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Assessment of genetically modified maize GA21 × T25 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2016-137)

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Abstract

Genetically modified maize GA21 × T25 was developed by crossing to combine two single events: GA21 and T25. The GMO Panel previously assessed the two single maize events and did not identify safety concerns. No new data on the single maize events were identified that could lead to modification of the original conclusions on their safety. The molecular characterisation, comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment indicate that the combination of the single maize events and of the newly expressed proteins in maize GA21 × T25 does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that maize GA21 × T25, as described in this application, is as safe as its conventional counterpart and the non-GM reference varieties tested, and no post-market monitoring of food and feed is considered necessary. In the case of accidental release of viable maize GA21 × T25 grains into the environment, this would not raise environmental safety concerns. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of maize GA21 × T25. Post-market monitoring of food and feed is not considered necessary. The GMO Panel concludes that maize GA21 × T25 is as safe as its conventional counterpart and the non-GM reference varieties tested, with respect to potential effects on human and animal health and the environment.

Keywords: GM, genetic engineering, maize (*Zea mays*), GA21, T25, PAT, mEPSPS

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Summary

Following the submission of application EFSA-GMO-DE-2016-137 under Regulation (EC) No 1829/2003 from Syngenta Crop Protection NV/SA (referred to hereafter as 'the applicant'), the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') was asked to deliver a Scientific Opinion on the safety of genetically modified (GM) herbicide-tolerant maize (*Zea mays*) GA21 × T25, according to Regulation (EU) No 503/2013. The scope of application EFSA-GMO-DE-2016-137 is for import, processing and food and feed uses within the European Union (EU) of maize GA21 × T25, and does not include cultivation in the EU. Maize GA21 × T25 was produced by crossing to combine two single maize events: GA21, expressing mEPSPS to confer tolerance to glyphosate-containing herbicides; T25, expressing PAT to confer tolerance to glufosinate ammonium-containing herbicides.

The GMO Panel evaluated maize GA21 × T25 with reference to the scope and appropriate principles described in its applicable guidelines for the risk assessment of GM plants and the post-market environmental monitoring. The GMO Panel considered the information submitted in application EFSA-GMO-DE-2016-137, additional information provided by the applicant during the risk assessment, the scientific comments submitted by the Member States and the relevant scientific literature. For application EFSA-GMO-DE-2016-137, previous assessments of the two single events (GA21 and T25) provided a basis for the assessment of maize GA21 × T25. No safety concerns were identified by the GMO Panel in the previous assessments. No safety issue concerning the two single maize events was identified by the updated bioinformatic analyses, nor reported by the applicant since the publication of the previous GMO Panel scientific opinions. Therefore, the GMO Panel considers that its previous conclusions on the safety of the single maize events remain valid.

For maize GA21 × T25, the risk assessment included the molecular characterisation of the inserted DNA and analysis of protein expression. An evaluation of the comparative analysis of agronomic, phenotypic and compositional characteristics was carried out, and the safety of the newly expressed proteins and the whole food and feed were evaluated with respect to potential toxicity, allergenicity and nutritional characteristics. Environmental impacts and post-market environmental monitoring (PMEM) plan were also evaluated. The molecular characterisation data establish that the events GA21 and T25 combined in maize GA21 × T25 have retained their integrity. Protein expression analysis showed that the levels of the newly expressed proteins are similar in maize GA21 × T25 and in the single events.

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic–phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis. The comparative analysis of agronomic and phenotypic characteristics and grain and forage composition identified no differences between maize GA21 × T25 and its conventional counterpart that required further assessment except for the change in early stand count. This change was further assessed for environmental impact and raised no concern. The molecular characterisation, the comparative analysis and the outcome of the toxicological, allergenicity and nutritional assessment indicate that the combination of the single maize events and of the newly expressed proteins in maize GA21 × T25 does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that maize GA21 × T25, is as safe as its conventional counterpart and the selected commercial non-GM maize reference varieties (referred to hereafter as non-GM reference varieties). Considering the combined events and their potential interactions, the outcome of the comparative analysis and the routes and levels of exposure, the GMO Panel concludes that maize GA21 × T25 would not raise safety concerns in the case of accidental release of viable GM maize grains into the environment.

Given the absence of safety concerns for foods and feeds from maize GA21 × T25, the GMO Panel considers that post-market monitoring (PMM) of these products is not necessary. The PMEM plan and reporting intervals are in line with the intended uses of maize GA21 × T25.

Considering the results of the literature searches, the GMO Panel does not identify any safety issue pertaining to the intended uses of maize GA21 × T25. The GMO Panel concludes that maize GA21 × T25, as described in this application, is as safe as its conventional counterpart and the non-GM reference varieties tested, with respect to potential effects on human and animal health and the environment.

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1. Introduction

The scope of the application EFSA-GMO-DE-2016-137 is for food and feed uses, import and processing of the genetically modified (GM) herbicide-tolerant maize GA21 × T25 and does not include cultivation in the European Union (EU).

1.1. Background

On 14 November 2016, the European Food Safety Authority (EFSA) received from the Competent Authority of Germany application EFSA-GMO-DE-2016-137 for authorisation of maize GA21 × T25 (Unique Identifier MON-ØØØ21-9 × ACS-ZMØØ3-2), submitted by Syngenta Crop Protection NV/SA (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003.[1] Following receipt of application EFSA-GMO-DE-2016-137, EFSA informed EU Member States (MS) and the European Commission, and made the application available to them. Simultaneously, EFSA published summary of the application.[2]

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003 and Regulation (EU) No 503/2013,[3] with the EFSA guidance documents, and, when needed, asked the applicant to supplement the initial application. On 23 February 2017, EFSA declared the application valid.

From validity date, EFSA and the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application EFSA-GMO-DE-2016-137. Such time limit was extended whenever EFSA and/or GMO Panel requested supplementary information to the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and European Commission (for further details, see the Section 'Documentation', below). In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC.[4] The EU Member States had 3 months to make their opinion known on application EFSA-GMO-DE-2016-137 as of date of validity.

1.2. Terms of reference as provided by the requestor

According to Articles 6 and 18 of Regulation (EC) No 1829/2003, EFSA and its GMO Panel were requested to carry out a scientific risk assessment of maize GA21 × T25 in the context of its scope as defined in application EFSA-GMO-DE-2016-137.

According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the EFSA overall opinion in accordance with Articles 6(5) and 18(5). In addition to the present scientific opinion, EFSA was also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No 1829/2003, but not to give an opinion on them because they pertain to risk management.[5]

2. Data and methodologies

2.1. Data

The GMO Panel based its scientific assessment of maize GA21 × T25 on the valid application EFSA-GMO-DE-2016-137, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by EU MS and literature searches. As part of this comprehensive information package, the GMO Panel received additional unpublished studies submitted

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.
[2] Available online: https://open.efsa.europa.eu/study-inventory/EFSA-Q-2016-00775.
3 Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L157, 8.6.2013, p. 1–48.
[4] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.
[5] These particulars are available online at: https://open.efsa.europa.eu/study-inventory/EFSA-Q-2016-00775.

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

2.2. Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 1829/2003, the applicable guidelines (i.e. EFSA GMO Panel, 2010a, 2011a,b, 2015a, 2017a; EFSA Scientific Committee, 2011) and explanatory notes and statements (i.e. EFSA, 2010, 2014, 2017, 2019a, 2019b; EFSA GMO Panel, 2010b) for the risk assessment of GM plants.

For this application, in the context of the contracts OC/EFSA/GMO/2018/02-lot 1, EOI/EFSA/SCIENCE/2020/01 – CT01GMO and OC/EFSA/GMO/2021/06, the contractors performed preparatory work for the evaluation of the methods applied for the statistical analysis of agronomic, phenotypic and composition, the 90-day toxicity study on the maize T25 and the bioinformatic analyses, respectively.

3. Assessment

3.1. Introduction

Maize GA21 × T25 was produced by crossing to combine two single maize events: GA21, expressing mEPSPS to confer tolerance to glyphosate-containing herbicides, T25, expressing PAT to confer tolerance to glufosinate ammonium-containing herbicides.

Both two single events were assessed previously (see Table 1) and no safety concerns were identified.

