

SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-NL-2007-39) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON89034 x MON88017 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto¹

EFSA Panel on Genetically Modified Organisms (GMO)^{2, 3}

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ABSTRACT

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

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

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⁴ In section References on page 25, the EFSA GMO Panel corrected the list of references (some references were not listed). The changes do not affect the overall conclusions of the scientific opinion. To avoid confusion, the original version of the scientific opinion has been removed from the website, but is available on request as is a version showing the changes made. Suggested citation: EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on application (EFSA-GMO-NL-2007-39) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON89034 x MON88017 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2010; 8(3):1564. [27 pp.]. doi:10.2903/j.efsa.2010.1564. Available online: www.efsa.europa.eu

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

KEY WORDS

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

SUMMARY

Following the submission of an application (EFSA-GMO-NL-2007-39) under Regulation (EC) No 1829/2003 from Monsanto, the Panel on Genetically Modified Organisms was asked to deliver a scientific opinion on herbicide tolerant and insect resistant genetically modified (GM) maize MON89034 x MON88017 (Unique identifier MON-89034-3 × MON-88017-3) for food and feed uses, import and processing.

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

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

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

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

The application EFSA-GMO-NL-2007-39 concerns food and feed uses, import and processing, but excludes cultivation in the EU. Therefore, there is no requirement for scientific assessment of possible environmental effects associated with the cultivation of maize MON89034 x MON88017. There are no indications of an increased likelihood of establishment and spread of feral maize plants in case of accidental release into the environment of viable maize MON89034 x MON88017 grains during transportation and processing for food and feed uses, except in the presence of glyphosate. Taking into account the scope of the application, both the rare occurrence of feral maize plants and the low levels of exposure through other routes indicate that the risk to non-target organisms is extremely low. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize MON89034 x MON88017. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

In conclusion, the EFSA GMO Panel considers that the information available for maize MON89034 x MON88017 addresses the scientific comments raised by the Member States and that the maize MON89034 x MON88017 as described in this application is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of its intended uses. The EFSA GMO Panel concludes that maize event MON89034 x MON88017 is unlikely to have any adverse effect on human and animal health and the environment, in the context of its intended uses.

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48 BACKGROUND

49 On 12/02/2007, the European Food Safety Authority (EFSA) received from the Competent Authority
50 of The Netherlands an application (Reference EFSA-GMO-NL-2007-39), for authorisation of the
51 insect resistant and herbicide tolerant genetically modified (GM) maize MON89034 x MON88017
52 (Unique Identifier MON-89034-3 × MON-88017-3), submitted by Monsanto within the framework
53 of Regulation (EC) No 1829/2003 on genetically modified food and feed. After receiving the
54 application EFSA-GMO-NL-2007-39 and in accordance with Articles 5(2)(b) and 17(2)(b) of
55 Regulation (EC) No 1829/2003, EFSA informed Member States and the European Commission, and
56 made the summary of the application publicly available on the EFSA website. EFSA initiated a formal
57 review of the application to check compliance with the requirements laid down in Articles 5(3) and
58 17(3) of Regulation (EC) No 1829/2003. On 23/08/2007 and 11/09/2007, EFSA received additional
59 information requested under completeness check (requested on 01/08/2007 and 05/09/2007
60 respectively). On 20/09/2007, EFSA declared the application as valid in accordance with Articles 6(1)
61 and 18(1) of Regulation (EC) No 1829/2003.

62 EFSA made the valid application available to Member States and the European Commission, and
63 consulted nominated risk assessment bodies of Member States, including national Competent
64 Authorities within the meaning of Directive 2001/18/EC following the requirements of Articles 6(4)
65 and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member State bodies
66 had three months after the date of acknowledgement of the valid application (19/12/2007) within
67 which to make their opinion known.

68 The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out a
69 scientific assessment of the GM maize MON89034 x MON88017 for food and feed uses, import and
70 processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. When
71 carrying out the safety assessment, the EFSA GMO Panel took into account the appropriate principles
72 described in the Guidance Documents of the Scientific Panel on Genetically Modified Organisms for
73 the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006c) and for
74 the risk assessment of genetically modified plants containing stacked transformation events (EFSA,
75 2007), the scientific comments of Member States and the additional information provided by the
76 applicant. Further information from applications for placing the single events MON89034 and
77 MON88017 on the market under EU regulatory procedures was taken into account where appropriate.

