Vitenskapskomiteen for mattrygghet
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VKM Report 2016: 13

Final health- and environmental risk assessment of genetically modified maize MON 89034 x MON 88017

Scientific opinion on insect-resistant and herbicide tolerant, genetically modified maize MON 89034 x MON 88017 from Monsanto for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (EFSA/GMO/NL/2007/39)

Opinion of the Panel on Genetically Modified Organisms of the Norwegian Scientific Committee for Food Safety

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Scientific opinion on insect-resistant and herbicide tolerant, genetically modified maize MON 89034 x MON 88017 from Monsanto for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (Application EFSA/GMO/NL/2007/39)

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Acknowledgment

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed the Panel on Genetically Modified Organisms (GMO) to answer the request from the Norwegian Food Safety Authority and the Norwegian Environment Agency. Project leaders from the VKM secretariat have been Anne-Marthe Jevnaker, Ville Erling Sipinen and Merethe Aasmo Finne.

Monica Sanden, The National Institute of Nutrition and Seafood Research, was acknowledged for her valuable work on this opinion (Not a full member of the VKM GMO Panel at the time).

Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Scientific Committee for Food Safety (VKM) has been requested by the Norwegian Environment Agency and the Norwegian Food Safety Authority (NFSA) to conduct final food/feed and environmental risk assessments for all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are authorized in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC. The request covers scope(s) relevant to the Gene Technology Act. The request does not cover GMOs that VKM already has conducted its final risk assessments on. However, the Agency and NFSA requests VKM to consider whether updates or other changes to earlier submitted assessments are necessary.

The insect-resistant and glyphosate-tolerant genetically modified maize MON 89034 x MON 88017 from Monsanto (Unique Identifier MON-89Ø34-3 × MON-88Ø17-3) was approved under Regulation (EC) No 1829/2003 in the EU for food and feed uses, import and processing on 17th of June 2011 (Commission Decision 2011/366/EC).

Genetically modified maize MON 890314 x MON 88017 has previously been risk assessed by the VKM Panel on Genetically Modified Organisms (GMO), commissioned by the Norwegian Food Safety Authority and the Norwegian Environment Agency related and to the EFSA public hearing of the applications EFSA/GMO/NL/2007/39 and EFSA/GMO/BE/2009/71 in 2007 and 2009/2010 (VKM 2008a, VKM 2010a). In addition, the parental lines MON 89034 and MON 88017 have been evaluated by the VKM GMO Panel as single events and as a component of several stacked GM maize events (VKM 2007a,b, VKM 2008b, VKM 2009a,b,c, VKM 2010b,c, VKM 2012, VKM 2013, VKM 2014).

The food/feed and environmental risk assessment of the maize MON 89034 x MON 88017 is based on information provided by the applicant in the applications EFSA/GMO/NL/2007/39 EFSA/GMO/BE/2009/71 and scientific comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment also considered other peer-reviewed scientific literature when relevant.

The VKM GMO Panel has evaluated MON 89034 x MON 88017 with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed. The Norwegian Scientific Committee for Food Safety has also decided to take account of the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2011a), the environmental risk assessment of GM plants (EFSA 2010), selection of

comparators for the risk assessment of GM plants (EFSA 2011b) and for the post-market environmental monitoring of GM plants (EFSA 2011c).

The scientific risk assessment of maize MON 89034 x MON 88017 include molecular characterisation of the inserted DNA and expression of novel proteins, comparative assessment of agronomic and phenotypic characteristics, nutritional assessments, toxicology and allergenicity, unintended effects on plant fitness, potential for gene transfer, effects on biogeochemical processes and interactions between the GM plant and target and non-target organisms.

It is emphasised that the VKM mandate does not include assessments of contribution to sustainable development, societal utility and ethical considerations, according to the Norwegian Gene Technology Act and Regulations relating to impact assessment pursuant to the Gene Technology Act. These considerations are therefore not part of the risk assessment provided by the VKM Panel on Genetically Modified Organisms. Likewise, the VKM mandate does not include evaluations of herbicide residues in food and feed from genetically modified plants.The hybrid maize MON 89034 x MON 88017 has been produced by conventional crosses between inbred lines containing MON 89034 and MON 88017 events to combine resistance to certain coleopteran and lepidopteran pests, and to confer tolerance towards glyphosate-containing herbicides. Maize MON 89034 was developed to provide protection against specific lepidopteran target pest, including *Ostrinia nubilalis, Spodoptera* spp. and *Agrotis ipsilon.* Protection is achieved through expression in the plant of two insecticidal Cry proteins, Cry1A.105 and Cry2Ab2, derived from *Bacillus thuringiensis* subsp. *aizawai* and *kurstaki.*

Maize MON 88017 was developed to express a modified Cry3Bb1 insecticidal protein, derived from *B. thuringiensis* subsp. *kumamotoensis*, which confers protection against coleopteran target pests belonging to the genus *Diabrotica* such as Western corn rootworm (*D. virgifera virgifera*). MON 88017 is also developed to provide tolerance to the herbicidal active substance glyphosate by the introduction of a gene coding for the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), from *Agrobacterium tumefaciens* strain CP4 (CP4 EPSPS).

Molecular characterisation

Southern and PCR analyses indicate that the recombinant inserts in the single maize events MON 89034 and MON 88017 are retained in the stacked event MON 89034 x MON 88017. Genetic stability of the inserts has previously been demonstrated in the single events. The levels of Cry1A.105, Cry2Ab2, CP4 EPSPS and Cry3Bb1 proteins in grain and forage from the stacked event are comparable to the levels in the corresponding single events. Phenotypic analyses also indicate stability of the insect resistance and herbicide tolerance traits of the stacked event.

Based on current knowledge and the previous assessments of the parental maize events, the VKM GMO Panel considers the molecular characterisation of maize MON 89034 x MON 88017 satisfactory.

Comparative assessment

Comparative analyses of maize MON 89034 x MON 88017 and its conventional counterpart have been performed by the applicant during field trials located at representative sites and environments in USA during 2004, and in Europe in 2007. Several different conventional maize varieties were included in the field trials and used as references. With the exception of small variations, and the insect resistance and herbicide tolerance conferred by the Cry3Bb1, Cry1A105, Cry2Ab2, and CP4 EPSPS proteins, the results from these studies showed no biologically relevant differences between the maize stack MON 89034 x MON 88017 and its conventional counterpart.

Based on the assessment of available data, the VKM GMO Panel concludes that maize MON 89034 x MON 88017 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, except for the new proteins.

Food and feed safety assessment

A whole food feeding study performed on broilers indicates no adverse health effects of maize MON 89034 x MON 88017, and shows that it is nutritionally equivalent to conventional maize varieties. The Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS proteins do not show relevant sequence resemblance to other known toxins or IgE-allergens, nor have they been reported to cause IgE-mediated allergic reactions. However, some studies have indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize MON 89034 x MON 88017 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS proteins will cause toxic or IgE-mediated allergic reactions to food or feed derived from maize MON 89034 x MON 88017 compared to conventional maize.

Environmental risk

Considering the intended uses of maize MON 89034 x MON 88017, excluding cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize MON 89034 x MON 88017.

Maize MON 89034 x MON 88017 has no altered survival, multiplication or dissemination characteristics, and there are no indications of an increased likelihood of spread and establishment of feral maize plants in the case of accidental release into the environment of seeds from maize MON 89034 x MON 88017. Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation. The VKM GMO Panel considers the risk of gene flow from occasional feral GM maize plants to conventional maize varieties to be negligible in Norway. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered by the GMO Panel to be an issue.

Overall conclusion

Based on current knowledge, the VKM GMO Panel concludes that maize MON 89034 x MON 88017 is compositionally, nutritionally, agronomically and phenotypically equivalent to its conventional counterpart except for the new proteins. It is unlikely that the Cry1A.105, Cry2Ab2, CryBb1 and CP4 EPSPS proteins will cause an increased risk of toxic or IgE-mediated allergic reactions to food or feed based on maize MON 89034 x MON 88017 compared to conventional maize varieties.

The VKM GMO Panel concludes that maize MON 89034 x MON 88017, based on current knowledge, is comparable to conventional maize varieties concerning environmental risk in Norway with the intended usage.

Keywords

VKM, Norwegian Scientific Committee for Food Safety, maize, *Zea mays* L., genetically modified maize MON 89034 x MON 88017 EFSA/GMO/NL/2007/39, insect-resistance, herbicide-tolerance, *cry1A.105, cry2Ab2, cry3Bb1, cp4 epsps*, glyphosate, food/feed safety assessment, environmental risk assessment, Regulation (EC) No 1829/2003, Directive 2001/18.

Norsk sammendrag

I forbindelse med forberedelse til implementering av forordning 1829/2003 i norsk rett, er Vitenskapskomiteen for mattrygghet (VKM) bedt av Miljødirektoratet og Mattilsynet om å utarbeide endelige helse- og miljørisikovurderinger av alle genmodifiserte organismer (GMOer) og avledete produkter som inneholder eller består av GMOer som er godkjent under forordning 1829/2003 eller direktiv 2001/18, og som er godkjent for ett eller flere bruksområder som omfattes av genteknologiloven. Miljødirektoratet og Mattilsynet har bedt VKM om endelige risikovurderinger for de EU-godkjente søknader hvor VKM ikke har avgitt endelige risikovurderinger. I tillegg er VKM bedt om å vurdere hvorvidt det er nødvendig med oppdatering eller annen endring av de endelige helse- og miljørisikovurderingene som VKM tidligere har levert.

