SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 25 September 2019

doi: 10.2903/j.efsa.2019.5844

Statement complementing the EFSA Scientific Opinion on application (EFSA-GMO-UK-2006-34) for authorisation of food and feed containing, consisting of and produced from genetically modified maize 3272

EFSA Panel on Genetically Modified Organisms (GMO),
Hanspeter Naegeli, Jean-Louis Bresson, Tamas Dalmay, Ian Crawford Dewhurst,
Michelle M Epstein, Leslie George Firbank, Philippe Guerche, Jan Hejatko,
Francisco Javier Moreno, Ewen Mullins, Fabien Nogué, Nils Rostoks,
Jose Juan Sánchez Serrano, Giovanni Savoini, Eve Veromann, Fabio Veronesi,
Fernando Álvarez, Michele Ardizzone, Anna Lanzoni, Jose Ángel Gómez Ruiz,
Giacomo De Sanctis, Antonio Fernández Dumont, Andrea Gennaro and Franco Maria Neri

Abstract

Following a request from the European Commission, the GMO Panel assessed additional information related to the application for authorisation of food and feed containing, consisting of and produced from genetically modified (GM) maize 3272 (EFSA-GMO-UK-2006-34). The applicant conducted new agronomic, phenotypic and compositional analysis studies on maize 3272 and assessed the allergenic potential of AMY797E protein, addressing elements that remained inconclusive from previous EFSA opinion issued in 2013. The GMO Panel is of the opinion that the agronomic and phenotypic characteristics as well as forage and grain composition of maize 3272 do not give rise to food and feed safety, and nutritional concerns when compared to non-GM maize. Considering the scope of this application and the characteristics of the trait introduced in this GM maize, the effect of processing and potential safety implications of specific food or feed products remain to be further investigated. Regarding the allergenic potential of AMY797E protein and considering all possible food and feed uses of maize 3272, the Panel concludes that the information provided does not fully address the concerns previously raised by the Panel in 2013. Owing to the nature and the knowledge available on this protein family, it is still unclear whether under specific circumstances the alpha-amylase AMY797E has the capacity to sensitise certain individuals and to cause adverse effects. To further support the safety of specific products of maize 3272, the applicant provided thorough information relevant for the allergenicity assessment of dried distiller grains with solubles (DDGS), which is the main product of interest for importation into the EU. Having considered the information provided on this product, the Panel is of the opinion that under the specific conditions of use described by the applicant, DDGS produced from maize 3272 does not raise concerns when compared to DDGS from non-GM maize.

Keywords: GMO, maize (*Zea mays*), 3272, thermotolerant alpha-amylase, food and feed safety

Requestor: European Commission

Question number: EFSA-Q-2017-00341

Correspondence: gmo@efsa.europa.eu

Panel members: Jean-Louis Bresson, Tamas Dalmay, Ian Crawford Dewhurst, Michelle M Epstein, Leslie George Firbank, Philippe Guerche, Jan Hejatko, Francisco Javier Moreno, Ewen Mullins, Hanspeter Naegeli, Fabien Nogué, Nils Rostoks, Jose Juan Sánchez Serrano, Giovanni Savoini, Eve Veromann and Fabio Veronesi.

Acknowledgements: The Panel wishes to thank the members of its Working Groups for the preparatory work on this scientific opinion and EFSA staff members Sylvie Mestdagh and Irene Muñoz Guajardo for the support provided to this scientific opinion.

Suggested citation: EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Bresson J-L, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Moreno FJ, Mullins E, Nogué F, Rostoks N, Sánchez Serrano JJ, Savoini G, Veromann E, Veronesi F, Álvarez F, Ardizzone M, Lanzoni A, Gómez Ruiz JÁ, De Sanctis G, Fernández Dumont A, Gennaro A and Neri FM, 2019. Scientific Opinion on the statement complementing the EFSA Scientific Opinion on application (EFSA-GMO-UK-2006-34) for authorisation of food and feed containing, consisting of and produced from genetically modified maize 3272. EFSA Journal 2019;17(11):5844, 15 pp. https://doi.org/10.2903/j.efsa.2019.5844

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

The EFSA Panel on Genetically Modified Organisms (GMO) has previously issued an opinion on maize 3272 in 2013. This scientific opinion was inconclusive because of insufficient data provided for the comparative assessment and lack of further studies on the *de novo* sensitisation potential of the newly expressed protein AMY797E.

On 4 April 2017, the European Commission requested the GMO Panel to complement its original scientific opinion on application EFSA-GMO-UK-2006-34 for placing on the market maize 3272 for food and feed uses, import and processing under Regulation (EC) No 1829/2003[1], taking into consideration new additional information provided by the applicant relevant for the comparative analysis of maize 3272 as well as for assessing the allergenic potential of the AMY797E protein.

The GMO Panel assessed the information provided by the applicant and concludes that the agronomic and phenotypic characteristics as well as forage and grain composition of maize 3272 do not give rise to food and feed safety, and nutritional concerns when compared to non-genetically modified (non-GM) maize. Considering the scope of this application and the characteristics of the trait introduced in this GM maize, the effect of processing and potential safety implications of specific food or feed products remain to be further investigated.

In relation to the allergenic potential of AMY797E protein and considering all possible food and feed uses of maize 3272, the GMO Panel concludes that the information provided does not fully address the concerns previously raised by the Panel in 2013. Owing to the nature and the knowledge available on this protein family (or functional class of enzymes), it is still unclear whether under specific circumstances the alpha-amylase AMY797E has the capacity to sensitise certain individuals and to cause adverse effects. In addition, the applicant provided thorough information relevant for the allergenicity assessment of a specific product, the dried distiller grains with solubles (DDGS) which is the main product of interest for importation into the European Union. Having considered the information provided on this product, the GMO Panel is of the opinion that under the specific conditions of use described by the applicant, DDGS produced from maize 3272 does not raise concerns when compared to DDGS from non-GM maize.

