SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-CZ-2008-62) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Dow AgroSciences and Monsanto[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This scientific opinion is an evaluation of a risk assessment for placing on the market the genetically modified (GM) insect resistant and herbicide tolerant maize MON 89034 x 1507 x MON 88017 x 59122 for food and feed uses, import and processing. Maize MON 89034 x 1507 x MON 88017 x 5912 was produced by conventional crossing and the F_1 plant is hemizygous for all newly introduced genes. The maize contains *cry1A.105*, *cry2Ab2*, *cry1F*, *pat*, *cry3Bb1*, CP4 *epsps*, *cry34Ab1* and *cry35Ab1* genes conferring resistance against certain lepidopteran and coleopteran target pests and tolerance to glufosinate-ammonium- and glyphosate-based herbicides. The maize events MON 89034, 1507, MON 88017 and 59122 crossed together to create maize MON 89034 x 1507 x MON 88017 x 59122, behave as independent genetic loci. The F_2 grain harvested from maize MON 89034 x 1507 x MON 88017 x 59122 is expected to contain a mixture of MON 89034 x 1507 x MON 88017 x 59122 and all combinations of the individual events which will be imported and processed for food and feed uses. The EFSA GMO Panel has evaluated the risk assessment with respect to safety concerns which might arise through any potential combination of the following events MON 89034, 1507, MON 88017, and 59122 in maize MON 89034 x 1507 x MON 88017 x 59122 and in its segregating progeny.
Molecular analyses indicated that the structure of the inserts in the single events was retained in maize MON 89034 x 1507 x MON 88017 x 59122. Updated bioinformatic analyses of the flanking sequences and the open reading frames spanning the insert-plant DNA junctions did not raise any safety concern. Levels of the newly expressed proteins in maize MON 89034 x 1507 x MON 88017 x 59122 were demonstrated to be comparable with those of the single events. Comparative analyses established that maize MON 89034 x 1507 x

[1] On request from the Competent Authority of the Czech Republic on an application (EFSA-GMO-CZ-2008-62) submitted by Dow AgroSciences and Monsanto, Question No EFSA-Q-2008-764, adopted on 8 September 2010.

[2] Panel members: Hans Christer Andersson, Salvatore Arpaia, Detlef Bartsch, Josep Casacuberta, Howard Davies, Patrick du Jardin, Gerhard Flachowsky, Lieve Herman, Huw Jones, Sirpa Kärenlampi, Jozsef Kiss, Gijs Kleter, Harry Kuiper, Antoine Messéan, Kaare Magne Nielsen, Joe Perry, Annette Pöting, Jeremy Sweet, Christoph Tebbe, Atte Johannes von Wright, and Jean-Michel Wal. Correspondence: gmo@efsa.europa.eu

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MON 88017 x 59122 does not differ compositionally, agronomically and phenotypically from its conventional counterpart, and is equivalent to commercial maize varieties, except for the newly introduced traits. The safety assessment identified no concerns regarding potential toxicity and allergenicity of maize MON 89034 x 1507 x MON 88017 x 59122. A feeding study on broiler chickens confirmed the nutritional equivalence of maize MON 89034 x 1507 x MON 88017 x 59122 to its conventional counterpart and commercial maize varieties. Considering the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122, which excludes cultivation within the European Union, no scientific assessment of potential environmental effects associated with cultivation of this maize was required. In case of accidental release of viable grains produced by maize MON 89034 x 1507 x MON 88017 x 59122 into the environment during transportation and processing, there are no indications of an increased likelihood of establishment or survival of feral maize plants, except in the presence of glufosinate-ammonium- and/or glyphosate-based herbicides and/or under infestation by target pests. It is highly unlikely that the recombinant DNA will be transferred and establish itself in the genome of bacteria in the environment or human and animal digestive tracts.
In conclusion, the EFSA GMO Panel considers that the maize MON 89034 x 1507 x MON 88017 x 59122, as described in this application, is as safe as its conventional counterpart and commercial maize varieties with respect to potential effects on human and animal health and the environment. In addition, the EFSA GMO Panel is of the opinion that crossing of maize events MON 89034, 1507, MON 88017 and 59122 to produce maize MON 89034 x 1507 x MON 88017 x 59122 does not result in interactions between the events which would affect the safety of maize MON 89034 x 1507 x MON 88017 x 59122 with respect to potential effects on human and animal health and on the environment, in the context of its intended uses. Based on the data provided for maize stack MON 89034 x 1507 x MON 88017 x 59122, the single maize events and for the two parental double stacks 1507 x 59122 and MON 89034 x MON 88017, the EFSA GMO Panel is of the opinion that there is no biological reason to expect that any of the other sub-combinations of the individual events present in its segregating progeny would raise a safety concern. The EFSA GMO Panel concludes that maize MON 89034 x 1507 x MON 88017 x 59122 is unlikely to have adverse effects on human and animal health and the environment, in the context of its intended uses.

KEY WORDS

GMO, maize (*Zea mays*), MON 89034 x 1507 x MON 88017 x 59122, insect resistance, herbicide tolerance, risk assessment, food and feed safety, environment, import and processing, Regulation (EC) No 1829/2003.

SUMMARY

Following the submission of an application (EFSA-GMO-CZ-2008-62) under Regulation (EC) No 1829/2003 from Dow AgroSciences and Monsanto, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on the safety of insect resistant and herbicide tolerant genetically modified (GM) maize MON 89034 x 1507 x MON 88017 x 59122[4] and all sub-combinations of the individual events as present in its segregating progeny[5] for food and feed uses, import and processing.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-CZ-2008-62, additional information supplied by the applicants, scientific comments submitted by the Member States, and relevant scientific publications. Further information from applications for placing on the market under EU regulatory procedures the single maize events MON 89034, 1507, MON 88017 and 59122, and the two parental double stacks MON 89034 x MON 88017 and 1507 x 59122 was taken into account. The scope of the application EFSA-GMO-CZ-2008-62 is for food and feed uses, import and processing of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny, and all derived products, but excludes cultivation in the EU. The EFSA GMO Panel evaluated maize MON 89034 x 1507 x MON 88017 x 59122 with reference to the intended uses and appropriate principles described in its Guidance Documents for the risk assessment of GM plants and derived food and feed, and for the risk assessment of GM plants containing stacked transformation events. The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and expression of the corresponding proteins. An evaluation of the comparative analyses of composition, agronomic and phenotypic traits was undertaken, and the safety of the new proteins, both individually and in combination, and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional quality. An evaluation of environmental impacts and the post-market environmental monitoring plan was undertaken.

The single maize events MON 89034, 1507, MON 88017, and 59122 and the two double stacks 1507 x 59122 and MON 89034 x MON 88017, were the subject of previous risk assessment evaluations by the EFSA GMO Panel. No new genes in addition to those occurring in maize MON 89034, 1507, MON 88017 and 59122 have been introduced in maize MON 89034 x 1507 x MON 88017 x 59122. Maize MON 89034 x 1507 x MON 88017 x 59122 was produced by conventional crossing of inbred lines containing the maize stacks 1507 x 59122 and MON 89034 x MON 88017, to combine resistance against certain lepidopteran and coleopteran target pests and tolerance to glufosinate-ammonium- and glyphosate-based herbicides.

Molecular analysis confirmed that maize MON 89034, 1507, MON 88017 and 59122 inserts are present and that their structures are retained in maize MON 89034 x 1507 x MON 88017 x 59122. The result of the updated bioinformatic analyses of the flanking sequences and the open reading frames spanning the insert-plant DNA junctions did not reveal a safety concern. The overall levels of Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1 proteins were comparable to those in the respective single events MON 89034, 1507, MON 88017 and 59122.

Previous evaluations showed that the single maize events (MON 89034, 1507, MON 88017 and 59122) and the two double stacks (1507 x 59122 and MON 89034 x MON 88017) do not differ compositionally, agronomically and phenotypically from their respective conventional counterparts,

[4] Unique identifier MON-89Ø34-3 x DAS-Ø15Ø7-1 x MON-88Ø17-3 x DAS-59122-7

[5] Sub-combinations of the individual events exclude all single events. Sub-combinations not previously evaluated by the EFSA GMO Panel are MON 89034 x 1507 x MON 88017, MON 89034 x 1507 x 59122, MON 89034 x MON 88017 x 59122, 1507 x MON 88017 x 59122, MON 89034 x 1507, MON 89034 x 59122, MON 88017 x 59122, 1507 x MON 88017; sub-combinations previously evaluated by the EFSA GMO Panel are 1507 x 59122 and MON 89034 x MON 88017

and that the single events and the two double stacks are equivalent to commercial maize varieties except for the introduced traits. In this application, results of the comparative analyses indicated that maize MON 89034 x 1507 x MON 88017 x 59122 does not differ compositionally, agronomically and phenotypically from its conventional counterpart, and is equivalent to commercial maize varieties, except for the newly introduced traits. The safety of the proteins Cry1A.105 and Cry2Ab2 expressed in maize MON 89034, proteins Cry1F and PAT expressed in maize 1507, proteins Cry3Bb1 and CP4 EPSPS expressed in maize MON 88017, and proteins Cry34Ab1, Cry35Ab1 and PAT expressed in maize 59122 have been assessed previously, and no safety concerns were identified for humans and animals. In addition, the EFSA GMO Panel considers that it is unlikely that the overall toxicity and allergenicity of the whole maize MON 89034 x 1507 x MON 88017 x 59122 has been changed. A feeding study with broiler chickens confirmed that the nutritional properties of grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 are not different from those of its conventional counterpart and commercial maize varieties. Potential interactions between the maize events with respect to potential effects on human and animal health were the focus of the assessment on food/feed safety issues. On the basis of the known functional characteristics and modes of action of the newly expressed proteins (Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1), the EFSA GMO Panel considers it unlikely that interactions between these proteins would occur that would raise any safety concern. Based on the assessment of data provided for the maize stack MON 89034 x 1507 x MON 88017 x 59122, for the single maize events MON 89034, 1507, MON 88017, and 59122, and for the two double stacks 1507 x 59122 and MON 89034 x MON 88017, the EFSA GMO Panel considered the other sub-combinations of the individual events not previously assessed and identified no biological reason to expect that any of the other sub-combinations of these single events would raise a safety concern. In conclusion, the EFSA GMO Panel is of the opinion that maize MON 89034 x 1507 x MON 88017 x 59122 and any sub-combinations of the individual events as present in its segregating progeny are as safe and as nutritious as the conventional counterpart and commercial maize varieties, and concludes that these maize and derived products are unlikely to have adverse effects on human and animal health, in the context of its intended uses.

