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SCIENTIFIC OPINION

Scientific Opinion on an application (EFSA-GMO-NL-2009-70) for the placing on the market of genetically modified drought tolerant maize MON 87460 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto[1]

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

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ABSTRACT

Maize MON 87460 was developed through *Agrobacterium*-mediated transformation and expresses the cold shock protein B (CspB) from *Bacillus subtilis* and neomycin phosphotransferase II (NPTII) from *Escherichia coli* to reduce yield loss under water-limited conditions. Maize MON 87460 contains a single copy of the *csp*B and *npt*II expression cassettes. Bioinformatic analysis of the flanking sequences and the open reading frames spanning the junctions created by the transformation did not raise safety issues. Comparative analyses established that, besides the expression of the CspB and NPTII proteins, some differences were observed in the composition of forage and grain produced from maize MON 87460 compared with its conventional counterpart, when grown under well-watered conditions. Given the magnitude of these changes and the characteristics of these endpoints, the EFSA GMO Panel concluded that the observed differences do not raise safety concerns for humans and animals. Under stressful conditions, maize MON 87460 can show enhanced agronomic performance characteristics and some differences in chemical composition in comparison with its conventional counterpart. Given the intended trait, the observed differences were not unexpected, and did indicate no safety concerns. The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the CspB and NPTII proteins, or of maize MON 87460. Maize MON 87460 is as nutritious as any other maize and can be used in the same way. In cases of spillage, there are no indications of increased likelihood of the establishment or survival of feral maize plants MON 87460. Risks associated with a theoretically possible horizontal gene transfer from maize MON 87460 to bacteria have been analysed in detail, including different scenarios of integration, and did not raise safety concerns for the intended uses of maize MON 87460. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of maize MON 87460.

[1] On request from the Competent Authority of the Netherlands for an application (EFSA-GMO-NL-2009-70) submitted by Monsanto, Question No EFSA-Q-2009-00661, adopted on 18 October 2012.

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KEY WORDS

GMO, maize (*Zea mays*), MON87460, MON 87460, drought tolerance, risk assessment, food and feed safety, environmental safety, food and feed uses, import, processing, Regulation (EC) No 1829/2003

SUMMARY

Following the submission of an application (EFSA-GMO-NL-2009-70) under Regulation (EC) No 1829/2003 from Monsanto, 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 the genetically modified (GM) drought tolerant maize MON 87460[4] for food and feed uses, import and processing of maize MON 87460 and all derived products but excluding cultivation in the European Union (EU).

In delivering its Scientific Opinion, the EFSA GMO Panel considered: the application EFSA-GMO-NL-2009-70; additional information supplied by the applicant; scientific comments submitted by the Member States; and relevant scientific publications.

The EFSA GMO Panel evaluated maize MON 87460 with reference to the intended uses and principles described in its risk assessment and monitoring guidelines. The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and expression of the target proteins. An evaluation of the comparative analysis of composition and agronomic and phenotypic traits was undertaken, and the safety of the newly expressed proteins and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional quality. An evaluation of environmental impacts and of the post-market environmental monitoring plan was undertaken.

Maize MON 87460 was developed through *Agrobacterium*-mediated transformation and expresses the cold shock protein B (CspB) from *Bacillus subtilis* and neomycin phosphotransferase II (NPTII) from Tn5 of *Escherichia coli*. Maize MON 87460 was developed to provide reduced yield loss under conditions in which water is limited compared with conventional maize. The CspB protein is an RNA chaperone associated with enhanced abiotic stress tolerance in bacteria and plants, through its interaction with RNA secondary structures, limiting their misfolding and allowing cells to maintain cellular functions under various stress conditions. In maize MON 87460, this genetic modification aims to reduce yield loss caused by drought stress.

The molecular characterisation data establish that maize MON 87460 contains a single copy of the *csp*B and *npt*II expression cassettes, and lacks other sequences from the transformation vector. Bioinformatic analysis of the flanking sequences and the open reading frames spanning the junctions created by the transformation did not raise safety issues. The stability of the inserted DNA was confirmed over multiple generations. The levels of the CspB and NPTII protein from maize MON 87460 grown in field studies performed under different environmental conditions, including water-limited conditions, were assessed.

The EFSA GMO Panel compared the composition and phenotypic and agronomic characteristics of maize MON 87460 with those of its conventional counterpart and assessed all statistically significant differences identified. The EFSA GMO Panel concludes that, besides the expression of the CspB and NPTII proteins, some differences were observed in the composition of forage and grain produced from maize MON 87460, compared with its conventional counterpart, when grown under well-watered conditions. Given the magnitude of these changes and the characteristics of these endpoints, the EFSA GMO Panel concludes that the observed differences do not raise safety concerns for humans and animals. The EFSA GMO Panel notes that under water-limited and other stressful conditions, maize MON 87460 can show enhanced agronomic performance characteristics (e.g. yield) and some differences in chemical composition in comparison with its conventional counterpart. Given the intended trait, the observed differences were not unexpected, and did indicate no safety concerns.

The safety assessment of the newly expressed protein and the whole crop included an analysis of data from analytical and bioinformatics studies, as well as *in vitro* pepsin and pancreatin resistance tests with the CspB protein and a subchronic 90-day rat feeding study. The NPTII protein has been evaluated previously and did not raise safety concerns. The EFSA GMO Panel concludes that maize

[4] Unique identifier MON-8746Ø-4.

MON 87460 is as safe as its conventional counterpart; there is no evidence that the genetic modification might significantly change the overall allergenicity of maize MON 87460. The results of the study on chickens for fattening concerning zootechnical performance support the conclusion that maize MON 87460 can be used as other maize sources as a feedingstuff in animal nutrition. The EFSA GMO Panel considers that maize MON 87460 is as safe and as nutritious as its conventional counterpart and commercial varieties, and concluded that this maize and derived products are unlikely to have adverse effects on human and animal health, in the context of their intended uses.

The application EFSA-GMO-NL-2009-70 covers the import and processing of maize MON 87460 for food and feed uses but excludes its cultivation in the EU. Therefore, there is no requirement for scientific information on the possible environmental effects associated with the cultivation of maize MON 87460. There are no indications of an increased likelihood of the establishment and spread of feral maize plants in cases of accidental release into the environment of viable grains from maize MON 87460 during transport and processing. Considering the intended uses of maize MON 87460 as food and feed, interactions with the biotic and abiotic environment are not considered to be an issue owing to the low levels of exposure. Risks associated with a theoretically possible horizontal transfer from maize MON 87460 *npt*II and *csp*B genes to bacteria have been analysed in detail, including different scenarios of integration, and did not raise safety concerns for the intended uses of maize MON 87460. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize MON 87460. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

In conclusion, the EFSA GMO Panel considers that the information available for maize MON 87460 addresses scientific issues indicated by its risk assessment and monitoring guidelines and the scientific comments raised by the Member States, and that maize MON 87460, as described in this application, is as safe as its conventional counterpart and non-GM reference varieties with respect to potential effects on human and animal health and the environment, in the context of its intended uses.

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BACKGROUND

On 29 May 2009, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands an application (Reference EFSA-GMO-NL-2009-70), for authorisation of genetically modified (GM) drought tolerant maize MON 87460 (Unique Identifier MON-8746Ø-4), submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on GM food and feed.[5] After having received the application EFSA-GMO-NL-2009-70 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed Member States and the European Commission, and made the summary of the application available to the public on the EFSA website. 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. On 1 December 2009 and 8 January 2010, EFSA received additional information requested under completeness check (requested on 9 July 2009 and 14 December 2009). On 28 January 2010, EFSA declared the application as formally 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 State bodies had three months after the date of receipt of the valid application (until 28 April 2010) within which to make their opinion known.

The EFSA Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel) carried out an evaluation of the scientific risk assessment of the maize MON 87460 for food and feed uses, import and processing, in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. When carrying out the safety evaluation, the EFSA GMO Panel took into account the principles described in its risk assessment and monitoring guidelines (EFSA, 2006a, 2011b), the scientific comments of the Member States and the additional information provided by the applicant and relevant scientific publications.

The EFSA GMO Panel requested from the applicant additional information on 12 May 2010, 20 December 2010, and 8 July 2011. The applicant provided the requested information on 4 October 2010, 18 April 2011 and 30 April 2012, respectively. After receipt and assessment of the full data package, the EFSA GMO Panel finalised its risk assessment on maize MON 87460.

In giving its Scientific Opinion on maize MON 87460 to the European Commission, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of 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.

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 risk assessment of maize MON 87460 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

[5] 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, pp. 1–2).

[6] Directive 20010/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release of genetically modified organisms and repealing Council Directive 90/220/EEC (OJ L 106, 17/04/2001, pp. 1–38).

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 a Scientific Opinion on information required under Annex II to the Cartagena Protocol, nor on the 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.

Being outside the remit of the Regulation (EC) 1829/2003, the EFSA GMO Panel did not conclude on the efficacy of the drought tolerance trait introduced in maize MON 87460 under stressed conditions.

ASSESSMENT

1. Introduction

Maize MON 87460 was evaluated with reference to its intended uses and taking account of the principles described in the risk assessment and monitoring guidelines of the EFSA Scientific Panel on Genetically Modified organisms (GMO Panel) (EFSA, 2006a,b, 2010b, 2011b). The evaluation of the risk assessment presented here is based on the information provided in the application relating to maize MON 87460 submitted in the European Union (EU), including additional information from the applicant, as well as issues raised by the Member States and relevant scientific publications.

2. Issues raised by Member States

The scientific comments raised by the Member States are addressed in Annex G of the EFSA overall opinion[7] and have been considered in this Scientific Opinion.

3. Molecular characterisation

Maize MON 87460 was developed through *Agrobacterium*-mediated transformation of conventional maize variety LH59 and expresses a *csp*B gene from *Bacillus subtilis* encoding a cold shock protein B (CspB) and the *npt*II gene from *Escherichia coli* encoding the neomycin phosphotransferase II (NPTII) protein conferring resistance to kanamycin and related antibiotics. The latter was used as a marker to facilitate the selection process of transformed plant cells. The CspB protein is an RNA chaperone associated with enhanced abiotic stress tolerance in bacteria and plants, through its interaction with RNA secondary structures, limiting their misfolding and allowing cells to maintain cellular functions under various stress conditions (Phadtare et al., 2002a,b; Castiglioni et al., 2008). In maize MON 87460, this genetic modification aims to reduce yield loss caused by drought stress. *Bacillus subtilis* is a common soil bacterium, expressing a 67-amino acid CspB chaperone protein. Maize MON 87460 was genetically transformed to express an identical chaperone protein, with the exception of one amino acid change resulting from the DNA cloning procedure. The plant-expressed protein was named CspB-L2V, accordingly.

3.1. Evaluation of relevant scientific data

3.1.1. Transformation process and vector constructs

Maize MON 87460 was obtained by *Agrobacterium*-mediated transformation of isolated immature embryos from the conventional maize LH59 variety, using the binary plasmid vector PV-ZMAP595 and the *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*) strain ABI.[8]

Two expression cassettes are located between the right and left borders of the transfer DNA (T-DNA), driving the expression of the *csp*B gene and *npt*II gene in plant tissues.[9] The *csp*B expression cassette contains the promoter, leader and first intron of the rice (*Oryza sativa*) actin 1 gene (*act*1), the *csp*B coding sequence, and the 3′ non-translated sequence of the transcript 7 gene (T-tr*7*) from *A. tumefaciens*. The *csp*B coding sequence is translated into the CspB-L2V protein, which differs from the *B. subtilis* CspB protein by one leucine-to-valine substitution at amino acid position 2. This sequence modification was intentionally introduced to facilitate the construction of the plasmid vector PV-ZMAP595 for plant transformation.

The *npt*II expression cassette contains the *npt*II coding sequence under the regulation of a 35S promoter from the *Cauliflower mosaic virus* (P35S) and the 3′ non-translated sequence from the *A. tumefaciens* nopaline synthase (T-*nos*) gene; the *npt*II expression cassette is flanked by two *loxP* sites, allowing its potential excision in the presence of the Cre recombinase from the corresponding

[7] http://registerofquestions.efsa.europa.eu/
[8] Technical Dossier/Section C1.
[9] Technical Dossier/Section C2.

Cre/*lox* site-specific recombination system of bacteriophage origin. However, the *npt*II cassette was not excised in maize MON 87460. In addition, bioinformatic analysis was conducted and indicated that, on a theoretical basis, double homologous recombination could occur owing to the presence of sequences from *A. tumefaciens*.[10] The impact of the *lox* sequences and other bacterial sequences on plant to bacteria gene transfer is described in Section 6.1.1.2.

