European Food Safety Authority

The EFSA Journal (2009) 1192, 1-27

SCIENTIFIC OPINION

Application (Reference EFSA-GMO-CZ-2006-33) for the placing on the market of the insect-resistant and glyphosate-tolerant genetically modified maize MON 88017 x MON 810, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto[1]

Scientific Opinion of the Panel on Genetically Modified Organisms

(Question No EFSA-Q-2006-020)

Adopted on 2 July 2009

PANEL MEMBERS*

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SUMMARY

Following a request from Monsanto within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed, the Panel on Genetically Modified Organisms was asked to deliver a scientific opinion on the authorisation of the insect-resistant, glyphosate-tolerant genetically modified maize MON 88017 x MON 810 (Unique Identifier MON88Ø17-3 x MON-ØØ81Ø-6).

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-CZ-2006-33, additional information provided by the applicant (Monsanto) and the scientific comments submitted by the Member States. Further information from applications for placing the single insert lines MON 88017 and MON 810 on the market under EU regulatory procedures was taken into account where appropriate. The scope of application EFSA-GMO-CZ-2006-33 is for food and feed uses, import and processing of genetically modified maize MON 88017 x MON 810 and all derived products, but excluding cultivation in the EU.

[1] For citation purposes: Scientific Opinion of the Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-CZ-2006-33) for the placing on the market of the insect-resistant and glyphosate-tolerant genetically modified maize MON 88017 x MON 810, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto *The EFSA Journal* (2009) 1192, 1-27

* (minority opinion) This opinion is not shared by 0 members of the Panel. / (conflict of interest) 0 members of the Panel did not participate in (part of) the discussion on the subject referred to above because of possible conflicts of interest.

The EFSA GMO Panel assessed maize MON 88017 x MON 810 with reference to the intended uses and the appropriate principles described in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The scientific assessment included molecular characterisation of the inserted DNA and expression of the new proteins. A comparative analysis of agronomic traits and composition was undertaken and the safety of the newly expressed proteins and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment of environmental impacts and the post-market environmental monitoring plan were also undertaken.

Maize MON 88017 was developed to express a modified Cry3Bb1 protein derived from *Bacillus thuringiensis* subsp. *kumamotoensis* rendering maize MON 88017 resistant to certain coleopteran pests and the CP4 EPSPS protein derived from *Agrobacterium* sp. strain CP4 which provides tolerance to glyphosate. Maize MON 810 expresses the Cry1Ab insecticidal protein, derived from *Bacillus thuringiensis* subsp. *kurstaki*, which confers protection against lepidopteran pests such as the European corn borer (*Ostrinia nubilalis*) and species belonging to the genus *Sesamia*.

Maize MON 88017 x MON 810 was produced by crosses between maize inbred lines containing MON 88017 and MON 810 events to combine resistance to certain coleopteran (MON 88017 trait) and lepidopteran (MON 810 trait) pests and to confer tolerance to glyphosate (MON 88017 trait).

The molecular characterisation data established that the structure of the individual inserts was retained in the hybrid maize MON 88017 x MON 810. Appropriate analyses of the integration sites in maize MON 88017 x MON 810, including flanking regions, was carried out. The bioinformatic analysis demonstrated the absence of any potential new ORFs coding for known toxins or allergens.

Based on results of the comparative analysis the EFSA GMO Panel concludes that maize MON 88017 x MON 810 is compositionally, phenotypically and agronomically equivalent to the non-genetically modified (GM) counterpart and conventional maize varieties, except for the presence of Cry3Bb1, CP4 EPSPS and Cry1Ab proteins in maize MON 88017 x MON 810. Based on the assessment of data available, including the additional information provided by the applicant in response to the EFSA GMO Panel's requests for maize MON 88017 x MON 810, for the single events and for appropriate non-GM controls, the EFSA GMO Panel has found no indication that crossing of MON 88017 with MON 810 maize results in an interaction between the single events which causes compositional or agronomic changes. The Cry3Bb1 and CP4 EPSPS proteins expressed in the parental maize line MON 88017, and the Cry1Ab protein expressed in the parental maize MON 810 have been assessed previously and no safety concerns were identified. Given all the information provided, the EFSA GMO Panel concludes that interactions between the single events that might impact on food and feed safety are unlikely. The nutritional value of maize MON 88017 x MON 810 has been investigated in a feeding study with broilers which confirmed that the nutritional properties of maize MON 88017 x MON 810 would be no different from those of conventional maize. In conclusion, the EFSA GMO Panel considers that maize MON 88017 x MON 810 is as safe and as nutritious as its non-GM counterpart and that the overall allergenicity of the whole plant is not changed.

The application EFSA-GMO-CZ-2006-33 concerns food and feed uses, import and processing. Therefore, there is no requirement for scientific assessment of possible environmental effects

associated with the cultivation of maize MON 88017 x MON 810. There are no indications of increased likelihood of establishment or survival of feral maize plants in case of accidental release into the environment of maize MON 88017 x MON 810 viable grains during transportation and processing. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize MON 88017 x MON 810.

In conclusion, the EFSA GMO Panel considers that the information available for maize MON 88017 x MON 810 addresses the scientific comments raised by the Member States and that it is as safe as its non-genetically modified counterpart with respect to potential effects on human and animal health or the environment. Therefore the EFSA GMO Panel concludes that maize MON 88017 x MON 810 is unlikely to have any adverse effect on human or animal health or on the environment in the context of its intended uses.

Key words: **GMO, maize (*Zea mays*), MON 88017, MON 810, MON 88017 x MON 810, glyphosate-tolerant, insect-resistant, Cry3Bb1, Cry1Ab, CP4 EPSPS, food safety, feed safety, human and animal health, environment, import, processing, Regulation (EC) No 1829/2003.**

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BACKGROUND

On 3 January 2006, EFSA received from the Competent Authority of the Czech Republic an application (Reference EFSA-GMO-CZ-2006-33) for authorisation of the insect resistant glyphosate tolerant genetically modified maize MON 88017 x MON 810 (Unique Identifier MON-88Ø17-3 x MON-ØØ81Ø-6), submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed (EC, 2003) for food and feed uses, import and processing.

After receiving the application EFSA-GMO-CZ-2006-33 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the Member States as well as the European Commission and made the summary of the dossier publicly available 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 2 February 2007, EFSA received additional information requested under completeness check (requested on 25 January 2007) and on 21 February 2007 EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC (EC, 2001) following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member State bodies had three months after the date of receipt of the valid application (until 21 May 2007) within which to make their opinion known.

The EFSA GMO Panel carried out a scientific assessment of genetically modified maize MON 88017 x MON 810 taking into account the appropriate principles described in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006a).

On 26 March 2007 and 13 March 2008 the EFSA GMO Panel asked for additional data on maize MON 88017 x MON 810. The applicant provided additional information on 10 December 2007, 16 April 2008 and 13 October 2008. After receipt and assessment of the full data package, the EFSA GMO Panel finalised its risk assessment of maize MON 88017 x MON 810.

The EFSA GMO Panel carried out a scientific assessment of the GM maize MON 88017 x MON 810 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, taking into consideration the scientific comments of the Member States and the additional information provided by the applicant.

In giving its opinion on GM maize MON 88017 x MON 810 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 receipt of the valid application. As additional information was requested by the EFSA GMO Panel, the time limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, the EFSA opinion shall include a report describing the assessment of the food and feed and stating the reasons for its opinion and the information on which its opinion is based. This document 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 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 88017 x MON 810 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)(e) of Regulation (EC) No 1829/2003.