Den insektsresistente og glyfosattolerante maishybriden MON 89034 x MON 88017 fra Monsanto (unik kode MON-89Ø34-3 × MON-88Ø17-3) ble godkjent i EU til import, videreforedling og til bruk som mat og fôr under forordning 1829/2003, den 17. juni 2011 (Kommisjonsbeslutning 2011/366/EU).

I forbindelse med EFSAs offentlige høring av søknadene EFSA/GMO/NL/2007/39 og EFSA/GMO/BE/2009/71 i 2007 og 2009/2010 har VKMs faggruppe for genmodifiserte organismer tidligere vurdert maishybriden med hensyn på mulig helse- og miljørisiko (VKM 2008a, VKM 2010b). VKMs faggruppe for GMO har også risikovurdert foreldrelinjene MON 89034 og MON 88017 og maishybrider der disse inngår som en av foreldrelinjene (VKM 2007a,b, VKM 2008b, VKM 2009a,b,c, VKM 2010b,c, VKM 2012, VKM 2013, VKM 2014, VKM 2016).

Risikovurderingen av den genmodifiserte maislinjen er basert på uavhengige vitenskapelige publikasjoner og dokumentasjon som er gjort tilgjengelig på EFSAs GMO Extranet. Vurderingen er gjort i henhold til tiltenkt bruk i EU/EØS-området, og i overensstemmelse med miljøkravene i genteknologiloven med forskrifter, først og fremst forskrift om konsekvensutredning etter genteknologiloven. Videre er kravene i forordning 1829/2003/EF, utsettingsdirektiv 2001/18/EF (vedlegg 2,3 og 3B) og veiledende notat til Annex II (2002/623/EF), samt prinsippene i EFSAs retningslinjer for risikovurdering av genmodifiserte planter og avledete næringsmidler (EFSA 2006, 2010, 2011a,b,c) lagt til grunn for vurderingen.

Den vitenskapelige vurderingen omfatter transformeringsprosess og vektorkonstruksjon, karakterisering og nedarving av genkonstruksjonen, komparativ analyse av ernæringsmessig kvalitet, mineraler, kritiske toksiner, metabolitter, antinæringsstoffer, allergener og nye proteiner. Videre er agronomiske egenskaper, potensiale for utilsiktede effekter på fitness (overlevelse og konkurransedyktighet), genoverføring og effekter på ikke-målorganismer vurdert. Det presiseres at VKMs mandat ikke omfatter vurderinger av etikk, bærekraft og samfunnsnytte, i henhold til kravene i den norske genteknologiloven og dens

konsekvensutredningsforskrift. Disse aspektene blir derfor ikke vurdert av VKMs faggruppe for genmodifiserte organismer. Vurderinger av mulige plantevernmiddelrester i den genmodifiserte planten som følge av endret sprøytemiddelbruk faller per i dag utenfor VKMs ansvarsområde og er derfor heller ikke vurdert. F1-hybriden MON 89034 x MON 88017 er resultat av konvensjonelle kryssinger mellom innavlede maislinjer med eventene MON 89034 og MON 88017. Kryssingene er utført for å utvikle en maishybrid med resistens mot visse skadegjørere i sommerfuglordenen Lepidoptera og billeslekten *Diabroticia*, samt toleranse mot herbicider med virkestoff glyfosat. Den genmodifiserte maislinjen MON 89034 er fremkommet ved *Agrobacterium*-mediert transformasjon av umodne maisceller. MON 89034-plantene har fått satt inn et rekombinant DNA-fragment med to genekspresjonskassetter, inneholdende genene *cry1A.105* og *cry2Ab2*. *Cry1A.105* er et syntetisk gen, som er sammensatt av sekvenser fra genene *cry1Ac*, *cry1Ab* og *cry1F* fra *Bacillus thuringiensis* subsp. *aizawai. Cry2Ab*-genet stammer fra *B. thuringiensis* subsp. *kurstaki*. *Cry1A.105*- og *cry2Ab2*-genene koder for δ-endotoksiner, som gir plantene resistens mot enkelte arter i ordenen Lepidoptera, eksempelvis europeisk maispyralide (*Ostrinia nubilalis*), *Spodoptera* spp. og stort jordfly (*Agrotis ipsilon*)

Den genmodifiserte maislinjen MON 88017 uttrykker Cry3Bb1- og CP4-EPSPS-proteiner, som er resultat av introduksjon av genene *cry3Bb1* og *cp4-epsps* fra jordbakteriene *B. thuringiensis* subsp. *kumamotoensis* og *Agrobacterium tumefaciens*. Cry3Bb1-proteinet gir plantene beskyttelse mot angrep fra arter i billeslekten *Diabrotica*. *Cp4-epsps*-genet koder for enzymet 5-enolpyruvylsikimat-3-fosfatsyntetase, som omdanner fosfoenolpyruvat og sikimat-3-fosfat til 5-enolpyruvylsikimat-3-fosfat, en viktig metabolitt i syntesen av aromatiske aminosyrer. I motsetning til plantens enzym er det bakterielle enzymet også aktivt ved nærvær av N-fosfonometylglycin (glyfosat). De transgene plantene vil derfor tolerere høyere doser av herbicider med virkestoff glyfosat sammenlignet med konkurrerende ugras.

Molekylær karakterisering

Southern- og PCR- analyser viser at de rekombinante gensekvensene som ble satt inn i maislinjene MON 89034 og MON 88017 er bevart i den kryssede maishybriden MON 89034 x MON 88017. Genetisk stabilitet av de innsatte sekvensene har tidligere blitt vist for mais MON 89034 og MON 88017. Nivåene av Cry1A.105, Cry2Ab2, CP4 EPSPS og Cry3Bb1 – protein målt i korn og vegetativt vev fra mais MON 89034 x MON 88017 samsvarer med nivåene i de respektive foreldrelinjene. Fenotypiske analyser viser at egenskapene for insektsresistens og herbicidtoleranse er stabile også i MON 89034 x MON 88017. VKMs faggruppe for GMO anser den molekylære karakteriseringen av mais MON 89034 x MON 88017 som tilfredsstillende.

Komparative analyser

Komparative analyser av mais MON 89034 x MON 88017 og konvensjonell kontroll har blitt utført av søker under feltforsøk i representative dyrkningsområder i USA i 2004, og i Europa i 2007. Flere konvensjonelle maissorter var inkludert i feltforsøkene og brukt som referanser. Med unntak av små variasjoner, insekts-resistens og herbicidtoleransen mediert av Cry3Bb1-, Cry1A105-, Cry2Ab2-, og CP4 EPSPS- proteinene, viste resultatene ingen biologisk relevante forskjeller mellom maishybriden MON 89034 x MON 88017 og konvensjonell kontroll.

Basert på vurdering av tilgjengelige data konkluderer VKMs faggruppe for GMO at mais MON 89034 x MON 88017 er vesentlig lik konvensjonell kontroll med hensyn til næringsstoffsammensetning og agronomiske og fenotypiske egenskaper, med unntak av de nye proteinene.

Helserisiko

I en fôringsstudie utført på broilere indikeres det ikke helseskadelige effekter av mais MON 89034 x MON 88017, og studien viser at den er ernæringsmessig lik konvensjonell mais. Proteinene Cry1A.105, Cry2Ab2, Cry3Bb1 og CP4 EPSPS viser ingen relevante sekvenslikheter med andre kjente toksiner eller IgE-avhengige allergener, og er heller ikke rapportert å ha forårsaket IgE-medierte allergiske reaksjoner. Enkelte studier har derimot indikert at Cry-proteiner potensielt kan forsterke allergiske reaksjoner (virke som adjuvans).

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at mais MON 89034 x MON 88017 er ernæringsmessig lik konvensjonell mais, og at det er lite sannsynlig at proteinene Cry1A.105, Cry2Ab2, Cry3Bb1 eller CP4 EPSPS vil føre til økt risiko for toksiske eller IgE-medierte allergiske reaksjoner fra mat eller fôr basert på mais MON 89034 x MON 88017 sammenliknet med konvensjonelle maissorter.

Miljørisiko

Søknaden gjelder godkjenning av maishybrid MON 89034 x MON 88017 for import, prosessering og til bruk i næringsmidler og fôrvarer, og omfatter ikke dyrking. Med bakgrunn i tiltenkt bruksområde er miljørisikovurderingen avgrenset til mulige effekter av utilsiktet frøspredning i forbindelse med transport og prosessering, samt indirekte eksponering gjennom gjødsel fra husdyr fôret med genmodifisert mais.

Det er ingen indikasjoner på økt sannsynlighet for spredning, etablering og invasjon av maislinjen i naturlige habitater eller andre arealer utenfor jordbruksområder som resultat av frøspill i forbindelse med transport og prosessering. Risiko for utkryssing med dyrkede sorter vurderes av GMO panelet til å være ubetydelig. Ved foreskreven bruk av maislinjen MON 89034 x MON 88017 antas det ikke å være risiko for utilsiktede effekter på målorganismer, ikke-målorganismer eller på abiotisk miljø i Norge.