Table 1: Single maize events previously assessed by the GMO Panel

Events	Application or mandate	References
GA21	EFSA-GMO-UK-2005-19 and EFSA-GMO-RX-GA21 EFSA-GMO-UK-2008-60 EFSA-GMO-RX-005	EFSA (2007) EFSA GMO Panel (2011) EFSA GMO Panel (2017b)
T25	EFSA-GMO-RX-T25 and EFSA-GMO-NL-2007-46	EFSA GMO Panel (2013)

3.2. Updated information on GA21 and T25 events[6]

Since publication of the scientific opinions on GA21 and T25 maize events by the GMO Panel (see Table 1), no safety issue concerning the two single events has been reported by the applicant.

EFSA assessed updated nucleotide sequence information for maize event GA21 and confirmed that the conclusions of the original risk assessments of maize event GA21 remain valid (Table 1; EFSA GMO Panel, 2015b).

According to the Implementing Regulation EC 503/2013, sequencing information of the two events provided in the stack was assessed and confirmed the previously assessed sequences of the single events (see Section 3.4.2).

Updated bioinformatic analyses for events GA21 and T25 confirmed that no known endogenous genes were disrupted.

Updated bioinformatic analyses of the amino acid sequence of the newly expressed mEPSPS and PAT proteins confirmed previous results indicating no significant similarities to known toxins and allergens. Updated bioinformatic analyses of the newly created open reading frames (ORFs) within the inserts or spanning the junctions between the insert and the flanking regions for events GA21 and T25 confirmed previous analyses (see Table 1). These analyses indicate that the production of a new peptide showing significant similarities to toxins or allergens for any of the events in maize GA21 × T25 is highly unlikely.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination, the applicant performed a sequence identity analysis with microbial DNA for maize events GA21 and T25. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.7.1.2.

[6] Dossier: Part II – Section 1.2; additional information: 3/7/2019, 18/8/2022, 18/11/2022.

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

3.3. Systematic literature review[7]

The GMO Panel assessed the applicant's literature searches on maize GA21 × T25, which include a scoping review, according to the guidelines given in EFSA (EFSA, 2010, 2019b).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application EFSA-GMO-DE-2016-137. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for maize GA21 × T25 at present.

The GMO Panel considered the overall quality of the performed literature searches acceptable. The literature searches did not identify any relevant publications on maize GA21 × T25. Considering the result of the literature searches, the GMO Panel does not identify any safety issues pertaining to the intended uses of maize GA21 × T25.

3.4. Molecular characterisation[8]

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

3.4.1. Genetic elements and biological function of the inserts

Maize events GA21 and T25 were combined by crossing to produce maize GA21 × T25. The structure of the inserts introduced into maize GA21 × T25 is described in detail in the respective EFSA scientific opinions (see Table 1) and no new genetic modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 2.

Intended effects of the inserts in maize GA21 × T25 are summarised in Table 3. Based on the known biological function of the newly expressed proteins (see Table 3), no interactions at biological level are foreseen.

Table 2: Genetic elements in the expression cassettes of the events stacked in maize GA21 × T25

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
GA21	Actin 1 (*Oryza sativa*)	Actin 1 (*Oryza sativa*)	OTP (*Helianthus annuus*)	mEPSPS (*Zea mays*)	*nos* (*Agrobacterium tumefaciens*)
T25	35 S (CaMV)	–	–	pat (*Streptomyces viridochromogenes*)	35 S (CaMV)

CaMV: cauliflower mosaic virus; UTR: untranslated region.
–: when no element was specifically introduced to optimise expression or to target proteins to subcellular compartments.

Table 3: Characteristics and intended effects of the events stacked in maize GA21 × T25

Event	Protein	Donor organism and biological function	Intended effects in GM plant
GA21	mEPSPS	Based on a gene from *Zea mays*. 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Hermann, 1995).	The amino acid sequence of the maize EPSPS enzyme was modified to render the maize tolerant to glyphosate. Expression of mEPSPS confers tolerance to glyphosate-based herbicides (Lebrun et al., 2003).

[7] Dossier: Part II – Section 7; additional information: 7/12/2018, 21/3/2019 and 1/9/2022.
[8] Dossier: Part II – Section 1.2; additional information: 29/05/2017, 21/12/2017, 20/02/2018, 3/7/2019, 18/8/2022, 18/11/2022.

Event	Protein	Donor organism and biological function	Intended effects in GM plant
T25	PAT	Based on a gene from *Streptomyces viridochromogenes* strain Tü494. Phosphinothricin-acetyl-transferase (PAT) acetylates L-glufosinate-ammonium (Wohlleben et al., 1988).	T25 maize expresses a synthetic version of the PAT protein, which inactivates glufosinate-ammonium, rendering the plant tolerant to the herbicide (Droge-Laser et al., 1994).

3.4.2. Integrity of the events in maize GA21 × T25

The genetic stability of the inserted DNA over multiple generations in the single maize events GA21 and T25 was demonstrated previously (see Table 1 and Section 3.2). Integrity of these events in maize GA21 × T25 was demonstrated by Southern analyses followed by sequence analysis, showing that the sequences of the events (inserts and their flanking regions) in maize GA21 × T25 are identical to the sequences already assessed (see Table 1 and Section 3.2), thus confirming that the integrity of these events was maintained in maize GA21 × T25.

3.4.3. Information on the expression of the insert

Protein levels of mEPSPS and PAT were analysed by an enzyme-linked immunosorbent assay (ELISA) in material harvested in a field trial across three locations in the United States during the 2012 growing season. Samples analysed included leaf (BBCH 16 and BBCH 63–65), root (BBCH16 and BBCH 63–65), whole plant (BBCH 16 and BBCH 63–65), pollen (BBCH 63–65) and grains (BBCH 87–99 and senescence) from plants not treated with intended herbicides.[9]

In order to assess the changes in protein expression levels which may result from potential interactions between the events, protein levels were determined for maize GA21 × T25 and the corresponding single events in different parts of the plant.

The levels of the newly expressed proteins in maize GA21 × T25 and the corresponding singles were comparable in all tissues (Appendix B). Therefore, there is no indication of an interaction that may affect the levels of the newly expressed proteins in this stack.

3.4.4. Conclusion on molecular characterisation

The molecular data establish that the events stacked in maize GA21 × T25 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in maize GA21 × T25 and in the single events. Therefore, there is no indication of an interaction that may affect the integrity of the events and the levels of the newly expressed proteins in this stack.

Based on the known biological function (see Table 3) of the newly expressed proteins, no foreseen interaction at biological level is expected.

3.5. Comparative analysis[10]

3.5.1. Overview of studies conducted for the comparative analysis

Application EFSA-GMO-DE-2016-137 presents data on agronomic and phenotypic characteristics as well as on forage and grain composition of maize GA21 × T25 (see Table 4). In addition, the application contains data on characteristics of seed from maize GA21 × T25.

[9] BBCH scale describes phenological stages (Meier, 2001). BBCH 16, BBCH 63–65 and BBCH 87–99 correspond to approximately V6, R1 and R6 stages of maize development, respectively.

[10] Dossier: Part II – Section 1.3; additional information: 31/5/2017, 26/9/2017, 30/10/2017, 9/7/2018 and 3/7/2019.

Table 4: Main comparative analysis studies to characterise maize GA21 × T25 provided in application EFSA-GMO-DE-2016-137

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic and phenotypic analysis	Field study, USA, 2012 and 2016, thirteen field trial sites[(a)]	NP2460 × NP2171	6[(b)]
Compositional analysis	Field study, USA, 2012, eight field trial sites[(c)]		6[(b)]

GM: Genetically modified.

(a): A field trial site is a combination location/year. There was a total of 11 locations, two of which were used in two different seasons. Eight locations were used only in 2012: Atlantic, IA; Seymour, IL; York, NE; Delavan, WI; Bagley, IA; Larned, KS; Hereford, PA; and Wyoming, IL. Two locations were used both in 2012 and 2016: Stewardson, IL and Richland, IA. A location was used only in 2016: Germansville, PA. Two field trials were compromised by extreme weather events and partially removed from the study: for Stewardson, IL (2012), the data were collected only prior to pollen shed and silking because of a severe drought; for Hereford, PA (2012), the endpoints collected at harvest were discarded because of high grain moisture, likely caused by an early frost.