The application EFSA-GMO-CZ-2008-62 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 MON 89034 x 1507 x MON 88017 x 59122. 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 grains produced by maize MON 89034 x 1507 x MON 88017 x 59122 (including all sub-combinations of the individual events) during transportation and processing, except in the presence of glufosinate-ammonium- and/or glyphosate-based herbicides and/or under infestation by target pests. Taking into account the scope of the application, the rare occurrence of feral maize plants and the low levels of exposure through other routes, the risk to non-target organisms is extremely low. 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 tracts. The scope of the post-market environmental monitoring plan provided by the applicants is in line with the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicants in the general surveillance plan. The EFSA GMO Panel recommends that appropriate management systems should be in place to restrict seeds of maize MON 89034 x 1507 x MON 88017 x 59122 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

In conclusion, the EFSA GMO Panel considers that the information available for maize MON 89034 x 1507 x MON 88017 x 59122 addresses the scientific comments raised by the Member States and that the maize MON 89034 x 1507 x MON 88017 x 59122, as described in this application, is as safe as its conventional counterpart and commercial maize varieties with respect to potential effects on human and animal health and the environment. In addition, the EFSA GMO Panel is of the opinion

that crossing of maize events MON 89034, 1507, MON 88017 and 59122 to produce maize MON 89034 x 1507 x MON 88017 x 59122 does not result in interactions between the events which would affect the safety of maize MON 89034 x 1507 x MON 88017 x 59122 with respect to potential effects on human and animal health and on the environment, in the context of its intended uses. Based on the data provided for maize stack MON 89034 x 1507 x MON 88017 x 59122, the single maize events MON 89034, 1507, MON 88017, 59122, and for the two double stacks 1507 x 59122 and MON 89034 x MON 88017, the EFSA GMO Panel is of the opinion that there is no biological reason to expect that any of the other sub-combinations[5] of the individual events present in the segregating progeny would raise a safety concern. The EFSA GMO Panel concludes that maize MON 89034 x 1507 x MON 88017 x 59122 is unlikely to have adverse effects on human and animal health and the environment, in the context of its intended uses.

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BACKGROUND

On 28 October 2008, the European Food Safety Authority received from the Competent Authority of the Czech Republic an application (Reference EFSA-GMO-CZ-2008-62) for authorisation of genetically modified (GM) maize MON 89034 x 1507 x MON 88017 x 59122[6], submitted by Dow AgroSciences and Monsanto within the framework of Regulation (EC) No 1829/2003 on GM food and feed. After receiving the application EFSA-GMO-CZ-2008-62 and in accordance with Articles 5(2)(b) and 17(2)(b) of the Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission, and made the summary of the application publicly available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of the Regulation. On 19 December 2008 and 12 February 2009, EFSA received additional information requested under completeness check (requested on 5 December 2008 and 14 January 2009). On 3 March 2009, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

Concerning grain, in their letter of 26 May 2010, the applicants confirmed that the scope of this application covers the F_2 grain produced by hybrid F_1 maize MON 89034 x 1507 x MON 88017 x 59122[7]. The applicants indicated that the single maize events MON 89034, 1507, MON 88017 and 59122 crossed together to create maize MON 89034 x 1507 x MON 88017 x 59122, behave as independent genetic loci. Since maize grain is the product of fusion of gametes formed after segregation of genetic components according to Mendelian laws, the F_2 grain harvested from maize MON 89034 x 1507 x MON 88017 x 59122 and imported into the EU, is expected to contain a mixture of maize MON 89034 x 1507 x MON 88017 x 59122 (31.6%), the four triple stacks MON 89034 x 1507 x MON 88017, MON 89034 x 1507 x 59122, MON 89034 x MON 88017 x 59122 and 1507 x MON 88017 x 59122 (10.5% each), the six double stacks MON 89034 x 1507, MON 89034 x 59122, MON 89034 x MON 88017[8], MON 88017 x 59122, 1507 x 59122[9] and 1507 x MON 88017 (3.5% each), the single events (1.2% each) and negative segregant grain (0.4%). This mixture is referred to hereafter as "segregating progeny".

Each event present in maize MON 89034 x 1507 x MON 88017 x 59122 has been previously assessed by EFSA without raising safety concerns (EFSA, 2004, 2005a, 2005b, 2007b, 2008, 2009b, 2009c). The EFSA GMO Panel Guidance Document for the risk assessment of genetically modified plants containing stacked transformation events (EFSA, 2007a) states that a single risk assessment of a stack could cover all combinations with fewer of these events if the single events have been risk assessed. The risk assessment should focus on the intactness and stability of events combined by crossing, the expression of the traits, and the potential interactions between the stacked events. Therefore, EFSA was asked to deliver a scientific opinion on the safety of insect resistant and herbicide tolerant GM maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny[10].

EFSA made the valid application available to the Member States and the European Commission, and consulted nominated risk assessment bodies of the Member States, including national Competent

6 Unique identifier MON-89Ø34-3 x DAS-Ø15Ø7-1 x MON-88Ø17-3 x DAS-59122-7

7 The F1 hybrid maize plants of MON 89034 x 1507 x MON 88017 x 59122 are hemizygous for all newly introduced traits

8 Previously evaluated by EFSA (2009)

9 Previously evaluated by EFSA (2010)

10 For regulatory purposes, the term "sub-combinations of the individual events as present in the segregating progeny" as used throughout this opinion, excludes all single events, although it is recognised that the latter will also occur in the F2 grain harvested from maize MON 89034 x 1507 x MON 88017 x 59122. The unique identifiers of these sub-combinations are MON-89Ø34-3 x DAS-Ø15Ø7-1 x MON-88Ø17-3; MON-89Ø34-3 x DAS-Ø15Ø7-1 x DAS-59122-7; MON-89Ø34-3 x MON-88Ø17-3 x DAS-59122-7; DAS-Ø15Ø7-1 x MON-88Ø17-3 x DAS-59122-7; MON-89Ø34-3 x DAS-Ø15Ø7-1; MON-89Ø34-3 x DAS-59122-7; MON-89Ø34-3 x MON-88Ø17-3; DAS-Ø15Ø7-1 x MON-88Ø17-3; DAS-Ø15Ø7-1 x DAS-59122-7; and MON-88Ø17-3 x DAS-59122-7

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 States had three months after the date of receipt of the valid application (until 3 June 2009) within which to make their opinion known.

The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out an evaluation of the scientific risk assessment of the GM maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny, for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The EFSA GMO Panel carried out the safety evaluation in accordance with the appropriate principles described in the EFSA GMO Panel Guidance Documents for the risk assessment of GM plants and derived food and feed (EFSA, 2006a), and for the risk assessment of GM plants containing stacked transformation events (EFSA, 2007a). Accordingly, the EFSA GMO Panel took into account risk assessments provided by applicants for the placing on the market of the single maize events MON 89034, 1507, MON 88017 and 59122, as well as the maize double stacks MON 89034 x MON 88017 and 1507 x 59122 under EU regulatory procedures, for which EFSA has given an opinion (EFSA, 2004, 2005a, 2005b, 2007b, 2008, 2009a, 2009b, 2009c, 2010). In addition, the scientific comments of the Member States, the additional information provided by the applicants, and relevant scientific publications were taken into consideration.

On 20 May 2009, 11 September 2009, 5 & 29 October 2009 and on 11 March 2010, the EFSA GMO Panel requested additional information from the applicants. The applicants provided the requested information on 23 June 2009, 15 September 2009, 20 November 2009, 2 February 2010 and 31 March/3 May 2010, respectively. Additional information was provided by the applicant on 25 September 2009 and 2 February 2010 (spontaneous submission). On 2 and 26 July 2010, EFSA requested additional information from the applicants. The applicants provided the requested information on 19 and 30 July 2010.

In giving its scientific opinion on maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny, to the European Commission, the Member States and the applicants, 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 assessment of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny, 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 (GM) maize MON 89034 x 1507 x MON 88017 x 59122[6] and all sub-combinations of the individual events as present in its segregating progeny[10] were evaluated with reference to their intended uses, taking account of the appropriate principles described in the EFSA GMO Panel Guidance Documents for the risk assessment of GM plants and derived food and feed (EFSA, 2006a) and for the risk assessment of GM plants containing stacked transformation events (EFSA, 2007a). The evaluation of the risk assessment presented here is based on the information provided in the application relating to maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny submitted in the EU, as well as scientific comments submitted by the Member States and relevant scientific publications. Furthermore, information from applications for the placing on the market of the single maize events (MON 89034, 1507, MON 88017 and 59122) and maize double stacks (1507 x 59122 and MON 89034 x MON 88017) under the EU regulatory framework was taken into account (EFSA, 2004, 2005a, 2005b, 2007b, 2008, 2009a, 2009b, 2009c, 2010).

2. Issues raised by the Member States

The comments raised by the Member States are addressed in Annex G of the EFSA overall opinion[11] and have been considered in this scientific opinion.

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Method of production of maize MON 89034 x 1507 x MON 88017 x 59122

Maize MON 89034 x 1507 x MON 88017 x 59122 was produced by conventional crossing and no new genetic modification was involved. The four inserts that are present in maize MON 89034 x 1507 x MON 88017 x 59122 were derived from maize lines containing the single events: MON 89034, 1507, MON 88017 and 59122. Each of these GM maize events has been the subject of a previous opinion of the EFSA GMO Panel (EFSA, 2004, 2005a, 2005b, 2007b, 2008, 2009a, 2009b).

The maize MON 89034 x 1507 x MON 88017 x 59122 assessed in this application is hemizygous for all newly introduced genes and was produced from a cross between homozygous MON 89034 x MON 88017 in the inbred line HCL301 and homozygous 1507 x 59122 in the inbred line 5XH751. It is mainly the F_2 grain produced by maize MON 89034 x 1507 x MON 88017 x 59122, and not other plant components, that will be imported into the EU. The applicants indicated that the inserts of the events behave as independent genetic loci[12]. The F_2 grain produced by self fertilisation of maize MON 89034 x 1507 x MON 88017 x 59122 imported to the EU is expected to contain a mixture of maize MON 89034 x 1507 x MON 88017 x 59122 (31.6%), the four triple stacks MON 89034 x 1507 x MON 88017, MON 89034 x 1507 x 59122, MON 89034 x MON 88017 x 59122 and 1507 x MON 88017 x 59122 (10.5% each), the six double stacks MON 89034 x 1507, MON 89034 x 59122, MON 89034 x MON 88017, MON 88017 x 59122, 1507 x 59122 and 1507 x MON 88017 (3.5% each), the single events (1.2% each) and negative segregant grain (0.4%).

[11] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2008-764
[12] Additional information 30 March 2010

3.1.2. Summary of the evaluation of the single maize events

Maize MON 89034

Maize MON 89034 was developed through *Agrobacterium tumefaciens* (renamed *Rhizobium radiobacter*)-mediated transformation using the binary plasmid vector PV-ZMIR245 containing two separate T-DNAs. One T-DNA, designated as T-DNA I, contains the *cry1A.105* and the *cry2Ab2* expression cassettes providing increased resistance to certain lepidopteran target pests such as European corn borer (*Ostrinia nubilalis*), fall armyworm (*Spodoptera* ssp), black cutworm (*Agrotis ipsilon*) and corn earworm (*Helicoverpa zea*). The other 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 crossing 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).

Molecular characterisation data established that maize MON 89034 contains a single copy of T-DNA I and that T-DNA II and vector backbone sequences are absent (EFSA, 2008). The structure of the insert in maize MON 89034 was determined by Southern analyses 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 T-DNA right border region has been replaced by a T-DNA left border region. Sequence comparison between the flanking regions of the maize MON 89034 and the corresponding genomic region of conventional maize indicated that the pre-insertion locus was preserved, except for the deletion of 57 bp and the addition of 10 bp. Updated bioinformatic analyses indicate that no known endogenous maize coding sequences or regulatory sequences have been disrupted by the insert[13]. Updated bioinformatic analyses also revealed no biologically relevant similarities to allergens or toxins for any of the putative peptides that might be produced from open reading frames (ORFs) spanning the junction regions[14]. Southern analyses of maize MON 89034 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations.