78 On 23/10/2007, 19/11/2007, 10/12/2007, 18/12/2008, 28/01/2009, 08/04/2009, 29/05/2009,
79 30/06/2009, 05/11/2009, the EFSA GMO Panel requested from the applicant additional information.
80 The applicant provided the requested information on 30/10/2007, 30/11/2007 and on 07/12/2007,
81 17/04/2008 (spontaneously submitted), 06/10/2008, 08/01/2009, 19/02/2009, 03/06/2009, 30/06/2009,
82 08/09/2009, 11/11/2009. After receipt and assessment of the full data package the EFSA GMO Panel
83 finalised its risk assessment on maize MON89034 x MON88017.

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

86 In giving its scientific opinion on GM maize MON89034 x MON88017 to the European Commission,
87 the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation
88 (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of six months from the
89 acknowledgement of the valid application. As additional information was requested by the EFSA
90 GMO Panel, the time limit of six months was extended accordingly, in line with Articles 6(1), 6(2),
91 18(1), and 18(2) of Regulation (EC) No 1829/2003.

92 According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report
93 requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the EFSA overall
94 opinion in accordance with Articles 6(5) and 18(5).

95 **TERMS OF REFERENCE**

96 The EFSA GMO Panel was requested to carry out a scientific risk assessment of maize MON89034 x
97 MON88017 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6)
98 of Regulation (EC) No 1829/2003. Where applicable, any conditions or restrictions which should be
99 imposed on the placing on the market and/or specific conditions or restrictions for use and handling,
100 including post-market monitoring requirements based on the outcome of the risk assessment and, in
101 the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of
102 particular ecosystems/environment and/or geographical areas should be indicated in accordance with
103 Articles 6(5)(e) and 18(5)(e) of Regulation (EC) No 1829/2003.

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

109

110 Assessment

111 **1. Introduction**

112 The genetically modified maize MON89034 x MON88017 (Unique Identifier MON-89034-3 ×
113 MON-88017-3) was assessed with reference to its intended uses, taking account of the appropriate
114 principles described in the Guidance Documents of the Scientific Panel on Genetically Modified
115 Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA,
116 2006b) and for the risk assessment of genetically modified plants containing stacked transformation
117 events (EFSA, 2006a). The risk assessment presented here is based on the information provided in the
118 application relating to maize MON89034 x MON88017 submitted in the EU including additional
119 information from the applicant and information on the single events, as well as scientific comments
120 that were raised by the Member States.

121 **2. Issues raised by Member States**

122 The scientific comments raised by Member States are addressed in details in Annex G of the EFSA
123 overall opinion⁵ and have been considered throughout this EFSA GMO Panel scientific opinion.

124 **3. Molecular characterisation**

125 **3.1. Evaluation of relevant scientific data**

126 **3.1.1. Method of production of maize MON89034 × MON88017**

127 Conventional breeding methods were used to produce maize MON89034 × MON88017 and no new
128 genetic modification was involved. The two inserts that are present in maize MON89034 ×
129 MON88017 were derived from maize lines containing two independent events: MON89034 and
130 MON88017. Each of these GM maize events was the subject of an earlier safety evaluation and
131 separate opinions for each of them have been published (EFSA, 2008, 2009a). Maize MON89034 ×
132 MON88017 combines resistance to certain lepidopteran (*Ostrinia nubilalis*, *Spodoptera* spp., *Agrotis*
133 *ipsilon*) and coleopteran (*Diabrotica* spp.) pests and tolerance to glyphosate-containing herbicides.

