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Assessment of genetically modified maize 4114 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2014-123)

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Abstract

Maize 4114 was developed through *Agrobacterium tumefaciens*-mediated transformation to provide protection against certain lepidopteran and coleopteran pests by expression of the Cry1F, Cry34Ab1 and Cry35Ab1 proteins derived from *Bacillus thuringiensis*, and tolerance to the herbicidal active ingredient glufosinate-ammonium by expression of the PAT protein derived from *Streptomyces viridochromogenes*. The molecular characterisation data did not identify issues requiring assessment for food/feed safety. None of the compositional, agronomic and phenotypic differences identified between maize 4114 and the non-genetically modified (GM) comparator(s) required further assessment. There were no concerns regarding the potential toxicity and allergenicity of the newly expressed proteins Cry1F, Cry34Ab1, Cry35Ab1 and PAT, and no evidence that the genetic modification might significantly change the overall allergenicity of maize 4114. The nutritional value of food/feed derived from maize 4114 is not expected to differ from that derived from non-GM maize varieties and no post-market monitoring of food/feed is considered necessary. In the case of accidental release of viable maize 4114 grains into the environment, maize 4114 would not raise environmental safety concerns. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of maize 4114. The genetically modified organism (GMO) Panel concludes that maize 4114 is as safe as the non-GM comparator(s) and non-GM 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), 4114, Cry1F, Cry34Ab1, Cry35Ab1, PAT, insect-resistant, herbicide tolerance, Regulation (EC) No 1829/2003

Requestor: Competent Authority of the Netherlands

Question number: EFSA-Q-2014-00850

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Panel members: Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Mikolaj 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.

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Summary

Following the submission of the application EFSA-GMO-NL-2014-123 under Regulation (EC) No 1829/2003 from Pioneer Overseas Corporation, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA) (GMO Panel) was asked to deliver a Scientific Opinion on the safety of genetically modified (GM) maize (*Zea mays L.*) 4114 (Unique Identifier DP-ØØ4114-3). The scope of the application EFSA-GMO-NL-2014-123 is for import, processing and food and feed uses of maize 4114 within the European Union (EU), but excludes cultivation in the EU.

The GMO Panel evaluated maize 4114 with reference to the scope of the application 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 proteins; the comparative analyses of compositional, agronomic and phenotypic characteristics; the safety of the newly expressed proteins and of the whole food/feed with respect to potential toxicity, allergenicity, nutritional characteristics and dietary exposure; the environmental risk assessment and the post-market environmental monitoring (PMEM) plan.

Maize 4114 was developed through *Agrobacterium tumefaciens*-mediated transformation of immature maize embryos of maize line PHWWE to express the Cry1F (truncated version), Cry34Ab1 and Cry35Ab1 proteins providing protection against specific lepidopteran and coleopteran pests, and the PAT protein conferring tolerance to the herbicidal active ingredient glufosinate-ammonium. The molecular characterisation data established that maize 4114 contains a single insert consisting of the *cry1F*, *cry34Ab1*, *cry35Ab1* and *pat* expression cassettes, and genetic stability was demonstrated. No other parts of the plasmid used for transformation were detected in maize 4114. Bioinformatic analyses did not indicate significant similarities to toxins and allergens. The Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins were detected and quantified in different tissues.

The comparative assessment of agronomic and phenotypic characteristics did not identify differences between maize 4114 and its non-GM comparator(s) requiring further assessment. There were no concerns regarding the potential toxicity and allergenicity of the newly expressed proteins Cry1F, Cry34Ab1 and Cry35Ab1 and PAT, and no evidence that the genetic modification might significantly change the overall allergenicity of maize 4114. The nutritional value of food/feed derived from maize 4114 is not expected to differ from that of food/feed derived from non-GM maize varieties and no post-market monitoring of food/feed is considered necessary.

Considering the introduced traits, the outcome of the comparative analysis, the routes and levels of exposure, the GMO Panel concludes that maize 4114 would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment. The PMEM plan and reporting intervals are in line with the intended uses of maize 4114.

Based on the relevant scientific publications retrieved through systematic literature searches, the GMO Panel did not identify any safety issues pertaining to the intended uses of maize 4114. In the context of PMEM, the applicant should improve the literature searches according to the GMO Panel recommendations.

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-NL-2014-123, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. The GMO Panel concludes that maize 4114, as described in this application, is as safe as its non-GM comparator(s) and the tested non-GM reference varieties with respect to potential effects on human and animal health and the environment.

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1. Introduction

The scope of application EFSA-GMO-NL-2014-123 is for food and feed uses, import and processing of maize 4114 and does not include cultivation in the European Union (EU).

Maize 4114 was developed to confer resistance against specific lepidopteran and coleopteran pests by the expression of the *cry1F*, *cry34Ab1* and *cry35Ab1* genes derived from *Bacillus thuringiensis* (*Bt*) and tolerance to the herbicidal active ingredient glufosinate-ammonium by expression of the *PAT* gene derived from *Streptomyces viridochromogenes*.[1]

1.1. Background

On 27 November 2014, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands application EFSA-GMO-NL-2014-123 for authorisation of maize 4114 (Unique Identifier DP-ØØ4114-3), submitted by Pioneer Overseas Corporation (hereafter referred as the applicant) within the framework of Regulation (EC) No 1829/2003 on genetically modified (GM) food and feed.[2]

After receiving the application EFSA-GMO-NL-2014-123, and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission, and made the summary of the application available to the public 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 Regulation (EC) No 1829/2003 and Regulation (EU) No 503/2013[4].

EFSA requested additional information under completeness check on 20 January 2015 and received it on 2 March 2015 and 24 April 2015. On 30 March 2015, EFSA declared the application valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[5] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003 to request their scientific opinion. Member States had 3 months after the opening of the Member State commenting period (until 11 September 2015) to make their opinion known.

The genetically modified organism (GMO) Panel requested additional information from the applicant on 23 April 2015 (EURL-GMFF), 11 May 2015, 17 July 2015, 3 December 2015, 12 February 2016, 29 June 2016, 18 July 2016 (EURL-GMFF), 5 December 2016, 14 March 2017, 24 May 2017 and 25 September 2017. The applicant provided the requested information on 1 June 2016 (EURL-GMFF), 10 June 2016 (EURL-GMFF), 23 September 2015, 15 December 2015, 12 April 2016, 4 August 2016, 12 September 2016 (EURL-GMFF), 06 February 2017, 15 May 2017, 28 September 2017 and 23 November 2017, respectively. The applicant also spontaneously submitted additional sequencing information on 15 May 2017.

In the frame of the contracts OC/EFSA/GMO/2013/01 and OC/EFSA/GMO/2014/01, contractors performed preparatory work and delivered reports on the methods applied by the applicant in performing bioinformatics and statistical analyses, respectively.

As provided by the EFSA's Catalogue of services, a clarification teleconference between EFSA and the applicant took place on 11 April 2017.

In giving its scientific opinion on maize 4114 to the European Commission, 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 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] Dossier Part II – Section 1.2.2.1.

[2] 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.

[3] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2014-00850

[4] Regulation (EU) No 503/2013 of 3 April 2013 on application for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 604/2004 and (EC) No 1981/2006.

[5] 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.

1.2. Term of References as provided by the requestor

The GMO Panel was requested to carry out a scientific risk assessment of maize 4114 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 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.

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-2014-123, 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 a scientific risk assessment of maize 4114 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 Regulation (EU) No 503/2013 and the appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed (EFSA GMO Panel, 2011a), the environmental risk assessment (ERA) of GM plants (EFSA GMO Panel, 2010a) and the post-market environmental monitoring (PMEM) of GM plants (EFSA GMO Panel, 2011b).

The GMO Panel took into account the criteria included in the 'Explanatory statement for the applicability of the Guidance of the EFSA Scientific Committee on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed for GMO risk assessment' (EFSA 2014), to perform the assessment of the 90-day feeding study provided.

The GMO Panel assessed the applicant's systematic literature searches in accordance with the guidelines on literature search given in EFSA (2010) for the initial search, and in EFSA (2017) for the latest update.

