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SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-DE-2011-95) for the placing on the market of genetically modified maize 5307 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Crop Protection AG[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2,3]

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ABSTRACT

Maize 5307 was developed by *Agrobacterium tumefaciens*-mediated transformation to express two proteins: eCry3.1Ab, conferring resistance to certain coleopteran pests, and phosphomannose isomerase (PMI), used as selection marker. The molecular characterisation showed relevant similarities between the amino acid sequence of PMI and a known allergen, and between the amino acid sequence of eCry3.1Ab and a potential toxin. Some agronomic and phenotypic differences between maize 5307 and its conventional counterpart were observed (higher 'heat units to 50 % pollen shed', grain moisture, plant height, grain yield); however, the EFSA GMO Panel considered that these do not give rise to food/feed or environmental safety concerns. No differences in the compositional data requiring further safety assessment were identified. There were no concerns regarding the potential toxicity and allergenicity of the PMI protein. The EFSA GMO Panel could not conclude on the safety of the eCry3.1Ab protein due to the inadequate 28-day toxicity study provided. The outcome of a broiler feeding study with maize 5307 was not assessed by the EFSA GMO Panel, due to study weaknesses. There are no indications of an increased likelihood of the establishment and spread of feral maize plants. Interactions with the biotic and abiotic environment were not considered to be a relevant issue. Risks associated with the unlikely but theoretically possible horizontal gene transfer of recombinant genes from maize 5307 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, in the absence of an appropriate assessment of eCry3.1Ab, the EFSA GMO Panel is not in a position to complete its food/feed risk assessment of maize 5307. However, the EFSA GMO Panel concludes that the maize 5307 is unlikely to have any adverse effect on the environment in the context of its scope.

KEY WORDS

GMO, maize 5307, food and feed safety, environment, import and processing, eCry3.1Ab, PMI, Regulation (EC) No 1829/2003

[1] On request from the Competent Authority of Germany for an application (EFSA-GMO-DE-2011-95) submitted by Syngenta Crop Protection AG, Question No EFSA-Q-2011-00310, adopted on 16 April 2015.

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SUMMARY

Following the submission of an application (EFSA-GMO-DE-2011-95) under Regulation (EC) No 1829/2003 from Syngenta Crop Protection AG, 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 5307 (Unique Identifier SYN-Ø53Ø7-1). The scope of application EFSA-GMO-DE-2011-95 is for import, processing, and food and feed uses of maize 5307 within the European Union (EU), as for any non-GM maize, but excludes cultivation in the EU.

The EFSA GMO Panel evaluated maize 5307 with reference to the scope and appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed, the environmental risk assessment of GM plants and the post-market environmental monitoring of GM plants. The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and analysis of the expression of the corresponding proteins. An evaluation of the comparative analyses of the compositional, agronomic and phenotypic characteristics was undertaken, and the safety of the newly expressed proteins and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional wholesomeness. An evaluation of environmental impacts and the post-market environmental monitoring plan was also undertaken.

Maize 5307 was developed by *Agrobacterium tumefaciens*-mediated transformation of the conventional maize line NP2222. It expresses the chimeric eCry3.1Ab protein (based on a modified Cry3A from *Bacillus thuringiensis* subsp. *tenebrionis* and the Cry1Ab from *B. thuringiensis* subsp. *kurstaki* strain HD-1), which confers resistance to certain coleopteran pests, and the phosphomannose isomerase (PMI) protein, which is used as a marker for the selection of transformants. The molecular characterisation data established that the genetically modified maize 5307 contains a single insertion consisting of two intact expression cassettes (*ecry3.1Ab* and *pmi*). No other parts of the plasmid used for transformation are present in maize 5307. Bioinformatic analyses revealed sequence identities greater than 35 % with allergens in putative translation products of open reading frames (ORFs) newly created by the genetic modification. The likelihood that these ORFs are both transcribed and translated in maize 5307 is negligible. Bioinformatic analyses revealed relevant similarities between the amino acid sequence of PMI and a known allergen, and between the amino acid sequence of eCry3.1Ab and a potential toxin. These were further assessed for their relevance for food and feed safety. The stability of the inserted DNA was confirmed over several generations and a Mendelian inheritance pattern was demonstrated. The levels of the eCry3.1Ab and PMI proteins in maize 5307 were obtained and reported adequately.

Based on the agronomic and phenotypic characteristics of maize 5307 tested under field conditions, some differences were noted in maize 5307 compared with its conventional counterpart (i.e. higher 'heat units to 50 % pollen shed', higher grain moisture and higher plant height in 2007 field trials; higher grain yield in 2008 field trials). The EFSA GMO Panel is of the opinion that these do not give rise to any food and feed or environmental safety concerns. No differences requiring further assessment with regard to safety by the EFSA GMO Panel were identified at analyses of compositional data of forage or grains obtained from maize 5307.

No safety concerns were identified regarding the potential toxicity and allergenicity of the newly expressed protein PMI. The 28-day rat oral toxicity study on eCry3.1Ab, provided to support the safety assessment of this newly expressed protein, was not considered adequate by the EFSA GMO Panel (i.e. use of datasets from two separate experiments and low number of animals per gender per group). Therefore, the EFSA GMO Panel could not conclude on the safety of the eCry3.1Ab protein. The EFSA GMO Panel could not evaluate the outcome of a feeding study in broilers with maize 5307 because of weaknesses in the study conduct and reporting.

The application GMO-DE-2011-95 concerns food and feed uses and import and processing. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of maize 5307. There are no indications of an increased likelihood of the establishment and

spread of feral maize plants. Considering the scope of application EFSA-GMO-DE-2011-95, interactions with the biotic and abiotic environment were not considered to be a relevant issue. The EFSA GMO Panel also concludes that, considering the scope of application EFSA-GMO-DE-2011-95, the unlikely but theoretically possible horizontal gene transfer of recombinant genes from maize 5307 to bacteria does not give rise to any environmental safety concern. The post-market environmental monitoring plan and reporting intervals are in line with the scope of application EFSA-GMO-DE-2011-95.

In delivering its scientific opinion, the EFSA GMO Panel took into account application EFSA-GMO-DE-2011-95, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. In conclusion, the EFSA GMO Panel could not complete the food and feed safety assessment of maize 5307 due to the lack of an appropriate assessment of the eCry3.1Ab protein. However, the EFSA GMO Panel concludes that maize 5307 is unlikely to have any adverse effect on the environment in the context of the scope of application EFSA-GMO-DE-2011-95.

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BACKGROUND

On 7 April 2011, the European Food Safety Authority (EFSA) received from the German Competent Authority an application (Reference EFSA-GMO-DE-2011-95) for authorisation of GM maize 5307 (Unique Identifier SYN-Ø53Ø7-1), submitted by Syngenta Crop Protection AG within the framework of Regulation (EC) No 1829/2003 on GM food and feed.[4]

After receiving the application EFSA-GMO-DE-2011-95 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.[5] 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 31 May 2011, EFSA received additional information requested under completeness check (13 May 2011). On 21 June 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 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[6] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had three months after the date of receipt of the valid application (until 21 September 2011) to make their opinion known.

The EFSA GMO Panel carried out an evaluation of the scientific risk assessment of maize 5307 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The EFSA 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 of GM plants (EFSA GMO Panel, 2010c) and on the post-market environmental monitoring of GM plants (EFSA GMO Panel, 2011b). Furthermore, the EFSA GMO Panel also took into consideration the scientific comments of Member States, the additional information provided by the applicant and the relevant scientific publications.

On 13 September 2011, 14 October 2011, 25 June 2013, 27 May 2014, 09 September 2014, 29 October 2014, 16 February 2015 and on 27 February 2015, the EFSA GMO Panel requested additional information from the applicant. The applicant provided the requested information on 03 October 2011, 29 January 2013, 08 August 2013, 12 June 2014, 18 September 2014, 11 December 2014 and on 24 March 2015, respectively. The applicant also spontaneously provided additional information on 28 July 2014. After evaluation of the full data package, the EFSA GMO Panel finalised its risk assessment of maize 5307.

In giving its scientific opinion on maize 5307 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 six months from the acknowledgement of the valid application. As additional information was requested by the EFSA GMO Panel, the time limit of six months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

[4] 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.

[5] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2011-00310

[6] 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.

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).

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific assessment of maize 5307 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 EFSA GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the EFSA 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.

ASSESSMENT

1. Introduction

The genetically modified (GM) maize 5307 (Unique Identifier SYN-Ø53Ø7-1) was assessed with respect to the scope of application EFSA-GMO-DE-2011-95, taking into account the appropriate principles described in the applicable guidance documents (EFSA, 2006a, b; EFSA GMO Panel, 2010c); and, whenever possible, also the current guidance documents (EFSA GMO Panel, 2011a, b). The risk assessment presented here is based on the information provided in the application relating to maize 5307 submitted in the EU, scientific comments raised by the Member States and relevant scientific publications.