134 **3.1.2. Summary of the evaluation of the single events**

135 **Maize MON89034**

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

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

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

152 Sequence comparison between the corresponding genomic region of conventional maize and the
153 flanking regions of the maize MON89034 indicated that the pre-insertion locus was preserved except
154 for the deletion of 57 bp and the addition of 10 bp. An updated bioinformatic analysis was performed
155 (Tu and Silvanovich, 2009a, b). The data confirmed that no known endogenous maize coding
156 sequences or regulatory sequences have been disrupted by the insert. Updated bioinformatic analysis
157 also revealed no biologically relevant homologies to allergens or toxins for any of the putative
158 polypeptides that might be produced from ORFs spanning the junction regions (Girault and McClain,
159 2008; Tu and Silvanovich, 2009c, d, h).

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

162 **Maize MON88017**

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

169 Similarity searches revealed that the flanking regions of the insert in maize MON88017 show
170 significant level of identity to maize genomic DNA sequences and indicated that the pre-insertion
171 locus was preserved except for the deletion of 26 bp and the addition of 20 bp. An updated
172 bioinformatic analysis was performed (Tu and Silvanovich, 2009e, f). The data indicated that the
173 insert is located approximately 100 bp upstream of a region corresponding to a maize full-length
174 cDNA potentially coding for a protein with sequence similarity to putative purine permeases. This
175 analysis confirmed previous bioinformatic analyses. Phenotypic, agronomic and compositional
176 analyses showed that MON88017 is equivalent to conventional maize, except for the expected traits,
177 indicating that the insertion of the transgene has not altered the expression of an essential gene that
178 would raise a safety concern. Updated bioinformatic analysis also revealed no biologically relevant
179 similarity to allergens or toxins for any of the putative peptides that might be produced from open
180 reading frames spanning the junction regions (Girault *et al.*, 2008; Tu and Silvanovich, 2009g, i).

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

183 **3.1.3. Transgene constructs in maize MON89034 x MON88017**

184 Maize MON89034 x MON88017 has been obtained by conventional crossing of MON89034 and
185 MON88017. No new genetic modification has been introduced in the stacked maize line. The integrity
186 of the individual inserts present in this maize was investigated using Southern analyses. This involved
187 the use of DNA probes specific for MON89034 and MON88017 inserts and restriction enzymes
188 informative of the structure of both events, including the junctions with the host genomic DNA and
189 confirmed the integrity of the single events when combined in maize MON89034 x MON88017.

190 **3.1.4. Information on the expression of the inserts**

191 The levels of the newly expressed proteins Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS in
192 MON89034 × MON88017 were analysed by validated enzyme-linked immunosorbent assays
193 (ELISA). Tissue samples for analysis were collected from five field trials conducted in USA during
194 2005 (Hartmann *et al.*, 2006, b). The trials were located within the major maize-growing regions of the
195 USA and provided a variety of environmental conditions. At each site, maize MON89034 ×
196 MON88017, an appropriate conventional counterpart, MON89034 and MON88017 were planted
197 using a randomized complete block design.

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

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

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

204

205 **3.1.5. Inheritance and stability of inserted DNA**

206 The genetic stability of the inserted DNA in events MON89034 and MON88017 was demonstrated
207 previously (EFSA, 2008, 2009a). In maize MON89034 × MON88017 the two inserts are combined.
208 The Southern analyses show that the integrity of the inserts present in the single events is retained in
209 MON89034 × MON88017 (Groat *et al.*, 2006). Furthermore, each of the traits has been conserved in
210 this maize.

211 **3.2. Conclusion**

212 As conventional breeding methods were used in the production of maize MON89034 × MON88017,
213 no additional genetic modification was involved. Southern analyses demonstrated that the structures of
214 the MON89034 and MON88017 events were retained in maize MON89034 × MON88017.

215 The expression levels of Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS proteins in the grains of
216 maize MON89034 × MON88017 have been demonstrated to be comparable with those of the single
217 events.

218 The EFSA GMO Panel concludes that the molecular characterisation of maize MON89034 x
219 MON88017 does not indicate safety concerns.