The comments raised by Member States are addressed in Annex G of EFSA's overall opinion[6] and were taken into consideration during the scientific risk assessment.

3. Assessment

3.1. Systematic literature review[7]

The GMO Panel assessed the systematic literature searches provided by the applicant on maize 4114 according to the guidelines given in EFSA (2010, 2017).

A systematic literature review as referred to in Regulation EU No 503/2013 has not been provided in support of the risk assessment of application EFSA-GMO-NL-2014-123, because of the limited number of relevant publications available on maize 4114.

Although the overall quality of the performed literature searches is acceptable, the GMO Panel considers that the searches on maize 4114 could be improved. The GMO Panel, therefore, recommends the applicant to: use truncation consistently; adapt the search for each database used, particularly in relation to using database-specific subject headings when available (in addition to text

[6] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2014-00850
[7] Dossier: Part II— Section 7; Additional information: 12/4/2016 and 28/09/2017.

words); and report the number of publications retrieved for each single subsearch performed (or search lines).

The GMO Panel considered the relevant publications retrieved through the literature searches and their implications for risk assessment, and addressed those in the related sections below, as appropriate.

3.2. Molecular characterisation

3.2.1. Transformation process and vector constructs[8]

Maize 4114 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*) mediated transformation. Immature embryos of maize line PHWWE were inoculated with the *A. tumefaciens* strain LBA4404 containing the PHP27118 transformation vector.

The PHP27118 transformation vector includes one T-DNA, which contains three expression cassettes conferring insect resistance and one expression cassette conferring herbicide tolerance. The genetic elements of each expression cassette are depicted below:

- *cry1F* expression cassette: the promoter, the 5′ untranslated region and the intron region of the polyubiquitin (*ubi*ZM1) gene from *Zea mays*; the codon-optimised truncated *cry1F* coding sequence from *Bacillus thuringiensis* var. *aizawai*; and the terminator sequence from the *A. tumefaciens* pTi15955 ORF25.
- *cry34Ab1* expression cassette: the promoter, the 5′ untranslated region and the intron region of the *ubi*ZM1 gene from *Zea mays*; the codon-optimised *cry34Ab1* coding sequence from *B. thuringiensis* strain PS149B1; and the terminator region of the proteinase inhibitor II (*pin*II) gene from *Solanum tuberosum*.
- *cry35Ab1* expression cassette: the promoter and leader sequence from a *Triticum aestivum* peroxidase gene; the codon-optimised *cry35Ab1* coding sequence from *B. thuringiensis* strain PS149B1; and the terminator region of the *pin*II gene from *S. tuberosum*.
- *pat* expression cassette: the 35S promoter from *Cauliflower Mosaic Virus* (CaMV), the codon-optimised *pat* coding sequence from *Streptomyces viridochromogenes*; and the 35S terminator from CaMV.

The vector backbone contained elements necessary for the maintenance and selection of the plasmid in bacteria.

3.2.2. Transgene constructs in the GM plant[9]

Molecular characterisation of maize 4114 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.

Southern analyses indicated that maize 4114 contains a single insert consisting of a single copy of the T-DNA in the same configuration as in the PHP27118 transformation vector. The insert and copy number were confirmed by the hybridisation signals generated with two restriction enzymes, together with 11 probes covering the T-DNA region. The absence of vector backbone sequences was tested by Southern analysis, using one restriction enzyme with 45 backbone-specific probes.

The nucleotide sequence of the entire insert of maize 4114 together with approximately 2 kb of both 5′ and 3′ flanking regions (2,398 and 2,405 bp, respectively) of maize 4114 were determined. The results were in line with those shown by the Southern blot analyses. The insert of 11,949 bp is identical to the T-DNA of PHP27118 transformation vector, except for the following changes: a 29 bp deletion in the right border and a 24 bp deletion in the left border. In addition, a 24 bp insertion composed of 15 bp of a T-DNA polylinker region and 9 bp internal sequence of *cry1F* gene is present at the 5′ end of the inserted T-DNA (nucleotides 1–24).

The possible interruption of known endogenous maize genes by the insertion in maize 4114 was evaluated by bioinformatic analyses of the preinsertion locus and of the genomic sequences flanking the insert.[10] BLASTn search against EST database and BLASTx search against the protein database

[8] Dossier: Part II – Section 1.2.1.2.
[9] Dossier: Part II – Section 1.2.2.2; Additional information 15/12/2015.
[10] Dossier: Part II – Section 1.2.2.1; Additional information 28/9/2017.

revealed significant similarities to a hypothetical glutaredoxin-like (GRX-like) protein sequence in the 5′ flanking genomic region. The insertion site is located in the upstream region of the hypothetical gene. Therefore, the insertion is unlikely to have interrupted the coding sequence of the hypothetical GRX-like protein.

The expression of this predicted gene in leaves and developing seeds of wild type plants analysis was not detected by northern analysis. However, even if the insertion would have altered the expression of this predicted gene, there are no indications from comparative agronomic-phenotypic performance and compositional analyses of any unintended effect caused by the insertion.

The results of segregation analysis (see Section 3.2.5) confirmed the presence of a single insert located in the nuclear genome.

Updated bioinformatic analyses of the amino acid sequence of the newly expressed Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins revealed no significant similarities to known toxins and allergens.[10] In addition, updated bioinformatics analyses of the newly created open reading frames (ORFs) within the insert and spanning the junctions between the insert and genomic DNA did not indicate significant similarities to toxins and allergens.[10]

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis to microbial DNA for event 4114.[10] The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.5.1.2.

3.2.3. Protein characterisation and equivalence[11]

Maize 4114 expresses four new proteins: a truncated version of the Cry1F protein and full-length Cry34Ab1, Cry35Ab1 and PAT proteins. The GMO Panel has previously assessed these proteins (EFSA, 2005, 2007, 2009a).

Western blot analysis was employed to demonstrate the equivalence between Cry1F, Cry34Ab1, Cry35Ab1 and PAT, produced in maize 4114 with plant-derived proteins in previously assessed maize events 1507, 59122 and 1507 × 59122 as well as their corresponding *Escherichia coli*-produced proteins. These data showed that they all migrated close to their expected molecular weight of ~ 68.2 kDa, ~ 13.6 kDa, ~ 43.8 kDa and ~ 20.6 kDa for truncated-Cry1F, Cry34Ab1, Cry35Ab1 and PAT, respectively, and were comparably immunoreactive to protein specific antibodies.

3.2.4. Information on the expression of the insert[12]

Protein levels of Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from replicated field trials across eight locations in the USA during the 2011 (six field sites) and 2012 (two field sites) growing seasons. Samples analysed included leafs (V6, V9, R1, R4 and R6), roots (V6, V9, R1, R4 and R6), whole plant (V9, R1 and R6), stalk (R1), forage (R4), grain (R6) and pollen (R1) from both not-treated and treated with glufosinate. The mean values, standard deviations and ranges of protein expression levels in grains and forage of the Cry1F, Cry31Ab1, Cry35Ab1 and PAT proteins are summarised in Tables 1 and 2.

Table 1: Means, standard deviations and ranges of protein levels in grains (n = 22) and forage (n = 24) (μg/g dry weight) from maize 4114 in field trials performed in 2011

	Not-treated	Treated
Grain		
Cry1F	3.0[(a)] ± 1.0[(b)] (1.8–5.7)[(c)]	3.1 ± 1.0 (1.3–4.8)
Cry34Ab1	35.0 ± 10.0 (17.0–51.0)	36.0 ± 14.0 (17.0–69.0)
Cry35Ab1	0.94 ± 0.49 (0.45–2.9)	1.0 ± 0.41 (0.3–1.9)
PAT	< 0.069 (LLOQ)	< 0.069 (LLOQ)

[11] Dossier: Part II – Section 1.2.2.1; Annex 3_PHI-2013-215.
[12] Dossier: Part II – Section 1.2.2.3.

	Not-treated	Treated
Forage		
Cry1F	11.0 ± 2.9 (7.6–18.0)	11.0 ± 3.0 (7.0–18.0)
Cry34Ab1	110.0 ± 26.0 (54.0–160.0)	110.0 ± 24.0 (68.0–150.0)
Cry35Ab1	20.0 ± 4.8 (12.0–32.0)	19.0 ± 3.5 (12.0–28.0)
PAT	2.0 ± 0.60 (1.0–3.2)	2.0 ± 0.67 (0.92–3.0)

LLOQ: Lower limit of quantification.
(a): Mean.
(b): Standard deviation.
(c): Range.