Maize 5307 expresses a chimeric Cry protein, designated eCry3.1Ab, which is based on a modified Cry3A protein (mCry3A) derived from *Bacillus thuringiensis* subsp. *tenebrionis* and the Cry1Ab from *B. thuringiensis* subsp. *kurstaki* strain HD-1. The protein confers resistance to certain coleopteran pests. The expression of the phosphomannose isomerase (PMI) protein is used as a marker for the selection of transformants.

The genetic modification in maize 5307 is intended to improve agronomic performance only and is not intended to influence the nutritional properties, the processing characteristics or the overall use of maize as a crop.

The scope of application EFSA-GMO-DE-2011-95 is for import, processing, and food and feed uses of maize 5307 and does not include cultivation in the European Union (EU). Thus, maize 5307 will be imported into the EU for food or feed uses in the same way as any commercial maize variety. Possible food and feed products include starch, syrup, ethanol, maize oil, flakes, coarse and regular grits, coarse and dusted meal, flour, maize germ meal, maize gluten and maize gluten meal.

2. Issues raised by the Member States

The comments raised by Member States are addressed in Annex G of the European Food Safety Authority (EFSA) overall opinion[7] and were taken into consideration during the evaluation of the risk assessment.

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Transformation process and vector constructs

Immature embryos of a proprietary maize (*Zea mays* L.) line, NP2222, were transformed with the plasmid vector pSYN12274 using *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*) strain LBA4404. The regeneration of the transformed tissue was achieved after a callus phase.[8]

The plasmid pSYN12274 includes one T-DNA that contains the *ecry3.1Ab* gene expression cassette, providing expression of modified eCry3.1Ab protein, and the *pmi* gene expression cassette, providing expression of a PMI protein.[9]

The T-DNA present in plasmid pSYN12274 contains the following elements between its respective right and left border region:

- *ecry3.1Ab* gene expression cassette consisting of the CMP promoter from *Cestrum Yellow Leaf Curling Virus*; coding sequence for the engineered eCry3.1Ab protein; terminator sequence from

[7] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2011-00310
[8] Dossier: Part I—Section C1.
[9] Dossier: Part I—Sections C2, C3, D1.

the nopaline synthase (*nos*) gene from *A. tumefaciens*, providing a polyadenylation signal. The e*cry3.1Ab* gene is based on the *cry1Ab* and the modified *cry3A* (m*cry3A*) genes derived from *B. thuringiensis* and consists of a fusion between the 5′ end of the m*cry3A* domain (459 amino acids) and the 3′ end of the *cry1Ab* domain (172 amino acids). Upstream of the m*cry3A* domain, a 67-bp oligomer extension was introduced during the engineering process and is translated into 22 amino acid residues at the N terminus of the chimeric eCry3.1Ab.[10]

- *pmi* gene expression cassette consisting of the promoter and first intron from the maize polyubiquitin gene (*ZmUbiInt*); *pmi* gene from *Escherichia coli* encoding PMI, allowing selection of transformants using mannose as the sole carbon source; terminator sequence from the nopaline synthase (*nos*) gene from *A. tumefaciens*, providing a polyadenylation signal.

Additional functional elements in the plasmid vector positioned outside the T-DNA (and not expected to be transferred into the maize genome), were: *ori*VS1 and *repA*, the origin of replication and replicase genes, respectively, both derived from the *Pseudomonas aeruginosa* plasmid pVS1 and required for the maintenance of the plasmid vector in *Agrobacterium*; *virG*, regulator of virulence in *A. tumefaciens*; *ori*ColE1, the origin of replication required for the maintenance of pSYN12274 in *E. coli*; *aad*A from *E. coli* transposon Tn*7*, coding for a streptomycin adenyltransferase enzyme conferring resistance to spectinomycin and streptomycin for selection of the plasmid in *E. coli* and *Agrobacterium*.

3.1.2. Transgene constructs in the genetically modified plant[11]

The DNA sequences inserted in maize 5307 were characterised by Southern blot analysis, polymerase chain reaction (PCR) and sequencing of both the insert and flanking regions.

Southern analysis indicated that maize 5307 contains a single insert with one copy of the intact *ecry3.1Ab* and *pmi* expression cassettes. The insert and copy number were confirmed by multiple restriction enzyme/probe combinations covering the T-DNA region and the flanking regions. No signal was observed, with the probe corresponding to the pSYN12274 vector backbone.

The nucleotide sequences of the entire insert, as well as 1 kb of both 5′ and 3′ flanking regions were determined from maize 5307. The sequence of the insert confirmed the conclusions drawn from the Southern analyses. Comparison with the sequence of pSYN12274 indicated that the insert in maize 5307 contained a single nucleotide difference 48 bp upstream of the CMP promoter. Furthermore, the entire right border including 3 bp of non-coding sequence at the 5′ end of the insert and 8 bp of the left border, were absent. None of the differences influenced the functionality of the insert. The possible interruption of known endogenous maize genes by the insertion in maize 5307, was evaluated by bioinformatic analyses of the pre-insertion locus and the genomic sequences flanking the insert. Comparison of the sequences of the flanking regions in maize 5307 with the conventional maize genomic sequences indicated a 33-bp deletion of maize DNA at the insertion site. BLASTN searches were performed against a plant EST (Expressed Sequence Tag) database and a non-redundant nucleotide database and BLASTX searches against a non-redundant amino acid database. These bioinformatic analyses did not reveal the interruption of any known endogenous gene in the maize 5307 flanking regions.[12] BLASTN analysis of the flanking sequences did reveal alignments with multiple maize BAC clones and suggested that the insert is located in a repetitive region of the maize genome.

The results of segregation (see Section 3.1.4.) and bioinformatic analyses established that the insert is located in the nuclear genome.[13]

[10] Dossier: Part I—Section C3.
[11] Dossier: Part I—Section D2.
[12] Dossier: Part I —Section D2.4; additional information: 12/06/2014, 28/07/2014 and 24/03/2015.
[13] Dossier: Part I —Sections D2.4, D5; additional information: 12/06/2014 and 28/07/2014.

In order to assess whether the open reading frames (ORFs) present within the insert and spanning the junction sites give rise to any safety issues, their putative translation products were compared with databases for similarities to known allergens and toxins relevant for humans and/or animals using suitable algorithms. By using an 80-amino-acid sliding window approach, a sequence identity greater than 35 % between putative translated products of five ORFs and known allergens were found by the applicant. Three ORFs are not in the codon frame intended to be expressed, do not include an ATG start codon and do not have known promoters in close proximity. Another ORF is not in the codon frame intended to be expressed and does not include an ATG start codon. Although the fifth ORF is in the codon frame intended to be expressed and has an ATG start codon, the applicant determined that the ATG start codon is upstream of the transcription start site. The transcription start site was determined in Stavolone et al. (2003) and Sahoo et al. (2014). Based on all available information, the EFSA Panel on Genetically Modified Organisms (GMO Panel) is of the opinion that the likelihood that these ORFs are both transcribed and translated in maize 5307 is negligible.

No significant similarities with known allergens were found with the eCry3.1Ab protein. There was a match of eight identical amino acids between the sequence encoding PMI and α-parvalbumin from *Rana* sp. CH2001. This is discussed further in Section 5.1.4.

Bioinformatic analyses revealed no relevant similarities between the amino acid sequence of PMI and known toxic proteins.The applicant identified relevant similarities between the amino acid sequence of eCry3.1Ab and parasporins, which might act as cytotoxic proteins on mammalian cells, mainly tumoral. The assessment of the toxicity of eCry3.1Ab for humans and animals is discussed in Section 5.1.2.

3.1.3. Information on the expression of the insert[14]

Levels of the eCry3.1Ab and PMI proteins were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from replicated field trials across four locations in the USA in 2008. Samples analysed included leaf, root and whole plants at four growth stages (whorl, anthesis, maturity and senescence), grain samples at maturity and senescence, and pollen samples at anthesis. Data on forage was not provided. The mean values and ranges of the eCry3.1Ab and PMI protein levels in mature grains (n = 5 per location) are summarised. The mean eCry3.1Ab level across all sites was 6.19 µg/g dry weight (dw) (SD = 1.87), with a range of 2.37–9.64 µg/g dw. The mean value for PMI levels was 2.08 µg/g dw (SD = 0.49) with a range of 1.04–3.82 µg/g dw.