220 **4. Comparative analysis**

221 **4.1. Evaluation of relevant scientific data**

222 **4.1.1. Summary of the previous evaluation of the single events**

223

224 **Maize MON89034**

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

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

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

242

243 **Maize MON88017**

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

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

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

267 **4.1.2. Choice of conventional counterpart and additional comparators and production of** 268 **material for the compositional assessment**

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

278 In the context of previous applications, analytical data on materials obtained from field trials with the
279 single maize events (MON89034 and MON88017) and the respective appropriate conventional
280 counterparts and commercial non-GM maize varieties were provided by the applicant (see section
281 4.1.1). The EFSA GMO Panel previously evaluated these data and concluded that the maize events
282 MON89034 and MON88017 (the latter treated and untreated with the respective target herbicide) were
283 compositionally and agronomically equivalent to their respective conventional counterparts, except for
284 the newly introduced traits (EFSA, 2008, 2009a). The EFSA GMO Panel noted the fact that treatment
285 of the single maize event MON88017 with the target herbicide to which it is tolerant did not affect its
286 agronomic and compositional characteristics compared to untreated maize MON88017 plants (EFSA,
287 2009a). The EFSA GMO Panel, therefore, accepts the design of field trials with maize MON89034 x
288 MON88017 without inclusion of the single events and treatment with the target herbicide.

289 **4.1.3. Compositional analysis**

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

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

326 **4.1.4. Agronomic traits and GM phenotype**

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

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

345 **4.2. Conclusion**

346 Based on the results of the comparative analysis, it is concluded that maize MON89034 x MON88017
347 is compositionally and agronomically equivalent to its conventional counterpart and commercial non-
348 GM maize varieties, except for the presence of Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS
349 proteins in maize MON89034 x MON88017. Based on the assessment of the data available, the EFSA
350 GMO Panel has found no indication that crossing maize MON89034 with MON88017 maize results in
351 an interaction between the single events which causes compositional or agronomic changes.

352

353 **5. Food/Feed safety assessment**

354 **5.1. Evaluation of relevant scientific data**

355 **5.1.1. Summary of the previous evaluation of the single events**

356

357 **Maize MON89034**

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

369 It was concluded that maize MON89034 is as safe as conventional maize and that the overall
370 allergenicity of the whole plant is not changed. Maize MON89034 and derived products are unlikely
371 to have any adverse effects on human and animal health in the context of its intended use (EFSA,
372 2008).

373

374 **Maize MON88017**

375 Analogues of the newly expressed Cry3Bb1 and CP4 EPSPS proteins in MON88017 maize were
376 obtained from recombinant strains of *E. coli* and used for safety testing after their equivalence to the
377 plant-expressed proteins had been demonstrated experimentally. Neither proteins showed toxicity in
378 acute oral toxicity studies in mice, nor did they show relevant similarities to known toxic or allergenic
379 proteins in bioinformatics-supported comparisons of their amino acid sequences. Cry3Bb1 and CP4
380 EPSPS proteins were also rapidly degraded during incubations with simulated gastric fluid containing
381 the digestive enzyme pepsin.

382 The safety of the whole food/feed derived from MON88017 was tested in a 90 day rat feeding study
383 with diets containing 33% grains from maize MON88017. No indications of adverse effects were
384 observed in this study. Also a nutritional, 42-day broiler chicken feeding study was carried out with
385 diets containing between 55 and 60% grains from maize MON88017, showing that the latter was
386 nutritionally equivalent to conventional maize (EFSA, 2009a).

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

391 **5.1.2. Product description and intended use**

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

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

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

399 **5.1.3. Effect of processing**

400 Since maize MON89034 x MON88017 is compositionally equivalent to its conventional counterpart
401 (see Section 4.2), except for the newly expressed proteins, the effect of processing on maize
402 MON89034 x MON88017 is not expected to be different compared to that on conventional maize.

403 **5.1.4. Toxicology**

404 5.1.4.1. Toxicological assessment of expressed novel proteins in MON89034 x MON88017

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

421 5.1.4.2. Toxicological assessment of new constituents other than proteins

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

425 5.1.4.3. Toxicological assessment of the whole GM food/feed

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

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

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

442 **5.1.5. Allergenicity**

443 The strategies used when assessing the potential allergenic risk focus on the characterisation of the
444 source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation
445 or to elicit allergic reactions in already sensitised persons and whether the transformation may have
446 altered the allergenic properties of the modified food. A weight-of-evidence approach is
447 recommended, taking into account all of the information obtained with various test methods, since no
448 single experimental method yields decisive evidence for allergenicity (CAC, 2003; EFSA, 2006).