Samlet vurdering

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO, at mais MON 89034 x MON 88017 er vesentlig lik konvensjonell kontroll med hensyn til næringsstoffsammensetning og ernæringsmessige, agronomiske og fenotypiske egenskaper, med unntak av de nye proteinene. Det lite sannsynlig at proteinene Cry1A.105, Cry2Ab2, Cry3Bb1 eller CP4 EPSPS vil føre til økt risiko for toksiske eller IgE-medierte allergiske reaksjoner fra mat eller fôr basert på mais MON 89034 x MON 88017 sammenliknet med konvensjonelle maissorter.

VKMs faggruppe for genmodifiserte organismer konkluderer at mais MON 89034 x MON 88017, ut i fra dagens kunnskap og tiltenkt bruksområde, tilsvarer konvensjonell mais når det gjelder mulig miljørisiko i Norge.

Abbreviations and explanations

ALS	Acetolactate synthase, an enzyme that catalyses the first step in the synthesis of the branched-chain amino acids, valine, leucine, and isoleucine
ARMG	Antibiotic resistance marker gene
BC	Backcross. Backcross breeding in maize is extensively used to move a single trait of interest (e.g. disease resistance gene) from a donor line into the genome of a preferred or "elite" line without losing any part of the preferred lines existing genome. The plant with the gene of interest is the donor parent, while the elite line is the recurrent parent. BC_1, BC_2 etc. designates the backcross generation number.
BLAST	Basic Local Alignment Search Tool. Software that is used to compare nucleotide (BLASTn) or protein (BLASTp) sequences to sequence databases and calculate the statistical significance of matches, or to find potential translations of an unknown nucleotide sequence (BLASTx). BLAST can be used to understand functional and evolutionary relationships between sequences and help identify members of gene families.
bp	Basepair
Bt	*Bacillus thuringiensis*
CaMV	Cauliflower mosaic virus
Codex	Set by The Codex Alimentarius Commission (CAC), an intergovernmental body to implement the Joint FAO/WHO Food Standards Programme. Its principle objective is to protect the health of consumers and to facilitate the trade of food by setting international standards on foods (i.e. Codex Standards).
CP4 EPSPS	Glyphosate-tolerant EPSPS, encoded by the *cp4 epsps* gene cassette.
cp4 epsps	DNA sequence, derived from *Agrobacterium* sp. Strain CP4, encoding the CP4 EPSPS protein.
Cry	Any of several proteins that comprise the crystal found in spores of *Bacillus thuringiensis*. Activated by enzymes in the insects midgut,

	these proteins attack the cells lining the gut, and subsequently kill the insect.
Cry1A.105	Chimeric protein comprised of domains from the naturally occurring Cry1Ab, Cry1F, and Cry1Ac proteins of *Bacillus thuringiensis*
Cry2Ab2	A Cry2 class crystal protein from *Bacillus thuringiensis* subsp. *kurstaki*
Cry3	A class of *Bacillus thuringiensis* crystal proteins with insecticidal activity against coleopteran species.
Cry3Bb1	Coding sequence for the Cry3Bb1 protein
Cry3Bb1	Protein with activity against coleopteran insects, produced by *B. thuringiensis* subsp. *kumamotoensi.*
CTP	Chloroplast transit peptide
DAP	Days after planting
DNA	Deoxyribonucleic acid
DT50	Time to 50% dissipation of a protein in soil
DT90	Time to 90% dissipation of a protein in soil
dw	Dry weight
dwt	Dry weight tissue
EC	European Commission
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	Environmental risk assessment
E-score	Expectation score
EU	European Union
fa	Fatty acid

FAO	Food and Agriculture Organisation
FIFRA	US EPA Federal Insecticide, Fungicide and Rodenticide Act
Fitness	Describes an individual's ability to reproduce successfully relative to that of other members of its population.
fw	Fresh weight
fwt	Fresh weight tissue
GAT	Glyphosate N-acetyltransferase
GLP	Good Laboratory Practice
Glyphosate	Broad-spectrum systemic herbicide
GM	Genetically Modified
GMO	Genetically Modified Organism
GMP	Genetically Modified Plant
H	Hybrid
ha	Hectare
ILSI	International Life Sciences Institute
IPM	Integrated Pest Management
IRM	Insect Resistance Management
Locus	The position/area that a given gene occupies on a chromosome
LOD	Limit of detection
LOQ	Limit of quantification
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization-Time Of Flight. A mass spectrometry method used for detection and characterisation of biomolecules, such as proteins, peptides, oligosaccharides and oligonucleotides, with molecular masses between 400 and 350,000 Da.

MCB	Mediterranean corn borer, *Sesamia nonagrioides*
mRNA	Messenger RNA
MT	Norwegian Food Safety Authority (Mattilsynet)
NDF	Neutral detergent fibre, measure of fibre used for animal feed analysis. NDF measures most of the structural components in plant cells (i.e. lignin, hemicellulose and cellulose), but not pectin.
Northern blot	Northern blot is a technique used to study gene expression by detection of RNA or mRNA separated in a gel according to size.
NTO	Non-target organism
Nicosulfuron	Herbicide for maize that inhibits the activity of acetolactate synthase
Near-isogenic lines	Term used in genetics/plant breeding, and defined genetic lines that are identical except for differences at a few specific locations or genetic loci.
OECD	Organisation for Economic Co-operation and Development
ORF	Open Reading Frame, in molecular genetics defined as a reading frame that can code for amino acids between two stop codons (without stop codons).
OSL	Over season leaf
OSR	Over season root
OSWP	Over season whole plant
pat	*Phosphinothricin-Acetyl-Transferase* gene
PAT	Phosphinothricin-Acetyl-Transferase protein
PCR	Polymerase chain reaction, a technique to amplify DNA by copying it
R0	First transformed generation, parent
RNA	Ribonucleic acid
RP	Recurrent parent

SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis. Technique to separate proteins according to their approximate size
SAS	Statistical Analysis System
SD	Standard deviation
Southern blot	Method used for transfer of electrophoresis-separated DNA fragments to a filter membrane and possible subsequent fragment detection by probe hybridisation
T-DNA	Transfer DNA, the transferred DNA of the tumour-inducing (Ti) plasmid of some species of bacteria such as *Agrobacterium tumefaciens* and *A. rhizogenes*, into plant's nuclear genome. The T-DNA is bordered by 25-base-pair repeats on each end. Transfer is initiated at the left border and terminated at the right border and requires the *vir* genes of the Ti plasmid.
TI	Trait integrated
TMDI	Theoretical Maximum Daily Intake
U.S. EPA	United States Environmental Protection Agency.
Maize growth stages	*Vegetative* VE: emergence from soil surface V1: collar of the first leaf is visible V2: collar of the second leaf is visible Vn: collar of the leaf number 'n' is visible VT: last branch of the tassel is completely visible *Reproductive* R0: Anthesis or male flowering. Pollen shed begins R1: Silks are visible R2: Blister stage. The kernels are filled with a clear nourishing endosperm fluid and the embryo can be seen R3: Milk stage. The kernels endosperm is milky white. R4: Dough stage. The kernels endosperm has developed to a white paste R5: Dent stage. If the genotype is a dent type, the grains are dented R6: Physiological maturity

Western blot	Technique used to transfer proteins separated by gel electrophoresis by 3-D structure or denatured proteins by the length of the polypeptide to a membrane, where they might be identified by antibody labelling.
WHO	World Health Organisation
ZM	*Zea maize* L.

Background

On 12 February 2007, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands an application (Reference EFSA/GMO/NL/2007/39) for authorisation of the insect-resistant and herbicide tolerant genetically modified (GM) maize MON 89034 x MON 88017 (Unique Identifier MON-89Ø34-3 × MON-88Ø17-3), submitted by Monsanto within the framework of Regulation (EC) No 1829/2003.

The scope of the application covers:

- Food
  - ✓ GM plants for food use
  - ✓ Food containing or consisting of GM plants
  - ✓ Food produced from GM plants or containing ingredients produced from GM plants
- Feed
  - ✓ GM plants for feed use
  - ✓ Feed containing or consisting of GM plants
  - ✓ Feed produced from GM plants

- GM plants for environmental release
  - ✓ Import and processing (Part C of Directive 2001/18/EC)

After receiving the application EFSA/GMO/NL/2007/39 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the EU- and EFTA Member States (MS) and the European Commission and made the summary of the dossier publicity available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of regulation (EC) No 1829/2003. On 20 September 2007, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003. EFSA made the valid application available to Member States and the EC and consulted nominated risk assessment bodies of the MS, including the Competent Authorities within the meaning of Directive 2001/18/EC (EC 2001), following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1929/2003, to request their scientific opinion. Within three months following the date of validity, all MS could submit via the EFSA GMO Extranet to EFSA comments or questions on the valid application under assessment. The VKM GMO Panel assessed the application in connection with the EFSA official hearing, and submitted a preliminary opinion in May 2008 (VKM 2008a). EFSA published its scientific opinion 30 March 2010 (EFSA 2010b), and maize MON 89034 x MON 88017 was approved for food and feed uses, import and processing in 17 June 2011 (Commission Decision 2011/366/EC). An application for authorisation of maize MON 89034 x MON 88017 for cultivation in the EU was submitted by Monsanto in June 2009 (EFSA/GMO/BE/2009/71). On 4 November 2009 EFSA declared the application as valid, and made the valid application available to Member States and the European Commission. VKM participated in the 90 days public consultation, and

submitted a preliminary environmental risk assessment report in April 2010 (VKM 2010a). On 20 August 2013 the application was, however, withdrawn by the applicant.

