REGULATED PRODUCTS SAFETY ASSESSMENT

Assessment of the Safety the Genetically Modified Maize MON 89034 X 1507 X MIR162 X NK603 X DAS-40278-9 for Food and Feed Uses Under Assimilated Regulation (EC) No. 1829/2003 (RP2152)

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

Keywords: Regulated Products, Safety assessment, Genetically Modified Organisms

https://doi.org/10.46756/001c.131770

FSA Research and Evidence

The Food Standards Agency (FSA) and Food Standards Scotland (FSS) received an application from Corteva Agriscience LLC ("the applicant") under assimilated Regulation (EC) No. 1829/2003 for maize MON 89034 x 1507 x MIR162 x NK603 x DAS-40278-9 (referred to hereafter as 'five-event stack maize'). The FSA and FSS reviewed the submitted evidence, including the 2022 European Food Safety Authority (EFSA) opinion, to assess the safety of five-event stack maize in terms of the UK.

The five-event stack maize is modified by crossing to combine five single events: MON 89034, 1507, MIR162, NK603 and DAS-40278-9. EFSA previously assessed the five single maize events and 16 of the sub combinations whilst the UK was a Member State of the EU, and did not identify safety concerns. This combination is intended to confer herbicide tolerance and insect-resistance to maize (*Zea mays L.*).

The scope of the application is for the authorisation for import, processing, and food and feed use of the five-event stack maize.

The application does not cover cultivation and no maize MON 89034 x 1507 x MIR162 x NK603 x DAS-40278-9 will be grown in the UK.

The FSA and FSS agreed with the EFSA Scientific Opinion confirming that the five-event stack maize, as described in this application, is unlikely to have any adverse effect on human or animal health, or on the environment, in the context of its intended uses in GB.

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

This is a joint FSA and FSS publication.

1. Introduction

The Food Standards Agency (FSA) and Food Standards Scotland (FSS) received an application (RP2152) for the authorisation of genetically modified maize MON 89034 x 1507 x MIR162 x NK603 x DAS-40278-9 (unique identifier: MON-89Ø34-3 x DAS-Ø15Ø7-1 x SYN-IR162-4 x MON-ØØ6Ø3-6 x DAS-4Ø278-9), submitted by Corteva Agriscience LLC (represented by Corteva Agriscience UK Limited, Cpc2 Capital Park, Fulbourn, Cambridge, United Kingdom) (hereafter referred to as "the applicant"), according to assimilated Regulation (EC) No. 1829/2003.

To ensure the regulatory systems of the FSA/FSS are risk proportionate, and resources are used effectively, the FSA and FSS have used the evidence submitted by the applicant and other information in the public domain, including the EFSA Scientific Opinion, to provide a summary assessment of the evidence of safety presented in this report. In carrying out the assessment, the FSA/FSS reviewed the information provided by the EFSA Scientific Opinion and the dossier provided to FSA/FSS by the applicant.

The EFSA Scientific Opinion (2022) has been reviewed by the FSA/FSS, and it has been verified that the standard approach taken, when compared to the relevant guidance applied in GB, has been followed and the conclusions made are consistent with the data summarised in the opinion. This assessment represents the opinion of FSA/FSS on the safety of genetically modified maize MON 89034 x 1507 x MIR162 x NK603 x DAS-40278-9.

This five-event stack maize was produced by crossing to combine five single maize events: MON 89034 expressing Cry1A.105 and Cry2Ab2 (for protection against certain lepidopteran pests), 1507 expressing Cry1F (for protection against certain lepidopteran pests) and PAT protein (for tolerance to glufosinate-ammonium-containing herbicides), MIR162 expressing Vip3Aa20 (for protection against certain lepidopteran pests) and PMI (selectable marker), NK603 expressing CP4 EPSPS (for tolerance to glyphosate-containing herbicides) and DAS-40278-9 expressing AAD-1 (to confer tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and the aryloxyphenoxypropionate (AOPP) containing herbicides). This

combination is intended to confer resistance to certain lepidopteran pests and tolerance to glyphosate, glufosinateammonium,2,4-D and the AOPP-based herbicides to maize (*Zea mays L.*).

