SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-NL-2007-39) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON89034 x MON88017 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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This opinion, published on 27th May 2010, replaces the earlier version published on 30st March 2010[4].

ABSTRACT

This opinion reports on an evaluation of a risk assessment for placing on the market the genetically modified herbicide tolerant and insect resistant maize MON89034 x MON88017 for food and feed uses, import and processing. Conventional breeding methods were used in the production of maize MON89034 x MON88017 from inbred lines of the respective parental events. The structural integrity of the inserts in the single events as well as the phenotypes were retained in the hybrid. The expression levels of the Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS proteins in maize MON89034 x MON88017 were demonstrated to be comparable with those of the single events. The comparative analysis of compositional, phenotypic and agronomic characteristics of this GM maize indicated equivalence with its conventional counterpart and commercial non-GM maize varieties except for the expression of the target proteins, providing resistance to certain lepidopteran and coleopteran pests and tolerance to glyphosate herbicide. The safety assessment identified no concerns regarding potential toxicity and allergenicity of maize MON89034 x MON88017. A feeding study on broiler chickens confirmed the nutritional equivalence of this GM maize to its conventional counterpart and commercial non-GM maize varieties. Considering the intended uses of maize MON89034 x MON88017, which excludes cultivation within the European Union, no scientific assessment of potential environmental effects associated with cultivation of maize MON89034 x MON88017 was required. In case of accidental release of viable maize grain of MON89034 x MON88017 into the

1 On request from the Competent Authority of the Netherlands on an application (EFSA-GMO-NL-2007-39) submitted by Monsanto, Question No EFSA-Q-2007-056, adopted on 10 March 2010.

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3 Acknowledgement: The EFSA GMO Panel wishes to thank the members of the Working Group on Molecular characterisation, Food and Feed and Environment for the preparation of this opinion, Ilona Kryspin-Sorensen as external expert and EFSA's staff members Anna Christodoulidou, Karine Lheureux and Nancy Podevin for the support provided to this EFSA scientific output.

4 In section References on page 25, the EFSA GMO Panel corrected the list of references (some references were not listed). The changes do not affect the overall conclusions of the scientific opinion. To avoid confusion, the original version of the scientific opinion has been removed from the website, but is available on request as is a version showing the changes made.

Suggested citation: EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on application (EFSA-GMO-NL-2007-39) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON89034 x MON88017 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2010; 8(3):1564. [27 pp.]. doi:10.2903/j.efsa.2010.1564.Available online: www.efsa.europa.eu

environment during transportation and processing, there are no indications of increased likelihood of establishment or survival of feral maize plants except in the presence of the glyphosate herbicides. In conclusion, the EFSA GMO Panel considers that the information available for maize MON89034 x MON88017 addresses the scientific comments raised by the Member States and that the maize MON89034 x MON88017 as described in this application is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of its intended uses. The EFSA GMO Panel concludes that maize event MON89034 x MON88017 is unlikely to have any adverse effect on human and animal health and the environment, in the context of its intended uses.

KEY WORDS

GMO, maize (*Zea mays*), maize MON89034 x MON88017, insect resistant, herbicide tolerant, risk assessment, food and feed safety, environment, import and processing, Regulation (EC) No 1829/2003.

SUMMARY

Following the submission of an application (EFSA-GMO-NL-2007-39) under Regulation (EC) No 1829/2003 from Monsanto, the Panel on Genetically Modified Organisms was asked to deliver a scientific opinion on herbicide tolerant and insect resistant genetically modified (GM) maize MON89034 x MON88017 (Unique identifier MON-89Ø34-3 × MON-88Ø17-3) for food and feed uses, import and processing.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-NL-2007-39, additional information supplied by the applicant and scientific comments submitted by Member States. Further information from applications for placing the single events MON89034 and MON88017 on the market under EU regulatory procedures was taken into account where appropriate. The scope of application EFSA-GMO-NL-2007-39 is for food and feed uses, import and processing of maize MON89034 x MON88017 and all derived products, but excludes cultivation in the EU. The EFSA GMO Panel assessed maize MON89034 x MON88017 with reference to the intended uses and appropriate principles described in the Guidance Documents of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed and for the risk assessment of genetically modified plants containing stacked transformation events. The scientific assessment included molecular characterisation of the inserted DNA and expression of target proteins. A comparative analysis of agronomic traits and composition was undertaken, and the safety of the new protein and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment of environmental impacts and the post-market environmental monitoring plan were undertaken.

Maize MON89034 and MON88017 have been developed for protection respectively against specific lepidopteran (*Ostrinia nubilalis*, *Spodoptera* spp., *Agrotis ipsilon*) and coleopteran (*Diabrotica* spp.) pests and for tolerance to glyphosate herbicides. Lepidopteran resistance is achieved by expression of the Cry1A.105 and Cry2Ab2 proteins derived from *B. thuringiensis* subsp. *kurstaki* in maize MON89034 and coleopteran resistance by expression of Cry3Bb1 protein from B. *thuringiensis* subsp. *kumamotoensis*) in maize MON88017, while tolerance to glyphosate is conferred by expression of CP4 EPSPS protein from a transgene derived from *Agrobacterium tumefaciens (*renamed *Rhizobium radiobacter)* strain CP4 in maize MON88017.

Molecular analysis of the DNA present in maize MON89034 x MON88017 confirmed that maize MON89034 and MON88017 inserts are present and that their structures are retained. With regard to the expression of Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS proteins, the overall protein levels were comparable between maize MON89034 x MON88017 and the respective single events.

Based on results of the comparative analysis the EFSA GMO Panel concludes that maize MON89034 x MON88017 is compositionally, phenotypically and agronomically equivalent to its conventional counterpart and commercial non-GM maize varieties, except for the presence of Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS proteins in maize MON89034 x MON88017. 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 MON89034 x MON88017, for the single events and for its conventional counterpart and commercial non-GM maize varieties, the EFSA GMO Panel has found no indication that crossing of MON89034 with MON88017 maize results in an interaction between the single events which causes compositional, phenotypic or agronomic changes. The Cry1A.105 and Cry2Ab2 expressed in the parental maize line MON89034 and the Cry3Bb1 and CP4 EPSPS proteins expressed in the parental maize line MON88017 have been assessed previously and no safety concerns were identified. Given all of the information provided, the EFSA GMO Panel concludes that there is no evidence for interactions between the single events that might impact on food and feed safety. The nutritional value of maize MON89034 x MON88017 has been investigated in a feeding study with broilers which confirmed that the nutritional properties of maize MON89034 x MON88017 would be no different from those of its conventional counterpart and commercial non-GM maize varieties.

The application EFSA-GMO-NL-2007-39 concerns food and feed uses, import and processing, but excludes cultivation in the EU. Therefore, there is no requirement for scientific assessment of possible environmental effects associated with the cultivation of maize MON89034 x MON88017. There are no indications of an increased likelihood of establishment and spread of feral maize plants in case of accidental release into the environment of viable maize MON89034 x MON88017 grains during transportation and processing for food and feed uses, except in the presence of glyphosate. Taking into account the scope of the application, both the rare occurrence of feral maize plants and the low levels of exposure through other routes indicate that the risk to non-target organisms is extremely low. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize MON89034 x MON88017. 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 MON89034 x MON88017 addresses the scientific comments raised by the Member States and that the maize MON89034 x MON88017 as described in this application is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of its intended uses. The EFSA GMO Panel concludes that maize event MON89034 x MON88017 is unlikely to have any adverse effect on human and animal health and the environment, in the context of its intended uses.