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

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Groups on Molecular Characterisation, Food/Feed and Environment, as well as the *ad hoc* experts: Sue Hartley and Ilona Kryspin-Sørensen, and the following members of its staff: Zoltán Divéki, Karine Lheureux and Ellen Van Haver for the preparation of this opinion.

ASSESSMENT

1. Introduction

The genetically modified maize MON 88017 x MON 810 (Unique Identifier MON88Ø17-3 x MON-ØØ81Ø-6) was assessed with reference to its intended uses, taking account of the appropriate principles described in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006a) and the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events (EFSA, 2007). The risk assessment presented here is based on the information provided in the application relating to maize MON 88017 x MON 810 submitted in the EU including additional information from the applicant, information on the single events, as well as scientific comments that were raised by the Member States.

2. Issues raised by Member States

Issues raised by Member States (MS) are addressed in Annex G of the overall opinion.

3. Molecular characterisation

3.1 Evaluation of relevant scientific data

3.1.1. Method of production of maize MON 88017 x MON 810

Conventional breeding methods were used to produce maize MON 88017 x MON 810 and no new genetic modification was involved. The two inserts that are present in maize MON 88017 x MON 810 were derived from maize lines containing two independent events: MON 88017 and MON 810. Each of these GM maize events was the subject of an earlier safety evaluation and separate opinions for each of them have been published (EFSA, 2009a, b). Maize MON 88017 x MON 810 combines the lepidopteran and coleopteran protection traits, and the tolerance to herbicides containing glyphosate.

3.1.2. Summary of the evaluation of the single events

MON 88017

Maize MON 88017 was developed through *Agrobacterium*-mediated transformation using the PV-ZMIR39 plasmid and as a result expresses the *cry3Bb1* and CP4 *epsps* genes conferring resistance to coleopteran insect pests (*Diabrotica* spp.) and resulting in tolerance towards glyphosate-containing herbicides, respectively.

Molecular characterisation data established that MON 88017 contains one copy of the T-DNA and that vector backbone sequences are absent.
Similarity searches revealed that the flanking regions of the insert in maize MON 88017 show significant level of identity to maize genomic DNA sequences and indicated that the pre-insertion locus was preserved except for the deletion of 26 bp and the addition of 20 bp. An updated bioinformatic analysis was performed. The data indicated that the insert lies 174 bp upstream of a region showing high sequence similarity to the expressed sequence tags annotated as corresponding to putative purine permeases. Phenotypic, agronomic and compositional analyses

showed that MON 88017 is equivalent to conventional maize, except for the expected traits, indicating that the insertion of the transgene has not altered the expression of an essential gene and the insertion of the transgene *per se* does not pose a safety concern. Bioinformatic analysis also revealed no biologically relevant similarity to allergens or toxins for any of the putative polypeptides that might be produced from open reading frames spanning the junction regions.
Southern analysis of MON 88017 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations.

MON 810

Maize MON 810 was developed through particle bombardment using plasmid PV-ZMGT10 (which was not integrated in the plant) and plasmid PV-ZMBK07 which contains a *cry1Ab* expression cassette.

Molecular characterisation data established that MON 810 contains one truncated copy of PV-ZMBK07 and as a result expresses the *cry1Ab* gene conferring resistance to lepidopteran pests. MON 810 contains the *cry1Ab* cassette at a single locus and vector backbone sequences are absent.
Similarity searches revealed that the flanking regions of the insert in maize MON 810 show significant identity to maize genomic DNA sequences and indicated that the pre-insertion locus was preserved except for the addition of 400 bp of maize DNA at the 3' flank and 1000 bp of maize DNA at the 5' flank. An updated bioinformatic analysis was performed. The data indicated that no known endogenous ORFs or regulatory sequences have been disrupted in flanking regions adjacent to the insert. Bioinformatic analysis also revealed no biologically relevant similarity to allergens or toxins for any of the putative polypeptides that might be produced from ORFs spanning the junction regions. Southern analysis of MON 810 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations.

3.1.3. Transgenic constructs in maize MON 88017 x MON 810

Maize MON 88017 x MON 810 has been obtained by conventional crossing of MON 88017 with MON 810. No new genetic modification has been introduced in the stacked maize line. The integrity of the individual inserts present in this maize was investigated using Southern analyses. This involved the use of DNA probes specific for the MON 88017 and MON 810 inserts and enzymatic digestions informative of the structure of both events, including the junctions with the host genomic DNA. The predicted DNA hybridisation patterns from each single event were retained in the MON 88017 x MON 810 hybrid, demonstrating that integrity of the inserts was maintained.

3.1.4. Information on the expression of the inserts

The levels of newly expressed proteins Cry3Bb1, Cry1Ab, and CP4 EPSPS in forage and grains of maize MON 88017 x MON 810 were assessed by enzyme-linked immunosorbent assay (ELISA). Tissue samples for analysis were collected from field trials conducted in the USA during 2002. The trials were located within the major maize-growing region of the USA and provided a variety of environmental conditions. At each site, maize MON 88017 x MON 810, and maize MON 88017 or MON 810 were planted. The scope of the application covers food and feed uses and import and processing, therefore only protein expression data related to the grains is considered relevant, which are summarised in Table 1. Levels of proteins in the stacked line are comparable to levels in the single events and do not pose any safety concerns.

Table 1. Protein expression levels in MON 88017 x MON 810, MON 88017 and MON 810 maize grains (µg / g dry weight)

		MON 88017 x MON 810	MON 88017	MON 810
CP4 EPSPS	mean (SD)	4.3 (1.6)	5.8 (0.97)	--
	range	2.2 - 6.2	4.1 - 7.1	
Cry3Bb1	mean (SD)	9.3 (3.4)	15 (3.6)	--
	range	3.9 - 13	10 - 22	
Cry1Ab	mean (SD)	0.39 (0.13)	--	0.43 (0.091)
	range	0.16 - 0.63		0.27 - 0.54

3.1.5. Inheritance and stability of inserted DNA

The genetic stability of the inserted DNA in events MON 88017 and MON 810 was demonstrated previously (EFSA 2009a,b). In the maize MON 88017 x MON 810 inserts are combined. The Southern data presented show that both events are present and the structure of each insert is retained. Furthermore, each of the traits has been conserved in this maize.

3.2. Conclusion

As conventional breeding methods were used in the production of maize MON 88017 x MON 810, no additional genetic modification was involved. Southern analyses demonstrated that the structures of the MON 88017 and MON 810 events were retained in maize MON 88017 x MON 810.

The expression levels of Cry3Bb1, Cry1Ab and CP4 EPSPS proteins in the grains of maize MON 88017 x MON 810 have been demonstrated to be comparable with those of the single events.

The EFSA GMO Panel concludes that the molecular characterisation does not indicate safety concerns.