All five single events, nine two-event stacks, six three-event stacks, one four-event stack and 16 of the sub combinations were previously assessed by EFSA and authorised for use in the EU while the UK was a Member State (EFSA, 2004a, 2004b, EFSA, 2005b, 2005a, EFSA, 2006, EFSA, 2007, EFSA, 2008, EFSA, 2009; EFSA GMO Panel 2009a, 2009b, EFSA GMO Panel, 2010a, 2010b, EFSA GMO Panel, 2011a, 2011b, EFSA GMO Panel, 2012, EFSA GMO Panel, 2016, EFSA GMO Panel, 2017, GMO Panel, 2018a, 2018b, EFSA GMO Panel, 2019a, 2019g, 2019f, 2019d, 2019b, 2019e, EFSA GMO Panel, 2021a, 2021c, 2021d) as shown in Table 1, and for this reason these provided a basis for the assessment of the five-event stack maize and all its 26 sub combinations. No concerns for human or animal health or environmental safety were identified. The five-event stack maize and its 26 sub combinations have also been assessed and authorised in the EU, with an EFSA opinion (EFSA-GMO-NL-2018-151) published in 2022 (EFSA, 2022).

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

Event	Application	EFSA opinion date
MON 89034	EFSA-GMO-NL-2007-37 EFSA-GMO-RX-015	EFSA (2008) EFSA GMO Panel (2019a)
1507	EFSA-Q-2004-011 EFSA-GMO-NL-2004-02 EFSA-Q-2006-00330 EFSA-GMO-RX-1507 EFSA-GMO-RX-001	EFSA (2004a) EFSA (2005b) EFSA (2005a) EFSA (2009) EFSA GMO Panel (2017)
MIR162	EFSA-GMO-DE-2010-82	EFSA GMO Panel (2012)
NK603	EFSA-Q-2003-002 EFSA-Q-2003-003 EFSA-GMO-NL-2005-22 EFSA-GMO-RX-NK603	EFSA (2004b) EFSA (2007) EFSA (2009a) EFSA (2009a)
DAS-40278-9	EFSA-GMO-NL-2010-89	EFSA GMO Panel (2016)
1507 x NK603	EFSA-GMO-UK-2004-05 EFSA-GMO-RX-008 EFSA EFSA-GMO-NL-2015-127	EFSA (2006) EFSA GMO Panel (2018a) EFSA GMO Panel (2021a)
1507 x MON 89034	EFSA-GMO-CZ-2008-62 EFSA-GMO-NL-2017-139	EFSA GMO Panel (2010a, 2011a) EFSA GMO Panel (2021c)
1507 x MIR162	EFSA-GMO-DE-2010-86 EFSA-GMO-NL-2017-139	EFSA GMO Panel (2018b) EFSA GMO Panel (2021c)