Maize 1507

Maize 1507 was generated by particle bombardment. As a result of the genetic modification, maize 1507 expresses a truncated *cry1F* gene from *Bacillus thuringiensis* subsp. *aizawai*, conferring resistance to certain lepidopteran target pests such as the European corn borer, and the *pat* gene from *Streptomyces viridochromogenes* that renders it tolerant to glufosinate-ammonium-based herbicides.

Molecular analyses showed that maize 1507 contains one copy of the DNA fragment used for transformation (containing the *cry1F* and phosphinothricin acetyltransferase (*pat)* genes) and additionally, partial fragments of the *cry1F* and *pat* genes, and that these fragments are present at a single locus in the nuclear genome (EFSA, 2004, 2005a, 2005b, 2009a). The structure of the insert in maize 1507 was determined by Southern analyses and DNA sequencing. Morisset et al. (2009) showed that the 35S promoter of maize 1507 contains a single nucleotide difference, compared to the reported sequence of the DNA fragment used for transformation. Following a request from the EFSA GMO Panel, the applicant has clarified that this difference was present in plants at early stages of product development and is present in all maize 1507 lines and stacks that have been evaluated by the EFSA GMO Panel[15]. Updated bioinformatic analyses confirmed that in addition to the intact genes, the insert in maize 1507 includes DNA sequences originating from the fragment used for transformation, as well as maize chloroplast DNA sequences[13] (EFSA, 2004). Analyses of DNA

[13] Additional info June 2009
[14] Additional info June 2009 & September 2009
[15] Spontaneous additional information September 2009 and additional info February 2010

sequences flanking both ends of the insert showed that they are maize genomic DNA. Updated bioinformatic analyses of these flanking sequences suggest that the insert in maize 1507 is flanked by a putative RIRE2 retrotransposon (downstream) and a Huck1 retrotransposable element (upstream). Transcript and bioinformatic analyses[16] of ORFs spanning all junction regions between genomic and insert DNA, as well as junction regions between partial fragments of *cry1F* and *pat* genes were performed and no novel putative proteins with sequence similarity to known toxins or allergens were identified. Southern analyses of maize 1507 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations.

Maize MON 88017

Maize MON 88017 was developed through *A. tumefaciens*-mediated transformation using the PV-ZMIR39 plasmid and as a result expresses a CP4 5-enolpyruvyl-3-phosphoshikimic acid synthase gene (*epsps)* and a modified *B. thuringiensis* subsp. *kumamotoensis cry3Bb1* gene resulting in tolerance to glyphosate-based herbicides and conferring resistance to certain coleopteran target pests such as corn rootworms (*Diabrotica* spp.), respectively.

Molecular characterisation data established that maize MON 88017 contains one copy of the T-DNA and that vector backbone sequences are absent (EFSA, 2009b). 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. Updated bioinformatic analyses 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[17], further confirming previously obtained results (EFSA, 2009b). Phenotypic, agronomic and compositional analyses showed that maize MON 88017 is equivalent to conventional maize (see sections 4.1.3 and 4.1.4), except for the newly expressed proteins, indicating that the insertion of the transgene has not caused a modification which would raise a safety concern. Updated bioinformatic analyses also revealed no biologically relevant similarity to allergens or toxins for any of the putative peptides that might be produced from ORFs spanning the junction regions[18]. Southern analyses of maize MON 88017 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations.

Maize 59122

Maize 59122 was developed through *A. tumefaciens*-mediated transformation and as a result expresses the *cry*34*Ab1* and *cry*35*Ab1* genes from *B. thuringiensis* strain PS149B1, conferring resistance to certain coleopteran target pests such as corn rootworms (*Diabrotica* spp.), and the *pat* coding sequence from *S. viridochromogenes* resulting in tolerance to glufosinate-ammonium-based herbicides.

Molecular characterisation data established that maize 59122 contains a single insert of the T-DNA, and that vector backbone sequences are absent (EFSA, 2007b). Bioinformatic analyses revealed that flanking regions of maize 59122 show significant homology to maize genomic DNA and expressed sequence tags (EST). Updated bioinformatic analyses indicated that the DNA in maize 59122 was inserted 1032 bp downstream from the coding region of a maize pentatricopeptide repeat (PPR) protein, the empty pericarp 4 (*emp*4)[19]. This PPR protein is essential for seed development in maize (Gutierrez-Marcos et al., 2007). In maize 59122, seed development is not affected (see section 4.1.4 & 6.1.2.1). Updated bioinformatic analyses of ORFs spanning the two junction regions were performed

[16] Additional info September 2009
[17] Additional info June 2009
[18] Additional info June 2009 & September 2009
[19] Additional info November 2009

and no novel ORFs with sequence similarity to known toxins or allergens were identified[19]. Southern analyses of maize 59122 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations.

3.1.3. Transgene constructs in MON 89034 x 1507 x MON 88017 x 59122

The integrity of the individual inserts present in maize MON 89034 x 1507 x MON 88017 x 59122 was investigated using Southern analyses[20]. This involved the use of DNA probes specific for the single inserts and restriction enzyme digestions informative of the structure of all events, including the junctions with the host genomic DNA. The predicted DNA hybridisation patterns from each single event were retained in maize MON 89034 x 1507 x MON 88017 x 59122, demonstrating that the integrity of the inserts was maintained.

3.1.4. Information on expression of the inserts

The levels of newly expressed proteins Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1 in maize MON 89034 x 1507 x MON 88017 x 59122 were analysed by enzyme-linked immunosorbent assays (ELISA)[21]. Tissue samples for analyses were collected from five locations in USA during 2006. The trials were located within the major maize-growing region of the USA and provided a variety of environmental conditions. Each trial included appropriate comparators (see section 4.1.2) and protein expression levels were determined in leaves, roots, forage, whole plant, pollen, and grain. The scope of the application covers food and feed uses, import and processing of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny, and excludes cultivation. Therefore, protein expression data related to the grain (F_2 generation) produced by maize MON 89034 x 1507 x MON 88017 x 59122[22] are considered most relevant, and are summarised in Table 1. Levels of proteins in the grain (F_2 generation) produced by maize MON 89034 x 1507 x MON 88017 x 59122 are comparable to those in the single events, although the mean level of Cry1A.105 was lower in maize MON 89034 compared to maize MON 89034 x 1507 x MON 88017 x 59122. The levels of the newly expressed proteins do not pose a safety concern (also see section 5.1.4.1, 5.1.5.1 and 6.1.2). The same conclusions were reached by the EFSA GMO Panel for the parental maize stacks 1507 x 59122 (EFSA, 2009c) and MON 89034 x MON 88017 (EFSA, 2010).

[20] Technical Dossier/ Section D2
[21] Technical Dossier/ Section D3
[22] Additional info March 2010

Table 1. Summary of protein levels in grain produced by maize MON 89034 x 1507 x MON 88017 x 59122, MON 89034, 1507, MON 88017 and 59122 (µg/g dry weight)

		MON 89034 x 1507 x MON 88017 x 59122	MON 89034	1507	MON 88017	59122
Cry1A.105	mean	4.3	2.8			
	range	[3.4 - 4.9]	[1.7 - 3.5]	--	--	--
Cry1F	mean	3.3		3.2		
	range	[2.1 - 7.4]	--	[2.4 - 4.6]	--	--
Cry2Ab2	mean	5.7	5.6			
	range	[4.1 - 7.5]	[2.7 - 7.1]	--	--	--
Cry3Bb1	mean	18			20	
	range	[10 - 26]	--	--	[12 - 38]	--
Cry34Ab1	mean	63				67
	range	[48 - 94]	--	--	--	[44 - 102]
Cry35Ab1	mean	1.69				1.86
	range	[1.24 - 2.31]	--	--	--	[1.18 - 2.65]
CP4 EPSPS	mean	5.2			4.9	
	range	[3.5 - 7.1]	--	--	[3.3 - 7.4]	--
PAT	mean	LOQ				LOQ
	range	[LOD - 0.10]	--	LOD	--	[LOQ - 0.09]

LOD, values below limit of detection; LOQ, values below limit of quantification and above LOD; --, not applicable

3.1.5. Inheritance and stability of inserted DNA

The genetic stability of the inserted DNA in the single events MON 89034, 1507, MON 88017 and 59122 was demonstrated previously (EFSA, 2005a, 2007b, 2008, 2009b). The Southern analyses data show that the four events are present in maize MON 89034 x 1507 x MON 88017 x 59122 and that the structure of each insert is retained[23].

3.2. Conclusion

Maize MON 89034 x 1507 x MON 88017 x 59122 was produced by conventional crossing, no additional genetic modification was involved. Southern analyses demonstrated that the structures of maize events MON 89034, 1507, MON 88017 and 59122 were retained in maize MON 89034 x 1507 x MON 88017 x 59122. Results of the updated bioinformatic analyses of the flanking sequences and the ORFs spanning the newly created DNA junctions did not indicate any safety concern. The levels of Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1 proteins of maize MON 89034 x 1507 x MON 88017 x 59122 have been demonstrated to be comparable with those of the single events. Molecular characterisation data do not indicate safety concerns arising from combining the single events MON 89034, 1507, MON 88017 and 59122 to produce the maize stack MON 89034 x 1507 x MON 88017 x 59122. Based on these data it is also unlikely that safety concerns would arise from the segregating progeny of maize MON 89034 x 1507 x MON 88017 x 59122.

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

[23] Technical Dossier/ Section D5

4. Comparative analysis

4.1. Evaluation of relevant scientific data

4.1.1. Summary of the previous evaluation of the single maize events

Maize MON 89034

Forage and grain of maize MON 89034 and its conventional counterpart were obtained from field trials carried out in the USA in 2004 and in Argentina for the season 2004-2005. Both cultivation periods included field trials at five different locations, all being representative of the maize growing regions of the respective countries. The trials used agronomic practices representative of the respective regions. In addition to maize MON 89034 and its conventional counterpart, a total of fifteen commercial maize varieties were included in the field trial to estimate the naturally occurring variation in composition expected for the various analytes in conventional maize. In the field trials performed to study the agronomic and phenotypic characteristics, in total twenty-three commercial maize varieties were used to describe the natural variation in studied parameters.

With regard to agronomic and phenotypic characteristics, no consistent differences were observed between maize MON 89034 and its conventional counterpart grown in the various field trials. With regard to compositional analyses, statistical differences between maize MON 89034 and its conventional counterpart were identified, but these were not consistently found across the different field trial locations. All of the observed differences were small and fell within the natural variation found in the commercial maize varieties grown in the study. Furthermore, the composition of maize MON 89034 fell within the natural variation as reported in the literature and in the ILSI crop composition database (ILSI, 2006).

Based on these data and in line with its previous opinion (EFSA, 2008) the EFSA GMO Panel considers that maize MON 89034 does not differ from its conventional counterpart with regard to compositional, phenotypic and agronomic characteristics and is equivalent to commercial maize varieties, except for the newly expressed Cry1A.105 and Cry2Ab2 proteins.