(b): The non-GM reference varieties used in the 2012 field trials were the hybrids: H-6044, NK Symba, NK Thermo, X36344, H-7191 and H-7540. The non-GM reference varieties used in the 2016 field trials were the hybrids: NK Thermo, X36344 and H-7191.

(c): The field trials were conducted in 2012 in: Atlantic, IA; Richland, IA; Seymour, IL; York, NE; Delavan, WI; Bagley, IA; Larned, KS; and Wyoming, IL.

3.5.2. Experimental field trial design and statistical analysis

At each field trial site, the following materials were grown in a randomised complete block design with four replicates: maize GA21 × T25 not exposed to the intended herbicides, maize GA21 × T25 exposed to the intended herbicides, the comparator maize NP2460 × NP2171 and six non-GM reference varieties.

The agronomic/phenotypic and compositional data were analysed as specified by GMO Panel (EFSA GMO Panel, 2010b, 2011a). This includes, for each of the two treatments of maize GA21 × T25, the application of a difference test (between the GM maize and the comparator) and an equivalence test (between the GM maize and the set of non-GM reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[11]

3.5.3. Suitability of selected test materials

3.5.3.1. Selection of the test materials

To obtain maize GA21 × T25, the single events GA21 and T25 were introgressed in the genetic background of the non-GM maize inbred lines NP2460 and NP2171, respectively, before crossing.

The comparator used in the studies was obtained by crossing the non-GM maize inbred lines NP2460 and NP2171. As documented by the pedigree, the GMO Panel considers the selected comparator the conventional counterpart for the comparative analysis.

Maize GA21 × T25 and its conventional counterpart, both with a comparative relative maturity (CRM) ranging from 105 to 107, are appropriate for growing in a range of environments across North America.

Non-GM reference varieties with a CRM ranging from 79 to 102 were selected by the applicant. Six reference varieties were grown at each site of the field trials conducted in 2012, whereas three were grown at each site of the 2016 field trials (see Table 4). The GMO Panel noted that the CRM of the selected non-GM reference varieties was lower (i.e. the growing cycle was shorter) than the optimal range for the chosen sites. The lower CRM of the non-GM reference varieties was accepted by the GMO Panel based on the specific management practices (late sowing) applied at the field trial sites (see Section 3.5.4.3).

3.5.3.2. Seed production and quality

The seeds of maize GA21 × T25 and its conventional counterpart used in the field trials (see Table 4) were produced from plants free of diseases, harvested and stored under similar conditions.

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

The seed lots were verified for their purity via event-specific quantitative polymerase chain reaction analysis. The seeds were tested for their germination capacity at six different temperatures.[12] Maize GA21 × T25, its conventional counterpart and three additional non-GM reference varieties[13] were compared for germination capacity[14] and the results of these studies indicate that the seed germination of maize GA21 × T25 is not different than that of its conventional counterpart and of the non-GM reference varieties. The GMO Panel considers that the starting seed used as test material in the agronomic, phenotypic and compositional studies was of suitable quality.

3.5.3.3. Conclusion on suitability

The GMO Panel is of the opinion that maize GA21 × T25 and its conventional counterpart were properly selected and are of sufficient quality. Therefore, these test materials are considered suitable for the comparative analysis. The selected non-GM reference varieties were assessed considering the specific management practices applied at the field trials sites (see Section 3.5.4.3).

3.5.4. Representativeness of the receiving environments

3.5.4.1. Selection of field trial sites

The selected field trials sites were located in commercial maize-growing regions of United States. The soil types of the selected fields were diverse,[15] representing regions of diverse environmental conditions for maize cultivation (Sys et al., 1993). The GMO Panel considers that the selected sites, including the subset chosen for the compositional analysis, reflect commercial maize-growing regions in which the test materials are likely to be grown.

3.5.4.2. Meteorological conditions

Maximum and minimum mean temperatures and sum of precipitations were provided on a monthly basis. Some exceptional weather conditions were reported at three of the selected sites.[16] However, due to the lack of major impacts on plant growth at these sites, the GMO Panel considers that the exceptional weather conditions did not invalidate the selection of the field trial sites for the comparative analyses. In addition, some extreme weather events were recorded at two of the selected sites.[17] The quality of the field trial sites was compromised; thus, only the endpoints collected before the occurrence of the extreme weather events were included in the statistical analysis.

3.5.4.3. Management practices

The field trials included plots containing maize GA21 × T25, plots with its conventional counterpart and plots with non-GM reference varieties, managed according to local agricultural practices. In addition, the field trials included plots containing maize GA21 × T25 managed following the same agricultural practices, plus exposed to the intended glufosinate ammonium- and glyphosate-containing herbicides. The glufosinate ammonium-containing herbicide was applied at BBCH 15–16[18] growth stage and glyphosate-containing herbicide at BBCH 17–18 growth stage.[19]

At some field trial sites,[20] sowing occurred relatively later than usual, close to the limit of the typical range, resulting in a shorter and/or shifted growing cycle. The additional information indicated that the shorter and/or shifted growing cycle was unlikely to affect the representativeness of field trial

[12] Tested temperature regime: continuous temperature of 10°C, 25°C and 30°C and three cyclical temperatures of 16 and 8 h at 10°C/20°C, 10°C/30°C and 20°C/30°C.
[13] Maize non-GM reference hybrids: NK Octet, NK Lucius and Cisko.
[14] GM hybrid maize GA21 × T25 showed a mean germination of 99.3%, 99.5%, 99.5%, 100%, 99%, 99.5% while its conventional counterpart showed a mean of 99.3%, 99.8%, 99.3%, 99.5%, 99.5%, 99.3% while the non-GM reference hybrids showed a mean of 85%, 99%, 99.8%, 98.1%, 99.8%, 99.2% at constant 10°C, constant 25°C, constant 30°C, alternating 10°C/20°C, alternating 10°C/30°C, alternating 20°C/30°C.
[15] Soil types of the field trials were clay loam, silty clay loam, loam and silt loam; average temperatures and sum of precipitations during the usual crop growing season ranged, respectively, from 18.0°C to 24.5°C and from 185.0 mm to 785.1 mm.
[16] Winds associated with storms occurred at Atlantic, Iowa and Hereford, Pennsylvania in 2012 and at Stewardson, Illinois in 2016.
[17] Two field trials established in Stewardson, Illinois and Hereford, Pennsylvania in 2012 were compromised, respectively, by severe drought prior to pollen shed and silking, and high grain moisture at harvest likely due to an early frost.
[18] BBCH scale describes phenological stages (Meier, 2001) and BBCH 15–16 corresponds to approximately V3–V4 stages of maize development.
[19] BBCH 17–18 corresponds to approximately V5–V6 stages of maize development
[20] Three field trials located in Atlantic, Iowa; Seymour, Illlinois; and Wyoming, Illinois.

conditions. In light of the delayed sowing of the test materials, the GMO Panel considers that despite the difference in CRM between maize GA21 × T25, its conventional counterpart and the non-GM reference varieties (see Section 3.5.3.1), the test materials can be considered acceptable. Indeed, the shorter growing cycle combined with a relatively late sowing resulted in a similar crop development (e.g. time to pollen shed) of the test materials at the selected sites.

The GMO Panel considers that the management practices including sowing, harvesting and application of plant protection products were acceptable for the selected receiving environments.

3.5.4.4. Conclusion on representativeness

The GMO Panel concludes that the geographical locations, soil types, meteorological conditions and most of the management practices are typical for receiving environments where the tested materials could be grown.

3.5.5. Agronomic and phenotypic analyses

Nine agronomic and phenotypic endpoints[21] plus information on abiotic stressors, disease incidence and insect damage were collected from the field trials (see Table 4).

The endpoint 'total lodged plants' was not analysed with formal statistical methods (see Section 3.5.2) because of lack of variability in the data.