1507 x DAS-40278-9	EFSA-GMO-NL-2013-112 EFSA-GMO-NL-2013-113	EFSA GMO Panel (2019g) EFSA GMO Panel (2019f)
MON 89034 x NK603	EFSA-GMO-NL-2007-38 EFSA-GMO-NL-2016-134	EFSA GMO Panel (2009b) EFSA GMO Panel (2019d)
MON 89034 x DAS-40278-9	EFSA-GMO-NL-2013-112 EFSA-GMO-NL-2013-113	EFSA GMO Panel (2019g) EFSA GMO Panel (2019f)
MON 89034 x MIR162	EFSA-GMO-NL-2016-131 EFSA-GMO-NL-2016-134 EFSA-GMO-NL-2017–144	EFSA GMO Panel (2019b) EFSA GMO Panel (2019d) EFSA GMO Panel (2019e)
NK603 x MIR162	EFSA-GMO-NL-2016-131 EFSA-GMO-NL-2016-134 EFSA-GMO-NL-2015-127	EFSA GMO Panel (2019b) EFSA GMO Panel (2019d) EFSA GMO Panel (2021a)
NK603 x DAS-40278-9	EFSA-GMO-NL-2013-112 EFSA-GMO-NL-2019–164	EFSA GMO Panel (2019g) EFSA GMO Panel (2021d)
1507 x NK603 x MON 89034	EFSA-GMO-NL-2009-65 EFSA-GMO-NL-2013-112	EFSA GMO Panel (2010b, 2011b) EFSA GMO Panel (2019g)
1507 x NK603 x MIR162	EFSA-GMO-NL-2015-127	EFSA GMO Panel (2021a)
1507 x NK603 x DAS-40278-9	EFSA-GMO-NL-2013-112	EFSA GMO Panel (2019g)
1507 x MON 89034 x DAS-40278-9	EFSA-GMO-NL-2013-112 EFSA-GMO-NL-2013-113	EFSA GMO Panel (2019g) EFSA GMO Panel (2019f)
NK603 x MON 89034 x MIR162	EFSA-GMO-NL-2016-131 EFSA-GMO-NL-2016-134	EFSA GMO Panel (2019b) EFSA GMO Panel (2019d)
NK603 x MON 89034 x DAS-40278-9	EFSA-GMO-NL-2013-112	EFSA GMO Panel (2019g)
MON 89034 x 1507 x NK603 9 DAS-40278-9	EFSA-GMO-NL-2013-112	EFSA GMO Panel (2019g)

The FSA/FSS reviewed the information in the EFSA opinion about the composition and agronomic characteristics of the stack, the potential for interactions between the individual events, DNA sequencing and updated bioinformatics analyses, and toxicological studies provided by the applicant as part of application RP2152. As the single events and 16 sub combinations were previously safety assessed and authorised, this 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 assimilated Regulation (EU) No 503/2013 (EC, 2013).

Maize is one of the most important crops worldwide and is grown over a wide range of climatic conditions, well-suited for warm, temperate climates. Maize, grown on 15 million hectares in the EU (14% of the EU's arable land, and 8% of worldwide maize acreage), is the leading cereal in terms of global production volumes. Its principal use is animal feed (83%), followed by starch manufacturing (15%) and cornmeal (2%). The methods of production and manufacturing are well known and have a long history of safe use. Silage maize is cultivated for feed and is mainly used on-farm. Grain maize is used for feed (poultry, corn-cob-mix for pigs), food (maize-meal-products, snacks, cornflakes, oil) or for industrial purposes and non-food products (starch, paper, industrial alcohol). The genetic modification in the five-event stack maize does not impact the production or manufacturing processes currently used for maize. The scope of the application is for the authorisation for import, processing, and food and feed use of the five-event stack maize. The application does not cover cultivation and therefore no five-event stack maize will be grown in the UK.

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

2. Details of other regulator's opinions

2.1. Molecular characterisation

The five-event stack maize was obtained by conventional crossing of the GM maize plants containing single events MON 89034, 1507, MIR162, NK603 and DAS-40278-9, and no vector has been used to produce this maize. The structure of the inserts introduced into five-event stack maize are described in previous EFSA assessments (Table 1) and no new genetic modifications were involved.

The only potential interactions at the biological level are among the Cry proteins or among the Vip3Aa20 and the Cry proteins in susceptible insects, which will be addressed in section 2.4 (Environmental risk assessment).

The five events contained in the five-event stack maize, the resultant proteins produced, and the traits conferred are as follows:

- MON 89034 expresses Cry1A.105 (a modified version of the Cry1A-type protein) and Cry2Ab2 proteins, both toxic to certain lepidopteran larvae feeding on maize. These

proteins are produced by crystal protein (*cry*) genes from *Bacillus thuringiensis* subsp. kurstaki and subsp. aizawai., which is an insect pathogen.