[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.

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

On 4 April 2017, the European Food Safety Authority (EFSA) received from the European Commission (EC) a mandate for the assessment of additional information relevant for the comparative assessment of maize 3272 and the allergenic potential of AMY797E protein provided by Syngenta. EFSA was asked to complement the GMO Panel scientific opinion on maize 3272 taking into consideration this additional information. EFSA acknowledged the receipt of the mandate on 2 May 2017. EFSA requested EC to extend the deadline for the finalisation of the mandate on 31 October 2018 and on 24 July 2019. The EC accepted EFSA's requests on 11 December 2018 and on 9 August 2019, respectively.

In 2013, the Panel on Genetically Modified Organisms of the European Food Safety Authority (GMO Panel) adopted a scientific opinion on application EFSA-GMO-UK-2006-34 for placing on the market of genetically modified maize 3272 for food and feed uses, import and processing under Regulation (EC) No 1829/2003[1] by Syngenta (EFSA GMO Panel, 2013). The opinion was inconclusive because of insufficient data provided for the comparative assessment and lack of further studies on the *de novo* sensitisation potential of the newly expressed protein AMY797E.

According to the mandate received from EC (mandate Q-2017-00341), this statement complements the EFSA opinion on maize 3272 (EFSA GMO Panel, 2013) in accordance to Articles 6(5), 6(6), 18(5) and 18(6) of Regulation (EC) No 1829/2003.

2. Data and methodologies

2.1. Data

In delivering this statement, the GMO Panel took into account the supplementary studies provided by the applicant in the context of this mandate, additional information provided by the applicant during the risk assessment, relevant scientific information publicly available and the EFSA Scientific Opinion on application EFSA-GMO-UK-2006-34 (EFSA GMO Panel, 2013).

2.2. Methodologies

The GMO Panel carried out a scientific risk assessment of the supplementary studies taking into account the appropriate principles described in its guidelines for the risk assessment of genetically modified (GM) plants and derived food and feed (EFSA GMO Panel, 2006, 2011; EFSA, 2017).

3. Assessment

3.1. Introduction

This statement has been produced in response to a mandate from the European Commission (mandate EFSA-Q-2017-00341) for the assessment of additional information related to the application for authorisation of food and feed containing, consisting of and produced from GM maize 3272 (EFSA-GMO-UK-2006-34). Maize 3272 contains a single insert consisting of the *amy797E* and the *pmi* cassettes, expressing a thermotolerant alpha-amylase (AMY797E) and a phosphamannose isomerase (PMI). The GMO Panel has previously issued an opinion on maize 3272 (EFSA GMO Panel, 2013) being inconclusive because of insufficient data provided for the comparative assessment and lack of further studies on the *de novo* sensitisation potential of the newly expressed protein AMY797E. The applicant has now addressed these aspects conducting new studies analysing the agronomic, phenotypic and compositional characteristics of maize 3272 and the allergenic potential of AMY797E.

It is noted that despite the scope of this application is for all food and feed uses (referred to hereafter as full scope), the applicant highlights that maize 3272 is specifically designed for ethanol production in the US. More recently, the applicant is investigating its potential utility as a feed enhancement for cattle. The intention of the company is to import into the European Union (EU) only dried distiller grains with solubles (DDGS) produced from maize 3272, a by-product of the ethanol production process. Therefore, grains derived from maize 3272 are not meant for importation into the EU. Considering that maize 3272 is not a commodity corn being a speciality maize, the applicant developed a grain production system under a closed loop system managed through contracts and stewardship programmes.[2]

[2] Enogen Value Tracker.

This is a relevant aspect as the main focus of the applicant in the additional information has been to assess DDGS at a larger extent than in the original application. The safety assessment presented here considered a full scope scenario, as requested by the mandate, as well as, the peculiarities of this crop not being a commodity crop, designed and managed for specific purposes, and where only certain products are intended for import into the EU.

The assessment of the information related to the comparative analysis of the agronomic and phenotypic characteristics and of the forage and grain composition is covered in Sections 3.2 and 3.3.2, while the allergenicity assessment is discussed in Section 3.3.1. The applicant provided a detailed documentation on the DDGS production process mainly discussed in Section 3.3.1. Nevertheless, additional considerations on the impact of other processing conditions relevant for risk assessment under a full scope situation were also provided by the applicant and are further discussed in Section 3.3.2.

3.2. Comparative analysis

3.2.1. Choice of comparator and production of material for the comparative assessment

Mandate EFSA-Q-2017-00341 presents data[3] on agronomic and phenotypic characteristics, as well as on forage and grain composition, of maize event 3272 derived from field trials performed in the US in 2014.[4]

Table 1: Overview of comparative assessment studies with maize event 3272 provided in the mandate EFSA-Q-2017-00341

Study focus	Study details	Comparator	Non-GM commercial reference varieties[(a)]
Agronomic, phenotypic and compositional characteristics	Field trials, 2014, US, nine locations[(b)]	NP2222/NP2391	Six

GM: genetically modified.
(a): Reference varieties: NK Kansas, NK Octet, NK Lucius, Cisko, SY Provial and SY Generoso.
(b): At the field trial located at Bagley (IA), a high incidence of plant lodging was reported. Upon request by the GMO Panel, it was clarified that a storm with strong winds occurred at the end of the growing season and did not compromise the validity of this location for the comparative analysis.

The field trials with maize event 3272 were conducted in major maize-growing areas of the US, representing regions of diverse agronomic practices and environmental conditions. At each site, the following materials were grown in a randomised complete block design with four replicates: maize event 3272, the conventional counterpart (NP2222/NP2391) and six different non-GM maize reference varieties, all treated (sprayed) with plant protection products (PPP) according to local requirements.