TABLE OF CONTENTS

BACKGROUND

On 12/02/2007, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands an application (Reference EFSA-GMO-NL-2007-39), for authorisation of the insect resistant and herbicide tolerant genetically modified (GM) maize MON89034 x MON88017 (Unique Identifier MON-89Ø34-3 × MON-88Ø17-3), submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed. After receiving the application EFSA-GMO-NL-2007-39 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 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 23/08/2007 and 11/09/2007, EFSA received additional information requested under completeness check (requested on 01/08/2007 and 05/09/2007 respectively). On 20/09/2007, 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 Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC 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 acknowledgement of the valid application (19/12/2007) within which to make their opinion known.

The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out a scientific assessment of the GM maize MON89034 x MON88017 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 assessment, the EFSA GMO Panel took into account the appropriate principles described in the Guidance Documents of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006c) and for the risk assessment of genetically modified plants containing stacked transformation events (EFSA, 2007), the scientific comments of Member States and the additional information provided by the applicant. Further information from applications for placing the single events MON89034 and MON88017 on the market under EU regulatory procedures was taken into account where appropriate.

On 23/10/2007, 19/11/2007, 10/12/2007, 18/12/2008, 28/01/2009, 08/04/2009, 29/05/2009, 30/06/2009, 05/11/2009, the EFSA GMO Panel requested from the applicant additional information. The applicant provided the requested information on 30/10/2007, 30/11/2007 and on 07/12/2007, 17/04/2008 (spontaneously submitted), 06/10/2008, 08/01/2009, 19/02/2009, 03/06/2009, 30/06/2009, 08/09/2009, 11/11/2009. After receipt and assessment of the full data package the EFSA GMO Panel finalised its risk assessment on maize MON89034 x MON88017.

The single events MON89034 and MON88017 have been the subjects of earlier assessments and have received an EFSA scientific opinion in favour of their authorisation (EFSA, 2008, 2009a).

In giving its scientific opinion on GM maize MON89034 x MON88017 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 MON89034 x MON88017 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.

Assessment

1. Introduction

The genetically modified maize MON89034 x MON88017 (Unique Identifier MON-89Ø34-3 × MON-88Ø17-3) was assessed with reference to its intended uses, taking account of the appropriate principles described in the Guidance Documents of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006b) and for the risk assessment of genetically modified plants containing stacked transformation events (EFSA, 2006a). The risk assessment presented here is based on the information provided in the application relating to maize MON89034 x MON88017 submitted in the EU including additional information from the applicant and information on the single events, as well as scientific comments that were raised by the Member States.

2. Issues raised by Member States

The scientific comments raised by Member States are addressed in details in Annex G of the EFSA overall opinion[5] and have been considered throughout this EFSA GMO Panel scientific opinion.

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Method of production of maize MON89034 × MON88017

Conventional breeding methods were used to produce maize MON89034 × MON88017 and no new genetic modification was involved. The two inserts that are present in maize MON89034 × MON88017 were derived from maize lines containing two independent events: MON89034 and MON88017. 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, 2008, 2009a). Maize MON89034 × MON88017 combines resistance to certain lepidopteran (*Ostrinia nubilalis*, *Spodoptera* spp., *Agrotis ipsilon*) and coleopteran (*Diabrotica* spp.) pests and tolerance to glyphosate-containing herbicides.

3.1.2. Summary of the evaluation of the single events

Maize MON89034

Maize MON89034 was developed through *Agrobacterium*-mediated transformation using the binary plasmid vector PV-ZMIR245 containing two separate T-DNAs. The first T-DNA, designated as T-DNA I, contains the *cry1A*.105 and the *cry2Ab2* expression cassettes providing increased protection to lepidopteran pests such as European corn borer (*Ostrinia nubilalis*) fall armyworm (*Spodoptera* ssp.), black cutworm (*Agrotis ipsilon*) and corn earworm (*Helicoverpa zea*). The second T-DNA, designated as T-DNA II, contains the *npt*II expression cassette that encodes neomycin phosphotransferase that confers tolerance to certain antibiotics such as neomycin and kanamycin. The use of the two-T-DNA approach facilitates integration of the two different T-DNAs at genetic loci which can be segregated by breeding. Conventional breeding was used to isolate plants that contain the *cry1A*.105 and the *cry2Ab2* expression cassettes (T-DNA I) but do not contain the *npt*II expression cassette (T-DNA II). This was confirmed by molecular analysis (EFSA, 2008).

[5] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-056

Molecular characterisation data established that MON89034 contains one copy of T-DNA I and that T-DNA II and vector backbone sequences are absent. The structure of the insert in maize MON89034 was determined by Southern analysis and DNA sequencing. Data indicate that the *Cauliflower mosaic virus e35S* promoter that regulates expression of the *cry1A.105* gene has been truncated and that the right border region of the T-DNA has been replaced by a left border region.

Sequence comparison between the corresponding genomic region of conventional maize and the flanking regions of the maize MON89034 indicated that the pre-insertion locus was preserved except for the deletion of 57 bp and the addition of 10 bp. An updated bioinformatic analysis was performed (Tu and Silvanovich, 2009a, b). The data confirmed that no known endogenous maize coding sequences or regulatory sequences have been disrupted by the insert. Updated bioinformatic analysis also revealed no biologically relevant homologies to allergens or toxins for any of the putative polypeptides that might be produced from ORFs spanning the junction regions (Girault and McClain, 2008; Tu and Silvanovich, 2009c, d, h).

Southern analysis of maize MON89034 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations.

Maize MON88017

Maize MON88017 was developed through *Agrobacterium*-mediated transformation using the PV-ZMIR39 plasmid and as a result expresses the modified *B. thuringiensis* (subsp. *kumamotoensis*) *cry3Bb1* and CP4 *epsps* genes conferring resistance to coleopteran insect pests (*Diabrotica* spp.) and resulting in tolerance to glyphosate-containing herbicides, respectively. Molecular characterisation data established that MON88017 contains one copy of the T-DNA and that vector backbone sequences are absent (EFSA, 2009a).

Similarity searches revealed that the flanking regions of the insert in maize MON88017 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 (Tu and Silvanovich, 2009e, f). The data indicated that the insert is located approximately 100 bp upstream of a region corresponding to a maize full-length cDNA potentially coding for a protein with sequence similarity to putative purine permeases. This analysis confirmed previous bioinformatic analyses. Phenotypic, agronomic and compositional analyses showed that MON88017 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 that would raise a safety concern. Updated bioinformatic analysis also revealed no biologically relevant similarity to allergens or toxins for any of the putative peptides that might be produced from open reading frames spanning the junction regions (Girault *et al.*, 2008; Tu and Silvanovich, 2009g, i).

Southern analysis of MON88017 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations.

3.1.3. Transgene constructs in maize MON89034 × MON88017

Maize MON89034 × MON88017 has been obtained by conventional crossing of MON89034 and MON88017. 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 MON89034 and MON88017 inserts and restriction enzymes informative of the structure of both events, including the junctions with the host genomic DNA and confirmed the integrity of the single events when combined in maize MON89034 × MON88017.

3.1.4. Information on the expression of the inserts

The levels of the newly expressed proteins Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS in MON89034 × MON88017 were analysed by validated enzyme-linked immunosorbent assays (ELISA). Tissue samples for analysis were collected from five field trials conducted in USA during 2005 (Hartmann *et al.*, 2006, b). The trials were located within the major maize-growing regions of the USA and provided a variety of environmental conditions. At each site, maize MON89034 × MON88017, an appropriate conventional counterpart, MON89034 and MON88017 were planted using a randomized complete block design.

The scope of the application covers food and feed uses and import and processing and excludes cultivation. Therefore protein expression data related to the grains are considered most relevant and are summarized in Table 1. Levels of proteins in the stacked line are comparable to those in the single events.