Four genetic elements exist outside of the T-DNA borders that are essential for the maintenance and selection of the vector ZMAP595 in bacteria and that are not expected to be transferred into the maize genome: *oriV*, origin of replication for the maintenance of the plasmid in *Agrobacterium*; *rop*, coding sequence of repressor of primer protein for the maintenance of plasmid copy number in *E. coli*; *ori*, origin of replication from pBR322 for the maintenance of the plasmid in *E. coli*; and *aad*A, a bacterial promoter and coding sequence from transposon Tn*7* that codes for a 3′-adenylyltransferase enzyme conferring spectinomycin and streptomycin resistances, used as selection marker in *E. coli* prior to plant transformation.

3.1.2. Transgene constructs in maize MON 87460

Molecular analyses were performed to characterise the DNA integrated in maize MON 87460.[11] In order to determine the copy number of the T-DNA and the genetic elements it contained, Southern blot analyses were performed using: (1) DNA extracted from maize MON 87640 and from a conventional maize with a similar genetic background; (2) three restriction enzyme combinations; and (3) sets of probes covering the entire plasmid PV-ZMAP595 (T-DNA and backbone sequences). The molecular characterisation showed that maize MON 87640 contains a single insert, comprising both the *csp*B and *npt*II expression cassettes, and no backbone sequences derived from the vector outside the T-DNA could be detected in the maize genome as the result of transformation.[12]

Sequence analysis of overlapping polymerase chain reaction (PCR) fragments covering the full length of the T-DNA insert in maize MON 87460 indicated that the right border region is absent. In addition, 733 base pairs (bp) of the rice actin 1 promoter are deleted, resulting in the removal of regulatory elements (McElroy et al., 1990; Wang et al., 1992) and leaving approximately 115 bp of the proximal part of the promoter.[13] The truncated rice actin 1 promoter in maize MON 87460 was designated as P-Ract1^{87460}. The analysis demonstrated the integrity and expected organisation of the other genetic elements of the T-DNA insert in maize MON 87460, compared with the T-DNA of the donor plasmid PV-ZMAP595.

To determine the DNA sequence at the pre-insertion site, PCR amplification was performed on parental (LH59) genomic DNA using primers designed from the 5′ and 3′ flanking sequences of the MON 87460 T-DNA insert. Sequence comparison between the pre-insertion site in the comparator (LH59) and the MON 87460 T-DNA flanking regions identified a 22 bp deletion at the integration site.

To assess whether the insertion of the T-DNA in maize MON 87460 disrupted any endogenous genes of maize, 1121 bp of the 5′ flanking region and 784 bp of the 3′ flanking region were used to search nucleotide and protein sequence databases, using the BLASTN and BLASTX algorithms. There is no evidence that the insert in maize MON 87460 disrupts any known maize coding sequences.[14] The results also confirmed that the insert is located in the nuclear genome. This could also be deduced from the observed Mendelian segregation of the integrated DNA.

In order to assess whether the insertion resulted in the creation of novel open reading frames (ORFs) at the junctions with the flanking DNA regions, the DNA sequence spanning the 5′ and 3′ junctions of

[10] Additional information October 2010.
[11] Technical Dossier/Section D2/Skipwith et al. (2007).
[12] Additional information October 2010 and Zong et al. (2010).
[13] Additional information October 2010.
[14] Technical Dossier/Section D2/Tu and Silvanovich (2009c)/Additional information October 2010.

the maize MON 87460 insertion site was analysed for the presence of all ORFs defined between translation termination codons in the six possible frames and originating or terminating within the maize MON 87460 insertion site.[15] Their putative translation products were used to search updated toxin and allergen databases by the FASTA algorithm. In addition, the presence of eight-amino acid perfect matches between the known allergens of the database and the potential translation products from the ORFs was examined. No alignment met or exceeded the threshold for potential allergenicity (EFSA, 2010), and no relevant similarities to known toxic proteins were shown, supporting the conclusion that the ORFs would not raise safety issues.

3.1.3. Information on the expression of the insert

The scope of the application covers food and feed uses, import and processing of maize MON 87460. CspB and NPTII proteins were quantified using enzyme-linked immunosorbent assay (ELISA) for different developmental stages and tissue types from maize grown in the field in the USA (season 2006) and Chile (season 2006–2007).[16] In the USA, plants were grown at six sites corresponding to a range of environmental conditions relevant to maize cultivation, and plants were grown under normal agronomic practices (i.e. with no water shortage).[17] Three field trial sites were in Chile and a strip-plot design was used to assess maize grown under two irrigation regimes (well watered and water limited).[18] The water-limited plots were managed to impose drought stress by withholding irrigation from the late vegetative phase through to the early grain fill stage, to assess changes in CspB and NPTII protein levels under different soil moisture conditions.

The CspB protein was expressed at a low level in all tissues relevant to the scope of this application, and the levels of the CspB protein tended to decline over the growing season. For grain the expression levels varied between 0.02 and 0.10 µg/g dry weight, and for forage between 0.04 and 0.22 µg/g dry weight (see Table 1). No obvious difference was observed in CspB protein levels in tissues collected from plants grown under well-watered or water-limited conditions for any of the analysed tissues (Table 1).

Table 1: Ranges of CspB and NPTII levels in maize MON 87460 (µg/g dry weight)

Protein	Plant part	USA 2006	Chile 2006–2007 well watered	Chile 2006–2007 water limited
CspB	Grain	0.05–0.10	0.03–0.08	0.02–0.05
	Forage	0.04–0.17	0.08–0.14	0.07–0.22
NPTII	Grain	<LOQ	<LOQ	<LOQ
	Forage	0.05–0.20	0.13–0.19	0.12–0.22

LOQ: limit of quantification.

In the NPTII expression analysis, the protein was found to be expressed at a low level in forage, whereas the levels of the NPTII protein in grain tissue samples were below the NPTII assay limit of quantification (Table 1).

3.1.4. Inheritance and stability of inserted DNA

The stability of the insert was demonstrated over seven backcrossed generations containing maize MON 87460 using Southern blot analysis.[19] Segregation analyses demonstrated the expected inheritance and stability of the inserted sequences across multiple generations. The EFSA GMO Panel considers that, should instability leading to loss of the trait(s) occur, no safety issue would arise.

[15] Technical Dossier/Section D2/Silvanovich and Tu (2009).
[16] Technical Dossier/Section D3.
[17] Mozaffar and Silvanovich (2008a).
[18] Shi et al. (2008a).
[19] Technical Dossier/Section D5.

3.2. Conclusion

The molecular characterisation data establish that the maize MON 87460 contains one copy of the *csp*B and *npt*II expression cassettes. No other parts of the plasmid used for transformation are present in the transformed plant. The expression of the genes introduced by genetic modification has been adequately analysed. The results of the bioinformatic analyses of the inserted DNA and the flanking regions did not raise safety issues. The stability of the inserted DNA was confirmed over several generations and a Mendelian inheritance pattern was demonstrated. The EFSA GMO Panel considers this to be an adequate analysis that does not raise safety issues.

4. Comparative analysis

4.1. Evaluation of relevant scientific data

Table 2 provides an overview of the various studies that have been carried out for the comparative analysis of maize MON 87460 versus its conventional counterpart and non-GM maize commercial varieties (referred to hereafter as "commercial varieties"). These studies comprise compositional studies, agronomic and phenotypic field studies, stress response studies, persistence and invasiveness assessments, and pollen morphology, pollen viability and seed germination tests.[20] For the studies conducted under field conditions, the EFSA GMO Panel considers the number of growing seasons and the selection of locations included in the experimental design of the comparative assessment to be adequate (for an overview, see Table 2). Therefore, data from these studies, taken together, in the EFSA GMO Panel's Scientific Opinion, are considered acceptable for the comparative analyses of maize MON 87460 and its conventional counterpart.

4.1.1. Choice of comparator

Maize lines DM1718 and H1548126 were used as comparators in the compositional studies, agronomic and phenotypic field studues, stress response studies, persistence and invasiveness assessments, and pollen morphology, pollen viability and seed germination tests (Table 2). The EFSA GMO Panel concludes that both comparators had genetic backgrounds comparable to those of the respective lines of maize MON 87460 used in the field studies, as evidenced by the corresponding pedigrees.[21] Therefore, these two lines can be regarded as conventional counterparts.

[20] Technical Dossier/Section D7.1 and D7.2/Additional information October 2010.
[21] Additional information October 2010.

1 **Table 2: Overview of comparative assessment studies with maize MON 87460 provided with application EFSA-GMO-NL-2009-70**

Study type	Study focus	Study details	Conventional counterpart	Number of commercial varieties	Reference	Section in this Scientific Opinion
Field	Composition	USA 2006 Six sites under water conditions typical of local agronomic practices	H1548126	18	Harrigan et al. (2008a)	4.1.2
Field	Composition	Chile 2006–2007 Three sites under well-watered and water-limited conditions	DM1718	12	Alba et al. (2008); Harrigan et al. (2008b)	4.1.2
Field	Agronomic and phenotypic characteristics	USA 2006 Eight sites under well-watered conditions	H1548126	19	Sammons et al. (2009)	4.1.3.1
Field	Agronomic and phenotypic characteristics	USA 2006 Five sites under water conditions typical of local agronomic practices	H1548126	15	Whitsel and Clark (2008)	4.1.3.1
Field	Agronomic and phenotypic characteristics	Chile 2006–2007 Three sites under well-watered and water-limited conditions	DM1718	16	Eberle (2009a)	4.1.3.1
Field	Agronomic and phenotypic characteristics	USA 2007 Ten sites under well-watered conditions	DM1718	11	Rosenbaum et al (2008)	4.1.3.1
Field	Agronomic and phenotypic characteristics	USA 2007 Three sites under well-watered conditions, of which one was also under water-limited conditions	DM1718	12	Eberle (2009b)	4.1.3.1
Field	Agronomic and phenotypic characteristics	USA 2007 Three sites under well-watered and water-limited conditions	DM1718	7	Sammons et al. (2008)	4.1.3.1
Field	Agronomic and phenotypic characteristics	USA 2003, 2007 One site under water-limited conditions (USA, 2003); one site under well-watered and water-limited conditions (USA, 2007)	LH59R1 x LH200; DM1718	0; 0	Luethy (2009)	4.1.3.1
Greenhouse	Stress response to drought	Exposure to drought treatment	H1548126	0	Chomet et al. (2008)	4.1.3.2
Greenhouse	Stress response to drought	Exposure to drought treatments (4)	DM1718	0	Eberle et al. (2009)	4.1.3.2

Study type	Study focus	Study details	Conventional counterpart	Number of commercial varieties	Reference	Section in this Scientific Opinion
Greenhouse	Stress response to salt	Exposure to salt treatments (4)	DM1718	0	Whitsel (2008b)	4.1.3.2
Growth chamber	Stress response to heat	Exposure to heat treatments (4)	DM1718	0	Eberle (2008b)	4.1.3.2
Growth chamber	Stress response to cold	Exposure to cold treatments (4)	DM1718	0	Eberle (2008a)	4.1.3.2
Field	Persistence	USA 2006–2007 Three sites	H1548126	6	Whitsel (2008a)	6.1.1.1
Field	Persistence and invasiveness	USA 2007 Four sites in unmanaged environments	H1548126	7	Rosenbaum and Eberle (2008)	6.1.1.1
Growth chamber	Seed germination	Field-collected seed (USA 2006, three sites)	H1548126	9	Whitsel (2007)	6.1.1.1
Laboratory	Pollen morphology and viability	Field-collected pollen (USA 2007, one site under well-watered and water-limited conditions)	DM1718	4	Whitsel and Sammons (2008)	6.1.1.2

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4.1.2. Compositional analysis[22]

4.1.2.1. Studies under local agronomic practices or well-watered conditions

Compositional data were collected from field studies in the USA during the growing season 2006 and in Chile during the growing season 2006–2007 (Table 2).

In the USA field study, maize MON 87460 was grown in replicated plots at six sites together with its conventional counterpart (H1548126) and 18 commercial varieties (three per site).[23] The water management regime was applied according to local agronomic practice.[24] Both forage and grain harvested from maize MON 87460, its conventional counterpart and the commercial varieties were assessed by proximate analysis (protein, fat, ash, moisture, carbohydrates by calculation) and for specific fibre fractions (acid-detergent fibre, neutral-detergent fibre), as well as for calcium and phosphorus. The analysis of grains also included total dietary fibre, amino acids, fatty acids, other minerals (Na, K, Mg), trace elements (Cu, Fe, Mn, Zn), vitamins (B1, B2, B6, E, niacin, folic acid), and secondary metabolites (*p*-coumaric acid, ferulic acid, furfural, raffinose, phytic acid). The 77 endpoints analysed are in line with the recommendations for key compositional endpoints in new varieties of maize (OECD, 2002).