449 5.1.5.1. Assessment of allergenicity of the newly expressed proteins

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

457 5.1.5.2. Assessment of allergenicity of the whole GM plant

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

471 **5.1.6. Nutritional assessment of GM food/feed**

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

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

478 females) each received one of six maize-containing diets, *i.e.* with maize MON89034 x MON88017,
479 its conventional counterpart and four commercial non-GM maize varieties. Each group had been
480 subdivided into ten pens of ten animals per pen, with five pens each for male and female, adding up to
481 50 animals per sex within each group of 100 animals. The content of maize in the diets varied between
482 55% maize in starter diets to 59% in grower/finisher diets. Whilst maize grains and diets were
483 analyzed for chemical composition, the grains were also analyzed for potential presence of pesticide
484 and mycotoxin residues, and for the presence of transgenic DNA using PCR analysis. The
485 measurements that were performed during the feeding experiment included the feed consumption,
486 body weight, morbidity, and mortality of the animals. After the experiment, the animals were analyzed
487 post-mortem for carcass characteristics, including the weights of the carcass and various carcass parts,
488 as well as the composition of the meat of thighs and breast (fat, moisture, protein). Following a request
489 from the EFSA GMO Panel the applicant has performed a direct comparison of the test and control
490 chicken for each observed parameter. No statistically significant differences for the tested parameters
491 were observed between the group fed maize MON89034 x MON88017 and its conventional
492 counterpart, apart from a minor but statistically significant difference in relative (%) breast weights for
493 which female chicken fed maize MON89034 x MON88017 showed a higher value than animals fed
494 the control diet. Additional, small statistically significant differences were observed in thigh protein in
495 females and breast moisture in males. The difference in relative breast weight was not observed in
496 absolute breast weights. In the absence of any other treatment-related effects on performance, the
497 EFSA GMO Panel does not consider these statistically significant differences to be of biological
498 relevance. The EFSA GMO Panel concludes that the results of this 42-days chicken feeding study
499 show that maize MON89034 x MON88017 is nutritionally equivalent to its conventional counterpart
500 and commercial non-GM maize varieties.

501 **5.1.7. Post-market monitoring of GM food/feed**

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

507 **5.2. Conclusion**

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

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

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

529

530 **6. Environmental risk assessment and monitoring**

531 **6.1. Evaluation of relevant scientific data**

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

538 As the scope of the present application excludes cultivation, environmental concerns related to the use
539 of glyphosate herbicides on maize MON89034 x MON88017 apply only to imported and processed
540 maize products that may have been treated with those herbicides in countries of origin. The EFSA
541 GMO Panel is aware that the risk assessment of active substances falls within the scope of Directive
542 91/414/EEC concerning the placing of plant protection products on the market.

543 **6.1.1. Evaluation of the single events MON88017 and MON89034**

544 In its previous scientific opinions, the EFSA GMO Panel was of the opinion that both the single maize
545 events MON89034 and MON88017 assessed in their respective applications as safe as their
546 conventional counterpart and that the placing on the market of maize MON89034 and MON88017, for
547 import and processing for food and feed uses, is unlikely to have an adverse effect on human or animal
548 health, or on the environment (EFSA, 2008, 2009a). Furthermore, post-market environmental
549 monitoring plans for MON89034 and MON88017, including general surveillance, were proposed by
550 the applicant and considered in line with EFSA GMO Panel opinion on post-market environmental
551 monitoring (EFSA, 2008, 2009a).

552

553 **6.1.2. Environmental risk assessment**

554 6.1.2.1. Unintended effects on plant fitness due to the genetic modification

555 Maize is highly domesticated and generally unable to survive in the environment without cultivation.
556 Maize plants are not winter hardy in many regions of Europe, they have lost their ability to release
557 seeds from the cob and they do not occur outside cultivated land or disturbed habitats in agricultural
558 landscapes of Europe, despite cultivation for many years. In cultivation, grains shed during harvest
559 may survive overwinter in some milder regions, germinate and appear as volunteers in subsequent
560 crops. The occurrence of maize volunteers was reported in Spain and other European regions (Gruber
561 et al., 2008) and many of them grow weakly and flower asynchronously with the maize crop
562 (Palau-delmas et al., 2009).