Table 1: Summary of protein expression levels in maize MON89034 × MON88017, MON89034 and MON88017 grains (µg/g dry weight)

	MON89034 x MON88017	**MON89034**	**MON88017**
Cry1A.105 mean range	5.6 [1.9-7.5]	5.8 [4.5-6.8]	--
Cry2Ab2 mean range	1.3 [0.8-1.9]	1.3 [0.8-1.9]	--
Cry3Bb1 mean range	4.1 [1.3-9.7]	--	4.4 [2.9-6.5]
CP4 EPSPS mean range	3.4 [2.2-4.7]	--	3.3 [1.8-4.8]

3.1.5. Inheritance and stability of inserted DNA

The genetic stability of the inserted DNA in events MON89034 and MON88017 was demonstrated previously (EFSA, 2008, 2009a). In maize MON89034 × MON88017 the two inserts are combined. The Southern analyses show that the integrity of the inserts present in the single events is retained in MON89034 × MON88017 (Groat *et al.*, 2006). 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 MON89034 × MON88017, no additional genetic modification was involved. Southern analyses demonstrated that the structures of the MON89034 and MON88017 events were retained in maize MON89034 × MON88017.
The expression levels of Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS proteins in the grains of maize MON89034 × MON88017 have been demonstrated to be comparable with those of the single events.

The EFSA GMO Panel concludes that the molecular characterisation of maize MON89034 x MON88017 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

Maize MON89034

Forage and grains of maize MON89034 and the same tissues from the conventional counterpart with a comparable genetic background were obtained from field trials carried out in the year 2004 in the USA and in the season 2004-2005 in Argentina. Both trials included five different locations representative of the respective geographical region. The trials used agronomic practices which were also representative of these regions. In addition to its conventional counterpart, a total of 15 commercial non-GM maize varieties were included in the field trial to estimate the naturally occurring variation in composition expected for the various analytes in conventional maize.

With regard to agronomic and phenotypic characteristics no consistent differences were observed between maize MON89034 and its conventional counterpart grown in the various field trials. With regard to compositional analyses, statistical difference between MON89034 and its conventional counterpart were identified but were not consistent across the different trial sites. All of the observed differences were small and fell within the natural variation found in the commercial non-GM maize varieties grown at these sites. Furthermore, the composition of maize MON89034 fell within natural variation as reported in the literature and crop composition databases (ILSI, 2006).

The EFSA GMO Panel concluded that maize MON89034 is equivalent to its conventional counterpart with regard to compositional, phenotypic and agronomic characteristics except for the expression of the target traits (EFSA, 2008).

Maize MON88017

Forage and grains of maize MON88017 plants sprayed with glyphosate and the same tissues from its conventional counterpart with a comparable genetic background were obtained from field trials carried out at three locations in the USA in 2002 and at four locations in Argentina in 2003-2004. Also commercial non-GM maize varieties were grown alongside maize MON88017 and its conventional counterpart in the same locations. The level of several compounds (vitamin B1, oleic acid, and linoleic acid) showed statistically significant differences between maize MON88017 and its conventional counterpart in the across-location and single site analysis during one of the seasons. However, these differences did not occur in the other season and were within the range of each constituent determined in non-GM varieties and/or obtained from historical data or information in the literature. Additional data from field trials in Europe were provided by the applicant at the request of the EFSA GMO Panel. In these cases, MON88017 not treated with glyphosate was grown at three locations in Germany and at three locations in Spain in 2007. Various statistically significant differences were observed between MON88017 and its conventional counterpart, none of which occurred within all locations and all of which were within the range of commercial non-GM maize varieties. Based on these data, the EFSA GMO Panel concluded that maize MON88017 is compositionally equivalent to its conventional counterpart and commercial non-GM maize varieties, except for the presence of Cry3Bb1 and CP4 EPSPS proteins in maize MON88017 due to the genetic modification.

No consistent differences were observed in the analysis of agronomic and phenotypic characteristics of MON88017 compared to its conventional counterpart and commercial non-GM maize varieties over several seasons and no consistent differences were observed in each season and at all locations. The

EFSA GMO Panel concluded that maize MON88017 is equivalent to its conventional counterpart and commercial non-GM maize varieties with regard to phenotypic characteristics and agronomic performance except for expression of the introduced trait (EFSA, 2009a).

4.1.2. Choice of conventional counterpart and additional comparators and production of material for the compositional assessment

The comparative compositional, phenotypic, and agronomic analysis of maize MON89034 x MON88017 and its conventional counterpart was performed in field trials at five locations in the USA in 2004. The combined event MON89034 x MON88017 had been obtained by crossing two inbred lines containing the single events MON89034 and MON88017. Also grown at the same locations were commercial non-GM maize varieties, three varieties at each location, amounting to a total of 15 different varieties across locations. All replicates at the same location underwent similar agronomic treatments. From each replicate, samples of grains and forage were analyzed for composition. The grain samples were additionally checked for the presence or absence of recombinant DNA by PCR analysis.

In the context of previous applications, analytical data on materials obtained from field trials with the single maize events (MON89034 and MON88017) and the respective appropriate conventional counterparts and commercial non-GM maize varieties were provided by the applicant (see section 4.1.1). The EFSA GMO Panel previously evaluated these data and concluded that the maize events MON89034 and MON88017 (the latter treated and untreated with the respective target herbicide) were compositionally and agronomically equivalent to their respective conventional counterparts, except for the newly introduced traits (EFSA, 2008, 2009a). The EFSA GMO Panel noted the fact that treatment of the single maize event MON88017 with the target herbicide to which it is tolerant did not affect its agronomic and compositional characteristics compared to untreated maize MON88017 plants (EFSA, 2009a). The EFSA GMO Panel, therefore, accepts the design of field trials with maize MON89034 x MON88017 without inclusion of the single events and treatment with the target herbicide.

4.1.3. Compositional analysis

The compositional analysis of maize forage included the following parameters: proximate (moisture, ash, total fat, crude protein; carbohydrates by calculation), fibre [acid detergent fibre (ADF) and neutral detergent fibre (NDF)], calcium, and phosphorus. Grains were additionally analyzed for total dietary fibre (TDF), amino acids, fatty acids, minerals, vitamins, and secondary metabolites (phytic acid, raffinose, furfuraldehyde, ferulic acid, and p-coumaric acid). The levels of these constituents found in maize MON89034 x MON88017, its conventional counterpart, and the commercial non-GM maize varieties, were also compared with background data on levels of these parameters reported in the literature and available in the ILSI Crop Composition database (ILSI, 2006). The across-location statistical analysis of the comparison between levels in maize MON89034 x MON88017 and its conventional counterpart showed that various parameters were statistically significantly different. In forage, the level of protein was higher in maize MON89034 x MON88017 than in its conventional counterpart. The level of protein was statistically significantly increased in grains, while carbohydrates were slightly decreased. In grains, also fifteen amino acids showed statistically significantly higher values for maize MON89034 x MON88017 as compared to its conventional counterpart if these values were calculated on a dry-weight basis. However, if calculations were based on the percentage of these amino acids as components of the total amino acid pool, no statistically significant differences could be observed in the comparison of amino acid values between maize MON89034 x MON88017 and its conventional counterpart. The EFSA GMO Panel therefore considers the elevated level of several amino acids on a dry-weight basis to be related to the increased level of protein. In grains, the fatty acid stearic acid occurred at a statistically significantly increased level, while the levels of oleic acid and eicosenoic acid were slightly decreased. Also levels of calcium, manganese, ferulic acid and p-coumaric acid were statistically significantly increased in maize MON89034 x MON88017 as compared to its conventional counterpart. Various other constituents were statistically significantly

increased or reduced at single locations but not at all locations. Moreover, the average values showing these differences were within the ranges of commercial non-GM maize varieties and also within the background ranges published in the literature and a crop composition database (ILSI, 2006). The EFSA GMO Panel considered the observed compositional differences between maize MON89034 x MON88017 and its conventional counterpart in the light of the field trial design, biological variation and level of the studied compounds in commercial non-GM maize varieties, and concludes that maize MON89034 x MON88017 is compositionally equivalent to its conventional counterpart and commercial non-GM maize varieties except for the introduced traits. Given these outcomes and the fact that compositional data on the single events grown during multiple seasons have already shown these to be compositionally equivalent to their conventional counterparts and commercial non-GM maize varieties, the EFSA GMO Panel does consider the data from one season comparing maize MON89034 x MON88017 with its conventional counterpart and commercial non-GM maize varieties as sufficient.