The parental lines MON 89034 and MON 88017 have also been evaluated by the VKM GMO Panel as single events and as a component of several stacked GM maize events (VKM 2007a,b, VKM 2008b, VKM 2009a,b,c, VKM 2010b,c, VKM 2012, VKM 2013, VKM 2014).

Terms of reference

The Norwegian Environment Agency has the overall responsibility for processing applications for the deliberate release of genetically modified organisms (GMOs). This entails inter alia coordinating the approval process, and to make a holistic assessment and recommendation to the Ministry of the Environment regarding the final authorization process in Norway. The Directorate is responsible for assessing environmental risks on the deliberate release of GMOs, and to assess the product's impact on sustainability, benefit to society and ethics under the Gene Technology Act.

The Norwegian Food Safety Authority (NFSA) is responsible for assessing risks to human and animal health on deliberate release of GMOs pursuant to the Gene Technology Act and the Food Safety Act. In addition, the NFSA administers the legislation for processed products derived from GMO and the impact assessment on Norwegian agriculture according to sector legislation.

The Norwegian Environment Agency

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Environment Agency, by letter dated 13 June 2012 (ref. 2008/4367/ART-BI-BRH), requests the Norwegian Scientific Committee for Food Safety, to conduct final environmental risk assessments for all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are authorized in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC. The request covers scope(s) relevant to the Gene Technology Act.

The request does not cover GMOs that the Committee already has conducted its final risk assessments on. However, the Norwegian Environment Agency requests the Committee to consider whether updates or other changes to earlier submitted assessments are necessary.

The basis for evaluating the applicants' environmental risk assessments is embodied in the Act Relating to the Production and Use of Genetically Modified Organisms etc. (the Norwegian Gene Technology Act), Regulations relating to impact assessment pursuant to the Gene Technology Act, the Directive 2001/18/EC on the deliberate release of genetically modified organisms into the environment, Guidance note in Annex II of the Directive 2001/18 (2002/623/EC) and the Regulation 1829/2003/EC. In addition, the EFSA guidance documents on risk assessment of genetically modified plants and food and feed from the GM plants (EFSA 2010, 2011a), and OECD guidelines will be useful tools in the preparation of the Norwegian risk assessments.

The risk assessments' primary geographical focus should be Norway, and the risk assessments should include the potential environmental risks of the product(s) related to any changes in agricultural practices. The assignment covers assessment of direct environmental

impact of the intended use of pesticides with the GMO under Norwegian conditions, as well as changes to agronomy and possible long-term changes in the use of pesticides.

The Norwegian Food Safety Authority

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Environment Agency has requested the Norwegian Food Safety Authority (NFSA) to give final opinions on all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are authorized in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC within the Authority's sectoral responsibility. The request covers scope(s) relevant to the Gene Technology Act.

The Norwegian Food Safety Authority has therefore, by letter dated 13 February 2013 (ref. 2012/150202), requested the Norwegian Scientific Committee for Food Safety (VKM) to carry out final scientific risk assessments of 39 GMOs and products containing or consisting of GMOs that are authorized in the European Union.

The assignment from NFSA includes food and feed safety assessments of genetically modified organisms and their derivatives, including processed non-germinating products, intended for use as or in food or feed.

In the case of submissions regarding genetically modified plants (GMPs) that are relevant for cultivation in Norway, VKM is also requested to evaluate the potential risks of GMPs to the Norwegian agriculture and/or environment. Depending on the intended use of the GMP(s), the environmental risk assessment should be related to import, transport, refinement, processing and cultivation. If the submission seeks to approve the GMP(s) for cultivation, VKM is requested to evaluate the potential environmental risks of implementing the plant(s) in Norwegian agriculture compared to existing varieties (e.g. consequences of new genetic traits, altered use of pesticides and tillage). The assignment covers both direct and secondary effects of altered cultivating practices.

VKM is further requested to assess risks concerning coexistence of cultivars. The assessment should cover potential gene flow from the GMP(s) to conventional and organic crops as well as to compatible wild relatives in semi-natural or natural habitats. The potential for establishment of volunteer populations within the agricultural production systems should also be considered. VKM is also requested to evaluate relevant segregation measures to secure coexistence during agricultural operations up to harvesting. Post-harvest operations, transport, storage are not included in the assignment.

Evaluations of suggested measures for post-market environmental monitoring provided by the applicant, case-specific monitoring and general surveillance, are not covered by the assignment from the Norwegian Food Safety Authority.

Assessment

1 Introduction

The hybrid maize MON 89034 x MON 88017 was produced by conventional crosses between inbred lines containing MON 89034 and MON 88017 events to combine resistance to certain lepidopteran and coleopteran pests, and to confer tolerance towards glyphosate-containing herbicides. The parental line MON 89034 was developed to provide protection against certain lepidopteran insect larvae, including European corn borer (*Ostrinia nubilalis*), fall armyworm (*Spodoptera* ssp.), black cutworm (*Agrotis ipsilon*) and corn earworm (*Helicoverpa zea*). Insect protection is achieved through expression in the plant of two insecticidal Cry proteins, Cry1A.105 and Cry2Ab2, derived from *Bacillus thuringiensis*, a common soil bacterium. Cry1A.105, encoded by the *cry1A.105* gene, is a chimeric protein made up of different functional domains derived from three wild-type Cry proteins from *B. thuringiensis* subspecies *kurstaki* and *aizawai*. The Cry2Ab2 protein is encoded by the *cry2Ab2* gene derived from *B. thuringiensis* subspecies *kurstaki*. The mode of action of the Cry proteins is to bind selectively to specific receptors on the epithelical surface of the midgut of larvae of susceptible insect species, leading to death of larvae through pore formation, cell burst and subsequently septicaemia (ref. EFSA 2011d). The parental line MON 88017 expresses the *cry3Bb1* gene from *Bacillus thuringiensis* subsp. *kumamotoensis,* (strain EG4691), conferring resistance to certain coleopteran target pests belonging to the genus *Diabrotica,* such as the larvae of western corn rootworm (*D. virgifera virgifera*), northern corn rootworm (*D. barberi*) and the southern corn rootworm (*D. undecimpunctata howardi*). Maize MON 88017 has also been modified to provide tolerance to the broad spectrum herbicide glyphosate. Glyphosate is normally phytotoxic to a broad range of plants. Its mode of action occurs by binding to and inactivating the EPSPS protein, which is a key enzyme in the shikimate pathway that leads to the biosynthesis of the aromatic amino acids tyrosine, tryptophan and phenylalanine (Dill 2005; Duke & Powles, 2008b). The disruption of this pathway and the resulting inability to produce key amino acids prevents growth and ultimately leads to plant death. However, in case of maize MON 88017, a gene has been introduced that codes for the expression of the CP4 EPSPS protein, which is insensitive towards inhibition by glyphosate. This protein is similar to the native EPSPS found in wild-type plants, but it is not inactivated by glyphosate thus allowing the crop to be protected from the recommended dosages of glyphosate. The genetic modification in maize MON 89034 x MON 88017 is intended to improve agronomic performance only, and is not intended to influence the nutritional properties, the processing characteristics and the overall use of maize as a crop. Maize stack MON 89034 x MON 88017 (Unique Identifier MON-89Ø34-3 × MON-88Ø17-3) has been evaluated with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and

Regulation (EC) No 1829/2003 on genetically modified food and feed. The Norwegian Scientific Committee for Food Safety has also decided to take account of the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2011a), the environmental risk assessment of GM plants (EFSA 2010), the selection of comparators for the risk assessment of GM plants (EFSA 2011b), and for the post-market environmental monitoring of GM plants (EFSA 2011c). The food/feed and environmental risk assessment of the genetically modified maize MON 89034 x MON 88017 is based on information provided by the applicant in the applications EFSA/GMO/NL/2007/39 and EFSA/GMO/BE/2009/71 and scientific opinions and comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment is also based on a review and assessment of relevant peer-reviewed scientific literature. It is emphasised that the VKM mandate does not include assessments of contribution to sustainable development, societal utility and ethical considerations, according to the Norwegian Gene Technology Act and Regulations relating to impact assessment pursuant to the Gene Technology Act. These considerations are therefore not part of the risk assessment provided by the VKM Panel on Genetically Modified Organisms.

2 Molecular characterisation

2.1 Evaluation of relevant scientific data

2.1.1 Method of production of maize MON 89034 x MON 88017

The stacked maize MON 89034 x MON 88017 was developed through conventional breeding by crossing the single maize events MON 89034 and MON 88017. Maize MON 89034 x MON 88017 combines the insect resistance of maize MON 89034 with the glyphosate tolerance and insect resistance of maize MON 88017, conferred through the expression of the *cry1A.105*, *cry2Ab2*, *cp4 epsps* and *cry3Bb1* genes, respectively.