3.1.4. Inheritance and stability of inserted DNA[15]

Stable integration of the insert was confirmed by Southern analysis over four maize 5307 generations and the insert followed the Mendelian inheritance pattern of a single locus. This was supported by real-time PCR analyses of the *ecry3.1Ab* and *pmi* genes. Stability in expression was demonstrated by ELISA analyses of eCry3.1Ab and PMI protein levels over four generations.

3.2. Conclusion

The molecular characterisation data provided by the applicant establish that the genetically modified maize 5307 contains a single insertion consisting of two intact expression cassettes (*ecry3.1Ab* and *pmi*). No other parts of the plasmid used for transformation are present in the transformed plant. Bioinformatic analyses of the ORFs spanning the junction sites within the insert or between the insert and genomic DNA did not give rise to safety issues. Sequence identities greater than 35 % with allergens were found in putative translation products of ORFs newly created by the genetic modification, but the likelihood that these ORFs are both transcribed and translated in maize 5307 is negligible.

[14] Dossier: Part I—Section D3; additional information: 12/06/2014.
[15] Dossier: Part I—Sections D2.3, D5.

Bioinformatic analyses revealed relevant similarities between the amino acid sequence of PMI and a known allergen. Relevant similarities were also identified between eCry3.1Ab and potential cytotoxic proteins. These are further assessed for their relevance for food and feed safety in Section 5. The stability of the inserted DNA was confirmed over several generations and a Mendelian inheritance pattern was demonstrated. The levels of the eCry3.1Ab and PMI proteins in maize 5307 were obtained and reported adequately.

4. Comparative analysis

4.1. Evaluation of relevant scientific data

4.1.1. Choice of comparator

Field trials for the comparative compositional analysis of maize 5307 and its conventional counterpart were carried out at six locations in the USA in 2008[16], at eight locations in the USA in 2009[17] and at eight locations in Argentina during the 2011/2012 growing season.[18] The agronomic and phenotypic characteristics of maize 5307 and its conventional counterpart were evaluated at field trials performed at five locations in the USA in 2007[19] and at 12 locations in the USA in 2008.[20] The locations selected in the USA and in Argentina represent major maize-growing areas. The conventional counterpart in these field trials was the non-GM maize NP2171/NP2460, which has a similar genetic background to maize 5307, as shown in the pedigree chart.[21] In addition, in 2009, eight commercial non-GM varieties[22] were grown at eight locations in the USA (Table 1).[23] Grain and forage samples were harvested for compositional analysis.

Table 1: Overview of comparative assessment studies with maize 5307

Study focus	Study details	Conventional counterpart	Non-GM maize varieties
Composition of forage/harvested grain	2008, six locations in the USA	1 (NP2171/NP2460)	–
Composition of forage/harvested grain	2009, eight locations in the USA	1 (NP2171/NP2460)	–
Composition of forage/harvested grain	2011/2012, eight locations in Argentina[24]	1 (NP2171/NP2460)	–
Agronomic and phenotypic characteristics	2007, five locations in the USA	1 (NP2171/NP2460)	–
Agronomic and phenotypic characteristics	2008, 12 locations in the USA	1 (NP2171/NP2460)	–
Establishment of natural compositional variation	2009, eight locations in the USA[25]	–[26]	8[17]

[16] Stanton (MN), Janesville (WI), New Haven (IN), Shirley (IL), Marshall (MO), Bloomington (IL).
[17] York (NE), Swanton (OH), Deerfield (MI), Richland (IA), Seymour (IL), York (NE), Kimballton (IA), Elk Horn (IA).
[18] Tacuari, Berdier, Chacabuco, San Patricio, Carmen de Areco, Arroyo Dulce, Arrecifes, El Crisol.
[19] Brookings (SD), Waldorf (MN), Corwith (IA), Green Valley (IL), El Paso (IL).
[20] Brookings (SD), Minnesota Lake (MN), Northfield (MN), Janesville (WI), New Haven (IN), Beaver Crossing (NE), El Paso (IL), Bloomington (IL), Shirley (IL), St. Joseph (IL), La Salle (IL), Marshall (MO).
[21] Dossier: Part I—Section D7.2/Appendix 27, Vol. 1.
[22] The eight commercial non-GM maize varieties were NK KANSAS, NK SYMBA, X36344, NK THERMO, H-7191, H-6044, H-6218, H-7540.
[23] York (NE), Fulton (OH), Lenawee (MI), Richland (IA), Seymour (IL), York (NE), Audobon (IA), Shelby (IA).
[24] Additional information: 29/01/13, Appendix B5.
[25] Dossier : Part I—Section D7.2/Appendix 30.
[26] Neither maize 5307 nor the conventional counterpart was included in these field trials.

4.1.2. Agronomic and phenotypic characteristics

Based on data collected in the USA at five locations in 2007 and at 12 locations in 2008, the applicant performed a comparative assessment of 17 phenotypic and agronomic characteristics and two disease resistance traits of maize 5307 and its conventional counterpart in 2007 and three additional phenotypic and agronomic characteristics (% snapped plants, early root lodging, push test) to the 17 in 2008[27]. As one location planted in 2007 had a significantly delayed planting date, the data were analysed excluding this location. The applicant analysed the data for each season in an across-site analysis using an analysis of variance (ANOVA), which identified statistically significant differences between maize 5307 and its conventional counterpart for the endpoints 'heat units to 50 % pollen shed', plant height and grain moisture in 2007 and grain yield in 2008 (Table 2). In all cases the values were slightly higher for maize 5307 in comparison to the conventional counterpart. When the field trials in 2007 were analysed without the Corwith, IA, location, for which the applicant claimed there was a one-month delay in planting compared with the other locations, the parameter 'plant height' was no longer statistically significantly different. When analysed by a *t*-test at each site separately, grain yield was statistically significantly different at one location in 2008, whereas grain moisture showed differences at two locations in each of the 2007 and 2008 growing seasons. The EFSA GMO Panel is of the opinion that these differences do not give rise to any food and feed safety concerns. The potential impact of the differences on the environment is further discussed in Section 6.1.1.1.

Table 2: Statistically significant agronomic and phenotypic differences across locations in field trials in 2007 and 2008 (the mean values, together with the standard error of the means, are given).

	2007 season		2008 season	
	Maize 5307	**Conventional counterpart**	**Maize 5307**	**Conventional counterpart**
Grain moisture (%)	17.9 ± 0.2*	16.9 ± 0.2	20.8 ± 0.1	20.3 ± 0.1
Plant height (cm)	216 ± 1*	210 ± 1	247 ± 2	239 ± 2
Heat units to 50 % pollen shed	1230 ± 1*	1247± 1	1330 ± 2	1325 ± 2
Grain yield (kg/ha)**	9380 ± 160	9660 ± 160	9510 ± 150*	8700 ± 150

* Significance assigned at $p < 0.05$ level.
** Data converted to metric units from original results of measurements provided by the applicant in bushels/acre (Bu/A): maize 5307 149.5 ± 2.52 Bu/A vs. conventional counterpart 154 ± 2.52 Bu/A in 2007; maize 5307 151.6 ± 2.34 Bu/A vs. conventional counterpart 138.6 ± 2.40 Bu/A in 2008. One bushel of maize (US trade unit) corresponds to 56 lbs (25 kg) of maize.

4.1.3. Compositional analysis

The compounds selected to be analysed in grain and forage followed the OECD recommendations (OECD, 2002). In forage, nine parameters (protein, fat, ash, moisture, carbohydrates by calculation, acid detergent fibre, neutral detergent fibre, calcium, phosphorus) were analysed and, in grain, 59 parameters were analysed.[28] Thirteen additional fatty acids were measured but not statistically analysed because levels were below the limit of quantification. For each season, the statistical analysis

[27] The following agronomic/phenotypic parameters were measured: number of barren plants, number of plants with dropped ears, number of emerged plants, early emergence vigour, early growth vigour, ear height, % grain moisture, % snapped plants, early root lodging, plant population at harvest, heat units to 50 % silking, heat units to 50 % pollen shed, late season intactness, leaf colour rating, late root lodging, plant height, push test, stalk lodging, test weight, grain yield.

[28] For the compositional analysis, the following parameters were measured in grain: moisture, protein, fat, ash, carbohydrates, acid detergent fibre (ADF), neutral detergent fibre (NDF), total dietary fibre (TDF), starch, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, sodium, zinc, β-carotene, thiamine, riboflavin, niacin, pyridoxine, folic acid, α-tocopherol, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, eicosenoic acid, behenic acid, ferulic acid, furfural, inositol, *p*-coumaric acid, phytic acid, raffinose, trypsin inhibitor.

was a combined-site ANOVA to compare maize 5307 with its conventional counterpart. In the 2008 field trials, the levels of 16 components (selenium, sodium, furfural and 13 fatty acids) were below the limit of quantification in the majority of the analyses and were excluded from the statistical comparison, while in the 2009 field trials, the levels of 18 components (selenium, sodium, furfural, raffinose and 14 fatty acids) were below the limit of quantification for the majority of the analyses and excluded from statistical comparison. Moisture in grain was not statistically analysed due to mechanical drying.