4.1.4. Agronomic traits and GM phenotype

As previously mentioned in section 4.1.2, an analysis of the agronomic and phenotypic characteristics of maize MON89034 x MON88017, its conventional counterpart maize, and non-GM maize varieties were carried out during field trial at five locations in the USA in 2004. The following parameters, were measured and statistically analyzed: early stand count; seedling vigour; days to 50% silking; days to 50% pollen shed; plant height; ear height; staygreen; dropped ears; stalk lodging; root lodging; final stand count; grain moisture; test weight; and yield. In the statistical analysis, the outcomes for maize MON89034 x MON88017 were compared with those for its conventional counterpart. The outcomes for the commercial non-GM maize varieties were used to create a tolerance interval with which the results for maize MON89034 x MON88017 could be compared. In the overall statistical analysis of average values across locations, several small but statistically significant differences were observed between MON89034 x MON88017 and its conventional counterpart, including a higher number of days until 50% of the plants had developed silk, a lower number stalk-lodged plants, and a higher yield of grains in maize MON89034 x MON88017. None of the differences observed across locations occurred at each location.

In the absence of consistent unexpected differences between the studied maize plants, the EFSA GMO Panel concluded that no agronomic differences specific for maize MON89034 x MON88017 as compared to its conventional counterpart and commercial non-GM maize varieties have been observed except for the introduced herbicide tolerance and insect resistance traits.

4.2. Conclusion

Based on the results of the comparative analysis, it is concluded that maize MON89034 x MON88017 is compositionally and agronomically equivalent to its conventional counterpart and commercial non-GM maize varieties, except for the presence of Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS proteins in maize MON89034 x MON88017. Based on the assessment of the data available, the EFSA GMO Panel has found no indication that crossing maize MON89034 with MON88017 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

Maize MON89034

Maize MON89034 expresses the Cry1A.105 and Cry2Ab2 proteins. Escherichia coli-produced Cry1A.105 and Cry2Ab2 proteins were used for safety studies after it had been demonstrated experimentally that they are equivalent to those that are present in maize event MON89034. No toxicity of the Cry1A.105 and Cry2Ab2 proteins were observed in acute oral toxicity studies in mice. Both proteins were shown to be quickly degraded in simulated gastric fluid, and a little less quickly in simulated intestinal fluid. In bioinformatics studies, the amino acid sequence of Cry1A.105 and Cry2Ab2 showed no similarity either to proteins that are known to be toxic to humans and other mammals or to allergens. In a 90-day feeding study in rats with grain material from maize MON89034, no treatment-related adverse effects were observed, and a 42-day feeding study on broiler chickens showed that maize MON89034 is nutritionally equivalent to its conventional counterpart and commercial non-GM maize varieties included in the study.

It was concluded that maize MON89034 is as safe as conventional maize and that the overall allergenicity of the whole plant is not changed. Maize MON89034 and derived products are unlikely to have any adverse effects on human and animal health in the context of its intended use (EFSA, 2008).

Maize MON88017

Analogues of the newly expressed Cry3Bb1 and CP4 EPSPS proteins in MON88017 maize were obtained from recombinant strains of *E. coli* and used for safety testing after their equivalence to the plant-expressed proteins had been demonstrated experimentally. Neither proteins 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 MON88017 was tested in a 90 day rat feeding study with diets containing 33% grains from maize MON88017. 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 MON88017, showing that the latter was nutritionally equivalent to conventional maize (EFSA, 2009a).

The GMO Panel concluded that maize MON88017 is as safe as its conventional counterpart and commercial non-GM maize varieties and considered it unlikely that the overall allergenicity of the whole plant is changed. Maize MON88017 and derived products are unlikely to have any adverse effect on human and animal health in the context of the intended uses (EFSA, 2009a).

5.1.2. Product description and intended use

The scope of application EFSA-GMO-NL-2007-39 includes the import and processing of maize MON89034 x MON88017 and its derived products for use as food and feed. Thus, the possible uses of

maize MON89034 x MON88017 include the production of animal feed, but it also includes food products such as, starch, syrups and oils.

Maize MON89034 x MON88017 is intended to improve agronomic performance only and is not intended to influence the nutritional properties, processing characteristics and overall use of maize as a crop.

5.1.3. Effect of processing

Since maize MON89034 x MON88017 is compositionally equivalent to its conventional counterpart (see Section 4.2), except for the newly expressed proteins, the effect of processing on maize MON89034 x MON88017 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 MON89034 x MON88017

As summarized in section 5.1.1, the EFSA GMO Panel has previously evaluated the safety of the newly expressed Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS proteins, which are present in maize MON89034 x MON88017, and for which the EFSA GMO Panel has not identified any safety concern (EFSA, 2008, 2009a). In its evaluations of the safety of the single events MON89034 and MON88017, the EFSA GMO Panel considered a range of data on these newly expressed proteins, including their resistance to in-vitro degradation by proteolytic enzymes, acute toxicity studies, and similarity of the amino acid sequences of these proteins to those of known toxins based on bioinformatics-supported sequence comparisons. At the request of the EFSA GMO Panel, the applicant provided results of updated bioinformatic comparisons of the Cry1A105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS with known toxic proteins for the current evaluation. The outcomes of these bioinformatics-supported comparisons did not show any relevant similarities. In addition, the EFSA GMO Panel is not aware of any other new information that would change the conclusions of its previous opinions. Based on the known function and mode of action of the newly expressed proteins Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS, the EFSA GMO Panel considers the occurrence of interactions among these proteins unlikely.

5.1.4.2. Toxicological assessment of new constituents other than proteins

No new constituents besides the Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS proteins are expressed in maize MON89034 x MON88017. Moreover, in the compositional analysis of this maize, no relevant changes in its composition were detected.

5.1.4.3. Toxicological assessment of the whole GM food/feed

As described in the summaries of the EFSA's GMO Panel's previous assessments of the single maize events MON89034 and MON88017 (see section 5.1.1), the EFSA GMO Panel also considered the outcomes of 90-day rat feeding studies with grains of these events. No adverse effects were observed in these studies. The EFSA GMO Panel also found, in more general terms, these single events to be safe for human and animal consumption. No new genes in addition to those present in the parental maize varieties have been introduced in maize MON89034 x MON88017. In the current assessment, neither the structural integrity of the insert in maize MON89034 x MON88017 maize nor the protein expression levels have been found to be changed in comparison to the single events MON89034 and MON88017 (section 3.2). Moreover, the composition of maize MON89034 x MON88107 has been found to be equivalent to its conventional counterpart and commercial non-GM maize varieties

(section 4.1.3). The EFSA GMO Panel considered all the data available for maize MON89034 x MON88017, and the newly expressed proteins Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS, and is of the opinion that interactions between the single maize events that might impact on the food and feed safety of maize MON89034 x MON88017 are unlikely.

Therefore, the EFSA GMO Panel does not consider additional animal safety studies with the whole GM food/feed necessary.

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 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, since no single experimental method yields decisive evidence for allergenicity (CAC, 2003; EFSA, 2006).