Table 2: Means, standard deviations and ranges of protein levels in grains (n = 8) and forage (n = 8) (μg/g dry weight) from maize 4114 in field trials performed in 2012

	Not-treated	Treated
Grain		
Cry1F	1.8[(a)] ± 0.51[(b)] (0.81–2.4)[(c)]	2.2 ± 0.49 (1.6–3.0)
Cry34Ab1	49.0 ± 14.0 (22.0–63.0)	51.0 ± 13.0 (36.0–75.0)
Cry35Ab1	0.52 ± 0.25 (0.14–0.81)	0.68 ± 0.33 (0.29–1.2)
PAT	< 0.069 (LLOQ)	< 0.069 (LLOQ)
Forage		
Cry1F	7.3 ± 0.86 (6.0–8.4)	8.0 ± 1.1 (6.4–9.8)
Cry34Ab1	88 ± 16 (66–120)	78 ± 8.6 (60–88)
Cry35Ab1	15 ± 3.7 (9.6–20.0)	15 ± 3.7 (10.0–20.0)
PAT	1.9 ± 0.56 (1.1–2.8)	2.1 ± 0.78 (1.0–3.2)

LLOQ: Lower limit of quantification.
(a): Mean.
(b): Standard deviation.
(c): Range.

3.2.5. Inheritance and stability of inserted DNA[13]

Genetic stability of the maize 4114 insert was assessed by PCR analysis of genomic DNA from five different generations. The PCR analyses were specific for event 4114 and for the *cry1F*, *cry34Ab1*, *cry35Ab1* and *pat* genes, respectively. The obtained data were sufficient to conclude that all the plants tested retained the single copy of the insert, which was stably inherited in subsequent generations.

Phenotypic stability was observed by segregation analysis of the herbicide tolerance trait in plants from five generations of maize 4114. The results supported the presence of a single insertion, segregating in a Mendelian fashion.

3.2.6. Conclusion on molecular characterisation

The molecular characterisation data establish that maize 4114 contains a single insert consisting of one copy of the *cry1F, cry34Ab1, cry35Ab1 and pat* expression cassettes. Bioinformatic analyses of the sequences encoding the newly expressed proteins and other ORFs present within the insert, or

[13] Dossier: Part II – Section 1.2.2.4 and Annex 11.

spanning the junctions between the insert and genomic DNA, did not indicate significant similarities to known toxins and allergens. The stability of the inserted DNA and of the introduced herbicide tolerance trait was confirmed over several generations. The Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins were expressed and the methodology used to quantify their levels was considered adequate. The protein characterisation data comparing Cry1F, Cry34Ab1, Cry35Ab1 and PAT produced in maize 4114 with plant-derived proteins in previously assessed maize events 1507, 59122 and 1507 × 59122 as well as with the corresponding *E. coli*-produced proteins indicate that these proteins are equivalent.

3.3. Comparative analysis

3.3.1. Choice of comparator and production of materials for the comparative assessment[14]

Application EFSA-GMO-NL-2014-123 presents data on agronomic and phenotypic characteristics as well as on forage and grain composition of maize 4114 derived from field trials performed in the US and Canada, and from a seed germination study performed under environmentally controlled conditions (Table 3).

Table 3: Overview of comparative analysis studies with maize 4114 provided in application EFSA-GMO-NL-2014-123

Study focus	Study details	Comparator	Commercial non-GM reference varieties[(a)]
Agronomic and phenotypic characteristics	Field trials, 2011/2012, USA and Canada (10 sites)[(b)]	PH705 × PHW2Z[(c)] PH12SG × PHW2Z[(d)]	12
	Field trials, 2014, USA, (eight sites)	PHR1J × PHW2Z	20
	Seed germination study, controlled conditions (three temperature regimes)	PHTFE × PHNAR	2
Compositional analysis	Field trials, 2011/2012, study, USA and Canada (ten sites)[(b)]	PH705 × PHW2Z[(c)] PH12SG × PHW2Z[(d)]	12

GM: Genetically modified.
(a): Three and four different reference varieties were grown at each site in the field studies conducted in 2011–2012 and 2014, respectively.
(b): Agronomic, phenotypic and compositional data were obtained from the same study.
(c): Used in the 2011 field trials.
(d): Used in the 2012 field trials.

The field trials were conducted in major maize growing areas of the US and Canada,[15] 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 4114 not treated with the intended herbicide (maize 4114/not-treated), maize 4114 treated with glufosinate (maize 4114/treated), a non-GM comparator, and several commercial non-GM maize reference varieties (i.e. three in the 2011/2012 study and four in the 2014 study). All materials were treated (sprayed) with required maintenance pesticides (including conventional herbicides) according to local requirements. In total, 12 and 20 non-GM maize reference varieties were included across the field trial sites performed in 2011/2012 and 2014, respectively.[16]

Maize 4114 was introgressed via backcrossing into different inbred lines (Table 3). At each site/study, the non-GM comparator had a genetic background similar to the maize 4114 hybrid used, as documented by the pedigree. The GMO Panel considered the selected non-GM comparator to be suitable.

[14] Dossier: Part II – Section 1.3.1 and 1.3.2; Additional information: 23/9/2015.
[15] The sites for the field trials conducted in 2011 and 2012 were in Bagley (IA, USA, 2012), Bradford (IL, USA, 2011), Branchton (ON, Canada, 2012), Carlyle (IL, USA, 2011 and 2012), Hinton (OK, USA, 2011), Jefferson (IA, USA, 2011), Wall (TX, USA, 2011), York (NE, USA, 2011 and 2012). The sites for the field trials conducted in 2014 were in Geneva (MN, USA), Germansville (PA, USA), Jefferson (IA, USA), Larned (KS, USA), Lebanon (IN, USA), Speer (IL, USA), Stewardson (IL, USA), York (NE, USA).
[16] The varieties used in the field trials conducted in 2011 and 2012 were 35T06, 35T36, P0751, 36B08, Po537, 34K77, X03A115, X7N790, X03A194, X1069G, X6M501, and X7K512. The varieties used in the field trials conducted in 2014 were 36K67, P0423, 35T06, 35F38, XL5246, 35K02, 34N61, XL5354, 34Y02, 34B39, P0965, XL5435, 34F06, 34H31, XL6077, P1184, XL6272, XL6175, P1319 and P1395.

3.3.2. Statistical analysis of field trial data

The statistical analysis of agronomic, phenotypic and compositional data from the field trials followed the recommendations of the GMO Panel (EFSA GMO Panel, 2010b, 2011a) and complied with Regulation (EU) No 503/2013. This included, for each of the two treatments of maize 4114, 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 maize reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[17]

Here, and in the rest of this scientific opinion, the term 'non-GM comparator' is used to refer to all genetic backgrounds (PH705 × PHW2Z, PH12SG × PHW2Z, PHR1J × PHW2Z, PHTFE × PHNAR, Table 1) used in the comparative assessment studies with maize 4114.

3.3.3. Agronomic and phenotypic characteristics[18]

3.3.3.1. Agronomic and phenotypic characteristics tested under field conditions

Fourteen and 15 agronomic and phenotypic end points were analysed in total in the 2011/2012 and 2014 field trials, respectively.[19] Of those, 'pollen viability – colour and shape at 120 min', 'insect damage', 'stalk lodging' and 'root lodging' measured in the 2014 field trials were not analysed with formal statistical tests because of high discreteness and lack of variability in the data.

The outcome of the test of difference and test of equivalence is listed below:

- For maize 4114/not-treated, the test of difference identified statistically significant differences for 'early population', 'time to silking', 'pollen viability – shape at 120 min and pollen colour at 30 and 120 min', 'plant height', 'ear height', 'insect damage', 'root lodging' and 'final population' in the 2011/2012 field trials, and for, 'ear height', in the 2014 field trials, all falling under equivalence category I (full equivalence).
- For maize 4114/treated, the test of difference identified statistically significant differences for 'early population', 'plant height', 'ear height', 'insect damage', 'root lodging', and 'final population' in the 2011/2012 field trials, and for 'pollen viability – colour at 60 min', 'stay green' and 'yield' in the 2014 field trials; all falling under equivalence category I (full equivalence), except 'pollen viability – colour at 60 min' for which the test of equivalence could not be applied.