4. Comparative Analysis

4.1. Evaluation of relevant scientific data

4.1.1 Summary of the previous evaluation of the single events

MON 88017

Forage and grains of maize MON 88017 sprayed with glyphosate and the same tissues from non-GM control maize with a comparable genetic background were obtained from field trials carried out in three locations in the USA in 2002 and in four locations in Argentina in 2003-2004. Also reference maize lines were grown alongside the test and control maize in the same locations. Whilst several compounds (vitamin B1, oleic acid, and linoleic acid) showed statistically significant differences in the across-locations and each single-location analysis during a single season, these differences did not occur in the other season and were within the range of reference lines and/or historical and literature ranges. Additional data from another field trial in Europe were provided by the applicant at the request of the EFSA GMO Panel, namely of MON 88017 not treated with glyphosate and grown in three locations in Germany and in three locations in Spain in 2007. Various statistically significant differences were observed between MON 88017

and the non-GM control maize, none of which occurred within each location and all of which were within the range of reference varieties. Based on these data, the EFSA GMO Panel concluded that maize MON 88017 is compositionally equivalent to its non-GM counterpart and conventional maize varieties, except for the presence of Cry3Bb1 and CP4 EPSPS proteins in maize MON 88017 due to the genetic modification.

In the analysis of agronomic and phenotypic characteristics of MON 88017 compared to the non-GM comparator over several seasons, no consistent changes in the same direction (*i.e.* decrease or increase) were observed in each season and in all locations. The EFSA GMO Panel concluded that maize MON 88017 is equivalent to its non-GM counterpart with regard to phenotypic characteristics and agronomic performance except for the introduced trait (EFSA, 2009a).

MON 810

The original field trials with maize MON 810 were performed in the USA in 1994 (6 sites) and in France in 1995 (4 sites). As these field trials were not replicated, only the combined data were statistical analysed. The non-GM maize control material was maize MON 818 in all 1994 field trials and maize MON 820 in the 1995 field trials. Both control materials were similar in pedigree to the tested maize MON 810. Only grain material was analysed from the field trials in 1994, whereas both grain material and forage was analysed from the field trials performed in 1995. The set of compounds analysed in grain material was proximates, 18 amino acids, 9 fatty acids, carbohydrates (5 compounds or fractions), vitamins (3 tocopherols), minerals (calcium and phosphorous), and anti-nutrients (phytic acid). Forage was analysed for proximates, and neutral and acidic fibre. Leaf, forage and grains were also analysed for the expression of the Cry1Ab protein. In total 44 compounds were analysed.

To support the original compositional data, data on forage and grain material collected from field trials with 3 different stacked GM maize events where maize MON 810 was one of the parental GM maize lines were provided. The studies were on MON 810 x MON 863 grown at 4 replicated sites in Argentina in 1999, MON 810 x NK603 grown at 3 replicated sites in France in 2000, and MON 810 x MON 863 x NK603 grown at 4 replicated sites in Argentina during the season 2002-2003.

The compositional analysis of grains of maize MON 810 and its control line MON 818 harvested in 1994 showed that all the analysed values were within the ranges reported in the literature, except for histidine, cystine and calcium levels. The level of histidine and cystine were higher than reported in the literature in both studied materials (maize MON 810 and its control). On the other hand, the calcium levels in both materials were below the levels reported in the literature for maize. However, notably the levels for all 3 compounds did not deviate from those reported by the applicant to occur in another conventional maize variety with a similar genetic background. For the data of the field trials performed in 1995, statistical differences in constituent levels between maize MON 810 and its control (MON 820) were observed for 5 compounds (increased grain moisture and palmitic acid content, and reduced levels of methionine and tryptophan, as well as increased crude protein in forage) which in no case confirmed findings from the 1994 trial. Based on these data, the EFSA GMO Panel concluded that maize MON 810 was compositionally equivalent to the non-GM maize counterparts MON 820 and MON 818 and to conventional maize varieties except for the presence of the Cry1Ab protein.

The EFSA GMO Panel has already assessed the agronomic and phenotypic characteristics of maize MON 810 in relation to an appropriate non-GM maize control having a comparable genetic background in connection with giving its opinions on several stacked GM maize events

(EFSA, 2005a,b,c,d,e). The information available in the renewal applications gave no reason to change the opinion that maize MON 810 is agronomically and phenotypically equivalent to currently grown non-GM maize varieties, with the exception of the insect resistance conferred by the Cry1Ab protein (EFSA, 2009b).

4.1.2. Choice of comparator and production of material for the compositional assessment

Maize MON 88017 x MON 810 and the non-GM controls were grown in three replicated field sites across the USA (Iowa, Illinois, Nebraska[2]) during the 2002 growing season. Maize MON 88017 x MON 810 was treated with glyphosate and compared with a conventional control non-GM line with a comparable genetic background to maize MON 88017 x MON 810. Twelve different reference maize hybrids (four references per site) were grown in parallel to study the natural variation. In addition, maize MON 88017 x MON 810 was compared to the parental maize lines MON 810 and MON 88017, the latter being treated with glyphosate, which is representative of the agricultural practice regarding weed control.

4.1.3 Compositional analysis

The chemical analytical data on composition of forage and grain material from maize MON 88017 x MON 810, the non-GM control with a genetic background similar to maize MON 88017 x MON 810 except for the introduced traits, MON 88017, MON 810, and several conventional reference maize hybrids were provided from material collected during the 2002 USA field trials. The compounds analysed followed the recommendation of OECD (2002).

Compositional analysis of forage samples included proximates (fat, protein, ash and moisture, total carbohydrate), acid detergent fibre (ADF), neutral detergent fiber (NDF) and minerals (phosphorus and calcium).

Compositional analyses of grain samples included proximates, ADF, NDF, total dietary fibre (TDF), amino acids, fatty acids (C8-C22), minerals (calcium copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), vitamins (B1, B2, B6, E, niacin and folic acid), and secondary metabolites (phytic acid, raffinose, furfural, ferulic acid, and p-coumaric acid).

In summary, the compositional comparison between forage and grains of MON 88017 x MON 810 and non-GM control maize showed no statistically significant differences for any parameters in forage when data from all locations were combined. In grains, the statistically significant differences that were observed in the combined-location analysis included increased levels of alanine, linoleic acid (C18:3), arachidic acid (C20:0), and ferulic acid, as well as decreased levels of eicosenoic acid (C20:1), copper, potassium, and vitamin B2, in maize MON 88107 x MON 810 as compared to its control. Except for eicosenoic acid, none of the statistically significant differences occurred in each of the three locations. All the average values showing statistically significant differences were within the 99%-tolerance interval of reference values and also fell within the historic and literature ranges.

The comparison between forage and grains of maize MON 88017 x MON 810 and MON 810 in the combined sites showed various statistically significant differences in grains. These statistically significant differences included increased levels of arachidic acid (C20:0), alanine, and ferulic acid, as well as decreased levels of eicosenoic acid (C20:1) in maize MON 88017 x

[2] Grain samples from the Ohio field site were not analysed in this study due to the unintended presence of DNA from other test or control substances.

MON 810 as compared to MON 810. The average values and the range of these parameters fell within the tolerance intervals defined by the reference varieties and also fell within historic and literature ranges.

The comparison of forage and grain composition between maize MON 88017 x MON 810 and MON 88017 treated with glyphosate showed various statistically significant differences in grains. These statistically significant differences included increased levels of total fat, arachidic acid (C20:0), alanine, vitamin B1, and ferulic acid, as well as decreased levels of eicosenoic acid (C20:1) and p-coumaric acid. Except for vitamin B1, none of these statistically significant differences were observed in each location. The average values and the range of these parameters, including vitamin B1, fell within the tolerance intervals defined by the reference varieties and also within the historic ranges.