The comparator used in the field trials is a non-GM maize hybrid (NP2222/NP2391) with a genetic background similar to that of maize event 3272 as documented by the pedigree, and was therefore considered to be the appropriate conventional counterpart.

The statistical analysis of the agronomic, phenotypic and compositional data from the 2014 field trials followed the recommendations of the GMO Panel (EFSA GMO Panel, 2010a, 2011). This includes the application of a difference test (between the GM maize and conventional counterpart) and an equivalence test (between the GM maize and the set of non-GM commercial reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[5]

[3] Additional information: 24/5/2017.
[4] The sites for the agronomic, phenotypic and compositional field trials were located at Lime Springs (IA); Richland (IA); Germansville (PA); Bagley (IA); Delavan (WI); Carlyle (IL); York (NE); Atlantic (IA); and Stewardson (IL).
[5] 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).

3.2.2. Agronomic and phenotypic analysis

Agronomic and phenotypic characteristics tested under field conditions

The agronomic and phenotypic endpoints evaluated in the 2014 field trials were: early stand count (pre-thinning), early stand count (post-thinning), days to 50% pollen shed,[6] days to 50% silking,[6] plant height, final stand count, total lodged plants, grain yield, grain moisture and grain test weight. Two of the endpoints (early stand count post-thinning and total lodged plants) for which the observations were highly discrete were not considered for formal statistical analysis.

Of the remaining eight endpoints, statistically significant differences were found for early stand count (pre-thinning), days to 50% pollen shed and days to 50% silking, which all fell under equivalence category I.

3.2.3. Compositional analysis

Maize 3272 grains and forage harvested from the field trials in the US in 2014 (Table 1) were analysed for 82 constituents (9 in forage and 73 in grain), including the key constituents recommended by OECD (2002). The statistical analysis was not applied to 16 grain constituents,[7] because more than half of the observations were below the limit of quantification, and to moisture levels in grain, as the grains were dried before the analytical measurements.

The statistical analysis was applied to the remaining 66 constituents (9 in forage[8] and 57 in grain[9]). Significant differences between maize 3272 and its conventional counterpart were identified for 14 grain endpoints, which all fell under equivalence category I or II except for ferulic acid levels in grain, which fell under equivalence category IV (Table 2). No significant differences were found between maize 3272 and its conventional counterpart for any of the endpoints analysed in forage.

Table 2: Outcome of the comparative compositional analysis in grains and forage for maize 3272. The table shows the number of endpoints in each category

		Test of difference[(a)]	
		Not different	**Significantly different**
Test of equivalence[(b)]	Category I/II	50	13[(c)]
	Category III/IV	–	1[(d)]
	Not categorised	2[(e)]	–
	Total endpoints	66	

(a): Comparison between maize 3272 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): Endpoints with significant differences between maize 3272 and its conventional counterpart falling in equivalence category I–II. For grain: ash, TDF, manganese, zinc, pyridoxine, palmitic acid (16:0), stearic acid (18:0), arachidic acid (20:0), eicosenoic acid (20:1), behenic acid (22:0), methionine, tryptophan and p-coumaric acid. For forage: none.
(d): Ferulic acid in grains of maize 3272 was found significantly different than in its conventional counterpart and fell under equivalence category IV. Results for ferulic acid are reported in Table 3.
(e): Endpoints not categorised for equivalence and with no significant differences between maize 3272 and its conventional counterpart: inositol and trypsin inhibitor in grain.

[6] Additional information: 4/5/2018.
[7] 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)).
[8] Protein, moisture, neutral detergent fibre (NDF), acid detergent fibre (ADF), total fat ash, calcium, phosphorus, and carbohydrates.
[9] Proximates (moisture, protein, total fat, ash, carbohydrates), fibre fractions (ADF, NDF, total detergent fibre (TDF)), starch, amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine), fatty acids (palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), behenic acid (C22:0)), vitamins (vitamin A (β-carotene), vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B6 (pyridoxine), vitamin E (α-tocopherol), vitamin B3 (niacin) and vitamin B9 (folic acid)), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc) and other compounds (inositol, phytic acid, trypsin inhibitor, raffinose, ferulic acid and *p*-coumaric acid).

The GMO Panel assessed all significant differences between maize 3272 and its conventional counterpart, taking into account potential impact on plant metabolism and the natural variability observed for the set of non-GM commercial reference varieties. Mean estimates for the endpoint showing a significant difference between maize 3272 and its conventional counterpart and falling under category IV are given in Table 3.

Table 3: Quantitative results (estimated means and equivalence limits) for the endpoint with a significant difference between maize 3272 and its conventional counterpart and falling under category IV in the test of equivalence (see Table 2)

Endpoint (mg/kg DW)	Maize 3272	Conventional counterpart	Non-GM reference varieties	
			Mean	Equivalence limits
Ferulic acid	3360	3298[(a)]	2180	1870–2560

DW: dry weight; GM: genetically modified.
(a): Mean values for ferulic acid in conventional counterpart were out of the equivalence limits derived from the selected non-GM reference varieties.

3.2.4. Conclusions of the comparative assessment

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

- none of the differences identified in the agronomic and phenotypic characteristics tested between maize 3272 and its conventional counterpart needs further assessment for environmental safety;
- none of the differences identified in forage and grain composition between maize 3272 and its conventional counterpart needs further assessment regarding food and feed safety, except for higher ferulic acid levels in grains of maize 3272 that is assessed in Section 3.3.2.