- 1507 expresses Cry1F (a truncated version protein) and Phosphinothricin-acetyl-transferase (PAT) proteins. Cry1F is a protein toxic to certain lepidopteran larvae feeding on maize produced from crystal protein (*cry*) genes, present in *Bacillus thuringiensis* subsp. aizawai. PAT protein enzyme acetylates L-glufosinate ammonium, conferring tolerance to glufosinate ammonium-based herbicides, and it is from *Streptomyces Viridochromogenes*.

- MIR162 expresses Vip3Aa20 and phosphomannose isomerase (PMI) proteins. Vip3Aa20 is a protein toxic to certain lepidopteran larvae feeding on maize and it is produced by a gene from *Bacillus thuringiensis* strain AB88. PMI is produced by a gene from *E. coli* and catalyses the isomerisation of mannose-6-phosphate to fructose-6-phosphate and plays a role in the metabolism of mannose. PMI is used as selectable marker and normally inhibits root growth, respiration and germination. Transformed cells expressing PMI are able to utilise mannose as a carbon source.

- NK603 expresses the bacterial CP4 EPSPS (5-enolpyruvyl-shikimate-3-phosphate synthase) protein and CP4 EPSPS L214P (a modified version of the bacterial CP4 EPSPS), which confers tolerance to glyphosate-containing herbicides as it has lower affinity towards glyphosate than the plant endogenous enzyme. The protein is produced by a gene from *Agrobacterium* strain CP4. EPSPS is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms.

- DAS-40278-9 expresses Aryloyankanoate dioxygenase (AAD-1) protein which degrades the 2,4-dichlorophenoxyacetic acid (2,4-D) and phenoxy auxin and aryloxyphenoxypropionate herbicides (AOPP) herbicides, conferring tolerance to these. The gene producing this protein is from *Sphingobium herbicidovorans*.

The genetic stability of the inserted DNA over multiple generations in the single maize events was previously demonstrated (Table 1).

The applicant demonstrated integrity of single events in the five-event stack maize through the Southern blot analyses; in addition, the sequence of the events (inserts and their flanking regions) was determined in the five-event stack maize and confirmed as 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 the five-event stack maize raised no safety concerns.

The applicant used Enzyme-linked immunosorbent assays (ELISA) to determine Cry1A.105, Cry2Ab2, Cry1F, Vip3Aa20, PAT, CP4 EPSPS, AAD-1 and PMI protein levels for the five-event stack and the corresponding single events in different parts of the plant (leaves, roots, pollen, forage and grains), both those treated and not treated with glyphosate, glufosinate-ammonium, haloxyfop and 2,4-D containing herbicides. The material was collected from field trials across eight locations in Argentina in 2015–2016.

The levels of all the newly expressed proteins in the five-event maize stack and the corresponding singles were comparable in all tissues and no indication emerged of an interaction affecting the levels of the newly expressed proteins in this stack.

The expression levels of the transgenic proteins were determined by the applicant using suitable methodologies and no biologically relevant changes in protein expression were observed between the five-event maize stack and in the single event maize lines.

The FSA/FSS agree with EFSA that the applicant demonstrated the equivalence of the single events within the five-event maize stack to those already assessed by EFSA and given a positive opinion while the UK was an EU Member State.

2.2. Comparative analysis

All individual events in the five-event maize stack were previously assessed, whereby equivalence with conventional counterpart and non-GM reference varieties was demonstrated for all single events.

In addition to the information already available, the applicant provided a comparative assessment of the five-event maize stack. The five-event maize stack was equivalent to the conventional counterpart and to reference varieties for its agronomic characteristics and composition.

Ten sites for agronomic and 8 sites for compositional analysis in United States of America (USA) were tested by the applicant during 2020. Each field trial site utilised a randomised complete block design with four blocks, each containing: 1) the five-event stack maize not exposed to the intended

herbicide; 2) the five-event stack maize exposed to the intended herbicides; 3) the comparator SLB01 x PH184C; and 4) four non-GM reference varieties.