The EFSA GMO Panel concludes that expression of the newly introduced genes in maize MON 88017 x MON 810 did not result in any effect on the chemical composition and that maize MON 88017 x MON 810 is compositionally equivalent to its non-GM counterpart and conventional maize except for the presence of Cry3Bb1, Cry1Ab, or CP4 EPSPS proteins.

4.1.4. Agronomic traits and GM phenotype

During field trials in 2002, maize MON 88017 x MON 810 and its non-GM counterpart were grown in four replicated field trials across the USA (Iowa, Illinois, Nebraska and Ohio). The agronomic data on seedling vigor, early stand count, days to 50% pollen shed, days to 50% silking, stay green and plant height were collected at each production site and across the locations. There were no statistically significant differences observed between maize MON 88017 x MON 810 and its non-GM counterpart in stay green and seedling vigor. Additional measurements included biotic and abiotic stressors at each location, as well as grain weight.

Various statistically significant differences between maize MON 88017 x MON 810 and the corresponding non-GM counterpart were observed, whilst each of these observed differences occurred only in one location but not in the analysis of the combined data. The values of MON 88017 x MON 810 were still within the background range defined by reference maize varieties grown at the same locations. The data on biotic and abiotic stressors did not show any conspicuous differences between test and control maize besides a difference in corn rootworm infestation in one location, which obviously relates to the corn-rootworm-resistance trait introduced into MON 88017 and to the heterogenous dispersal of Western corn rootworm. The EFSA GMO Panel found it unlikely that these observed differences are of biological significance and concludes that maize MON 88017 x MON 810 is equivalent to its non-GM counterpart and conventional maize with regard to phenotypic characteristics and agronomic performance.

4.2 Conclusion

Based on the results of the comparative analysis, it is concluded that maize MON 88017 x MON 810 is compositionally and agronomically equivalent to its non-GM counterpart and conventional maize, except for the presence of Cry3Bb1, CP4 EPSPS and Cry1Ab proteins in maize MON 88017 x MON 810. Based on the assessment of the data available, the EFSA GMO Panel has found no indication that crossing of MON 88017 and MON 810 maize results in an interaction between the single events which causes compositional or agronomic changes.

5. Food/feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Summary of the previous evaluation of the single events

MON 88017

Analogues of the newly expressed Cry3Bb1 and CP4 EPSPS proteins in MON 88017 maize were obtained from recombinant strains of *Escherichia coli* and used for safety testing after their equivalence to the plant-expressed proteins had been demonstrated experimentally. Both proteins neither showed toxicity in acute oral toxicity studies in mice, nor did they show relevant similarities to known toxic or allergenic proteins in bioinformatics-supported comparisons of their amino acid sequences. Cry3Bb1 and CP4 EPSPS proteins were also rapidly degraded during incubations with simulated gastric fluid containing the digestive enzyme pepsin.

The safety of the whole food/feed derived from MON 88017 was tested in a 90-days rat feeding study with diets containing a maximum of 33% grains from maize MON 88017. No indications of adverse effects were observed in this study. Also a nutritional, 42-day broiler chicken feeding study was carried out with diets containing between 55 and 60% grains from maize MON 88017, showing that the latter was nutritionally equivalent to conventional maize (EFSA, 2009a).

MON 810

Given the low expression level of Cry1Ab protein in maize MON 810, Cry1Ab was produced in a recombinant *Escherichia coli* strain. As the Cry1Ab protein produced by MON 810 is converted to the trypsin-resistant core protein by digestive proteases, the trypsin resistant core protein (HD-1t), obtained through trypsinolysis of the *E. coli*-produced Cry1Ab protein, was used for safety assessment. The identity and the equivalence of the *E. coli*-expressed trypsin-resistant core protein to the core protein that remains after trypsin-mediated proteolysis of the Cry1Ab protein present in maize MON 810 have been demonstrated.

Extensive *in vivo* experience, backed by *in vitro* studies, have led to the conclusion that both the Cry1Ab protein expressed in *Bacillus thuringensis* and the Cry1Ab protein expressed in plants are highly selective and do not target mammalian organisms. Therefore, the EFSA GMO Panel has accepted the use of the trypsin-resistant core of Cry1Ab protein derived from *E. coli* for the safety testing of the trypsin-resistant core of the Cry1Ab protein present in maize MON 810.

The Cry1Ab protein induced no adverse effects in an acute oral toxicity study in mice, nor in a repeated dose toxicity study in rats. In addition, this protein is rapidly degraded under simulated gastric conditions. The Cry1Ab protein shows no homology with known toxic proteins and/or allergens. In a 90-day feeding study in rats receiving diets containing MON 810 maize grains, no indications of adverse effects were observed. In addition, a 42-day broiler feeding study provided evidence of nutritional equivalence of MON 810 maize grains to grains of conventional maize. Based on these data, the EFSA GMO Panel was of the opinion that maize MON 810 is as safe as its non-GM counterparts and that the overall allergenicity of the whole plant was not changed through the genetic modification. Furthermore, the Cry1Ab protein has been extensively assessed in previous opinions of the EFSA GMO Panel and found to be safe (EFSA 2005a,b,c,d,e; EFSA, 2009b).

5.1.2. Product description and intended use

The scope of application EFSA-GMO-CZ-2006-33 includes the import and processing of maize MON 88017 x MON 810 and its derived products for use as food and feed. Thus, the possible uses of maize MON 88017 x MON 810 include the production of animal feed, but it also includes valuable food products such as, starch, syrups and oils.

The genetic modification of maize MON 88017 x MON 810 is intended to improve agronomic performance only and is not intended to influence the nutritional properties, processing characteristics and overall use of maize MON 88017 x MON 810 as a crop.

5.1.3. Effects of processing

Since maize MON 88017 x MON 810 is compositionally equivalent to conventional maize (see Section 4.2), except for the newly expressed proteins (see Section 3.1.4), the effect of processing on maize MON 88107 x MON 810 is not expected to be different compared to that on conventional maize.

5.1.4. Toxicology

5.1.4.1. Toxicological assessment of expressed novel proteins in maize MON 88017 x MON 810

The Cry3Bb1 and CP4 EPSPS proteins expressed in the parental maize MON 88017, and the Cry1Ab protein expressed in the parental maize MON 810 have been assessed for their safety previously (EFSA, 2009a,b) and no safety concerns were identified. The EFSA GMO Panel is not aware of any new information that would change this conclusion.

No new genes in addition to those occurring in the parental maize varieties have been introduced in maize MON 88017 x MON 810.

The applicant also submitted an argumentation for the low likelihood of possible interactions between the newly expressed proteins Cry3Bb1, CP4 EPSPS and Cry1Ab in maize MON 88017 x MON 810, including the different modes of action, the absence of any reported adverse health effects, and the low expression levels of these proteins. It also refers to combinations of the newly expressed Cry3Bb1, Cry1Ab and CP4 EPSPS proteins in other stacked maize events, including MON 863 x MON 810 x NK603, MON 863 x MON 810, and MON 863 x NK603, which previously were assessed and given positive opinion by the EFSA GMO Panel (EFSA, 2005a,b,c,e).

Based on the data provided, the EFSA GMO Panel considers that interactions between the single events that might impact on food and feed safety are unlikely.

5.1.4.2. Toxicological assessment of new constituents other than proteins

No new constituents other than the Cry3Bb1, CP4 EPSPS and Cry1Ab proteins are expressed in maize MON 88017 x MON 810, and no relevant changes in the composition of maize MON 88017 x MON 810 were detected by the compositional analysis.