The results of the compositional analysis of maize MON 87460 were compared with those of the conventional counterpart via per-site and combined-site analysis of variance. Fifteen endpoints for which more than 50 % of the samples were below the limit of quantification were excluded from the statistical analysis. These endpoints included a range of fatty acids that constitute a minor fraction of total fatty acids, sodium and furfural. The applicant used the values obtained from the 18 commercial varieties to build a 99 % tolerance interval (with 95 % confidence) (Kotz, 2006) for each of the remaining 62 compositional endpoints, to provide an estimate of natural variation against which differences between maize MON 87460 and its conventional counterpart could be interpreted. The EFSA GMO Panel considers that this method provides an estimate of the variation between commercial genotypes that may not always be optimal. This depends on the formulae used to derive the estimate and on the design of the study. When none of the commercial varieties are grown at more than one site, an estimated tolerance interval may reflect not only variability between genotypes but also variability between sites, albeit to a limited extent (see Section 3.3.2 of EFSA, 2010a). The EFSA GMO Panel considers that the estimated tolerance interval allows the observed differences to be placed into the context of natural variability.

In the combined-site analysis of forage, no statistically significant differences were identified between maize MON 87460 and its conventional counterpart. In the combined-site analysis of grain, three statistically significant differences were observed when comparing maize MON 87460 with its conventional counterpart: ash (1.54 % vs 1.46 % dry weight, respectively), stearic acid (2.05 % vs 1.98 % of total fatty acids, respectively), and eicosenoic acid (0.18 % vs 0.19 % of total fatty acids, respectively). Values for these three endpoints in maize MON 87460 were all within their respective 99 % tolerance intervals. In the per-site analysis, stearic acid was not different at any site; but statistically significant differences were observed at only one site for ash and eicosenoic acid.

The field study conducted in Chile in 2006–2007 included maize MON 87460 grown in replicated plots at three sites together with its conventional counterpart (DM1718) and 12 commercial varieties (four per site).[25] Both well-watered and water-limited conditions, depending on whether irrigation was applied during specific late plant growth stages of maize (V10–R2), were applied.

Under well-watered conditions, both forage and grains were analysed for 77 compositional endpoints, fulfilling the recommendations for key compositional endpoints in new varieties of maize (OECD,

[22] Technical Dossier/Sections D7.2 and D7.3.
[23] Technical Dossier/Harrigan et al. (2008a).
[24] Additional information October 2012.
[25] Technical Dossier/Harrigan et al. (2008b).

2002). In addition, more specific compositional information on the levels of stress-related biochemical maize components, including plant hormones and organic osmolytes, were collected from this field study. The outcomes of these studies carried out under water-limited conditions are discussed in Sections 4.1.1.2 and 4.1.1.3.

Statistical analysis was applied to the compositional endpoints in the same manner as that for the study in the USA in 2006–2007: 16 endpoints for which more than 50 % of the samples were below the limit of quantification were excluded from the statistical analysis, and the values obtained from the 12 commercial varieties were used to build a 99 % tolerance interval (with 95 % confidence) for each of the remaining 61 compositional endpoints.

In the combined-site analysis of forage, no statistically significant differences were identified between maize MON 87460 and its conventional counterpart. In the combined-site analysis of grain, two statistically significant differences were observed when comparing maize MON 87460 with its conventional counterpart: total fat (3.89 % vs 3.72 % dry weight, respectively) and magnesium (0.12 % vs 0.11 % dry weight). Values for total fat and magnesium were all within their respective 99 % tolerance intervals. In the per-site analysis, significant differences were observed at only one site for both endpoints.

The observed differences in the various endpoints of the composition of maize MON 87460, compared with its conventional counterpart, do not raise safety concerns for humans and animals in the opinion of the EFSA GMO Panel, given the estimated natural variation of those endpoints and the magnitude of these differences in relation to the characteristics of the pertinent endpoint.

4.1.2.2. Studies under water-limited conditions

As described in Section 4.1.2.1, the field study in Chile was carried out under both well-watered and water-limited conditions, focusing on a comprehensive compositional analysis. Under water-limited conditions, both forage and grains were analysed for 77 compositional endpoints that are in line with the recommendations for key compositional endpoints in new varieties of maize (OECD, 2002).

In the combined-site analysis of forage, a statistically significant difference was identified for total fat (1.32 % vs 0.84 % dry weight, respectively) when comparing maize MON 87460 with its conventional counterpart. The value of total fat was within the 99 % tolerance interval. In the per-site analysis, this difference was not observed at any site.

In the combined-site analysis of grain, a small but significant decrease was identified for eicosenoic acid (both values equal to 0.18 % of total fatty acids after rounding to two decimals) when comparing maize MON 87460 with its conventional counterpart. The value of eicosenoic acid was within the 99 % tolerance interval. In the per-site analysis, this difference was observed at one site.

Based on the magnitude of these differences, the estimated natural variation and the characteristics of the pertinent endpoints, the EFSA GMO Panel concludes that these compositional differences between maize MON 87460 and its conventional counterpart do not raise safety concerns for humans or animals.

4.1.2.3. Analysis of stress-related compounds

As described in Section 4.1.2.1, stress-related compounds (organic osmolytes and plant hormones) were analysed on forage and grains obtained from the Chilean field study under well-watered and water-limited conditions.[26] The analysis of organic osmolytes included free proline, choline, glycine betaine and various carbohydrates (fructose, glucose, glycerol, mannitol, sorbitol, sucrose), while that of plant hormones included abscisic acid and salicylic acid. Mannitol and sorbitol in both forage and grains were below the limit of quantification in all samples and were therefore excluded from the statistical analysis.

[26] Technical Dossier/Alba et al. (2008).

Under well-watered conditions, in the combined-site analysis of forage, maize MON 87460 showed a statistically significantly higher level of abscisic acid compared with the conventional counterpart (37.03 vs 15.66 ppb fresh weight, respectively), which also fell outside the tolerance interval defined by the commercial varieties (upper boundary 33.02 ppb). In the per-site analysis, such an increase was observed at one site. In the combined-site analysis of grains, no statistically significant differences were identified between maize MON 87460 and its conventional counterpart. The observed difference for abscisic acid in the combined-site statistical analysis is not considered relevant by the EFSA GMO Panel, given the absence of other concomitant changes that would raise safety concerns for humans and animals.

Under water-limited conditions, in the combined-site analysis of forage, no significant differences were identified between maize MON 87460 and its conventional counterpart. In the combined-site analysis of grain, a statistically significant decrease was identified in the level of sucrose from maize MON 87460 compared with the conventional counterpart (1.63 % vs 1.86 % dry weight). The sucrose level in grains from maize MON 87460 fell within the tolerance interval. In the per-site analysis, sucrose in grains was significantly lower at two sites. The observed difference for sucrose is not considered to raise safety issues for human and animal health, given the magnitude of the change and the nature of this compound.

Having considered the total set of compositional data supplied and the observed compositional differences between maize MON 87460 and its conventional counterpart in the light of the field study design, the nature and magnitude of the differences and biological variation, the EFSA GMO Panel concludes that no biologically relevant differences were identified in the compositional characteristics of forage and grains produced by maize MON 87460 compared with its conventional counterpart, and that its composition falls within the estimated natural variation, except for the expression of the CspB and NPTII proteins.

The compositional differences between maize MON 87460 and its conventional counterpart under water-stressed conditions are not considered relevant for humans or animals by the EFSA GMO Panel, based on each particular endpoint studied and the magnitude of the observed difference in relation to the characteristics of the endpoint.

4.1.3. Agronomic traits and GM phenotype[27]

The agronomic and phenotypic characteristics of maize MON 87460 compared with its non-GM maize counterpart were analysed, both in field studies and under greenhouse or growth chamber conditions.

4.1.3.1. Agronomic and phenotypic field studies

Agronomic and phenotypic field studies with maize MON 87460 were carried out across 31 sites over two consecutive years: 13 US sites in 2006; three Chilean sites in 2006–2007; and 14 US sites in 2007 (Table 2). In all field studies, a randomised complete block or strip design with three replications was used. Fields studies were established using three different water management regimes, with field studies established under:

- well-watered conditions: 8 sites in 2006 and 10 in 2007 in the USA;
- water conditions typical of local agronomic practices: 5 sites in 2006 in the USA;
- well-watered and water-limited conditions in the same field: 3 sites in 2006–2007 in Chile and 7 sites in 2007 in the USA.

In some sites, the stress experienced by plants was varied by including both well-watered (irrigated) and water-limited conditions in the field trial. Under well-watered conditions, soil moisture levels

[27] Technical Dossier/Section D4.

were required to attain levels (e.g. 60–85 %) adequate for supporting maize production and hence non-limiting to yield potential. Under water-limited conditions, water management was transiently (e.g. from the V10–R2 until the R4 stage) adjusted during plant development so as to reduce the available soil moisture below 50 % (e.g. to 30–40 % soil moisture) as a means of causing stress. A further indicator of water stress was the change in certain endpoints in the commercial varieties grown in the same experiment, such as a decrease in plant height, ear height or yield, or an extended period until 50 % silking was achieved. Sufficiently large changes in these endpoints supported the decision to accept the field study conditions as being representative of water-limited conditions.

(a) Comparisons without deliberate application of water stress

Well-watered conditions were applied in two field studies in 8 and 10 replicated sites in the USA in 2006 and 2007, respectively.[28] In the 2006 field study, maize MON 87460 was grown together with a conventional counterpart (H1548126) and commercial varieties (four lines at each site, 19 different lines in total) at all sites.[29] In the 2007 field study, maize MON 87460 was grown together with a conventional counterpart (DM1718) and 11 commercial varieties.[30] Water management was carried out according to local agronomic practices in a parallel field study comparing maize MON 87460 with the same conventional counterpart (H1548126) and 15 commercial varieties in total at five replicated sites in the USA during the same year (2006).[31] The crop was irrigated at one site and rain fed in the four others. Both well-watered and water-limited conditions were applied in one field study with three sites in Chile during the 2006–2007 season[32], and in two field studies at four and three sites in the USA during the 2007 season.[33] In these field studies, maize MON 87460 was grown together with its conventional counterpart (DM1718) and varying numbers of commercial varieties (four lines per site).

In all field studies, information on phenotypic and agronomic characteristics of maize MON 87460 and its conventional counterpart was generated to compare their growth habit, vegetative vigour and reproduction characteristics. The endpoints that were statistically analysed in various field studies included commonly measured characteristics related to plant development, physiology, and agronomic performance. These endpoints included the following: seedling vigour, early stand count, days to 50 % pollen shed and silking, stay-green rating, ear height, plant height, dropped ears, stalk lodging, root lodging, final stand count, grain moisture, test weight, and yield. Visually observable responses to naturally occurring insects, diseases or abiotic stressors were also recorded in order to provide indications of altered stress responses in maize MON 87460 compared with its conventional counterpart.

In the across-site statistical analysis of the results of the well-watered field trials in the USA, maize MON 87460 showed a higher number of root-lodged plants than the conventional counterpart (5.6 vs 1.5 plants per plot), yet this difference fell within the range of values for the commercial varieties. This difference in the number of root-lodged plants was not observed in most sites in the per-site statistical analysis of root lodging data. For several other endpoints, including various phenotypic, agronomic, and arthropod-related endpoints, statistically significant differences were also observed in one or two individual sites, but not in all of them.[34]

In the parallel field study in the USA in 2006, in which the water management was according to local agronomic practices, no statistically significant differences were observed in the combined-site statistical analysis. In individual sites, several quantitative endpoints showed statistically significant differences, with each endpoint being different in not more than a single site (i.e. not in multiple sites). The qualitative analysis of arthropod damage showed a qualitative difference in grasshopper damage,

[28] Technical Dossier/Rosembaum et al. (2008) and Sammons (2009).
[29] Technical Dossier/Sammons et al. (2009).
[30] Technical Dossier/Rosembaum et al. (2008).
[31] Technical Dossier/Whitsel and Clark (2008).
[32] Technical Dossier/Eberle (2009a).
[33] Technical Dossier/Eberle (2009b), Rosenbaum et al. (2008) and Sammons (2008).
[34] Technical Dossier/Sammons et al. (2009).

being lower for maize MON 87460 than for its conventional counterpart at one site at one observation but not at the others, and still within the background range of observations in commercial varieties.[35]

Another field study in the USA was carried out at three sites in 2007, including two that contained well-watered plots and another one that contained both well-watered and water-limited plots (data from the latter are discussed in the subsection on water-stressed trials). In this study, maize MON 87460 showed a statistically significantly lower stay-green value under well-watered conditions in the combined-site statistical analysis, while the average values fell within the background range of reference values. The stay-green value also showed a statistically significant difference in two individual sites in the per-site statistical analysis, being higher for maize MON 87460 than the conventional counterpart at one of these sites and lower at another site. A statistically significant difference that was observed under well-watered conditions at an individual site but not in the combined-site statistical analysis was slightly elevated grain moisture in maize MON 87460.[36]

Under the well-watered conditions of another field study in eight sites in the USA in 2007, no statistically significant differences were found between maize MON 87460 and its conventional counterpart in the combined-site statistical analysis of the results. In the per-site statistical analysis, various endpoints showed statistically significant differences at individual sites (three sites at most), such as higher stay-green values in maize MON 87460 in three sites.[37]

Although the agronomic and phenotypic data derived from field studies showed a statistically significant higher number of root-lodged plants per plot and lower stay-green ratings for maize MON 87460 than its conventional counterpart under well-watered or typical watered conditions in the combined-site analyses performed per study, these differences were not consistently observed across studies and seasons, and fell in the range of values observed for the commercial varieties. Under water-limited conditions, maize MON 87460 exhibited lower yields than in well-watered conditions but higher yields in the combined-site analysis compared with its conventional counterpart, although these differences were not consistently observed across studies and seasons. No visually observable responses to naturally occurring insects and diseases were recorded in the field studies.