In the case of forage, no compositional differences were observed between maize 5307 and the conventional counterpart. The statistical analysis of the compositional data of grain harvested from field trials in the USA identified seven statistically significant differences between maize 5307 and the conventional counterpart in the 2008 season and 10 in the 2009 season. The level of five of these components were different in both seasons (16:0 palmitic acid, 18:0 stearic acid, 18:3 linolenic acid, 20:1 eicosenoic acid; and β-carotene) (Table 3). The endpoint values for compounds showing differences were within the ranges of the non-GM varieties grown in separate field trials in the USA in 2008 and 2009.

Table 3: Statistically significant compositional differences across locations for grain in the field trials in 2008 and 2009 (the mean values, together with the standard error of the means, are given).

	2008 season		2009 season		2009 season
	Maize 5307	**Conventional counterpart**	**Maize 5307**	**Conventional counterpart**	**Non-GM varieties range**
Fat (% dw)	4.54 ± 0.067	4.72 ± 0.067	4.26 ± 0.09*	4.58 ± 0.09	2.74–4.89
Palmitic acid (% total FA)	15.7 ± 0.1*	15.2 ± 0.1	15.2 ± 0.1*	14.9 ± 0.1	10.9–15.9
Stearic acid (% total FA)	1.74 ± 0.06*	1.81 ± 0.06	1.55 ± 0.02*	1.61 ± 0.02	1.38–2.08
Linolenic acid (% total FA)	1.60 ± 0.6*	1.50 ± 0.6	1.64 ± 0.01*	1.55 ± 0.01	1.31–2.14
Arachidic acid (% total FA)	0.392 ± 0.005	0.387 ± 0.005	0.363 ± 0.005*	0.369 ± 0.005	0.338–0.460
Eicosenoic acid (% total FA)	0.250 ± 0.003*	0.242 ± 0.003	0.249 ± 0.002*	0.243 ± 0.002	0.228–0.333
Copper (mg/kg dw)	1.52 ± 0.25	1.89 ± 0.25	1.25 ± 0.06*	1.43 ± 0.06	0.97–7.46
β-Carotene (mg/kg dw)	1.55 ± 0.05*	1.76 ± 0.05	1.51 ± 0.07*	1.68 ± 0.07	0.81–2.59
α-Tocopherol (mg/kg dwW)	9.3 ± 0.55	9.0 ± 0.55	8.5 ± 0.21*	8.0 ± 0.21	5.8–18.6
Riboflavin (mg/kg dw)	1.98 ± 0.1	1.98 ± 0.1	2.32 ± 0.07*	2.08 ± 0.07	1.07–3.46
Pyridoxine (mg/kg dw)	6.92 ± 0.17*	7.37 ± 0.17	5.58 ± 0.13	5.69 ± 0.13	3.30–8.58
Folic acid (mg/kg dw)	0.40 ± 0.02*	0.38 ± 0.02	0.41 ± 0.02	0.43 ± 0.02	0.17–0.52

* Significance assigned at $p < 0.05$ level.

Given that PMI is an enzyme involved in carbohydrate metabolism, information on the levels of specific compounds linked to the mode of action of PMI in maize 5307 and in its conventional counterpart was requested by the EFSA GMO Panel, in order to assess the likelihood of the occurrence of unintended effects. In response to this request[29], the applicant provided the results of an analysis of monosaccharides and disaccharides, sugar alcohols, and their phosphorylated forms, in grain derived from maize 5307 and its conventional counterpart grown in an additional field study at eight locations in Argentina during the 2011/2012 growing season. Various statistically significant increases (of approximately 15%) were observed in the content of several of these carbohydrates (mannose 6-phosphate, fructose 6-phosphate, fructose, myo-inositol, sucrose) in maize 5307 compared with the conventional counterpart in the combined-site analysis (and in no or a limited number of per-location statistical analyses for each of these parameters). These were not considered to be attributable to the newly expressed PMI enzyme, based, among other factors, on the lack of a plausible biochemical mechanism that could account for the presence and direction of the differences observed in these components, and on the absence of effects on other carbohydrates. These results are in line with previous assessments of GM plants expressing PMI proteins by the EFSA GMO Panel (EFSA, 2009a; EFSA GMO Panel, 2012).

The EFSA GMO Panel did not identify a significant difference in the composition of forage and grain of maize 5307 that needs further assessment regarding food and feed safety.

4.2. Conclusion

Based on the agronomic and phenotypic characteristics of maize 5307 tested under field conditions, some differences were noted in maize 5307 compared with its conventional counterpart (i.e. higher 'heat units to 50 % pollen shed', higher grain moisture and higher plant height in 2007 field trials; higher grain yield in 2008 field trials). These are addressed in Section 6.

The EFSA GMO Panel concluded that none of the differences identified in the composition, agronomic and phenotypic characteristics of grain and forage obtained from maize 5307 required further assessment regarding food and feed safety.

5. Food/feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Effect of processing

Based on the outcome of the comparative assessment, the effect of processing maize 5307 is not expected to be different from that of processing conventional maize.

5.1.2. Toxicology

Maize 5307 expresses two new proteins, eCry3.1Ab, conferring insect resistance, and PMI, used as a selection marker (see Section 3).

5.1.2.1. Protein used for safety assessment

Given the low expression levels of the eCry3.1Ab protein in maize 5307 and the consequent difficulty of extracting enough protein from the GM plant, sufficient amounts of the protein were produced in an *E. coli* system (strain DH5α). The structural and functional equivalence of the eCry3.1Ab protein derived from *E. coli* with that expressed in maize 5307 (leaves) was shown by immunoblotting, glycosylation analysis and insecticidal activity. Peptide mass mapping analysis was also performed on the *E. coli*- and the maize 5307-expressed proteins. Both eCry3.1Ab proteins react to the same antibody upon Western blot analysis and have an expected molecular weight of 73.7 kDa (plant derived) and 74.8 kDa (*E. coli* derived, confirmed by mass spectrometry), respectively. The higher

[29] Additional information: 29/01/2013

molecular weight of the *E. coli*-produced protein is due to seven additional amino acids introduced at the N-terminus to facilitate protein purification in the microbial system (histidine-tag). An immunologically positive band of 150 kDa was noted for the *E. coli*-produced protein, probably representing a dimer. Both proteins were not glycosylated and showed insecticidal activity. The peptide mass mapping analysis identified 76 % and 87 % of the predicted amino acid sequence of eCry3.1Ab for the maize 5307- and *E. coli*-produced proteins, respectively. N-terminal analysis confirmed the expected N-terminal amino acid sequence for both proteins. N-terminal acetylation, a common modification known for plant-expressed proteins, was observed in the maize 5307-derived eCry3.1Ab.

The EFSA GMO Panel accepts the use of the *E. coli*-produced eCry3.1Ab protein in the safety studies, as it was demonstrated to be equivalent to the plant-produced one.

The PMI protein expressed in maize 5307 has been assessed in the context of previous applications (EFSA GMO Panel, 2012; EFSA, 2013a,b). No safety concerns were identified by the EFSA GMO Panel for humans or animals.

5.1.2.2. Toxicological assessment of the newly expressed proteins

a) Bioinformatic analyses

Bioinformatic analyses of the amino acid sequence of the PMI protein revealed no significant similarities to proteins known to be toxic to human and animals.

As indicated in Section 3, the applicant identified significant similarities between the amino acid sequence of eCry3.1Ab and parasporin proteins. Parasporins are non-haemolytic and non-insecticidal *B. thuringiensis* (and related bacteria) parasporal proteins that show cytotoxic activity on mammalian cells (predominantly tumoral in origin). Cytotoxicity is achieved after proteolytic processing by apoptosis or plasma membrane increased permeability (Ohba, 2009).

b) *In vitro* degradation studies

The resistance of the eCry3.1Ab protein to degradation by pepsin was studied in solutions at pH ~ 1.2. The integrity of the test protein in samples taken at various time points was analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) followed by protein staining or by Western blotting. No intact protein (ca. 74 kDa) was seen after 30 seconds of incubation. Short fragments of 4 kDa and 5 kDa in the protein-stained SDS-PAGE gel were observed in samples exposed to pepsin for 10 minutes. By Western blot analysis, these fragments were found to be not immunologically reactive to a polyclonal antibody against eCry3.1Ab.