5.1.4.3. Toxicological assessment of the whole GM food/feed

Regarding human and animal consumption, the genetically modified maize events MON 88017 and MON 810 have previously been found as safe as the conventional counterpart (EFSA, 2009a,b). A molecular characterisation undertaken on maize MON 88017 x MON 810 identified no altered stability of the events (see Section 3.2) when these were brought together by crossing, and expression analysis of the proteins Cry3Bb1, CP4 EPSPS and Cry1Ab revealed no change in protein expression levels that could raise concerns for human and animal health. As the composition of maize MON 88017 x MON 810 is equivalent to that of non-GM maize varieties and also no indication for interaction between the single events was found, the EFSA GMO Panel is of the opinion that no additional animal safety studies are required.

5.1.5. Allergenicity

The strategies used when assessing the 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 on whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (EFSA, 2006a; CAC, 2003).

5.1.5.1. Assessment of allergenicity of the newly expressed proteins

The newly expressed proteins (Cry3Bb1, CP4 EPSPS and Cry1Ab) present in maize MON 88017 x MON 810 have been assessed previously and it was found unlikely that any of them are allergenic (EFSA, 2009a,b). Based on the information provided, the EFSA GMO Panel considers it unlikely that potential interactions occur that might change the allergenicity of the expressed proteins.

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

The issue of a potential for increased allergenicity of maize MON 88017 x MON 810 does not appear relevant to the EFSA GMO Panel since maize is not considered a common allergenic food. Food allergies to maize are of low frequency and mainly occur in populations of specific geographic areas. Rare cases of occupational allergy to maize dust have been reported. There is no reason to expect that the use of maize MON 88017 x MON 810 will significantly increase the intake and exposure to maize. Therefore a possible over-expression of any endogenous protein, which is not known to be allergenic, would be unlikely to alter the overall allergenicity of the whole plant or the allergy risk for consumers.

5.1.6. Nutritional assessment of GM food/feed

The applicant provided a 42-day feeding study with broiler chickens to analyse the nutritional value of grains from maize MON 88017 x MON 810 treated with glyphosate, in relation to grains from the non-GM control maize with comparable genetic background (LH59xLH198) and five conventional maize varieties. One hundred birds per treatment divided into 10 pens per treatment (5 males and 5 females) were fed diets containing approximately 55% (w/w) of maize grains during the first half and 60% during the second half of the experiment. Weight gain, feed consumption and carcass parameters (weight, weight of carcass parts and composition of breast and thigh meat) were measured. 27 parameters were studied for each comparison performed

between animals fed maize MON 88017 x MON 810 versus the non-GM control. Any statistical significant difference was evaluated against background variation observed in the five conventional maize lines.

There were no statistically significant differences in the performance parameters including body weight, total feed intake and feed conversion between the chickens fed MON 88017 x MON 810 and the non-GM control diet as well as the conventional reference diets.

Carcass measurements showed significant diet by gender differences in live weight, final body weight, fat pad weight and thigh weight. If analyzed for each gender separately, these parameters did not show statistically significant differences between the test (MON 88017 x MON 810) and control groups.

Thus the broiler feeding study supported the results of the comparative compositional analysis that showed that maize MON 88017 x MON 810 is compositionally and nutritionally equivalent to the non-GM maize counterpart and conventional maize.

5.1.7. Post-market monitoring of GM food/feed

The risk assessment concluded that there are no data to indicate that maize MON 88017 x MON 810 is any less safe than its non-GM counterpart and parental GM lines. In addition, maize MON 88017 x MON 810 is, from a nutritional point of view, equivalent to conventional maize. Given the intended use of maize MON 88017 x MON 810, the overall intake of maize is not expected to be different from that of conventional maize. Therefore, and in line with the Guidance document (EFSA, 2006a), the EFSA GMO Panel is of the opinion that post-market monitoring of the food/feed derived from maize MON 88017 x MON 810 is not necessary.

5.2. Conclusion

The Cry3Bb1 and CP4 EPSPS proteins expressed in the parental maize MON 88017, as well as the Cry1Ab protein present in maize MON 810 have been assessed previously and no safety concerns were identified.

Given all the information provided, the EFSA GMO Panel concludes that interactions between the single events that might impact on food and feed safety are unlikely when these are brought together by conventional crosses, and that the nutritional properties of maize MON 88017 x MON 810 would be no different from those of conventional maize.

In conclusion, the EFSA GMO Panel considers that maize MON 88017 x MON 810 is as safe and as nutritious as its non-GM counterpart and that the overall allergenicity of the whole plant is not changed and concludes that maize MON 88017 x MON 810 is unlikely to have any adverse effect on human and animal health in the context of its intended uses.

6. Environmental risk assessment and monitoring plan

6.1 Evaluation of relevant scientific data

The scope of the application is for food and feed uses, import and processing of maize MON 88017 x MON 810 and does not include cultivation. Considering the proposed uses of maize MON 88017 x MON 810, the environmental risk assessment is concerned with the exposure through manure and faeces from gastrointestinal tracts of animals fed maize MON 88017 x MON 810 and with the accidental release of maize MON 88017 x MON 810 viable grain into the environment during transportation and processing.

As the scope of the present application excludes cultivation, environmental concerns within the EU related to the use of glyphosate herbicides on maize MON 88017 x MON 810 do not apply.

Maize MON 88017 x MON 810 has been developed for protection respectively against specific coleopteran (*Diabrotica* spp.) and lepidopteran pests, including the European Corn Borer (*Ostrinia nubilalis*) and pink borers (*Sesamia* spp.*)* and tolerance to glyphosate. Insect resistance is achieved by expression of a modified Cry3Bb1 protein from a transgene derived from *Bacillus thuringiensis* subspecies *kumamotoensis* in maize MON 88017 and Cry1Ab protein from a transgene derived from *Bacillus thuringiensis* subsp. *kurstaki* in maize MON 810 and tolerance to glyphosate is conferred by expression of CP4 EPSPS protein from a transgene derived from *Agrobacterium sp.* strain CP4 in maize MON 88017 (see Section 3.2).

6.1.1. Evaluation of single maize events

In its previous scientific opinions, the EFSA GMO Panel was of the opinion that both the single maize events MON 88017 and MON 810 are as safe as conventional maize (EFSA, 2009a,b) and that the placing on the market of maize MON 88017 and MON 810, for import and processing for food and feed uses, is unlikely to have an adverse effect on human or animal health, or on the environment. Furthermore, post-market environmental monitoring plans, including general surveillance, were proposed by the applicant and accepted by the EFSA GMO Panel for maize MON 88017 and MON 810 (EFSA, 2009a,b).

6.1.2. Environmental risk assessment

6.1.2.1. Unintended effects on plant fitness due to the genetic modification

Maize is highly domesticated and generally unable to survive in the environment without cultivation. Maize plants are not winter hardy in many regions of Europe, they have lost their ability to release seeds from the cob and they do not occur outside cultivated land or disturbed habitats in agricultural landscapes of Europe, despite cultivation for many years.