(b) Studies with application of water stress

Yield- and physiological stress-related endpoints were measured in maize MON 87460 and a conventional counterpart (LH59 × LH200 in 2003; DM1718 in 2007) during a field study in the USA at one site with water-limited conditions in 2003 and at another site with both well-watered and water-limited conditions in 2007. In 2003, maize MON 87460 showed a higher leaf extension rate, while under water-limited conditions in 2007, maize MON 87460 showed higher yield, number of grains per ear, leaf extension rate and plant height. The higher yield in the last year appeared to relate to both a higher number of grains per ear and a (non-significant) higher kernel weight.[38]

Stay-green rating was statistically significantly lower for maize MON 87460 in the water-limited plots at one site in the experiment carried out at three sites in the USA in 2007, while also falling below the reference range established from the commercial varieties.[39]

No statistically significant differences between maize MON 87460 under both well-watered and water-limited conditions were observed in the combined-site statistical analysis of results from another field study at two sites in the USA in 2007, while a number of endpoints showed statistically significant differences at one site but not at the other in the per-site statistical analysis.[40]

[35] Technical Dossier/Whitsel and Clark (2008).
[36] Technical Dossier/Eberle (2009).
[37] Technical Dossier/Rosenbaum et al. (2008).
[38] Technical Dossier/Luethy (2009).
[39] Technical Dossier/Eberle (2009b).
[40] Technical dossier/Sammons et al. (2008).

In the Chilean field study carried out under water-limited conditions in 2006–2007, a statistically significantly increased yield was observed in the combined-site statistical analysis, as well as at one of the three sites in the per-site analysis. This difference could not be linked to changes in other agronomic data.[41]

4.1.3.2. Abiotic stress response studies under greenhouse and growth chamber conditions

The response of maize MON 87460 and its conventional counterpart to various types of abiotic stress was tested under greenhouse and growth chamber conditions.

- In two greenhouse studies, maize MON 87460 and its conventional counterpart (H1548126 and DM1718) were grown in pots and exposed to four different drought treatments (well-watered conditions and mild, moderate and severe drought conditions) at the V4 growth stage of development and then continued for 15 days. Eighty maize MON 87460 and 80 control plants were placed in a randomised complete block design with 20 replications.[42] Plants were subjected to a 6-day period of drought and subsequently allowed to recover, and various physiological endpoints related to photosynthesis (chlorophyll fluorescence), assimilation (CO_2 gas exchange, stomatal conductance), leaf extension rate (manual and potentiometric measurements), ion leakage from leaves, and relative water content of leaves were measured.

  Maize MON 87460 and its conventional counterpart, exposed to well-watered conditions and mild, moderate and severe drought conditions, exhibited a dose-dependent pattern of lower plant height, growth stage, and fresh and dry weight with increasing water stress. Depending on the treatment, differences (such as fewer leaves and lower fresh and dry weight in the well-watered treatment; reduced plant height, fewer leaves and higher leaf rolling score in the moderate drought treatment; lower leaf rolling score in the severe drought treatment) were observed between maize MON 87460 and the conventional counterpart.

- For the assessment of salt tolerance a similar approach was followed as for the assessment of drought tolerance: maize MON 87460 and its conventional counterpart (DM1718) received either no, mild, moderate or severe salt treatment under greenhouse conditions over 12 days (up to 600 mM $NaCl/CaCl_2$ or 58 g salt/pot in the most severely treated group).[43] The measures taken during the analyses were the same as for cold and heat stress testing (see below, excluding necrosis), while morphology was also recorded. Both maize MON 87460 and its conventional counterpart exhibited a dose-dependent pattern of lower plant height, growth stage, vigour and fresh and dry weight. Depending on the treatment, differences (such as lower dry weight in the mild salt treatment; increased vigour and chlorophyll content in the moderate salt treatment; lower plant height and decreased vigour in the severe salt treatment) between maize MON 87460 and the conventional counterpart were observed.

- Growth chamber studies were carried out to test the physiological behaviour of maize MON 87460 and its conventional counterpart (DM1718) under heat and cold stress.[44] Plants at the V3 growth stage were exposed to various heat or cold conditions (optimal, mild, moderate, or severe temperatures for 8 and 5 days, respectively) with 16-hour lighting periods. The heat conditions included optimal growth conditions (30 ºC during lighting periods/22 ºC for the remainder), and heat conditions ranging from mild to severe (47 ºC/35 ºC) over 5 days. Cold stress was tested in a growth chamber experiment with a similar design, with conditions ranging from optimal (30 ºC/22ºC) to severe (4 ºC/4 ºC) over 8 days of cold treatment. Maize MON 87460 and its conventional counterpart exhibited a dose-dependent pattern of lower plant height, growth stage, vigour and fresh and dry weight with decreasing or increasing temperatures. No significant differences were observed between maize MON 87460 and its conventional counterpart in the

[41] Technical dossier/Eberle (2009).
[42] Technical dossier/Chomet (2008) and Eberle (2009).
[43] Technical dossier/Whitsel (2008b).
[44] Technical dossier/Eberle (2008a,b).

mild, moderate or severe cold treatments.[45] Differences (such as greater number of leaves, increased dry weight, reduced chlorophyll content and greater fresh and dry weight in the optimal temperature treatment; reduced chlorophyll content in the mild treatment; reduced vigour in the severe treatment) were observed for plants exposed to high temperature heat stress.[46]

Given the intended trait, the observed differences were not unexpected, and did indicate no safety concerns.

4.2. Conclusion

Based on the results of a comparative analysis, the EFSA GMO Panel concludes that, besides the expression of the CspB and NPTII proteins, some differences were observed in the composition of forage and grain produced from maize MON 87460 compared with its conventional counterpart when grown under well-watered conditions. Given the magnitude of these changes and the characteristics of these endpoints, the EFSA GMO Panel concludes that the observed differences do not raise safety concern for humans or animals. The EFSA GMO Panel notes that under water-limited and other stressful conditions, maize MON 87460 can show enhanced agronomic performance characteristics and some differences in chemical composition in comparison with its conventional counterpart. Given the intended trait, the observed differences were not unexpected, and did not raise safety concerns.

5. Food/feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Product description and intended uses[47]

The scope of application EFSA-GMO-NL-2009-70 is for food and feed uses, import and processing of maize MON 87460 and all derived products (e.g. starch, syrups, ethanol, maize oil, flakes, coarse and regular grits, coarse and dusted meal, flour, maize germ meal, maize gluten feed, condensed steep water, and maize gluten meal).

The genetic modifications in maize MON 87460 are intended to improve agronomic performance only and are not intended to influence the nutritional properties, the processing characteristics and the overall use of maize as a crop.

5.1.2. Effect of processing[48]

Maize MON 87460 will be used for production and manufacturing of food and feed products in the same way as any other commercial maize variety. Some differences were observed in the composition of forage and grain produced from maize MON 87460, compared with its conventional counterpart, when grown under well-watered conditions. Given the magnitude of these changes and the characteristics of these endpoints, the EFSA GMO Panel concludes that the observed differences do not raise safety concerns for humans or animals (see Section 4.2), and the effect of processing on maize MON 87460 is not expected to be any different from that on conventional maize.

5.1.3. Toxicology[49]

5.1.3.1. Proteins used for the safety assessment

Owing to the relatively low expression level of the CspB protein in maize MON 87460 (see Section 3.1.3) and the difficulty of isolating a sufficient quantity of purified protein from maize MON 87460, the safety studies with the newly expressed protein were conducted with a CspB protein

[45] Technical Dossier/Eberle (2008a).
[46] Technical Dossier/Eberle (2008b).
[47] Technical Dossier/Section A5-A7.
[48] Technical Dossier/Section D7.6.
[49] Technical Dossier/Section D7.8/Additional information October 2010 and April 2011.

produced by a genetically modified strain of *E. coli*, in which the introduced *csp*B gene encoded an amino acid sequence that matched that of the CspB expressed in maize MON 87460.

The structural similarity and physicochemical and functional equivalence of the CspB protein produced by *E. coli* to that produced in grain of maize MON 87460 was demonstrated by *N*-terminal sequencing (Edman degradation), western blot analysis with CspB-specific antibodies, mobility in sodium dodecylsulphate–polyacrylamide gel electrophoresis (SDS-PAGE), analysis by matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS) of tryptic peptides produced from CspB, glycosylation analysis, and purity analysis.[50] A functionality assay was also carried out based on the capacity for resolving the secondary structures of nucleic acids of CspB.[51] The only difference identified was that the protein isolated from maize MON 87460 is missing the *N*-terminal methionine present in the *E. coli*-derived CspB protein. As shown by Bradshaw et al. (1998), this type of modification is commonly observed in proteins from both prokaryotic and eukaryotic organisms.

A study[52] was provided that compared the NPTII produced by leaves of maize MON 87460 with the NPTII reference standard produced by recombinant *E. coli* bacteria through western blotting, showing that both NPTII proteins displayed immunoreactivity towards specific antibodies and had the same apparent molecular weight.

Based on the identified similarity in structure, and the equivalence in physicochemical and functional properties between these proteins, the EFSA GMO Panel accepts the use of bacterially produced CspB and NPTII proteins for the degradation studies and safety testing of the newly expressed proteins present in maize MON 87460 and as a reference standard in ELISA to estimate expression levels in various tissues of maize MON 87460.

5.1.3.2. Toxicological assessment of the expressed novel proteins in maize MON 87460

Various studies have been performed to test the safety and potential toxicity of the newly expressed CspB protein in maize MON 87460. The CspB protein is a bacterial protein derived from *B. subtilis*, which is a microorganism of which certain strains are used for the manufacture of food enzymes and for the production of fermented soybean products. *B. subtilis* has been granted the status of "qualified presumption of safety (QPS)" under the condition that food-poisoning toxins are absent from the strains used (EFSA, 2011c). The QPS assessment was developed by EFSA to provide a generic risk assessment approach applicable across EFSA's scientific Panels for biological agents notified for intentional use in the whole food and feed chain. In essence, this approach comprises a safety assessment of a defined taxonomic group (e.g. a genus or group of related species) based on four pillars: establishing identity, body of knowledge, possible pathogenicity and end use. EFSA has previously applied the QPS principle to *B. subtilis*, for example the use of *B. subtilis* as a live feed additive (EFSA, 2008, 2011c, 2012). The safe use of *B subtilis* is traced back to the traditional fermentation of soybean to make natto (in Japan).

Cold shock proteins such as CspB occur in a range of prokaryotic and eukaryotic organisms, such as lactic acid bacteria present in food and plants. The amino acid sequences of these cold shock proteins show a relatively high degree of similarity, despite their phylogenetic distance, indicating that the sequence has been conserved relatively well, including the presence of certain RNA-binding segments. For example, the sequence of CspB is up to 79 % identical to that in various lactic acid bacteria. CspB has the ability to bind to RNA and single-stranded DNA, probably stabilising correctly folded RNA structures as "RNA chaperones", thereby enabling cellular functioning under stressful conditions.

A study on the presence of CspB in natto, soybeans fermented with *B. subtilis* strain natto, showed that CspB occurred at an average level of 12.5 µg/g in 12 commercial samples analysed for the presence of

[50] Technical Dossier/Burzio (2008a) and Chandu (2010)/Additional information April 2011.
[51] Technical Dossier/Burzio (2008c).
[52] Technical Dossier/Gu (2008).