In the application EFSA-GMO-DE-2011-95, the applicant presented an additional *in vitro* degradation study with the PMI protein that confirmed the outcome of a previous *in vitro* degradation study assessed by the EFSA GMO Panel (EFSA GMO Panel, 2012).

c) Acute toxicity

In an acute oral toxicity study in Crl:CD-1 (ICR) mice, the eCry3.1Ab protein expressed in *E. coli* induced no adverse effects after administration in a single dose of 1 720 mg/kg body weight. In the application EFSA-GMO-DE-2011-95, the applicant presented an additional acute toxicity study with the PMI protein that confirmed the outcome of a previous acute oral toxicity study assessed by the EFSA GMO Panel (EFSA GMO Panel, 2012).

The EFSA GMO Panel considers that acute toxicity testing of the newly expressed protein is of little value for the risk assessment of the repeated human and animal consumption of food and feed derived from GM plants.

d) 28-day repeated dose toxicity study

Following a request by the EFSA GMO Panel, the applicant provided a 28-day repeated dose oral toxicity study on eCry3.1Ab in rats.[30] This includes the results of two separate experiments, the second one being a repetition to collect missed data (liver weight). In the first experiment, five groups of Han Wistar Crl: WI (Han) rats (five per gender per group, housed at two and three per cage) were administered the eCry3.1Ab protein (produced in *E. coli*) by gavage at dose levels of 0.36, 3.36 or 33.6 mg/kg body weight (bw) per day, the vehicle, i.e. water for injection (vehicle control group), or bovine serum albumin (BSA, 33.6 mg/kg bw per day; protein control group). During the study, all animals were checked daily for mortality and clinical signs. Body weights and feed consumption were measured twice weekly, and water consumption was monitored weekly. Ophthalmoscopy was carried out before the start of the treatment (all animals) and during week 4 (high-dose group and both control groups). Detailed functional examinations (including motor activity) were conducted for all animals during week 4. After 28 days of treatment, blood samples were taken for haematology, coagulation and clinical chemistry analyses. The animals were necropsied and macroscopically examined; organ weights were determined and tissues for histopathology examination were taken in accordance with OECD Test Guideline (TG) 407 with the exception of liver weight. Histopathological examination was performed on both control groups and the high-dose group. As liver weight was not recorded, the study was repeated in accordance with the same overall design, but only liver weights were measured. Blood and liver samples were taken but not evaluated.

The EFSA GMO Panel requested a 28-day toxicity study of sufficient statistical power in rodents to support the safety assessment of eCry3.1Ab, particularly considering the lack of history of exposure to this protein in humans and animals. However, the study provided by the applicant is not considered adequate by the EFSA GMO Panel for both the following reasons:

- The use of datasets from two separate experiments does not allow the integrated interpretation of findings, specifically with reference to potential effects to the liver;
- The number of animals (five rats per gender per group, two or three per cage) is considered insufficient by the EFSA GMO Panel (see EFSA GMO Panel, 2011a); moreover, the dataset per treatment and gender is derived from a mixture of independent values (two cages) and dependent values (two or three animals per cage).

Therefore, the EFSA GMO Panel can not conclude on the safety of the newly expressed eCry3.1Ab protein.

5.1.2.3. Toxicological assessment of new constituents other than proteins

Maize 5307 does not show any compositional difference to its conventional counterpart that would require further assessment (see Section 4.1.3). No further food and feed safety assessment of components other than newly expressed proteins is required.

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

A 49-day feeding study using chickens for fattening (both sexes) was provided.[31] In this study, 540 broilers (Heritage, day-old) were randomly allocated into three diet treatment groups with 180 chicks per treatment (15 birds of the same sex per pen, and 12 pens per treatment including six with males and the other six with females). Maize 5307 was compared with its conventional counterpart and a non-GM commercial variety (NCSU 2007 maize). Grain was obtained from plants of maize 5307 and its conventional counterpart grown under the same standard local agricultural practices. Before formulating the diets, the grain was analysed for proximates, amino acids and mycotoxins. The chickens were fed starter (days 1–15), grower (days 16–34) and finisher (days 35–49) diets containing 52–54%, 56–58 % and 61–63 % of maize, respectively. The diets were adjusted according to the

[30] Additional information: 29/01/13.
[31] Dossier: Part I—Appendices 38 and 38.1.

standards of the Dutch Central Feed Bureau (CVB, 2001, 2002) and the National Research Council (NRC, 1994). The feed conversion ratio was calculated as feed consumed by live body weight per pen. The concentrations of the newly expressed proteins were determined in the grain and the pelleted diets by ELISA and, as expected, a reduction following feed processing was observed.[32] Feed and water were provided to the birds for *ad libitum* intake.

Chickens were observed twice daily for clinical signs; any deaths were recorded. Body weight and feed intake were measured on days 1, 16, 35 and 49. At day 50, two birds per pen were taken for post-mortem carcass evaluation (dressing percentage, weight of thighs, breast, wings, drums and abdominal fat). A two-way ANOVA (diet and gender) was applied, using the pen as the experimental unit, for performance parameters. A one-way ANOVA was used to analyse the gender-specific carcass parameters using individual data. A direct comparison was also made between the group fed the GM diet and that fed the conventional counterpart.

The EFSA GMO Panel could not evaluate the outcome of this study, because of weaknesses in the study conduct and reporting. This included (i) discrepancies between the number of animals included in the study and that for which results have been reported; (ii) insufficient details given for the statistical evaluation and taking feed samples; (iii) inconsistency in the content of eCry3.1Ab and PMI proteins in the different diets; and (iv) a marked difference between intended and analysed values of dietary protein.

5.1.4. 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 persons and on whether the transformation may have altered the allergenic properties of the modified plant.

5.1.4.1. 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 proteins, as no single piece of information or experimental method yields sufficient evidence to predict allergenicity (EFSA, 2006a; Codex Alimentarius, 2009; EFSA GMO Panel, 2011a).

The newly expressed eCry3.1Ab protein is based on fused partial sequences of the mCry3A and Cry1Ab proteins, which are both derived from *B. thuringiensis*, a bacterium not considered to be a common allergenic source. Bioinformatic analyses of the amino acid sequence of the eCry3.1Ab 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 performed analyses searching for matches of eight contiguous identical amino acid sequences between this newly expressed protein and known allergens, which confirmed the outcome of the above-mentioned bioinformatic analyses showing no similarities to known allergens. The study on resistance to degradation by proteolytic enzymes presented in the current application has been described in Section 5.1.2.2 and did not give rise to safety concerns.

The toxicity study with the eCry3.1Ab protein presented in this application was considered inadequate by the EFSA GMO Panel (see Section 5.1.2.2), and therefore potential adverse effects including those on the immune system cannot be assessed.

With regard to the PMI protein in maize 5307, the gene coding for this newly expressed protein was derived from *E. coli*, which is not considered to be a common allergenic source. Bioinformatic analyses of the amino acid sequence of the PMI protein using the criterion of 35 % identity in a

[32] Protein concentrations (µg/kg of dry weight) measured in the maize grain, starter diet, grower diet and finisher diet are, for eCry3.1Ab 4.71, 2.13, 0.34 and 0.38; and, for PMI 0.85, 0.67, below limit of detection (LoD), below LoD.
[33] Dossier: Part I—Section D7.9.1; additional information: 12/06/14, 28/07/14 and 24/03/15.

window of 80 amino acids revealed no significant similarities to known allergens. In addition, the applicant performed analyses searching for matches of eight contiguous identical amino acid sequences between this newly expressed protein and known allergens. An identical eight-amino-acid-long sequence match between the PMI protein and a frog allergen (i.e. α-parvalbumin from *Rana* sp. CH2001) was reported. This identical match in PMI was previously assessed by the EFSA GMO Panel and no safety concerns were identified (EFSA GMO Panel, 2012). The data assessed included an immunoblotting analysis provided by the applicant where serum from the same allergic individual reported in the literature to react to the frog leg allergen in *Rana* sp. CH2001 did not bind to PMI. The study on resistance to degradation by proteolytic enzymes presented in the current application confirmed the outcome of a previous *in vitro* degradation study assessed by the EFSA GMO Panel (EFSA GMO Panel, 2012; see also Section 5.1.2.2). There is no information available on the structure or function of the newly expressed PMI protein that would suggest an adjuvant effect resulting in or increasing an eventual immunoglobulin (Ig) E response to a bystander protein.

The EFSA GMO Panel has previously evaluated the safety of the PMI protein in the context of other applications and no concerns on allergenicity were identified (e.g. EFSA GMO Panel, 2012; EFSA 2013a,b).