3.3. Food and feed safety assessment

3.3.1. Allergenicity assessment of AMY797E protein

In 2013, the GMO Panel issued an opinion on the safety of maize 3272 expressing the AMY797E protein (EFSA GMO Panel, 2013). This protein is a thermotolerant enzyme which originates from alpha-amylases genes from archaeal sources, in particular from three hyper thermophilic microorganisms of the order Thermococcales.

In the context of the original application (EFSA-GMO-UK-2006-34), the GMO Panel requested for additional experimental data to the applicant to support the allergenicity assessment of the AMY797E protein owing to the fact that: (i) some alpha-amylases are known to be allergens (external report EFSA, 2009); (ii) the newly expressed AMY797E protein is an enzyme that is stable at high temperatures; and (iii) AMY797E originates from a source of which there is little information on its exposure to humans. Based on the information provided and owing to the absence of bibliographic and/or experimental data demonstrating the lack of *de novo* sensitisation capacity specific to the AMY797E protein, the GMO Panel could not conclude on the potential for *de novo* sensitisation of the newly expressed AMY797E protein (EFSA GMO Panel, 2013).

In the context of the current mandate (EFSA-Q-2017-00341), new complementary information on the safety profile of the AMY797E protein relevant for allergenicity assessment was made available. This additional information initially consisted of: (i) a claim by the applicant on a history of safe use of alpha-amylase enzymes derived from fungal, bacterial and archaeal sources, including Thermococcales; and (ii) occupational exposure survey data and other related information.[10] These two aspects will be discussed separately in Sections 3.3.1.1 and 3.3.1.2, respectively.

The GMO Panel and the scientific community at large acknowledge the difficulty in identifying adequate *in vitro* or *in vivo* methods for the assessment of the allergenic potential of proteins, in particular those investigating *de novo* sensitisation risk (FAO/WHO, 2001; EFSA GMO Panel, 2010b, 2017; Ladics et al., 2014; EFSA, 2018 Remington et al., 2018; Houben et al., 2019). The GMO Panel is aware of the fact that for allergenicity assessment *in vitro* cell-based assays or *in vivo* tests on animal

[10] Additional information: 24/4/2017.

models have not yet been validated for regulatory purposes. However, the GMO Panel may consider them as additional information, under specific circumstances (EFSA GMO Panel, 2011). Codex Alimentarius (2009) establishes the principles for the allergenicity assessment highlighting that if additional *in vitro* and/or *in vivo* methods are necessary, they should be scientifically sound. In line with these considerations, the need to develop more robust approaches appropriately designed for risk assessment purposes when testing the allergenic potential of novel proteins and more broadly on their effects on the immune system has been highlighted (EFSA GMO Panel, 2010b, 2017; EFSA, 2018).

3.3.1.1. A claim of history of safe use of alpha-amylase enzymes

In the context of this mandate (EFSA-Q-2017-00341), the applicant provided considerations on the history of safe use of alpha-amylase enzymes derived from fungal, bacterial and archaeal sources including Thermococcales under specific conditions. To support such statement, the GMO Panel requested the applicant to perform a scoping review on sensitising potential of alpha-amylases from these sources used in food or any other industrial areas and on related potential adverse effects encountered.[11] The information provided evidenced that (i) various alpha-amylases under specific conditions of exposure are sensitisers with the potential to cause adverse effects, and that (ii) only one paper has investigated the safety of an archaea-derived amylase focusing on general toxicology of the alpha-amylase. According to the applicant, the sensitisation to these enzymes appears to be linked to very specific exposure scenarios for few taxonomically limited alpha-amylases. The specific exposure conditions occur in occupational settings (e.g. bread baking and flour preparation, as well as detergent industry) and a consideration was made to the particular nature of airborne enzymes.

The GMO Panel agrees that one of the main concerns of this GM maize relates to the hypothesised sensitising potential through the respiratory route of the AMY797E protein at specific settings. The scoping review revealed that on one hand there is a vast amount of information showing that specific alpha-amylases are implicated in airborne sensitisation and in adverse health effects, i.e. allergy. On the other hand, no relevant information on the topic from alpha-amylases of archaeal sources is available from the literature.

The GMO Panel notes that alpha-amylases sensitisation potential and related adverse effects are mostly linked to fungi sources and, at a lesser extent, to bacterial species. The majority of the available evidence associates those effects to industrial enzymes and their use in the food industry, being particularly relevant for bakers (Baur et al., 1986, 1989; Brisman and Belin, 1991; Losada et al., 1992; Quirce et al., 1992; Sander et al., 1998; Brisman, 2002; Brisman et al., 2004; Baur, 2005; Budnik et al., 2017). Occupational respiratory sensitisation to alpha-amylases is well known and alpha-amylases are categorised as respiratory sensitisers.[12] Based on the information available, it is concluded that the most relevant exposure scenario for sensitisation to alpha-amylases is the respiratory route at the occupational settings. Specifically, for thermotolerant amylases the information publicly available on this aspect is very scarce. One paper has investigated the safety of an archaea-derived amylase focusing on general toxicology but not on occupational exposure (Landry et al., 2003). A case study report showed that a thermotolerant alpha-amylase derived from *Bacillus* sp. elicited allergic respiratory disorders in a worker employed in the detergent industry (Baur et al., 2013). Previously, this bacterial thermotolerant alpha-amylase was reported to be a potential respiratory allergen in an animal test using guinea pigs (Sarlo et al., 1997).

The GMO Panel also considered different routes of exposure for *de novo* sensitisation other than respiratory, such as skin and oral routes. In this context, contact dermatitis to alpha-amylases has also been reported (Morren et al., 1993; Vein, 2000; Meding et al., 2003; Hernández-Bel et al., 2011). In a case report, induction of oral angioedema due to an alpha-amylase after eating bread was described in an individual who was not related to either the bakery or the pharmaceutical industry (Moreno-Ancillo et al., 2004).