563 Applicant's field trials have shown that there are no indications of an altered fitness of the single
564 maize events MON88017 and MON89034 as compared to their conventional counterparts. In addition
565 to the field trials carried out with the single events MON88017 and MON89034 (EFSA, 2008, 2009a),
566 a series of field trials with maize MON89034 x MON88017 were conducted across 5 USA locations in
567 2004. Information on 14 phenotypic and agronomic characteristics was provided to assess agronomic

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

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

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

591 6.1.2.2. Potential for gene transfer

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

595

596

597 (a) Plant to bacteria gene transfer

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

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

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

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

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

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

632 (b) Plant to plant gene transfer

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

638 The flowering of occasional GM maize plants originating from accidental release occurring during
639 transportation and processing is unlikely to disperse significant amounts of GM maize pollen to other
640 maize plants. Field observations performed on maize volunteers after GM maize cultivation in Spain
641 revealed that maize volunteers had a low vigour, rarely had cobs and produced pollen that cross-
642 pollinated neighbour plants only at low levels (Palau-delmas et al., 2009).

643 Herbicide tolerance and insect resistance provide agronomic and selective advantages in areas where
644 glyphosate herbicides are applied and/or under infestation of target pests. Even though the occurrence
645 of some GM maize plants outside cropped area have been reported in Korea due to grain spillage
646 during import, transportation, storage, handling and processing (Kim CG et al., 2006, Lee et al., 2009,
647 Park KW et al., 2009), survival of maize plants outside cultivation in Europe is mainly limited by a
648 combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant
649 pathogens and frost. Since these general characteristics are unchanged in maize MON89034 x
650 MON88017, herbicide tolerance and insect resistance are not likely to provide selective advantages
651 outside cultivation or other areas where glyphosate herbicides could be applied and/or under
652 infestation of target pests in Europe. Therefore, as for any other maize varieties, these GM maize
653 plants would only survive in subsequent seasons in warmer regions of Europe and are not likely to
654 establish feral populations under European environmental conditions.

655 In conclusion, maize MON89034 x MON88017 has no altered survival, multiplication or
656 dissemination characteristics except when glyphosate herbicides are applied, and/or under infestation
657 of target pests. The EFSA GMO Panel is of the opinion that the likelihood of unintended
658 environmental effects as a consequence of spread of genes from this maize in Europe will not differ
659 from that of the single maize events MON88017 and MON89034, or of other conventional maize

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

662 6.1.2.3. Interactions of the GM plant with target organisms

663 The intended uses of maize MON89034 x MON88017 specifically exclude cultivation and the
664 environmental exposure of target organisms to maize MON89034 x MON88017 plants is limited to
665 the accidental release of viable grains into the environment during transportation and processing. The
666 EFSA GMO Panel considers that it would need successful establishment and spread of high numbers
667 of maize MON89034 x MON88017 plants to enable any significant interaction with target organisms,
668 which is very unlikely.

669 6.1.2.4. Interactions of the GM plant with non-target organisms

670 The intended uses of maize MON89034 x MON88017 specifically exclude cultivation so that
671 environmental exposure of non-target organisms to maize MON89034 x MON88017 plants is limited
672 to the accidental release of viable grains into the environment during transportation and processing.
673 The EFSA GMO Panel considers that it would need successful establishment and spread of high
674 numbers of maize MON89034 x MON88017 plants to enable any significant interaction with non-
675 target organisms, which is very unlikely.