Maize 1507

The whole crop and grain of maize 1507 and its conventional counterpart were collected for compositional analysis from field trials. These field trials were performed during three seasons and at different locations (six locations in Chile (1998-1999), three locations in France and Italy (1999), and six locations in France, Italy and Bulgaria (2000)). GM maize plants in the Chilean field trials were all treated with glufosinate-ammonium-based herbicides, while those in the European field trials were split into treated and untreated groups. On the basis of the results of compositional analysis of samples from three seasons and a representative range of environments, it was concluded by the EFSA GMO Panel that forage and grain of maize 1507 were compositionally equivalent to those of conventional maize, except for the presence of Cry1F and PAT proteins in maize 1507.

In addition, field trials carried out over several seasons and at different locations (USA in 1999, France, Italy, and Bulgaria in 2000, Spain in 2002) did not indicate any unexpected changes to agronomic and phenotypic characteristics (EFSA, 2005a, 2005b).

Based on these data and in line with its previous opinions (EFSA, 2005a, 2005b), the EFSA GMO Panel considers that maize 1507 does not differ from its conventional counterpart with regard to compositional, phenotypic and agronomic characteristics and is equivalent to commercial maize varieties, except for the newly expressed Cry1F and PAT proteins.

Maize MON 88017

Forage and grain of maize MON 88017 plants sprayed with glyphosate-based herbicides and the same tissues from its conventional counterpart were obtained from field trials carried out at three locations in the USA in 2002 and at four locations in Argentina in 2003-2004. Commercial maize varieties were also grown alongside maize MON 88017 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 MON 88017 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 commercial maize 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, MON 88017 not treated with glyphosate-based herbicides was grown at three locations in Germany and at three locations in Spain in 2007. Various statistically significant differences were observed between MON 88017 and its conventional counterpart, none of which occurred within all locations and all of which were within the range of commercial maize varieties. No consistent differences were observed in the analysis of agronomic and phenotypic characteristics of MON 88017 compared to its conventional counterpart and commercial maize varieties over several seasons and no consistent differences were observed in each season and at all locations.

Based on these data and in line with its previous opinion (EFSA, 2009b), the EFSA GMO Panel considers that maize MON 88017 does not differ from its conventional counterpart with regard to compositional, phenotypic and agronomic characteristics and is equivalent to commercial maize varieties, except for the newly expressed Cry3Bb1 and CP4 EPSPS proteins.

Maize 59122

Maize 59122 was compared with an appropriate non-GM control with comparable genetic background to maize 59122. Whole crops and maize tissues, including grain, were collected for compositional analysis from field trials. These field trials were carried out over several seasons and at different locations: six locations in Chile (2002-2003), three locations in the USA (2003), two locations in Canada (2003), three locations in Bulgaria (2003 and 2004), and three locations in Spain (2004). Maize 59122 plants treated with glufosinate-ammonium-based herbicides, untreated and the non-GM control maize were included in these field trials. On the basis of the results of compositional analysis of samples from a representative range of environments and several seasons, it was concluded by the EFSA GMO Panel that forage and grain of maize 59122 were compositionally equivalent to those of conventional maize, except for the presence of Cry34Ab1, Cry35Ab1 and PAT proteins in maize 59122 (EFSA, 2007b).

In addition, during these field trials over several seasons and at different locations agronomic and phenotypic data did not show indications for unexpected changes of agronomic characteristics and performance (EFSA, 2007b).

Based on these data and in line with its previous opinion (EFSA, 2007b), the EFSA GMO Panel considers that maize 59122 does not differ from its conventional counterpart with regard to compositional, phenotypic and agronomic characteristics and is equivalent to commercial maize varieties, except for the newly expressed Cry34Ab1, Cry35Ab1 and PAT proteins.

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

Given the outcomes of the risk assessment of the single maize events and the fact that compositional data on the single maize events grown during multiple seasons have already been assessed by the EFSA GMO Panel, the Panel considers the data from one season as sufficient for the evaluation of the

maize stack MON 89034 x 1507 x MON 88017 x 59122, and in line with its guidance on the assessment of stacked events (EFSA, 2007a).

For the comparative analyses of the phenotypic and agronomic characteristics, as well as the composition of forage (F_1 generation) and grain (F_2 generation), maize MON 89034 x 1507 x MON 88017 x 59122 and its conventional counterpart, *i.e.* maize XE6001, were grown in five locations in the USA in 2006. Commercial maize varieties were also included in the field trial design, with three varieties per location and fifteen different varieties in total. The field trial design in each location included three replicated blocks. All of these plots underwent similar agronomic treatments, except for treatment of plots of the GM maize MON 89034 x 1507 x MON 88017 x 59122 with target herbicides containing glufosinate-ammonium and glyphosate as active ingredients. Given the fact that previous assessments of the herbicide-tolerant single events MON 88017, 1507 and 59122 considered both plants treated with the target and conventional herbicides and plants treated with only conventional herbicides, the EFSA GMO Panel does not consider it necessary to ask for compositional data on maize MON 89034 x 1507 x MON 88017 x 59122 that was treated with conventional herbicides (*i.e.* not with the target herbicides). Single maize events were included for protein expression analysis only.

Given all information provided, including the fact that the single events have previously been shown to be compositionally, agronomically and phenotypically not different from their conventional counterparts and equivalent to commercial varieties (EFSA, 2004, EFSA, 2005b, EFSA, 2005a, EFSA, 2007b, EFSA, 2009c, EFSA, 2009b, EFSA, 2008, EFSA, 2010, EFSA, 2009a), the events have been molecular characterized (see section 3.2), and the functional characteristics and modes of actions of the newly expressed proteins are known, the EFSA GMO Panel accepts the field trial design used to assess maize MON 89034 x 1507 x MON 88017 x 59122.

Grain samples were also analysed for the presence of recombinant DNA by PCR. Due to the presence of recombinant DNA in grain of the conventional maize counterpart and one of the three commercial maize varieties at one location (which probably resulted from strong winds at the time of pollen shed), maize MON 89034 x 1507 x MON 88017 x 59122 and its conventional maize counterpart, as well as the specified sample of the commercial maize variety from this location were not included in the final analysis. In consequence, the number of samples of forage and grain of either maize MON 89034 x 1507 x MON 88017 x 59122 or its conventional maize counterpart amounted to twelve (three per location, four locations in total), whilst fourteen commercial maize varieties from five locations were included[24].

4.1.3. Compositional analysis

The compositional parameters for which forage and grain produced by maize MON 89034 x 1507 x MON 88017 x 59122, its conventional counterpart, and commercial maize varieties have been analysed are in line with those recommended by the Consensus Document on key compositional parameters of maize published by the OECD (2002). The analysis of forage included proximates (moisture, ash, fat, protein, carbohydrates by calculation), fibre (acid detergent fibre [ADF]; neutral detergent fibre [NDF]), calcium, and phosphorus, whilst that of grain included proximates, fibre (ADF, NDF, dietary fibre), amino acids, fatty acids, minerals, vitamins (thiamine [B1], riboflavin [B2], niacin, pyridoxamine [B6], folic acid, alpha-tocopherol [E]), provitamin A (beta-carotene), and secondary plant metabolites (phytic acid, raffinose, furfuraldehyde, ferulic acid, p-coumaric acid). Besides the information obtained from the commercial maize varieties included in the same field trial, also data from the literature and the ILSI Crop Composition database (ILSI, 2006) were used to establish the ranges normally observed in commercial maize for the various nutrients and anti-nutrients analyzed.

[24] Technical dossier/ Section D7.2

The outcomes of the comparison between maize MON 89034 x 1507 x MON 88017 x 59122 and its conventional counterpart across locations showed that six parameters showed statistically significant differences in grain produced by maize MON 89034 x 1507 x MON 88017 x 59122. Vitamin B1, oleic acid, and eicosenoic acid were slightly lower, whilst stearic acid, linolenic acid, and arachidic acid were slightly higher, in grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 compared to its conventional counterpart. In the per-location statistical analysis, those differences observed in the across-location analysis and a number of additional statistically significant differences between maize MON 89034 x 1507 x MON 88017 x 59122, its conventional counterpart and commercial maize varieties occurred in separate locations but not in all of them. For all parameters showing differences, the range of individual values of MON 89034 x 1507 x MON 88017 x 59122 was completely within the range of commercial maize varieties, except for three parameters (arachidic acid, vitamin B1, and folic acid), each of which showed a single sample in one location having a value slightly beyond this range, the average values of maize MON 89034 x 1507 x MON 88017 x 59122 were within the background range of literature and database values[25].

The EFSA GMO Panel considered the observed compositional differences between grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 and its conventional counterpart in the light of the field trial design, measured biological variation and the level of the studied compounds in commercial maize varieties, and concludes that forage and grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 do not differ compositionally from its conventional counterpart and are equivalent to commercial maize varieties, except for the newly introduced traits.

4.1.4. Agronomic traits and GM phenotype

Previous studies have shown that with the exception of the insect resistance traits in maize MON 89034, the combined traits of insect resistance and herbicide tolerance in maize 1507, MON 88017 and 59122, these GM maize are agronomically and phenotypically equivalent to their conventional counterparts (EFSA, 2004, 2005a, 2005b, 2007b, 2008, 2009a, 2009b).

In the present application, the analyses of agronomic and phenotypic characteristics of maize MON 89034 x 1507 x MON 88017 x 59122, its conventional counterpart and twelve commercial maize varieties included a range of parameters related to plant morphology, physiology, appearance and performance, including stressors and plant health. A number of parameters showed statistically significant differences in the per-location statistical analysis of the comparison between maize containing stack MON 89034 x 1507 x MON 88017 x 59122 and its conventional counterpart but this was not consistently observed in each location.

The EFSA GMO Panel concludes that, with regard to its agronomic performance and phenotypic characteristics, maize MON 89034 x 1507 x MON 88017 x 59122 does not differ from its conventional counterpart and is equivalent to commercial maize varieties, except for the newly introduced traits.

4.2. Conclusion

On the basis of the results of the comparative analysis, the EFSA GMO Panel concludes that maize MON 89034 x 1507 x MON 88017 x 59122, as assessed in this application, does not differ compositionally, phenotypically and agronomically from its conventional counterpart and is equivalent to commercial maize varieties, except for the presence of the newly expressed proteins (Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1). Furthermore, on the basis of the assessment of the data available including data on the F_2 grain, the EFSA GMO Panel is of the opinion that crossing MON 89034, 1507, MON 88017 and 59122 to produce maize MON 89034 x 1507 x MON 88017 x 59122 does not result in interactions between the

[25] Technical dossier/ Section D7.3 and additional info February 2010

maize events which cause compositional, agronomic or phenotypic changes that would raise a safety concern.

Furthermore, based on all data available, the EFSA GMO Panel is of the opinion that the same conclusions can be extended to any sub-combinations of the individual events as present in its segregating progeny.

5. Food/Feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Summary of the previous evaluation of the single maize events

Maize MON 89034

Maize MON 89034 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 were equivalent to those present in maize expressing the event MON 89034. 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 marginally less rapidly in simulated intestinal fluid. In bioinformatic studies, the amino acid sequence of Cry1A.105 and Cry2Ab2 showed no similarity either to proteins that are known to be allergens or toxic to humans and other animals (EFSA, 2008).

In a 90-day feeding study in rats with grain material from maize MON 89034 (33% of the feed), no treatment-related adverse effects were observed, and a 42-day feeding study on broiler chickens (55-59% of the feed) showed that maize MON 89034 does not differ nutritionally from its conventional counterpart and is equivalent to commercial maize varieties included in the study (EFSA, 2008).