Significant differences between maize 4114 and the non-GM comparator were not consistently observed across the different genetic backgrounds that were used in the various sites.

The average values for the end points that were not statistically analysed fell within the range of the non-GM reference varieties.

3.3.3.2. Agronomic and phenotypic characteristics tested under controlled conditions

The applicant reported data on seed characteristics of maize 4114. Seed germination of maize 4114 was compared with that of its non-GM comparator. Seeds were incubated under controlled conditions at three different temperature regimes and the numbers of germinated (normal and abnormal) and non-germinated (hard, imbibed and dead) seeds were counted. No statistically significant differences between maize 4114 and its non-GM comparator were observed at any temperature regime.

3.3.4. Compositional analysis

Maize 4114 grains and forage harvested from the field trials in North America in 2011/2012 (Table 4) were analysed for 84 constituents (9 in forage and 75 in grain). The analysis included the key constituents recommended by OECD (OECD, 2002). For 13 grain constituents,[20] more than half of the observations were below the limit of quantification.

[17] The results of the equivalence test are categorised into four possible outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence).

[18] Dossier: Part II – Sections 1.2.3.1 and 1.3.5; additional information: 23/9/2015 and 24/8/2016.

[19] Early population (count), seedling vigour (1–9 scale), time to silking (accumulated heat units), time to pollen shed (accumulated heat units), pollen viability (shape (% of pollen with collapsed walls) and colour (% of pollen yellow in colour) at 0, 30, 60 and 120 min), plant height (cm), ear height (cm), stay green (1–9 scale), disease incidence (1–9 scale), insect damage (1–9 scale), stalk lodging (%), root lodging (%), final population (count) and yield (ton/ha). The field trials conducted in 2014 included the end point 'yield' as requested by the GMO Panel.

[20] Caprylic acid (C8:0), lauric acid (C12:0), myristic acid (C14:0), heptadecenoic acid (C17:1), heptadecadienoic acid (C17:2), nonadecanoic acid (C19:0), eicosadienoic acid (C20:2), heneicosanoic acid (C21:0), tricosanoic acid (C23:0), vitamin B2 (riboflavin), β-tocopherol, δ-tocopherol and furfural.

The statistical analysis was applied to the remaining 71 constituents (9 in forage[21] and 62 in grain[22]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 4:

- For maize 4114/not-treated, all the 27 end points in grain with significant differences with its non-GM comparator fell under equivalence category I or II as also observed for two end points in forage.
- For maize 4114/treated, all the 33 endpoints in grain with significant differences with its non-GM comparator fell under equivalence category I or II as also observed for three end points in forage.

Table 4: Outcome of the comparative compositional analysis in grains and forage for maize 4114. The table shows the number of end points in each category

	Test of difference[a]			
	Not-treated[c]		**Treated[c]**	
	Not different	**Significantly different**	**Not different**	**Significantly different**
Test of equivalence[b]				
Category I/II	39	29[d]	32	36[d]
Category III/IV	–	–	–	–
Not categorised	1[e]	2[f]	2[e]	1[f]
Total end points	71			71

(a): Comparison between maize 4114 and its non-GM comparator.
(b): Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.
(c): Treated/not-treated with intended herbicide: glufosinate (see Section 3.3.1).
(d): End points with significant differences between maize 4114 and its non-GM comparator falling in equivalence category I-II (treated and not-treated).For grains, both treated and not-treated: moisture, crude protein, ash, carbohydrates (only treated), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), eicosenoic acid (C20:1), behenic acid (C22:0), isoleucine, leucine, lysine, serine, γ-tocopherol, total tocopherol, inositol and phytic acid. Only treated: lignoceric acid (C24:0), alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, methionine, phenylalanine, proline, threonine, tyrosine, valine, β-carotene and α-tocopherol. Not-treated: ADF, manganese, phosphorus, magnesium, potassium, vitamin B6 (pyridoxine), vitamin B9 (folic acid), ferulic acid.For forage, both treated and not-treated: crude fat and phosphorus. Only treated: carbohydrates.
(e): End points not categorised for equivalence and with significant differences between maize 4114 and its non-GM comparator: pantothenic acid (in grains of treated) and pantothenic acid and trypsin inhibitors (in grains of not-treated).
(f): End points not categorised for equivalence and with no significant differences between maize 4114 and its non-GM comparator: sodium and trypsin inhibitor (in grains of treated maize) and sodium (in grains of not-treated maize).

The GMO Panel assessed all significant differences between maize 4114 and its non-GM comparator, taking into account potential impact on plant metabolism and the natural variability observed for the set of non-GM commercial reference varieties. No endpoints showing significant differences between maize 4114 and its non-GM comparator and falling under category III/IV were identified.

[21] Crude protein, crude fat, crude fibre, acid detergent fibre (ADF), neutral detergent fibre (NDF), ash, carbohydrates, calcium and phosphorus.
[22] Proximates and fibre content (moisture, crude protein, crude fat, crude fibre, ADF, NDF, ash and carbohydrates), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine), fatty acids (palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), (9,15) isomer of linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), behenic acid (C22:0), lignoceric acid (C24:0), minerals (calcium, manganese, phosphorus, iron, magnesium, copper, potassium, sodium and zinc), vitamins (β-carotene, vitamin B1 (thiamine), vitamin B3 (niacin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), vitamin B9 (folic acid), α-tocopherol, γ-tocopherol and total tocopherol) and other compounds (p-coumaric acid, inositol, ferulic acid, phytic acid, raffinose and trypsin inhibitors).

3.3.5. Conclusion on comparative analysis

The GMO Panel concludes that all the compositional, agronomic and phenotypic changes identified with respect to the non-GM comparator and the non-GM commercial reference varieties do not need further assessment regarding food and feed safety and their environmental impact.

3.4. Food/feed safety assessment

3.4.1. Effects of processing

Based on the outcome of the comparative assessment (Section 3.3.5), processing of maize 4114 into food and feed products is not expected to result in products different from those of commercial non-GM maize varieties.

3.4.2. Influence of temperature and pH on newly expressed proteins

Effects of temperature and pH on Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins have been previously evaluated by the EFSA GMO Panel (EFSA, 2005, 2007, 2009a). No new studies were provided in the context of this application.

3.4.3. Toxicology

3.4.3.1. Testing of newly expressed proteins

Maize 4114 expresses four new proteins (Cry1F, Cry34Ab1, Cry35Ab1, and PAT) previously assessed by the GMO Panel (EFSA, 2005, 2007, 2009a). Western blot analysis demonstrated the equivalence between these proteins produced in maize 4114 with plant-derived Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins expressed in previously assessed maize events 1507, 59122 and 1507 × 59122 as well as their corresponding *E. coli*-produced proteins (Section 3.2.3). The GMO Panel accepts that the previous assessment of these proteins can be used for those newly expressed in maize 4114.

No safety concerns for humans and animals were identified for the Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins previously assessed by the GMO Panel (EFSA, 2005, 2007, 2009a). Updated bioinformatics analysis did not reveal similarities of the four proteins to known toxins. The applicant did not provide new studies on Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins. The GMO Panel is not aware of any new information that would change previous assessments. Based on known biological properties, no interactions between the four proteins newly expressed in maize 4114 which could raise safety concerns for food and feed are expected. The GMO Panel concludes that the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins do not raise toxicological concerns.

3.4.3.2. Testing of new constituents other than newly expressed proteins

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no new constituents other than newly expressed proteins have been identified in seed and forage from maize 4114. Therefore, no further food and feed safety assessment of components other than the newly expressed proteins is required.

3.4.3.3. Information on altered levels of food and feed constituents

None of the differences identified in seed and forage composition between maize 4114 and its non-GM comparator required further assessment.