The statistical analysis (see Section 3.5.2) was applied to eight endpoints, with the following results:

- For maize GA21 × T25 (treated with conventional herbicides), the test of difference identified statistically significant differences with its conventional counterpart for early stand count and final stand count. The endpoint 'early stand count' fell under equivalence category III,[22] while final stand count fell under category I.
- For maize GA21 × T25 (treated with intended herbicides), the test of difference identified a statistically significant difference for days to 50% pollen shed, which fell under equivalence category I.

Early stand count for maize GA21 × T25 (treated with conventional herbicides) was reduced with respect to its conventional counterpart and the non-GM reference varieties, which could indicate a differential establishment of the GM maize. As no further impact on the subsequent crop development endpoints was observed, the GMO Panel considers that this possible differential establishment does not affect the use of the field trials for the comparative analysis. Whether the difference can lead to an environmental adverse effect is considered in Section 3.7.1.1.

3.5.6. Compositional analysis

Forage and grains of maize GA21 × T25 harvested from eight sites (see Table 4) were analysed for 82 constituents (nine in forage and 73 in grains), including those recommended by OECD (OECD, 2002). The statistical analysis as described in Section 3.5.2 was not applied to 16 grain constituents because their concentration in more than half of the samples was below the limit of quantification.[23] Additionally, moisture levels in grains were not compared since ears were dried in the field or mechanically after being harvested.

[21] 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.

[22] Estimated mean values for early stand count (plants/m^2) were 8.36 (conventional counterpart) and 7.84 (GM maize/treated). The equivalence limits were (7.89, 8.73).

[23] Selenium, sodium, furfural and 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), pentadecenoic acid (15:1), heptadecanoic acid (17:0), heptadecenoic acid (17:1), γ-linolenic acid (18:3), eicosadienoic acid (20:2), eicosatrienoic acid (20:3) and arachidonic acid (20:4)).

The statistical analysis was applied to a total of 65 constituents (56 in grains[24] and nine in forage[25]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 5:

- For maize GA21 × T25 not treated with the intended herbicides, statistically significant differences with its conventional counterpart were found for 12 endpoints in grain. All these endpoints fell under equivalence category I or II.
- For maize GA21 × T25 treated with the intended herbicides, statistically significant differences with its conventional counterpart were found for 14 endpoints in grain. All these endpoints fell under equivalence category I or II.

Table 5: Outcome of the comparative compositional analysis in grain and forage for maize GA21 × T25. The table shows the number of endpoints in each category

		Test of difference[a]			
		Not treated[c]		Treated[c]	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence[b]	Category I/II	52	12[d]	50	14[d]
	Category III/IV	–	–	–	–
	Not categorised	1[e]	–	1[e]	–
	Total endpoints	65		65	

(a): Comparison between maize GA21 × T25 and its conventional counterpart.
(b): Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.
(c): Treated/not treated with the intended herbicides.
(d): Endpoints with significant differences between maize GA21 × T25 and its conventional counterpart falling in equivalence category I–II (treated and not treated). For forage, both treated and not treated: none. For grains, not treated only: Ash, calcium, phosphorus, potassium, α-tocopherol, tryptophan, oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), ferulic acid, p-coumaric acid and phytic acid. Treated: fat, ash, starch, phosphorus, potassium, pyridoxine, α-tocopherol, oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), eicosenoic acid (20:1), ferulic acid, phytic acid and copper.
(e): Endpoints not categorised for equivalence and without significant differences between maize GA21 × T25 and its conventional counterpart: For forage: none. For grains, both treated and untreated: trypsin inhibitor.

The GMO Panel assessed all the significant differences between maize GA21 × T25 and its conventional counterpart, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. No endpoints were identified that showed significant differences between maize GA21 × T25 and its conventional counterpart and fell under category III/IV.

3.5.7. Conclusion on comparative analysis

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

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

- None of the differences identified in agronomic and phenotypic characteristics between maize GA21 × T25 and its conventional counterpart needs further assessment regarding their

[24] Grain constituents included 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 (β-carotene, thiamine, riboflavin, niacin, pyridoxine, folic acid and α-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 (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)) and other compounds (ferulic acid, p-coumaric acid, inositol, phytic acid, trypsin inhibitor and raffinose).
[25] Forage constituents included moisture, protein, fat, ash, carbohydrates, acid detergent fibre (ADF), neutral detergent fibre (NDF), calcium and phosphorus.

potential environmental impact except for early stand count, which is further assessed in Section 3.4.4.
- None of the differences identified in forage and grain composition between maize GA21 × T25 and its conventional counterpart needs further assessment regarding food and feed safety.

3.6. Food/feed safety assessment[26]

3.6.1. Effects of processing

Maize GA21 × T25 will undergo existing production processes used for conventional crop. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of maize GA21 × T25 into food and feed products is not expected to result in products being different from those of conventional non-GM maize varieties.

3.6.2. Stability of newly expressed proteins

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

3.6.2.1. Effect of temperature and pH on newly expressed proteins

The effects of temperature and pH on the mEPSPS and PAT proteins have been previously evaluated by the GMO Panel (see Table 1). No new information has been provided in the context of this application.

3.6.2.2. *In vitro* protein degradation by proteolytic enzymes

The resistance to degradation by pepsin of the newly expressed mEPSPS and PAT proteins have been previously evaluated by the GMO Panel (see Table 1). No new information has been provided in the context of this application.

3.6.3. Toxicology

3.6.3.1. Testing of newly expressed proteins

Two proteins (mEPSPS and PAT) are newly expressed in maize GA21 × T25 (see Section 3.4.1).

The GMO Panel has previously assessed these proteins in the context of the single maize events (see Table 1), and no safety concerns were identified for humans and animals (i.e. farmed and companion animals). The GMO Panel is not aware of any new information that would change these conclusions. The potential for a functional interaction among the proteins newly expressed in maize GA21 × T25 has been assessed with regard to human and animal health.

The two enzymatic proteins catalyse distinct biochemical reactions, acting on unrelated substrates, and are not expected to interact.

The mEPSPS protein confers tolerance to glyphosate-containing herbicides acting on the shikimic acid pathway for the biosynthesis of aromatic amino acids in plants. The PAT protein confers tolerance to glufosinate ammonium-containing herbicides, acting by acetylation of glufosinate ammonium.

On the basis of the known biological function of the individual newly expressed proteins, there is currently no expectation for possible interactions relevant to the food and feed safety of this maize GA21 × T25.

The GMO Panel concludes that there are no safety concerns to human and animal health related to the newly expressed proteins mEPSPS and PAT in maize GA21 × T25.

[26] Dossier: Part II – Sections 1.4 and 2; additional information: 26/9/2017, 9/7/2018, 6/8/2018, 29/11/2018, 4/2/2020, 31/3/2021, 18/8/2022, 31/8/2022.

3.6.3.2. Testing of new constituents other than proteins

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no new constituents other than the newly expressed proteins have been identified in grain and forage from maize GA21 × T25. Therefore, no further food and feed safety assessment of components other than the newly expressed proteins is required.

3.6.3.3. Information on altered levels of food and feed constituents

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, none of the differences identified between maize GA21 × T25 and its conventional counterpart in grain and forage composition require further assessment.

3.6.3.4. Testing of the whole genetically modified food and feed

Based on the outcome of the molecular characterisation, comparative analysis and toxicological assessment, no indication of findings relevant to food and feed safety related to the stability and expression of the inserts or to interaction between the transformation events, and no modifications of toxicological concern in the composition maize GA21 × T25 have been identified (see Sections 3.4, 3.5 and 3.6.3). Therefore, animal studies with food/feed derived from maize GA21 × T25 are not considered necessary by the GMO Panel (EFSA GMO Panel, 2011a).[27]

In accordance with Regulation (EU) No 503/2013, the applicant provided a 90-day feeding study on whole food/feed from each of the maize single events composing maize GA21 × T25.