Concerning the elicitation phase, allergic reactions upon oral exposure to alpha-amylase in individuals sensitised via the respiratory route to the enzyme have been reported (Losada et al., 1992; Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004). In contrast, other studies have shown no clinical symptoms in allergic individuals following ingestion of specific alpha-amylases (Cullinan et al., 1997; Poulsen, 2004; Armentia et al., 2009).

[11] Additional information: 30/7/2018, 14/9/2018 and 2/10/2018.

[12] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

The GMO Panel notes that previous concerns on the potential capacity for *de novo* sensitisation of the AMY797E still remain not completely addressed. An aspect for concern is the potential of this protein to sensitise and provoke respiratory disorders in humans. Furthermore, elicitation of allergic reactions upon oral ingestion of maize 3272 in potentially sensitised individuals to the AMY797E protein is unlikely to occur but it cannot be excluded. In the case of animals, the available literature on allergy to alpha-amylases is more limited.

3.3.1.2. Occupational exposure survey

In the context of this mandate (EFSA-Q-2017-00341) and to support the safety of this product, the applicant initially provided workplace survey data on 202 workers exposed to maize 3272 in specific facilities of ethanol production. These data consisted of reports related to incidents of illness, in particular respiratory illness in workers from facilities processing maize 3272 and repeatedly exposed to the crop. The applicant reported no respiratory conditions or adverse effects linked to maize 3272 or AMY797E protein in the facilities investigated. According to the applicant, under the intended conditions of use, exposure via the respiratory route is the most likely source of human exposure to AMY797E protein expressed in maize 3272, being the formation of dust the most relevant aspect to consider.

Following a request from the GMO Panel, the applicant provided additional information clarifying aspects related to the different exposure situations and to the steps followed to collect and appraise the information presented in the occupational study.[13] In the US, the safety of workers is covered by the Occupational Safety and Health Administration (OSHA)[14] and requirements under the Environmental Protection Agency (EPA).[15] The applicant described the different exposure scenarios encountered by the workers considering the several phases in the entire process from the grower to the production of ethanol and DDGS which are intended for importation into the EU. The applicant mentions that DDGS are generally directed into feed manufacturing facilities and are included into feed formulas, being pelleting the most common process that reduces dust production (US Grains Council, 2018). Finally, these pellets are bagged and transported to the animal production facilities.

The applicant quantified the level and determined the enzymatic activity of AMY797E protein in DDGS from various facilities where GM maize containing maize 3272 event is used. As a comparative control element, the applicant also measured alpha-amylase activity from DDGS that underwent conventional ethanol production involving the use of non-GM maize and the external addition of a microbially produced alpha-amylase. From facilities where GM maize containing AMY797E protein is used, the data revealed the presence of AMY797E in two out of nine samples of DDGS. The applicant reported that the alpha-amylase activity in the DDGS from conventional ethanol production was higher when compared to the activity from DDGS produced from GM maize. A characterisation of the particle size and the presence of AMY797E protein in the dust of DDGS was also provided. The applicant estimated that less than 0.5% of the dust particle size is classified as respirable dust, while it was confirmed the presence of AMY797E in the dust from the two samples of DDGS where the protein was previously detected. Finally, the applicant also made reference to other enzymes (e.g. [redacted]) currently imported into the EU and sharing high sequence identity (up to 93%) with the AMY797E protein. The applicant argues that EU workers have been exposed to DDGS containing a very similar enzyme, in a similar manner to those workers that will be exposed to DDGS produced from maize 3272 if imported into the EU from the US. Furthermore, the applicant intends to monitor reporting systems in the US, including the stewardship programs already in place and will notify any adverse findings to regulatory authorities.

In summary and under the intended conditions of use, exposure to AMY797E protein in maize 3272 predominantly occurs via the respiratory route through dust in the US. The AMY797E protein has been detected in a limited number of DDGS samples which is the only product intended for import into the EU. No increase in the number of reported respiratory conditions from the use of maize 3272 by ethanol producers in the US have been reported. In 2019, maize 3272 has been used in 25 ethanol plants in the US. EU ethanol factory workers have been in contact with very similar enzymes, under similar exposure circumstances. The GMO Panel is of the opinion that under the specific conditions of use in the ethanol production facilities and subsequent steps described by the applicant and briefly presented above in this Section 3.3.1.2, DDGS produced from maize 3272 does not raise concerns when compared to DDGS from non-GM maize.

[13] Additional information: 4/6/2019.
[14] Available at: https://www.osha.gov
[15] Available at: https://www.epa.gov

Nevertheless, the GMO Panel also evaluated the usefulness of such information in the context of a full scope application covering all potential uses of maize 3272 and it is considered of limited value. The allergenic capacity of AMY797E has not been fully characterised and the information provided above on DDGS only reflects a portion of the market where maize 3272 could be used and a limited population that could be exposed to the AMY797E protein.

3.3.2. Nutritional assessment

3.3.2.1. Human and animal nutrition

The outcome of the comparative analysis showed significantly differences in ferulic acid content between maize 3272 and its conventional counterpart, falling under category IV in the test of equivalence (see Section 3.2). Ferulic acid (4-hydroxy-3-methoxycinnamic) is the most abundant phenolic compound in maize grain (Boz, 2015; Bento-Silva et al., 2018). No toxicity has been linked to the dietary intake of ferulic acid, being a phenolic compound easily absorbed and metabolised in humans and animals.

Based on this, the GMO Panel concludes that the observed increase of this compound in maize 3272 is not relevant from a nutritional and safety point of view for humans and animals.