3.4.3.4. Testing of the whole genetically modified food and feed

No substantial modifications in the composition of maize 4114, no indication of possible unintended effects and no indication of interactions relevant for food/feed safety were identified. Therefore, animal studies on food/feed derived from maize 4114 are not necessary (EFSA GMO Panel, 2011a). In accordance to Regulation (EU) No 503/2013, the applicant provided a 90-day oral repeated-dose toxicity study on whole food and feed from maize 4114 in rats. Animal feeding studies in broiler and channel catfish fed diets containing maize 4114 material were also provided in compliance with Regulation (EU) No 503/2013. All these studies were evaluated by the GMO Panel.

90-day feeding studies in rats[23]

Singly housed Crl:CD(SD) rats (12/sex per group) were allocated to six groups using a computerised, stratified randomisation on the basis of the group body weight mean within a sex.[24] Groups were fed test, control or reference diets containing approximately 32% milled maize grain from event 4114 either unsprayed (4114) or sprayed (4114 glufosinate (GLU)) with glufosinate (test items), or from an appropriate non-GM comparator (control item) or one of the three non-GM commercial varieties 32D78, 33N29 and 34P88 (reference items), respectively. The study provided was adapted from OECD TG 408 and complying with the principles of Good Laboratory Practice (GLP).

Event-specific PCR analysis on grains prior to processing into meal confirmed the molecular identity of maize 4114. The maize 4114 event was not detected by PCR in the non-GM comparator and commercial varieties. This was furthermore confirmed by ELISA analyses in grains used to determine the presence or absence of newly expressed proteins (Cry1F, Cry34Ab1, Cry35Ab1 and PAT). All six maize grain lots were characterised for proximates, amino acids, minerals, vitamins, secondary metabolites and antinutrients, pesticide residues and mycotoxins.

Balanced diets were prepared according to the specifications for PMI Certified Rodent LabDiet #5002, with maize-milled grains inclusion rate of 32% (w/w). Event-specific PCR and ELISA analyses confirmed that event 4114 was present only in the test diets. Homogeneity and short- and long-term stability of the test diets were considered adequate by ELISA determination of newly expressed proteins (Cry1F, Cry34Ab1, Cry35Ab1 and PAT).

Feed and water were provided ad libitum. Animals were checked twice daily for mortality and clinical signs. Detailed physical examinations were conducted weekly on all animals during the dosing period. Individual body weights were recorded pretreatment and then weekly during the dosing period. Final fasting body weights were recorded on the day of the scheduled necropsy. Feed consumption was determined weekly during the study. Ophthalmoscopy, functional observation battery and locomotor activity were recorded on all animals pretreatment and at the end of the study. Clinical pathology (i.e. haematology, clinical chemistry and coagulation, urine analyses) and necropsy examination with organs weighing were conducted at the end of the treatment period on all animals. The animals were fasted overnight prior to blood collection while in metabolism cages for urine collection. Organs and tissues from all sacrificed animals fed the test (4114 and 4114 GLU) and control (non-GM comparator) diets and from a female (animal no. 454) fed the reference diet 32D78, euthanised prior to the scheduled sacrifice for humane reasons were subjected to histopathological examination. Kidneys and gross lesions were also histopathologically examined in all animals. Upon completion of the histopathologic assessment, a histopathology peer review was conducted on selected animals and organs. Due to findings observed in the kidneys in this study, a pathology working group reviewed kidney slides from males fed the control and test (4114 and 4114 GLU) diets.

Mean and standard deviation were reported for all continuous end points for each group. In response to a request from EFSA,[25] for each end point showing statistical differences between the test and control group, the standardised effect size (SES) and associated 95% confidence interval were provided. Furthermore, for each gender, the applicant performed a power analysis for selected endpoints[26] using prespecified effect sizes defined on the basis of variance estimates and historical control data. For all end points, except final body weight and cumulative body weight gain, the power estimates were at least 80%.

The main statistical analysis compared rats consuming the two test diets (4114 and 4114 GLU) with those consuming the control diet. Individual animal was considered the experimental unit. The in-life and terminal body weights/gain, organ weights, feed consumption/efficiency and clinical pathology parameters were checked for homogeneity and normality[27] and analysed with either parametric ANOVA or non-parametric test (Dunn's test), as appropriate. Finally, incidence of functional observational battery (FOB) data was analysed with Fisher's exact test using a multiple comparisons correction (i.e. Bonferroni–Holm correction).

[23] Dossier: Part II – Section 1.4.4.1 (studies PHI-2011-055 and PHI-2013-232).
[24] On the basis of no statistically significant differences among groups identified.
[25] Additional information 6/2/2017 and 15/5/2017.
[26] Twelve end points were selected based on their likelihood to be impacted by altered test substance palatability, digestibility, or nutrient bioavailability: Body weight (final, non-fasted), Cumulative body weight gain, liver weight (absolute), liver weight, % body weight, kidney weight (absolute), kidney weight (% body weight), leukocyte count (absolute), lymphocyte count (absolute), cholesterol (CHOL), blood urea nitrogen (BUN), creatinine (CREA), alkaline phosphatase (ALKP).
[27] Levene's test for homogeneity and Shapiro–Wilk test for normality.

No test diet-related mortality was observed during the study. One female fed the reference diet 32D78 was euthanised on day 76 for humane reasons due to an ulcerated mammary gland mass, histologically diagnosed as a mammary gland adenocarcinoma.

Occasionally, clinical signs and ophthalmoscopy findings, compatible with the background of this strain of rats, were observed throughout all groups with similar or low incidence. The GMO Panel considered these findings isolated and not treatment-related.

No statistically significant differences in mean body weights at test day 91 and in the overall mean body weight gains calculated from test day 0 to 91, in mean daily feed consumption and in the overall mean daily feed efficiency from test day 0 to 91 were observed in males and females fed test diets (4114 and 4114 GLU), compared to the concurrent control. Statistical differences were observed at different times in males and females in body weights and mean daily feed efficiency; however, the GMO Panel considered that, in the absence of changes in the overall mean terminal body weight and body weight gain, the differences observed were not toxicologically relevant.

No statistically significant differences in FOB and locomotor activity parameters were observed between animals fed the test (4114 and 4114 GLU) and control diets.

Statistically significant higher mean haemoglobin and haematocrit values were observed in males fed the test diet (4114), compared to concurrent controls. These findings were not associated with changes in haematopoietic tissue histopathology (e.g. sternum bone marrow and spleen) and therefore not considered toxicologically relevant. No statistically significant differences in coagulation parameters (prothrombin time (PT) and activated partial thromboplastin time (APTT)) were observed in animals fed the test diets (4114 and 4114 GLU), compared to concurrent control. Statistically significant higher mean alkaline phosphatase (~ 20%) and creatinine (20%) were observed in males fed the test diet (4114 GLU), compared to concurrent control. These findings were not associated with changes in related end points (e.g. liver and kidney histopathology) and were not considered toxicologically relevant. Significant lower mean potassium (~ 7%) was observed in females fed the test diets (4114 and 4114 GLU), compared to concurrent control. This minimal difference is not considered to be toxicologically relevant. No statistically significant differences in urinalysis parameters were observed in animals fed the test diets (4114 and 4114 GLU), compared to concurrent control.

No statistically significant differences in final fasting body and organ weights were observed in animals fed the test diets (4114 and 4114 GLU), compared to concurrent control, except for a lower mean absolute (11%) and relative-to-brain (10%) weights of epididymides in males fed the test diet (4114 GLU); these differences were not considered to be toxicologically relevant because the mean relative-to-body weight of epididymides was not confirmed to be statistically different from concurrent control and the decrease observed was not associated with changes in related endpoints (e.g. testes and epididymides histopathology).

The macroscopic examination performed at necropsy on all animals revealed no gross pathological findings related to the administration of the test material in the diet. The microscopic examinations of selected organs and tissues did not identify relevant differences in the incidence and severity of the histopathological findings related to the administration of the test materials in the diet.