The two studies were conducted with two upper limit doses of 50%[28] and 41.5%.[29] Since 2019, a 50% maize incorporation rate is used as the high dose (EFSA, 2014; Steinberg et al., 2019, 2020; EFSA GMO Panel, 2021b,c, 2022a,b,c). While the GMO Panel is reviewing the evidence regarding test diets incorporating up to 50% and the potential to induce nutritional imbalance, currently the Panel considers that the upper limit of 41.5% is acceptable for the existing study.[30]

90-day feeding study on maize T25

A 90-day study on maize T25 has been previously assessed by the GMO Panel in the context of the single-event renewal application dossier (EFSA GMO Panel, 2013), and was not considered adequate because of the low number of experimental units per treatment (two cages per sex with five animals per cage), reducing the power of the statistical analysis; moreover, the study was conducted with grains which were harvested from T25 maize plants not treated with the intended herbicide; upon EFSA's request to fulfil the requirements of Regulation (EU) No 503/2013, the applicant provided a new 90-day toxicity study on T25 maize.[31] The GMO Panel has previously assessed this study (EFSA GMO Panel, 2021c) and concluded that it is in line with Regulation (EU) No 503/2013 and that no treatment-related adverse effects were observed in rats after feeding diets including 50% grains from T25 maize for 90 days.

90-day feeding study on maize GA21

A 90-day study on maize GA21 performed in 2005 has been previously assessed by the GMO Panel in the context of the single-event applications (EFSA, 2007; EFSA GMO Panel, 2011) and no adverse effects related to the administration of the GM diet had been identified. In the context of the assessment of this maize GA21 × T25, EFSA asked for additional information to confirm the adherence of this study to requirements of Regulation (EU) 503/2013, OECD TG 408 (OECD, 1998), EFSA

[27] A 90-day feeding study on whole food/feed from maize GA21 × T25 was provided. The study was considered by the GMO Panel. The GMO Panel noted that the submitted study report contained limited details about the materials and methods used for the statistical analysis and on the production of the test diets. As the study was not a requirement for the EU, clarification of the limitations was not sought. On evaluation of the available information, no treatment-related adverse effects were identified (see Appendix A).

[28] 90-day feeding study with maize T25 was completed in 2021.

[29] 90-day feeding study with maize GA21 was completed in 2005.

[30] Recent work (e.g. Steinberg, 2019; 2020) indicates that an acceptable upper limit for incorporation of maize into rodent diets is 50%. Many rodent studies evaluated by the GMO Panel were performed prior to 2019 and used upper incorporation rates of 33% or 41.5%. The GMO Panel considers that a 1.5-fold or 1.2-fold increase in incorporation rate is unlikely to identify any new hazards in the context of this application, and therefore there is no reason to repeat these older studies using the new upper incorporation rate of 50%. This approach is consistent with Directive 2010/63/EU on the protection of animals used for scientific purposes.

[31] Additional information 31/3/2021 and 18/8/2022; the same info was received in EFSA-GMO-NL-2019-164 as additional information 28/06/2021, and assessed by the GMO Panel.

Scientific Committee Guidance (2011) and EFSA (2014). The applicant provided details on the appropriateness of the test and control materials and on the experimental design, together with additional statistical analyses. The GMO Panel concludes that this study is in line with the legal requirements and confirms the original conclusions that there are no indications of adverse effects related to the 90-day administration to rats of diets including grains from maize GA21, up to 41.5% of inclusion rate.

3.6.4. Allergenicity

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

3.6.4.1. Assessment of allergenicity of the newly expressed proteins

The GMO Panel has previously evaluated the safety mEPSPS and PAT proteins individually, and no evidence of allergenicity was identified in the context of the applications assessed (see Table 1). No new information on allergenicity of the proteins newly expressed in this maize GA21 × T25 that might change the previous conclusions of the GMO Panel has become available. Based on the current knowledge, and as there is no evidence of allergenicity of the newly expressed proteins, there are no expected concerns of allergenicity as a consequence of their interaction in this maize GA21 × T25.

The GMO Panel has previously evaluated the safety of the newly expressed proteins, and no evidence of adjuvanticity was identified in the context of the applications assessed (see Table 1). The GMO Panel did not find indications that the proteins at the levels expressed in this maize GA21 × T25 might be adjuvants able to enhance an allergic reaction.

Furthermore, the applicant provided information on the safety of the mEPSPS and PAT proteins regarding their potential hazard to cause a celiac disease response. For such assessment, the applicant followed the principles described in the EFSA GMO Panel guidance document (EFSA GMO Panel, 2017a). The assessment of the mEPSPS protein identified no perfect or relevant partial matches with known celiac disease peptide sequences. The assessment of the PAT protein revealed partial matches containing the Q/E-X1-P-X2 motif and required further investigations. Based on additional considerations on position and nature of amino acids flanking the ELPA motif, such as the presence of two consecutive prolines and the charge and size of adjacent amino acids (EFSA GMO Panel, 2017a), the two relevant peptides containing the motif do not raise concern as they fail to mimic gluten sequences. Therefore, no indications of safety concerns were identified by the GMO Panel.

3.6.4.2. Assessment of allergenicity of the whole GM plant

The GMO Panel regularly reviews the available publications on food allergy to maize. However, maize is not considered a common allergenic food[32] (OECD, 2002). Therefore, the GMO Panel does not request experimental data to analyse the allergen repertoire of GM maize. In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (see Sections 3.4, 3.5 and 3.6), the GMO Panel identifies no indications of a potentially increased allergenicity of food and feed derived from this maize GA21 × T25 with respect to that derived from its conventional counterpart and the non-GM reference varieties tested.

3.6.5. Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013, the applicant provided dietary exposure estimates to mEPSPS and PAT proteins newly expressed in maize GA21 × T25. Dietary exposure was estimated based on protein expression levels reported in this application for maize GA21 × T25 treated with the

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

intended herbicides, the current available consumption data and feed practices, the foods and feeds currently available in the market and the described processing conditions.

For the purpose of estimating dietary exposure, the levels of newly expressed proteins in maize GA21 × T25 grains, forage and pollen were derived from replicated field trials (four replicates from three locations, n = 12) in the United States in 2021. Table 6 describes the protein expression levels used to estimate both human and animal dietary exposure.

Table 6: Mean values (n = 12, μg/g fresh weight) for mEPSPS and PAT proteins in grains, forage and pollen from GA21 × T25 maize treated with the intended herbicides[(a)]

Protein	Tissue/developmental stage		
	Grains/ BBCH 87–99 (μg/g fresh weight)	Pollen/BBCH 63–65 (μg/g dry weight)[(b)]	Forage/ BBCH 85 (μg/g fresh weight)
mEPSPS	8.61	< 37.5[(d)]	6.77
PAT[(c)]	0.217	< 0.240[(d)]	4.46

(a): Intended herbicides: glyphosate- and glufosinate ammonium-containing herbicides.
(b): Concentrations values in pollen were adjusted to 6% moisture content before using them to estimate dietary exposure to the different newly expressed proteins via the consumption of pollen supplements.
(c): In accordance with EFSA statement (EFSA, 2019a), the greatest means of NEP concentrations among growth stages of kernels were used to estimate dietary exposure. For PAT protein, the selected growth stage was BBCH 99.
(d): For PAT and mEPSPS proteins, all samples were reported as below LOD (0.240 μg/g dry weight and 37.5 μg/g dry weight, respectively).

3.6.5.1. Human dietary exposure

Chronic and acute estimations of dietary exposure to mEPSPS and PAT proteins newly expressed in maize GA21 × T25 were provided. The applicant followed the methodology described in the EFSA Statement 'Human dietary exposure assessment to newly expressed protein in GM foods' (EFSA, 2019a) to estimate human dietary exposure in average and high consumers making use of summary statistics of consumption.

Human dietary exposure was estimated across different European countries on different population groups: young population (infants, toddlers, 'other children'), adolescents, adult population (adults, elderly and very elderly) and special populations (pregnant and lactating women). Since no specific consumption data were available on commodities containing, consisting of or obtained from maize GA21 × T25 grains, a conservative scenario with 100% replacement of conventional maize by the GM maize was considered. Consumption figures for all relevant commodities (e.g. corn flakes, sweet corn, popcorn, etc.) were retrieved from the EFSA Comprehensive European Food Consumption Database (EFSA consumption database).[33] Corn oil, corn starch and corn syrup were excluded from the assessment since no proteins are expected to be present in these commodities.

Mean protein expression values on fresh weight basis are considered as the most adequate to estimate human dietary exposure (both acute and chronic) when working with raw primary commodities that are commonly consumed as processed blended commodities (EFSA, 2019a). Different recipes and factors were considered to estimate the amount of maize in the consumed commodities before assigning newly expressed protein levels to the relevant commodities.[34] No losses in the newly expressed proteins during processing were considered, except for the commodities mentioned above.