The FSA/FSS agree with EFSA that 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 the five-event stack maize could be grown.

The FSA/FSS agree with EFSA that there are no differences between the five-event stack maize and the conventional counterpart or the non-GM reference varieties that would raise safety concerns were observed.

The applicant assessed 11 agronomic and phenotypic endpoints, as well as abiotic stressors, diseases, and pest damage. The applicant applied statistical analysis to eight endpoints, because of lack of variability for the remaining three. Significant differences were observed between the five-event stack maize treated with conventional herbicides (early stand count, plant height, final stand count and 100-kernel weight) and the non-GM comparator. Significant differences were also observed between the five-event stack maize treated with the intended herbicides (plant height and 100-kernel weight) and the non-GM comparator. However, the applicant considers these differences not a concern because equivalence with the non-GM reference varieties was demonstrated for all these endpoints.

Eighty compositional constituents were analysed; however, statistical analysis was only performed on 71 of these.

Statistically significant differences were observed between five-event stack maize not treated with the intended herbicides and the non-GM comparator for 32 endpoints (one in forage and 31 in grains). All these endpoints fell under equivalence category I or II except for histidine, which fell under equivalence category III, and ash, behenic acid (C22:0), arginine, glycine, lysine, phosphorus, potassium and phytic acid, which fell under equivalence category IV, all in grain.

For the five-event stack maize treated with the intended herbicides, the test of difference identified statistically significant differences with the non-GM comparator for 39 endpoints (one in forage and 38 in grains). All these endpoints fell under equivalence category I or II except for histidine, which fell under equivalence category III, and ash, behenic acid (C22:0), arginine, glycine, phosphorus, potassium and phytic acid, which fell under equivalence category IV, all in grain.

EFSA considered a further assessment (shown in section 2.3.4 (Nutritional assessment)) regarding food and feed safety for the altered levels in grain of those components which fell in category III and IV. EFSA concluded that

the altered levels of components identified from the five-event stack are considered not to represent a concern for animal and human health. The FSA and FSS agreed with the EFSA Scientific Opinion, confirming that the five-event stack maize is equivalent to the conventional counterpart and the non-GM reference varieties.

2.3. Food/feed safety assessment

As the five-event stack maize will undergo established production processes used for conventional maize, and considering the outcome of the comparative assessment, processing of the five-event stack maize into food and feed products is not expected to result in products being different from those of conventional non-GM maize varieties, except for the newly expressed proteins.

EFSA previously evaluated (Table 1) the stability of the newly expressed proteins in the context of previous applications, whilst the UK was a Member State of the EU. The effects of temperature and pH and the resistance to degradation by pepsin of the newly expressed proteins were considered valid from EFSA and for this reason no new information has been provided in the context of this application.

2.3.1. Toxicological assessment

The newly expressed proteins (Cry1A.105, Cry2Ab2, Cry1F, Vip3Aa20, PAT, CP4 EPSPS, AAD-1 and PMI) have been previously evaluated and assessed by EFSA (Table 1) in the context of previous applications, whilst the UK was a Member State of the EU, and no safety concerns were identified for humans and animals.

EFSA assessed the potential interaction among the newly expressed proteins. Regarding the three insecticidal proteins Cry1A.105, Cry2Ab2 and Cry1F, EFSA considered that the gastrointestinal tract of mammals, including humans, lacks those receptors found in target insect species and with high specific affinity to Cry proteins. This consideration is similarly applied to the Vip3Aa20 as targets insects using a similar mechanism to Cry proteins. The four enzymatic proteins (PMI, PAT, CP4 EPSPS and AAD-1) catalyse distinct biochemical reactions, acting on unrelated substrates and are not expected to interact.

After evaluating the known biological function of the individual newly expressed proteins, EFSA concluded that there are no safety concerns to human and animal health related to the newly expressed proteins Cry1A.105, Cry2Ab2, Cry1F, Vip3Aa20, PAT, CP4 EPSPS, AAD-1 and PMI in the five-event stack maize.