In two males fed test diet (4114) bilateral, multiple, amphophilic-vacuolar type renal tubule adenomas and/or carcinoma associated with multifocal atypical tubule hyperplasia were diagnosed. No evidence of tubular epithelial cytotoxicity or preneoplastic tubular epithelial changes, as is typical of renal tubular neoplasia, was observed. Histopathological examination of the kidneys was not performed on three samples (two kidneys from a female given the test diet (4114); one kidney from a female given the control diet) due to a technical mistake that occurred after their weighing. The GMO Panel considered this not to impact the interpretation of the study, considering the normal weights measured, the absence of macroscopic appearance observed, and the sufficient number of kidneys examined.

The assessment of the renal findings was further supported by the contribution of an expert panel of pathologists, a review of the available literature on spontaneous renal tubule tumours in young rats and a new repeated-dose 90-day oral toxicity study with 4114 maize focused on the assessment of kidneys.[28] The neoplastic lesions were morphologically identical to specific hyperplastic and neoplastic lesions previously reported to occur spontaneously in multiple rat strains, including Crl:CD(SD)IGS rats. The young age of onset for the tumours in these two rats is typical of spontaneous renal tumours in genetically susceptible rats and not characteristic of chemically induced renal tumours. Furthermore, the spontaneous renal tubule tumours observed in the initial repeated-dose 90-day oral toxicity study

[28] Dossier: Part II – Section 1.4.4.1; Additional information 23/11/2017.

were not reproducible under equivalent study conditions, and with a larger number of animals. The GMO Panel concluded that the observed adenomas and carcinomas and renal tubule hyperplasias are spontaneous lesions unrelated to consumption of the test diet.

The GMO Panel noted that the applicant only tested one dose level. However, the dose tested was close to the highest possible without inducing nutritional imbalance according to the current knowledge, and in accordance to the limit test dose as described in OECD TG 408. This is considered not to compromise the study.

The GMO Panel concluded that no maize 4114-related adverse effects were observed in this study after a 90-day administration to rats of a diet formulated with 32% milled grain from maize.

42-day feeding study in broiler[29]

A total of 720 (360 per sex) 1-day-old chicken broilers (Ross 708) were randomly allocated to six dietary groups with 120 chicks per treatment (12 pens per treatment, 10 birds per pen, half for each sex) and fed-balanced diets[30] containing up to 72.8%[31] maize grain from event 4114, either unsprayed (4114) or sprayed (4114 GLU) with glufosinate (test diets), or from the non-GM comparator (control diet) or one of the three maize reference varieties 32D78, 33N29, 34P88 (reference diets). Diets (as mash feed) and water were offered ad libitum. No significant differences between the groups fed test (treated with the intended herbicide or not) and control diets were observed in mortality (about 2%), final body weight, weight gain, feed to gain ratio and yield of prechill organs and post-chilled carcass and cuttable part percentages, with the exception of higher breast and thigh percentages in test groups, compared to control (tailored mixed models of analysis of variance).

The GMO Panel concludes that administration of diets containing up to 72.8% grain from maize event 4114 to broilers did not cause adverse effects. Moreover, the measured performance end points were similar between groups fed-balanced diets containing GM and non-GM comparator.

Channel catfish study[32]

A total of 480 Channel catfish (sex undetermined) were randomly allocated to six dietary groups with 80 catfishes per treatment (four aquaria per treatment, 20 fish per aquaria) and fed-balanced diets formulated as sinking pellets, containing 30% ground maize grain from event 4114, either unsprayed (4114) or sprayed (4114 GLU) with glufosinate, or from the appropriate non-GM comparator or one of the three maize commercial varieties 32D78, 33N29, 34P88. No mortality was observed in fish fed the test diets, and no abnormal behaviour was observed between dietary groups, during the study. There were no statistically significant differences in overall weight gain per fish, total diet consumption per fish or diet conversion ratio among fish fed the reference, control and test diets.

The GMO Panel concludes that administration of balanced diets containing up to 30% maize grain 4114 and 4114 GLU to Channel catfish, up to 8 weeks, did not cause adverse effects. Moreover, the measured performance end points were similar between groups fed-balanced diets containing GM and non-GM maize grains (comparator and references).

3.4.4. Allergenicity

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

3.4.4.1. Assessment of allergenicity of the newly expressed proteins[33]

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

The *cry*1F, *cry*34Ab1 and *cry*35Ab1 genes and the *pat* gene originate from *B. thuringiensis* and *S. viridochromogenes*, respectively, which are not considered to be allergenic sources.

[29] Dossier: Part II – Section 1.6.2. This study was not conducted in accordance with GLP requirements.
[30] Starter (0–21 days), grower (22–35 days) and finisher (35–42 days) diets.
[31] 61.5% in starter diets; 66.3% in grower diets; 72.8% in finisher diets.
[32] Additional information 12/4/2016.
[33] Dossier: Part II – Section 1.5.1 and 1.5.3.

Updated bioinformatic analyses[34] of the amino acid sequences of the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no significant similarities to known allergens.

The EFSA GMO Panel has previously evaluated the safety of the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in the context of several applications (see Section 3.4.3.1), and no concerns on allergenicity were identified (EFSA, 2005, 2007, 2009a). The GMO Panel is not aware of any new information that would change these conclusions.

There is no information available on the structure or function of the newly expressed Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins that would suggest an adjuvant effect, of the individual proteins or their simultaneous presence in maize 4114 resulting in or enhancing an eventual specific immunoglobulin E (IgE) response to a bystander protein.

In the context of the present application, the EFSA GMO Panel considers that there are no indications that the newly expressed Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in maize 4114 may be allergenic.

3.4.4.2. Assessment of allergenicity of the whole GM plant or crop[35]

The EFSA GMO Panel regularly reviews the available publications on food allergy to maize. However, to date, maize has not been considered to be a common allergenic food[36] (OECD, 2002). Therefore, the EFSA GMO Panel did not request experimental data to analyse the allergen repertoire of GM maize.

Conclusions

In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (see Sections 3.2, 3.3 and 3.4), the EFSA GMO Panel found no reason of concern regarding the allergenicity of food and feed derived from maize 4114 with respect to that derived from its non-GM comparator.

3.4.5. Dietary exposure assessment to endogenous and new constituents

According to Regulation (EU) No 503/2013, the applicant provided a dietary exposure to the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins produced in maize 4114.

3.4.5.1. Human dietary exposure[37]

Acute and chronic dietary exposure to each of the four different newly expressed proteins expressed in maize 4114 (Cry1F, Cry34Ab1, Cry35Ab1 and PAT) were provided by the applicant. Dietary exposure was estimated across different European countries on different age classes: young population (toddlers, other children) and adult population (adolescents, adults, elderly and very elderly).

For the purpose of estimating dietary exposure, the highest mean protein expression levels between two different set of field trials performed in 2011 and 2012 (see Tables 1 and 2 in Section 3.2.4) were used as occurrence data. Using these data, levels of the newly expressed proteins in the consumed maize-based food commodities were calculated considering the effect of processing based on the protein content of the processed commodities; this is considered as a conservative approach since it was assumed that no degradation of the newly expressed proteins occurs during processing. Since no specific consumption data were available on consumption of commodities containing maize 4114, a conservative scenario with 100% replacement of conventional maize was considered. Consumption data of the relevant commodities (corn bread, corn flakes, corn milling products, cornmeal porridge, corn grain, corn snacks, sweet corn and popcorn) were retrieved from the available summary statistics of the EFSA Comprehensive European Food Consumption Database.[38] The EFSA consumption database contains information on food consumption data at individual level

[34] Additional information 28/9/2017.
[35] Dossier: Part II – Section 1.5.2.
[36] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.
[37] Additional information 9/3/2018.
[38] https://www.efsa.europa.eu/en/applications/gmo/tools

from the most recent national dietary surveys in different EU Member States (EFSA, 2011; Huybrechts et al., 2011; Merten et al., 2011).

Acute dietary exposure in high consumers was estimated by summing the exposure derived from the 95th percentile consumption for the dominant food commodity[39] among consumers only and those exposures derived from the mean consumption of the remaining food categories in the total population (EFSA, 2015). The highest acute dietary exposure was estimated in one dietary survey from adults (18–65 years), with estimates of 250 μg/kg bw per day for Cry34Ab1 protein, and 15 μg/kg bw per day, 4.8 μg/kg bw per day and 0.34 μg/kg bw per day for Cry1F, Cry35Ab1 and PAT, respectively. Overall, popcorn was the main contributor to the highest acute exposure estimates among high consumers.