3.3.2.2. Effects of processing

As previously described in Section 3.1, maize 3272 is intended to be cultivated and used in the US in the dry-grind fuel ethanol process or as potential feed enhancement for cattle. The by-products of the dry-grind fuel ethanol process, the DDGS, are intended to be imported in the EU as a feed product. The comparability of these by-products to those produced from conventional maize in terms of composition, in particular the carbohydrate profile, was already demonstrated (EFSA GMO Panel, 2013).

Considering that the scope of this application is for all food and feed uses, it was necessary to assess the possibility that maize 3272 grains might undergo production processes currently used for conventional maize grains.[13] Technological concerns have been previously raised (Waltz, 2011). Regulatory bodies have also mentioned the effect of this amylase on certain food properties (e.g. shelf life) due to the conversion of starch into dextrins and sugars of low molecular weight (FSANZ, 2007).[16] The presence of the thermo-tolerant AMY797E protein in maize 3272 might result in processed food (e.g. ready-to-eat-cereals) and feed (e.g. canned pet feed or by-products of the wet-milling) being different from those produced from conventional maize. Under certain processing conditions (e.g. temperature, moisture, pH), the AMY797E protein might cause the hydrolysis of maize starch into dextrins, maltose and other oligosaccharides, changing the composition and texture of the processed commodities as compared to those produced from conventional maize.

From a nutritional perspective, the glycaemic index of processed products from maize 3272 is expected to increase as compared to those from conventional maize. However, the overall impact on the overall glycaemic index in food is expected to be minor as this index is heavily influenced by many other carbohydrate-rich foods (e.g. bread, potatoes, pasta, rice, cereals, dairy products, fruits, vegetables) present in the diet. In feed, the glycaemic index is considered when diets and rations are calculated, therefore variations in the glycaemic index in specific feed does not represent, *per se,* a concern. It is also noted that a link between the release of reducing sugars through enzymatic activity and acrylamide content has been already demonstrated in potato products (De Wilde et al., 2005; Rosen et al., 2018). The formation of acrylamide in processed foods, once reducing sugars are present, requires further specific conditions, i.e. high temperature and low water content. These conditions might be present during the processing of maize 3272 grains to produce diverse food and feed products (e.g. fried/baked polenta, by-product feed products of the wet-milling). In the context of a full scope application, the potential safety implications of these food and feed products as compared to those from conventional maize were not further investigated as the safety profile of the AMY797E protein remains incomplete (see Sections 3.3.1 and 4).

4. Conclusions

The GMO Panel was asked to complement a previous EFSA Scientific Opinion (EFSA GMO Panel, 2013) considering new information provided by the applicant. The scope of this application is for authorisation of food and feed containing, consisting of and produced from GM maize 3272. The

[16] Available at: www.foodstandards.gov.au/code/applications/documents/FRR_A580_GM_Corn_Line_3272.pdf

information provided in this application included new studies on the agronomic, phenotypic and compositional characteristics of maize 3272 and additional data and considerations on the allergenic potential of the AMY797E protein.

The agronomic and phenotypic characteristics, as well as forage and grain composition of maize 3272 do not give rise to food and feed safety and nutritional concerns when compared to non-GM maize. Under the context of a full scope application, the effect of processing and potential safety implications of specific food or feed products remain to be further investigated.

In relation to the allergenic potential of AMY797E protein, the GMO Panel concludes that the information provided does not completely address its previous concerns (EFSA GMO Panel, 2013) in the context of a full scope application. Owing to the nature and the knowledge available on this protein family (or functional class of enzymes), it is still unclear whether under specific circumstances the alpha-amylase AMY797E has the capacity to sensitise certain individuals and to cause adverse effects.

In addition, the applicant provided thorough information relevant for the allergenicity assessment on a specific product, the DDGS, being the main interest of the company for importation into the EU. Having considered all the information supplied, the GMO Panel is of the opinion that under the specific conditions of use in the ethanol production facilities and subsequent steps described by the applicant, DDGS produced from maize 3272 does not raise safety concerns when compared to DDGS from non-GM maize.

Documentation provided to EFSA

1) Mandate from the European Commission (EC) to EFSA received on 4 April 2017 concerning a request to assess new additional information related to the application for authorisation of food and feed containing, consisting of and produced from genetically modified maize 3272 (EFSA-GMO-UK-2006-34).
2) Letter from EFSA to EC acknowledging the reception of the mandate, 2 May 2017.
3) Request for supplementary information to the applicant, 19 May 2017.
4) Reception of supplementary information from the applicant, 24 May 2017.
5) Request for supplementary information to the applicant, 28 July 2017.
6) Reception of supplementary information from the applicant, 30 August 2017.
7) Request for supplementary information to the applicant, 11 September 2017.
8) EFSA invitation to the applicant for a clarification tele-conference to be hold on 19 October 2017, 16 October 2017.
9) EFSA to applicant, follow up on the clarification tele-conference held on 19 October 2017, 25 October 2017.
10) Request for supplementary information to the applicant, 18 October 2017.
11) Reception of supplementary information from the applicant, 20 October 2017.
12) Request to extend the deadline for submission of the supplementary information requested by EFSA (11 September 2017), 27 October 2017.
13) EFSA's acceptance to extend the deadline for submission of the supplementary information requested (11 September 2017), 31 October 2017.
14) Request to extend the deadline for submission of the supplementary information requested by EFSA (18 October 2017), 1 December 2017.
15) EFSA's acceptance to extend the deadline for submission of the supplementary information requested (18 October 2017), 19 December 2017.
16) Request for supplementary information to the applicant, 8 March 2018.
17) Reception of supplementary and spontaneous information from the applicant, 2 May 2018.
18) Reception of supplementary information from the applicant, 4 May 2018.
19) Reception of supplementary information from the applicant, 30 July 2018.
20) Request for supplementary information to the applicant, 1 August 2018.
21) Reception of supplementary information from the applicant, 14 September 2018.
22) Request for supplementary information to the applicant, 24 September 2018.
23) Reception of supplementary information from the applicant, 2 October 2018.
24) EFSA request to EC to extend the deadline for finalisation of the mandate, 31 October 2018.
25) EFSA invitation to the applicant for a clarification tele-conference to be hold on 27 November 2018, 8 November 2018.
26) EFSA to applicant, follow up on the clarification tele-conference held on 27 November 2018, 19 December 2018.