5.1.5.1. Assessment of allergenicity of the newly expressed proteins

The proteins Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS in maize MON89034 x MON88017 have been evaluated previously and it was found unlikely that they are allergenic (EFSA, 2008). At the request of the EFSA GMO Panel, the applicant submitted new bioinformatics-supported comparisons of the amino acid sequences of the proteins Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS with the sequences of known allergens using an updated version of the FARRP Allergen database. Based on the information provided, the EFSA GMO Panel considers it unlikely that potential interactions occur that might change the allergenicity of the newly expressed proteins.

5.1.5.2. Assessment of allergenicity of the whole GM plant

The issue of a potential increased allergenicity of maize MON89034 x MON88017, as compared to the single maize events MON89034 and MON88017, and to conventional maize varieties, does not appear relevant to the EFSA GMO Panel since maize is not considered a common allergenic food. However rare cases of occupational allergy to maize dust have been reported in the literature. The EFSA GMO Panel is also aware that few cases of food allergy to maize have been specifically observed in some geographically restricted areas where maize is a particular common food and that, in the few cases reported, the major maize allergens have then been identified. In the context of the present application the EFSA GMO Panel considers it unlikely that any interactions between the newly expressed proteins and metabolic pathways of maize would alter the pattern of expression of endogenous proteins/potential allergens and thereby significantly change the overall allergenicity of the whole plant. In addition, given all the available information, the EFSA GMO Panel sees no reason to expect that the use of GM maize MON89034 x MON88017 would significantly increase the intake and exposure to maize.

5.1.6. Nutritional assessment of GM food/feed

For each of the single events MON89034 and MON88017, the EFSA GMO Panel has previously assessed data on nutritional feeding studies in food-producing animals, in particular the rapidly growing chicken broiler (see section 5.1.1). The EFSA GMO Panel has thus concluded that the outcomes of these tests confirm the nutritional equivalence of the single events to conventional maize.

In addition, the outcomes of a 42-day feeding study with maize MON89034 x MON88017 in chicken were provided in the frame of the current application. Groups of 100 chickens (50 males and 50

females) each received one of six maize-containing diets, *i.e.* with maize MON89034 x MON88017, its conventional counterpart and four commercial non-GM maize varieties. Each group had been subdivided into ten pens of ten animals per pen, with five pens each for male and female, adding up to 50 animals per sex within each group of 100 animals. The content of maize in the diets varied between 55% maize in starter diets to 59% in grower/finisher diets. Whilst maize grains and diets were analyzed for chemical composition, the grains were also analyzed for potential presence of pesticide and mycotoxin residues, and for the presence of transgenic DNA using PCR analysis. The measurements that were performed during the feeding experiment included the feed consumption, body weight, morbidity, and mortality of the animals. After the experiment, the animals were analyzed post-mortem for carcass characteristics, including the weights of the carcass and various carcass parts, as well as the composition of the meat of thighs and breast (fat, moisture, protein). Following a request from the EFSA GMO Panel the applicant has performed a direct comparison of the test and control chicken for each observed parameter. No statistically significant differences for the tested parameters were observed between the group fed maize MON89034 x MON88017 and its conventional counterpart, apart from a minor but statistically significant difference in relative (%) breast weights for which female chicken fed maize MON89034 x MON88017 showed a higher value than animals fed the control diet. Additional, small statistically significant differences were observed in thigh protein in females and breast moisture in males. The difference in relative breast weight was not observed in absolute breast weights. In the absence of any other treatment-related effects on performance, the EFSA GMO Panel does not consider these statistically significant differences to be of biological relevance. The EFSA GMO Panel concludes that the results of this 42-days chicken feeding study show that maize MON89034 x MON88017 is nutritionally equivalent to its conventional counterpart and commercial non-GM maize varieties.

5.1.7. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that maize MON89034 x MON88017 is any less safe than its conventional counterpart. In addition, maize MON89034 x MON88017 is, from a nutritional point of view, substantially equivalent to commercial non-GM maize. Therefore, and in line with the Guidance document (EFSA, 2006), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

5.2. Conclusion

The Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS proteins that are newly expressed in maize MON89034 x MON88017 have previously been assessed for their safety by the EFSA GMO Panel, as summarized in its previously published opinions on the single events MON89034 and MON88017. During these previous assessments, no adverse effects of these newly expressed proteins have been identified. In addition, the EFSA GMO Panel considers it unlikely that interactions among the newly expressed proteins will occur that may impact on the food and feed safety of maize MON89034 x MON88017. The EFSA GMO Panel bases its consideration on the data on the functional characteristics of these proteins, as well as the outcomes of the comparative analysis of compositional, phenotypic, agronomic and nutritional characteristics of the maize MON89034 x MON88017.

Besides the newly expressed proteins, the safety and nutritional properties of whole food and feed products derived from MON89034 x MON88017 have also been considered. Maize MON89034 x MON88017 was tested in a nutritional chicken feeding study, which shows that this maize is nutritionally equivalent to its conventional counterpart and commercial non-GM maize varieties. The EFSA GMO Panel concludes that the outcomes of the chicken feeding study further support the findings of the comparative analysis of composition confirming the nutritional equivalence of maize MON89034 x MON88017 to conventional counterpart and commercial non-GM maize varieties. The EFSA GMO Panel also considers that it is unlikely that the overall allergenicity of maize MON89034 x MON88017 has been altered. The EFSA GMO Panel is of the opinion that MON89034 x MON88017 is as safe as its conventional counterpart and commercial non-GM maize varieties, and

concludes that this maize and derived products are unlikely to have any adverse effects on human and animal health in the context of its intended use.

6. Environmental risk assessment and monitoring

6.1. Evaluation of relevant scientific data

The scope of the application is for food and feed uses, import and processing of maize MON89034 x MON88017 and does not include cultivation. Considering the proposed uses of maize MON89034 x MON88017, the environmental risk assessment is concerned with the exposure through manure and faeces from gastrointestinal tracts of animals fed maize MON89034 x MON88017 and with the accidental release into the environment of maize MON89034 x MON88017 grains during transportation and processing.

As the scope of the present application excludes cultivation, environmental concerns related to the use of glyphosate herbicides on maize MON89034 x MON88017 apply only to imported and processed maize products that may have been treated with those herbicides in countries of origin. The EFSA GMO Panel is aware that the risk assessment of active substances falls within the scope of Directive 91/414/EEC concerning the placing of plant protection products on the market.

6.1.1. Evaluation of the single events MON88017 and MON89034

In its previous scientific opinions, the EFSA GMO Panel was of the opinion that both the single maize events MON89034 and MON88017 assessed in their respective applications as safe as their conventional counterpart and that the placing on the market of maize MON89034 and MON88017, 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 (EFSA, 2008, 2009a). Furthermore, post-market environmental monitoring plans for MON89034 and MON88017, including general surveillance, were proposed by the applicant and considered in line with EFSA GMO Panel opinion on post-market environmental monitoring (EFSA, 2008, 2009a).

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. In cultivation, grains shed during harvest may survive overwinter in some milder regions, germinate and appear as volunteers in subsequent crops. The occurrence of maize volunteers was reported in Spain and other European regions (Gruber et al., 2008) and many of them grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009).

Applicant's field trials have shown that there are no indications of an altered fitness of the single maize events MON88017 and MON89034 as compared to their conventional counterparts**.** In addition to the field trials carried out with the single events MON88017 and MON89034 (EFSA, 2008, 2009a), a series of field trials with maize MON89034 x MON88017 were conducted across 5 USA locations in 2004. Information on 14 phenotypic and agronomic characteristics was provided to assess agronomic

performance of maize MON89034 x MON88017 in comparison with its conventional counterpart. These field trial data did not show changes in plant characteristics that indicate altered fitness and invasiveness of maize MON89034 x MON88017. In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of any scientific report that would indicate the potential for increased establishment and spread of maize MON89034 x MON88017 and any change in survival capacity, including over-wintering.