In the context of this application, the EFSA GMO Panel considered that there are no indications that the newly expressed PMI protein in maize 5307 may be allergenic.

5.1.4.2. Assessment of allergenicity of the whole GM plant[34]

To date, maize has not been considered to be a common allergenic food[35] (OECD, 2002), and therefore the EFSA GMO Panel did not request experimental data to analyse the allergen repertoire of GM maize. The EFSA GMO Panel regularly reviews the available publications on food allergy to maize (e.g. EFSA, 2013b).

For the allergenicity assessment of the whole GM plant in maize 5307, the EFSA GMO Panel took into account the data from the molecular characterisation, the comparative analysis and the assessment of the newly expressed proteins. Considering that the safety assessment of the eCry3.1Ab protein could not be completed (see Sections 5.1.2.2 and 5.1.4.1), no conclusions could be reached regarding the overall allergenicity of maize 5307.

5.1.5. Nutritional assessment of GM food/feed

The intended trait of maize 5307 is insect resistance, with no intention of altering the nutritional parameters. Comparison of the nutrients and anti-nutrients of maize 5307 with its conventional counterpart did not identify differences that would require further safety assessment (see Section 4.1.3). From these data, an impact on the nutritional value of maize 5307-derived food and feed is not expected.

5.1.6. Post-market monitoring of GM food/feed

As the EFSA GMO Panel could not complete the safety assessment of maize 5307, the EFSA GMO Panel is currently not in a position to formulate any recommendation for a potential post-market monitoring of maize 5307.

5.2. Conclusion

The EFSA GMO Panel could not complete the food and feed safety assessment of maize 5307 due to the lack of an appropriate assessment of the eCry3.1Ab protein.

[34] Dossier: Part I—Section D7.9.2.

[35] 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.

6. Environmental risk assessment and monitoring plan

6.1. Evaluation of relevant scientific data

Considering the scope of application EFSA-GMO-DE-2011-95, the environmental risk assessment (ERA) is mainly concerned with (i) exposure of bacteria to recombinant DNA in the gastrointestinal tract of animals fed GM material and bacteria present in environments exposed to faecal material, and (ii) accidental release into the environment of viable grains of maize 5307 during transport and processing.

6.1.1. Environmental risk assessment

6.1.1.1. Potential unintended effects on plant fitness due to the genetic modification[36]

Maize is highly domesticated and generally unable to survive in the environment without management intervention. Maize plants are not winter hardy in many regions of Europe; they have lost their ability to release seeds from the cob and they do not occur outside cultivated land or disturbed habitats in the agricultural landscapes of Europe, despite cultivation for many years. In cultivation, maize volunteers may arise under some environmental conditions (mild winters). Observations made on cobs, cob fragments or isolated grains shed in the field during harvesting indicated 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 Sections 4.1.1 and 4.1.2, field trials were carried out in the USA in 2007 and 2008 to assess the agronomic and phenotypic performance[37] of maize 5307 in comparison with its conventional counterpart. Several agronomic and phenotypic characteristics[38] were measured. Considering the scope of application EFSA-GMO-DE-2011-95, special attention is paid to those agronomic characteristics that may affect the survival, establishment and fitness of maize 5307 grains that could be accidentally released into the environment, e.g. early and final stand count, plant vigour, grain test weight and yield.

In the across-site analysis of 2007 field trial data, maize 5307 had a higher percentage grain moisture, higher heat units to 50 % pollen shed and a higher plant height than its conventional counterpart. In the across-site analysis of 2008 field trial data, grain yield for maize 5307 was significantly higher than for its conventional counterpart (see Table 2). Although the 2007 field trial data show some statistically significant differences in some parameters (i.e. grain moisture, heat units to 50 % pollen shed, plant height), the EFSA GMO Panel recognises that these differences are small in magnitude. The EFSA GMO Panel is thus of the opinion that they do not give rise to any environmental safety concerns. Moreover, the 2007 field trial data indicate no difference in grain yield for maize 5307 and its conventional counterpart.

The EFSA GMO Panel acknowledges the difference in grain yield between maize 5307 and its conventional counterpart in the 2008 field trial. The EFSA GMO Panel also notes that maize 5307 and its conventional counterpart did not differ for other agronomic parameters that are likely to indicate a change in fitness potential of the GM maize, such as, for example, number of emerged plants per plot prior to thinning, early growth vigour and number of plants at harvest.

Therefore, considering the scope of application EFSA-GMO-DE-2011-95 and the poor ability of maize to survive outside cultivated areas in case of accidental spillage, the EFSA GMO Panel

[36] Dossier: Part I—Section D7.
[37] Dossier: Part I—Sections D7.1, D7.2, D7.4 and Appendices 20 and 21; additional information: 29/01/2013.
[38] The following agronomic/phenotypic parameters were measured: number of barren plants, number of plants with dropped ears, number of emerged plants, early emergence vigour, early growth vigour, ear height, % grain moisture, % snapped plants, early root lodging, plant population at harvest, heat units to 50 % silking, heat units to 50 % pollen shed, late season intactness, leaf colour rating, late root lodging, plant height, push test, stalk lodging, test weight, grain yield.

concludes that the aforementioned differences do not indicate a change in the overall fitness, invasiveness or weediness of maize 5307 that would raise any relevant environmental safety concerns.

In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of any scientific report of increased spread and establishment of maize 5307 or maize with comparable properties or of any change in survival capacity, including overwintering.

Insect resistance against certain coleopteran target pests provides a potential agronomic advantage in cultivation conditions involving infestation by the target pests. However, survival of maize plants outside cultivation or other areas is limited mainly by a combination of low competitiveness, the absence of a dormancy phase and susceptibility to plant pathogens, herbivores and cold climatic conditions. Based on the inserted traits, the EFSA GMO Panel considers that these general characteristics are unchanged in maize 5307. Therefore, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects due to the accidental release into the environment of viable grains from maize 5307 will not differ from that of conventional maize varieties.

6.1.1.2. Potential for gene transfer[39]

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either horizontal gene transfer of DNA or vertical gene flow via seed spillage and cross-pollination.

(a) 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 microorganisms, especially 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 plants to bacteria) is not likely to occur at detectable frequencies under natural conditions (see EFSA, 2009a,b for further details).

Successful horizontal transfer would require stable insertion of the transgene sequences into a bacterial genome and a selective advantage to be conferred on the transformed host. The only mechanism known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes is homologous recombination. In the case of sequence similarity between the transgenic DNA and the natural variants of the gene in bacteria, recombination could result in gene replacement in bacteria. In the case of two pairs of sequences with sufficient length of identity and correct orientation, recombination could facilitate the transfer of insert sequences to bacterial recipients by double homologous recombination.

Maize 5307 contains several genetic elements of bacterial origin. These are the coding sequences of the *pmi* gene from *E. coli* and of *ecry3.1Ab*, a synthetic gene that is based on the *cry1Ab* and the modified *cry3A* (m*cry3A*) genes derived from *B. thuringiensis* and consists of a fusion between the 5′ end of the *mcry3A* domain (459 bp) and the 3′ end of the *cry1Ab* domain (172 bp). Furthermore, maize 5307 also contains two *nos*-terminator sequences originating from the Ti plasmid of *Agrobacterium tumefaciens*. Whereas *E. coli* is considered to be prevalent in the main receiving environment, i.e. the gastrointestinal (GI) tract of humans or animals, *B. thuringiensis* is not considered to be an inhabitant of the GI tract of humans and animals, and, thus, the presence of strains carrying *cry3A* or *cry1Ab* genes is expected to be very low. *B. thuringiensis* strains can be isolated from soil and are frequently found in material from the guts of various insects (Jensen et al., 2003). Equally, *A. tumefaciens*, or its

[39] Dossier: Part I—Section D9.3.

close relatives from the genus *Rhizobium*, is not expected to be prevalent in the GI tract. However, occurrence of the recombinant genes outside the immediate receiving environment, in habitats where *E. coli* may also occur and *B. thuringiensis* or *A. tumefaciens* is more abundant, cannot be ruled out (Hart et al., 2009) and is therefore also taken into account for assessing the risks associated with horizontal gene transfer.

On a theoretical basis (i.e. without any study providing experimental evidence for the occurrence of horizontal gene transfer in the case of GM food and feed derived from maize 5307 or any other GM plant), it can be assumed that, as an extremely rare event, homologous recombination may occur in the environment between nucleotide sequences of the recombinant *ecry3.1Ab* and *pmi* genes and their natural variants, as they may occur for *ecry3.1Ab* in certain *B. thuringiensis* strains and *pmi* in *E. coli* or other bacteria, with *pmi* genes providing sufficient sequence identity.