CspB through ELISA. Western blotting and *N*-terminal sequence analysis of CspB in two samples showed that CspB in natto had the expected size and *N*-terminal sequence.[53]

(a) Oral toxicity testing

The potential acute oral toxicity of CspB was tested in mice (strain CD-1). There were no adverse effects after administration of a single oral dose of CspB at 4.70 mg/kg body weight.[54]

Considering the knowledge available with respect to the protein's source, its function and its history of human/animal consumption, the EFSA GMO Panel considers that a repeated-dose oral toxicity study is not necessary.

(b) Bioinformatic studies

In a bioinformatics-supported study, the amino acid sequence of the CspB protein was compared with the sequences of toxic proteins and with proteins in general using the FASTA algorithm. No relevant similarities between CspB and known toxins could thus be established, while the comparison with general proteins revealed that CspB showed a high degree of similarity with cold shock proteins from a wide range of organisms.[55]

(c) Pepsin and pancreatin resistance tests

The resistance of CspB to proteolytic enzymes was tested *in vitro* using incubations of CspB in solutions with pepsin at pH 1.2 (10 units of pepsin per µg of CspB) and with pancreatin at neutral pH (pancreatin:CspB = 55.3 (w/w)). The intactness and formation of peptide fragments of CspB was followed by analysing the incubation mixtures sampled at different time points after initiation by SDS-PAGE and western blotting. It was observed that, in the presence of pepsin, the full-length CspB protein rapidly (within 30 seconds) degraded to below detectable levels (> 99 % degraded), except for a 2.5-kDa fragment which was still detectable after 1 hour of incubation. This fragment was determined to be derived from CspB as shown by *N*-terminal sequencing. When incubated with pancreatin, the full-length CspB was degraded to below detectable levels within 5 minutes. In subsequent incubations of CspB with pepsin and pancreatin, the 2.5-kDA fragment was observed to disappear within 30 seconds.[56]

(d) Toxicological assessment of the NPTII protein

A number of studies on the safety of the NPTII protein in this application have already been provided previously in the frame of applications for other GM crops expressing newly introduced genes encoding the NPTII protein, such as the studies on acute toxicity of the NPTII protein and its degradation by proteolytic enzymes, and they are therefore not considered further here by the EFSA GMO Panel. An updated bioinformatics study comparing the amino acid sequence of NPTII with sequences of toxic proteins failed to find relevant similarities, thus confirming previous outcomes.[57]

The safety of the NPTII protein, which is expressed in maize MON 87460 and serves a role as the transformation marker, has been the subject of previous evaluations by the EFSA GMO Panel of the safety of other GM crops that also express this protein (maize MON 863 and cotton MON 531 and MON 1445, and potato EH92-527-1). The safety data on NPTII provided with the application included data previously provided in the frame of other applications, such as an acute oral toxicity study and sensitivity of NPTII to degradation by proteolytic enzymes, besides an updated bioinformatics-supported comparison of the amino acid sequence of NPTII with those of toxic proteins.

[53] Technical Dossier/Schaffer (2007).
[54] Technical Dossier/CRO-2007-182 (2008).
[55] Technical Dossier/Tu and Silvanovich (2009a,b).
[56] Technical Dossier/Kapadia (2008).
[57] Technical Dossier/Tu (2009).

5.1.3.3. Toxicological assessment of new constituents other than proteins

No new constituents other than the CspB and NPTII proteins are expressed in maize MON 87460. No biologically relevant changes in the composition of maize MON 87460 were found (Section 4.1.2). Therefore, a toxicological assessment of new constituents is not applicable.

5.1.3.4. Toxicological assessment of the whole GM food/feed

Since no biologically relevant differences were identified in the compositional, agronomic and phenotypic characteristics of maize MON 87460 (Section 4.2), animal safety studies with the whole food/feed are not considered necessary by the EFSA GMO Panel. However, the applicant provided a report of a subchronic 90-day feeding study with maize MON 87460-containing diets in rats. The study design was adapted from the OECD technical guideline 408 for the testing of chemical substances in laboratory rodents for 90 days.

Three groups of 40 Sprague Dawley rats (strain CRL:CD[SD], 20 animals of each gender) were fed diets containing 33 % maize MON 87460, 11 % MON 87460 plus 22 % of its conventional counterpart (DM1718) or 33 % maize DM1718. Maize grain harvested from field study in Chile was used to formulate the diets. The dietary inclusion of maize MON 87460 was analytically confirmed by ELISA for the newly expressed CspB protein. The experimental diets were shown to be equivalent concerning nutrient composition and content of heavy metals, mycotoxins and pesticides.

The endpoints analysed during and after the experimental feeding period included clinical observations, mortality, body weights, feed consumption, clinical pathology (including haematology, coagulation, serum chemistry, and urinalysis), and macroscopic pathology (gross necropsy, organ weight determinations) and microscopic examinations. In the statistical analysis, each of both test groups (fed diets containing either 11 % or 33 % maize MON 87460) was compared with the group fed a diet containing 33 % DM1718.

All animals survived the treatment period and there were no relevant clinical signs. Body weights and feed consumption were comparable in all groups. Statistically significant differences that occurred only in the group fed 11 % maize MON 87460, i.e. higher mean serum alkaline phosphatase activity and lower urine specific gravity in females, are not considered treatment related by the EFSA GMO Panel, owing to the lack of a dose response. A significantly lower aspartate aminotransferase activity in females fed diets containing 33 % maize MON 87460 is not considered by the EFSA GMO Panel to be an indication of an adverse effect, of which increased activity would be an indicator. Mean sodium serum levels were slightly lower in females of the high-dose group but fell within the range of the historical control means. Males in the group fed a diet containing 33 % maize MON 87460 showed a significantly lower heart weight, both in absolute terms and as a relative ratio to brain weight but not in relation to body weight, and females showed a lower thyroid and parathyroid weight in relation to body weight. The mean values fell within the range of the historical control means. There were no relevant findings in the histopathological examinations of these organs. Macroscopic and microscopic examinations of other selected organs and tissues did not reveal changes related to administration of the test materials. The EFSA GMO Panel concludes that there are no indications of adverse effects in this study.

5.1.4. Allergenicity[58]

The strategies used when assessing potential allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons, and whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, as no single experimental method yields decisive evidence of allergenicity (EFSA, 2006a, 2010b; Codex Alimentarius, 2009).

[58] Technical Dossier/Section D7.9/Additional information October 2010.

5.1.4.1. Assessment of allergenicity of the newly expressed proteins

The source of CspB, *B. subtilis*, is not known to be an allergen in its own right. Using bioinformatics, the amino acid sequence of CspB was compared with the sequences of known allergenic and celiac-disease-causing proteins. The latter group of sequences had been obtained from an external dedicated database with records of individual sequences that had been peer reviewed for data supporting the allergenicity of the specific sequence. A FASTA algorithm was applied for alignment of the CspB with the database sequences, while the criteria for relevant alignments included a lower threshold of at least 35 % identity in an 80-amino-acid window or an expectation (E-) value below 1×10^{-5}. In addition, another algorithm was applied to search for matches of short identical segments consisting of eight contiguous amino acids. No positive results were returned from this bioinformatics-supported comparison.[59] CspB is rapidly hydrolysed by pepsin and pancreatin.[60]

The EFSA GMO Panel notes that a publication in the scientific literature describes the identification of a potential cold shock protein encoded by a gene isolated from the mould *Cladosporium herbarum*, which according to the authors is bound by immunoglobulin (Ig) E sera from donors allergic to this mould. This 73-amino-acid protein was observed to share 70% similarity with CspB from *B. subtilis*, while IgE serum reactivity with the latter was not tested.[61] At the request of the EFSA GMO Panel, the applicant was asked to comment on this issue. Based on the answer received and the data available, the EFSA GMO Panel considers that no further accounts of the allergenicity of this protein and other cold shock proteins exist, and that the protein has neither been included in the official allergen list of the WHO-IUIS (World Health Organization–Union of Immunological Sciences) nor in protein sequence databases. Moreover, a review of fungal allergens considers this mould protein (designated Cla h 8 CSP) and various other proteins from the same mould not to be major allergenic components in allergy to *C. herbarum*.[62] Further, the EFSA GMO Panel notes that fungal allergens rarely cause food allergy. The EFSA GMO Panel concludes that the risk of an allergic reaction caused by potential CspB cross-reactivity with a minor mould allergen is low.

The potential allergenicity of NPTII has previously been assessed during evaluations of other crops expressing this protein. NPTII was thus found unlikely to become an allergen. An updated bioinformatics-supported comparison of NPTII with allergens and celiac-disease-causing proteins also failed to find relevant similarities.[63]

Based on this information, the EFSA GMO Panel concludes that it is unlikely that these newly expressed proteins are allergenic.

5.1.4.2. Assessment of allergenicity of the whole GM plant or crop

According to the EFSA GMO Panel risk assessment guidelines (EFSA, 2006a, 2010b, 2011a), the applicant should test any potential change in the allergenicity of the whole GM plant by comparing the allergen repertoire with that of its appropriate comparator(s), when the plant receiving the introduced gene is known to be allergenic. In this context, maize is not considered to be a common allergenic food (EC, 2007).

The prevalence of food allergy to maize is low and appears to vary with the geographic location (Moneret-Vautrin et al., 1998; Pastorello et al., 2009; Fonseca et al., 2012). At least 23 IgE-binding proteins have been identified in maize, a number of which are recognised as allergens. Sixteen of these proteins have been reported to be stress related, with LTP (lipid transfer protein) being the most important allergen in the Mediterranean region (Pastorello et al., 2000; Pasini et al., 2002; Pastorello et al., 2009; Fonseca et al., 2012). In some studies, most individuals with a positive skin prick test (SPT) or having IgE antibodies against maize were suffering from a respiratory allergy and only a few

[59] Technical Dossier/Tu and Silvanovich (2009b).
[60] Technical Dossier/Kapadia (2008).
[61] Technical Dossier/Falsone (2002).
[62] Technical Dossier/Simon Nobbe et al. (2008).
[63] Technical Dossier/Tu (2009).

displayed a true food allergy upon oral challenge with maize products (Jones et al., 1995; Pasini et al., 2002). In another study of 27 patients with a claimed history of maize allergy one-half were found to be challenge-positive and thus had a food allergy to maize (Scibilia et al., 2008).

Compositional analysis of stress-related compounds in grains did not show significant changes that would suggest alterations in the expression of stress-related allergenic proteins.

Bioinformatics analyses of the DNA sequence at the insertion sites did not indicate (1) an insertion within or near a known endogenous gene (potential allergens); or (2) creation of ORFs at the insert–plant DNA junctions that are likely to be translated into allergenic peptides (Section 3.1.2).

In the context of the present application, there is no evidence that the genetic modification might significantly change the overall allergenicity of maize MON 87460.

5.1.5. Nutritional assessment of GM food/feed[64]

As only minor differences were observed in the composition of forage and grain produced from maize MON 87460 compared with its conventional counterpart, the EFSA GMO Panel considers that the nutritional properties are likely to be essentially the same as those of other maize.

Apart from these considerations, a 42-day feeding study with chickens was carried out. After a 7-day pre-period, 800 chickens for fattening (Ross × Ross 308, both genders) were distributed to eight treatments (5 replicates with 10 birds each of each gender per treatment) fed diets containing maize MON 87460, the conventional counterpart (DM1718) or maize from 6 commercial varieties. Animal housing and management of the study followed the principles and guidelines for care and use of agriculture animals in research (FASS, 1999). The diets were formulated according to nutrient requirements (NRC, 1994), adjusted for nutrient and energy content, contained 58.7–59.7 % maize in the starter period (days 1–21) and 62.7–63.7 % maize in the grower/finisher period. All corn sources and the diets were comprehensively analysed to ensure nutritionally equivalent concentrations. At the end of the study, body weight and feed intake were determined, the birds slaughtered (males on day 43, females on day 44), and carcass parameters determined. A two-factorial analysis of variance (factors: diet and sex) was used for statistical assessment of all endpoints. Afterwards adequate tests were used to compare individual treatments. Total losses during the experimental period amounted to an average of 6.9 % (maize MON 87460: 6.7 %).

Final body weight for both sexes was 2.71 kg, the treatments ranging between 2.65 an 2.72 kg (maize MON 87460: 2.71 kg). The feed intake varied between 4.19 and 4.38 kg/broiler (maize MON 87460: 4.38 kg) and the feed to gain ratio between 1.61 and 1.65. Feed to gain ratio for the group with maize MON 87460 was 1.64 and significantly higher than that of the control group (DM1718: 1.61). However, the difference is considered small by the EFSA GMO Panel and not indicative of a nutritional imbalance of biological relevance. No significant differences between the treatments were found concerning carcass quality.

