SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 26 October 2016

doi: 10.2903/j.efsa.2016.4633

Scientific Opinion on an application by DOW AgroSciences LLC (EFSA-GMO-NL-2010-89) for placing on the market the genetically modified herbicide-tolerant maize DAS-40278-9 for food and feed uses, import and processing under Regulation (EC) No 1829/2003

EFSA Panel on Genetically Modified Organisms (GMO),
Hanspeter Naegeli, Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Mikołaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli, Jean-Michel Wal, Fernando Álvarez, Michele Ardizzone, Antonio Fernández Dumont, Yi Liu, Franco Maria Neri and Matthew Ramon

Abstract

Maize DAS-40278-9 was developed by direct Whiskers-mediated transformation to express the aryloxyalkanoate dioxygenase-1 (AAD-1) protein, conferring tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (AOPP) herbicides. The molecular characterisation of maize DAS-40278-9 did not raise safety issues. The agronomic, phenotypic and compositional characteristics of maize DAS-40278-9 tested under field conditions revealed no differences between maize DAS-40278-9 and its non-genetically modified (GM) comparator that would give rise to food and feed or environmental safety concerns. There were no concerns regarding the potential toxicity and allergenicity of the newly expressed protein AAD-1, and no evidence that the genetic modification might significantly change the overall allergenicity of maize DAS-40278-9. The nutritional characteristics of maize DAS-40278-9 are not expected to differ from those of non-GM maize varieties and no post-market monitoring of food/feed is considered necessary. Maize DAS-40278-9 is as nutritious as its non-GM comparator and other non-GM commercial varieties. There are no indications of an increased likelihood of establishment and spread of occasional feral maize DAS-40278-9 plants, unless these plants are exposed to the intended herbicides. However, this will not result in different environmental impacts compared to conventional maize. Considering the scope of the application, interactions with the biotic and abiotic environment were not considered an issue. Risks associated with the unlikely but theoretically possible horizontal gene transfer from maize DAS-40278-9 to bacteria were not identified. The post-market environmental monitoring plan and reporting intervals are in line with the scope of the application. In conclusion, the EFSA GMO Panel considers that the information available for maize DAS-40278-9 addresses the scientific comments raised by the Member States and that maize DAS-40278-9, as described in this application, is as safe as the non-GM comparator and non-GM maize reference varieties with respect to potential effects on human and animal health and the environment in the context of the scope of this application.

Keywords: GMO, maize (*Zea mays*), DAS-40278-9, herbicide tolerance, AAD-1, Regulation (EC) No 1829/2003

Requestor: Competent Authority of the Netherlands

Question number: EFSA-Q-2010-01326

Correspondence: GMO@efsa.europa.eu

Panel members: Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Mikołaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Hanspeter Naegeli, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli and Jean-Michel Wal.

Acknowledgements: The Panel wishes to thank the following for the support provided: the members of the Working Group on Molecular Characterisation, Food and Feed Safety Assessment and Environmental Risk Assessment for the preparatory work on this scientific opinion.

Suggested citation: 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, Messéan A, Nielsen EE, Nogué F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Álvarez F, Ardizzone M, Fernández Dumont A, Liu Y, Neri FM and Ramon M, 2016. Scientific Opinion on an application by DOW AgroSciences LLC (EFSA-GMO-NL-2010-89) for placing on the market the genetically modified herbicide-tolerant maize DAS-40278-9 for food and feed uses, import and processing under Regulation (EC) No 1829/2003. EFSA Journal 2016;14(12):4633, 25 pp. doi:10.2903/j.efsa.2016.4633

ISSN: 1831-4732

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

Summary

Following the submission of an application (EFSA-GMO-NL-2010-89) under Regulation (EC) No 1829/2003[1] by Dow AgroSciences LLC, the EFSA Panel on Genetically Modified Organisms (GMO Panel) was asked to deliver a scientific opinion on the safety of the herbicide-tolerant genetically modified (GM) maize (*Zea mays* L.) DAS-40278-9 (Unique Identifier DAS-4Ø278-9). The scope of application EFSA-GMO-NL-2010-89 is for import, processing, and food and feed uses of maize DAS-40278-9 within the European Union (EU), but excludes cultivation in the EU.

The GMO Panel evaluated maize DAS-40278-9 with reference to the scope and appropriate principles described in its guidelines for the risk assessment of GM plants. The evaluation addressed the following components of the risk assessment: the molecular characterisation of the inserted DNA and analysis of the expression of the corresponding protein; the comparative analyses of compositional, agronomic and phenotypic characteristics; the safety of the newly expressed proteins and the whole food/feed with respect to potential toxicity, allergenicity and nutritional characteristics; the environmental risk assessment and the post-market environmental monitoring plan.

Maize DAS-40278-9 was developed by direct Whiskers-mediated transformation of immature maize embryos. It expresses the aryloxyalkanoate dioxygenase-1 (AAD-1) protein, which confers tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (AOPP) herbicides. The molecular characterisation data established that maize DAS-40278-9 contains one functional insert consisting of an intact *aad-1* expression cassette. No other parts of the plasmid used for transformation could be detected in maize DAS-40278-9. Bioinformatic analyses and genetic stability studies were performed and the results did not raise safety issues. The levels of the newly expressed protein present in maize DAS-40278-9 were obtained and reported adequately.

Based on the agronomic and phenotypic characteristics of maize DAS-40278-9 tested under field conditions, no relevant differences were observed between maize DAS-40278-9 and its non-GM comparator, except for 'plant height' and 'time to silking'. None of the differences identified in forage and grain composition between maize DAS-40278-9 and the non-GM comparator required further assessment for food and feed safety.

The safety assessment identified no concerns regarding the potential toxicity of the newly expressed AAD-1 protein in maize DAS-40278-9, considering the results of a subacute 28-day toxicity study where no adverse effects were observed at the highest dose tested and considering the results on the structural and functional properties of the AAD-1 protein, including bioinformatic analyses. The GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity with the AAD-1 protein or regarding the overall allergenicity of maize DAS-40278-9. Based on the comparative analysis, the nutritional characteristics of food and feed derived from maize DAS-40278-9 are not expected to differ from that of food and feed derived from non-GM maize varieties. The GMO Panel concludes that maize DAS-40278-9 is as safe and as nutritious as its non-GM comparator and the non-GM maize reference varieties. The GMO Panel considers that post-market monitoring of food/feed derived from maize DAS-40278-9 is not necessary, given the absence of safety concerns identified.

Due to the low survival capacity of maize, the observed differences in 'plant height' and 'time to silking' are unlikely to change the fitness (e.g. survival, fecundity, competitiveness) or invasiveness characteristics of maize DAS-40278-9 plants. In the case of accidental release into the environment of viable seeds of maize DAS-40278-9, there are no indications of an increased likelihood of establishment and spread of feral maize DAS-40278-9 plants, unless these plants are exposed to 2,4-D- or AOPP-containing herbicides. However, the GMO Panel is of the opinion that this will not result in different environmental impacts compared to conventional maize. Potential interactions with the biotic and abiotic environment were not considered to be an issue by the GMO Panel. Risks associated with an unlikely but theoretically possible horizontal gene transfer (HGT) from maize DAS-40278-9 to bacteria have not been identified. Therefore, considering the introduced traits, the outcome of the comparative analysis, the routes of exposure and the limited exposure levels, the GMO Panel concludes that maize DAS-40278-9 would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment. The post-market environmental monitoring (PMEM) plan provided by the applicant is in line with the scope of the application and the requirements of the GMO Panel for PMEM of GM plants. The GMO Panel agrees with the reporting intervals proposed by the applicant in the monitoring plan.

[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.

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-NL-2010-89, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. In conclusion, the GMO Panel considers that the information available for maize DAS-40278-9 addresses the scientific comments raised by the Member States and that maize DAS-40278-9, as described in this application, is as safe as the non-GM comparator and non-GM maize reference varieties with respect to potential effects on human and animal health and the environment in the context of the scope of this application.

Table of contents

1. Introduction

Maize DAS-40278-9 was developed to confer tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (AOPP) herbicides. Tolerance to 2,4-D and AOPP herbicides is achieved by the expression of an *aad-1* gene from *Sphingobium herbicidovorans* encoding the aryloxyalkanoate dioxygenase (AAD-1) enzyme.[2]

1.1. Background

On 11 November 2010, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands an application (Reference EFSA-GMO-NL-2010-89) for authorisation of genetically modified (GM) maize DAS-40278-9 (Unique Identifier DAS-4Ø278-9), submitted by Dow AgroSciences LLC within the framework of Regulation (EC) No 1829/2003 on GM food and feed.

After receiving the application EFSA-GMO-NL-2010-89, and in accordance with Articles 5(2)(b) and 17 (2)(b) of the Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission, and made the summary of the application publicly available on the EFSA website.[3] EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of the Regulation (EC) No 1829/2003. On 20 January 2011 and 18 February 2011, EFSA received additional information requested under completeness check (on 20 December 2010 and 9 February 2011, respectively). On 11 March 2011, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to the Member States and the European Commission, and consulted nominated risk assessment bodies of the Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[4] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member States had 3 months after the date of receipt of the valid application (until 11 June 2011) to make their opinion known.