27) EC acceptance of EFSA's request to extend the deadline for finalisation of the mandate, 11 December 2018.
28) Request for supplementary information to the applicant, 17 December 2018.
29) Reception of supplementary information from the applicant, 4 June 2019.
30) EFSA request to EC to extend the deadline for finalisation of the mandate, 24 July 2019.
31) EC acceptance of EFSA's request to extend the deadline for finalisation of the mandate, 9 August 2019.

References

Armentia A, Dias-Perales A, Castrodeza J, Duenas-Laita A, Palacin A and Fernandes S, 2009. Why can patients with baker's asthma tolerate wheat flour ingestion? Is wheat pollen allergy relevant? Allergologia et Immunopathologia, 37, 203–204.

Baur X, 2005. Enzymes as occupational and environmental respiratory sensitisers. International Archives of Occupational and Environmental Health, 78, 279–286.

Baur X and Czuppon AB, 1995. Allergic reaction after eating alpha-amylase (Asp o 2)-containing bred. A case report. Allergy, 50, 85–87.

Baur X, Fruhmann G, Haug B, Rasche B, Reiher W and Weiss W, 1986. Role of *Aspergillus* amylase in baker's asthma. The Lancet, 327, 43.

Baur X, Sauer W, Weiss W and Fruhmann G, 1989. Inhalant allergens in the modern baking industry. Journal of Immunology and Allergy Practice, 11, 13–15.

Baur X, Budnik LT and von Kirchbach G, 2013. Allergic asthma caused by exposure to bacterial alpha-amylase Termamyl®. American Journal of Industrial Medicine, 56, 378–380.

Bento-Silva A, Vaz Patto MC and do Rosário Bronze M, 2018. Relevance, structure and analysis of ferulic acid in maize cell walls. Food Chemistry, 246, 360–378.

Boz H, 2015. Ferulic acid in cereals—a review. Czech Journal of Food Science, 33, 1.

Brisman J, 2002. Baker's asthma. Occupational and Environmental Medicine, 59, 498–502.

Brisman J and Belin L, 1991. Clinical and immunological responses to occupational exposure to alpha-amylase inthe baking industry. British Journal of Industrial Medicine, 48, 604–608.

Brisman J, Nieuwenhuijsen MJ, Venables KM, Putcha V, Gordon S and Taylor AJ, 2004. Exposure–response relations for work related respiratory symptoms and sensitisation in a cohort exposed to alpha-amylase. Occupational and Environmental Medicine 61, 551–553.

Budnik LT, Scheer E, Burge PS and Baur X, 2017. Sensitising effects of genetically modified enzymes used in flavour, fragrance, detergence and pharmaceutical production: cross-sectional study. Occupational and Environmental Medicine, 74, 39–45.

Codex Alimentarius, 2009. Foods Derived from Modern Biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Rome. Available online: http://www.fao.org/docrep/011/a1554e/a1554e00.htm

Cullinan P, Cook A, Jones M, Cannon J, Fitzgerald B and Taylor AJ, 1997. Clinical responses to ingested fungal alpha-amylase and hemicellulase in persons sensitized to Aspergillus fumigatus? Allergy, 52, 346–349.

De Wilde T, De Meulenaer B, Mestdagh F, Govaert Y, Vandeburie S, Ooghe W, Fraselle S, Demeulemeester K, van Peteghem C, Calus A, Degroodt JM and Verhé R, 2005. Influence of storage practices on acrylamide formation during potato frying. Journal of Agricultural and Food Chemistry, 53, 6550–6557.

EFSA (European Food Safety Authority), 2009. External Scientific Report submitted to EFSA. Bibliographic review on the potential of microorganisms, microbial products and enzymes to induce respiratory sensitization. Question number EFSA-Q-2009-00789. EFSA supporting publications 2010:EN-75, 95 pp. https://doi.org/10.2903/sp.efsa.2010.en-75

EFSA (European Food Safety Authority), Devos Y, Guajardo IM, Glanville J and Waigmann E, 2017. Explanatory note on literature searching conducted in the context of GMO applications for (renewed) market authorisation and annual post-market environmental monitoring reports on GMOs authorised in the EU market. EFSA supporting publications 2017;14(4):EN-1207, 48 pp. https://doi.org/10.2903/sp.efsa.2017.en-1207

EFSA (European Food Safety Authority), Dumont AF, Lanzoni A, Waigmann E and Paoletti C, 2018. Relevance of new scientific information (Santos-Vigil et al., 2018) in relation to the risk assessment of genetically modified crops with Cry1Ac. EFSA supporting publication 2018:EN-1504, 13 pp. https://doi.org/10.2903/sp.efsa.2019.EN-1504

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2006. Guidance document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. EFSA Journal 2006:4(4);99, 100 pp. https://doi.org/10.2903/j.efsa.2006.99

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010a. Statistical considerations for the safety evaluation of GMOs. EFSA Journal 2010;8(1):1250, 59 pp. https://doi.org/10.2903/j.efsa.2010.1250

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010b. Draft Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. https://doi.org/10.2903/j.efsa.2010.1700

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011. Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011;9(5):2150, 37 pp. https://doi.org/10.2903/j.efsa.2011.2150