The herbicide tolerance trait can only be regarded as providing an agronomic advantage for this GM maize plant where and when glyphosate herbicides are applied. Similarly insect resistance against certain lepidopteran and coleopteran pests provides a potential advantage in cultivation under infestation conditions. However survival of maize outside of cultivation in Europe is mainly limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens and frost. Since these general characteristics are unchanged in maize MON 88017 x MON 810, herbicide tolerance and insect resistance are not likely to provide a selective advantage outside of cultivation in Europe. Therefore it is considered very unlikely that plants or volunteers of maize MON 88017 x MON 810, or its progeny will differ from conventional maize varieties in their ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

Applicant's field trials have shown that there are no indications of an altered fitness of the single maize events MON 88017 and MON 810 as compared to conventionally bred hybrids with similar genetic background (EFSA, 2009a,b). In addition to the field trials carried out with the single events, a series of additional field trials with maize MON 88017 x MON 810 were carried out across 4 locations in 2002 in USA. These field trial data do not show a change in fitness and invasiveness or weediness, except when glyphosate herbicides are applied and/or under

infestation conditions of specific target organisms. In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of any scientific report of increased spread and establishment of maize MON 88017 x MON 810 and any change in survival capacity, including over-wintering.

Since maize MON 88017 x MON 810 has no altered survival, multiplication or dissemination characteristics except when glyphosate herbicides are applied and/or under infestation conditions of target pests, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this maize event will not differ from that of maize MON 88017 or MON 810 or that of conventional maize varieties.

6.1.2.2. Gene transfer

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

Current scientific knowledge (see EFSA, 2009c for further details) suggests that gene transfer from GM plants to microorganisms under natural conditions is extremely unlikely, and that its establishment would occur primarily through homologous recombination in microorganisms.

Cry3Bb1, *cry1Ab* and *cp4 epsps* genes, as expressed in maize MON 88017 x MON 810, are of bacterial origin. As the functional genes are already present in microorganisms in the natural environment, homologous recombination and acquisition of these genes by microorganisms will not alter the gene pool of the natural microbial community.

In addition, the *cry3Bb1*, *cry1Ab* and *cp4 epsps* genes in maize MON 88017 x MON 810 are under the control of eukaryotic promoters with limited, if any, activity in prokaryotic organisms (EFSA, 2009a,b).

Transgenic DNA is a component of many food and feed products derived from GM maize. Therefore, microorganisms in the digestive tract of humans and animals (domesticated animals and other animals feeding on fresh and decaying GM plant material) may be exposed to transgenic DNA although DNA becomes degraded in the human or animal digestive tract.

In the case of accidental release and establishment of maize MON 88017 x MON 810 in the environment, exposure of microorganisms to transgenic DNA derived from GM maize plants would take place during natural decay of GM plant material and/or pollen in the soil of areas where GM plants establish.

Taking into account the microbial origin and/or nature of the *cry3Bb1*, *cry1Ab* and *cp4 epsps* genes and the lack of selective pressure in the intestinal tract and/or the environment, the likelihood that horizontal gene transfer would result in increased fitness on microorganisms or other selective advantages is very small. For this reason it is very unlikely that genes from maize MON 88017 x MON 810 would become established in the genome of microorganisms in the environment or human and animal digestive tract. In the very unlikely event that such a horizontal gene transfer would take place, no adverse effects on human and animal health or the

environment are expected, as no principally new traits would be introduced into or expressed by natural microbial communities.

b) Plant to plant gene transfer

The extent of cross-pollination of other maize varieties will mainly depend upon the scale of accidental release during transportation and processing. 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 GM maize plants originating from accidental release occurring during transportation and processing is unlikely to disperse significant amounts of GM maize pollen to other maize plants. Field observations performed on GM maize volunteers 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).

Herbicide tolerance and insect resistance provide agronomic advantages in cultivation where and when the specific herbicides are applied and/or under infestation conditions of the specific target organisms. However survival of maize outside of cultivation in Europe is mainly limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens and frost. Since these general characteristics of this GM maize are unchanged in maize MON 88017 x MON 810, herbicide tolerance and insect resistance are not likely to provide selective advantages outside cultivation in Europe. Therefore, as for any other maize varieties, GM plants would only survive in subsequent seasons in the warmer regions of Europe and are not likely to establish feral populations under European environmental conditions.

In conclusion, since maize MON 88017 x MON 810 has no altered survival, multiplication or dissemination characteristics except when cultivated in the presence of the glyphosate herbicides and/or under target pest infestation conditions, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this maize in Europe will not differ from that of maize MON 88017 and MON 810 or of other maize varieties.

6.1.2.3. Interactions between the GM plant and target organisms

The intended uses of maize MON 88017 x MON 810 specifically exclude cultivation and environmental exposure to maize MON 88017 x MON 810 plants is limited to the accidental release of viable grains into the environment during transportation and processing. The EFSA GMO Panel considers that it would need successful establishment and spread of high numbers of maize MON 88017 x MON 810 to enable any significant interaction with target organisms, which is very unlikely (see Section 6.1.1.1).

Environmental exposure to Cry3Bb1 and Cry1Ab proteins is otherwise limited to manure and faeces from the gastrointestinal tracts of animals fed maize MON 88017 x MON 810. Data supplied by the applicant suggest that only a small amount of the Cry3Bb1 and Cry1Ab proteins enter the environment due to low expression in grains. Moreover, most Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only a very low amount of Cry proteins would remain intact to pass out in faeces (Einspanier et al., 2004, Ahmad et al., 2005, Lutz et al., 2005, Lutz et al., 2006, Wiedemann et al., 2006, Guertler et al., 2008). It can thus be

concluded that the level of exposure of target organisms to the Cry3A protein is likely to be extremely low and of no biological relevance.

6.1.2.4. Interactions between the GM plant and non-target organisms

Considering the proposed uses of maize MON 88017 x MON 810, the environmental risk assessment is concerned with exposure through manure and faeces from the gastrointestinal tracts of animals fed on this GM maize and with accidental release into the environment of GM viable grains during transportation and processing.

The EFSA GMO Panel assessed whether the Cry3Bb1 and Cry1Ab proteins might potentially affect non-target organisms by entering the environment through manure and faeces from the gastrointestinal tracts of animals fed maize MON 88017 x MON 810. Due to the selectivity of the Cry proteins, non-target organisms most likely to be affected by the Cry3Bb1 and Cry1Ab proteins are those belonging to a similar taxonomic group as that of the target organisms.

Data supplied by the applicant on both single events (EFSA, 2009a,b) and on the hybrid in the present application and literature on Cry proteins and references therein suggest that only very low amounts of the Cry3Bb1 and Cry1Ab proteins enter the environment due to low expression in grains. Moreover, most Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only a very low amount of Cry proteins would remain intact to pass out in faeces (Einspanier et al., 2004, Ahmad et al., 2005, Lutz et al., 2005, Lutz et al., 2006, Wiedemann et al., 2006, Guertler et al., 2008). There would subsequently be further degradation of the Cry proteins in the manure and faeces due to microbial processes.

Exposure of soil and water environments to the Cry3Bb1 and Cry1Ab proteins from disposal of animal wastes or accidental spillage of maize grains is likely to be very low and localized (Hopkins and Gregorich, 2003, Baumgarte and Tebbe, 2005). While Cry proteins can bind to a certain extent to clay minerals and humic substances in soil, thereby potentially reducing their availability to microorganisms for degradation, a number of studies revealed that there is no persistence and accumulation of Cry proteins from GM crops in soil (Herman et al., 2001, Head et al., 2002, Herman et al., 2002, Hopkins and Gregorich, 2003, Ahmad et al., 2005, Baumgarte and Tebbe, 2005, Dubelman et al., 2005, Vaufleury et al., 2007, Icoz and Stotzky, 2008, Shan et al., 2008).