On 20 May 2011, 7 September 2012, 21 May 2014, 19 June 2014, 18 November 2014, 19 February 2015, 27 February 2015, 27 March 2015, 14 April 2015, 24 April 2015, 17 July 2015 and 19 October 2015, the GMO Panel requested additional information from the applicant. The applicant provided the requested information on 01 February 2012, 29 January 2014, 23 July 2014, 29 July 2014, 16 December 2014, 12 March 2015, 7 April 2015, 27 April 2015, 1 June 2015, 24 August 2015, 31 March 2016 and 9 August 2016. The applicant also spontaneously provided additional information on 5 April 2011, 11 April 2012, 2 July 2012, 24 August 2012, 29 January 2014, 23 July 2014, 31 March 2016 and 9 August 2016.

In giving its scientific opinion on maize DAS-40278-9 to the European Commission, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of 6 months from the acknowledgement of the valid application. As additional information was requested by the EFSA Panel on Genetically Modified Organisms (GMO Panel), the time limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1) and 18(2) of Regulation (EC) No 1829/2003.

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).

1.2. Terms of Reference as provided by the requestor

The EFSA GMO Panel was requested to carry out a scientific assessment of maize DAS-40278-9 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003.

Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of genetically modified organisms (GMOs) or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)(e) of Regulation (EC) No 1829/2003.

[2] Dossier: Part I – Section A5.
[3] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2010-01326
[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.

The GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the GMO Panel did not consider proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

2. Data and methodologies

2.1. Data

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-NL-2010-89, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications.

2.2. Methodologies

The GMO Panel carried out an evaluation of the scientific risk assessment of maize DAS-40278-9 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The GMO Panel took into account the appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed (EFSA, 2006a; EFSA GMO Panel, 2011a), the environmental risk assessment (ERA) of GM plants (EFSA GMO Panel, 2010c) and on the post-market environmental monitoring (PMEM) of GM plants (EFSA, 2006b; EFSA GMO Panel, 2011b).

The comments raised by the Member States are addressed in Annex G of the EFSA overall opinion[3] and were taken into consideration during the evaluation of the risk assessment.

3. Assessment

3.1. Molecular characterisation

3.1.1. Evaluation of relevant scientific data

3.1.1.1. Transformation process and vector constructs

Immature embryos of maize line Hi-II (a derivative of the A188 and B73 inbred maize lines) were transformed with an *Fsp*I fragment of plasmid pDAS1740 by direct Whiskers-mediated transformation (Petolino et al., 2003; Petolino and Arnold, 2009). The DNA fragment to be transformed was obtained by a *Fsp*I digestion of plasmid pDAS1740, which resulted in five fragments: a 6,236 bp fragment containing the *aad-1* expression cassette, providing tolerance to 2,4-D and AOPP herbicides, two fragments containing a portion of the ampicillin resistance gene of the vector backbone (about 1 kb each), and two minor fragments of 9 bp. Fragments were separated by column chromatography and the 6,236 bp *Fsp*I fragment was isolated and used for transformation.[5]

The 6,236 bp fragment consisted of the following genetic elements: the constitutive ZmUbi1 promoter from *Zea mays*; a synthetic, plant codon-optimised version of the *aad-1* gene from soil bacterium *S. herbicidovorans*; the 3′ untranslated region from the peroxidase gene from *Z. mays* (ZmPer 3′ untranslated region (UTR)), used as a terminator. The expression cassette was flanked by identical matrix attachment regions (RB7 MAR v3 and RB7 MAR v4) from *Nicotiana tabacum*, in order to increase expression of the *aad-1* gene.[6]

Additional functional elements of the *Fsp*I digested plasmid pDAS1740, not intended to be transferred into the maize genome, were plasmid backbone sequences of pUC19, including an ampicillin resistance gene.

3.1.1.2. Transgene constructs in the GM plant

Molecular characterisation of maize DAS-40278-9 was performed by Southern analysis, polymerase chain reaction (PCR) and DNA sequence analysis in order to determine insert copy number, size and organisation of the inserted sequences and to confirm the absence of plasmid backbone sequences. The approach used was acceptable both in terms of coverage and sensitivity.[7]

[5] Dossier: Part I – Section C1.
[6] Dossier: Part I – Section C3.
[7] Dossier: Part I – Section D2.

Southern analysis indicated that maize DAS-40278-9 contains a single insert, which consists of a single copy of the *aad-1* expression cassette from pDAS1740. The insert and copy number were confirmed by multiple restriction enzyme/probe combinations covering the 6,236 bp inserted fragment and flanking regions. PCR analyses confirmed the results obtained by Southern analyses. The absence of vector backbone sequences was demonstrated by Southern analysis using backbone-specific probes.

The insert and 5′ and 3′ flanking regions (1,873 and 1,868 bp, respectively) of maize DAS-40278-9 were sequenced. The sequence of the insert confirmed the presence of a 4,816 bp fragment of pDAS1740 which contains an intact *aad-1* expression cassette, a 249 bp partial MAR v3 on the 5′ terminus, and a 1,096 bp partial MAR v4 on the 3′ terminus. Sequence analysis revealed that the *aad-1* sequence in DAS-40278-9 maize is identical to the corresponding sequences in pDAS1740, except for a single base pair change (from T to C) in the non-coding region of the 3′UTR of the *aad-1* gene.[7]

The possible interruption of known endogenous maize genes by the insertion in DAS-40278-9 maize was evaluated by bioinformatic analyses of the pre-insertion locus and of the genomic sequences flanking the insert. Comparison of the sequences of the flanking regions in DAS-40278-9 with that of the pre-insertion locus indicated a 21 bp insertion as well as a 2 bp deletion at the 5′ junction in DAS-40278-9. A 1 bp insertion occurred at the 3′ junction.[7] The bioinformatic analyses of the DAS-40278-9 flanking regions indicated that the insert in DAS-40278-9 most likely integrated into a region containing sequences showing similarity to a Grande retrotransposon. No evidence was found for the interruption of known endogenous gene in the maize genome.[8]

The results of segregation (see Section 3.1.1.4) and bioinformatic analyses established that the insert is located in the nuclear genome.[9]

Updated bioinformatic analyses of the amino acid sequences of the newly expressed protein (AAD-1), according to EFSA guidance (EFSA GMO Panel, 2011a), did not indicate significant similarities to toxins and allergens.[10]

In addition, updated bioinformatic analyses of the newly created open reading frames (ORFs) within the insert and at its junction sites did not indicate significant similarities to toxins and allergens.[10]

3.1.1.3. Information on the expression of the insert

AAD-1 protein levels were analysed by an enzyme-linked immunosorbent assay (ELISA) in material harvested from replicated field trials across eight locations in the USA during the 2009 growing season.[11] Samples analysed included leafs (V2–V4, V9 and R1), roots and pollen (R1), forage (R4), whole plants (R6), and grain at maturity both those treated and non-treated with quizalofop, 2,4-D or a combination of the two. The mean values and ranges of the AAD-1 protein levels in grains and forage are summarised in Table 1.

Table 1: Protein expression data for AAD-1 in maize DAS-40278-9 (μg/g dry weight) grains and forage (number of grain and forage samples is 31 for unsprayed and 32 for herbicide-treated plants)

	Untreated	2,4-D-treated	Quizalofop-treated	2,4-D- and quizalofop-treated
Grains	3.95[(a)] ± 1.03[(b)] (1.76–6.31)[(c)]	4.16 ± 1.22 (1.97–8.18)	4.11 ± 0.55 (2.32–6.15)	3.85 ± 0.95 (1.64–6.46)
Forage	8.08 ± 1.65 (4.63–11.87)	8.74 ± 1.98 (ND–13.73)	8.03 ± 1.76 (4–12.12)	8.55 ± 2.06 (3.77–12.3)

2,4-D: 2,4-dichlorophenoxyacetic acid herbicide.
(a): Mean.
(b): Standard deviation.
(c): Range.

3.1.1.4. Inheritance and stability of inserted DNA

Genetic stability of the maize DAS-40278-9 insert was assessed by Southern analysis of genomic DNA from five different generations.[12] The restriction enzyme/probe combinations used were sufficient

[8] Dossier: Part I – Section D7; additional information: 9/8/2016.
[9] Dossier: Part I – Section D2, D5; additional information: 9/8/2016.
[10] Dossier: Part I – Section D2; additional information: 9/8/2016.
[11] Dossier: Part I – Section D3.
[12] Dossier: Part I – Section D5.

to conclude that all the plants tested retained the single copy of the insert and flanking regions, which were stably inherited in subsequent generations.

Phenotypic stability was observed by segregation analysis of the quizalofop tolerance trait of maize DAS-40278-9. The results supported the presence of a single insertion, segregating in a Mendelian fashion.

3.1.2. Conclusion

The molecular characterisation data establish that maize DAS-40278-9 contains a single insert consisting of one copy of the *aad-1* expression cassette. Bioinformatic analyses of the sequences encoding the newly expressed proteins and other ORFs present within the insert or spanning the junctions between the insert and genomic DNA did not raise safety issues. The stability of the inserted DNA and of the introduced herbicide tolerance trait was confirmed over several generations. The levels of the AAD-1 protein were obtained and reported adequately.

3.2. Comparative analysis

3.2.1. Evaluation of relevant scientific data

3.2.1.1. Choice of comparator and production of material for the comparative analysis[13]

Application EFSA-GMO-NL-2010-89 presents data on agronomic and phenotypic characteristics, as well as forage and grain composition, of maize DAS-40278-9 derived from field trials performed at eight sites in the USA in 2009 (Table 2).