The EFSA GMO Panel concluded that maize MON 89034 is as safe as conventional maize and that the overall allergenicity of the whole plant is not changed. Maize MON 89034 and derived products are unlikely to have adverse effects on human and animal health in the context of its intended use (EFSA, 2008).

Maize 1507

Maize 1507 expresses the Cry1F and PAT proteins. A trypsinised *Pseudomonas fluorescens*-produced Cry1F protein, identical to the truncated Cry1F protein expressed in 1507 maize, except for a phenylalanine instead of a leucine at position 604 and a C-terminal extension with seven amino acids residues (606-612: Ala-Glu-Tyr-Asp-Leu-Glu-Arg), was used for the safety testing instead of the maize-produced truncated Cry1F after it had been demonstrated experimentally that it was equivalent to that present in maize 1507. Similarly a PAT microbial protein was used for safety studies after it had been demonstrated experimentally that it was equivalent to the enzyme present in maize 1507. No toxicity of the Cry1F and PAT proteins were observed in acute oral toxicity studies in mice. No oral toxicity of maize 1507 was observed in a rat study where the experimental animals were fed *ad libitum* a diet containing up to 33% maize 1507. In addition, nutritional data comprising target animal feeding studies with maize grain on broiler chickens and dairy cows indicate that maize 1507 is nutritionally equivalent to conventional maize cultivars. The allergenicity risk assessment of the Cry1F and PAT proteins indicated a low probability of potential allergenicity. The allergenicity of the whole crop does not appear relevant to the EFSA GMO Panel since maize is not considered a common allergenic food. The GMO Panel concluded that the studies available support the findings of the molecular characterization and the compositional analysis and indicates maize 1507 to be as safe as its conventional counterparts (EFSA, 2004, 2005a, 2005b, 2009a).

The GMO Panel concluded that maize 1507 is as safe as its conventional counterpart and commercial maize varieties and considered it unlikely that the overall allergenicity of the whole plant is changed. Maize 1507 and derived products are unlikely to have adverse effects on human and animal health in the context of the intended uses (EFSA, 2004, 2005a, 2005b, 2009a).

Maize MON 88017

Analogues of the newly expressed Cry3Bb1 and CP4 EPSPS proteins in MON 88017 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. The proteins neither showed toxicity in acute oral toxicity studies in mice, nor did they show relevant similarities to known toxic or allergenic proteins in bioinformatic-supported comparisons of their amino acid sequences. Cry3Bb1 and CP4 EPSPS proteins were also rapidly degraded during incubation 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-day rat feeding study with diets containing 33% grain from maize MON 88017. No indications of treatment-related adverse effects were observed in this study. A nutritional, 42-day broiler chicken feeding study was also carried out with diets containing between 55 and 60% grain from maize MON 88017, showing that the latter was nutritionally equivalent to conventional maize (EFSA, 2009b).

The GMO Panel concluded that maize MON 88017 is as safe as its conventional counterpart and commercial maize varieties and considered it unlikely that the overall allergenicity of the whole plant is changed. Maize MON 88017 and derived products are unlikely to have adverse effects on human and animal health in the context of the intended uses (EFSA, 2009b).

Maize 59122

P. fluorescens-produced Cry34Ab1 and Cry35Ab1 proteins and *E. coli*-produced PAT protein were used for toxicity studies after it has been demonstrated experimentally that they are equivalent to those extracted from leaf material of maize event 59122. The newly expressed Cry34Ab1 and Cry35Ab1 proteins induced no adverse effects in acute and repeated dose oral toxicity studies in mice at high dose levels and they are rapidly degraded in simulated gastric fluid and inactivated during heat treatments. The PAT protein is expressed at very low levels in maize 59122 and it has also been proved to be safe in toxicity studies and it is rapidly degraded by proteases. The sequence of the Cry34Ab1, Cry35Ab1 and PAT proteins did not show any significant similarity with the sequences of known toxins or allergens. With regard to animal studies with the whole product, there were no indications of adverse effects in a 90-day subchronic toxicity study on rats fed diets containing maize 59122 grain. The allergenicity of the whole crop does not appear relevant to the EFSA GMO Panel since maize is not considered a common allergenic food. In addition, nutritional data comprising a target animal feeding study with maize 59122 grain on broiler chickens indicate that maize 59122 is nutritionally equivalent to the non-GM comparator. These animal studies therefore further supported the findings of the compositional analysis indicating no effect beyond the intended introduction of the Cry34Ab1, Cry35Ab1 and PAT proteins (EFSA, 2007b).

The GMO Panel concluded that maize 59122 is as safe as its conventional counterpart and commercial maize varieties and considered it unlikely that the overall allergenicity of the whole plant is changed. Maize 59122 and derived products are unlikely to have adverse effects on human and animal health in the context of the intended uses (EFSA, 2007b).

5.1.2. Product description and intended use

The scope of application EFSA-GMO-UK-2008-62 is for food and feed uses, import and processing of maize MON 89034 x 1507 x MON 88017 x 59122 and all derived products (*e.g.* starch, syrups,

ethanol, maize oil, flakes, coarse and regular grits, coarse and dusted meal, flour, maize germ meal, maize gluten feed, condensed steep water, and maize gluten meal).

The genetic modifications in maize MON 89034 x 1507 x MON 88017 x 59122 are intended to improve agronomic performance only and it is not intended to influence the nutritional properties, the processing characteristics, and overall use of maize as a crop.

5.1.3. Effect of processing

Since maize MON 89034 x 1507 x MON 88017 x 59122 does not differ compositionally from its conventional counterpart and is equivalent to commercial maize varieties, except for the newly expressed proteins (see section 4.2), the effect of processing on maize MON 89034 x 1507 x MON 88017 x 59122 is not expected to be different compared with conventional maize.

5.1.4. Toxicology

5.1.4.1. Toxicological assessment of expressed novel proteins in maize MON 89034 x 1507 x MON 88017 x 59122

No new genes in addition to those occurring in maize MON 89034, 1507, MON 88017 and 59122 have been introduced in maize MON 89034 x 1507 x MON 88017 x 59122. The proteins Cry1A.105 and Cry2Ab2 expressed in maize MON 89034, Cry1F and PAT expressed in maize 1507, Cry3Bb1 and CP4 EPSPS expressed in maize MON 88017, and Cry34Ab1, Cry35Ab1 and PAT expressed in maize 59122 have been assessed previously, and no safety concerns were identified for humans and animals (EFSA, 2004, 2005a, 2005b, 2007b, 2008, 2009a, 2009b). The EFSA GMO Panel is not aware of any new information that would change these conclusions.

While the EFSA GMO Panel has considered the bioinformatic studies in the recently published EFSA scientific opinions on the single maize events MON 89034 (EFSA, 2008), 1507 (EFSA, 2004, 2005a, 2005b, 2009a), and MON 88017 (EFSA, 2009b), the Panel requested from the applicants updated studies for the newly expressed proteins being present in maize 59122, *i.e.* Cry34Ab1, Cry35Ab1, and PAT. The applicants provided updated bioinformatic studies for all newly expressed proteins present in the four single maize events. No relevant similarities were identified between these newly expressed proteins and known toxins[26].

Determination of the levels of the newly expressed proteins in grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 showed comparable levels to those in the respective single maize events (see section 3.1.4). On the basis of the known functions and modes of action, the EFSA GMO Panel considers it unlikely that interactions between these newly expressed proteins (Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1) would occur that would raise any safety concern.

5.1.4.2. Toxicological assessment of new constituents other than proteins

No new constituents other than the Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1 proteins are expressed in maize MON 89034 x 1507 x MON 88017 x 59122. No biologically relevant changes in the composition of maize MON 89034 x 1507 x MON 88017 x 59122 were detected (see section 4.1.3). Therefore, a toxicological assessment of new constituents is not applicable.

5.1.4.3. Toxicological assessment of the whole GM food/feed

Maize MON 89034, 1507, MON 88017 and 59122 have previously been found as safe as their conventional counterparts for human and animal consumption (EFSA, 2004, 2005a, 2005b, 2007b,

[26] Additional info June 2009

2008, 2009a, 2009b). As described in section 5.1.1, the EFSA GMO Panel's assessment of the single maize events MON 89034, 1507, MON 88017, and 59122 also considered the outcomes of 90-days rat feeding studies with each of these single events, which did not show adverse treatment-related effects (EFSA, 2004, 2005a, 2005b, 2007b, 2008, 2009b). In the present assessment, no change in the structural integrity of the inserts in maize MON 89034 x 1507 x MON 88017 x 59122 was found when compared to the respective single events in the analysis of molecular characteristics, and protein levels of grain produced from maize MON 89034 x 1507 x MON 88017 x 59122 were shown to be comparable to those in the respective single maize events (see section 3.2). Moreover, the compositional, agronomic and phenotypic characteristics of maize MON 89034 x 1507 x MON 88017 x 59122 were not different from those of its conventional counterpart (see section 4.2). In addition, at the EFSA GMO Panel's request, the applicant provided an assessment of the potential interactions between the events combined within maize MON 89034 x 1507 x MON 88017 x 59122 that could impact on human and animal health[27]. The EFSA GMO Panel considered all the data available for maize MON 89034 x 1507 x MON 88017 x 59122 and the newly expressed proteins (Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1) and is of the opinion that interactions between the maize events that might impact on the food and feed safety of maize MON 89034 x 1507 x MON 88017 x 59122 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, 2006a).

5.1.5.1. Assessment of allergenicity of the newly expressed proteins

As described in section 5.1.1, the EFSA GMO Panel has previously assessed the potential allergenicity of the newly expressed proteins Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1 within the assessments of the single events MON 89034, 1507, MON 88017, and 59122. It was found unlikely that these proteins are allergenic (EFSA, 2004, 2005a, 2005b, 2007b, 2008, 2009a, 2009b). In addition, updated bioinformatic studies were provided by the applicants for all single events in 2009[28]. No relevant similarities between the amino acid sequences of the newly expressed proteins and known allergenic proteins could be identified in the outcomes of these bioinformatic studies, confirming the results of previous studies. The EFSA GMO Panel has, thus, concluded that it is unlikely that these newly expressed proteins are allergenic.

5.1.5.2. Assessment of allergenicity of the whole GM plant

The issue of a potentially increased allergenicity of maize MON 89034 x 1507 x MON 88107 x 59122 as compared to its respective single maize events, and 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 scientific literature (Bardana, 2008; Jeebhay and Quirce, 2007). 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 common food and that, in the few cases reported, the major maize allergens have 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

[27] Additional info June 2009
[28] Additional info June and September 2009

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 maize MON 89034 x 1507 x MON 88107 x 59122 would significantly increase the intake and exposure to maize.

5.1.6. Nutritional assessment of GM food/feed

For each of the single maize events, the EFSA GMO Panel has previously assessed data on nutritional feeding studies in food-producing animals, in particular the rapidly growing broiler chickens (EFSA, 2004, 2005a, 2005b, 2007b, 2008, 2009a, 2009b). The EFSA GMO Panel has concluded that the outcomes of these tests confirm these single events do not differ nutritionally from their conventional counterparts. Moreover, the compositional data summarized in section 4.1.3 show that maize MON 89034 x 1507 x MON 88017 x 59122 is compositionally equivalent to commercial maize varieties.

