comparisons between the CHT-GA21 x T25 and control, and between the IHT-GA21 x T25 maize and control. In addition, the zero estimate of variance occurred for this component only. Therefore, the outcomes for this component in both cases were categorised as no-difference from the conventional counterpart.

The mean levels of trypsin inhibitor in the CHT-GA21 x T25 and IHT-GA21 x T25 maize grain samples are within the ranges of trypsin inhibitor levels in the control and the reference grain, and the ranges of levels of trypsin inhibitor in CHT-GA21 x T25 maize and IHT-GA21 x T25 maize are

completely within the range of levels of the reference grain. Therefore, the levels of trypsin inhibitor in both CHT-GA21 x T25 maize and IHT-GA21 x T25 maize are not considered to raise a safety concern.

Trypsin inhibitors are naturally-occurring anti-nutritional factors that are found in a variety of plant food products including soybeans, cereal grains, sweet potatoes, beets, and sugar beets. Trypsin inhibitor activity in food and feed products has been shown to negatively impact protein digestibility and the bioavailability of amino acids in experimental animals. However, trypsin inhibitor activity is inactivated by heat, and therefore is effectively removed from maize and maize products after proper cooking and/or processing.

4.2.5. Conclusion on the comparative analysis

The FSA/FSS assessed the field trials (including locations and management practices) used to generate material for the comparative analyses and considered that the locations selected were representative of commercial maize production, and that the meteorological conditions and management practices used during the field trials were appropriate.

The FSA/FSS also assessed the results from the comparative analysis, including all the significant differences between GA21 x T25 maize and its conventional counterpart, and no differences between GA21 x T25 maize and the conventional counterpart or the non-GM reference varieties that would raise safety concerns were observed.

4.3. Food/feed safety assessment

The food/feed safety assessment covers the likelihood that the newly expressed protein, 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 newly introduced proteins. Any toxicological or allergenic risks that can be identified and any effects on nutritional quality were also assessed.

4.3.1. Effects of processing

In the UK and EU, most 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 GA21 x T25 maize. Considering the genetic modifications in GA21 x T25 maize, none of the processing outcomes are likely to be affected by the traits introduced in GA21 x T25 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 GA21 x T25 maize expresses the mEPSPS and PAT proteins. All newly expressed proteins have previously been assessed (EFSA-GMO-UK-2005-19, EFSA-GMO-UK-2008-60, EFSA-GMO-NL-2007-46), including analysis of their modes of action, specificity of their biological activity, thermal stability and resistance to proteolysis. The activities of mEPSPS and PAT are reduced upon heating, and it is not expected that interactions will occur between the newly expressed proteins. No safety concerns were raised by the comparative assessment or levels of expression of the newly introduced proteins and the genetic modifications do not alter how the maize will be processed or the effects of processing on the end products.

4.3.3. Toxicology assessment of the newly expressed proteins

The proteins expressed from the transgenes in GA21 x T25 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 GA21 x T25 maize, a repeated dose 28-day oral toxicity and 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.

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

mEPSPS and PAT proteins have independent biological roles in different metabolic pathways, making interactions between the two proteins and their products unlikely. A lack of interaction *in planta* is also supported by the crop compositional analysis, which demonstrates the similarity of GA21 x T25 maize to conventional maize. No unintended effects that could be attributable to interactions between mEPSPS and PAT have been identified in GA21 x T25 maize.

4.3.4. Toxicology assessment of new constituents other than the newly expressed proteins

The genetic modifications in GA21 x T25 maize do not aim to change the composition of the crop or processed products produced from it, with no compositional differences between GA21 x T25 maize, the conventional counterpart, and the reference varieties identified that raise safety concerns. Therefore no assessment of any constituents other than the newly expressed proteins was required.

4.3.5. Toxicology assessment 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 for GA21 x T25 maize. Feeding studies on the individual events were performed as part of previous single applications with no adverse effects observed.

90-day feeding study on GA21 x T25 maize

Despite the 90-day feeding study for GA21 x T25 maize providing limited information about the materials and methods used for the statistical analysis and on the production of the test diets, no adverse effects were observed. The FSA/FSS do not consider further animal studies with food/feed derived from maize GA21 x T25 necessary. In addition, the applicant provided previous 90-day feeding studies on whole food/feed from each of the maize single events composing maize GA21 x T25, which also showed no observed adverse effects.