Table 2: Overview of comparative assessment studies with maize DAS-40278-9 provided in application EFSA-GMO- NL-2010-89

Study focus	Study details	Comparators	Commercial reference varieties
Agronomic and phenotypic characteristics; composition	Field trials, 2009, USA (eight locations)	Non-GM comparator (XHH13 × 7SH382)	Six non-GM varieties

Non-GM: non-genetically modified.

Field trials for the agronomic, phenotypic and compositional assessment of maize DAS-40278-9 were conducted in major maize growing areas of the USA,[14] 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 DAS-40278-9 not treated with the intended herbicides (DAS-40278-9/untreated), DAS-40278-9 treated with 2,4-D (DAS-40278-9/2,4-D), DAS-40278-9 treated with quizalofop (DAS-40278-9/quizalofop), DAS-40278-9 treated sequentially with 2,4-D and quizalofop (DAS-40278-9/2,4-D+quizalofop), the non-GM comparator (XHH13 × 7SH382), and three commercial non-GM maize reference varieties. All materials were treated (sprayed) with required maintenance pesticides (including conventional herbicides) according to local requirements. In total, six non-GM maize reference varieties were included across all the field trials sites.[15] The non-GM comparator XHH13 × 7SH382 had a genetic background similar to maize DAS-40278-9 as documented by the pedigree.[13]

Statistical analysis of field trial data

The statistical analysis of the agronomic, phenotypic and compositional data from the 2009 field trials followed the recommendations of the GMO Panel (EFSA GMO Panel, 2010a, 2011a). This includes, for each of the four treatments of maize DAS-40278-9, the application of a difference test (between the GM maize and its non-GM comparator) 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).[16]

[13] Dossier: Part I – Section D7; additional information: 1/2/2012 and 23/7/2014.
[14] The sites were in Richland (IA), Carlyle (IL), Bradford (IL), Rockville (IN), La Plata (MO), Dudley (MO), York (NE) and Germansville (PA).
[15] Dekalb 6343, Croplan 691, LG seeds 2597, Northup King 72-G8, Midland/Phillips 7B15 and Pioneer 32T16.
[16] 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).

3.2.1.2. Agronomic and phenotypic characteristics[17]

In total, 28 agronomic and phenotypic endpoints were evaluated.[18]

For 16 agronomic/phenotypic endpoints[19] not fulfilling the requirements of the statistical tests described above (e.g. categorical endpoints), a non-parametric test of difference (Wilcoxon signed-rank test) was performed. No statistically significant differences were identified for any of the endpoints.

Of the 12 endpoints analysed with parametric statistics, the test of equivalence could not be applied to plant height because of the very small variation among the non-GM reference varieties. Plant height was found significantly different between maize DAS-40278-9 and the non-GM comparator for one of the four treatments (2,4-D+quizalofop).[20]

The combination of the test of difference and the test of equivalence could be applied to the remaining 11 endpoints, with the following results:

- The test of difference between phenotypic and agronomic characteristics of maize DAS-40278-9/untreated and the non-GM comparator identified statistically significant differences for two endpoints (early stand count at V1 and time to silking). The test of equivalence between maize DAS-40278-9/untreated and the non-GM maize reference varieties indicated that early stand count at V1 fell under equivalence category I and time to silking fell under equivalence category II.
- For DAS-40278-9/2,4-D, statistically significant differences were identified for seven endpoints (early stand count at V1 and V4, final stand count, yield, pollen colour at 30 min, pollen shape at 30 and 60 min). The test of equivalence showed that all these endpoints fell under equivalence category I.
- For DAS-40278-9/quizalofop, statistically significant differences were identified for three endpoints (yield, pollen colour at 30 min, and pollen shape at 60 min). The test of equivalence showed that all these endpoints fell under equivalence category I.
- For DAS-40278-9/2,4-D+quizalofop, statistically significant differences were identified for five endpoints (yield, pollen colour at 30 and 60 min, pollen shape at 30 and 60 min). The test of equivalence showed that all these endpoints fell under equivalence category I.

Time to silking for DAS-40278-9/untreated was found significantly different than the non-GM comparator and fell under equivalence category II.[21] Plant height for DAS-40278-9/2,4-D+quizalofop was significantly different than the non-GM comparator and could not be categorised for equivalence. The results for time to silking and plant height are further assessed for their potential environmental impact in Section 3.4.

3.2.1.3. Compositional analysis[22]

Maize forage and grain harvested from the field trials in the USA in 2009 were analysed for 82 compounds (nine compounds for forage[23] and 73 compounds for grain[24]). The compounds included the key constituents recommended by OECD (2002).

---

[17] Dossier: Part I – Sections D4, D7; additional information: 2/7/2012, 24/8/2012, 29/1/2014, 23/7/2014 and 7/4/2015.

[18] Early stand count (V1 and V4), seedling vigour, plant vigour (injury from each of five herbicide applications), time to silking, time to pollen shed, pollen viability (pollen shape and colour, both measured at 0, 30, 60 and 120 min), plant height, ear height, stalk lodging, root lodging, final stand count, days to maturity, stay green, disease incidence, insect damage, and yield.

[19] Seedling vigour, plant vigour (five endpoints), pollen shape (0 and 120 min), pollen colour (0 and 120 min), stalk lodging, root lodging, days to maturity, stay green, disease incidence, and insect damage.

[20] Mean values (cm): the non-GM comparator: 280.4; DAS-40278-9/2,4-D+quizalofop: 277.1.

[21] Mean values (heat units): non-GM comparator: 1262; DAS-40278-9/untreated: 1270.

[22] Dossier: Part I – Section D7; additional information: 1/2/2012, 11/4/2012, 2/7/2012, 24/8/2012, 29/1/2014, 23/7/2014, 12/3/2015 and 7/4/2015.

[23] Proximates (crude protein, total fat, ash and moisture), carbohydrates by calculation, fibre fractions (acid detergent fibre (ADF) and neutral detergent fibre (NDF)), calcium and phosphorus.

[24] Proximates (crude protein, total fat, ash, and moisture), fibre fractions (ADF, NDF and total dietary fibre), amino acids (alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine), fatty acids (caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), γ-linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4) and behenic acid (C22:0).), 10 minerals (calcium, phosphorous, potassium, sodium, iron, copper, magnesium, manganese, selenium and zinc), eight vitamins (vitamin B1 (thiamine), vitamin B2 (riboflavin) vitamin B3 (niacine), vitamin B6 (pyridoxine), vitamin B9 (folic acid), β-carotene, vitamin C and vitamin E), and other compounds (inositol, furfural, *p*-coumaric acid, ferulic acid, phytic acid, trypsin inhibitor and raffinose).

The GMO Panel considered the studies performed by the applicant concerning the substrate specificity of the newly expressed protein AAD-1, which showed that AAD-1 appears to be capable of oxidising the maize endogenous compounds *trans*-cinnamic acid and indole-3-acetic acid.[25] The GMO Panel noted that phenylalanine and coumaric acid, involved in the phenylpropanoid biosynthesis and metabolically related to *trans*-cinnamic acid, were included in the spectrum of compositional parameters.

Seventeen grain constituents[26] with more than 50% of the observations below the limit of quantification were excluded from the statistical analysis.

Of the remaining 65 compounds, the test of equivalence could not be applied to one forage constituent (total fat) and to five grain constituents[27] because of the very small variation among the non-GM reference varieties**.** Among those six constituents, arginine content was found significantly different than the non-GM comparator (Table 3).

Table 3: Compositional endpoints that are further considered based on the results of the statistical analysis: means (for the GM maize and the non-GM comparator) and equivalence limits (from the non-GM reference varieties) estimated from field trials data (USA 2009, Table 2)

Endpoint	Non-GM comparator XHH13 × 7SH382	Maize DAS-40278-9				Equivalence limits from non-GM reference varieties
		Untreated[(a)]	2,4-D[(b)]	Quizalofop[(c)]	2,4-D+ quizalofop[(d)]	
Glycine (% AA)	3.67	3.52*	3.55*	3.48*	3.51*	(3.54, 4.06)
Leucine (% AA)	13.06	13.34*	13.26*	13.40*	13.32*	(12.18, 13.33)
Methionine (% AA)	1.81	1.78	1.77	1.74*	1.75	(1.77, 2.2)
Phenylalanine (% AA)	5.35	5.40	5.43*	5.44*	5.41	(4.98, 5.32)
Carbohydrates (% DM)	83.65	82.91*	82.8*	82.74*	82.91*	(83, 86.75)
Crude protein (% DM)	10.85	11.56*	11.67*	11.69*	11.51*	(8.99, 11.23)
β-Carotene (mg/kg DM)	2.46	2.80*	2.88*	2.95*	2.80*	(0.61, 1.48)
Arginine (% AA)	4.64	4.47*	4.51*	4.4*	4.48*	–

For the GM maize, significantly different entries are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds: white (for equivalence categories I–II and for arginine, for which the test was not performed), light grey (equivalence category III) and dark grey (equivalence category IV).
% AA: percentage total amino acids; DM: dry matter; –: the test of equivalence was not applied because of the small variation among the non-GM reference varieties.
(a): maize DAS-40278-9 given no target herbicide treatment.
(b): maize DAS-40278-9 treated with 2,4-D.
(c): maize DAS-40278-9 treated with quizalofop.
(d): maize DAS-40278-9 treated sequentially with 2,4-D and quizalofop.