The highest acute dietary exposure (high consumers) was estimated in the age class 'Other children' with exposure estimates of 3.3 μg/kg body weight (bw) per day for PAT protein and 130.8 μg/kg bw per day for mEPSPS protein. The main contributor to the exposure in the dietary survey with the highest estimates was corn grains.

The highest chronic dietary exposure (high consumers) was estimated in the age class 'Infants', with exposure estimates of 1.8 μg/kg bw per day for PAT protein and 70.3 μg/kg bw per day for mEPSPS protein. The main contributor to the exposure in the dietary survey with the highest estimates was corn flakes.

[33] https://www.efsa.europa.eu/en/applications/gmo/tools. From version updated in March 2022.
[34] Example: 100 g of maize bread is made with approximately 74 g of maize flour, and a reverse yield factor of 1.22 from the conversion of maize grains into flour is used. This results in ~7.8 μg of mEPSPS per gram of maize bread as compared to the 8.61 μg/g reported as mean concentration in the maize grains.

An ad hoc dietary exposure scenario was provided for consumers of pollen supplements under the assumption that these supplements might be made of pollen from maize GA21 × T25 with expression levels at the respective LODs. Consumption data on pollen supplements are available for few consumers across seven different European countries.[32] The low number of consumers available adds uncertainty to the exposure estimations which should be interpreted with care, and it prevents from estimating exposure for high consumers of pollen supplements. In average consumers of pollen supplements, the highest acute dietary exposure would be 0.17 μg/kg bw per day for PAT protein and 26.1 μg/kg bw per day for mEPSPS protein, in the elderly population. Similarly, the highest chronic dietary exposure in average consumers would be 0.11 μg/kg bw per day for PAT protein to 17.4 μg/kg bw per day for mEPSPS protein, also in the elderly population.

3.6.5.2. Animal dietary exposure

Dietary exposure to mEPSPS and PAT proteins in maize GA21 × T25 was estimated across different animal species, as below described, assuming the consumption of maize products commonly entering the feed supply chain (i.e. maize grains, gluten feed, gluten meal, milled by-products, hominy meal, forage/silage and stover). A conservative scenario with 100% replacement of conventional maize products by maize GA21 × T25 products was considered.

Mean levels (fresh weight) of the newly expressed proteins in grain and forage from maize GA21 × T25 treated with the intended herbicides were used for animal dietary exposure are listed in Table 6.

Mean levels (fresh weight) of the newly expressed proteins in maize gluten feed and gluten meal, hominy meal and milled by-products were calculated to be, respectively, 2.13-, 6.38-, 1.18- and 0.894-fold than in grain, and in maize stover 0.861-fold than in forage, based on adjusting factors that take into account the protein content in these feed materials relative to maize grain and forage (see Appendix C – Table C.1), and assuming that no protein is lost during their production/processing.

The applicant estimated dietary exposure to mEPSPS and PAT proteins via the consumption of maize grains, gluten feed, gluten meal, milled by-products, hominy meal, forage/silage and stover, based on default values for animal body weight, daily feed intake and inclusion rates (percentage) of maize feedstuffs in diets/rations, as provided for the EU by OECD (2013). The total theoretical maximum contribution to the highest exposure to mEPSPS and PAT proteins was taken into account for each feedstuff, according to the *reasonable worst case diet/feed* (RWCF) approach described by OECD (2013).[35]

Estimated dietary exposure in the concerned animals is reported in Appendix C (see Table C.2).

3.6.6. Nutritional assessment of endogenous constituents

The intended trait of maize GA21 × T25 is herbicide tolerance, with no intention to alter nutritional parameters. Comparison of the composition of maize GA21 × T25 with its conventional counterpart and the non-GM reference varieties tested did not identify differences that would require further safety assessment. From these data, the GMO Panel concludes that maize GA21 × T25 is nutritionally equivalent to its conventional counterpart and the non-GM reference varieties used.

3.6.7. Post-market monitoring of GM food/feed

The GMO Panel concluded that maize GA21 × T25, as described in this application, does not raise any nutritional concern and is as safe as its conventional counterpart and the non-GM reference varieties tested. Therefore, the GMO Panel considers that post-market monitoring of food and feed from this GM maize, as described in this application, is not necessary.

3.6.8. Conclusions on the food/feed safety assessment

The GMO Panel did not identify indications of safety concerns regarding toxicity, allergenicity or adjuvanticity related to the presence of the newly expressed proteins (mEPSPS and PAT) in maize GA21 × T25, or regarding the overall toxicity and allergenicity of this GM maize. No interaction between the newly expressed proteins relevant for food and feed safety were identified. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of maize GA21 × T25 does not represent any nutritional concern, in the context of

[35] A full description of the model applied was provided in the study report #RIR-0010899

this application. The GMO Panel concludes that maize GA21 × T25 is nutritionally equivalent to, and as safe as, its conventional counterpart and the non-GM reference varieties tested, and no post-market monitoring of food and feed is considered necessary.

3.7. Environmental risk assessment and monitoring plan[36]

3.7.1. Environmental risk assessment

Considering the scope of the application EFSA-GMO-DE-2016-137, which excludes cultivation, the environmental risk assessment (ERA) of maize GA21 × T25 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable maize GA21 × T25 grains during transportation and/or processing (EFSA GMO Panel, 2010a).

3.7.1.1. Persistence and invasiveness of the GM plant

Maize is highly domesticated, not winter hardy in colder regions of Europe, and generally unable to survive in the environment without appropriate management. Survival is limited mainly by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens, herbivores and cold climate conditions (OECD, 2003), even though occasional feral GM maize plants may occur outside cultivation areas in the EU (e.g. Pascher, 2016). Field observations indicate that maize grains may survive and overwinter in some EU regions, resulting in volunteers in subsequent crops (e.g. Gruber et al., 2008; Palaudelmàs et al., 2009; Pascher, 2016). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). Thus, the establishment and survival of feral and volunteer maize in the EU is currently limited and transient.It is unlikely that the intended traits of maize GA21 × T25 and the observed reduction in early stand count (see Section 3.5.5) will provide a selective advantage to maize plants, except when they are exposed to glyphosate- and glufosinate ammonium-containing herbicides. However, this fitness advantage will not allow maize GA21 × T25 to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits and the observed difference in early stand count will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers it very unlikely that maize GA21 × T25 will differ from conventional maize hybrid varieties in its ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable maize GA21 × T25 grains.

3.7.1.2. Potential for gene transfer

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

Plant-to-microorganism gene transfer

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

The applicant submitted an updated bioinformatic analysis for each of the single events to assess the possibility for HGT by homologous recombination. The updated bioinformatic analyses for GA21 and T25 confirm the assessments provided in the context of previous applications (EFSA GMO Panel, 2019, 2021c).

[36] Dossier: Part II – Sections 5 and 6; additional information: 18/8/2022.

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

Therefore, the GMO Panel concludes that the unlikely, but theoretically possible, horizontal transfer of recombinant genes from maize GA21 × T25 to bacteria does not raise any environmental safety concern.

Plant-to-plant gene transfer

The potential for occasional feral maize GA21 × T25 stack maize plants originating from grain import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer were considered.

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

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

The potential of spilled maize grains to establish, grow and produce pollen is extremely low and transient (see Section 3.7.1.1). Therefore, the likelihood/frequency of cross-pollination between occasional feral GM maize plants resulting from grain spillage, and weedy or cultivated *Zea* plants is considered extremely low (EFSA, 2016, 2022). Even if cross-pollination would occur, the GMO Panel is of the opinion that environmental effects as a consequence of the spread of genes from occasional feral GM maize plants in Europe will not differ from that of conventional maize varieties for the reasons given in Section 3.7.1.1 even if exposed to the intended herbicides.

3.7.1.3. Interactions of the GM plant with target organisms

Taking the scope of application EFSA-GMO-DE-2016-137 (no cultivation) and the absence of target organisms into account, potential interactions of occasional feral maize GA21 × T25 plants arising from grain import spills with target organisms are not considered a relevant issue.