The herbicide tolerance trait can only be regarded as providing an agronomic advantage for this GM maize MON89034 x MON88017 plant where and when glyphosate herbicides are applied. Similarly insect resistance against certain lepidopteran and coleopteran target pests provides a potential advantage in cultivation under infestation of target pests. However survival of maize plants outside cultivation or other areas where glyphosate herbicides could be applied in Europe is mainly limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens, herbivores and cold climatic conditions. Since these general characteristics are unchanged in maize MON89034 x MON88017, herbicide tolerance and insect resistance are not likely to provide a selective advantage outside cultivation in Europe. Therefore it is considered very unlikely that maize MON89034 x MON88017 will differ from conventional maize varieties in their ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

Since maize MON89034 x MON88017 has no altered survival, multiplication or dissemination characteristics, except when glyphosate herbicides are applied and/or under infestation of target pests, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects due to the accidental release into the environment of viable maize MON89034 x MON88017 grains will not differ from that of the single events maize MON88017 or MON89034 or from that of conventional maize varieties.

6.1.2.2. Potential for 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

Genomic DNA is a component of many food and feed products derived from maize. It is well documented that DNA present in food and feed becomes substantially degraded in the process of digestion in the human or animal gastrointestinal tract. However, a low level of exposure of fragments of ingested DNA, including the recombinant fraction of such DNA, to micro-organisms in the digestive tract of humans, domesticated animals, and other animals feeding on maize MON89034 x MON88017 is expected (see section 5 of the scientific opinion).

Current scientific knowledge indicates that horizontal gene transfer of non-mobile DNA fragments between unrelated organisms (such as plants to micro-organisms) is extremely unlikely to occur under natural conditions (see EFSA, 2009bfor further details). In addition to the low concentration of DNA in the gastrointestinal tract and the lack of competence of most bacteria to take up foreign DNA, the major barrier to such horizontal transfer is the lack of sufficient DNA sequence similarity for homologous recombination to occur in bacteria.

The *cry1A*.105*, cry2Ab2, cry3Bb1* and CP4 *epsps* genes are of bacterial origin. Thus, in theory, the *cry1A*.105*, cry2Ab2, cry3Bb1* and CP4 *epsps* genes of the recombinant DNA insert could provide sufficient DNA similarity for homologous recombination with genes from environmental bacteria.

However, such hypothesized horizontal gene transfer event is not likely to be maintained in bacterial populations due to a predicted lack of efficient expression and no identified selective advantage for gene transfer recipients in the unlikely case of their expression.

In case of illegitimate recombination into genomes of bacteria in the environment, it is unlikely that recombinant genes (CP4 *epsps*) regulated by eukaryotic plant promoters in maize MON89034 x MON88017 would be expressed. The *cry1A.*105, *cry2Ab2* and *cry3Bb1* genes are regulated by plant virus promoters. The activity of these plant virus promoters in unrelated organisms such as bacteria cannot be excluded but in the unlikely event that the above mentioned genes and regulatory elements are taken up by bacteria, no selective advantage is anticipated because *cry* and CP4 *epsps* genes are distributed in various bacterial species in the natural environment. Thus, the hypothesized low level exposure of bacterial communities in the environment to the maize MON89034 x MON88017 *cry1A.*105, *cry2Ab2*, *cry3Bb1and* CP4 *epsps* genes must be seen in the context of the natural occurrence and level of exposure to alternative sources of genetically diverse *cry* and *epsps* genes to which bacterial communities are naturally exposed.

The wide environmental presence of genetically diverse natural variants of the recombinant DNA coding sequences, the use of regulatory sequences optimised for expression in eukaryotes, and the absence of an identified plausible selective advantage, suggest it is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tract.

(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, and the successful establishment and subsequent flowering of this GM maize plant. For maize, any vertical gene transfer is limited to other *Zea mays* plants as populations of sexually compatible wild relatives of maize are not known in Europe (Eastham and Sweet, 2002, OECD, 2003).

The flowering of occasional 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 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).

Herbicide tolerance and insect resistance provide agronomic and selective advantages in areas where glyphosate herbicides are applied and/or under infestation of target pests. Even though the occurrence of some GM maize plants outside cropped area have been reported in Korea due to grain spillage during import, transportation, storage, handling and processing (Kim CG et al., 2006, Lee et al., 2009, Park KW et al., 2009), survival of maize plants outside 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 MON89034 x MON88017, herbicide tolerance and insect resistance are not likely to provide selective advantages outside cultivation or other areas where glyphosate herbicides could be applied and/or under infestation of target pests in Europe. Therefore, as for any other maize varieties, these GM maize plants would only survive in subsequent seasons in warmer regions of Europe and are not likely to establish feral populations under European environmental conditions.

In conclusion, maize MON89034 x MON88017 has no altered survival, multiplication or dissemination characteristics except when glyphosate herbicides are applied, and/or under infestation 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 in Europe will not differ from that of the single maize events MON88017 and MON89034, or of other conventional maize

varieties and considers that maize MON89034 x MON88017 is unlikely to cause adverse effects, in the context of the intended uses.

6.1.2.3. Interactions of the GM plant with target organisms

The intended uses of maize MON89034 x MON88017 specifically exclude cultivation and the environmental exposure of target organisms to maize MON89034 x MON88017 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 MON89034 x MON88017 plants to enable any significant interaction with target organisms, which is very unlikely.

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

The intended uses of maize MON89034 x MON88017 specifically exclude cultivation so that environmental exposure of non-target organisms to maize MON89034 x MON88017 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 MON89034 x MON88017 plants to enable any significant interaction with non-target organisms, which is very unlikely.

In addition, the EFSA GMO Panel evaluated whether the Cry1A.105, Cry2Ab2 and Cry3Bb1 proteins might potentially affect non-target organisms by entering the environment through manure and faeces from the gastrointestinal tracts of animals fed maize MON89034 x MON88017. Due to the specific insecticidal selectivity of the Cry proteins, non-target organisms most likely to be affected by the Cry1A.105, Cry2Ab2 and Cry3Bb1 proteins belong to the same or closely related taxonomic groups as those of the target organisms.

Data supplied by the applicant suggest that only very low amounts of the Cry1A.105, Cry2Ab2 and Cry3Bb1 proteins enter the environment due to low expression in grains. Moreover, these Cry proteins are degraded by enzymatic activity in the gastrointestinal tract of animals fed GM maize or derived feed products (see section 5.1.1), meaning that only low amounts of these proteins would remain intact to pass out in faeces. This has been demonstrated for Cry1Ab (Einspanier et al., 2004, Ahmad et al., 2005, Lutz et al., 2005, Lutz et al., 2006, Wiedemann et al., 2006, Guertler et al., 2008, Paul et al., 2010). It is expected that there would subsequently be further degradation of Cry proteins in the manure and faeces due to intrinsic microbial proteolytic activity. Therefore, exposure of soil and aquatic environments to the Cry1A.105, Cry2Ab2 and Cry3Bb1 proteins from disposal of animal wastes or accidental spillage of maize grains is likely to be very low and localized. While Cry proteins may bind to a certain degree to clay minerals or humic substances in soil, thereby reducing their availability to micro-organisms for degradation, there are no indications of persistence and accumulation of Cry proteins from GM crops in soil (reviewed by (Icoz and Stotzky, 2008). More specifically, Cry3Bb1 of GM maize was found to be more rapidly degraded in soil compared to Cry1Ab under similar conditions (Baumgarte and Tebbe, 2005; Miethling-Graff et al., 2010).

Considering the scope of the application (that excludes cultivation) and the intended uses of maize MON89034 x MON88017, it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry1A.105, Cry2Ab2 and Cry3Bb1 proteins is likely to be very low and of no ecological relevance.

6.1.2.5. Interactions with the abiotic environment and biogeochemical cycles

Considering the scope of the application and the intended uses of maize MON89034 x MON88017 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.