For the remaining 59 endpoints, the results of the difference and equivalence tests were as follows:

- The test of difference between maize DAS-40278-9/untreated and the non-GM comparator identified statistically significant differences for 20 constituents[28] (16 in grain and four in

[25] Dossier: Part I – Section D7.8.1; additional information: 1/2/2012 and 1/6/2015.
[26] Caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), gamma-linolenic acid (C18:3), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4) and behenic acid (C22:0), sodium and furfural.
[27] Inositol, phytic acid, ash, selenium and arginine.
[28] Forage: carbohydrates, crude protein, calcium and phosphorus; grain: carbohydrates, crude protein, aspartic acid, glutamic acid, glycine, leucine, proline, threonine, valine, trypsin inhibitor, stearic acid (C18:0), moisture, calcium, niacin, β-carotene and ascorbic acid.

forage). The test of equivalence between maize DAS-40278-9/untreated and the set of non-GM maize reference varieties indicated that the level of 15 of the 20 constituents fell under equivalence category I or II, while the level of five grain constituents fell under equivalence category III or IV (Table 3).

- For DAS-40278-9/2,4-D, statistically significant differences were identified for 20 constituents[29] (16 in grain and four in forage). The test of equivalence showed that the level of 16 of the 20 constituents fell under equivalence category I or II, while the level of four grain constituents fell under equivalence category III or IV (Table 3).
- For DAS-40278-9/quizalofop, statistically significant differences were identified for 26 constituents[30] (21 in grain and five in forage). The test of equivalence showed that the level of 19 of the 26 constituents fell under equivalence category I or II, while the level of seven grain constituents fell under equivalence category III or IV (Table 3).
- For DAS-40278-9/2,4-D+quizalofop, statistically significant differences were identified for 14 constituents[31] (11 in grain and three in forage). The test of equivalence showed that the level of 10 of the 14 constituents fell under equivalence category I or II, while the level of four grain constituents fell under equivalence category III or IV (Table 3).

The GMO Panel considered the results for the two compounds metabolically related to *trans*-cinnamic acid; no differences were identified for coumaric acid, and the difference identified for phenylalanine (Table 3) does not pose a safety concern. Therefore, it is unlikely that the AAD-1 enzyme interacts with endogenous plant metabolism in a way which may pose a safety concern.

The GMO Panel assessed all the compositional differences between maize DAS-40278-9 and the non-GM comparator. After considering the biological role of the compounds and the magnitude and direction of the changes observed, the GMO Panel did not identify any need for further assessment with regard to food and feed safety.

3.2.2. Conclusion

The GMO Panel concludes that none of the differences identified in grain and forage composition between maize DAS-40278-9 and the non-GM comparator, and none of those identified in the agronomic and phenotypic characteristics, needs further assessment regarding food and feed safety.

Based on the agronomic and phenotypic characteristics of maize DAS-40278-9 tested under field conditions, none of the differences identified between maize DAS-40278-9 and the non-GM comparator are considered relevant except for plant height and time to silking, which are further assessed for their potential environmental impact in Section 3.4.

3.3. Food/feed safety assessment

3.3.1. Evaluation of relevant scientific data

3.3.1.1. Effects of processing[32]

Processed products

Based on the outcome of the comparative assessment (Section 3.2), processing of maize DAS-40278-9 into food and feed products is not expected to result in products different from those of commercial non-GM maize varieties.

Newly expressed proteins[33]

a) Effect of temperature on AAD-1 protein. The thermal stability of the bacterial AAD-1 protein was evaluated by heating protein solutions for 30 min at 50, 70 and 95°C in a buffer solution. At all heating

[29] Forage: carbohydrates, crude protein, calcium and phosphorus; grain: carbohydrates, crude protein, glycine, leucine, phenylalanine, threonine, raffinose, stearic acid (C18:0), arachidic acid (C20:0), moisture, calcium, iron, phosphorus, niacin, β-carotene and ascorbic acid.

[30] Forage: ash, carbohydrates, crude protein, calcium and phosphorus; grain: carbohydrates, crude protein, alanine, cystine, glutamic acid, glycine, histidine, leucine, methionine, phenylalanine, threonine, tryptophan, valine, trypsin inhibitor, stearic acid (C18:0), calcium, phosphorus, zinc, niacin, β-carotene and ascorbic acid.

[31] Forage: carbohydrates, crude protein and phosphorus; grain: carbohydrates, crude protein, aspartic acid, glycine, leucine, threonine, stearic acid (18:0), moisture, calcium, β-carotene and niacin.

[32] Dossier: Part I – Section D7.6.

[33] Dossier: Part I – Section D7.8.1; additional Information: 23/7/2014 and 27/4/2015.

conditions, the enzymatic activity of the protein was reduced to less than 3%. Only 1% of its immunoreactivity, as measured by a polyclonal antibody sandwich ELISA, was still observed at the temperatures tested. The molecular mass of the AAD-1 protein was unchanged, as indicated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

b) Effect of pH on the AAD-1 protein. The effect of pH on the bacterial AAD-1 protein *in vitro* activity was determined using 2,4-D as a substrate, revealing a pH optimum of 7.9 with progressive loss of activity at decreasing and increasing pH values. SDS-PAGE indicated that the AAD-1 protein concentration remained consistent over this pH range, suggesting that loss of enzymatic activity resulted from disruption of enzyme structure and not degradation of the AAD-1 protein.

3.3.1.2. Toxicology

Maize DAS-40278-9 expresses the new protein AAD-1 (Section 3.1.1).

Proteins used for safety assessment

Given the technical restraints in producing large enough protein quantities for safety testing from plants, AAD-1 protein was recombinantly produced in *Pseudomonas fluorescens.* The purified protein from the bacterial source and from maize DAS-40278-9 were characterised and compared in terms of their physicochemical, structural and functional properties.

AAD-1 characterisation and equivalence[34]

SDS-PAGE showed that both plant- and microbe-derived AAD-1 proteins migrated close to the expected molecular weight of ~ 33 kDa and were comparably immunoreactive to AAD-1 protein specific antibodies as shown by western blot analysis. In addition, glycosylation detection analysis demonstrated that the plant- and microbe-derived AAD-1 proteins were not glycosylated. Amino acid sequence analysis by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) and electrospray ionisation liquid chromatography/mass spectrometry (ESI-LC/MS) showed that both proteins matched the expected AAD-1 sequence. These data also revealed that the N-terminal methionine of both proteins was truncated. Additional variants were observed for the plant protein with another three short truncations up to alanine 4. A small portion of the plant protein (3%) was also N-acetylated. The C-termini were identical for both proteins and fully matched the theoretical AAD-1 sequence. Functional equivalence was demonstrated by a biochemical *in vitro* activity assay which showed that both proteins had comparable activity and specificity for the intended herbicides. Furthermore, the microbial AAD-1 protein was screened for its ability to utilise certain endogenous plant substrates. The data demonstrated that AAD-1 activity is unlikely to have a metabolic impact within transgenic plants. In addition, to justify the appropriateness of the microbial recombinant AAD-1 protein used in a 28-day oral toxicity study in mice,[35] equivalence with the DAS-40278-9 expressed AAD-1 protein was demonstrated by MS analysis and enzymatic activity assay.

Based on these data, the GMO Panel accepts the use of the microbial recombinant AAD-1 proteins for the safety studies.

Toxicological assessment of newly expressed protein

The AAD-1 protein has never been assessed by the GMO Panel.

a) Bioinfomatic studies[36]

Bioinformatic analyses of the amino acid sequence of the AAD-1 protein expressed in maize DAS-40278-9 revealed no relevant similarities to proteins known to be toxic to humans and animals (Section 3.1.1.2).

b) *In vitro* degradation studies[37]

The resistance to degradation by pepsin of the bacterial AAD-1 protein was investigated in solutions at pH ~ 1.2. The integrity of the test protein in probes taken at various time points was analysed by SDS-PAGE followed by protein staining or western blot. The AAD-1 protein was degraded by pepsin within 30 sec.

[34] Dossier: Part I – Section D7.8.1; additional information: 1/2/2012, 1/6/2015 and 9/8/2016.
[35] Additional information: 9/8/2016.
[36] Dossier: Part I – Section D7.8.1; additional information: 29/1/2014.
[37] Dossier: Part I – Section D7.9.1; additional information: 29/7/2014.

c) Acute oral toxicity testing[38]

The bacterial-derived AAD-1 protein was administrated by oral gavage at a dose of 2,000 mg/kg body weight (bw) to male and female Crl:CD1(ICR) mice. No effects related to the AAD-1 protein were observed.

The GMO Panel is of the opinion that acute toxicity testing of the newly expressed proteins is of little additional value for the risk assessment of the repeated consumption of food and feed from GM plants by humans and animals.

d) 28-day repeated dose toxicity studies[39]

The applicant provided a 28-day oral repeated dose toxicity study in mice to investigate the potential toxicity of the AAD-1 protein. However, the GMO Panel did not consider the overall study design adequate to identify the potential hazard of the AAD-1 protein, because of the low doses of AAD-1 protein tested (highest dose level approximately 45 mg/kg bw per day) and the limited number of animals used in treatment groups (5 per sex per group) to ensure an adequate statistical power (EFSA GMO Panel, 2011a). Moreover, the GMO Panel noted that analyses of coagulation were not performed. The GMO panel requested a 28-day toxicity study in rodents, to support the safety assessment of the AAD-1 protein, with a sufficient number of animals, selecting the doses according to the OECD TG 407 in order to induce adverse effects at the highest dose, or following a limit test approach, if toxicity is not expected.