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

Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled maize GA21 × T25 grains is limited and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, the GMO Panel considers that potential interactions of maize GA21 × T25 with non-target organisms do not raise any environmental safety concern.

3.7.1.5. Interactions with abiotic environment and biogeochemical cycles

Given that environmental exposure to spilled grains or occasional feral maize GA21 × T25 plants arising from grain import spills is limited and because most proteins are degraded before entering the environment through faecal material of animals fed GM maize, the GMO Panel considers that potential interactions with the abiotic environment and biogeochemical cycles do not raise any environmental safety concern.

3.7.2. Post-market environmental monitoring

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

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

As the ERA did not identify potential adverse environmental effects from maize GA21 × T25, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for maize GA21 × T25 includes: (1) the description of a monitoring approach involving operators (federations involved in import and processing), reporting to the applicant, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by CropLife Europe for the collection of information recorded by the various operators; and (3) the review of relevant scientific publications retrieved from literature searches (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis for the duration of the authorisation period.

The GMO Panel considers that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize GA21 × T25. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

3.7.3. Conclusion of the environmental risk assessment and monitoring plan

The GMO Panel concludes that it is unlikely that the maize GA21 × T25 would differ from conventional maize varieties in its ability to persist under European environmental conditions. Considering the scope of the application EFSA-GMO-DE-2016-137, interactions of occasional feral maize GA21 × T25 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from maize GA21 × T25 to bacteria does not indicate a safety concern. Therefore, considering the combined traits and their interactions, the outcome of the agronomic and phenotypic analysis, the routes and levels of exposure, the GMO Panel concludes that maize GA21 × T25 would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of maize GA21 × T25.

4. Overall conclusions

The GMO Panel was asked to carry out a scientific assessment of maize GA21 × T25 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

No new information was identified on the two single maize events (GA21 and T25) that would lead to a modification of the original conclusions on their safety.

The molecular characterisation, the comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment indicate that the combination of the single maize events and of the newly expressed proteins in maize GA21 × T25 does not give rise to food/feed safety and nutritional concerns. The GMO Panel concludes that maize GA21 × T25, as described in this application, does not raise any nutritional concern and is as safe as its conventional counterpart and the selected non-GM reference varieties.

The GMO Panel concludes that there is a very low likelihood of environmental effects resulting from the accidental release of viable grains from maize GA21 × T25 into the environment.

Based on the results of the literature searches, the GMO Panel did not identify any safety issues pertaining to the intended uses of maize GA21 × T25.

In addition, the GMO Panel considered the additional unpublished studies listed in Appendix A. This new information does not raise any concern for human and animal health and the environment regarding maize GA21 × T25. Given the absence of safety and nutritional concerns for foods and feeds from maize GA21 × T25, the GMO Panel considers that PMM of these products is not necessary. The PMEM plan and reporting intervals are in line with the intended uses of maize GA21 × T25. In conclusion, the GMO Panel considers that maize GA21 × T25, as described in this application, is as safe as its conventional counterpart and the selected non-GM reference varieties with respect to potential effects on human and animal health and the environment.

Documentation as provided to EFSA

- Letter from the Competent Authority of Germany, received on 14 November 2016 concerning a request for authorisation of the placing on the market of genetically modified maize GA21 × T25 submitted in accordance with Regulation (EC) No 1829/2003 by Syngenta Crop Protection NV/SA (EFSA-GMO-DE-2016-137; EFSA-Q-2016-00775)
- The application was made valid on 23 February 2017.

- Additional information (1) was requested on 2 March 2017
- Additional information (1) was received on 31 May 2017
- Additional information (2) was requested on 10 April 2017
- Additional information (2) was received on 29 May 2017
- Additional information (3) was requested on 6 July 2017
- Additional information (3) was received on 26 September 2017
- Additional information (4) was requested on 1 September 2017
- Additional information (4) was received on 30 October 2017
- Additional information (5) was requested on 23 November 2017
- Additional information (5) was received on 21 December 2017
- Additional information (6) was requested on 22 December 2017
- Additional information (6) was received on 20 February 2018
- Additional information (7) was requested on 8 March 2018
- Additional information (7) was received on 9 July 2018
- Additional information (8) was requested on 20 March 2018
- Additional information (8) was received on 6 August 2018
- Additional information (9) was requested on 30 July 2018
- Additional information (9) was received on 29 November 2018
- Additional information (10) was requested on 31 July 2018
- Additional information (10) was received on 7 December 2018
- Additional information (11) was requested on 14 September 2018
- Additional information (11) was received on 31 March 2021
- Additional information (12) was requested on 25 January 2019
- Additional information (12) was received on 21 March 2019
- Additional information (13) was requested on 3 April 2019
- Additional information (13) was received on 3 July 2019
- Additional information (14) was requested on 26 July 2019
- Additional information (14) was received on 4 February 2020
- Additional information (15) was requested on 8 February 2021
- Additional information (15) was received on 31 August 2022
- Additional information (16) was requested on 3 June 2022
- Additional information (16) was received on 18 August 2022
- Additional information (17) was requested on 10 November 2022
- Additional information (17) was received on 22 November 2022
- Supplementary information was provided on voluntary basis on 17 October 2022

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Abbreviations

bw	body weight
CaMV	cauliflower mosaic virus
CRM	comparative relative maturity

DNA	deoxyribonucleic acid
dw	dry weight
ELISA	enzyme-linked immunosorbent assay
EU	European Union
GLP	good laboratory practice
GM	genetically modified
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
LOD	limit of detection
LOQ	limit of quantification
mEPSPS	modified 5-enolpyruvylshikimate-3-phosphat synthase
MS	Member States
NEP	newly expressed protein
OECD	Organisation for Economic Co-operation and Development
ORFs	open reading frames
PAT	phosphinothricin-acetyl-transferase
PMEM	post-market environmental monitoring
PMM	post market monitoring
USA	United States of America
UTR	untranslated region

Appendix A – List of additional studies

List of additional studies performed by or on behalf of the applicant with regard to the evaluation of the safety of maize GA21 × T25 for humans, animal or the environment

Study identification	Title
Report No: TK0105357 A1	Comparison of Transgenic Protein Concentrations in GA21 × T25, Event GA21, and Event T25 Maize Tissues Amendment 1
Report No: TK0290363	EFSA Statistical Analysis of the Comparison of Transgenic Protein Concentrations in GA21 × T25, Event GA21, and Event T25 Maize Tissues Supplement to TK0105357 A1
Report No: TK0105376	Summary of Forage and Grain Composition Data from Event GA21 and GA21 × T25 Hybrid Maize Report
Report No: TK0105370	Compositional Analysis of Forage and Grain from GA21 × T25 Maize Grown During 2012 in the USA Assessment
Report No: TK0156409 A1	EFSA Statistical Analysis of Forage and Grain Composition Data from GA21 × T25 Maize Grown During 2012 in the USA Report Amendment 1
Report No: TK0224881	Seed Germination and Dormancy Test for GA21 × T25
Report No: TK0156408 A1	EFSA Statistical Analysis of Agronomic Performance of GA21 × T25 Maize Grown During 2012 in the USA Amendment 1
Report No: TK0224882	Pollen Viability and Morphology of GA21 × T25 Maize
Report No: 35857[(a)]	GA21 × T25 Maize Grain – A 13 Week Oral (Dietary) Toxicity Study of GA21 × T25 Maize Grain in Rats Final Report
Report No: TK0105365	Agronomic Performance of GA21 × T25 Maize Grown During 2012 in the USA Assessment
Report No: TK0218864 A1	GA21 × T25 Maize Insert and Flanking Sequence Analysis of Event GA21 in GA21 × T25 Maize Final Report Amendment 1
Report No: TK0105360 A2	GA21 × T25 Maize Comparative Southern Blot Analyses Final Report Amendment 2
Report No: 1781.4134[(a)]	GA21 × T25 Evaluation of the GA21 × T25 Transgenic Maize Grain in a Broiler Chicken Feeding Study Final Report
Report No: TK0224881	Seed Germination and Dormancy Test for GA21 × T25 Maize Final Report

(a): The GMO Panel notes that the submitted study report contained limited details about the materials and methods used for the statistical analysis and on the production of the test diets. As the study was not a requirement for the EU, clarification of the limitations was not sought. On evaluation of the available information, no treatment-related adverse effects were identified.