Considering the scope of the application (that excludes cultivation) and the intended uses of maize MON 88017 x MON 810, it can be concluded that the level of exposure of potentially sensitive non-target organisms to the Cry3Bb1 and Cry1Ab proteins expressed in maize MON 88017 x MON 810 in combination with the CP4 EPSPS protein is likely to be very low and of no biological relevance.

6.1.2.5. Interactions with the abiotic environment and on biogeochemical processes

Considering the scope of the application and the intended uses of maize MON 88017 x MON 810 and due to the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles were not considered an issue by the EFSA GMO Panel.

6.1.3. Post-market environment 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 which 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 the EFSA GMO Panel. However, the EFSA GMO Panel gives its opinion on the scientific quality of the monitoring plan provided by the applicant (EFSA, 2006a,b). The potential exposure to the environment of maize MON 88017 x MON 810 would be through manure and faeces from the gastrointestinal tracts of animals fed maize MON 88017 x MON 810 or through accidental release into the environment of GM viable grains during transportation and processing.

No specific environmental impact of maize MON 88017 x MON 810 was indicated by the environmental risk assessment and thus no case-specific monitoring is required.

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 applicants, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment, and (2) a coordinating system newly established by EuropaBio for the collection of the information recorded by the various operators (Lecoq et al., 2007, Windels et al., 2008); (3) the use of networks of existing surveillance systems. The applicant proposes to submit a general surveillance report on an annual basis and a final report at the end of the consent.

The EFSA GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicant is in line with the intended uses of maize MON 88017 x MON 810 since the environmental risk assessment does not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan. The EFSA GMO Panel advises that appropriate management systems should be in place to prevent seeds of maize MON 88017 x MON 810 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

6.2 Conclusion

The scope of the application is for food and feed uses, import and processing of maize MON 88017 x MON 810 and excludes cultivation. Considering the proposed uses of maize MON 88017 x MON 810, the environmental risk assessment is concerned with exposure through manure and faeces from gastrointestinal tracts of animals fed maize MON 88017 x MON 810 and with the accidental release into the environment of maize MON 88017 x MON 810 viable grains during transportation and processing.

There are no indications of increased likelihood of establishment or survival of feral maize plants in case of accidental release into the environment of MON 88017 x MON 810 viable grains during transportation and processing for food and feed uses. Taking into account the scope of the

application, both the rare occurrence of sporadic feral plants and the low levels of exposure through other routes indicate that the risk to non-target organisms is considered negligible.

The scope of the monitoring plan provided by the applicant is in line with the intended uses of maize MON 88017 x MON 810 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. Furthermore the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out a scientific risk assessment of the maize MON 88017 x MON 810 for food and feed uses, import and processing.

The EFSA GMO Panel is of the opinion that the molecular characterisation provided for maize MON 88017 x MON 810 produced by conventional breeding is sufficient for the safety assessment. The bioinformatic analysis of the inserted DNA and the flanking regions of the single events MON 88017 and MON 810 do not raise any safety concern. The expression of the genes introduced by the genetic modification has been sufficiently analysed and proved to be comparable to the ones found in the single events. The stability of the genetic modifications has been demonstrated over several generations in the single events, and southern analysis confirmed that their structures were maintained in the hybrid. The EFSA GMO Panel considers that the molecular characterisation does not indicate any safety concern.

Based on the results of the comparative analysis it was concluded that maize MON 88017 x MON 810 is compositionally and agronomically equivalent to conventional maize, except for the presence of Cry3Bb1, CP4 EPSPS and Cry1Ab proteins in maize MON 88017 x MON 810. Based on the assessment of data available, including the additional information provided by the applicant in response to the EFSA GMO Panel's request, for maize MON 88017 x MON 810, for the single events and for appropriate non-GM controls, the EFSA GMO Panel has found no indication that crossing of MON 88017 and MON 810 results in an interaction between the single events which causes compositional or agronomic changes. The Cry3Bb1 and CP4 EPSPS proteins expressed in the parental maize line MON 88017, and the Cry1Ab protein expressed in the parental maize MON 810 have been assessed previously and no safety concerns were identified. Given all the information provided, the EFSA GMO Panel concludes that interactions between the single events that might impact on food and feed safety are unlikely and that the nutritional properties of maize MON 88017 x MON 810 would be not different from those of conventional maize. In conclusion, the EFSA GMO Panel considers that maize MON 88017 x MON 810 is as safe and as nutritious as its non-GM counterpart and that the overall allergenicity of the whole plant is not changed and concludes that maize MON 88017 x MON 810 is unlikely to have any adverse effect on human and animal health in the context of its intended uses.

Considering the intended uses of maize MON 88017 x MON 810, which exclude cultivation, there is no requirement for scientific assessment of possible environmental effects associated with the cultivation of this GM maize. In case of accidental release into the environment of maize MON 88017 x MON 810 viable grains during transportation and processing, there are no indications of increased likelihood of establishment or survival of feral maize plants. Also, the low levels of environmental exposure through other routes indicate that the risk to target and non-target organisms is likely to be extremely low. The scope of the post-market environmental

monitoring plan provided by the applicant is in line with the intended uses of maize MON 88017 x MON 810.

In conclusion, the EFSA GMO Panel considers that information available for maize MON 88017 x MON 810 addresses the comments raised by the Member States and considers it unlikely that maize MON 88017 x MON 810 will have any adverse effect on human and animal health or on the environment in the context of its intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the Czech Republic, dated 03 January 2006, concerning a request for placing on the market of maize MON 88017 x MON 810 in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 18 January 2006, from EFSA to the Competent Authority of the Czech Republic.

3. Letter from EFSA to the applicant, dated 25 January 2007, requesting additional information under completeness check.

4. Letter from the applicant to EFSA, dated 2 February 2007, providing additional information under completeness check.

5. Letter from EFSA to the applicant, dated 21 February 2007, delivering the 'Statement of Validity' for application EFSA-GMO-CZ-2006-33, maize MON 88017 x MON 810 submitted by Monsanto Europe, S.A. under Regulation (EC) No 1829/2003.

6. Letter from EFSA to the applicant, dated 26 March 2007, stopping the clock .

7. Letter from the applicant to EFSA, dated 10 December 2007, providing additional information.

8. Letter from EFSA to the applicant, dated 13 March 2008, requesting additional information and maintaining the clock stopped.

9. Letter from the applicant to EFSA, dated 16 April 2008, providing additional information.

10. Letter from the applicant to EFSA, dated 18 April 2008, providing the timeline for submission of response.

11. Letter from the applicant to EFSA, dated 13 October 2008, providing additional information.

12. Letter from EFSA to the applicant, dated 08 April 2009, restarting the clock.

REFERENCES

Ahmad A, Wilde GE and Zhu KY, 2005. Detectability of coleopteran-specific Cry3Bb1 protein in soil and its effect on nontarget surface and below-ground arthropods. *Environmental Entomology* 34, 385-394.

Baumgarte S and Tebbe CC, 2005. Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. *Molecular Ecology* 14, 2539-2551.

CAC, 2003. Codex principles and guidelines on foods derived from biotechnology. Joint FAO/WHO Food Standards Programme, Food and Agriculture Organisation, Rome.

Dubelman S, Ayden BR, Bader BM, Brown CR, Jiang CJ and Vlachos D, 2005. Cry1Ab protein does not persist in soil after 3 years of sustained Bt corn use. *Environmental Entomology* 34, 915-921.