Following the EFSA request, the applicant provided a new 28-day oral repeated dose toxicity study in mice, conducted in accordance with the OECD TG 407 and in compliance with the principles of Good Laboratory Practice (GLP). Groups of singly caged Crl:CD1(ICR) mice (12 per sex per group) were administered by gavage the AAD-1 protein, at a targeted dose of 1,000 mg/kg bw per day (AAD-1 protein group); the vehicle alone (vehicle control group) and bovine serum albumin (BSA) at a targeted nominal dose of 1,000 mg/kg bw per day (BSA control group). The ADD-1 and BSA protein formulations were prepared daily; for the AAD-1 protein group, the concentration of the AAD-1 protein in the test material (63.5%) was taken into account to meet the targeted dose. Feed and water were provided *ad libitum*. Animals were checked daily for mortality and general clinical signs; detailed clinical observations were conducted on all animals pretreatment and then weekly. Ophthalmoscopy was carried out before the start and at the end of the treatments. Body weights were recorded on test day 1, 2, 3, 4, 8, 15, 22 and 29 (terminal body weight) and body weight gains were calculated relative to test day 1. Feed consumption was determined on test days 1–2, 2–3, 3–4, 4–8, 8–15 and 22–29. At the end of the treatment period, haematological and clinical chemistry analyses were performed. All animals were sacrificed and underwent a complete necropsy examination with selected organs weighed. Organs and tissues from all animals were subjected to a comprehensive histological examination.

The GMO Panel noted that the AAD-1 protein formulations were prepared from the powdered test material stored at approximately 4°C until use. Stability tests on the powdered test material (i.e. the lyophilized AAD-1 protein) were not performed as part of this study and, according to the study report, the test material stability under storage conditions was unknown. Therefore, the concentration of AAD-1 protein of 63.5% in the powdered test material following storage has not been confirmed.

The GMO Panel noted that haematology and clinical chemistry analyses were conducted on six mice/gender per group and coagulation (prothrombin time) was conducted on the remaining six animals in each group. The reasoning provided by the applicant was practical limitations in obtaining sufficient quantities of blood from mice for both haematology, clinical chemistry and coagulation examinations in the same animal. However, it is well known that when mice are used as the test animal, additional animals may be needed in each dose group, to conduct all required determinations. The GMO Panel also noted that the animals were not fasted prior to necropsy and blood collection, as recommended in the OECD TG 407.

The AAD-1 protein group was statistically compared to the BSA control group; the latter was also compared to the vehicle control group in order to assess potential effects of the higher protein intake. All the parameters examined statistically were first tested for equality of variance using Bartlett's test (for sexes combined), and possibly scale-transformed if the test was significant.[40] Two different statistical analyses were then performed: a two-way analysis of variance (ANOVA; factors: sex and

[38] Dossier: Part I – Section D7.8.1.
[39] Dossier: Part I – Section D7.8.1; additional information: 9/8/2016.
[40] $\alpha = 0.01$.

dose for the sexes combined), followed by a one-way ANOVA (factor: dose for sexes separated) if a significant result[41] was obtained in the former. The same parameters were also tested using Bartlett's test (for sexes separated) and analysed with a one-way ANOVA (factor: dose for sexes separated). Comparisons of individual dose groups to the control group were made with Dunnett's test.[41] For body weight gains, globulin, albumin/globulin ratio, RBC indices and differential WBC counts, only descriptive statistics were reported.

The results of the dose confirmation analyses from the first mix revealed that the average recoveries for AAD-1 protein and BSA in 0.5% METHOCEL were 75.1% and 79.4%, respectively, based on nominal dosing suspensions at 100 mg/mL; the average recoveries were therefore within the acceptable experimental variation (70–120%). The GMO Panel noted that, based on the measured concentration of AAD-1 protein in the dosing suspension, the actual dose administered in the first mix was 751 mg/kg bw per day. The results of the homogeneity analyses indicated that the preparations were homogeneously mixed.

The few statistically significant differences between the BSA control and vehicle control groups in the examined parameters were considered by the GMO Panel to be within normal biological variability; therefore, both the vehicle control and the BSA control groups were considered suitable to be used as the control groups for the comparison and evaluation of data from the AAD-1 protein group.

No AAD-1 protein-related mortality, clinically relevant findings and ophthalmic changes were found. No statistically significant differences in body weight or body weight gain were observed in the AAD-1 protein group compared to the BSA control group. Statistically significantly lower feed consumption (day 4–8) was observed in females of the AAD-1 protein group when compared with the BSA control group. This difference was considered by the GMO Panel to be an incidental finding as being transient, only observed in one gender and not associated with significant differences in body weights and body weight gains.

Haematology analysis showed statistically significantly lower haemoglobin (Hb) levels in males of the AAD-1 protein group when compared with the BSA control group. The difference was small; the mean level was very close to the mean level in the vehicle control group and fell within the historical control range. In the absence of changes in other haematological parameters, the GMO Panel considered that this difference was not toxicologically relevant.

Clinical chemistry analysis showed a statistically significantly higher serum alkaline phosphatase (ALP) activity in males of the AAD-1 protein group when compared with the BSA control group. The GMO Panel noted that the activities in individual animals varied considerably in all three groups and the mean value was close to the historical control range.[42] Therefore, this difference was not considered to be toxicologically relevant.

There were no other significant differences in haematological and clinical chemistry parameters, and in the prothrombin time. However, the GMO Panel noted that the haematological, clinical chemistry and coagulation examinations were only performed on six animals per gender per group, and thus, the EFSA recommendation to use a higher number of animals was not fulfilled for the examination of these parameters.

Organ weight determinations showed no statistically significant differences, except for a higher absolute and relative prostate weight in males treated with the AAD-1 protein compared with the BSA control group. The mean values of the absolute and relative prostate weights were very close to the historical control ranges; the differences were not associated with macroscopic and/or microscopic findings in the prostate gland or with changes in other organs and tissues of the male genital system. Therefore, the GMO Panel considered that the differences were not toxicologically relevant.

Macroscopic examinations at necropsy revealed no gross pathological findings related to the treatment with the AAD-1 protein. Microscopic examinations of selected organs and tissues identified no treatment-related differences in the incidence and severity of the histopathological findings between the groups.

The GMO Panel concluded that there were no adverse effects after a 28 day administration of the AAD-1 protein to mice at the dose tested (751 mg/kg bw per day). However, the GMO Panel noted that the haematological, clinical chemistry and coagulation examinations were only performed on 6 animals per gender per group, and thus, the EFSA recommendation to use a higher number of animals was not fulfilled for the examination of these parameters.

[41] $\alpha = 0.05$.

[42] Historical control mean values were obtained from six 28-day mouse studies between 2012 and 2016, as stated in the study report.

Toxicological assessment of components other than newly expressed proteins

No new constituents other than AAD-1 protein are expressed in maize DAS-40278-9 and no relevant changes in the composition of GM maize were detected in the comparative compositional analysis (see Section 3.2.1.3).

3.3.1.3. Animal studies with the food/feed derived from GM plants

None of the observed differences in the composition of the food/feed derived from maize DAS-40278-9 (Section 3.2.1.3) required further assessment regarding food and feed safety. Therefore, no animal studies on the food/feed derived from maize DAS-40278-9 were required (EFSA, 2006a; EFSA GMO Panel, 2011a). However, the applicant provided a broiler study which was considered by the EFSA GMO Panel.

A 42-day feeding study with a total of 600 chickens (half male and half female) for fattening (day-old Ross 708) was provided.[43] The birds were randomly allocated to five dietary treatment groups with 120 chicks per treatment (six pens/treatment per gender, 10 birds per pen). Birds were fed diets containing maize DAS-40278-9 (AAD-1 verified by ELISA), and compared to those fed diets containing the non-GM comparator or three non-GM commercial maize varieties (Dekalb 6343, NK 72-G8 and Pioneer 32T16 maize). Maize DAS-40278-9 was not treated with intended herbicides. The starter (0–14 days), grower (15–28 days) and finisher (29–42 days) diets consisted of 50%, 55% and 60% maize meal, respectively. Other component was a commercial soybean meal (46% protein). Before feed formulation, all maize and soybean meal varieties were analysed for proximates, ADF, NDF, minerals, amino acids, fatty acids, some anti-nutrients and mycotoxins. The diets were isonitrogenous and isocaloric (confirmed by analysis). The AAD-1 protein was present throughout the study, and at the end of the study it was reduced approximately to 30% of AAD-1.[44] Feed in mash form and water were provided *ad libitum*.

Chickens were observed twice daily for mortality and clinical signs; deaths were recorded and necropsy performed on all birds found dead. Pen body weights and feed intake were measured on day 1, 14, 28 and 42 and feed conversion ratio was calculated. At the end of the trial, four birds per pen were taken for carcass evaluation (yield, dressing percentage, weight of thighs, breast, wings, legs, abdominal fat and whole liver). ANOVA (pen was considered as the experimental unit, dietary treatment and gender as fixed effects) was applied to determine statistical differences between groups. Four pair-wise comparisons between the GM group and each of the non-GM groups were made by Dunnett's test.

Overall mortality was low (< 2%) with no significant differences between the groups. No significant treatment-by-sex interactions were detected for final weight, weight gain and feed intake. Overall, no significant differences were found in final body weight, feed intake, and feed to gain ratio between the treatment groups. Final body weight was higher in males (average ca 2.8 kg) than females (average ca 2.5 kg). Feed to gain ratio was similar (average 1.65 for females and 1.61 for males). Carcass characteristics and liver weight did not show significant differences between animals fed diets containing the GM and the non-GM comparator.