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

682 Data supplied by the applicant suggest that only very low amounts of the Cry1A.105, Cry2Ab2 and
683 Cry3Bb1 proteins enter the environment due to low expression in grains. Moreover, these Cry proteins
684 are degraded by enzymatic activity in the gastrointestinal tract of animals fed GM maize or derived
685 feed products (see section 5.1.1), meaning that only low amounts of these proteins would remain intact
686 to pass out in faeces. This has been demonstrated for Cry1Ab (Einspanier et al., 2004, Ahmad et al.,
687 2005, Lutz et al., 2005, Lutz et al., 2006, Wiedemann et al., 2006, Guertler et al., 2008, Paul et al.,
688 2010). It is expected that there would subsequently be further degradation of Cry proteins in the
689 manure and faeces due to intrinsic microbial proteolytic activity. Therefore, exposure of soil and
690 aquatic environments to the Cry1A.105, Cry2Ab2 and Cry3Bb1 proteins from disposal of animal
691 wastes or accidental spillage of maize grains is likely to be very low and localized. While Cry proteins
692 may bind to a certain degree to clay minerals or humic substances in soil, thereby reducing their
693 availability to micro-organisms for degradation, there are no indications of persistence and
694 accumulation of Cry proteins from GM crops in soil (reviewed by Icoz and Stotzky, 2008). More
695 specifically, Cry3Bb1 of GM maize was found to be more rapidly degraded in soil compared to
696 Cry1Ab under similar conditions (Baumgarte and Tebbe, 2005; Miethling-Graff et al., 2010).

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

701 6.1.2.5. Interactions with the abiotic environment and biogeochemical cycles

702 Considering the scope of the application and the intended uses of maize MON89034 x MON88017
703 and due to the low level of exposure to the environment, potential interactions with the abiotic
704 environment and biogeochemical cycles were not considered an issue by the EFSA GMO Panel.

705

706 **6.1.3. Post-market environment monitoring**

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

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

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

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

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

733 **6.2. Conclusion**

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

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

747 The scope of the monitoring plan provided by the applicant is in line with the intended uses of maize
748 MON89034 x MON88017, since the environmental risk assessment did not cover cultivation and

749 identified no potential adverse environmental effects. Furthermore, the EFSA GMO Panel agrees with
750 the reporting intervals proposed by the applicant in the general surveillance plan.

751 **OVERALL CONCLUSIONS AND RECOMMENDATIONS**

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

754 The EFSA GMO Panel is of the opinion that the molecular characterisation data provided for maize
755 MON89034 x MON88017 produced by conventional breeding are adequate to perform this part of the
756 safety assessment. The bioinformatic analysis of the inserted DNA and the flanking regions of the
757 single events MON89034 and MON88017 does not raise safety concerns. The expression of
758 Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS proteins in maize MON89034 x MON88017 has
759 been sufficiently analysed and the stability of the genetic modification has been demonstrated. The
760 EFSA GMO panel considers that the molecular characterisation does not indicate any safety concern.

761 Based on the results of the comparative analysis it was concluded that maize MON89034 x
762 MON88017 is compositionally and agronomically equivalent to its conventional counterpart and
763 commercial non-GM maize varieties, except for the presence of Cry1A.105, Cry2Ab2, Cry3Bb1 and
764 CP4 EPSPS proteins expressed in maize MON88017 x MON89034. Based on the assessment of data
765 available, including the additional information provided by the applicant in response to the EFSA
766 GMO Panel's request, for maize MON89034 x MON88017, for the single events and for appropriate
767 comparator(s), the EFSA GMO Panel has found no indication that crossing of MON89034 and
768 MON88017 results in an interaction between the single events which causes compositional or
769 agronomic changes. The Cry1A.105 and Cry2Ab2 proteins expressed in the parental maize
770 MON89034 and the Cry3Bb1 and CP4 EPSPS proteins expressed in the parental maize MON88017
771 have been assessed previously and no safety concerns were identified. Given all the information
772 provided, the EFSA GMO Panel concludes that interactions between the single events that might
773 impact on food and feed safety are unlikely and that the nutritional properties of maize MON89034 x
774 MON88017 would not be different from those of its conventional counterpart and commercial non-
775 GM maize varieties. The EFSA GMO Panel considers that maize MON89034 x MON88017 is as safe
776 and as nutritious as its conventional counterpart and commercial non-GM maize varieties and that the
777 overall allergenicity of the whole plant is not changed.