The comparative analysis and molecular characterisation confirmed that no new constituents other than newly expressed proteins have been identified in seed and forage from five-event stack maize.

The altered levels of components (ash, behenic acid (C22:0), arginine, glycine, histidine, phosphorus, potassium, phytic acid (both treated and not treated), lysine (not treated) and pyridoxine (treated)) identified in seed and forage from the five-event stack are considered not to represent a toxicological concern and therefore, no further toxicological assessment is needed.

Molecular characterisation, comparative analysis and toxicological assessment confirmed the safety of the five-event stack maize; therefore, animal studies on food/feed derived from this five-stack maize are not necessary. In accordance with Regulation (EU) No 503/2013, the applicant provided a 90-day oral repeated-dose toxicity study in rats on whole food and feed from each of the maize single events composing the five-event stack maize. EFSA previously concluded that these studies are in line with Regulation (EU) No 503/2013 and do not show adverse effects related to diets incorporating the single-event maize MON 89034 (EFSA GMO Panel, 2019b), 1507 (EFSA GMO Panel, 2021a, 2021c), MIR162 (EFSA GMO Panel, 2019f), NK603 (EFSA GMO Panel, 2019f) and DAS-40278-9 (EFSA GMO Panel, 2021d).

The FSA and FSS agreed with the EFSA Scientific Opinion, confirming that the five-event stack maize does not show any toxicological concern for animal and human health.

2.3.2. Allergenicity assessment

A weight-of-evidence approach was used by the applicant to determine any potential allergenicity. EFSA previously evaluated the safety of newly expressed proteins individually, and no evidence of allergenicity was identified in the context of the applications assessed (Table 1). No new information on allergenicity of the proteins newly expressed in this five-event stack maize that might change the previous conclusions of the GMO had become available when it was assessed by EFSA in 2022. Based on the knowledge available at the time, there was no evidence of allergenicity of the newly expressed proteins and no expected concerns of allergenicity as a consequence of their interaction in the five-event stack maize (EFSA, 2022).

In addition, no information available on the structure or function of the newly expressed proteins would suggest an adjuvant effect of these proteins in the five-event stack maize.

The applicant provided information about the Cry1A.105, Cry2Ab2, Cry1F, Vip3Aa20, PAT, CP4 EPSPS, AAD-1 and PMI proteins with respect to coeliac disease.

The assessment of the Cry2Ab2, Vip3Aa20, CP4 EPSPS (including its variant CP4 EPSPS L214P) and AAD-1 proteins found no sequences with perfect or relevant partial matches with known coeliac disease peptide sequences.

For Cry1F, Cry1A.105, PAT and PMI proteins, partial matches containing the Q/E-X1-P-X2 motif required further investigations; however, several of these partial matches were previously assessed by the EFSA GMO Panel (2019d, 2019c, 2021d, 2021b). After additional consideration, on the position and nature of amino acids flanking the motifs, EFSA concluded that no indications of safety concern with regard to coeliac disease were identified.

Because maize is not considered a common allergenic food EFSA did not request experimental data. Based on the information provided, the FSA and FSS agreed with the EFSA Scientific Opinion, concluding that in the context of this application there are no indications of potentially increased allergenicity of food and feed derived from this five-event stack maize with respect that that derived from the non-GM comparator and the non-GM reference varieties tested.

2.3.3. Exposure assessment

The human and animal dietary exposure to Cry1A.105, Cry2Ab2, Cry1F, Vip3Aa20, PAT, CP4 EPSPS, AAD-1 and PMI proteins were also estimated by the applicant based on the five-event stack maize treated with the intended herbicides, the current available consumption data and feed practices, the foods and feeds currently available in the market, and the described processing conditions.

Human dietary exposure was estimated using a conservative scenario with 100% replacement of conventional maize by the GM maize and for all relevant commodities (e.g. corn flakes, sweet corn, popcorn, etc.), excluding corn oil, corn starch and corn syrup because no proteins are expected to be present in these commodities. Most relevant food commodities in terms of contribution to the exposure were corn grains and sweet corn.