The GMO Panel concludes that administration of diets containing up to 60% of maize meal DAS-40278-9 to broilers, up to 42 days, did not cause adverse effects. Moreover, the measured performance endpoints were similar between groups fed balanced diets containing GM and non-GM maize.

3.3.1.4. Allergenicity[45]

The strategies to assess the potential risk of allergenicity focus on the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and on whether the transformation may have altered the allergenic properties of the modified plant.

[43] Dossier: Part I – Section D7.8.4.
[44] AAD-1 protein mean concentration in the meal was 3.59 mg/kg fresh weight; at the beginning of the experiment, the diets contained 2.12, 2.77 and 2.70 mg/kg fresh weight; at the end of the study, the diets contained 0.52, 1.11 and 0.7 mg/kg fresh weight (starter, grower and finisher, respectively).
[45] Dossier: Part I – Section D7.9; additional information: 29/1/14, 16/12/2014 and 9/8/2016.

Assessment of allergenicity of the newly expressed proteins[46]

A weight-of-evidence approach was followed, taking into account all of the information obtained on the newly expressed protein, since no single piece of information or experimental method yield sufficient evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel, 2010b, 2011a).

The *aad-1* gene originates from *S. herbicidovorans*, a soil microorganism which is not considered to be a common allergenic source.

Updated bioinformatic analyses of the amino acid sequences of the AAD-1 protein, using the criterion of 35% identity in a window of 80 amino acids, revealed no significant similarities to known allergens. In addition, the applicant also performed analyses searching for matches of eight contiguous identical amino acid sequences between the AAD-1 protein and known allergens, which confirmed the outcome of the previous bioinformatic analyses.

The study on resistance to degradation of the AAD-1 protein by proteolytic enzymes has been described in Section 3.3.1.2.

There is no information available on the structure or function of the newly expressed AAD-1 protein that would suggest an adjuvant effect resulting in or increasing an eventual immunoglobulin E (IgE) response to a bystander protein.

In the context of the present application, the GMO Panel considers that there are no indications that the newly expressed AAD-1 protein in maize DAS-40278-9 may be allergenic.

Assessment of allergenicity of GM food/feed[47]

To date, maize has not been considered to be a common allergenic food[48] (OECD, 2002), and therefore, the GMO Panel did not request experimental data to analyse the allergen repertoire of GM maize. The GMO Panel regularly reviews the available publications on food allergy to maize.

In the context of the present application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed protein (Sections 3.1, 3.2 and 3.3), the GMO Panel identified no indications of a potentially increased allergenicity of maize DAS-40278-9 food and feed with respect to food and feed from the non-GM comparator.

3.3.1.5. Nutritional assessment of GM food/feed

The intended trait of maize DAS-40278-9 is herbicide tolerance, with no intention to alter the nutritional parameters. The outcome of the compositional analysis of maize DAS-40278-9 did not identify differences that would require a nutritional assessment as regards food and feed (Section 3.2.1.3). The introduction of food and feed products derived from maize DAS-40278-9 into the food and feed supply is expected to have no adverse nutritional impact, as compared to the non-GM comparator and commercial reference varieties.

3.3.1.6. Post-market monitoring of GM food/feed

There was no indication that maize DAS-40278-9 is any less safe than the non-GM comparator. Maize DAS-40278-9 is as nutritious as non-GM commercial varieties. Therefore, and in line with the guidance documents (EFSA, 2006a; EFSA GMO Panel 2011a), the GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

3.3.2. Conclusion

The safety assessment identified no concerns regarding the potential toxicity of the newly expressed AAD-1 protein in maize DAS-40278-9, considering the results of a subacute 28-day toxicity study where no adverse effects were observed at the highest dose tested and considering the results on the structural and functional properties of the AAD-1 protein, including bioinformatic analyses. The GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity with the AAD-1 protein or regarding the overall allergenicity of maize DAS-40278-9. Based on the comparative analysis, the nutritional characteristics of food and feed derived from maize DAS-40278-9 are not expected to differ from that of food and feed derived from non-GM maize varieties.

[46] Dossier: Part I – Section D7.9.1; additional information: 29/1/14, 16/12/2014 and 9/8/2016.
[47] Dossier: Part I – Section D7.9.2.
[48] Directive 2007/68/EC of the European Parliament and of the Council of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. OJ L 310, 27.11.2007, p. 11–14.

3.4. Environmental risk assessment and monitoring plan

3.4.1. Evaluation of relevant scientific data

Considering the scope of application EFSA-GMO-NL-2010-89 (which excludes cultivation), the ERA of maize DAS-40278-9 is mainly concerned with: (1) the exposure of bacteria to recombinant DNA in the gastrointestinal tract of animals fed GM material and bacteria present in environments exposed to their faecal material (manure and faeces); and (2) the accidental release into the environment of viable maize DAS-40278-9 grains during transportation and processing.

3.4.1.1. Environmental risk assessment

Persistence and invasiveness of the GM plant[49]

Maize is highly domesticated, not winter hardy in colder regions of Europe, and generally unable to survive in the environment without appropriate management. Occasional maize plants may occur outside cultivation areas but survival is limited mainly by a combination of low competitiveness, the absence of a dormancy phase and susceptibility to plant pathogens, herbivores and cold climate conditions (OECD, 2002). In fields, maize volunteers may arise under some environmental conditions (mild winters). Observations done in the field during harvesting indicate that grain may survive and overwinter in some regions, resulting in volunteers in subsequent crops. The occurrence of maize volunteers has been reported in Spain and other European regions (e.g. Gruber et al., 2008). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009).

As mentioned in Section 3.2.1.3, phenotypic and agronomic characteristics of maize DAS-40278-9 were evaluated in a field trial across eight locations in the USA in 2009. Maize DAS-40278-9 was considered equivalent to the non-GM maize reference varieties for eight out of the ten endpoints for which a significant difference was observed between the GM maize and its comparator. For the remaining two endpoints (i.e. 'plant height' and 'time to silking'), the equivalence test could not be performed for 'plant height' because of lack of variation in the commercial non-GM maize reference varieties, and the 'time to silking' of maize DAS-40278-9 fell under equivalence category II (i.e. equivalent to the non-GM maize reference varieties more likely than not).

The difference observed for 'plant height' between maize DAS-40278-9/2,4-D+quizalofop and its non-GM comparator was considered not relevant in terms of increased fitness potential given its nature and magnitude.

The equivalence test for the endpoint 'time to silking' indicates that equivalence with non-GM reference varieties is more likely than non-equivalence. For the correlated endpoints that are likely to indicate a change in fitness potential of the GM maize, such as, for example, yield, equivalence with non-GM reference varieties was demonstrated. The observed difference in 'time to silking' might therefore be an indication of unintended effects due to the genetic modification. Differences in seed lot quality could also explain such observations; however, the information included in the dossier does not indicate such an effect.

In the case of accidental release into the environment of maize DAS-40278-9, there are no indications of an increased likelihood of establishment and spread of occasional feral maize DAS-40278-9 plants. Should these plants be exposed to 2,4-D- or AOPP-containing herbicides, they are likely to exhibit a selective advantage that could increase their transient local occurrence. However, this will not result in different environmental impacts compared to conventional maize.

In addition to the data presented by the applicant, the GMO Panel is not aware of any scientific report of increased spread, establishment and survival capacity of maize DAS-40278-9 or maize with comparable properties.

Therefore, the GMO Panel concludes that it is unlikely that maize DAS-40278-9 would differ from conventional maize varieties in its ability to survive until subsequent season under European environmental conditions, if there was accidental release of viable GM maize grains into the environment. The occurrence of GM maize plants in the environment will thus be limited.

[49] Dossier: Part I – Section D4, D9.1 and D9.2.

Effects of gene transfer[50]

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 vertical gene transfer via seed spillage followed by cross-pollination.

1) Plant-to-bacteria gene transfer

Genomic DNA is a component of many food and feed products derived from maize. It is well documented that DNA present in food and feed becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, a low level of exposure of fragments of ingested DNA, including the recombinant fraction of such DNA, to bacteria in the digestive tract of humans, domesticated animals, and other environments exposed to the GM plant or plant material is expected.

Current scientific knowledge of recombination processes in bacteria suggests that horizontal transfer of non-mobile, chromosomally located DNA fragments between unrelated organisms (such as from plants to bacteria) is not likely to occur at detectable frequencies under natural conditions (for further details see EFSA, 2009).

A successful HGT would require stable insertion of the recombinant DNA sequences into a bacterial genome and a selective advantage to be conferred to the transformed host. The only mechanism known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes, is homologous recombination. This requires the presence of stretches of DNA sequences that are similar in the recombining DNA molecules. The similarity between the plant and bacterial sequences can be situated in the coding region of a recombinant protein (transgene) or in the border regions of the recombinant gene cassettes inserted into the plant genome. In the case of sequence identity with the transgene itself, recombination would result in gene replacement. In the case of identity with border regions, recombination could result in the insertion of additional DNA sequences in bacteria and thus confer the potential for new properties.

The coding sequence of the AAD-1 protein, as inserted in maize DAS-40278-9, is a synthetic gene derived from the *aad-1* gene of *S. herbicidovorans*, but codon-optimised with a higher G+C level for better expression in plant cells. None of the genetic elements of the DAS-40278-9 insert is of bacterial origin. *S. herbicidovorans* and other closely related bacteria have been isolated for their capacity to degrade 2,4-D or related compounds by dioxygenase activities (Horvath et al., 1990). Their occurrence in the main receiving environments, i.e. the gastrointestinal tract of humans or animals, is expected to be low or non-existent. However, occurrence of the recombinant genes outside their immediate receiving environment (e.g. through manure and faeces) in habitats of *S. herbicidovorans* and related bacteria is therefore also considered here.