Chronic dietary exposure in high consumers was estimated following the same approach as for acute dietary exposure (EFSA, 2015), but using the available summary statistics for chronic consumption.[1] The highest chronic dietary exposure was estimated in one dietary survey from toddlers (1–3 years) with estimates of 170 μg/kg bw per day for Cry34Ab1 protein and 10 μg/kg bw per day, 3.2 μg/kg bw per day and 0.22 μg/kg bw per day for Cry1F, Cry35Ab1 and PAT, respectively. Overall, corn snacks and popcorn were the main contributor to the chronic exposure estimates among high consumers.

3.4.5.2. Animal dietary exposure[40]

Daily dietary exposure (DDE) to each of the four newly expressed proteins in maize 4114 (Cry1F, Cry34Ab1, Cry35Ab1 and PAT) was provided by the applicant across different livestock animal species (poultry, swine, cattle and sheep) based on estimates, as provided for the EU by OECD (OECD, 2009), for animal body weight, daily feed intake and the inclusion rates (percentage) of maize grain and forage/silage in animal diets. A conservative scenario with 100% replacement of the conventional maize (grain and/or forage/silage) was considered. The highest mean protein expression levels in maize grain and forage between two different set of field trials performed 2011 and 2012 (see Tables 1 and 2 in Section 3.2.4) was used as occurrence data. Among the four newly expressed proteins, Cry34Ab1 showed the highest estimated DDE (mg/kg bw): 2.52 in broiler chickens (4114 maize grain replacement), 2.54 in dairy cattle (4114 maize forage replacement) and 3.13 in dairy cattle (combination of 4114 maize grain and forage replacement). The highest estimated DDEs to the other three newly expressed proteins were 0.05, 0.45 and 0.29 mg/kg body weight, respectively, for PAT, Cry35Ab1 and Cry1F in cattle (combination of 4114 maize grain and forage replacement).

3.4.6. Nutritional assessment of GM food/feed

The intended trait of maize 4114 is insect resistance and herbicide tolerance, with no intention to alter nutritional parameters. Comparison of the seed and forage composition of maize 4114 with the non-GM comparator and the non-GM commercial reference varieties did not identify differences that would require a nutritional assessment as regards food and feed (see Section 3.3.5). From these data, the GMO Panel concludes that the nutritional impact of maize 4114-derived food and feed is expected to be similar to that from the non-GM comparator and non-GM commercial reference varieties.

3.4.7. Post-market monitoring of GM food/feed

The food/feed products derived from maize 4114 are as safe and nutritious as those derived from the non-GM comparator. Therefore, the GMO Panel considers that the post-market monitoring (EFSA GMO Panel, 2011a) of the food and feed derived from maize 4114 is not necessary.

3.4.8. Conclusion on the food/feed safety assessment

The GMO Panel did not identify safety concerns regarding the toxicity and allergenicity of the proteins Cry1F, Cry34Ab1, Cry35Ab1 and PAT produced in maize 4114 and found no evidence that the genetic modification might significantly change the overall allergenicity of maize 4114. Based on the outcome of the comparative assessment, the GMO Panel concludes that the nutritional impact of maize 4114-derived food and feed is expected to be similar to that from the non-GM comparator and non-GM commercial reference varieties. The GMO Panel concludes that maize 4114 is as safe and nutritious as the non-GM comparator and the non-GM reference varieties tested.

[39] Dominant food commodity refers to the food that will lead to the highest exposure among all consumed foods.
[40] Dossier: Part II – Section 2.

3.5. Environmental risk assessment and monitoring plan

Considering the scope of application EFSA-GMO-NL-2014-123, which excludes cultivation, the ERA of maize 4114 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable maize 4114 grains during transportation and/or processing (EFSA GMO Panel, 2010a).

3.5.1. Environmental risk assessment

3.5.1.1. Persistence and invasiveness of the GM plant[41]

Maize is highly domesticated, not winter hardy in colder regions of Europe, and generally unable to survive in the environment without appropriate management. Occasional feral GM maize plants may occur outside cultivation areas in the EU (e.g. Pascher, 2016), 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, 2003). Field observations indicate that maize grains may survive and overwinter in some EU regions, resulting in volunteers in subsequent crops (e.g. Gruber et al., 2008; Palaudelmàs et al., 2009; Pascher, 2016). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). Thus, the establishment and survival of feral and volunteer maize in the EU is currently limited and transient.

It is unlikely that the intended traits of maize 4114 will provide a selective advantage to maize plants, except when they are exposed to glufosinate-containing herbicides or infested by insect pests that are susceptible to the Cry1F, Cry34Ab1 or Cry35Ab1 proteins. However, this fitness advantage will not allow the GM plant to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers it very unlikely that maize 4114 will differ from conventional maize hybrid varieties in its ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable GM maize grains.

3.5.1.2. Potential for gene transfer[42]

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

Plant to microorganism gene transfer

Genomic DNA can be a component of food and feed products derived from maize. It is well documented that such DNA becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, bacteria in the digestive tract of humans and domesticated animals and in other environments may be exposed to fragments of DNA, including the recombinant fraction of such DNA.

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, 2009b).

The only mechanism known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes is HR. This requires the presence of at least two stretches of DNA sequences that are similar in the recombining DNA molecules. In the case of sequence identity with the transgene itself, recombination would result in gene replacement. In the case of identity with two or more regions flanking recombinant DNA, recombination could result in the insertion of additional DNA sequences in bacteria and thus confer the potential for new properties.

[41] Dossier: Part II – Section 5.3.1.
[42] Dossier: Pa rt II – Section 5.3.1 and 5.3.2.

Maize 4114 contains genetic elements originating or derived from bacteria, i.e. (1) the truncated *cry1F* gene from *B. thuringiensis* var. *aizawai*; (2) the terminator sequence from the *A. tumefaciens* pTi15955 ORF25 in the *cry1F* cassette; (3) the codon-optimised *cry34Ab1* gene from *B. thuringiensis* strain PS149B1; (4) the codon-optimised *cry35Ab1* gene from *B. thuringiensis* strain PS149B1; and (5) the codon-optimised *pat* gene from *S. viridochromogenes*.

Bioinformatic analysis of the inserted DNA confirmed that all three *cry* genes and the *pat* genes derived from bacteria were codon-optimised and thus showed insufficient DNA sequence identity to facilitate HR with DNA from environmental bacterial genes. However, a 100% sequence identity with bacterial DNA was found for 714 nucleotides from the ORF25 terminator which could facilitate HR.

A. tumefaciens occurs in soil, water and in the plant rhizosphere. It is therefore not expected to be prevalent in the main receiving environments, i.e. the gastrointestinal tract of humans or animals. A recombination within the ORF25 terminator region would only result in DNA sequence replacement and thus not confer any novel trait or selective advantage.

In summary, given the nature of the recombinant DNA, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT.

Plant to plant gene transfer

The potential for occasional feral maize 4114 plants originating from grain import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer were considered.

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

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

The potential of spilled maize grains to establish, grow and produce pollen is extremely low and transient (see Section 3.5.1.1). The likelihood/frequency of cross-pollination between occasional feral GM maize plants resulting from grain spillage, and weedy or cultivated *Zea* species is considered extremely low (EFSA, 2016). Even if cross-pollination would occur, the GMO Panel is of the opinion that the likelihood of environmental effects as a consequence of the spread of genes from occasional feral GM maize plants in Europe will not differ from that of conventional maize varieties for the reasons given in Section 3.5.1.1, even in the case of treatment with the intended herbicide.

3.5.1.3. Interactions of the GM plant and target organisms[43]

Taking the scope of application EFSA-GMO-NL-2014-123 (no cultivation) into account, potential interactions of occasional feral maize 4114 plants arising from grain import spills with target organisms are not considered a relevant issue.

3.5.1.4. Interactions between the GM plant and non-target organisms[44]

Given that environmental exposure of non-target organisms to spilled maize 4114 grains or occasional feral GM maize plants arising from spilled GM grains is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the GM plant with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern. Interactions that may occur between the Cry proteins will not alter this conclusion.