An ad hoc dietary exposure scenario was carried out for consumers of pollen supplements, assuming that these were made from the five-event stack maize. The low number of consumers available adds uncertainty to the exposure estimations, which should be carefully interpreted.

A conservative scenario with 100% replacement of conventional maize feed products by the five-event stack maize products was used to identify animal exposure across different animal species.

The FSA and FSS agreed with the EFSA Scientific Opinion, concluding that human and animal exposure to the five-event stack maize does not show any safety concern for animal and human health.

2.3.4. Nutritional assessment

As the intended trait of genetically modified maize is protection against lepidopteran pests and tolerance to herbicides, no nutritional effects are expected. However EFSA considered in the nutritional assessment those components (ash, behenic acid, arginine, glycine, histidine, phosphorus, potassium, phytic acid (all treated and not treated plants with the intended herbicides), lysine (not treated) and pyridoxine (treated)) that could not be categorised during composition analysis because they showed significant difference in maize grains from the comparator and a lack of equivalence with the set of non-GM reference varieties.

Histidine and lysine are considered as indispensable amino acids and their small increase (2% for lysine and 5% for histidine) does not represent a nutritional concern. Potassium and phosphorus are the most abundant minerals in maize and their increase (6–8% and 8–9% respectively) probably explains the higher levels of ash observed as compared to the comparator. Potassium is an essential compound involved in many different physiological processes and as no tolerable upper intake level (UL) has been set for potassium, its increase is not a nutritional concern. The increase in phosphorus is very likely related to the higher levels of phytic acid (between 12% and 13%), a primary storage form of phosphorus in seeds responsible of protection against oxidative stress.

Phytic acid is typically considered as an antinutrient in human nutrition but other dietary sources (other cereals, legumes, oil seeds and nuts) are available. In addition, phytic acid in maize is mainly located in the germ, which is usually removed during maize milling, or decreased by other processing methods.

However, because the phytic acid slightly differs from the conventional counterpart and some of the selected non-GM reference varieties, and because similar and higher values are described in the literature, EFSA concluded that the increased level of phytic acid in the five-event stack GM maize is not a nutritional concern.

Considering the magnitude of the decrease for vitamin B6 (8%), and its presence in many different foods (grains, pulses, nuts, seeds, potatoes and meat and meat products), this decrease is not considered of nutritional concern.

The increase of 3–5% of behenic acid (C22:0) normally present at very low levels in maize (< 0.3% of total fatty acids (FA)), and the relevance of this saturated fatty acid in the total FA content of maize grains, are not considered nutritionally a concern.

Regarding animal nutrition, because they usually have very balanced diets the changes observed in the components in the five-events stack maize do not represent a nutritional concern.

The FSA and FSS agreed with the EFSA Scientific Opinion, confirming the five-event stack maize is not nutritionally disadvantageous for animal and human health.

2.3.5. Conclusions on the food/feed assessment

EFSA concluded that the newly expressed proteins Cry1A.105, Cry2Ab2, Cry1F, PAT, Vip3Aa20, PMI, CP4 EPSPS and AAD-1 proteins newly expressed in the five-event stack maize do not raise safety concerns for human and animal health.

Bioinformatics analyses of the sequences of newly expressed proteins found no evidence of potential toxicity or allergenicity concerns, and the stability of the proteins to temperature, pH, and *in vitro* digestion did not raise safety concerns.

EFSA evaluated the human and animal dietary exposure to the five-event stack maize, and found no safety concerns. The comparative assessment confirmed the equivalence between the five-event stack maize and the conventional counterpart and therefore the five-event stack maize does not represent a safety, or nutritional, concern.

EFSA concluded that the five-event stack maize does not show any toxicological concern for animal and human health.