90-day feeding study on maize T25

A 90-day feeding study on the T25 single event maize previously assessed in the context of the single-event renewal application (EFSA GMO Panel, 2013) was not considered adequate because of a low number of experimental units per treatment, and grains used were harvested from T25 maize plants not treated with the intended herbicide. An updated 90-day feeding study on the T25 single event maize was then provided. This was requested by EFSA to fulfil the requirements of Regulation (EU) No 503/2013, during their own assessment for the renewal of T25 maize, and

reviewed by FSA/FSS. The FSA/FSS concluded that no treatment-related adverse effects were observed in rats after feeding diets including 50% glyphosate sprayed or unsprayed grains from T25 maize for 90 days.

90-day feeding study on maize GA21

A 90-day study on the GA21 single event maize previously assessed in the context of the single-event application (EFSA GMO Panel, 2011, EFSA, 2013) did not show adverse effects related to the administration of the GM diet. In the context of the assessment of the stacked maize GA21 x T25, EFSA required additional information to confirm the adherence of this study to requirements of Regulation (EU) 503/2013, OECD TG 408 (OECD, 1998) (EFSA Scientific Committee, 2011 and (EFSA, 2014). The applicant provided further details on the experimental design, and additional statistical analyses. The FSA/FSS concluded that this study is compliant with the legal requirements, and confirmed that there are no adverse effects related to the 90-day administration to rats fed diets containing glyphosate sprayed or unsprayed GA21 maize grain, up to 41.5% of inclusion rate. Toxicological testing of newly expressed proteins was conducted as part of the previous EU applications, showing no adverse effects. In addition, the 90-day feeding study performed on GA21 x T25 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.

4.3.6. Allergenicity assessment

In accordance with assimilated Regulation (EU) No. 503/2013, the applicant used a weight-of-evidence approach to assess the allergenicity potential of mEPSPS and PAT proteins as no single method is sufficient to predict allergenicity (Codex Alimentarius, 2009). Both newly expressed proteins were assessed in the EU while the UK was a Member State, and they 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. The applicant assessed the non-IgE-mediated adverse immune reactions to both proteins expressed in GA21 x T25 maize using *in silico* approaches in line with 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 FSA/FSS 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 potential exposure in the European Union (EU)/United Kingdom (UK) to mEPSPS and PAT proteins from consumption of maize grain or maize by-products containing GA21 x T25 maize was estimated. For both acute (single day) and chronic (lifetime) exposure, the amounts of mEPSPS and PAT that could be consumed by humans per kilogram of body weight (kg/bw) per day in both the EU and the UK were conservatively calculated in the following groups: young population (infants, toddlers, “other children”), adolescents, adult population (adults, elderly and very elderly) and special populations (pregnant and lactating women). The anticipated human dietary intake of GA21 x T25 maize is considered to be negligible, based on data available on the consumption of maize and maize-derived products in the EU and the UK. Therefore, no nutritional impact is expected and the risk to consumers is considered negligible.

The theoretical maximum (worst case) acute exposure to the mEPSPS protein from consumption of GA21 x T25 maize and GA21 x T25 maize-derived products is 26.38 µg/kg/bw in adults and 61.50 µg/kg/bw in children when compared across all of the countries in the EU that are represented in the EFSA comprehensive database.

The theoretical maximum (worst case) acute exposure to the mEPSPS protein from consumption of GA21 x T25 maize and GA21 x T25 maize by-products is 4.3 µg/kg/bw in adults and 12 µg/kg/bw in children when compared across all the UK nations, represented in the FSA database, National Diet & Nutrition Survey (NDNS).

The highest chronic exposure to the mEPSPS protein from consumption of GA21 x T25 maize and GA21 x T25 maize by-products is 13.69 µg/kg bw/d in adults and 48.45 µg/kg bw/d in children when compared across all of the countries in the EU that are represented in the EFSA comprehensive database. mEPSPS was non-quantifiable in an enzyme-linked immunosorbent assay, and therefore the assay's limits of detection and quantitation were used to calculate theoretical chronic exposure.

The highest chronic exposure to the mEPSPS protein from consumption of GA21 x T25 maize and GA21 x T25 maize by-products is 1.2 µg/kg bw/d in adults and 3.5 µg/kg bw/d in children when compared across all the UK nations, represented in the FSA database, NDNS.

The theoretical maximum acute exposure to the PAT protein from consumption of GA21 x T25 maize and GA21 x T25 maize by-products is 0.720 µg/kg bw in adults and 1.670 µg/kg bw in children when compared across all of the countries in the EU that are represented in the EFSA comprehensive database.