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2013. Scientific Opinion on application (EFSA-GMO-UK-2006-34) for the placing on the market of genetically modified maize 3272 with a thermotolerant alpha-amylase, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Crop Protection AG. EFSA Journal 2013;11(6):3252, 27 pp. https://doi.org/10.2903/j.efsa.2013.3252

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Guerche P, Jones H, Manachini B, Messean A, Nielsen EE, Nogue F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Eigenmann P, Epstein M, Hoffmann-Sommergruber K, Koning F, Lovik M, Mills C, Moreno FJ, van Loveren H, Selb R and Fernandez-Dumont A, 2017. Guidance on allergenicity assessment of genetically modified plants. EFSA Journal 2017;15(5):4862, 49 pp. https://doi.org/10.2903/j.efsa.2017.4862

FAO/WHO, 2001. Evaluation of Allergenicity of Genetically Modified Foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology. Food and Agricultural Organization/World Health Organization. Rome, Italy.

Hernández-Bel P, de la Cuadra J, García R and Alegre V, 2011. Protein contact dermatitis: review of 27 cases. Actas Dermosifiliogr, 102, 336–343.

Houben G, Blom M, Alvito P, Assunção R, Crevel R, Fæste CK, Le TM, Madsen CB, Remington B, Stroheker T, Vassilopoulou E, Verhoeckx K, Žiarovská J and Constable A, 2019. Defining the targets for the assessment of IgE-mediated allergenicity of new or modified food proteins. Food and Chemical Toxicology, 127, 61–69.

Kanny G and Moneret-Vautrin DA, 1995. Alpha-amylase contained in bred can induce food allergy. Journal of Allergy and Clinical Immunology, 95, 132–133.

Ladics GS, Fry J, Goodman R, Herouet-Guicheney C, Hoffmann-Sommergruber K, Madsen CB, Penninks A, Pomés A, Roggen EL, Smit J and Wal JM, 2014. Allergic sensitization: screening methods. Clinical and Translational Allergy, 4, 13.

Landry TD, Chew L, Davis JW, Frawley N, Foley HH, Stelman SJ, Thomas J, Wolt J and Hanselman DS, 2003. Safety evaluation of an α-amylase enzyme preparation derived from the archaeal order Thermococcales as expressed in *Pseudomonas fluorescens* biovar I. Regulatory Toxicology and Pharmacology 37, 149–168.

Losada E, Hinojosa M, Quirce S, Sanchez-Cano M and Moneo I, 1992. Occupational asthma caused by alpha-amylase inhalation: clinical and immunologic findings and bronchial response patterns. Journal of Allergy and Clinical Immunology, 89, 118–125.

Meding B, Wrangsjo K, Brisman J and Jarvholm B, 2003. Hand eczema in 45 bakers - a clinical study. Contact Dermatitis, 48, 7–11.

Moreno-Ancillo A, Domínguez-Noche C, Gil-Adrados AC and Cosmes PM, 2004. Bread eating induced oral angioedema due to alpha-amylase allergy. Journal of Investigational Allergology and Clinical Immunology, 14, 346–347.

Morren MA, Janssens V, Dooms-Gossens A, Van Hoeyveld E, Cornelis A, De Wolf-Peeters C and Heremans A, 1993. alpha-Amylase, a flour additive: an important cause of protein contact dermatitis in bakers. Journal of the American Academy of Dermatology, 29, 723–728.

Poulsen LK, 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel food. Molecular Nutrition and Food Research, 48, 413–423.

Quirce S, Cuevas M, Dıez-Gomez M, Fernandez-Rivas M, Hinojosa M, Gonzalez R and Losada E, 1992. Respiratory allergy to Aspergillus-derived enzymes in bakers' asthma. Journal of Allergy and Clinical Immunology, 90, 970–978.

Remington B, Broekman HCH, Blom WM, Capt A, Crevel RWR, Dimitrov I, Faeste CK, Fernandez-Canton R, Giavi S, Houben GF, Glenn KC, Madsen CB, Kruizinga AK and Constable A, 2018. Approaches to assess IgE mediated allergy risks (sensitization and cross-reactivity) from new or modified dietary proteins. Food and chemical toxicology, 112, 97–107.

Rosen C, Sun S, Olsen N, Thornton M, Knowles L and Knowles NR, 2018. Impact of agronomic and storage practices on acrylamide in processed potatoes. American Journal of Potato Research, 95, 319–327.

Sander I, Raulf-Heimsoth M, Siethoff C, Lohaus C, Meyer HE and Baur X, 1998. Allergy to Aspergillus-derived enzymes in the baking industry: identification of beta-xylosidase from *Aspergillus niger* as a new allergen (Aspn 14). Journal of Allergy and Clinical Immunology, 102, 256–264.

Sarlo K, Fletcher ER, Gaines WG and Ritz HL, 1997. Respiratory allergenicity of detergent enzymes in the guinea pig intratracheal test: Association with sensitization of occupationally exposed individuals. Fundamental and Applied Toxicology, 39, 44–52.

US Grains Council. 2018. DDGS User's Handbook, 4th Ed. U.S. Grains Council, Washington, DC. 34 chapters, 376 pages. Available online: https://grains.org/wp-content/uploads/2018/05/USGC-DDGS-Handbook-2018-WEB.pdf

Vein NK, 2000. Bakers. In: Kanerva L, Elsner P, Wahlberg JE, Maibach HI, eds. Handbook of Occupational Dermatology. Springer-Verlag, Berlin.

Waltz E, 2011. Amylase corn sparks worries. Nature Biotechnology, 29, 294.

Abbreviations

DDGS	dried distiller grains
GM	genetically modified
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
OECD	Organisation for Economic Co-operation and Development
PMI	phosphamannose isomerase