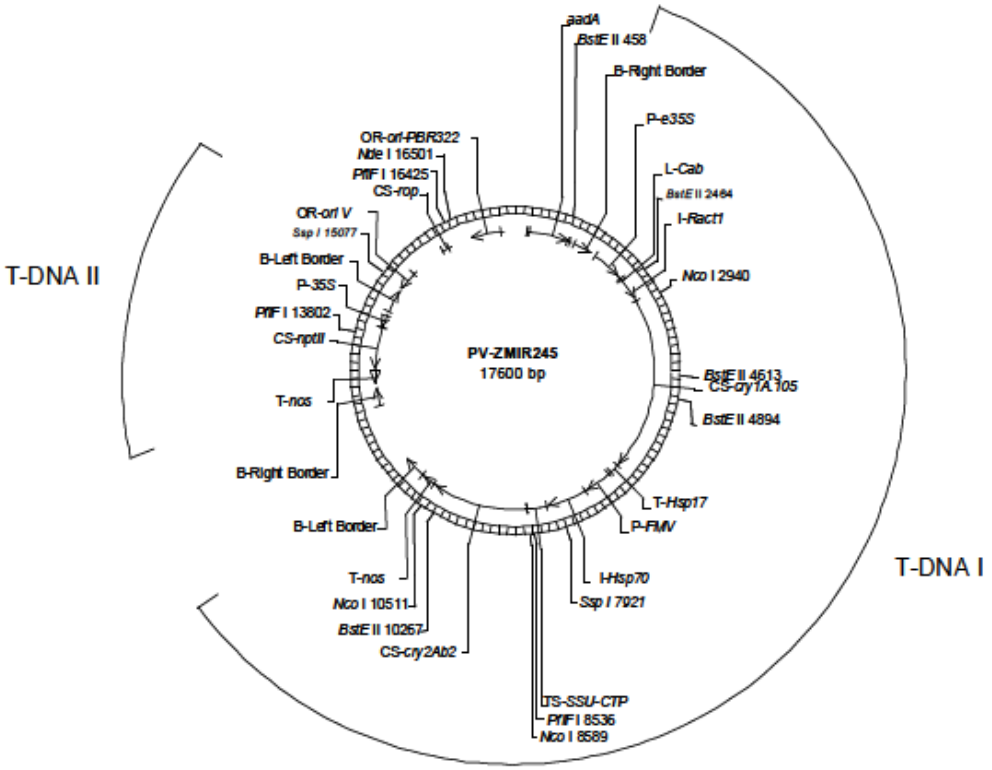
2.1.2 Summary of previous evaluation of the single events

2.1.2.1 Maize MON 89034

Maize event MON 89034 produces the Cry1A.105 and Cry2Ab2 insecticidal proteins that confer tolerance to certain lepidopteran insect pests, and was developed through *Agrobacterium*-mediated transformation of the proprietary inbred maize line LH172 with the transformation vector PV-ZMIR245. The plasmid vector PV-ZMIR245 (Figure 1) contains two separate transfer DNAs (T-DNAs) that were transferred to the genome of immature plant embryos from maize LH172. The first T-DNA, designated as T-DNA I, contains the *cry1A.105* and the *cry2Ab2* coding sequences and components necessary to regulate their expression in the maize. The second T-DNA, designated as T-DNA II, contains the *nptII* coding sequence and regulatory components. The *nptII* gene encodes the neomycin phosphotransferase enzyme that confers tolerance to certain antibiotics such as neomycin, kanamycin and paromomycin, and was used as a selectable marker gene. The *nptII* gene was subsequently removed during development through selective breeding of transformed plants, and is not present in maize event MON 89034. The absence of the *nptII* gene and the NPTII protein was confirmed by both Southern blot and ELISA analyses. The Cry1A.105 protein is a modified *Bacillus thuringiensis* (Bt) Cry1A protein with an amino acid sequence identity to Cry1Ab, Cry1Ac and Cry1F proteins of 90.0%, 93.6% and 76.7%, respectively. Expression of *cry1A.105* is regulated by P-e35S - the promoter and leader for the cauliflower mosaic virus (CaMV) 35S RNA, and the 3' nontranslated region of the coding sequence for wheat heat shock protein 17.3 (T-*Hsp17*), which terminates transcription. Cry2Ab2 is a member of the Cry2Ab class of proteins that share more than 95% amino acid sequence homology, and is a variant of the wild-type Cry2Ab2 protein isolated from *Bacillus thuringiensis* subsp. *kurstaki*. The *cry2Ab2* gene is regulated by the 35S promoter from figwort mosaic virus (P-*FMV*), and the 3' nontranslated region of the nopaline synthase (T-*nos*) from *Agrobacterium tumefaciens,* which terminates transcription. With the use of PCR, sequence analyses, restriction enzymes and Southern blot analyses the applicant has characterised the DNA

insert and its flanking sequences in MON 89034, assessed the integrity of the insert and the insert number (number of insertions of the integrated DNA within the maize genome), the copy number (the number of copies of the integrated DNA within one locus), the presence or absence of the elements of T-DNA II, the presence or absence of the *nptII* coding sequence and the presence or absence of plasmid backbone sequences. The results showed that T-DNA I was inserted into the maize genome at a single locus, that the insert contained single functional copies of the *cry1A.105* and *cry2Ab2* coding sequences, that no additional elements were detected other than those present in T-DNA I, and that it was unlikely that any endogenous genes were disrupted at the insertion site. Cry1A.105 and Cry2Ab2 protein levels were determined by enzyme-linked immunosorbent assay (ELISA) in various tissues of MON 89034 collected from US, Argentinean and European field trials conducted in 2005, 2004 and 2007, respectively. In tissues harvested throughout the growing season in the USA, Cry1A.105 protein levels across all sites ranged from 27 - 850 µg/g dwt in leaf, 20 - 570 µg/g dwt in whole plant and 6.2 - 110 µg/g dwt in root. In forage, pollen and grain, Cry1A.105 levels ranged from 20 - 56 µg/g dwt, 8.5 - 16 µg/g dwt, and 4.7 - 7.0 µg/g dwt, respectively. Cry2Ab2 levels across all sites ranged from 48-270 µg/g dwt in leaf, 5-230 µg/g dwt in whole plant, and 13-100 µg/g dwt in root. In forage, pollen and grain, Cry2Ab2 levels ranged from 15 - 55 µg/g dwt, 0.49 - 0.79 µg/g dwt, and 0.77 - 2.1 µg/g dwt, respectively. The means for Cry1A.105 protein levels across all sites in Argentina were 2.6 µg/g dwt in grain, 30 µg/g dwt in forage, 7.7 µg/g dwt in pollen, 260 µg/g dwt in OSL-1 (overseason leaf-1), 200 µg/g dwt in OSL-4, 28 µg/g dwt in forage root, and 19 µg/g dwt in stover. In tissues harvested throughout the growing season, mean Cry1A.105 protein levels across all sites ranged from 160 – 260 µg/g dwt in leaf, 22 – 71 µg/g dwt in root, and 48 – 170 µg/g dwt in whole plant. The means for Cry2Ab2 protein levels across all sites were 0.95 µg/g dwt in grain, 45 µg/g dwt in forage, 0.56 µg/g dwt in pollen, 120 µg/g dwt in OSL-1, 270 µg/g dwt in OSL-4, 31 µg/g dwt in forage root, and 44 µg/g dwt in stover. In tissues harvested throughout the growing season, mean Cry2Ab2 protein levels across all sites ranged from 120 – 270 µg/g dwt in leaf, 23 – 48 µg/g dwt in root, and 61 – 98 µg/g dwt in whole plant. The mean levels of Cry1A.105 in MON 89034 from the European field trials maize were highest in tissue samples from whole plants early in the growth season (V2-V4 stage; 240 µg/g dwt), with the mean level in pollen and grain being 24 µg/g dwt and 3.4 µg/g dwt, respectively. The mean Cry1A.105 protein levels across all sites was 130 µg/g dwt in OSL-1, 44 µg/g dwt in OSR-1 (overseason root-1), 7.4 µg/g dwt in forage-root, 60 µg/g dwt in OSWP-3 (overseason whole plant-3), 31 µg/g dwt in forage, 24 µg/g dwt in pollen, and 3.4 µg/g dwt in grain. The mean Cry2Ab2 protein levels in MON 89034 across all field sites were 250 µg/g dwt in leaf samples from growth stages V6-V8, 30 µg/g dwt in forage root, 49 µg/g dwt in forage, 0.59 µg/g dwt in pollen and 1.8 µg/g dwt in grain. In tissues harvested throughout the growing season, mean Cry2Ab2 protein levels at all sites ranged from 71-250 µg/g dwt in leaf, 23-33 µg/g dwt in root and 48-150 µg/g dwt in whole plant. The results show that the overall range of the observed protein levels for Cry1A.105 and Cry2Ab2 were all spanning the range of the relative control in the US, Argentinean and European field trials. Potential for novel open reading frames (ORFs) that may produce proteins with similarities to known allergens and toxins was assessed for 10 putative sequences within the DNA spanning the 5' and 3' junctions between the DNA insert in MON 89034 and the maize

genomic DNA. According to the applicant, the analyses did not disclose any biologically relevant sequence similarities between allergens, toxins or other biologically active proteins with any of the 10 sequences tested – new potentially harmful fusion proteins are therefore not expected to be produced in maize MON 89034. Several analyses over multiple generations with Southern blot, ELISA, PCR and Chi-square analysis have been performed by the applicant to demonstrate the stability of the genetic and phenotypic changes in MON 89034. According to the applicant, these analyses are consistent with a single site of insertion for the *cry1A.105* and *cry2Ab2* gene sequences, and show comparable levels of the Cry1A.105 and Cry2Ab2 proteins.