The *nos*-terminator sequences present in maize 5307, each with a length of 200 bp, may facilitate double homologous recombination with corresponding *nos* genes on Ti plasmids of environmental *A. tumefaciens* strains. Theoretically, such a recombination could result in the acquisition of the *pmi* gene on natural Ti plasmids and a possible decrease in the expression potential of the *nos* gene due to the replacement of part of its termination sequence by the *pmi* gene. Due to the conjugative gene transfer system encoded by the Ti plasmid, i.e. the *tra*-system, the potential for transfer of the *pmi* gene to other bacteria that can serve as recipients of the Ti plasmid could be enhanced (Zatyka and Thomas, 1998). Such bacteria would thereby gain the genetic potential to utilise mannose as a carbon and energy source. As the *nos* sequence-flanked *pmi* gene present in maize 5307 is contained within the *vir*-system, environmental *A. tumefaciens* with a recombinant Ti plasmid would also gain the capacity to transfer the *pmi* gene to plant cells where it could be integrated into the plant genome together with the genes converting the plant cells to crown gall tumour cells. The crown gall tumour cells would thereby receive the genetic potential to produce the enzyme phosphomannose isomerase and, upon expression, gain the capacity to convert mannose 6-phosphate to fructose 6-phosphate, which could then be metabolised though gluconeogenesis (Zhengquan He et al., 2004), whereas non-transformed cells cannot metabolise mannose 6-phosphate.

In addition to homology-based recombination processes, 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, 2009b). Illegitimate recombination events have not been detected in studies that have exposed bacteria to high concentrations of GM plant DNA (EFSA, 2009b). Thus, this process, in comparison to homologous recombination, is not considered to contribute significantly to horizontal gene transfer events. In comparison to the above-described homology-facilitated recombination processes, the contribution of illegitimate recombination is extremely low.

Both protein-encoding genes from bacteria are regulated in maize 5307 by promoters optimised for expression in plants: the e*cry3.1Ab* gene of maize 5307 by a eukaryotic plant promoter (derived from the *Arabidopsis thaliana RbcS4*) and the *pmi* gene by the promoter of the *Zea mays* polyubiquitin gene. The expression of the *prRBCS4–cry3.1Ab* and *ZmUbiInt–man*A constructs in bacteria is unknown, but generally the expression level of eukaryotic promoters in bacteria is inefficient (Warren et al., 2008). Therefore, the acquisition of the *pmi* gene by bacterial recipients, including those that would receive a recombinant Ti plasmid (see above) is unlikely to confer a high level of expression of the enzyme phosphomannose isomerase. Plant cells transformed with the Ti plasmid containing the *pmi* gene could, however, considering the plant-derived promoter, gain the capacity to utilise mannose for growth. However, the replacement of part of the *nos* terminator sequence by the *pmi* gene would result in decreased expression of nopaline synthase, which would decrease the utilisation of nopaline by the plant cells.

The following potential environmental implications are considered:

(1) Substitutive recombination between partial sequences of the synthetic *ecry3.1Ab* gene or the *pmi* gene with natural variants, as they may occur in habitats receiving DNA of maize 5307 and bacteria would only replace natural variants (substitutive recombination) and are therefore unlikely to provide any new property connected to a selective advantage for the recipient organisms (EFSA, 2009b).

(2) Bacterial recipients of Ti plasmids with the *pmi* gene could gain the genetic potential to utilise mannose as a carbon and energy source, but due to the plant-specific promoter, expression of this gene is expected to be low. Furthermore, phosphomannose isomerases are expected to occur in many different environmental bacteria and fungi and, thus, the trait is expected to be common in receiving environments. Therefore, the ecological implications of bacterial recipients with a poorly expressed *pmi* gene on Ti plasmids are expected to be very low.

(3) Plant tumour cells infected by crown gall disease via the *vir*-system of *A. tumefaciens* Ti plasmids, including the pmi gene, would gain a selective advantage to utilise mannose as a carbon source, which would coincide with decreased utilisation of nopaline. The major carbon source of plant cells originates from photosynthetically assimilated carbon that is transported via the phloem, mainly in the molecular form of sucrose and amino acids but not mannose, to the plant cells. Thus, a selective advantage of utilising mannose is not apparent. Furthermore, the potential utilisation of mannose by the plants would be accompanied by a loss of capacity to utilise nopaline. Therefore, the risk associated with an additional *pmi* gene in the *nos* terminator in crown gall tumour cells is regarded as being very low.

The EFSA GMO Panel concludes that the *ecry3.1Ab* and *pmi* genes from maize 5307 may, on a theoretical basis, be transferred by homologous recombination to environmental strains of *B. thuringiensis* or *E. coli*, where they could replace, in the case of the *pmi* gene, natural variants of the gene or, in the case of the *ecry3.1Ab* gene, partial regions of the *cry1Ab* or *cry3A* genes. As *B. thuringiensis* is not considered to be a member of the gut microbial community, its exposure to recombinant DNA of maize 5307 is considered to be very low, while an abundance of *pmi* gene variants in *E. coli* or related enterobacteriaceae can be expected. Due to the occurrence of natural variants of the *cry* and *pmi* genes in the environment, low-level gene replacement by horizontal gene transfer is not regarded as conferring a novel selective advantage. Similarly, the substitutive recombination of *nos* sequences, as present in DNA of maize 5307, with *nos* sequences present in strains of *A. tumefaciens*, which are not expected to be prevalent in the main receiving environment, would not confer a new trait. On a theoretical basis, horizontal gene transfer events may result in *A. tumefaciens* strains with Ti plasmids carrying the *pmi* gene in the *nos* terminator sequence and, thus, allow further transfer of the *pmi* gene via the *tra*-system to other bacteria or via the *vir*-system to crown gall cells in plants. While these transfers potentially may confer a new trait to bacterial or plant recipients, due to conferring the capacity to utilise mannose, the EFSA GMO Panel did not consider this altered metabolic potential to be a selective advantage and, thus, of concern due to (i) the wide abundance of natural variants of phosphomannose isomerases in environmental microbial communities, and (ii) the fact that plant tumour cells do not receive significant amounts of mannose under natural conditions.

Considering the scope of application EFSA-GMO-DE-2011-95, the EFSA GMO Panel concluded that the unlikely but theoretically possible horizontal gene transfer of recombinant genes from maize 5307 to bacteria does not raise any environmental safety concerns.

(b) Plant-to-plant gene transfer

Considering the scope of application EFSA-GMO-DE-2011-95 and the physical characteristics of maize grain, possible pathways of gene dispersal are grain spillage and the dispersal of pollen from occasional feral GM maize plants originating from accidental grain spillage during transport and processing.

The extent of cross-pollination with other maize varieties will depend mainly on the scale of accidental release during transport and processing, and on the successful establishment and subsequent flowering of this GM maize plant. For maize, any vertical gene transfer is limited to other *Zea mays* plants, as populations of sexually compatible wild relatives of maize are not known in Europe (Eastham and Sweet, 2002; OECD, 2003).

The flowering of occasional feral GM maize plants originating from accidental release occurring during transport and processing is unlikely to disperse significant amounts of GM maize pollen to 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).

Although the occurrence of some GM maize plants outside cropped areas has been reported in Korea due to grain spillage during import, transport, storage, handling and processing (Kim et al., 2006; Lee et al., 2009; Park et al., 2010), survival of maize plants outside cultivation in Europe is mainly limited by a combination of low competitiveness, the absence of a dormancy phase and susceptibility to plant pathogens, herbivores and frost. As these general characteristics are unchanged in maize 5307, insect resistance is not likely to provide a selective advantage outside cultivation or under infestation by target pests in Europe. Therefore, as for any other maize varieties, these GM maize plants would only survive in subsequent seasons in warmer regions of Europe and are not likely to establish feral populations under European environmental conditions.

The EFSA GMO Panel takes into account the fact that this application does not include cultivation of maize 5307 within the EU, so that the likelihood of cross-pollination between cultivated maize and the occasional feral maize plants resulting from grain spillage is considered extremely low.

In conclusion, as maize 5307 has no altered survival, multiplication or dissemination characteristics, except under infestation by target pests, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of the spread of genes, resulting from imports of this maize in Europe, will not differ from that of conventional maize varieties.

6.1.1.3. Interactions of the genetically modified plant with target organisms[40]

Considering the scope of application EFSA-GMO-DE-2011-95, and the low level of exposure to the environment, potential interactions of the GM plant with target organisms were not considered a relevant issue by the EFSA GMO Panel.

6.1.1.4. Interactions of the genetically modified plant with non-target organisms[41]

Considering the scope of application EFSA-GMO-DE-2011-95, and the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms were not considered a relevant issue by the EFSA GMO Panel.