705

706 ### 6.1.3. Post-market environment monitoring

707 The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are (1) to
708 confirm that any assumption regarding the occurrence and impact of potential adverse effects of the
709 GMO, or its use, in the environmental risk assessment are correct and (2) to identify the occurrence of
710 adverse effects of the GMO, or its use, on human health or the environment which were not
711 anticipated in the environmental risk assessment.

712 Monitoring is related to risk management, and thus a final adoption of the monitoring plan falls
713 outside the mandate of the EFSA GMO Panel. However, the EFSA GMO Panel gives its opinion on
714 the scientific quality of the monitoring plan provided by the applicant (EFSA, 2006c). The potential
715 exposure to the environment of maize MON89034 x MON88017 would be mainly through manure
716 and faeces from the gastrointestinal tracts of animals fed maize MON89034 x MON88017 and/or
717 through accidental release into the environment of viable GM maize grains during transportation and
718 processing.

719 No specific environmental impact of maize MON89034 x MON88017 was indicated by the
720 environmental risk assessment and thus no case-specific monitoring is required.

721 The general surveillance plan proposed by the applicant includes (1) the description of an approach
722 involving operators (federations involved in maize import and processing), reporting to the applicants,
723 via a centralised system, any observed adverse effect(s) of GMOs on human health and the
724 environment, and (2) a coordinating system established by EuropaBio for the collection of the
725 information recorded by the various operators (Lecoq et al., 2007, Windels et al., 2008); (3) the use of
726 networks of existing surveillance systems. The applicant proposes a general surveillance report on an
727 annual basis and a final report at the end of the consent.

728 The EFSA GMO Panel is of the opinion that the scope of the monitoring plan provided by the
729 applicant is in line with the intended uses of maize MON89034 x MON88017 since the environmental
730 risk assessment does not cover cultivation and identified no potential adverse environmental effects.
731 The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general
732 surveillance plan.

733 ## 6.2. Conclusion

734 The scope of the application includes food and feed uses, import and processing of maize MON89034
735 x MON88017 and excludes cultivation. Considering the intended uses of maize MON89034 x
736 MON88017, the environmental risk assessment is concerned with indirect exposure through manure
737 and faeces from gastrointestinal tracts of animals fed maize MON89034 x MON88017 and with the
738 accidental release into the environment of maize MON89034 x MON88017 grains during
739 transportation and processing.

740 There are no indications of an increased likelihood of establishment and spread of feral maize plants in
741 case of accidental release into the environment of viable maize MON89034 x MON88017 grains
742 during transportation and processing for food and feed uses, except in the presence of the herbicide.
743 Taking into account the scope of the application, both the rare occurrence of feral maize plants and the
744 low levels of Cry1A.105, Cry2Ab2, Cry3Bb1 protein exposure in maize MON89034 x MON88017
745 grains or through other routes indicate that the risk to non-target organisms is considered extremely
746 low.

747 The scope of the monitoring plan provided by the applicant is in line with the intended uses of maize
748 MON89034 x MON88017, 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.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out a scientific risk assessment of the maize MON89034 x MON88017 for food and feed uses, import and processing.

The EFSA GMO Panel is of the opinion that the molecular characterisation data provided for maize MON89034 × MON88017 produced by conventional breeding are adequate to perform this part of the safety assessment. The bioinformatic analysis of the inserted DNA and the flanking regions of the single events MON89034 and MON88017 does not raise safety concerns. The expression of Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS proteins in maize MON89034 × MON88017 has been sufficiently analysed and the stability of the genetic modification has been demonstrated. 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 MON89034 x MON88017 is compositionally and agronomically equivalent to its conventional counterpart and commercial non-GM maize varieties, except for the presence of Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS proteins expressed in maize MON88017 x MON89034. 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 MON89034 x MON88017, for the single events and for appropriate comparator(s), the EFSA GMO Panel has found no indication that crossing of MON89034 and MON88017 results in an interaction between the single events which causes compositional or agronomic changes. The Cry1A.105 and Cry2Ab2 proteins expressed in the parental maize MON89034 and the Cry3Bb1 and CP4 EPSPS proteins expressed in the parental maize MON88017 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 MON89034 x MON88017 would not be different from those of its conventional counterpart and commercial non-GM maize varieties. The EFSA GMO Panel considers that maize MON89034 x MON88017 is as safe and as nutritious as its conventional counterpart and commercial non-GM maize varieties and that the overall allergenicity of the whole plant is not changed.

Considering the intended uses of maize MON89034 x MON88017, which exclude cultivation, there is no requirement for scientific assessment of potential environmental effects associated with the cultivation of this GM maize. In case of accidental release into the environment of viable maize MON89034 x MON88017 grains during transportation and processing, there are no indications of an increased likelihood of establishment and spread of feral maize plants, except in the presence of the herbicide. Also, the low levels of environmental exposure to these GM maize plants and the Cry1A.105, Cry2Ab2, Cry3Bb1 proteins through other routes indicate that the risk to non-target organisms is extremely low. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize MON89034 x MON88017.

In conclusion, the EFSA GMO Panel considers that the information available for maize MON89034 x MON88017 addresses the scientific comments raised by the Member States and that the maize MON89034 x MON88017 as described in this application is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of its intended uses. The EFSA GMO Panel concludes that maize event MON89034 x MON88017 is unlikely to have any adverse effect 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 MS, dated 12 February 2007, concerning a request for placing on the market of genetically modified MON89034 x MON88017 maize in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 16 February 2007, from EFSA to the Competent Authority of the Netherlands.

3. Letter from EFSA to applicant, dated 1 August 2007, requesting additional information under completeness check

4. Letter from applicant to EFSA, dated 23 August 2007, providing additional information under completeness check.

5. Letter from EFSA to applicant, dated 5 September 2007, requesting additional information under completeness check

6. Letter from applicant to EFSA, dated 11 September 2007, providing additional information under completeness check.

7. Letter from EFSA to applicant, dated 20 September 2007, delivering the 'Statement of Validity' for application EFSA-GMO-NL-2007-39, MON89034 x MON88017 maize submitted by MONSANTO under Regulation (EC) No 1829/2003.

8. Letter from EFSA to applicant, dated 21 September 2007, requesting additional information and stopping the clock (JRC).

9. Letter from applicant to EFSA, dated 26 September 2007, providing the additional copies for the valid version.

10. Letter from EFSA to applicant, dated 23 October 2007, requesting additional information and stopping the clock (EFSA).

11. Letter from applicant to EFSA, dated 10 December 2007, providing the Monitoring plan.

12. Letter from EFSA to applicant, dated 8 January 2008, restarting the clock (JRC) and maintaining the clock stopped (EFSA).

13. Letter from applicant to EFSA, dated 17 April 2008, providing spontaneously info (updated Monitoring plan).

14. Letter from EFSA to applicant dated 19 November 2008, maintaining the clock stopped (3).

15. Letter from EFSA to applicant, dated 15 November 2008, restarting the clock.

16. Letter from EFSA to applicant, dated 18 December 2008, requesting additional information and stopping the clock (3).

17. Letter from EFSA to applicant dated 8 April 2009, requesting additional information and maintaining the clock stopped (4).

18. Letter from applicant to EFSA, dated 17 April 2009, providing the timeline for submission of response.

19. Letter from EFSA to applicant dated 29 May 2009, requesting additional information and maintaining the clock stopped (5).

20. Letter from applicant to EFSA, dated 3 June 2009, providing additional information (requested by EFSA on 8th April).

21. Letter from applicant to EFSA, dated 30 June 2009, providing additional information (requested by EFSA on 29 May).

22. Letter from EFSA to applicant dated 30 June 2009, requesting additional information and maintaining the clock stopped (6).