A circular map of the plasmid vector PV-ZMIR245 used in *Agrobacterium*-mediated transformation to develop MON 89034 is shown. PV-ZMIR245 contains two T-DNA regions designated as T-DNA I and T-DNA II. In this procedure, only the DNA present between the left and right borders was transferred into the host maize cells.

Figure 1. Map of the plasmid PV-ZMIR245

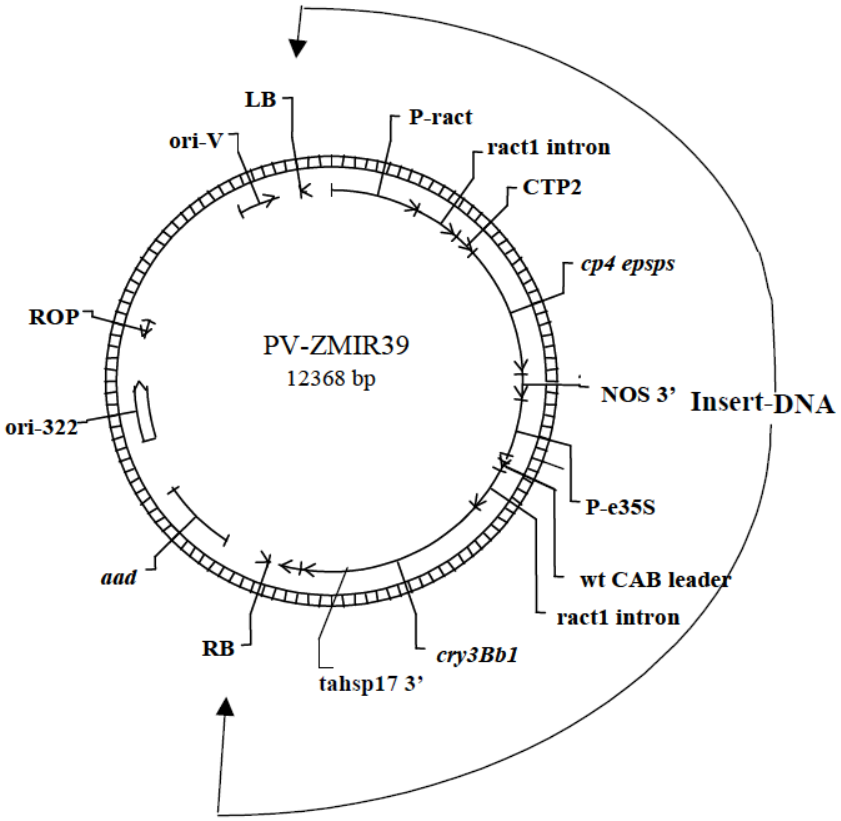
2.1.2.2 Maize MON 88017

Genetically modified maize MON 88017 was developed to produce a modified Cry3Bb1 protein, derived from *Bacillus thuringiensis* subsp. *kumamotoensis* providing protection against certain coleopteran insect pests, and the CP4 EPSPS protein derived from *Agrobacterium* sp. strain CP4 which provides tolerance to glyphosate.

The plasmid vector PV-ZMIR39 (Figure 2) was used in the transformation of maize embryonic cells to produce MON 88017. PV-ZMIR39 is a disarmed, binary *Agrobacterium tumefaciens* transformation vector that contains both left and right transfer-DNA (T-DNA) border sequences to facilitate transformation. The T-DNA region contains the *cp4 epsps* and *cry3Bb1* gene expression cassettes, and is the portion of plasmid PV-ZMIR39 that is integrated into the maize genome during the transformation process.

The *cp4 epsps* coding sequence derived from *Agrobacterium* sp. strain CP4, a common soil bacterium, has been sequenced and shown to encode a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids. In the plant gene expression cassette, the *cp4 epsps* coding sequence is joined to a DNA sequence coding for the chloroplast transit peptide 2 (CTP2) isolated from the *Arabidopsis thaliana epsps* gene. This transit peptide directs the CP4 EPSPS protein to the chloroplast, the location of EPSPS in plants and the site of aromatic amino acid biosynthesis. The *ctp2-cp4 epsps* coding sequence is under the control of the rice actin 1 sequence containing the promoter (*P-ract1*) and first intron (*ract1* intron) introduced upstream of the *ctp2* sequence. The *cp4 epsps* sequence is joined to the NOS 3' sequence from *Agrobacterium tumefaciens* that provides the transcription termination and the mRNA polyadenylation signal.

The *cry3Bb1* coding sequence from the wild-type *Bacillus thuringiensis* (subsp. *kumamotoensis*) strain EG4691 was modified to encode six specific amino acid substitutions, resulting in the synthetic MON 88017 *cry3Bb1* coding sequence present in plasmid vector PV-ZMIR39. It encodes a variant of the wild-type Cry3Bb1 protein with which it shares an amino acid sequence identity of 99.1%, differing by six of 652 amino acid residues. According to the applicant, the Cry3Bb1 proteins in MON 88017 have been extensively characterised. The synthetic MON 88017 *cry3Bb1* gene expression cassette that produces the MON 88017 Cry3Bb1 protein consists of the P-e35S promoter, the wt CAB leader, and the intron from the *ract1* gene joined to the synthetic MON 88017 *cry3Bb1* coding sequence at the 5' end. Joined to the 3' end of the synthetic MON 88017 *cry3Bb1* coding sequence is the *tahsp17* 3' sequence, which ends transcription and provides the signal for mRNA polyadenylation.



A circular map of the plasmid vector PV-ZMIR39 used in *Agrobacterium*-mediated transformation to create MON 88017. In this procedure, only the DNA present between the right and left borders (*i.e.*, RB and LB) was transferred into the host maize cells.

Figure 2. Map of the plasmid PV-ZMIR39

Southern analyses of genomic DNA digests with two different restriction enzymes (SacI and XbaI) and four different probes spanning the entire length of the insert showed the presence of a single copy of the introduced DNA at a single insertion locus. The intactness of the two inserts was examined by Southern analysis and confirmed by PCR amplification of seven overlapping regions of DNA that span the entire length of the insert. These PCR fragments were sequenced and used to confirm the identity between the sequences inserted in MON 88017 and the corresponding sequences of the PV-ZMIR39 plasmid. The absence of vector backbone sequences in MON 88017 plants was established by Southern analysis with two probes that covered the entire vector backbone. Samples for protein analysis were collected from field trials conducted at three locations in USA during the 2002 growing season and four locations in Argentina in 2003/2004. The levels of the Cry3Bb1 protein showed a decline in leaf, whole plant and root tissues collected over the growing season. Across the developmental stages examined, the mean Cry3Bb1 protein levels ranged between 260-570 µg/g dw in leaf, 220-500 µg/g dw in whole plant and 100-370 µg/g dw in root tissues. In the other tissues analysed across all sites, mean Cry3Bb1 protein levels were: 15 µg/g dw in grain (range 10-22 µg/g dw), 25 µg/g dw in pollen (range 17-32 µg/g dw), 380 µg/g dw in silk (300-500 µg/g dw) and 88 µg/g dw in stover (range 71-110 µg/g dw). The mean CP4

EPSPS protein levels across all sites ranged between 150-220 µg/g dw in over-season leaf and 70-150 µg/g dw in roots. In the other tissues analysed, mean CP4 EPSPS protein levels were 390 µg/g dw in pollen, 57 µg/g dw in forage and 5.8 µg/g dw in grain. CP4 EPSPS levels were not measured in whole plant, silk and stover. The mean protein levels observed for both Cry3Bb1 and CP4 EPSPS in grain tissues from MON 88017 grown in four Argentinean locations were 11 µg/g dw (range 8.0-19) and 4.6 µg/g dw (range 3.5-7.5), respectively.

Another field study was conducted during the 2006 growing season at seven locations in Europe. The mean Cry3Bb1 protein levels in MON 88017 across all sites were 8.7 µg/g dw in grain, 13 µg/g dw in pollen, 22 µg/g dw in senescent root, 160 µg/g dw in silk, and 30 µg/g dw in forage root. In tissues harvested throughout the growing season, mean Cry3Bb1 protein levels in MON 88017 across all sites ranged from 200 – 300 µg/g dwt in leaf, 75 - 160 µg/g dw in root, and 210 - 250 µg/g dw in whole plant. Measurements of Cry3Bb1 protein in tissue samples from the control substances were below the Cry3Bb1 assays LOQ and LOD for each tissue type. The mean CP4 EPSPS protein levels in MON 88017 across all sites were 3.9 µg/g dwt in grain, 280 µg/g dw in pollen, 14 µg/g dwt in senescent root, and 16 µg/g dwt in forage root. In tissues harvested throughout the growing season, mean CP4 EPSPS protein levels in MON 88017 across all sites ranged from 120 – 190 µg/g dwt in leaf, 22 - 50 µg/g dwt in root, and 130 - 160 µg/g dwt in whole plant. Measurements of CP4 EPSPS protein in tissue samples from the control substances were below the CP4 EPSPS assay LOQ and LOD for each tissue type.