On a theoretical basis (i.e. without any study providing experimental evidence for the occurrence of HGT in the case of GM food and feed derived from maize DAS-40278-9 or any other GM plant), and provided that plant codon optimisation would not strongly decrease the DNA sequence identity of the *aad-1* gene to corresponding sequences in bacteria, it can be assumed that, as an extremely rare event, homologous recombination may occur which would transfer the *aad-1* gene of maize DAS-40278-9 to bacteria in the environment. Such recombination event would be substitutive, replacing natural variants of the *aad-1* gene and, thus, not confer a novel trait. Such transfer would unlikely provide a new selective advantage for recipient bacteria (EFSA, 2009).

In addition to homology-based recombination processes, non-homologous (illegitimate) recombination that does not require the presence of DNA similarity between the recombining DNA molecules is theoretically possible. However, the transformation rates for illegitimate recombination were considered to be 10^{10}-fold lower than for homologous recombination (Hülter and Wackernagel, 2008; EFSA, 2009) and have not never been detected for GM plants and bacteria, even in studies that have directly exposed bacteria to high concentrations of GM plant DNA (EFSA, 2009).

The GMO Panel concludes that the *aad-1* gene from maize DAS-40278-9 may, on a theoretical basis, be transferred by homologous recombination to *S. herbicidovorans* and other bacteria with sufficient DNA sequence identity. However, since these bacteria are not considered to be members of the gut microbiota, exposure to recombinant DNA of maize DAS-40278-9 is considered to be very low.

Owing to the occurrence of bacteria with natural variants of the *aad-1* gene in the environment including soil, a low level of gene replacement in *S. herbicidovorans* or related bacteria is not considered a safety concern. Considering its intended use as food and feed and the above assessment,

[50] Dossier: Part I – Section D6.

the GMO Panel has therefore not identified a concern associated with a HGT from maize DAS-40278-9 to bacteria.

2) Plant-to-plant gene transfer

Considering the scope of application EFSA-GMO-NL-2010-89 and the biology of maize, the potential of occasional feral GM maize plants originating from accidental spillage of imported grains to transfer recombinant DNA to sexually cross-compatible plants is assessed. As pointed out above (Section 3.4.1.1), occurrence of feral GM maize is expected to be limited.

The extent of cross-pollination to other maize varieties will mainly depend on the scale of accidental release during transportation and processing and on successful establishment and subsequent flowering of the GM maize plant. For maize, vertical gene transfer is limited to other *Zea* species. Populations of sexually compatible indigenous wild relatives of maize are not known in Europe (Eastham and Sweet, 2002; OECD, 2003), therefore vertical gene transfer is not considered to be an environmental issue in the EU.

The flowering of occasional feral GM maize plants originating from accidental release during transportation and processing is unlikely to lead to dispersal of significant amounts of GM maize pollen onto other maize plants. Field observations performed on maize volunteers after GM maize cultivation in Spain revealed that maize volunteers had a low vigour, rarely had cobs and produced pollen that cross-pollinated neighbouring plants only at low levels (Palaudelmàs et al., 2009). Thus, the likelihood of cross-pollination between cultivated maize and the occasional feral maize plants resulting from grain spillage is considered extremely low.

In conclusion, the GMO Panel is of the opinion that the likelihood of spread of genes from this GM maize in Europe will not differ from that of conventional maize varieties, even in the case of treatment with the intended herbicide.

Interactions of the GM plant and target organisms[51]

Considering the scope of application EFSA-GMO-NL-2010-89 and the absence of target pests, potential interactions of the GM plant with target organisms are not considered a relevant issue by the GMO Panel.

Interactions between the GM plant and non-target organisms[52]

Considering the scope of application EFSA-GMO-NL-2010-89 and the low level of exposure to the environment, potential interactions of spilled grains or occasional feral maize DAS-40278-9 plants arising from grain import spills with non-target organisms are not considered a relevant issue by the GMO Panel.

Interactions with the abiotic environment and biogeochemical cycles[53]

Considering the scope of application EFSA-GMO-NL-2010-89 and the low level of exposure to the environment, potential interactions of spilled grains or occasional feral maize DAS-40278-9 plants arising from grain import spills with the abiotic environment and biogeochemical cycles are not considered a relevant issue by the GMO Panel.

3.4.1.2. Post-market environmental monitoring[54]

The objectives of a 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 which 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 content of the PMEM plan provided by the applicant (EFSA, 2006b; EFSA GMO Panel, 2011b).

The PMEM plan proposed by the applicant for maize DAS-40278-9 includes: (1) the description of a monitoring approach involving operators (federations involved in maize import and processing), reporting to applicants, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system newly established by EuropaBio for the

[51] Dossier: Part I – Section D9.4.
[52] Dossier: Part I – Section D9.5.
[53] Dossier: Part I – Section D9.8 and D10.
[54] Dossier: Part I – Section D11.

collection of the information recorded by the various operators; and (3) the use of networks of existing surveillance systems (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis and a final report at the end of the consent period.

The GMO Panel considers the scope of the PMEM plan provided by the applicant is consistent with the scope of maize DAS-40278-9. As the ERA does not cover cultivation and did not identify potential adverse environmental effects from maize DAS-40278-9, no case-specific monitoring is necessary. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

3.4.2. Conclusion

No safety concerns with regard to the environment from the import and processing of maize DAS-40278-9 were identified. There are no indications of an increased likelihood of establishment and spread of occasional feral maize DAS-40278-9 plants in the case of accidental release into the environment of viable GM maize grains, unless these plants are exposed to 2,4-D- or AOPP-containing herbicides. The GMO Panel is of the opinion that this will not result in different environmental impacts compared to conventional maize. The unlikely, but theoretically possible, horizontal transfer of the recombinant gene from maize DAS-40278-9 to bacteria does not raise any environmental safety concern. Considering the scope of the application, potential interactions of maize DAS-40278-9 with the biotic and abiotic environment were not considered a relevant issue by the GMO Panel. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of maize DAS-40278-9 and the GMO Panel guidelines on the PMEM of GM plants (EFSA, 2006b; EFSA GMO Panel, 2011b).

4. Conclusions

The EFSA GMO Panel was asked to carry out a scientific assessment of maize DAS-40278-9 for import, processing, and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation data provided for maize DAS-40278-9 did not raise safety issues.

The GMO Panel concluded that none of the differences identified in the compositional, agronomic and phenotypic characteristics of maize DAS-40278-9 required further assessment regarding food and feed safety.

No concerns regarding the potential toxicity or allergenicity of the newly expressed AAD-1 protein were identified, and no evidence that the genetic modification might significantly change the overall allergenicity of maize DAS-40278-9 was found. The nutritional value of food and feed derived from maize DAS-40278-9 is not expected to differ from that of food and feed derived from non-GM maize varieties. The GMO Panel concludes that maize DAS-40278-9, assessed in this application, is as safe and as nutritious as its non-GM comparator and the non-GM maize reference varieties tested.

Considering the scope of the maize DAS-40278-9 application, which excludes cultivation, there is no requirement for a scientific assessment of possible environmental effects associated with the cultivation of this GM maize. The GMO Panel concluded that there is a very low likelihood of environmental effects resulting from the accidental release of viable seeds from maize DAS-40278-9 into the environment. The unlikely, but theoretically possible, horizontal transfer of the recombinant gene from maize DAS-40278-9 to bacteria does not raise any environmental safety concern. Considering the scope of the application, potential interactions of maize DAS-40278-9 with the biotic and abiotic environment were not considered a relevant issue by the GMO Panel. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of maize DAS-40278-9 and the GMO Panel guidelines on the PMEM of GM plants (EFSA, 2006b; EFSA GMO Panel, 2011b).

In conclusion, the GMO Panel considers that the information available for maize DAS-40278-9 addresses the scientific comments raised by the Member States and that maize DAS-40278-9, as described in this application, is as safe as the non-GM comparator and other non-GM maize varieties with respect to potential effects on human and animal health and the environment in the context of the scope of this application.

Documentation provided to EFSA

1) Letter from the Competent Authority of the Netherlands received on 11 November 2010 concerning a request for placing on the market of herbicide-tolerant genetically modified maize DAS-40278-9, application EFSA-GMO-NL-2010-89, submitted by DOW AgroSciences LLC in accordance with Regulation (EC) No 1829/2003.