In summary, the results of the study on chickens for fattening concerning zootechnical performance support the conclusion that maize MON 87460 can be used in the same way as other maize sources as a feedingstuff in animal nutrition.

5.1.6. Post-market monitoring of GM food/feed[65]

The risk assessment concluded that no data have emerged to indicate that maize MON 87460 is any less safe than its conventional counterpart. In addition, maize MON 87460 is as nutritious as commercial varieties. Therefore, and in line with its risk assessment guidelines (EFSA, 2011a), the EFSA GMO Panel considers that post-market monitoring of the GM food/feed is not necessary.

[64] Technical Dossier/Section D7.10.
[65] Technical Dossier/Section D7.11.

5.2. Conclusions

An appropriate set of data of has been considered by the EFSA GMO Panel for the evaluation of the safety of both the newly expressed proteins and the whole food/feed derived from maize MON 87460, as summarised in Section 5.1.

The newly expressed protein CspB occurs naturally in *B. subtilis*, of which some strains have applications in the production of food or food constituents. Moreover, proteins very similar to CspB occur in a wide range of organisms, including microorganisms such as lactic acid bacteria used for food fermentation. CspB was found to occur, for example, in a fermented soybean food. The amino acid sequence of CspB did not show any relevant similarity with known toxins. The full-length CspB protein was rapidly degraded by proteolytic enzymes. The newly expressed NPTII has previously been evaluated for its safety in the frame of previous applications for crops expressing this protein (e.g. maize MON 863, cotton 531 and 1445, and potato EH92-527-1) and no safety issues were identified. An updated bioinformatics comparison of NPTII with toxins revealed no new information and further confirmed the previous conclusions on its safety.

For the assessment of potential allergenicity of CspB, the internationally harmonised weight-of-evidence approach was applied. The source of CspB, *B. subtilis*, has no history of allergenicity. CspB is rapidly hydrolysed by pepsin and pancreatin. Its amino acid sequence did not show relevant similarities with allergens.

Diets containing grain derived from maize MON 87460 were fed to rats during a subchronic feeding study, and no indications of toxicity were found. In addition, there is no evidence that the genetic modification might significantly change the overall allergenicity of maize MON 87460. The compositional data indicating the nutritional equivalence of maize MON 87460 were further corroborated by the outcomes of a nutritional feeding study in chickens.

In conclusion, the EFSA GMO Panel considers that maize MON 87460 is as safe and as nutritious as its conventional counterpart and non-GM commercial varieties, in the context of its intended use.

6. Environmental risk assessment and monitoring plan

6.1. Evaluation of relevant scientific data

6.1.1. Environmental risk assessment

The scope of the application is for food and feed uses, import and processing of maize MON 87460 and does not include cultivation. Considering the intended uses of maize MON 87460, the environmental risk assessment is concerned with the accidental release into the environment of viable grains from maize MON 87460 during transport and processing, and with the exposure through manure and faeces from animals fed maize MON 87460.

6.1.1.1. Effects on plant fitness due to the genetic modification

A series of agronomic and phenotypic field studies with maize MON 87460 was carried out using three different water management regimes, with field studies established under: (1) well-watered conditions; (2) water conditions typical of local agronomic practices; and (3) well-watered and water-limited conditions in the same field (Section 4.1.3.1; Table 2). Under water-limited conditions, maize MON 87460 exhibited lower agronomic performance characteristics (e.g. yield) than in well-watered conditions but showed enhanced agronomic performance characteristics across locations compared with its conventional counterpart, although these differences were not consistently observed across studies and seasons. No biologically relevant differences in visually observable responses to naturally occurring insects and diseases were recorded in the field studies.

Abiotic (drought, cold, heat and salt) stress tolerance of maize MON 87460 was evaluated under greenhouse or growth chamber conditions in various studies (Section 4.1.3.2; Table 2). Depending on

the treatment, differences were observed between maize MON 87460 and its conventional counterpart. Given the intended trait, the observed differences were not unexpected, and did indicate no safety concerns.

An additional field study with maize MON 87460, its conventional counterpart (H1548126), and six non-GM maize commercial varieties was conducted at three locations during 2006–2007 in the USA to assess the persistence (overwintering and volunteer potential) of maize MON 87460, using a randomised complete block design with three replications (Table 2).[66] In this field study, seeds were planted in autumn (November 2006) and the occurrence of volunteer maize plants was surveyed in the autumn of 2006 and spring of 2007, while the fields were maintained according to local agricultural practice. No volunteer maize plants were observed at any site or observation time.

In 2007, additional field studies with maize MON 87460 and its conventional counterpart (H1548126) and non-GM maize commercial varieties (three per location, seven different lines in total) were carried out at four locations in the USA to assess the persistence and invasiveness (competitive) ability of plants from F_2 seed of maize MON 87460 (Table 2).[67] A randomised complete block design with three replications was implemented. Each location was unmanaged and received no agricultural inputs, allowing maize MON 87460, its comparator and non-GM maize commercial varieties to compete with existing vegetation and abiotic and biotic stressors present in each environment. Endpoints analysed included early stand count, vigour (at five different growth stages), late vegetative plant height, final stand count, final plant height, number of ears per plant and per plot, and number of seeds per plot. For most endpoints, no differences were observed between maize MON 87460 and its conventional counterpart; for early and final stand count greater values were reported for maize MON 87460 than for its conventional counterpart. In one location, no maize seedlings emerged at all, while in one out of the three remaining locations, maize plants reached the seed-setting stage of development. In the latter location, both early and final stand counts were statistically significantly higher for maize MON 87460 than for the conventional counterpart. The average replacement values (ratio of the number of seed produced to the number of seeds sown) for seed produced from all maize varieties grown in this location were low (< 1), indicating that less seed was produced in the location than had previously been sown there, and that the maize population was declining.

Seed germination tests with seeds harvested from maize MON 87460, its conventional counterpart (H1548126), and non-GM maize commercial varieties that had been grown in three locations in the USA in 2006 were performed to evaluate seed characteristics under growth chamber conditions (Table 2).[68] Seeds were incubated in germination chambers in the dark using different temperature regimes: temperatures ranging between 5 ºC and 30 ºC or temperatures alternating between 10 ºC (16 hours) and 20 ºC (8 hours), and between 10 ºC and 30 ºC, over 12 days. Other seeds were subject to a temperature regime alternating between 20 ºC and 30 ºC, according to the Association of Official Seed Analysts (AOSA) protocol, over 7 days. Endpoints analysed included the number of germinated seed (including a distinction between normal and abnormal germinated seed for seeds incubated according to the AOSA protocol), hard seed, dead seed and firm swollen seed. No statistically significant differences in germination characteristics (dead, germinated, viable swollen and viable hard) were found between maize MON 87460 and its comparator. In the combined-location statistical analysis, no statistically significant differences were observed between maize MON 87460 and its conventional counterpart. Because of a significant location × genotype interaction for germinated and viable firm swollen seed at 10 ºC, the results for these endpoints were statistically analysed on a per-location basis. It showed that the number of germinated seed was higher, while the number of viable firm swollen seed was lower, for maize MON 87460 than its conventional counterpart in seed from one location, with the values for maize MON 87460 falling within the background range of values for the non-GM maize commercial varieties.

[66] Technical Dossier/Whitsel (2008a).
[67] Technical Dossier/Rosenbaum and Eberle (2008).
[68] Technical Dossier/Whitsel (2007).

The breeding pedigree of maize MON 87460 and its conventional counterparts provided by the applicant confirmed that the comparators used in the persistence and invasiveness assessments, and seed germination tests had a comparable genetic background with maize MON 87460 (Table 2).[69] Therefore, these two lines can be regarded as conventional counterparts.

Overall, the data presented in the application do not show biologically relevant differences in plant characteristics that indicate altered fitness, persistence or invasiveness of maize MON 87460 plants, compared with its conventional counterpart (see also Section 4.1.3). Under water-limited conditions, maize MON 87460 exhibited lower agronomic performance characteristics (e.g. yield) than in well-watered conditions but enhanced agronomic performance characteristics across locations compared with its conventional counterpart, although these differences were not consistently observed across studies and seasons. Further, no biologically relevant differences in biotic and abiotic stress responses were found between maize MON 87460 and its conventional counterpart after exposure to a range of stress levels imposed during early vegetative growth stages. Therefore, the EFSA GMO Panel considers it very unlikely that the establishment, spread and survival of maize MON 87460 would be increased by the drought tolerance trait. 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; furthermore, they have lost their ability to release seeds from the cob and they do not occur outside cultivated land or disturbed habitats in 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, indicate that grains 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 (Gruber et al., 2008). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). 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 cold climatic conditions. Since these general characteristics are unchanged in maize MON 87460, drought tolerance alone is not likely to provide a selective advantage outside cultivation in Europe. Therefore, it is considered very unlikely that maize MON 87460 will differ from conventional maize varieties in its ability to survive into subsequent seasons or to establish feral populations under European environmental conditions.

The EFSA GMO Panel is not aware of any scientific report of increased establishment, spread or any change in survival capacity including overwintering of maize MON 87460 or maize with comparable properties.

Maize MON 87460 can show enhanced agronomic performance characteristics (e.g. yield) compared with its conventional counterpart under water-limited conditions but has no other altered survival, multiplication or dissemination characteristics. Therefore, the EFSA GMO Panel considers that the likelihood of unintended environmental effects owing to the accidental release into the environment of viable grains from maize MON 87460 will not differ from that of its conventional counterpart and non-GM maize commercial varieties.

6.1.1.2. Gene transfer[70]

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

(a) Plant to bacteria gene transfer

[69] Additional information October 2010.
[70] Technical Dossier/Additional information October 2010 and April 2012.

DNA of plants could hypothetically be acquired by bacteria through horizontal gene transfer. Current scientific evidence indicates that the transfer of genes derived from GM plants into bacteria and their stable integration either has not occurred or, if it has occurred, it has been below the limit of detection in all the studies performed (see EFSA, 2009 and references therein).

The probability of horizontal gene transfer of plant DNA (including the insert DNA) to exposed bacteria is determined by the following factors: (1) the amount and quality of plant DNA accessible to bacteria in receiving environments; (2) the presence of bacteria with a capacity to develop competence for natural transformation, i.e. to take up extracellular DNA; (3) the ability for genetic recombination by which the plant DNA can be incorporated and thus stabilised in the bacterial genome (including chromosomes and plasmids); and (4) the expression and the function of the protein in the bacterial recipient and potential for selection of the acquired transgene-encoded traits.

The EFSA GMO Panel considers the exposure of bacteria to the insert DNA of maize MON 87460 (containing the *npt*II and *csp*B genes) and the barriers to and the impact of hypothetical horizontal gene transfer in receiving environments. Special emphasis was put on whether horizontal gene transfer of the *npt*II gene of maize MON 87460 could lead to kanamycin- and neomycin-resistant bacteria emerging in some environments, especially the gastrointestinal tract or faeces, under selective conditions (usage of the corresponding antibiotics[71]), and could contribute to the environmental prevalence of *npt*II genes.

(i) Exposure to DNA

The scope of this application is for food and feed uses, import and processing and excludes cultivation in the EU. Therefore, the route of DNA exposure is through consumption of maize MON 87460 material. Furthermore, exposure may occur via accidental spillage into the environment of maize MON 87460 grains during transport and processing. Of all the maize commodities imported into the EU, whole maize grains and maize flour are those that most conceivably could contain DNA fragments of sufficient size to encompass full-length gene sequences. In the other maize commodities, such as maize gluten feed and meal, dregs from brewing and distilling and maize oil, the plant DNA is not detectable or intensively degraded to fragments with estimated lengths < 1500 bp (Rausch and Belyea, 2006; Rizzi et al., 2012).[72] Therefore, the possible source of full-length genes from maize MON 87460 to bacteria would mainly be limited to unprocessed whole grain, partially digested or spilled during transit, and to maize flour.

DNA present in food and feed becomes substantially further degraded through digestion in the human or animal gastrointestinal tract by host and microbial factors, and the likelihood that a full-length gene sequence would persist is very low in the lower intestinal tract (see references in Rizzi et al., 2008, 2012; EFSA, 2009).

Because seed spillage is a random event, predicting levels of exposure through this route is difficult. However, the vast majority of plant DNA is expected to be degraded after soil entry by microbial DNases in the soil environment. Plant DNA is considered a non-persistent component of the DNA pool in soil (Levy-Booth et al., 2007; Gulden et al., 2008). Thus, extracellular DNA (including the insert DNA of maize MON 87460) in gastrointestinal tracts, soil or other environments is present transiently, and mainly as short fragments at relatively low concentrations.