However, the EFSA GMO Panel evaluated whether the eCry3.1Ab protein might potentially affect non-target organisms by entering the environment through faecal material of animals fed this GM maize. Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only a very low amount of these proteins would remain intact to pass out in faeces. This has been demonstrated for Cry1Ab (Einspanier et al., 2004; Lutz et al., 2005, 2006; Wiedemann et al., 2006; Guertler et al., 2008; Paul et al., 2010) and Cry1Ab/Ac fusion protein (Xu et al., 2009). There would, subsequently, be further degradation of the protein in the faecal material due to microbiological proteolytic activity. In addition, there would be further degradation of Cry proteins in soil reducing the possibility for exposure of potentially sensitive non-target organisms. While Cry proteins may bind to clay minerals and humic substances in soil, thereby reducing their availability to microorganisms for

[40] Dossier: Part I—Section D9.4.
[41] Dossier: Part I—Section D9.5.

degradation, there are no indications of persistence and accumulation of Cry proteins from GM crops in soil (reviewed by Icoz and Stotzky, 2008).

The EFSA GMO Panel is not aware of evidence of released Cry proteins from GM plants causing significant negative effects on soil micro- or macroorganisms.

Considering the scope of application EFSA-GMO-DE-2011-95, it can be concluded that the exposure of potentially sensitive non-target organisms to the eCry3.1Ab protein is likely to be very low and of no biological relevance.

6.1.1.5. Interactions with the abiotic environment and biochemical cycles[42]

Considering the scope of application EFSA-GMO-DE-2011-95, and the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles were not considered a relevant issue by the EFSA GMO Panel.

6.1.2. Post-market environmental monitoring[43]

The objectives of a post-market environmental monitoring (PMEM) plan according to Annex VII of Directive 2001/18/EC are: (i) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct; and (ii) 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 also related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of the EFSA GMO Panel. However, the EFSA GMO Panel gives its opinion on the scientific quality of the PMEM plan provided by the applicant (EFSA, 2006b; EFSA GMO Panel, 2011b).

The PMEM plan proposed by the applicant includes (i) the description of an 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; (ii) a coordinating system established by EuropaBio for the collection of the information recorded by the various operators; and (iii) the use of networks of existing surveillance systems (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes submitting a PMEM report on an annual basis.

The EFSA GMO Panel is of the opinion that the PMEM plan provided by the applicant, including the reporting intervals, is in line with the scope of application EFSA-GMO-DE-2011-95, as the ERA did not cover cultivation and identified no potential adverse environmental effects. No case-specific monitoring is necessary. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

6.2. Conclusion

There are no indications of an increased likelihood of establishment and spread of feral maize plants. Considering the scope of application EFSA-GMO-DE-2011-95, interactions with the biotic and abiotic environment were not considered to be a relevant issue. The EFSA GMO Panel also concludes that, considering the scope of application EFSA-GMO-DE-2011-95, the unlikely but theoretically possible horizontal gene transfer of recombinant genes from maize 5307 to bacteria does not give rise to any environmental safety concerns. The PMEM plan and reporting intervals are in line with the scope of application EFSA-GMO-DE-2011-95.

[42] Dossier: Part I—Section D9.7.
[43] Dossier: Part I— Section D11.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was asked to carry out a scientific risk assessment of maize 5307 for import, processing, and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation data provided for maize 5307 raised issues to be further assessed for food and feed safety. The EFSA GMO Panel identified relevant similarities between the amino acid sequence of PMI and a known allergen, and between the amino acid sequence of eCry3.1Ab and a potential toxin.

Differences observed in the agronomic and phenotypic characteristics of maize 1507 tested under field conditions and compared with its conventional counterpart did not give rise to any food and feed or environmental safety concerns. No differences requiring further assessment with regard to safety by the EFSA GMO Panel were identified from analyses of compositional data of forage or grains obtained from maize 5307 and its conventional counterpart.

No safety concerns were identified regarding the potential toxicity and allergenicity of the newly expressed protein PMI. The 28-day rat oral toxicity study on eCry3.1Ab, provided to support the safety assessment of this newly expressed protein, was not considered adequate by the EFSA GMO Panel. Therefore, the EFSA GMO Panel cannot conclude on the safety of the eCry3.1Ab protein. The EFSA GMO Panel could not evaluate the outcome of a feeding study in broilers with maize 5307 because of study weaknesses.

The application GMO-DE-2011-95 concerns food and feed uses and import and processing. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of maize 5307. There are no indications of an increased likelihood of establishment and spread of feral maize plants. Considering the scope of application EFSA-GMO-DE-2011-95, interactions with the biotic and abiotic environment were not considered to be a relevant issue. The EFSA GMO Panel also concludes that, considering the scope of application EFSA-GMO-DE-2011-95, the unlikely but theoretically possible horizontal gene transfer of recombinant genes from maize 5307 to bacteria does not give rise to any environmental safety concerns. The PMEM plan and reporting intervals are in line with the scope of application EFSA-GMO-DE-2011-95.

In conclusion, the EFSA GMO Panel could not complete the food and feed safety assessment of maize 5307 due to the lack of an appropriate assessment of the eCry3.1Ab protein. However, the EFSA GMO Panel concludes that maize 5307 is unlikely to have any adverse effect on the environment in the context of the scope of application EFSA-GMO-DE-2011-95.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of Germany, received on 7 April 2011, concerning a request for placing on the market of genetically modified maize 5307, submitted by Syngenta Crop Protection AG in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 27 April 2011, from EFSA to the German Competent Authority.

3. Letter from EFSA to applicant, dated 13 May 2011, requesting additional information under completeness check.

4. Letter from applicant to EFSA, received on 31 May 2011, providing additional information under completeness check.

5. Letter from EFSA to applicant, dated 21 June 2011, delivering the 'Statement of Validity' of application for the authorisation of genetically modified maize 5307, application EFSA-GMO-DE-2011-95, submitted by Syngenta Crop Protection AG in accordance with Regulation (EC) No 1829/2003.

6. Letter from EFSA to applicant, dated 15 July 2011, requesting additional information and stopping the clock on behalf of the DG JRC/EURL-GMFF.

7. Letter from applicant to EFSA, received on 2 August 2011, providing a timeline for submission of responses.

8. Letter from EFSA to applicant, dated 13 September 2011, requesting additional information and maintaining the clock stopped.

9. Letter from applicant to EFSA, received on 3 October 2011, providing additional information.

10. Letter from EFSA to applicant, dated 14 October 2011, requesting additional information and maintaining the clock stopped.

11. Letter from applicant to EFSA, received on 7 December 2011, providing a timeline for submission of responses.

12. Letter from DG JRC/EURL-GMFF to EFSA, received on 14 September 2012, asking EFSA to re-start the clock.

13. Letter from EFSA to applicant, dated 15 November 2012, requesting additional information and maintaining the clock stopped on behalf of the DG JRC/EURL-GMFF.

14. Letter from applicant to EFSA, received on 29 November 2012, requesting extension of the timeline for submission of responses.

15. Letter from applicant to EFSA, received on 29 January 2013, providing additional information.

16. Letter from EFSA to applicant, dated 25 June 2013, requesting additional information and maintaining the clock stopped.

17. Letter from applicant to EFSA, received on 8 August 2013, providing additional information.

18. Letter from EFSA to applicant, dated 17 October 2013, re-starting the clock.

19. Letter from EFSA to applicant, dated 17 January 2014, re-starting the clock for DG JRC/EURL-GMFF and corrigendum of EFSA letter to applicant, dated 17 October 2013.

20. Letter from EFSA to applicant, dated 27 May 2014, requesting additional information and stopping the clock.

21. Letter from applicant to EFSA, received on 12 June 2014, providing the additional information requested.

22. Letter from EFSA to applicant, dated 2 July 2014, re-starting the clock.

23. Letter from applicant to EFSA, received on 28 July 2014, submitting additional information spontaneously.

24. Letter from EFSA to applicant, dated 9 September 2014, requesting additional information and stopping the clock.

25. Letter from applicant to EFSA, received on 18 September 2014, providing additional information.

26. Letter from EFSA to applicant, dated 29 October 2014, requesting additional information and maintaining the clock stopped.

27. Letter from applicant to EFSA, received on 11 December 2014, providing additional information.

28. Letter from EFSA to applicant, dated 16 February 2015, requesting additional information and maintaining the clock stopped.

29. Letter from EFSA to applicant, dated 27 February 2015, requesting additional information and maintaining the clock stopped

30. Letter from applicant to EFSA, received on 24 March 2015, providing additional information.

31. Letter from EFSA to applicant, dated 14 April 2015, re-starting the clock.

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