The theoretical maximum acute exposure to the PAT protein from consumption of GA21 x T25 maize and GA21 x T25 maize by-products is 0.11 µg/kg bw in adults and 0.30 µg/kg bw in children when compared across all of the UK nations, represented in the FSA database, NDNS.

The highest chronic exposure to the PAT protein from consumption of GA21 x T25 maize and GA21 x T25 maize by-products is 0.37 µg/kg bw/d in adults and 1.32 µg/kg bw/d in children when compared across all of the countries in the EU that are represented in the EFSA comprehensive database.

The highest chronic exposure to the PAT protein from consumption of GA21 x T25 maize and GA21 x T25 maize by-products is 0.03 µg/kg bw/d in adults and 0.087 µg/kg bw/d in children when compared across all of the UK nations, represented in the FSA database, NDNS.

The estimated potential dietary exposures to both proteins in GA21 x T25 maize grain for livestock animals were calculated as follows: total intake of mEPSPS ranged from less than 0.2207 mg/kg bw/day in breeding swine (approximately 0.008% of daily total protein intake) to less than 0.8717 mg/kg bw/day in lambs (approximately 0.01% of daily total protein intake). Total intake of PAT ranged from 0.0116 mg/kg bw/day in breeding swine (approximately 0.0004% of daily total protein intake) to 0.0458 mg/kg bw/day in lambs (approximately 0.0005% of daily total protein intake). The consumption of GA21 x T25 maize is not expected to pose a risk to livestock animals.

4.3.8. Nutritional assessment

As the introduced traits in GA21 x T25 maize are for agronomic purposes only and not intended to change the nutritional properties of the food/feed, no changes to the nutritional value of GA21 x T25 maize or its processed products is expected. As supported by the comparative analysis of composition, the consumption of GA21 x T25 maize does not raise nutritional concerns compared to the conventional counterpart.

4.3.9. Conclusion of the food/feed safety assessment

The FSA/FSS assessed the food/feed safety of the genetically modified GA21 x T25 maize in terms of 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 GA21 x T25 maize was not different to conventional maize. The FSA/FSS concluded that based on the comparative and compositional analysis, GA21 x T25 maize is not nutritionally disadvantageous, and is as safe as conventional maize varieties.

4.4. Environmental risk assessment and monitoring plan

4.4.1. Environmental risk assessment

The environmental risk assessment of GA21 x T25 maize is within the remit of Defra and the Advisory Committee on Releases to the Environment (ACRE); 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 GA21 x T25 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 GA21 x T25 maize to persist under GB environmental conditions, interactions of feral GA21 x T25 maize with the environment, and the potential for horizontal gene transfer (HGT) to the environment. ACRE concluded that GA21 x T25 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:

<https://www.gov.uk/government/publications/acre-advice-applications-to-market-gm-soybeans-and-maize>

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 GA21 x T25 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 GA21 x T25 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

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 GA21 x T25 were validated.

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

https://gmo-crl.jrc.ec.europa.eu/method-validation/details/all/2029/GA21_xT25

6. Overall conclusions and recommendations

The FSA/FSS was asked to assess the data submitted for the authorisation for import, processing, and food and feed use of genetically modified GA21 x T25 maize in accordance with assimilated Regulation (EU) No. 1829/2003.

GA21 x T25 maize contains *mEPSPS* and *pat* transgenic genes. The corresponding proteins produced confer 1) herbicide tolerance to glyphosate (mEPSPS) and 2) herbicide tolerance to glufosinate ammonium (PAT). The molecular characterisation data established that GA21 x T25 maize contains 2 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 trials were

appropriate. The FSA/FSS also assessed the results from the comparative analysis, including all the significant differences between GA21 x T25 maize and its conventional counterpart, and found no safety concerns when compared to reference varieties.

The food/feed safety of the newly expressed proteins 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, GA21 x T25 maize does not cause any nutritional concerns.

The FSA/FSS 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 GA21 x T25 maize would raise any safety concerns. The FSA/FSS therefore concludes that GA21 x T25 maize is as safe as its conventional counterpart with respect to its potential effects on human and animal health.