23. Letter from applicant to EFSA, dated 3 August 2009, providing the timeline for submission of response.

24. Letter from applicant to EFSA, dated 18 August 2009, providing a new timeline for submission of response.

25. Letter from applicant to EFSA, dated 8 September 2009, providing additional information (requested by EFSA on 30th June).

26. Letter from EFSA to applicant, dated 15 October 2009, restarting the clock.

27. Letter from EFSA to applicant, dated 5 November 2009, requesting additional information and stopping the clock (7).

28. Letter from applicant to EFSA, dated 11 November 2009, providing additional information.

29. Letter from EFSA to applicant, dated 11 January 2010, 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. C. C. Tebbe. 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. Mol. Ecol. 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.

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

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 99**,** 1-100.

EFSA, 2007. Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of GM Plants containing stacked transformation events. The EFSA journal 512**,** 1-5.

EFSA, 2008. Scientific opinion of the GMO Panel on application (Reference EFSA-GMO-NL-2007-37) for the placing on the market of the insect-resistant genetically modified maize MON89034, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. The EFSA journal 909**,** 1-29.

EFSA, 2009a. Scientific opinion of the GMO Panel on application (Reference EFSA-GMO-CZ-2005-27) for the placing on the market of the insect-resistant and herbicide-tolerant genetically modified maize MON88017, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 1075**,** 1-28.

EFSA, 2009b. 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 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.

Girault, R. and McClain, J. S, 2008. Updated bioinformatics evaluation of DNA sequences flanking the 5' and 3' junctions of the inserted DNA in MON 89034: assessment of putative polypeptides. Monsanto Technical Report, MSL0021368.

Girault, R., McClain, J. S. and Silvanovich, A, 2008. Updated bioinformatics evaluation of DNA sequences flanking the 5' and 3' junctions of the inserted DNA in MON 88017 corn: assessment of putative polypeptides. Monsanto Technical Report, MSL0021190.

Groat, J.R., Wolff, B.J., Rice, J.F. and Masucci, J.D. (2006) Confirmation of the integrity of corn MON 89034 x MON 88017 by Southern blot analysis. Monsanto Technical Report, MSL 20145 (CBI[6]).

Gruber S, Colbach N, Barbottin A and Pekrun C, 2008. Post-harvest gene escape and approaches for minimizing it. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 3, 17 pp.

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.

Hartmann, A. J., Niemeyer, K. E. and Silvanovich, A, 2006a. Assessment of the Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS Protein Levels in Tissues of Insect-Protected Corn MON 89034 × MON 88017 Produced in 2005 U.S. Field Trials. Monsanto Technical Report, MSL 0020286 (CBI).

Hartmann, A. J., Niemeyer, K. E. and Silvanovich, A, 2006b. Assessment of the Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS protein levels in selected tissues of insect-protected corn MON 89034 x MON 88017 produced in 2005 U.S. field trials. Monsanto Technical Report, MSL 0020479 (CBI).

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

ILSI, 2006. International Life Sciences Institute Crop Composition Database Version 3.0. http://www.cropcomposition.org

Kim CG, Yi H, Park S., Yeon J.E. , Kim D.Y., Kim D.I., Lee K.H., Lee T.C., Paek I.S., Yoon WK., Jeong S.-C. and H.M K, 2006. Monitoring the occurrence of genetically modified soybean and maize around cultivated fields and at a grain receiving port in Korea. Journal of Plant Biology 49, 218-298.

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.

Lee B, Kim C, Park J, Park K, Kim H, Yi H, Jeong S, Yoon W and Kim H, 2009. Monitoring the occurrence of genetically modified soybean and maize in cultivated fields and along the transportation routes of the Incheon Port in South Korea. Food Control 20, 250-254.

Lutz B, Wiedemann S and Albrecht C, 2006. Degradation of transgenic Cry1Ab DNA and protein in Bt-176 maize during the ensiling process. J Anim Physiol Anim Nutr (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. J Agric Food Chem 53, 1453-6.

[6] Indicated by the applicant as containing confidential business information (Regulation (EC) No 1829/2003 Article 30)

Miethling-Graff, R., S. Dockhorn, C.C. Tebbe. 2010. Release of the recombinant Cry3Bb1 protein of *Bt* maize MON88017 into field soil and detection of effects on the diversity of rhizosphere bacteria. European Journal of Soil Biology 46: 41-48.

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 and Messeguer J, 2009. Effect of volunteers on maize gene flow. DOI:10.1007/s11248-009-9250-7.Park KW, Lee B, Kim CG, Kim D-Y, Park J-Y, Ko EM, Jeong S-C, Choi KH, Yoon WK and Kim HM, 2009. Monitoring the occurrence of genetically modified maize at a grain receiving port and along transportation routes in the Republic of Korea. Food Control.

Park KW, Lee B, Kim C-G, Kim DY, Park J-Y, Ko EM, Jeong S-C, Choi KH, Yoon WK, Kim HM, 2009. Monitoring the occurrence of genetically modified maize at a grain receiving port and along transportation routes in the Republic of Korea. Food Control, DOI:10.1016/j.foodcont.2009.07.006.

Paul V, Guertler P, Wiedemann S and Meyer HHD, 2010. Degradation of Cry1Ab protein from genetically modified maize (MON810) in relation to total dietary feed proteins in dairy cow digestion. Transgenic Research. 10.1007/s11248-009-9339-z.

Tu, H. and Silvanovich, A, 2009a. Updated bioinformatics evaluation of the DNA sequences flanking the insertion site in MON 89034: BLASTn analysis using the GenBank non-redundant nucleotide database. Monsanto Technical Report, RAR-09-300.

Tu, H. and Silvanovich, A, 2009b. Updated bioinformatics evaluation of the DNA sequences flanking the insertion site in MON 89034: BLASTn and BLASTx analyses. Monsanto Technical Report, RAR-09-241.

Tu, H. and Silvanovich, A, 2009c. Updated bioinformatics evaluation of DNA sequences flanking the 5' and 3' junctions of the inserted DNA in MON 89034 utilizing the AD_2009 database: assessment of putative polypeptides. Monsanto Technical Report, RAR-09-334.

Tu, H. and Silvanovich, A, 2009d. Updated bioinformatics evaluation of DNA sequences flanking the 5' and 3' junctions of the inserted DNA in MON89034 using the TOX_2009 database: assessment of putative polypeptides. Monsanto Technical Report, RAR-09-173.

Tu, H. and Silvanovich, A, 2009e. Updated bioinformatics evaluation of the DNA sequences flanking the insertion site in MON 88017: BLASTn and BLASTx analyses. Monsanto Technical Report, RAR-09-240.

Tu, H. and Silvanovich, A, 2009f. Updated bioinformatics evaluation of the DNA sequences flanking the insertion site in MON 88017: BLASTn analysis using the GenBank non-redundant nucleotide database. Monsanto Technical Report, RAR-09-299.

Tu, H. and Silvanovich, A, 2009g. Updated bioinformatics evaluation of DNA sequences flanking the 5' and 3' junctions of the inserted DNA in MON 88017 utilizing the AD_2009 and TOX_2009 databases: assessment of putative polypeptides. Monsanto Technical Report, RAR-09-336.

Tu, H. and Silvanovich, A., 2009h. Updated bioinformatics evaluation of the transfer DNA and the associated 5' and 3' genomic junctions in MON 89034 utilizing the PRT_2009 database. Monsanto Technical Report, RAR-09-470, 1-237.

Tu, H. and Silvanovich, A., 2009i. Updated bioinformatics evaluation of the transfer DNA and the associated 5' and 3' genomic junctions in MON 88017 utilizing the PRT_2009 database. Monsanto Technical Report, RAR-09-469, 1-373.

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 Fur Verbraucherschutz Und Lebensmittelsicherheit-Journal of Consumer Protection and Food Safety 3**,** 14-16.