Eastham K and Sweet J, 2002. Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. European Environment Agency (EEA).

EC, 1991. Council Directive of 15 July 1991 concerning the placing of plant protection products on the market (91/414/EEC). *Official Journal of the European Union* L 230, 19.8.1991, 1-32.

EC, 2003. Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. *Official Journal of the European Union* L268, 1-23.

EFSA, 2005a. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Notification (Reference C/DE/02/9) for the placing on the market of insect-protected genetically modified maize MON 863xMON 810, for import and processing, under Part C of Directive 2001/18/EC from Monsanto. *The EFSA Journal* (2005), 251, 1-22, http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_opinion_ej251_mon863x810_en1,1.pdf

EFSA, 2005b. Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-DE-2004-03) for the placing on the market of insect protected genetically modified maize MON 863 x MON 810, for food and feed use, under Regulation (EC) No 1829/2003 from Monsanto. *The EFSA Journal* (2005), 252, 1-23. http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_opinion_ej252_mon863x810_2_en1.pdf

EFSA, 2005c. Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-BE-2004-07) for the placing on the market of insect-protected glyphosate-tolerant genetically modified maize MON 863 x MON 810 x NK603, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 from Monsanto. *The EFSA Journal* (2005), 256, 1-25. http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_opinion_ej256_mon863xMON 810xnk603_en1,0.pdf

EFSA, 2005d. Opinion of the Scientific Panel on genetically modified organisms [GMO] related to the notification (Reference C/GB/02/M3/3) for the placing on the market of glyphosate-tolerant and insect-resistant genetically modified maize NK603 x MON 810, for import and processing, under Part C of Directive 2001/18/EC from Monsanto. *The EFSA Journal* (2005), 308, 1-22. http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_op_ej308_maize%20NL603xMON 810_en,0.pdf

EFSA, 2005e. Opinion of the Scientific Panel on genetically modified organisms [GMO] on an application (Reference EFSA-GMO-UK-2004-06) for the placing on the market of insect-protected glyphosate-tolerant genetically modified maize MON 863 x NK603, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 from Monsanto. *The EFSA Journal* (2005), 255, 1-25. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620770806.htm

EFSA, 2006a. Guidance document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. The *EFSA Journal* (2006) 99, 1-100. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620770806.htm

EFSA, 2006b. Opinion of the Scientific Panel on Genetically Modified Organisms on the Post Market Environmental Monitoring (PMEM) of genetically modified plants. *The EFSA Journal* (2006) 319, 1-27. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620769727.htm

EFSA, 2007. Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events. *The EFSA Journal* (2007) 512, 1-5. http://www.efsa.europa.eu/cs/BlobServer/Scientific_Document/gmo_guidance_ej512_GM_stacked_events_en.pdf

EFSA, 2009a. Scientific Opinion of the Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-CZ-2005-27) for the placing on the market of the insect-resistant and herbicide-tolerant genetically modified maize MON 88017, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. *The EFSA Journal* (2009) 1075, 1-28. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902517555.htm

EFSA, 2009b. Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMO-RX-MON810) for the renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON 810; (2) feed consisting of and/or containing maize MON 810, including the use of seed for cultivation; and of (3) food and feed additives, and feed materials produced from maize MON810, all under Regulation (EC) No 1829/2003 from Monsanto. *The EFSA Journal* (2009) 1149, 1-27.

EFSA, 2009c. Statement of EFSA on the consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in

Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants". *The EFSA Journal* (2009) 1108, 1-8.

Einspanier R, Lutz B, Rief S, Berezina O, Zverlov V, Schwarz W and Mayer J, 2004. Tracing residual recombinant feed molecules during digestion and rumen bacterial diversity in cattle fed transgene maize. *European Food Research and Technology* 218, 269-273.

Guertler P, Lutz B, Kuehn R, Meyer HHD, Einspanier R, Killermann B and Albrecht C, 2008. Fate of recombinant DNA and Cry1Ab protein after ingestion and dispersal of genetically modified maize in comparison to rapeseed by fallow deer (Dama dama). *European Journal of Wildlife Research* 54, 36-43.

Head G, Surber JB, Watson JA, Martin JW and Duan JJ, 2002. No detection of Cry1Ac protein in soil after multiple years of transgenic Bt cotton (Bollgard) use. *Environmental Entomology* 31, 30-36.

Herman RA, Evans SL, Shanahan DM, Mihaliak CA, Bormett GA, Young DL and Buehrer J, 2001. Rapid degradation of Cry1F delta-endotoxin in soil. *Environmental Entomology* 30, 642-644.

Herman RA, Wolt JD and Halliday WR, 2002. Rapid degradation of the Cry1F insecticidal crystal protein in soil. *Journal of Agricultural and Food Chemistry* 50, 7076-7078.

Hopkins DW and Gregorich EG, 2003. Detection and decay of the Bt endotoxin in soil from a field trial with genetically modified maize. *European Journal of Soil Science* 54, 793-800.

Icoz I and Stotzky G, 2008. Fate and effects of insect-resistant Bt crops in soil ecosystems. *Soil Biology & Biochemistry* 40, 559-586.

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

Lutz B, Wiedemann S and Albrecht C, 2006. Degradation of transgenic Cry1Ab DNA and protein in Bt-176 maize during the ensiling process. *Journal of Animal Physiology and Animal Nutrition* (Berl) 90, 116-23.

Lutz B, Wiedemann S, Einspanier R, Mayer J and Albrecht C, 2005. Degradation of Cry1Ab protein from genetically modified maize in the bovine gastrointestinal tract. *Journal of Agriculture and Food Chemistry* 53, 1453-6.

OECD, 2002. Consensus Document on compositional considerations for new varieties of maize (Zea mays): Key food and feed nutrients, anti-nutrients and secondary plant metabolites (ENV/JM/MONO(2002)25), 6, 1-42.

OECD, 2003. Consensus document on the biology of Zea mays subsp. Mays (Maize). Series on Harmonisation of Regulatory Oversignt in Biotechnology. OECD.

Palaudelmàs, M., Peñas, G., Melé, E., Serra, J., Salvia, J., Pla, M., Nadal, A., Messeguer, J., 2009. Effect of volunteers on maize gene flow. *Transgenic Research*, pre-print published online, DOI:10.1007/s11248-009-9250-7.

Shan G, Embrey SK, Herman RA and McCormick R, 2008. Cry1F protein not detected in soil after three years of transgenic Bt corn (1507 corn) use. *Environmental Entomology* 37, 255-262.

Vaufleury Ad, Kramarz PE, Binet P, Cortet J, Caul S, Andersen MN, Plumey E, Coeurdassier M and Krogh PH, 2007. Exposure and effects assessments of Bt-maize on non-target organisms (gastropods, microarthropods, mycorrhizal fungi) in microcosms. *Pedobiologia* 51, 185-194.

Wiedemann S, Lutz B, Kurtz H, Schwarz FJ and Albrecht C, 2006. In situ studies on the time-dependent degradation of recombinant corn DNA and protein in the bovine rumen. *Journal of Animal Science* 84, 135-144.

Windels P, Alcalde E, Lecoq E, Legris G, Pleysier A, Tinland B and Wandelt C, 2008. General Surveillance for Import and Processing: the EuropaBio approach. *Journal für Verbraucherschutz und Lebensmittelsicherheit-Journal of Consumer Protection and Food Safety* 3, 14-16.