Abbreviations

Abbreviation	Definition
ACRE	Advisory Committee on Releases to the Environment
ADF	Acid Detergent Fiber
BLAST	Basic Local Alignment Search Tool
BLASTP	Basic Local Alignment Search Tool for Proteins
BLASTX	Basic Local Alignment Search Tool for Translated Nucleotides
bp	Base Pair
BW	Body Weight
CaMV	Cauliflower Mosaic Virus
DNA	Deoxyribonucleic Acid
EC	European Commission
EFSA	European Food Safety Authority
ELISA	Enzyme-linked Immunosorbent Assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	Environmental Risk Assessment
EST	Expressed Sequence Tags
EU	European Union
FSA	Food Standards Agency
FSS	Food Standard Scotland
FW	Fresh Weight
g	Gram
GM	Genetically Modified
GMO	Genetically Modified Organism
HLA-DQ2/ HLA-DQ8	Celiac Disease Genes
HGT	Horizontal Gene Transfer

Abbreviation	Definition
IgE	Immunoglobulin E
IHT	Intended Herbicide-Treated
LOD	Limit of Detection
LLOQ	Lower Limit of Quantification
mEPSPS	double-mutated 5-enolpyruvylshikimate-3-phosphate synthase
mg	Milligram
NDF	Neutral Detergent Fibre
NDNS	National Diet & Nutrition Survey
NOS	Transcription Terminator
OECD	Organisation for Economic Co-operation and Development
ORFs	Open Reading Frames
OTP	Optimised Transit Peptide
PAT	Phosphinothricin-Acetyl-Transferase
PCR	Polymerase Chain Reaction
PMEM	Post-Market Environmental Monitoring
PMI	Phosphomannose-isomerase
SGF	Simulated Mammalian Gastric Fluid
SD	Standard Deviation
SEM	Standard Error of the Mean
T-DNA	Transfer-Deoxyribonucleic Acid
TDF	Total Dietary Fiber

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Appendix 1. Interpretation of Comparative assessment results

1. List of 65 tested analytes for the comparative compositional analysis:

9 Analytes in forage:

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

56 Analytes in grain:

- Proximates and fibre content: protein, fat, ash, carbohydrates, acid detergent fibre (ADF), neutral detergent fibre (NDF) and total detergent fibre (TDF)
- Starch
- Minerals: calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc
- Vitamins: α -tocopherol, β -carotene, thiamine, riboflavin, niacin, pyridoxine, folic acid
- Amino acids: alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine
- Fatty acids: palmitic acid (16:0), palmitoleic acid (16:1), 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)
- Secondary metabolites and anti-nutrients: ferulic acid, *p*-coumaric acid, inositol, phytic acid, trypsin inhibitor and raffinose.

Categories reported in [Table 5](#) for CHT^c GA21 x T25 maize

Category I (equivalence demonstrated)

- i. Not different^d

In forage: Moisture, protein, fat, ash, carbohydrates, ADF, NDF, phosphorus

In grains: Protein, fat, carbohydrates, ADF, NDF, TDF, starch, iron, magnesium, zinc, β -carotene, thiamine HCl, riboflavin, niacin, pyridoxine HCl, folic acid, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, valine, palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), arachidic acid (20:0), eicosenoic acid (20:1), behenic acid (22:0), inositol, raffinose

ii. Significantly different ^e

In grains: Ash, calcium, phosphorus, potassium, tryptophan, α -tocopherol, linoleic acid (18:2), linolenic acid (18:3), ferulic acid, *p*-coumaric acid, phytic acid

Category II (equivalence more likely than not)

i. Not different ^f

In forage: Calcium

In grains: Copper, manganese, methionine

ii. Significantly different ^g

In grains: Oleic acid (18:1)

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
None

Not categorised

i. Not different ^h

In grains: Trypsin inhibitor

- ii. Significantly different
None

Categories reported in [Table 5](#) for IHT^c GA21 x T25 maize

Category I (equivalence demonstrated)

- i. Not differentⁱ

In forage: Moisture, protein, fat, ash, carbohydrates, ADF, NDF, phosphorus

In grains: Protein, carbohydrates, ADF, NDF, TDF, calcium, iron, magnesium, zinc, beta carotene, thiamine HCl, riboflavin, niacin, folic acid, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), arachidic acid (20:0), behenic acid (22:0), *p*-coumaric acid, inositol, raffinose

- ii. Significantly different^l

In grains: Fat, ash, starch, phosphorus, potassium, pyridoxine HCl, α -tocopherol, linoleic acid (18:2), linolenic acid (18:3), eicosenoic acid (20:1), ferulic acid, phytic acid

Category II (equivalence more likely than not)

- i. Not different^m

In forage: Calcium

In grains: Manganese

- ii. Significantly differentⁿ

In grains: Copper, oleic acid (18:1)

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
None

Not categorised

- i. Not different ^o

In grains: Trypsin inhibitor

- ii. Significantly different
None