Appendix B – Protein expression data

Means, standard deviation and ranges of protein levels (μg/g dry weight) from maize GA21 × T25, GA21 and T25 (not treated) from field trials performed across three locations in the USA in 2012 (N = 15)[(g)]

Protein	Event(s)	Leaf (BBCH16)	Leaf (BBCH63–65)	Root (BBCH16)	Root (BBCH63–65)	Whole plant (BBCH16)	Whole plant (BBCH63–65)	Pollen (BBCH63–65)	Grain (BBCH87–99)	Grain (senescence)
mEPSPS	**GA21 × T25**	116[(a)] ± 16.6[(b)] (83.5–149.0)[(c)]	105 ± 19.8 (66.0–136)	57.0 ± 13.34 (35.4–75.4)	27.4 ± 8.80 (< LOQ[(d)]–42.2)	113 ± 26.0 (74.3–153)	73.3 ± 28.35 (39.0–117)	96.4 ± 8.43 (< LOD[(d)]–103)	6.76 ± 1.345 (< LOQ[(d)]–9.09)	< LOQ[(e)]
	GA21	111 ± 14.5 (80.5–132.0)	100 ± 25.2 (58.6–142)	55.5 ± 10.73 (36.6–72.9)	26.9 ± 6.86 (< LOQ[(d)]–38.9)	130 ± 21.4 (85.2–174)	70.3 ± 24.05 (26.6–116)	146 ± 64.9 (< LOD[(d)]–240)	8.76 ± 2.551 (< LOQ[(d)]–12.4)	< LOQ[(e)]
PAT	**GA21 × T25**	68.3 ± 5.72 (58.5–79.4)	41.5 ± 8.75 (32.9–61.8)	23.0 ± 3.87 (16.5–29.3)	10.1 ± 6.14 (0.630–20.9)	49.3 ± 11.38 (20.2–66.2)	21.9 ± 5.98 (15.6–36.0)	< LOD[(f)]	0.241 ± 0.1086 (0.0915–0.439)	0.105 ± 0.0512 (0.0514–0.246)
	T25	63.9 ± 9.05 (47.1–79.0)	35.8 ± 7.87 (23.9–50.0)	23.4 ± 4.45 (14.0–29.9)	9.56 ± 6.724 (2.70–22.3)	53.2 ± 8.08 (37.8–66.6)	23.2 ± 6.35 (12.2–36.6)	< LOD[(f)]	0.214 ± 0.0760 (0.113–0.390)	0.124 ± 0.0445 (0.0522–0.211)

(a): Mean.
(b): Standard deviation.
(c): Range.
(d): Some, but not all, sample results were below the limit of quantification (LOQ = 12.8 μg/g for roots, LOQ = 4.0 μg/g for grains) or below the limit of detection (LOD = 37.50 μg/g for pollen).
(e): All samples resulted below the limit of quantification (LOQ = 4.0 μg/g).
(f): All samples resulted below the limit of detection (LOD = 0.025 μg/g).
(g): N = 14 for PAT in T25 for BBCH63-65 whole plant, BBCH63-65 root, BBCH63-65 leaf, BBCH87-99 grain; N = 13 in T25 for BBCH63-65 pollen and grain (senescence).

Appendix C – Animal dietary exposure

Table C.1: Derived NEP concentrations for maize products and maize stover

Feed material	Protein content (%)	References	Conversion factor for protein content
Grain[(a)]	9.4	Heuzé et al., 2017a	–
Gluten feed[(b)]	20	Kulp, 2000	2.13
Gluten meal[(b)]	60	Kulp, 2000	6.38
Hominy meal	11.1	Stock et al., 1999	1.18
Milled by-products[(c)]	8.4	Kulp, 2000	0.894
Forage[(a)]	7.9	Heuzé et al., 2017b	–
Stover	6.8	Heuzé et al., 2019	0.861

(a): Measured protein concentrations in grain and in forage (see also Table 6) are included here for clarity of derivations; all values reported in the table were rounded to three significant figures. The full unrounded values were used during calculation.

(b): The GMO Panel notes that protein content for gluten feed and gluten meal as reported in Kulp (2000) refers to as fed (i.e. 10% of moisture).

(c): The applicant did not provide a definition of milled by-products for feed use; they refer to coarse grits, meal and flour which, according to Kulp (2000), are dry milling products. In particular for the estimation of ADE, the consumption of coarse grits (protein content 8.4%) was considered by the applicant, as the by-product with the highest protein content (i.e. 6.6% for flour, and 7.2% for meal). However, the GMO Panel considers this approach not appropriate for animal feeding, because milled by-products for feed use could include many more ingredients with different and higher protein content.

Table C.2: Dietary exposure to mEPSPS and PAT proteins (mg/kg bw per day) in selected animals, based on the consumption of maize products

Animal species BW (kg)/total diet intake (kg dw)	Feed material	IR%	Dietary exposure (µg/kg bw per day)	
			mEPSPS	PAT
Beef cattle 500/12	Gluten Meal	15	0.495	0.0125
	Forage/Silage	80	0.325	0.214
	Grain	80	0.188	0.00473
	Gluten Feed	30	0.330	0.00831
	Hominy Meal	0	0	0
	Milled by-products	30	0.0652	0.00164
	Stover	25	0.0421	0.0278
Dairy cattle 650/25	Gluten Meal	20	1.06	0.0266
	Forage/Silage	60	0.391	0.257
	Grain	30	0.113	0.00285
	Gluten Feed	30	0.528	0.0133
	Hominy Meal	0	0	0
	Milled by-products	30	0.104	0.00263
	Stover	20	0.054	0.0356
Rams/Ewes 75/2.5	Gluten Meal	30	1.37	0.0346
	Forage/Silage	0	0	0
	Grain	30	0.0978	0.00247
	Gluten Feed	30	0.458	0.0115
	Hominy Meal	0	0	0
	Milled by-products	30	0.0905	0.00228
	Stover	0	0	0

Animal species BW (kg)/total diet intake (kg dw)	Feed material	IR%	Dietary exposure (μg/kg bw per day)	
			mEPSPS	PAT
Lambs 40/1.7	Gluten Meal	30	1.75	0.0442
	Forage/Silage	30	0.216	0.142
	Grain	30	0.125	0.00314
	Gluten Feed	30	0.584	0.0147
	Hominy Meal	0	0	0
	Milled by-products	30	0.115	0.00291
	Stover	0	0	0
Breeding Swine 260/6	Gluten Meal	10	0.317	0.0515
	Grain	70	0.158	0.00398
	Gluten Feed	20	0.211	0.00533
	Hominy Meal	0	0	0.00799
	Milled by-products	75	0.157	0
	Forage/Silage	20	0.0781	0.00395
	Stover	20	0.0324	0.0214
Finishing Swine 100/3	Gluten Meal	10	0.412	0.0104
	Forage/Silage	0	0	0
	Grain	70	0.205	0.00518
	Gluten Feed	20	0.275	0.00693
	Hominy Meal	0	0	0
	Milled by-products	75	0.204	0.00513
	Stover	0	0	0
Broiler Hens 1.7/0.12	Gluten Meal	10	0.970	0.0244
	Forage/Silage	0	0	0
	Grain	70	0.483	0.0122
	Gluten Feed	10	0.323	0.00815
	Hominy Meal	0	0	0
	Milled by-products	60	0.383	0.00966
	Stover	0	0	0
Layer Hens 1.9/0.13	Gluten Meal	10	0.940	0.0237
	Forage/Silage	10	0.116	0.0763
	Grain	70	0.469	0.0118
	Gluten Feed	0	0	0
	Hominy Meal	20	0.158	0.00398
	Milled by-products	50	0.310	0.00780
	Stover	0	0	0
Turkey 7/0.5	Gluten Meal	10	0.981	0.0247
	Forage/Silage	0	0	0
	Grain	50	0.349	0.00881
	Gluten Feed	0	0	0
	Hominy Meal	20	0.165	0.00416
	Milled by-products	50	0.323	0.00815
	Stover	0	0	0