(ii) Bacterial DNA uptake and stabilisation

The potential to develop competence for natural transformation is widely distributed among bacteria of different taxonomic affiliation and environmental prevalence (see Rizzi et al., 2008; EFSA, 2009; Rizzi et al., 2012; Seitz and Blokesch, 2012). Some studies have shown that introduced bacteria can be transformed naturally in the oral cavity of humans and animals (see Andersen et al., 2001; Hay et al.,

[71] The indicated uses of kanamycin or neomycin or similar substances include: gut irrigation; the treatment of encephalopathy in humans (neomycin); treatment of diarrhoea in farm animals; and aerosol administration for respiratory infections in humans and animals (EFSA, 2009).
[72] Additional information April 2012.

2002; EFSA, 2009), but competence development and transformation of bacteria by genomic DNA of plants has not been observed in the lower gastrointestinal tract even with optimised model systems providing a selective advantage (Nordgård et al., 2007; Rizzi et al., 2008; EFSA, 2009; Nordgård et al., 2012). Furthermore, not all species have the same degree of competence. Current knowledge suggests that most Enterobacteriaceae are not naturally transformable (Johnsborg et al., 2007; Sinha and Redfield, 2012).

Once the plant DNA is taken up by a bacterial cell, it must integrate into the recipient genome to persist during host replication. On a theoretical basis, this stabilisation would be based on non-homologous recombination events that do not require similarity between the recombining plant and bacterial DNA molecules. Non-homologous recombination has rarely been described in bacteria (de Vries et al., 2004; Hulter and Wackernagel, 2008; EFSA, 2009), and has not been detected in studies that have exposed bacteria to high concentrations of DNA from GM plants (EFSA, 2009). As non-homologous recombination is a major barrier for horizontal gene transfer, non-homologous recombination scenarios for the integration for *npt*II and *csp*B genes in maize MON 87460 are not further considered here.

While integration via non-homologous recombination is most unlikely, gene integration via other mechanisms may be facilitated by the gene context (i.e. the surrounding/neighbouring sequences) of the transgene(s) in the plant (EFSA, 2009). Maize MON 87460 contains DNA sequences that might enhance stabilisation of the *npt*II and *csp*B genes in the genome of specific groups of bacteria. Therefore, the risk assessment below considers three different scenarios for horizontal gene transfer (termed hereafter as scenarios of integration) of the transgenes of maize MON 87460 to bacteria in the environment (Section 3.2): (1) acquisition of the *npt*II gene through recombination at the *lox*P sites into bacteria providing Cre or Cre-like recombinases; (2) acquisition of the *npt*II gene through double homologous recombination involving bacterially derived sequences to *Agrobacterium* strains containing an octopine-type Ti-plasmid; and (3) gene substitution of the *csp*B and *npt*II genes through homologous recombination.

1. Assessment of the stabilisation of the *npt*II gene from maize MON 87460 to bacteria through Cre-meditated site-specific recombination at the *loxP* sites

In maize MON 87460, the *npt*II gene is flanked by two *loxP* sites, being part of the bacteriophage P1-related site-specific recombination system. The possibility that P1-mediated recombination could enhance the probability of horizontal transfer of the *loxP-npt*II-*loxP* cassette to bacterial cells is investigated below.

The bacteriophage P1 site-specific recombination system consists of two components: *loxP* is the site on the phage DNA at which recombination occurs and Cre is the phage-encoded protein that carries out this recombination between two *loxP* sites regardless of whether the conformation of the double-stranded DNA (dsDNA), is supercoiled, a relaxed circle or linear (Ambreski and Hoess, 1984).

Uptake of DNA by natural transformation typically results in single-stranded DNA (ssDNA) passing the bacterial membrane (Krüger and Stingl, 2011). The fate of this ssDNA in the cell after transformation is still unclear. Reannealing of complementary ssDNA fragments in the bacterial cytoplasm can theoretically occur (Saunders and Guild, 1981; Lorenz and Wackernagel, 1994; Domingues et al., 2012). Complementary DNA strands have been reported as dsDNA within the bacterial cell wall and within the transformed bacterial cell (Sun et al., 2009).

To become a substrate for Cre-recombinases, DNA must be double stranded (Guo et al., 1997). Double-stranded DNA, however, would be vulnerable to the action of restriction/modification systems, including the phage-related system typical of bacteriophage P1 and related phages (Łobocka et al., 2004), competing with the recombination process.

The potential for horizontal transfer of the *loxP-npt*II-*loxP* fragment in maize MON 87460 to bacteria depends on the presence of Cre or Cre-like recombinases in the exposed competent bacterial cells, which is related to the presence of P1 or P1-like bacteriophages. Bacteriophage P1 is capable of plaque formation in several species within the Enterobacteriaceae. The Enterobacteriaceae encompass

a diversity of bacterial species belonging to the gut microbiota, including pathogenic *E. coli*, *Salmonella* and *Shigella*. Studies indicate that bacteriophage P1 and related sequences are not only present in the gut but also in other environments (Jensen et al., 1998; Breitbart et al., 2002; Balding et al., 2005; Hazen et al., 2007).

Site-specific Cre-mediated recombination has been shown to occur between sites of varying degrees of sequence similarity to *loxP* with a high frequency *in vitro* (Hoess et al., 1982; Sauer, 1992, 1996; Thyagarajan et al., 2000; Corneille et al., 2003). Cryptic sites containing as few as 14 out of 34 bases in common with *loxP* have been shown to be effective (Sauer, 1996; Siegel et al., 2001; Chatterjee et al., 2010). On the contrary, Adams et al. (2002) found that Cre recombinase substrate specificity in *E. coli* is much higher *in vivo* than *in vitro*. This is in agreement with the reports that bacteriophage P1 rarely integrates into the chromosome at the *loxB* site of its host in its lysogenic mode (Hoess et al., 1982; Asteri et al., 2011; Popa et al., 2011) but maintains itself as an autonomous single-copy plasmid. It has been suggested that DNA structure, ionic conditions *in vivo* and/or the influence of host protein factors might have an impact (Seveno et al., 2002; MacDonald et al., 2008). The main action of Cre recombinase is excision that occurs several magnitudes more frequently than insertion (Sauer and Henderson, 1990; Missirlis et al., 2006).

Because chromosomal insertion of P1 or P1-like bacteriophages at the *loxB* site is rarely encountered, it is assumed that recombination of the *loxP*-*npt*II-*loxP* fragment would also preferentially occur into the *loxP* site of the P1 circular bacteriophage. However, insertion into the P1 circular molecule creates an extra *loxP* site that would lead to instability of the insertion because of the excision activity of Cre (Sauer and Henderson, 1990; Missirlis et al., 2006). Excision would lead to a circular small molecule encoding *npt*II that is expected to be lost during bacterial replication.

Integration of the *loxP*-*npt*II-*loxP* into the genome would be unlikely because of the preferential insertion into the *loxP* site of the P1 or P1-like bacteriophage and would coincide with the chromosomal insertion of P1 into the *loxB* site. The insertion of P1 in the *loxB* site of *E. coli* would create *loxR* and *loxL* sites. The *loxR* site has low recombination potential, whereas the *loxL* site is highly recombinogenic. In the case in which chromosomal insertion of the *loxP*-*npt*II-*loxP* fragment would occur, it would be preferentially at the *loxL* site. When introduced into the *loxL* site, the insertion would be unstable because two highly recombinogenic sites would be created in which excision would be the main activity (Sauer and Henderson, 1990; Missirlis et al., 2006). Excision would lead to a circular small molecule encoding *npt*II lacking a replication origin, which is expected to be lost during bacterial replication.

2. Assessment of the stabilisation of the *npt*II gene from maize MON 87460 to bacteria through double homologous recombination involving bacteria-derived sequences flanking the *npt*II coding sequence in maize MON 87460

Homologous recombination facilitates the integration of non-mobile, chromosomal DNA fragments into bacterial genomes (EFSA, 2009 and references therein). This process depends on the presence of stretches of identical DNA sequences between the recombining DNA molecules.

For maize MON 87460, the probability of transfer of *npt*II by homologous recombination, through the sequences present in the *npt*II flanking regions as shown by bioinformatic analyses, should be limited to *A. tumefaciens*. *A. tumefaciens* commonly occurs in agricultural soils and has been reported to be naturally competent in soil (Bertolla and Simonet, 1999; Demanèche et al., 2001). BLASTn analysis[73] revealed the possibility of a double homologous recombination between sequences upstream and downstream of the *npt*II gene in maize MON 87460 with the same sequences present on the octopine-type Ti-plasmid of *A. tumefaciens*. The sequences involved are: (1) upstream of the *npt*II the T-tr*7* – intervening sequence (612 bp); and (2) downstream of the left border – intervening sequence (367 bp). Homologous recombination between these sequences and the homologous sequences in the *A. tumefaciens* Ti-plasmid would result in the insertion of the *npt*II expression cassette (P35S/*npt*II/T-nos) and the concomitant loss of the gene *5* coding sequence of the Ti-plasmid.

[73] Additional information October 2010.

3. Assessment of gene substitution of the *npt*II and *csp*B genes from maize MON 87460 to bacteria through homologous recombination

The *npt*II and *csp*B genes in maize MON 87460 are derived from *E. coli* and *B. subtilis*, respectively, and their presence in environmental bacteria with homologous DNA sequences of both genes can be expected, so that theoretically recombination between these genes from maize MON 87460 and members of natural microbial communities could take place.

(iii) Likelihood of expression and selective advantage

*npt*II gene: The expression of the acquired DNA is a prerequisite to produce a risk-relevant change in the phenotype of the transformed bacteria. If the *npt*II cassette from maize MON 87460 is transferred to bacterial cells, the expression of the gene cannot be excluded because the 35S promoter (Section 3.1.1) has been shown to be functional in some bacteria (Assaad and Signer, 1990; Lewin et al., 1998; Jacob et al., 2002).

A positive directional selection is considered to be required for rare horizontal gene transfer events to represent biologically meaningful scenarios in the risk assessment, as bacterial communities are continually exposed to a high diversity of other sources of DNA in the environment. However, there is limited information about the spatial and temporal variability in the selective conditions that would favour antibiotic-resistant bacteria, and in the occurrence, transferability and distribution of *npt*II genes in different environments. Also, there is a lack of experimental data on horizontal gene transfer from maize MON 87460.

For the *npt*II gene of maize MON 87460, owing to the alternative gene transfer scenarios described above, both gene substitution and acquisition of the gene by recipients with the *npt*II gene would be possible. The presence of *npt*II genes in bacteria in environments exposed to maize MON 87460 in the context of its intended uses can be expected, but in those recipients the substitution of their natural *npt*II gene by the *npt*II gene of maize MON 87460 (scenario 3, see above) would not confer a novel trait, and thus not provide an additional selective advantage.

In contrast, the acquisition of the *npt*II gene by bacteria without *npt*II genes (scenarios 1 and 2, see above) could confer resistance to kanamycin or neomycin, and thus provide a selective advantage in habitats in which these antibiotics would be present, i.e. the gastrointestinal tract of animals receiving kanamycin or neomycin orally (EFSA, 2009), or soils supplied with faecal matter containing antibiotic residues in sufficient concentration (Nap et al., 1992).

For the specific case of *A. tumefaciens* (scenario 2, see above), a double homologous recombination would lead to the loss of gene *5* from the Ti-plasmid. This deletion should cause a selective disadvantage for *A. tumefaciens* as the tumour induction on plants will be impaired (Körber et al., 1991). In addition, further dissemination of the Ti plasmid to bacteria would be limited to the relatives of *Agrobacterium* within the Rhizobiaceae owing to the host range specificity of the Ti plasmid (Holsters et al., 1978; Cook et al., 1997; Teyssier-Cuvelle et al., 1999).

In the case of scenario 1, the EFSA GMO Panel considers that the stabilisation of the *loxP-npt*II-*loxP* fragment due to the Cre recombination system present in bacteria containing a P1 or P1-like bacteriophage is unlikely. Even in the case that integration would occur, as the main action of the Cre recombinase is excision, this would result in the formation of a circular small molecule encoding *npt*II, which would be expected to be lost during bacterial replication owing to the absence of an origin of replication.

The contribution of horizontal gene transfer of the recombinant *npt*II gene to the development and proliferation of antibiotic-resistant bacteria should be seen in the context of the naturally ongoing resistance gene transfer between bacteria, which is several orders of magnitude more frequent (Brigulla and Wackernagel, 2010). The frequency of horizontal gene transfer of the recombinant *npt*II gene must likewise be regarded relative to the natural distribution and prevalence of *npt*II genes on mobile genetic elements in bacteria. Bacteria carrying the *npt*II gene on mobile genetic elements are found in various environments, although with large spatial and temporal fluctuations (EFSA, 2009). Moreover, resistance genes other than *npt*II also lead to the distribution and prevalence of kanamycin-

and neomycin-resistant bacteria in various environments. Considering the naturally occurring processes of horizontal gene transfer among bacteria populations and the prevalence of *npt*II and kanamycin-/neomycin-resistant bacteria, the contribution of a theoretically possible transfer of *npt*II from maize MON 87460 to the environmental prevalence of kanamycin- and neomycin-resistant bacteria can, if it exists at all, be only extremely low.