The FSA/FSS agree with all the conclusions of EFSA which are applicable to GB, and confirm that maize MON 89034 x 1507 x MIR162 x NK603 x DAS-40278-9 does not raise any safety concerns in terms of human and animal health.

2.4. Environmental risk assessment

As the application does not cover cultivation, EFSA's environmental risk assessment focussed on the accidental release of the five-event stack maize into the environment, and exposure of micro-organisms to recombinant DNA from the five-event stack maize. The establishment and survival of volunteer maize plants in the EU is limited and transient, and it is unlikely that the traits inserted into the five-event stack maize will affect the ability of the maize plants to survive. In addition, there is no evidence that the genetic modifications made to the five-event stack maize

increases the likelihood of horizontal gene transfer to micro-organisms in the environment. Therefore, the FSA and FSS agreed with the EFSA Scientific Opinion, concluding that it is unlikely that the five-event stack maize would differ from conventional maize in its ability to persist under European conditions.

3. Other regulator's opinion and conclusions

The EFSA GMO Panel concluded that taking into account the previous assessment and the new information, the five-event stack maize, as assessed in the scientific opinion on application EFSA-GMO-NL-2018-151 and in the supplementary toxicity study, is as safe as its non-GM comparator and the non-GM reference varieties tested and does not represent a nutritional concern in humans and animals, within the scope of this application.

4. Uncertainties and limitations

No specific uncertainties or limitations were flagged in the assessment by EFSA. The FSA and FSS did not identify further uncertainties or limitations to be considered for this assessment.

5. FSA & FSS conclusions for GB assessment

The FSA and FSS assessed the EFSA opinion (EFSA, 2022) for stacked maize MON 89034 x 1507 x MIR162 x NK603 x DAS-40278-9 and confirmed that this is adequate and relevant for GB risk analysis.

The conclusions of the EFSA opinion have been reviewed in detail by FSA and FSS and are considered appropriate and consistent, including the uncertainties and limitations identified in the opinion which are applicable to GB. Sufficient evidence has been demonstrated to conclude without further questions or risk assessment.

The environmental risk assessment of stacked maize MON 89034 x 1507 x MIR162 x NK603 x DAS-40278-9 is within the remit of Advisory Committee on Releases to the Environment (ACRE), and their assessment will form part of the final scientific assessment published by FSA/FSS.

ACRE concluded that stacked maize MON 89034 x 1507 x MIR162 x NK603 x DAS-40278-9 would not raise safety concerns in the event of accidental release of viable seeds or propagating material into the environment.

ACRE's advice is available at the following link:

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

6. Outcome of the assessment

The FSA and FSS have reviewed the applicant's dossier, supporting documentation, and most notably the EFSA opinion (EFSA-GMO-NL-2018-151), and consider that there is sufficient evidence to conclude the safety assessment of stacked maize MON 89034 x 1507 x MIR162 x NK603 x DAS-40278-9 without obtaining further information or conducting a further risk assessment.

The FSA and FSS conclude that genetically modified stacked maize MON 89034 x 1507 x MIR162 x NK603 x DAS-40278-9 is as safe as its non-GM conventional comparator with respect to its potential effects on human and animal health, and the environment.

In making this assessment, the FSA and FSS were able to rely on sufficient scientific evidence provided by the EFSA Opinion and by the applicant, to make a conclusion on safety with no further questions to the applicant, and therefore no further risk assessment activities are necessary.

The FSA and FSS review did not find any issues of divergence from the EFSA guidance (EFSA NDA Panel, 2016) or mutual approaches or new scientific issues for consideration.

There were no other specific issues that would require an assessment for the UK or the nations of the UK.

Abbreviations

Abbreviation	Definition
AAD-1	aryloyankanoate dioxygenase
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	environmental risk assessment
FA	fatty acid
GM	genetically modified
GMO	genetically modified organism
ORF	open reading frame
PAT	phosphinothricin acetyltransferase
PCR	polymerase chain reaction
PMI	phosphomannose isomerase
USA	United States of America

Published: March 28, 2025 GMT.

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