The results from the 2006 field trials indicate that the levels of the Cry3Bb1 and CP4 EPSPS proteins show a decline in samples collected over the growing seasons, similar to that reported for maize MON 88017 grown in the USA in 2002. This is in agreement with the published results of field trials conducted with MON 88017 in Germany in 2005-2007 (Nguyen & Jehle 2009). The results also showed that the means and ranges of Cry3Bb1 and CP4 EPSPS proteins in maize MON 88017 grown in Europe were generally lower than those observed in samples collected from maize MON 88017 grown in 2002 in the USA.

The stability of the integrated DNA in MON 88017 has been established over multiple generations. The results are consistent with the finding of a single locus of insertion of the *cry3Bb1* and *cp4 epsps* genes that segregate according to Mendel's laws of genetics. The stability of the insert has been demonstrated through seven generations of cross-fertilisations and three generations of self-pollinations.

2.1.3 Transgene constructs in MON 89034 x MON 88017 maize

The MON 89034 x MON 88017 maize was obtained by conventional crossing between two genetically modified maize events: MON 89034 and MON 88017 maize. No new genetic modification was used in the development of the MON 89034 x MON 88017 maize. A detailed molecular analysis was conducted to investigate the copy number, structure and organisation of the inserts found in MON 89034 x MON 88017 maize. The integrity of the individual inserts present in this maize was investigated with Southern analyses. This involved the use of DNA probes specific for the MON 89034 and MON 88017 inserts and enzymatic digestions informative of the structure of both events, including the junctions with the host genomic DNA. The predicted DNA hybridisation patterns from each single event were retained in the MON 89034 x MON 88017 hybrid. The results obtained from Southern Blot analyses indicate molecular equivalence, and identical copy number of the inserts present in MON 89034 x MON 88017 maize to those present MON 89034 and MON 88017.

2.1.4 Information on the expression of the inserts

A study was conducted to estimate the amount of Cry1A.105, Cry2Ab2, CP4 EPSPS and Cry3Bb1 protein present in maize tissues collected from MON 89034 x MON 88017 grown in five field trials in the USA during the 2005 growing season (Hartmann et al. 2006). These field trials were located within the major maize-growing region of the USA and provided a variety of environmental conditions. At each site, three replicated plots of MON 89034 x MON 88017, MON 89034 and MON 88017, as well as the conventional control, were planted in a randomised complete block field design. Young leaf, young root, over season whole plant, forage, forage root, pollen, and grain tissues were collected from each replicated plot at all field sites. The samples from young leaf (over season leaf; OSL-1) and young root (over season root; OSR-1) were collected at the V2 – V4 growth stage and the OSWP-3 samples were collected at the V10 – V12 growth stage. ELISA methods were developed and validated for each protein. Protein levels for all ten tissues types were calculated on a microgram (µg) per gram (g) fresh weight (fwt) basis. Moisture content was then measured for all tissue types and all protein levels were converted and reported on a dry weight (dwt) basis. Levels of proteins are summarised in Table 1-4. The mean Cry1A.105 protein levels in MON 89034 x MON 88017 across all sites were 430 µg/g dwt in OSL-1, 83 Vg/g dwt in OSR-1, 13 µg/g dwt in forage-root, 140 µg/g dwt in OSWP-3, 48 µg/g dwt in forage, 16 µg/g dwt in pollen, and 5.6 µg/g dwt in grain. The mean Cry2Ab2 protein levels in MON 89034 x MON 88017 across all sites were 170 µg/g dwt in OSL-1, 53 µg/g dwt in OSR-1, 24 µg/g dwt in forage-root, 54 µg/g dwt in OSWP-3, 44 µg/g dwt in forage, 0.62 µg/g dwt in pollen, and 1.3 µg/g dwt in grain. The mean Cry3Bb1 protein levels in MON 89034 x MON 88017 across all sites were 220 µg/g dwt in OSL-1, 200 µg/g dwt in OSR-1, 69 µg/g dwt in forage-root, 160 µg/g dwt in OSWP-3, 50 µg/g dwt in forage, 15 µg/g dwt in pollen, and 4.1 µg/g dwt in grain. The mean CP4 EPSPS protein levels in MON 89034 x MON 88017 across all sites were 200 µg/g dwt in OSL-1, 75 µg/g dwt in OSR-1, 30 µg/g dwt in forage-root, 150 µg/g dwt in OSWP-3, 55 µg/g dwt in forage, 320 µg/g dwt in pollen, and 3.4 µg/g dwt in grain. Overall, the ranges across all sites for the Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS protein levels in MON

89034 x MON 88017 were comparable to the corresponding ranges in either MON 89034 or MON 88017.

Table 1. Summary of the levels of Cry1A.105 protein in maize tissues collected from MON 89034 x MON 88017 and MON 89034 produced in field trails in USA conducted in 2005.

Tissue Type[1]	MON 89034 × MON 88017		MON 89034	
	Mean (SD)[2] Range[3] (µg/g fwt)[4]	Mean (SD) Range (µg/g dwt)[5]	Mean (SD) Range (µg/g fwt)	Mean (SD) Range (µg/g dwt)
OSL-1	70 (8.3) 58 – 87	430 (71) 310 - 550	62 (13) 49 – 91	380 (93) 250 - 600
OSR-1	9.4 (1.4) 7.7 – 13	83 (18) 57 – 130	8.4 (0.89) 7.1 – 10	75 (16) 49 – 99
Forage-Root	2.5 (0.36) 1.9 – 3.3	13 (3.5) 8.1 – 18	2.1 (0.33) 1.4 – 2.6	12 (3.2) 6.0 – 17
OSWP-3	15 (2.8) 9.8 – 20	140 (28) 89 – 180	11 (2.6) 7.0 – 16	110 (27) 58 – 170
Forage	16 (3.9) 10 – 24	48 (13) 31 – 84	13 (3.8) 7.6 – 25	39 (9.5) 20 – 60
Pollen	8.6 (1.7) 6.2 – 11	16 (1.7) 14 – 20	6.5 (1.6) 4.0 – 9.1	12 (1.6) 8.9 – 15
Grain	4.9 (1.1) 1.6 – 6.5	5.6 (1.3) 1.9 – 7.5	5.0 (0.69) 3.8 – 5.9	5.8 (0.79) 4.5 – 6.8

[1] Tissues were collected at the following growth stages (Ritchie *et al.*, 1997): OSL-1 and OSR-1: V2 – V4; OSWP-3: V10 – V12; Forage and Forage-Root: early dent; Pollen: at pollination; and Grain: at physiological maturity

[2] The mean and standard deviation were calculated across sites (n=15, except MON 89034 × MON 88017 OSL-1, n=18 and all lines of forage, n=30).

[3] Minimum and maximum values were determined for each tissue type across sites.

[4] Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis.

[5] Protein levels are expressed as µg/g on a dry weight (dwt) basis. The dry weight values were calculated by dividing the fwt by the dry weight conversion factors obtained from moisture analysis data.

Table 2. Summary of the levels of Cry2Ab2 protein in maize tissues collected from MON 89034 x MON 88017 and MON 89034 produced in field trails in USA conducted in 2005.

Tissue Type[1]	MON 89034 × MON 88017		MON 89034	
	Mean (SD)[2] Range[3] (µg/g fwt)[4]	Mean (SD) Range (µg/g dwt)[5]	Mean (SD) Range (µg/g fwt)	Mean (SD) Range (µg/g dwt)
OSL-1	27 (9.6) 16 – 43	170 (69) 78 – 280	29 (8.1) 19 – 47	180 (65) 93 – 300
OSR-1	6.0 (2.7) 2.8 – 12	53 (27) 27 – 110	5.9 (1.4) 4.0 – 9.5	53 (16) 31 – 95
Forage-Root	4.4 (1.4) 2.6 – 7.6	24 (9.3) 12 – 40	4.1 (1.4) 2.3 – 6.6	21 (5.8) 14 – 33
OSWP-3	5.6 (1.7) 3.6 – 8.4	54 (15) 30 – 76	5.8 (1.1) 3.8 – 7.5	55 (12) 35 – 82
Forage	14 (2.4) 10 – 19	44 (7.4) 30 – 57	12 (3.9) 6.4 – 18	38 (13) 15 – 54
Pollen	0.33 (0.053) 0.25 – 0.43	0.62 (0.13) 0.50 – 0.96	0.35 (0.081) 0.22 – 0.47	0.65 (0.087) 0.51 – 0.85
Grain	1.2 (0.23) 0.71 – 1.6	1.3 (0.26) 0.82 – 1.9	1.1 (0.27) 0.71 – 1.6	1.3 (0.31) 0.82 – 1.9

1 Tissues were collected at the following growth stages (Ritchie *et al.*, 1997): OSL-1 and OSR-1: V2 – V4; OSWP-3: V10 – V12; Forage and Forage-Root: early dent; Pollen: at pollination; and Grain: at physiological maturity

2 The mean and standard deviation were calculated across sites (n=15).

3 Minimum and maximum values were determined for each tissue type across sites.

4 Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis.