*csp*B gene: Regarding the *csp*B gene, which is regulated by an eukaryotic plant promoter and contains a plant intron in maize MON 87460, it is unlikely that it would be expressed in bacteria. In *B. subtilis* and also in other bacteria, of which some may occur in environments exposed to maize MON 87460 in the context of its intended use (e.g. the gastrointestinal tract), the *csp*B gene encodes for a cold shock protein that may enhance the viability of its owners under certain conditions of stress, e.g. at low temperatures. However, for bacteria transformed with the *csp*B gene from maize MON 87460, no selective advantage is anticipated because recombination would result only in the replacement of the gene in a natural host and thus no novel property would be conferred.

(iv) Risk conclusion

The EFSA GMO Panel concludes that adverse effects on human and animal health and the environment resulting from the transfer of the *npt*II and *csp*B genes present in maize MON 87460 to bacteria are unlikely, because of a highly limited potential for gene transfer. Taking into account the different exposure routes, this conclusion is mainly based on the following assessment: (1) the integration of the *npt*II and *csp*B genes through non-homologous recombination is most unlikely (EFSA, 2009); (2) enhanced horizontal transfer of the *npt*II gene due to Cre-*lox*-mediated recombination is unlikely; (3) the stabilisation of the *npt*II gene into bacterial cells by double homologous recombination of *A. tumefaciens* sequences flanking the *npt*II gene, and subsequent dissemination in the environment, are unlikely; and (4) the unlikely but theoretically possible transfer of the *npt*II and *csp*B genes in maize MON 87460 to bacteria via homology-based gene substitution does not raise concerns owing to the lack of an additional selective advantage that would be provided to the recipients in the receiving environments. The probability of horizontal gene transfer of the insert DNA of maize MON 87460 remains several orders of magnitude lower than the gene transfer efficiencies between bacteria. Therefore, its contribution to the increased prevalence of *npt*II genes is considered negligible by the EFSA GMO Panel. In summary, the analysis of horizontal gene transfer from maize MON 87460 to bacteria did not indicate a risk to human or animal health or to the environment in the context of its intended uses.

(b) Plant-to-plant gene transfer

Considering the intended uses of maize MON 87460 and the physical characteristics of maize seeds, possible pathways of gene dispersal are (accidental) grain spillage during transport and processing and the dispersal of pollen from occasional feral GM maize plants originating from grain spillage.

Although GM maize plants outside cropped areas have been reported in Korea, as a result of grain spillage during import, transport, storage, handling and processing (Kim et al., 2006; Lee et al., 2009; Park et al., 2010), the 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 MON 87460, drought tolerance is not likely to provide selective advantages outside cultivation in Europe. Therefore, as for any other maize varieties, GM maize plants would survive in subsequent seasons only in warmer regions of Europe and are not likely to establish feral populations under European environmental conditions.

The extent of cross-pollination with other maize varieties will mainly depend on the scale of accidental release during transport and processing and on successful establishment and subsequent flowering of the resulting GM maize plants. 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 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 neighbour plants only at low levels (Palaudelmàs et al., 2009).

Pollen morphology and viability from maize MON 87460, its conventional counterpart (DM1718) and four non-GM maize commercial varieties were measured in pollen obtained from a field study carried out under both well-watered and water-limited conditions in one location in the USA in 2007.[74] Measures analysed included pollen viability, diameter and morphology. No statistically significant differences were observed between maize MON 87460 and its conventional counterpart.

The breeding pedigree of maize MON 87460 and its conventional counterpart provided by the applicant confirmed that the comparator used in the pollen morphology and viability test had a comparable genetic background with maize MON 87460 (Table 2).[75] Therefore, this line can be regarded as a conventional counterpart.

The EFSA GMO Panel took into account that this application does not include cultivation of maize MON 87460 within the EU, so the likelihood of cross-pollination between cultivated maize and the occasional feral maize MON 87460 plants resulting from grain spillage is considered extremely low.

In conclusion, maize MON 87460 can show enhanced agronomic performance characteristics under water-limited conditions but has no other altered survival, multiplication or dissemination characteristics. Therefore, the EFSA GMO Panel considers that the likelihood of unintended environmental effects as a consequence of the spread of genes from this maize in Europe will not differ from that of its conventional counterpart and non-GM commercial maize varieties.

6.1.1.3. Interactions of the GM plant with target organisms

Interactions of maize MON 87460 with target organisms are not considered an issue by the EFSA GMO Panel as there are no target organisms.

6.1.1.4. Interactions of the GM plant with non-target organisms

Owing to the intended uses of maize MON 87460, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms are not considered an issue by the EFSA GMO Panel.

6.1.1.5. Interactions with the abiotic environment and biochemical cycles

Owing to the intended uses of maize MON 87460, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with the abiotic environment and biogeochemical cycles are not considered an issue by the EFSA GMO Panel.

6.1.2. Post-market environmental monitoring

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are: (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment 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 environmental risk assessment.

Monitoring is related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gave its opinion on the scientific content of the monitoring plan provided by the applicant (EFSA, 2006b, 2011b).

[74] Technical Dossier/Whitsel and Sammons (2008).
[75] Additional information April 2010.

The scope of the monitoring plan provided by the applicant is in line with the intended uses of maize MON 87460. As the scope of the application EFSA-GMO-NL-2009-70 does not include cultivation, the environmental risk assessment is concerned with the accidental release into the environment of viable grains of maize MON 87460 during transport and processing for food and feed uses and with exposure through manure and faeces from animals fed maize MON 87460 grains. The environmental risk assessment identified no potential adverse effects to the environment. Therefore, no case-specific monitoring is necessary.

The general surveillance plan proposed by the applicant includes: (1) the description of an approach involving operators (federations involved in maize import and processing), reporting to the applicants, 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 the information recorded by the various operators; and (3) the use of networks of existing surveillance systems (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a general surveillance report on an annual basis and a final report at the end of the consent period.

The EFSA GMO Panel considers that the scope of the monitoring plan provided by the applicant is in line with the intended uses of maize MON 87460, as the environmental risk assessment does not cover cultivation and identified no potential adverse environmental effects. In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in the case of accidental release of viable grains of maize MON 87460. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

6.2. Conclusion

The scope of the application includes import and processing for food and feed uses of maize MON 87460 and excludes cultivation. Considering the intended uses of maize MON 87460, the environmental risk assessment is concerned with the accidental release into the environment of viable grains from maize MON 87460 during transport and processing for food and feed uses, and with the exposure through manure and faeces from animals fed maize MON 87460.

In the case of accidental release into the environment of viable maize MON 87460 grains, there are no indications of an increased likelihood of establishment and spread of feral maize MON 87460 plants. Considering the intended uses of maize MON 87460 as food and feed, interactions with the biotic and abiotic environment are not considered to be an issue owing to the low levels of exposure. Risks associated with a theoretically possible horizontal transfer from maize MON 87460 *npt*II and *csp*B genes to bacteria have been analysed in detail, including different scenarios of integration, and did not raise safety concerns for the intended uses of maize MON 87460.

The scope of the post-market environmental monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of maize MON 87460 and the EFSA GMO Panel guidelines on the post-market environmental monitoring of GM plants (EFSA, 2006b, 2011b). In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in cases of accidental release of viable grains of maize MON 87460. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

The EFSA GMO Panel recommends that appropriate management systems should be in place to restrict seeds of maize MON 87460 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel considers that the molecular characterisation data provided for maize MON 87460 are sufficient to conclude that maize MON 87460 contains a single copy of the *csp*B and

*npt*II expression cassettes, and lacks other sequences from the transformation vector. Bioinformatic analysis of the flanking sequences and the ORFs spanning the junctions created by the transformation did not raise safety issues. The stability of the inserted DNA was confirmed over multiple generations. The levels of the CspB and NPTII protein from field studies under different environmental conditions, including conditions in which water was limited, were assessed.

The EFSA GMO Panel compared the composition and phenotypic and agronomic characteristics of maize MON 87460 with those of its conventional counterpart and non-GM maize commercial varieties and assessed all statistically significant differences identified. Based on the results of a comparative analysis, the EFSA GMO Panel concludes that, besides the expression of the CspB and NPTII proteins, some differences were observed in the composition of forage and grain produced from maize MON 87460 compared with its conventional counterpart, when grown under well-watered conditions. Given the magnitude of these changes and the characteristics of these endpoints, the EFSA GMO Panel concludes that the observed differences do not raise safety concerns for humans and animals. The EFSA GMO Panel notes that under water-limited and other stressful conditions, maize MON 87460 can show enhanced agronomic performance characteristics and some differences in chemical composition in comparison with its conventional counterpart. Given the intended trait, the observed differences were not unexpected and did not indicate safety concerns.

The CspB protein is rapidly hydrolysed by pepsin and pancreatin. Bioinformatics-supported studies demonstrated that the CspB protein shows no homology to known toxic and allergenic proteins. There is no evidence that the genetic modification might significantly change the overall allergenicity of maize MON 87460. No indication of toxicity was found in a subchronic 90-day rat feeding study with diets containing grain from maize MON 87460. The results of the study on chickens for fattening concerning zootechnical performance support the conclusion that maize MON 87460 can be used in the same way as other maize sources as a feedingstuff in animal nutrition. The NPTII protein has been evaluated previously and did not raise safety concerns.

The EFSA GMO Panel considers that maize MON 87460 is as safe and as nutritious as its conventional counterpart and commercial varieties, and concluded that this maize and its derived products are unlikely to have adverse effects on human and animal health, in the context of their intended uses.

The application EFSA-GMO-NL-2009-70 is for the import and processing of maize MON 87460 for food and feed uses but excludes cultivation in the EU. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of maize MON 87460. There are no indications of an increased likelihood of establishment and spread of feral maize plants in the case of accidental release into the environment of viable grains from maize MON 87460 during transport and processing. Considering the intended uses of maize MON 87460 as food and feed, interactions with the biotic and abiotic environment are not considered to be an issue owing to the low levels of exposure. Risks associated with a theoretically possible horizontal transfer from maize MON 87460 of *npt*II and *csp*B genes to bacteria have been analysed in detail, including different scenarios of integration, and did not raise safety concerns for the intended uses of maize MON 87460. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize MON 87460. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

In conclusion, the EFSA GMO Panel considers that the information available for maize MON 87460 addresses scientific issues indicated by its risk assessment and monitoring guidelines and the scientific comments raised by the Member States, and that maize MON 87460, as described in this application, is as safe as its conventional counterpart and non-GM reference varieties with respect to potential effects on human and animal health and the environment, in the context of its intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the Netherlands, received 29 May 2009, concerning a request for placing on the market of maize MON 87460 in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 19 June 2009, from EFSA to the Competent Authority of the Netherlands.

3. Letter from EFSA to applicant, dated 9 July 2009, requesting additional information under completeness check.

4. Letter from applicant to EFSA, received 1 December 2009, providing additional information under completeness check.

5. Letter from EFSA to applicant, dated 14 December 2009, requesting additional information under completeness check.

6. Letter from applicant to EFSA, received 8 January 2010, providing additional information under completeness check.

7. Letter from EFSA to applicant, dated 28 January 2010, delivering the "Statement of Validity" for application EFSA-GMO-NL-2009-70, maize MON 87460 submitted by Monsanto under Regulation (EC) No 1829/2003.

8. Letter from EFSA to applicant, dated 12 May 2010, requesting additional information and stopping the clock.

9. Letter from applicant to EFSA, received 29 June 2010, providing the timeline for submission of response.

10. Letter from applicant to EFSA, received 4 October 2010, providing additional information.

11. Letter from EFSA to applicant, dated 20 December 2010, requesting additional information and maintaining the clock stopped.

12. Letter from applicant to EFSA, received 10 February 2011, providing the timeline for submission of response.

13. Letter from applicant to EFSA, received 18 April 2011, providing additional information.

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

15. Letters from applicant to EFSA, received 7 September 2011 and 27 January 2012, providing the timeline for submission of response.

16. Letter from applicant to EFSA, received 30 April 2012, providing additional information.

17. Letter from EFSA to applicant, dated 11 September 2012, re-starting the clock.

18. Letter from applicant to EFSA, received 3 October 2012, providing additional information spontaneously.

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