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

787 In conclusion, the EFSA GMO Panel considers that the information available for maize MON89034 x
788 MON88017 addresses the scientific comments raised by the Member States and that the maize
789 MON89034 x MON88017 as described in this application is as safe as its conventional counterpart
790 with respect to potential effects on human and animal health and the environment in the context of its
791 intended uses. The EFSA GMO Panel concludes that maize event MON89034 x MON88017 is
792 unlikely to have any adverse effect on human and animal health and the environment, in the context of
793 its intended uses.

794

795 **DOCUMENTATION PROVIDED TO EFSA**

- 796 1. Letter from the Competent Authority of the MS, dated 12 February 2007, concerning a request for
797 placing on the market of genetically modified MON89034 x MON88017 maize in accordance
798 with Regulation (EC) No 1829/2003.
- 799 2. Acknowledgement letter, dated 16 February 2007, from EFSA to the Competent Authority of the
800 Netherlands.
- 801 3. Letter from EFSA to applicant, dated 1 August 2007, requesting additional information under
802 completeness check
- 803 4. Letter from applicant to EFSA, dated 23 August 2007, providing additional information under
804 completeness check.
- 805 5. Letter from EFSA to applicant, dated 5 September 2007, requesting additional information under
806 completeness check
- 807 6. Letter from applicant to EFSA, dated 11 September 2007, providing additional information under
808 completeness check.
- 809 7. Letter from EFSA to applicant, dated 20 September 2007, delivering the ‘Statement of Validity’
810 for application EFSA-GMO-NL-2007-39, MON89034 x MON88017 maize submitted by
811 MONSANTO under Regulation (EC) No 1829/2003.
- 812 8. Letter from EFSA to applicant, dated 21 September 2007, requesting additional information and
813 stopping the clock (JRC).
- 814 9. Letter from applicant to EFSA, dated 26 September 2007, providing the additional copies for the
815 valid version.
- 816 10. Letter from EFSA to applicant, dated 23 October 2007, requesting additional information and
817 stopping the clock (EFSA).
- 818 11. Letter from applicant to EFSA, dated 10 December 2007, providing the Monitoring plan.
- 819 12. Letter from EFSA to applicant, dated 8 January 2008, restarting the clock (JRC) and maintaining
820 the clock stopped (EFSA).
- 821 13. Letter from applicant to EFSA, dated 17 April 2008, providing spontaneously info (updated
822 Monitoring plan).
- 823 14. Letter from EFSA to applicant dated 19 November 2008, maintaining the clock stopped (3).
- 824 15. Letter from EFSA to applicant, dated 15 November 2008, restarting the clock.
- 825 16. Letter from EFSA to applicant, dated 18 December 2008, requesting additional information and
826 stopping the clock (3).
- 827 17. Letter from EFSA to applicant dated 8 April 2009, requesting additional information and
828 maintaining the clock stopped (4).
- 829 18. Letter from applicant to EFSA, dated 17 April 2009, providing the timeline for submission of
830 response.
- 831 19. Letter from EFSA to applicant dated 29 May 2009, requesting additional information and
832 maintaining the clock stopped (5).

- 833 20. Letter from applicant to EFSA, dated 3 June 2009, providing additional information (requested by
834 EFSA on 8th April).
- 835 21. Letter from applicant to EFSA, dated 30 June 2009, providing additional information (requested
836 by EFSA on 29 May).
- 837 22. Letter from EFSA to applicant dated 30 June 2009, requesting additional information and
838 maintaining the clock stopped (6).
- 839 23. Letter from applicant to EFSA, dated 3 August 2009, providing the timeline for submission of
840 response.
- 841 24. Letter from applicant to EFSA, dated 18 August 2009, providing a new timeline for submission of
842 response.
- 843 25. Letter from applicant to EFSA, dated 8 September 2009, providing additional information
844 (requested by EFSA on 30th June).
- 845 26. Letter from EFSA to applicant, dated 15 October 2009, restarting the clock.
- 846 27. Letter from EFSA to applicant, dated 5 November 2009, requesting additional information and
847 stopping the clock (7).
- 848 28. Letter from applicant to EFSA, dated 11 November 2009, providing additional information.
- 849 29. Letter from EFSA to applicant, dated 11 January 2010, restarting the clock.

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