3.5.1.5. Interactions with the abiotic environment and biogeochemical cycles[45]

Given that environmental exposure to spilled grains or occasional feral maize 4114 plants arising from grain import spills is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the GM plant

[43] Dossier: Part II – Section 5.3.3.
[44] Dossier: Part II – Section 5.3.4.
[45] Dossier: Part II – Section 5.3.6.

with the abiotic environment and biogeochemical cycles are not considered by the GMO Panel to raise any environmental safety concern.

3.5.2. Post-market environmental monitoring[46]

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

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

As the ERA did not identify potential adverse environmental effects from maize 4114, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for maize 4114 includes: (1) the description of a monitoring approach involving operators (federations involved in import and processing), reporting to the applicant, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by EuropaBio for the collection of information recorded by the various operators; and (3) the review of relevant scientific publications retrieved from literature searches (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis and a final report at the end of the authorisation period.

The GMO Panel considers that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize 4114. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

In the context of PMEM, the applicant should improve the literature searches according to the GMO Panel recommendations given in Section 3.1.

3.5.3. Conclusion on the environmental risk assessment and monitoring plant

The GMO Panel concludes that it is unlikely that maize 4114 would differ from conventional maize varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-NL-2014-123, interactions of occasional feral maize 4114 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from maize 4114 to bacteria does not indicate a safety concern. Therefore, considering the introduced traits, the outcome of the comparative analysis, and the routes and levels of exposure, the GMO Panel concludes that maize 4114 would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of maize 4114.

4. Conclusions

The GMO Panel was asked to carry out a scientific assessment of maize 4114 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation data and bioinformatics analyses did not identify issues requiring further assessment for food/feed safety.

The GMO Panel concludes that none of the differences identified in the agronomic, phenotypic and compositional characteristics of maize 4114 required further assessment regarding environmental and food and feed safety.

The GMO Panel did not identify safety concerns regarding the toxicity and allergenicity of the proteins Cry1F, Cry34Ab1, Cry35Ab1 and PAT expressed in maize 4114 and found no evidence that the genetic modification might significantly change the overall allergenicity of maize 4114. The nutritional impact of maize 4114-derived food and feed is expected to be similar to that from the non-GM comparator and non-GM commercial reference varieties.

The GMO Panel concludes that there is a very low likelihood of environmental effects resulting from the accidental release of viable grains from maize 4114 into the environment. The PMEM plan and reporting intervals are in line with the intended uses of maize 4114.

[46] Dossier: Part II – Section 6.

Based on the relevant publications identified through the literature searches, the GMO Panel did not identify any safety issues pertaining to the intended uses of maize 4114. In the context of PMEM, the applicant should improve the literature searches according to the GMO Panel recommendations.

In conclusion, the GMO Panel considers that maize 4114, 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.

Documentation requested and provided to EFSA

1) Letter from the Competent Authority of the Netherlands received on 27 November 2014 concerning a request for placing on the market of genetically modified maize 4114 submitted by Pioneer Overseas Corporation in accordance with Regulation (EC) No 1829/2003 (application reference EFSA-GMO-NL-2014-123).
2) Acknowledgement letter dated 1 December 2014 from EFSA to the Competent Authority of the Netherlands.
3) Letter from EFSA to applicant dated 20 January 2015 requesting additional information under completeness check.
4) Letter from applicant to EFSA received on 2 March 2015 providing additional information under completeness check.
5) Letter from EFSA to applicant dated 30 March 2015 delivering the 'Statement of Validity' of application EFSA-GMO-NL-2014-123 for placing on the market of genetically modified maize 4114 submitted by Pioneer Overseas Corporation in accordance with Regulation (EC) No 1829/2003.
6) Email from EFSA to applicant dated 22 April 2015 requesting missing parts of the application.
7) Email from applicant to EFSA received on 24 April 2015 providing missing parts of the application.
8) Letter from EURL to EFSA dated 22 April 2015 requesting EFSA to stop the clock on behalf of the EURL.
9) Letter from EFSA to applicant dated 23 April 2015 requesting additional information for EURL and stopping the clock.
10) Email from applicant to EFSA received on 24 April 2015 providing additional information under completeness check.
11) Letter from EFSA to applicant dated 11 May 2015 requesting additional information and maintaining the clock stopped.
12) Letter from EFSA to applicant dated 17 July 2015 requesting additional information and maintaining the clock stopped.
13) Letter from applicant to EFSA received on 23 September 2015 providing additional information.
14) Letter from applicant to EFSA received on 23 September 2015 providing additional information.
15) Letter from EFSA to applicant dated 3 December 2015 requesting additional information and maintaining the clock stopped.
16) Letter from applicant to EFSA received on 15 December 2015 providing additional information.
17) Letter from EFSA to applicant dated 12 February 2016 requesting additional information and maintaining the clock stopped.
18) Letter from applicant to EFSA received on 12 April 2016 providing additional information.
19) Letter from EURL to EFSA dated 15 June 2016 requesting EFSA to re-start the clock on behalf of the EURL.
20) Letter from EFSA to applicant dated 20 June 2016 re-starting the clock.
21) Letter from EFSA to applicant dated 29 June 2016 requesting additional information and stopping the clock.
22) Letter from EURL to EFSA dated 15 July 2016 requesting EFSA to stop the clock on behalf of the EURL.
23) Letter from applicant to EFSA received on 4 August 2016 providing additional information.
24) Letter from EURL to EFSA dated 14 September 2016 requesting EFSA to re-start the clock on behalf of the EURL.

25) Letter from EFSA to applicant dated 15 September 2016 re-starting the clock.
26) Letter from EFSA to applicant dated 5 December 2016 requesting additional information and stopping the clock.
27) Letter from applicant to EFSA received on 6 February 2017 providing additional information.
28) Email from EFSA to applicant dated 9 February 2017 re-starting the clock on 6 February 2017.
29) Letter from EFSA to applicant dated 14 March 2017 requesting additional information and stopping the clock.
30) Letter from applicant to EFSA received on 15 May 2017 providing additional information.
31) Email from EFSA to applicant dated 15 May 2017 re-starting the clock.
32) Letter from applicant to EFSA received on 15 May 2017 providing sequencing information spontaneously.
33) Letter from EFSA to applicant dated 24 May 2017 requesting additional information and stopping the clock.
34) Letter from applicant to EFSA received on 6 July 2017 requesting an extension of the deadline to submitted additional information requested.
35) Email from EFSA to applicant dated 17 July 2017 accepting the deadline extension demand to submit additional information requested.
36) Letter from EFSA to applicant dated 25 September 2017 requesting additional information and maintaining the clock stopped.
37) Letter from applicant to EFSA received on 28 September 2017 providing additional information.
38) Letter from applicant to EFSA received on 23 November 2017 providing additional information.
39) Email from EFSA to applicant dated 23 November 2017 re-starting the clock.
40) Letter from EFSA to applicant dated 19 January 2018 requesting additional information and stopping the clock.
41) Letter from applicant to EFSA received on 9 March 2018 providing additional information.
42) Email from EFSA to applicant dated 3 March 2018 re-starting the clock.
43) Letter from applicant to EFSA received on 9 April 2018 regarding confidentiality parts of the application.
44) Letter from applicant to EFSA received on 24 April 2018 providing a Public Access version of the application.

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Abbreviations

ADF	acid detergent fibre
ALKP	alkaline phosphatase
APTT	activated partial thromboplastin time
BUN	blood urea nitrogen
bw	body weight
CaMV	*Cauliflower Mosaic Virus*
CHOL	cholesterol
CREA	creatinine
DDE	Daily dietary exposure
ELISA	enzyme-linked immunosorbent assay
GLP	Good Laboratory Practice
GLU	glufosinate
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
GRX	glutaredoxin
HGT	horizontal gene transfer
HR	homologous recombination
NDF	neutral detergent fibre
NEP	newly expressed proteins
ORFs	open reading frames
OECD	Organisation for Economic Co-operation and Development
PAT	phosphinothricin acetyltransferase
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
PT	prothrombin time
SES	standardised effect size