A 42-day broiler chicken feeding study with adjusted diets containing grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 was evaluated in the frame of the current application[29]. Both male and female chicken received adjusted diets containing 61-64% of one of eight maize lines, *i.e.* grain produced by maize MON 89034 x 1507 x MON 88017 x 59122, its conventional counterpart (XE6001), and six commercial maize varieties. One hundred animals, including fifty animals of each gender that were divided over ten pens of ten animals each, were used for each dietary treatment. Both maize grain and adjusted diets were analysed for chemical composition, whilst the grain was also analysed for potential presence of pesticide and mycotoxin residues. During the experiment, animals were checked for feed consumption, body weight and mortality; at the end of the experiment, they were checked for carcass characteristics, including the weight of the carcass and carcass parts, and the composition of the meat of thighs and breast (fat, moisture, protein). No statistically significant differences were observed between the group fed adjusted diets containing grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 and its conventional counterpart, except for a higher absolute and relative (%) fat pad weight in the group fed GM-maize as compared to that fed control maize (47 *v.* 43 g; 1.9 *v.* 1.7% of live weight). However, these differences were not observed in the comparison between the group fed GM-maize and each of the groups fed commercial maize varieties. The observed differences in fat pad weights were also observed in female chicken fed with GM maize compared with non-GM maize when analyzed in a by-gender statistical analysis. In the absence of any other treatment-related effects on performance, the EFSA GMO Panel does not consider the statistically significant difference in fat pad weights to be of biological relevance. The broiler chicken feeding study supported the results of the comparative compositional analysis and confirmed that grains produced by maize MON 89034 x 1507 x MON 88017 x 59122 are nutritionally equivalent to grains of the conventional counterpart and six commercial maize varieties.

5.1.7. Post-market monitoring of GM food/feed

No biologically relevant compositional, agronomic and phenotypic changes were identified in maize MON 89034 x 1507 x MON 88017 x 59122 when compared with its conventional counterpart and commercial maize varieties. Furthermore, the overall intake or exposure is not expected to change because of the introduction of maize MON 89034 x 1507 x MON 88017 x 59122 into the market. The EFSA GMO Panel therefore considers maize MON 89034 x 1507 x MON 88017 x 59122 to be as safe as its conventional counterpart and that post-market monitoring (EFSA, 2006a) of the food/feed derived from maize MON 89034 x 1507 x MON 88017 x 59122 is not necessary.

5.2. Conclusion

The proteins Cry1A.105 and Cry2Ab2 expressed in maize MON 89034, Cry1F and PAT expressed in maize 1507, Cry3Bb1 and CP4 EPSPS expressed in maize MON 88017, Cry34Ab1, Cry35Ab1 and PAT expressed in maize 59122, have previously been assessed (EFSA, 2004, 2005a, 2005b, 2007b,

[29] Technical Dossier/ Section D7.10

2008, 2009a, 2009b) and no safety concerns were identified for human and animals. No new genes in addition to those occurring in the single event maize (MON 89034, 1507, MON 88017, 59122) have been introduced in the maize stack MON 89034 x 1507 x MON 88017 x 59122. Based on the molecular characteristics of these events (see section 3.2), the known functional characteristics and modes of action of the newly expressed proteins and the outcomes of the comparative analyses of compositional, agronomic and phenotypic characteristics (see section 4.2), the EFSA GMO Panel considers it unlikely that interactions between the maize events will occur that could impact on the food and feed safety and the nutritional properties of this maize. The EFSA GMO Panel considers it unlikely that the overall allergenicity MON 89034 x 1507 x MON 88017 x 59122 of maize has been altered. Grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 was tested in a nutritional broiler chicken feeding study, which shows that this maize does not differ nutritionally from its conventional counterpart and is equivalent to commercial maize varieties.

Based on data on the maize stack MON 89034 x 1507 x MON 88017 x 59122, data on the single maize events MON 89034, 1507, MON 88017, and 59122, and the two double stacks 1507 x 59122 and MON 89034 x MON 88017, the EFSA GMO Panel identified no biological reason to expect that any of the other sub-combinations of the individual events, as present in its segregating progeny, would raise a safety concern[30]. In conclusion, the EFSA GMO Panel is of the opinion that maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny are as safe and as nutritious as its conventional counterpart and commercial maize varieties, and concludes that these maize and derived products are unlikely to have adverse effects 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 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny, and does not include cultivation. Considering the proposed uses of maize MON 89034 x 1507 x MON 88017 x 59122, the environmental risk assessment is concerned with the exposure through manure and faeces from animals fed grain (F_2 generation) produced by maize MON 89034 x 1507 x MON 88017 x 59122 and with the accidental release into the environment of viable grains produced by maize MON 89034 x 1507 x MON 88017 x 59122 (which include its segregating progeny, see section 3.1) during transportation and processing.

6.1.1. Evaluation of single maize events and maize stacks

In its previous scientific opinions, the EFSA GMO Panel was of the opinion that the single maize events MON 89034, 1507, MON 88017 and 59122 and the two double stacks 1507 x 59122 and MON 89034 x MON 88017 are as safe as their conventional counterparts, and that the placing on the market of maize MON 89034, 1507, MON 88017, 59122, 1507 x 59122 and MON 89034 x MON 88017 for food and feed uses, import and processing is unlikely to have an adverse effect on human or animal health, or on the environment (EFSA, 2004, 2005a, 2005b, 2007b, 2008, 2009a, 2009b, 2009c, 2010). Furthermore, post-market environmental monitoring plans for maize MON 89034, 1507, MON 88017, 59122, 1507 x 59122 and MON 89034 x MON 88017, including general surveillance, were proposed by the applicants and considered in line with the EFSA GMO Panel scientific opinion on post-market environmental monitoring (EFSA, 2006b).

[30] Sub-combinations not previously assessed MON 89034 x 1507 x MON 88017, MON 89034 x 1507 x 59122, MON 89034 x MON 88017 x 59122, 1507 x MON 88017 x 59122, MON 89034 x 1507, MON 89034 x 59122, MON 88017 x 59122, 1507 x MON 88017

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 management intervention. Maize plants are not winter hardy in many regions of Europe, they have lost their ability to release seeds from the cob and they do not occur outside cultivated land or disturbed habitats in agricultural landscapes of Europe, despite cultivation for many years. In cultivation, maize volunteers may arise under some environmental conditions (mild winters). Observations made on cobs, cob fragments or isolated grains shed in the field during harvesting indicated that grain may survive and overwinter in some regions, resulting in volunteers in subsequent crops. The occurrence of maize volunteers was reported in Spain and other European regions (e.g. Gruber et al., 2008). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009).

Previous field trials have shown that there are no indications of altered fitness of the single maize events MON 89034, 1507, MON 88017 and 59122 and the two double stacks 1507 x 59122 and MON 89034 x MON 88017, as compared to their conventional counterparts. In addition to the field trials carried out with the single events and maize stacks (EFSA, 2004, 2005a, 2005b, 2007b, 2008, 2009a, 2009b, 2009c, 2010), a series of field trials with maize MON 89034 x 1507 x MON 88017 x 59122 was conducted at four locations within major maize-growing regions of the USA in 2006[31]. Information on phenotypic and agronomic characteristics was provided to assess the agronomic performance of maize MON 89034 x 1507 x MON 88017 x 59122 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 MON 89034 x 1507 x MON 88017 x 59122 plants, though there is a potential for enhanced biomass production when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by target pests. On the basis of the available data on the single events and maize stacks (1507 x 59122, MON 89034 x MON 88017 and MON 89034 x 1507 x MON 88017 x 59122), the EFSA GMO Panel considers it very unlikely that the segregating progeny of MON 89034 x 1507 x MON 88017 x 59122 would have any increased persistence and invasiveness in EU receiving environments. In addition, the EFSA GMO Panel is not aware of any scientific report of increased establishment, spread or any change in survival capacity including overwintering of maize MON 89034 x 1507 x MON 88017 x 59122, or maize with comparable properties such as single events and sub-combinations of maize MON 89034 x 1507 x MON 88017 x 59122.

The herbicide tolerance traits can only be regarded as providing a potential agronomic advantage for maize MON 89034 x 1507 x MON 88017 x 59122 plants and all sub-combinations of the individual events expressing the herbicide tolerance genes where and when glufosinate-ammonium- and/or glyphosate-based herbicides are applied. Similarly, insect resistance against certain lepidopteran and coleopteran target pests provides a potential agronomic advantage in cultivation under infestation by target pests. However, survival of maize outside cultivation or other areas where glufosinate-ammonium- and/or glyphosate-based 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 MON 89034 x 1507 x MON 88017 x 59122, 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 MON 89034 x 1507 x MON 88017 x 59122 and its segregating progeny will differ from conventional maize varieties in their ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

[31] Technical dossier/ Section D4

Since maize MON 89034 x 1507 x MON 88017 x 59122 has no altered survival, multiplication or dissemination characteristics, except when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by 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 grains from maize MON 89034 x 1507 x MON 88017 x 59122 (which include all sub-combinations of the individual events) will not differ from that of the single maize events (MON 89034, 1507, MON 88017 and 59122), the two double stacks (1507 x 59122 and MON 89034 x MON 88017), or from 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

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 human or animal gastrointestinal tracts. However, a low level of exposure of fragmented products of the ingested DNA, including their recombinant fraction, to microorganism in the digestive tracts of humans, domesticated animals, and other animals feeding on grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 is expected (see section 5.1.1).

Current scientific knowledge indicates that horizontal gene transfer of non-mobile DNA fragments between unrelated organisms (such as plants to microorganisms) is not expected to occur under natural conditions (see EFSA, 2009d for further details). The concentration of extracellular DNA fragments in gastrointestinal tracts is relatively low and most bacteria lack competence to take up and recombine foreign DNA. The *cry1A.105*, *cry2Ab2*, *cry1F*, *pat*, *cry3Bb1*, CP4 *epsps*, *cry34Ab1* and *cry35Ab1* genes in maize MON 89034 x 1507 x MON 88017 x 59122 are all derived from bacterial genes. Thus, in theory, the *cry1A.105*, *cry2Ab2*, *cry1F*, *pat*, *cry3Bb1*, CP4 *epsps*, *cry34Ab1* and *cry35Ab1* genes of the recombinant DNA inserts could provide sufficient DNA similarity for homologous recombination to take place in bacteria. However, as discussed further below, such a hypothesised 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 non-homologous recombination into environmental bacterial genomes, it is unlikely that recombinant genes (*cry1F*, CP4 *epsps*, *cry34Ab1* and *cry35Ab1*) regulated by eukaryotic plant promoters in maize MON 89034 x 1507 x MON 88017 x 59122 would be expressed. The *cry1A.105*, *cry2Ab2*, *pat* and *cry3Bb1* genes are regulated by plant virus promoters. The activity of 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*, *pat* and *epsps* genes are already occurring in various bacterial species in the environment. Thus, the hypothesised low level exposure of bacterial communities to the maize MON 89034 x 1507 x MON 88017 x 59122 (and all sub-combinations of the individual events) *cry1A.105*, *cry2Ab2*, *cry1F*, *pat*, *cry3Bb1*, CP4 *epsps*, *cry34Ab1* and/or *cry35Ab1* genes must be seen in the context of the natural occurrence and level of exposure to alternative sources of genetically diverse *cry*, *pat* 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 that would be provided to receiving bacteria,

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 tracts (EFSA, 2009d).