2) Acknowledgement letter dated 7 December 2010 from EFSA to the Dutch Competent Authority.
3) Letter from EFSA to applicant dated 20 December 2010 requesting additional information under completeness check.
4) Letter from applicant to EFSA received on 20 January 2011 providing additional information under completeness check.
5) Letter from EFSA to applicant dated 9 February 2011 requesting additional information under completeness check.
6) Letter from applicant to EFSA received on 18 February 2011 providing additional information under completeness check.
7) Letter from EFSA to applicant dated 11 March 2011 delivering the 'Statement of Validity' of application for the authorisation herbicide-tolerant genetically modified maize DAS-40278-9, application EFSA-GMO-NL-2010-89, submitted by DOW AgroSciences LLC in accordance with Regulation (EC) No 1829/2003.
8) Letter from applicant to EFSA received on 5 April 2011 providing additional information spontaneously.
9) Letter from EFSA to applicant dated 11 May 2011 requesting additional information and stopping the clock on behalf of the DG JRC/EURL-GMFF.
10) Letter from EFSA to applicant dated 20 May 2011 requesting additional information and maintaining the clock stopped.
11) Letter from applicant to EFSA received on 6 July 2011 providing a timeline for submission of responses.
12) Letter from applicant to EFSA received on 7 December 2011 changing the previous timeline for submission of responses.
13) Letter from applicant to EFSA received on 1 February 2012 providing additional information.
14) Letter from EFSA to applicant dated 1 June 2012 re-starting the clock.
15) Letter from applicant to EFSA received on 2 July 2012 providing additional information spontaneously.
16) Letter from applicant to EFSA received on 24 August 2012 providing additional information spontaneously.
17) Letter from EFSA to applicant dated 7 September 2012 requesting additional information and stopping the clock.
18) Letter from applicant to EFSA received on 19 November 2012 providing a timeline for submission of responses.
19) Letter from applicant to EFSA received on 19 April 2013 changing the previous timeline for submission of responses.
20) Letter from applicant to EFSA received on 25 November 2013 changing the previous timeline for submission of responses.
21) Letter from applicant to EFSA received on 29 January 2014 providing additional information.
22) Letter from EFSA to applicant dated 21 May 2014 requesting additional information and maintaining the clock stopped.
23) Letter from EFSA to applicant dated 19 June 2014 requesting additional information and maintaining the clock stopped.
24) Letter from EFSA to applicant dated 7 July 2014 annulling question No 6 sent in the EFSA letter dated 21 May 2014 (Ref. EW/ZD/MR/lg(2014) 8784583).
25) Letter from EFSA to applicant dated 16 July 2014 requesting a timeline for submission of responses.
26) Letter from applicant to EFSA received on 23 July 2014 providing additional information.
27) Letter from applicant to EFSA received on 29 July 2014 providing additional information.
28) Letter from applicant to EFSA received on 4 November 2014 providing additional information spontaneously.
29) Letter from EFSA to applicant dated 18 November 2014 requesting additional information and maintaining the clock stopped.
30) Letter from applicant to EFSA received on 16 December 2014 providing additional information.

31) Letter from EFSA to applicant dated 19 February 2015 requesting additional information and maintaining the clock stopped.
32) Letter from EFSA to applicant dated 27 February 2015 requesting additional information and maintaining the clock stopped.
33) Letter from applicant to EFSA received on 12 March 2015 providing additional information (I).
34) Letter from applicant to EFSA received on 12 March 2015 providing additional information (II).
35) Letter from EFSA to applicant dated 27 March 2015 requesting additional information and maintaining the clock stopped.
36) Letter from applicant to EFSA received on 7 April 2015 providing additional information.
37) Letter from EFSA to applicant dated 14 April 2015 requesting additional information and maintaining the clock stopped.
38) Letter from EFSA to applicant dated 24 April 2015 requesting additional information and maintaining the clock stopped.
39) Letter from applicant to EFSA received on 27 April 2015 providing additional information.
40) Letter from applicant to EFSA received on 1 June 2015 providing additional information.
41) Letter from EFSA to applicant dated 17 July 2015 requesting additional information and maintaining the clock stopped.
42) Letter from applicant to EFSA received on 24 August 2015 providing additional information.
43) Letter from EFSA to applicant dated 19 October 2015 requesting additional information and maintaining the clock stopped.
44) Letter from EFSA to applicant dated 11 April 2016 requesting a timeline for responses regarding the additional information requested on 19 October 2015 and on 11 April 2016.
45) Letter from applicant to EFSA received on 13 April 2016 providing a timeline for submission of responses requested by EFSA on 11 April 2016.
46) Letter from EURL-GMFF dated 22 April 2016 requesting EFSA to stop the clock on behalf of EURL-GMFF.
47) Email from EFSA to applicant dated 26 April 2016 maintaining the clock stopped due to questions requested by EURL-GMFF.
48) Letter from applicant to EFSA received on 9 August 2016 providing additional information.
49) Letter from applicant to EFSA received on 9 August 2016 providing additional information spontaneously.
50) Email from EFSA to applicant dated 11 August 2016 maintaining the clock stopped pending additional information requested.
51) Letter from EURL to EFSA dated 7 September 2016 requesting EFSA to re-start the clock on behalf of EURL-GMFF.
52) Letter from EFSA to applicant dated 8 September 2016 re-starting the clock.

References

Codex Alimentarius, 2009. *Foods derived from modern biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme*. Rome, Italy. 85 pp.

Eastham K and Sweet J, 2002. Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. European Environment Agency, Environmental issue report, 28, 1–75. Available online: http://www.eea.europa.eu/publications/environmental_issue_report_2002_28

EFSA (European Food Safety Authority), 2006a. Guidance document for the risk assessment of genetically modified plants and derived food and feed by the Scientific Panel on Genetically Modified Organisms (GMO) - including draft document updated in 2008 (reference EFSA-Q-2003-005A). EFSA Journal 2006;4(4):99, 105 pp. doi:10.2903/j.efsa.2006.99

EFSA (European Food Safety Authority), 2006b. Opinion of the scientific panel on genetically modified organisms on the post-market environmental monitoring (PMEM) of genetically modified plants. EFSA Journal 2006;4(3):319, 27 pp. doi:10.2903/j.efsa.2006.319

EFSA (European Food Safety Authority), 2009. Statement of EFSA on the consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of antibiotic resistance genes as marker genes in genetically modified plants" and the Scientific Opinion of the GMO Panel on "Consequences of the opinion on the use of antibiotic resistance genes as marker genes in genetically modified plants on previous EFSA assessments of individual GM plants". EFSA Journal 2009;7(6):1108, 107 pp. doi:10.2903/j.efsa.2009.1108

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. doi:10.2903/j.efsa.2010.1250

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010b. 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. doi:10.2903/j.efsa.2010.1700

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010c. Guidance on the environmental risk assessment of GM plants. EFSA Journal 2010;8(11):1879, 111 pp. doi:10.2903/j.efsa.2010.1879

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

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011b. Guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. EFSA Journal 2011;9(8):2316, 40 pp. doi:10.2903/j.efsa.2011.2316

Gruber S, Colbach N, Barbottin A and Pekrun C, 2008. Post-harvest gene escape and approaches for minimizing it. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 3, 1–7.

Horvath M, Ditzelmüller G, Loidl M and Streichsbier F, 1990. Isolation and characterization of a 2-(2,4-dichlorophenoxy) propionic acid-degrading soil bacterium. Applied Microbiology and Biotechnology, 33, 213–216.

Hülter N and Wackernagel W, 2008. Double illegitimate recombination events integrate DNA segments through two different mechanisms during natural transformation of *Acinetobacter baylyi*. Molecular Microbiology, 67, 984–995.

Lecoq E, Holt K, Janssens J, Legris G, Pleysier A, Tinland B and Wandelt C, 2007. General surveillance: roles and responsibilities the industry view. Journal für Verbraucherschutz und Lebensmittelsicherheit-Journal of Consumer Protection and Food Safety, 2(S1), 25–28.

OECD (Organisation for Economic Co-operation and Development), 2002. Consensus Document on compositional considerations for new varieties of maize (*Zea mays*): key food and feed nutrients, anti-nutrients and secondary plant metabolites. Series on the Safety of Novel Food and Feeds (ENV/JM/MONO(2002)25), 6, 1–42.

OECD (Organisation for Economic Co-operation and Development), 2003. Consensus Document on the biology of *Zea mays* subsp. *mays* (Maize). Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO(2003)11), 27, 1–49.

Palaudelmàs M, Peñas G, Melé E, Serra J, Salvia J, Pla M, Nadal A and Messeguer J, 2009. Effect of volunteers on maize gene flow. Transgenic Research, 18, 583–594.

Petolino JF and Arnold NL, 2009. Whiskers-mediated maize transformation. Methods in Molecular Biology: Transgenic Maize, 526, 59–67.

Petolino JF, Welter M and Cai CQ, 2003. Whiskers-mediated transformation of maize. Molecular Methods of Plant Analysis, 23, 147–158.

Windels P, Alcalde E, Lecoq E, Legris G, Pleysier A, Tinland B and Wandelt C, 2008. General surveillance for import and processing: the EuropaBio approach. Journal of Consumer Protection and Food Safety, 3(S2), 14–16.

Abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid herbicide
AAD-1	aryloxyalkanoate dioxygenase-1
ADF	acid detergent fibre
ALP	alkaline phosphatase
ANOVA	analysis of variance
AOPP	aryloxyphenoxypropionate herbicide
BSA	bovine serum albumin
bw	body weight
DM	dry matter
ELISA	enzyme-linked immunosorbent assay
ERA	environmental risk assessment
ESI-LS	electrospray ionisation-liquid chromatography
EURL-GMFF	European Union Reference Laboratory for GM Food & Feed
FAO	Food and Agricultural Organisation of the United Nations
GLP	Good Laboratory Practice
GM	genetically modified
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
Hb	haemoglobin
HGT	horizontal gene transfer
IgE	immunoglobulin E
MALDI-TOF MS	matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry

MS	mass spectrometry
NDF	neutral detergent fibre
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
RBC	red blood cells
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
UTR	untranslated region
WBC	white blood cells
WHO	World Health Organisation
Zm	*Zea mays*