(b) Plant to plant gene transfer

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

The flowering of occasional feral GM maize plants originating from accidental release occurring during 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 glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by target pests. Even though the occurrence of some GM maize plants outside cropped area has been reported in Korea due to grain spillage during import, transportation, storage, handling and processing (Kim et al., 2006; Lee et al., 2009; Park 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, herbivores and frost. Since these general characteristics are unchanged in maize MON 89034 x 1507 x MON 88017 x 59122 and its segregating progeny, herbicide tolerance and insect resistance are not likely to provide selective advantages outside cultivation or other areas where glufosinate-ammonium- and/or glyphosate-based herbicides could be applied and/or under infestation by target pests in Europe. Therefore, as for any other maize varieties, these GM maize plants would only survive in subsequent seasons in warmer regions of Europe and are not likely to establish feral populations under European environmental conditions.

The EFSA GMO Panel takes into account that this application does not include cultivation of maize MON 89034 x 1507 x MON 88017 x 59122 within the EU so that the likelihood of cross-pollination between cultivated maize and the occasional feral maize plants resulting from grain spillage is considered extremely low. However, in countries cultivating maize MON 89034 x 1507 x MON 88017 x 59122 and producing seed for export, there is a potential for admixture in seed production and thus the introduction of GM seeds through this route. Hence, it is important that appropriate management systems are in place to restrict seeds of maize MON 89034 x 1507 x MON 88017 x 59122 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

In conclusion, maize MON 89034 x 1507 x MON 88017 x 59122 and its segregating progeny have no altered survival, multiplication or dissemination characteristics, except when glufosinate-ammonium- and/or glyphosate-based herbicides are applied, and/or under infestation by target pests. The EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes, resulting from imports of this maize and its segregating progeny in Europe, will not differ from that of the single events MON 89034, 1507, MON 88017 and 59122, or from that of conventional maize varieties.

6.1.2.3. Interactions of the GM plant with target organisms

The intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 specifically exclude cultivation, and the environmental exposure to maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events is limited to the accidental release of 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 89034 x 1507 x MON 88017 x 59122 plants or their segregating progeny 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 MON 89034 x 1507 x MON 88017 x 59122 specifically exclude cultivation, and the environmental exposure to maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events, as present in its segregating progeny, is limited to the accidental release of 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 89034 x 1507 x MON 88017 x 59122 plants or their segregating progeny to enable any significant interaction with non-target organisms, which is very unlikely.

In addition, the EFSA GMO Panel evaluated whether the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins might potentially affect non-target organisms by entering the environment through manure and faeces from animals fed grain produced by maize MON 89034 x 1507 x MON 88017 x 59122. Due to the specific insecticidal selectivity of the Cry proteins, non-target organisms most likely to be affected by the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins belong to the same or closely related taxonomic groups as those of the target organisms.

Data supplied by the applicants suggest that only low amounts of the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins enter the environment due to low expression in grain. Moreover, these Cry proteins are degraded by enzymatic activity in gastrointestinal tracts 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; Guertler et al., 2008; Lutz et al., 2006; Lutz et al., 2005; Paul et al., 2009; Wiedemann et al., 2006). 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, Cry1F, Cry2Ab2, Cry3Bb1 Cry34Ab1 and Cry35Ab1 proteins from disposal of animal wastes or accidental spillage of maize grains is likely to be very low and localised. While Cry proteins may bind to a certain degree to clay minerals or humic substances in soil, thereby reducing their availability to microorganisms for degradation, there are no indications of persistence and accumulation of Cry proteins from GM crops in soil (reviewed by Icoz and Stotzky, 2008). Compared to the Cry1Ab protein, the Cry3Bb1 protein of GM maize was found to be degraded more rapidly in soil 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 MON 89034 x 1507 x MON 88017 x 59122 (which include its segregating progeny), it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins is likely to be very low and of no ecological relevance.

6.1.2.5. Interactions with the abiotic environment and biochemical cycles

Considering the scope of the application (that excludes cultivation) and the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 (which include its segregating progeny), 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 environmental monitoring

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

Monitoring is also related to risk management, and, thus, a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific quality of the monitoring plan provided by the applicants (EFSA, 2006b). The potential exposure to the environment of maize MON 89034 x 1507 x MON 88017 x 59122 would be mainly through manure and faeces from animals fed grain produced by maize MON 89034 x 1507 x MON 88017 x 59122, and/or through accidental release into the environment of GM maize grains during transportation and processing.

No specific environmental impact of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny was indicated by the environmental risk assessment and, thus, no case-specific monitoring is required.

The general surveillance plan proposed by the applicants includes (1) the description of an approach involving operators (federations involved in maize import and processing), reporting to the applicants, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by EuropaBio for the collection of the information recorded by the various operators; and (3) the use of networks of existing surveillance systems (Lecoq et al., 2007; Windels et al., 2008). The applicants propose a general surveillance report on an annual basis and a final report at the end of the consent[32].

The EFSA GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicants is in line with the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events in the segregated progeny, as the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicants in the general surveillance plan.

6.2. Conclusion

The scope of the application includes food and feed uses, import and processing of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny, and excludes cultivation. Considering the intended uses, the environmental risk assessment is concerned with indirect exposure mainly through manure and faeces from animals fed grain produced by maize MON 89034 x 1507 x MON 88017 x 59122, and with the accidental release into the environment of viable grains from maize MON 89034 x 1507 x MON 88017 x 59122 (which include its segregating progeny) during transportation and processing.

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 grains from maize MON 89034 x 1507 x MON 88017 x 59122 during transportation and processing, except in the presence of glufosinate-ammonium- and/or glyphosate-based herbicides and/or under infestation by target pests. Taking into account the scope of the application, both the rare occurrence of feral maize plants and low levels of Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 protein exposure in maize MON 89034 x 1507 x MON 88017 x 59122 grains or through other routes indicate that the risk to

[32] Technical dossier/ Section D11

non-target organisms is extremely low. 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 tracts.

The scope of the monitoring plan provided by the applicants is in line with the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny, as 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 applicants in the general surveillance plan.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out an evaluation of a scientific risk assessment for food and feed uses, import and processing in accordance with Regulation (EC) No 1829/2003 of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny. The EFSA GMO Panel evaluated the risk assessment of maize MON 89034 x 1507 x MON 88017 x 59122, that was produced by conventional crossing of inbred lines containing events MON 89034 x MON 88017 and 1507 x 59122 to combine resistance against certain lepidopteran and coleopteran target pests and tolerance to glufosinate-ammonium- and glyphosate-based herbicides. The single maize events, MON 89034 x MON 88017 and 1507 x 59122 were the subject of previous evaluations by the EFSA GMO Panel. No new genetic modifications were introduced in maize MON 89034 x 1507 x MON 88017 x 59122. The evaluation of the risk assessment presented here is based on the information provided in the application relating to maize MON 89034 x 1507 x MON 88017 x 59122 submitted in the EU, including additional information provided by the applicants and information on the single maize events, as well as scientific comments raised by the Member States and relevant scientific publications. Further information from applications for placing the single maize events (MON 89034, 1507, MON 88017 and 59122) and two stacks (1507 x 59122 and MON 89034 x MON 88017) on the market under EU regulatory framework was taken into account (EFSA, 2004, 2005a, 2005b, 2007b, 2008, 2009a, 2009b, 2009c, 2010).

The EFSA GMO Panel is of the opinion that the molecular characterisation data provided for maize MON 89034 x 1507 x MON 88017 x 59122 are sufficient. The results of the bioinformatic analyses of the inserted DNA and the flanking regions of the single maize events MON 89034, 1507, MON 88017 and 59122 do not raise a safety concern. The levels of Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1 and Cry35Ab1 proteins in maize MON 89034 x 1507 x MON 88017 x 59122 have 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 a safety concern.

Previous evaluations of the single maize events (MON 89034, 1507, MON 88017, and 59122) and two double stacks (1507 x 59122 and MON 89034 x MON 88017) showed that they do not differ compositionally, agronomically and phenotypically from their respective conventional counterparts, and that the single events and the two double stacks are equivalent to commercial maize varieties except for the introduced traits. In this application, results of the comparative analyses indicated that maize MON 89034 x 1507 x MON 88017 x 59122 does not differ compositionally, agronomically and phenotypically from its conventional counterpart, and is equivalent to commercial maize varieties, except for the newly introduced traits. The safety of the proteins Cry1A.105 and Cry2Ab2 expressed in maize MON 89034, proteins Cry1F and PAT expressed in maize 1507, proteins Cry3Bb1 and CP4 EPSPS expressed in maize MON 88017, and proteins Cry34Ab1, Cry35Ab1 and PAT expressed in maize 59122 have been assessed previously, and no safety concerns were identified for humans and animals. In addition, the EFSA GMO Panel considers that it is unlikely that the overall toxicity and allergenicity of the whole maize MON 89034 x 1507 x MON 88017 x 59122 has been changed. A feeding study with broiler chickens confirmed that the nutritional properties of grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 are not different from those of its conventional counterpart and commercial maize varieties. Potential interactions between the maize events with respect to an effect on human and animal health were the focus of the assessment on food/feed safety issues. On the basis of the known functional characteristics and modes of action of the newly expressed proteins (Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1), the EFSA GMO Panel considers it unlikely that interactions between these proteins would occur that would raise any safety concern. Based on data provided on the maize stack MON 89034 x 1507 x MON 88017 x 59122, and data on the single maize events MON 89034, 1507, MON 88017, and 59122, and the two double stacks 1507 x 59122 and MON 89034 x MON 88017, the EFSA GMO Panel considered the other sub-combinations of the individual events not previously

assessed and identified no biological reason to expect that any of the other sub-combinations of the individual events as present in its segregating progeny would raise a safety concern. In conclusion, the EFSA GMO Panel is of the opinion that maize MON 89034 x 1507 x MON 88017 x 59122 and any sub-combinations of the individual events as present in its segregating progeny are as safe and as nutritious as the conventional counterpart and commercial maize varieties, and concludes that this maize and derived products are unlikely to have adverse effects on human and animal health, in the context of its intended uses.

Considering the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122, 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 viable grains of maize MON 89034 x 1507 x MON 88017 x 59122 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 glufosinate-ammonium- and/or glyphosate-based herbicides and/or under infestation by target pests. In addition, the low levels of environmental exposure to these GM maize plants and the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins through other routes indicate that the risk to non-target organisms is extremely low. 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 tracts. The scope of the post-market environmental monitoring plan provided by the applicants is in line with the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events as present in its segregating progeny.

The EFSA GMO Panel recommends that appropriate management systems should be in place to restrict seeds of maize MON 89034 x 1507 x MON 88017 x 59122 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

In conclusion, the EFSA GMO Panel considers that the information available for maize MON 89034 x 1507 x MON 88017 x 59122 addresses the scientific comments raised by the Member States and that maize MON 89034 x 1507 x MON 88017 x 59122, as described in this application, is as safe as its conventional counterpart and commercial maize varieties with respect to potential effects on human and animal health and the environment. In addition, the EFSA GMO Panel is of the opinion that crossing of maize events MON 89034, 1507, MON 88017 and 59122 to produce maize MON 89034 x 1507 x MON 88017 x 59122 does not result in interactions between the events which would affect the safety of maize MON 89034 x 1507 x MON 88017 x 59122 with respect to potential effects on human and animal health and on the environment, in the context of its intended uses. Based on the data provided for maize MON 89034 x 1507 x MON 88017 x 59122, the single maize events, and for two double stacks (1507 x 59122 and MON 89034 x MON 88017), the EFSA GMO Panel is of the opinion that there is no biological reason to expect that any of the other sub-combinations with two or three of the events maize MON 89034, 1507, MON 88017, and 59122 would raise a safety concern[33]. The EFSA GMO Panel concludes that maize MON 89034 x 1507 x MON 88017 x 59122 is unlikely to have adverse effects on human and animal health and the environment, in the context of its intended uses.

[33] Sub-combinations not previously evaluated by the EFSA GMO Panel are MON 89034 x 1507 x MON 88017; MON 89034 x 1507 x 59122; MON 89034 x MON 88017 x 59122; 1507 x MON 88017 x 59122; MON 89034 x 1507; MON 89034 x 59122; MON 88017 x 59122; and 1507 x MON 88017

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the Czech Republic, dated 28 October 2008, concerning a request for placing on the market of maize MON 89034 x 1507 x MON 88017 x 59122 in accordance with Regulation (EC) No 1829/2003.
2. Acknowledgement letter, dated 12 November 2008, from EFSA to the Competent Authority of the Czech Republic (Ref. PB/KL/shv (2008) 3453282).
3. Letter from EFSA to the applicants, dated 5 December 2008, requesting additional information under completeness check (Ref. PB/CE/md (2008) 3509767).
4. Letter from the applicants, dated 19 December 2008, providing additional information under completeness check.
5. Letter from EFSA to applicants, dated 14 January 2009, requesting additional information under completeness check (Ref. PB/CE/shv (2009) 3580358).
6. Letter from the applicants, dated 12 February 2009, providing additional information under completeness check.
7. Letter from EFSA to the applicants, dated 03 March 2009, delivering the 'Statement of Validity' for application EFSA-GMO-CZ-2008-62, maize MON 89034 x 1507 x MON 88017 x 59122 submitted by Dow AgroSciences and Monsanto under Regulation (EC) No 1829/2003 (Ref. PB/KL/CE/shv (2009) 3718306).
8. Letter from the applicants, dated 17 March 2009, providing EFSA with an updated version of the application EFSA-GMO-CZ-2008-62 submitted by Dow AgroSciences and Monsanto under Regulation (EC) No 1829/2003.
9. Letter from EFSA to the applicants, dated 20 May 2009, requesting additional information and stopping the clock (ref. PB/SM/ls (2009) 3980589).
10. Letter from the applicants to EFSA, dated 23 June 2009, providing additional information.
11. Letter from EFSA to the applicants, dated 11 September 2009, with request for additional information (ref. PB/KL/NP/lg (2009) 4246354).
12. Letter from the applicants to EFSA, dated 15 September 2009, providing additional information.
13. Letter from the applicants to EFSA, dated 25 September 2009, providing supplementary information spontaneously.
14. Letter from EFSA to the applicants, dated 5 October 2009, with request for clarification on the additional information (ref. PB/KL/NP/ls (2009) 4302847).
15. Letter from EFSA to applicant, dated 29 October 2009, requesting clarifications on the additional information sent spontaneously by the letter received 25 September 2009 (Ref. PB/KL/NP/ZD/lg (2009) 4390891).
16. Letter from the applicants to EFSA, dated 20 November 2009, providing additional information.
17. Letter from EFSA to the applicants, dated 29 January 2010, restarting the clock (Ref. PB/KL/NP/mt (2010) 4612365).
18. Letter from the applicants to EFSA, dated 2 February 2010, providing additional information related to 25 September 2009 spontaneously provided supplementary information.

19. Letter from EFSA to the applicants, dated 11 March 2010, requesting additional information and stopping the clock (ref. PB/KL/NP/YL/mt (2010) 4715435).

20. Letter from the applicants to EFSA, dated 31 March 2010 providing additional information.

21. Letter from the European Commission to the applicant, dated 26 April 2010, regarding a regulatory clarification of the applications submitted under Regulation (EC) No 1829/2003.

22. Letter from applicant to EFSA, received 3 May 2010, providing additional information.

23. Letter from EFSA to the applicants, dated 6 May 2010, restarting the clock (ref. PB/KL/NP/YL/shv (2010) 4844018).

24. Letter from the applicants, dated 16 June 2010, clarifying the scope of the application.

25. Letter from applicant to EFSA, received 24 June 2010, providing additional information spontaneously.

26. Letters from EFSA to the applicants, dated 2 and 26 July 2010, requesting updating of unique identifiers and stopping the clock (ref. PB/KL/NP/CE/mt (2010) 4971030 and PB/KL/NP/CE/mt (2010) 5017712 respectively).

27. Letters from the applicants, dated 19 and 30 July 2010, updating the unique identifiers.

28. Letter from EFSA to the applicants, dated 19 August 2010, restarting the clock (ref. PB/KL/AFD/lg (2010) 5063245).

REFERENCES

Bardana EJ Jr, 2008. 10. Occupational asthma. Journal of Allergy and Clinical Immunology, 121, S408-411; quiz S421.

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. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organisation: Rome. Available from: ftp://ftp.fao.org/codex/alinorm03/Al03_34e.pdf

Eastham K and Sweet J 2002. Genetically modified organisms (GMOs): The significance of gene flow through pollen transfer. European Environment Agency, Environmental issue report. 28, 1-75.

EFSA, 2004. Opinion of the Scientific Panel on genetically modified organisms on a request from the Commission related to the notification (Reference C/NL/00/10) for the placing on the market of insect-tolerant genetically modified maize 1507, for import and processing, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds. (Reference EFSA-Q-2004-011). The EFSA Journal, 2, 124.

EFSA, 2005a. Opinion of the Scientific Panel on genetically modified organisms [GMO] on an application (reference EFSA-GMO-NL-2004-02) for the placing on the market of insect-tolerant genetically modified maize 1507, for food use, under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred International/Mycogen Seeds (Reference EFSA-Q-2004-087). The EFSA Journal, 3, 182.

EFSA, 2005b. Opinion of the Scientific Panel on genetically modified organisms [GMO] related to the notification (Reference C/ES/01/01) for the placing on the market of insect-tolerant genetically modified maize 1507 for import, feed and industrial processing and cultivation, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds (Reference EFSA-Q-2004-072). The EFSA Journal, 3, 181.

EFSA, 2006a. Guidance document for the risk assessment of genetically modified plants and derived food and feed by the Scientific Panel on Genetically Modified Organisms (GMO) - including draft document updated in 2008 (reference EFSA-Q-2003-005A). The EFSA Journal, 4, 99.

EFSA, 2006b. Opinion of the Scientific Panel on genetically modified organisms [GMO] on the Post Market Environmental Monitoring (PMEM) of genetically modified plants (Reference EFSA-Q-2004-061). The EFSA Journal, 4, 319.

EFSA, 2007a. Guidance Document for the risk assessment of genetically modified plants containing stacked transformation events by the Scientific Panel on Genetically Modified Organisms (GMO) (Reference EFSA-Q-2003-005D). The EFSA Journal, 5, 512.

EFSA, 2007b. Opinion of the Scientific Panel on genetically modified organisms [GMO]on an application (Reference EFSA-GMO-NL-2005-12) for the placing on the market of insect-resistant genetically modified maize 59122, for food and feed uses, import and processing under Regulation (EC) No 1829/2003, from Pioneer Hi-Bred International, Inc. and Mycogen Seeds, c/o Dow Agrosciences LLC (Reference EFSA-Q-2005-045). The EFSA Journal, 5, 470.

EFSA, 2008. 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 (Reference EFSA-Q-2007-042). The EFSA Journal, 6, 909.

EFSA, 2009a. Application (EFSA-GMO-RX-1507) for renewal of authorisation for the continued marketing of existing products produced from maize 1507 for feed use, under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred International, Inc./Mycogen Seeds (Reference EFSA-Q-2007-144). The EFSA Journal, 7, 1138.

EFSA, 2009b. 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 (Reference EFSA-Q-2005-280). The EFSA Journal, 7, 1075.

EFSA, 2009c. Application (Reference EFSA-GMO-NL-2005-15) for the placing on the market of the insect-resistant and herbicide-tolerant genetically modified maize 1507 x 59122, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Mycogen Seeds, c/o Dow AgroSciences LLC and Pioneer Hi-Bred International, Inc. as represented by Pioneer Overseas Corporation (Reference EFSA-Q-2005-123). The EFSA Journal, 7, 1074.

EFSA 2009d. 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" (Reference EFSA-Q-2009-00589/00593). Available from: http://www.efsa.europa.eu/cs/BlobServer/Statm_of_Efsa/gmo_biohaz_st_ej1108_ConsolidatedARG_en,0.pdf

EFSA, 2010. 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 (Reference EFSA-Q-2007-056). The EFSA Journal, 8, 1564.

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.

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.

Gutierrez-Marcos JF, Dal Pra M, Giulini A, Costa LM, Gavazzi G, Cordelier S, Sellam O, Tatout C, Paul W, Perez P, Dickinson HG and Consonni G, 2007. *empty* pericarp4 encodes a mitochondrion-targeted pentatricopeptide repeat protein necessary for seed development and plant growth in maize. Plant Cell, 19, 196-210.

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. Available from: http://www.cropcomposition.org

Jeebhay MF and Quirce S, 2007. Occupational asthma in the developing and industrialised world: a review. International Journal of Tuberculosis and Lung Disease, 11, 122-133.

Kim CG, Yi H, Park S, Yeon JE, Kim DY, Kim DI, Lee KH, Lee TC, Paek IS, Yoon W, Jeong S-C and Kim HM, 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-Y, Park K, Kim H-J, Yi H, Jeong C-C, 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. Journal of Animal Physiology and Animal Nutrition (Berl), 90, 116-123.

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 Agricultural and Food Chemistry, 53, 1453-1456.

Miethling-Graff R, Dockhorn S and Tebbe CC, 2010. Release of the recombinant Cry3Bb1 protein of Bt maize MON 88017 into field soil and detection of effects on the diversity of rhizosphere bacteria. European Journal of Soil Biology, 46, 1-8.

Morisset D, Demsar T, Gruden K, Vojvoda J, Stebih D and Zel J, 2009. Detection of genetically modified organisms--closing the gaps. Nature Biotechnology, 27, 700-701.

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. OECD Series on the Safety of Novel Foods and Feeds, No. 6. ENV/JM/MONO(2002)25,

OECD 2003. Consensus document on the biology of *Zea mays* subsp. *mays* (Maize). OECD Series on Harmonisation of Regulatory Oversight in Biotechnology, No. 7. ENV/JM/MONO(2003)11,

Palaudelmàs M, Penas G, Mele E, Serra J, Salvia J, Pla M, Nadal A and Messeguer J, 2009. Effect of volunteers on maize gene flow. Transgenic Research, 18, 583-594.

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, 21, 456-461.

Paul V, Guertler P, Wiedemann S and Meyer HH, 2009. Degradation of Cry1Ab protein from genetically modified maize (MON810) in relation to total dietary feed proteins in dairy cow digestion. Transgenic Research, DOI:10.1007/s11248-009-9339-z,

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.