5. Protein levels are expressed as µg/g on a dry weight (dwt) basis. The dry weight values were calculated by dividing the fwt by the dry weight conversion factors obtained from moisture analysis data.

Table 3. Summary of the levels of Cry3Bb1 protein in maize tissues collected from MON 89034 x MON 88017 and MON 88017 produced in field trails in USA conducted in 2005.

Tissue Type[1]	MON 89034 × MON 88017		MON 88017	
	Mean (SD)[2] Range[3] (µg/g fwt)[4]	Mean (SD) Range (µg/g dwt)[5]	Mean (SD) Range (µg/g fwt)	Mean (SD) Range (µg/g dwt)
OSL-1	36 (8.5) 23 – 57	220 (66) 120 – 360	38 (6.9) 26 – 52	230 (56) 150 – 330
OSR-1	23 (7.1) 16 – 42[6]	200 (83) 120 – 420	18 (5.6) 12 – 35	160 (64) 98 – 350
Forage-Root	13 (2.6) 9.6 – 17	69 (21) 32 – 110	16 (3.6) 11 – 23	82 (23) 42 – 120
OSWP-3	17 (2.8) 11 – 24	160 (29) 110 – 240	16 (3.6) 6.4 – 21	150 (35) 58 – 210
Forage	16 (3.0) 12 – 22	50 (9.1) 37 – 70	18 (2.6) 13 – 21	54 (9.5) 35 – 70
Pollen	8.0(1.3) 6.3 – 11	15 (3.4) 11 – 24	6.8 (1.8) 4.3 – 9.7	13 (3.0) 8.9 – 19
Grain	3.5 (2.0) 1.2 – 8.3	4.1 (2.3) 1.3 – 9.7	3.8 (0.71) 2.5 – 5.6	4.4 (0.82) 2.9 – 6.5

[1] Tissues were collected at the following growth stages (Ritchie *et al.*, 1997): OSL-1 and OSR-1: V2 – V4; OSWP-3: V10 – V12; Forage and Forage-Root: early dent; Pollen: at pollination; and Grain: at physiological maturity

[2] Mean and standard deviation were calculated across sites (n=15) except MON 89034 × MON 88017 OSWP-3 and all lines for grain, n=30).

[3] Minimum and maximum values were determined for each tissue type across sites.

[4] Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis.

[5] Protein levels are expressed as µg/g on a dry weight (dwt) basis. The dry weight values were calculated by dividing the fwt by the dry weight conversion factors obtained from moisture analysis data.

[6] One sample from MON 89034 × MON 88017 at site IL-1 came up below the LOD for root tissue. This sample is not included in the average or range calculations.

Table 4. Summary of the levels of CP4 EPSPS protein in maize tissues collected from MON 89034 x MON 88017 and MON 88017 produced in field trails in USA conducted in 2005.

Tissue Type[1]	MON 89034 × MON 88017		MON 88017	
	Mean (SD)[2] Range[3] (µg/g fwt)[4]	Mean (SD) Range (µg/g dwt)[5]	Mean (SD) Range (µg/g fwt)	Mean (SD) Range (µg/g dwt)
OSL-1[6]	35 (6.6) 23 – 45	200 (32) 150 – 250	31 (3.6) 24 – 35	180 (29) 120 – 220
OSR-1[7]	8.3 (3.4) 4.9 – 16	75 (38) 36 – 160	6.4 (1.6) 3.9 – 9.4	57 (16) 31 – 86
Forage-Root	5.5 (1.4) 4.0 – 9.7	30 (11) 14 – 51	4.5 (0.73) 3.5 – 6.3	24 (5.7) 13 – 33
OSWP-3	16 (2.9) 11 – 22	150 (26) 100 – 190	14 (2.3) 7.6 – 17	130 (23) 69 – 160
Forage	18 (2.1) 14 – 21	55 (8.9) 38 – 69	18 (3.4) 12 – 25	56 (11) 39 – 73
Pollen	170 (47) 120 – 270	320 (89) 200 – 550	150 (39) 77 – 220	270 (66) 180 – 400
Grain	3.0 (0.60) 1.9 – 4.1	3.4 (0.68) 2.2 – 4.7	2.8 (0.71) 1.6 – 4.2	3.3 (0.81) 1.8 – 4.8

1 Tissues were collected at the following growth stages (Ritchie *et al*., 1997): OSL-1 and OSR-1: V2 – V4; OSWP-3: V10 – V12; Forage and Forage-Root: early dent; Pollen: at pollination; and Grain: at physiological maturity

2 The mean and standard deviation were calculated across sites (n=15, except MON 89034 × MON 88017 grain, n=17).

3 Minimum and maximum values were determined for each tissue type across sites.

4 Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis.

5 Protein levels are expressed as µg/g on a dry weight (dwt) basis. The dry weight values were calculated by dividing the fwt by the dry weight conversion factors obtained from moisture analysis data.

6. OSL-1 results reported from four sites, resulting in n=12.

7 One sample from MON 89034 × MON 88017at site IL-1 came up below the LOD for root tissue. This sample is not included in the average or range calculations

2.1.5 Inheritance and genetic stability of inserted DNA

The genetic stability of the inserted DNA in events MON 89034 and MON 88017 have previously been evaluated by the VKM GMO Panel (VKM 2008b, VKM 2010b). Southern blot analyses have shown that both events are present in the stacked event maize MON 89034 x MON 88017, and that the structure of each insert is retained. Transgenic protein levels, phenotypic characteristics and agronomic performance, also indicate that the integrity of the inserts inherited from the single events is preserved in the maize stack.

2.2 Conclusion

Southern and PCR analyses indicate that the recombinant inserts in the single maize events MON 89034 and MON 88017 are retained in the stacked event MON 89034 x MON 88017. Genetic stability of the inserts has previously been demonstrated in the single events. The levels of Cry1A.105, Cry2Ab2, CP4 EPSPS and Cry3Bb1 proteins in grain and forage from the stacked event are comparable to the levels in the corresponding single events. Phenotypic analyses also indicate stability of the insect resistance and herbicide tolerance traits of the stacked event.

Based on current knowledge and the previous assessments of the parental maize events, the VKM GMO Panel considers the molecular characterisation of maize MON 89034 x MON 88017 satisfactory.

3 Comparative assessment

3.1 Summary of the previous evaluations of the single events

3.1.1 Maize MON 89034

Comparative assessments of phenotypic, agronomic and ecological characteristics of MON 89034 maize was conducted in 2004-2005 at nine field locations within major US maize producing geographies, and in 2007 at eight field locations within two major European maize producing regions. No consistent compositional differences were observed between maize MON 89034 and non-transgenic maize. The reported differences in composition between MON 89034 and control maize was considered to reflect natural variation, and are not regarded as unintended effects resulting from the genetic modification. In the updated risk assessment of maize MON 89034 the VKM GMO Panel concludes that maize MON 89034 is compositionally, agronomically and phenotypically equivalent to conventional maize varieties, except for the presence of the insect resistance trait conferred by the Cry1A.105 and Cry2Ab2 proteins (VKM 2013).

3.1.2 Maize MON 88017

Phenotypic evaluation of maize MON 88017 and production of materials for the comparative assessments was conducted during field trials in the USA in 2001 and 2002 and in Argentina in 2003/2004. Supplementary compositional data were obtained from field trials in Europe during the 2006 growth season. In the 2001 and 2002 growing seasons, genetically modified maize MON 88017 was grown in field trials at 8 and 10 locations, respectively in major maize-growing areas of the USA. The test and control hybrids had a LH59 x LH198 genetic background and were tested as hybrid pairs. MON 88017 and conventional control maize were grown at four replicated field sites across Argentina during the 2003-2004 field season. Four commercially available maize hybrids were grown at each of the same field sites to provide a total of 16 different reference substances. In the 2006 growing season, MON 88017 and conventional control maize hybrids were grown at three northern European locations situated in Germany and at four southern European locations situated in Spain. In these field trials, the test hybrid MON 88017 was compared with conventional counterparts consisting of the varieties designed as DKC3945 and DKC5143. No consistent compositional differences were observed between maize MON 88017 and non-transgenic maize. In the updated risk assessment of maize MON 88017, the VKM GMO Panel concludes that maize MON 88017 is compositionally, agronomically and phenotypically equivalent to conventional maize varieties, except for the insect resistance conferred by the Cry3Bb1 protein and tolerance to glyphosate conferred by the CP4 EPSPS protein (VKM 2016).

3.2 Choice of comparator and production of material for the compositional assessment

Comparative assessments of compositional, phenotypic, agronomic and ecological characteristics of maize stack MON 89034 x MON 88017 and its conventional counterpart have been performed in field trials in the USA in 2004 and within major European maize producing regions in 2007 (Tech. Dossier: De Billot 2009).

Field trials in USA (2004)

Compositional, phenotypic and agronomic data were collected from five locations in USA in the 2004 growth season. According to the applicant, these locations provided a range of environmental and agronomic conditions representative of major USA maize-growing regions where commercial production of MON 89034 x MON 88017 would be expected. The stacked event MON 89034 x MON 88017 was obtained by crossing two inbred lines containing the single events MON 89034 and MON 88017 (Figure 3). A conventional maize line (LH198 x LH172) with a similar genetic background to the stacked event was included as a conventional control in the trials. Relatedness between the control and the stacked event is shown in Figure 3.

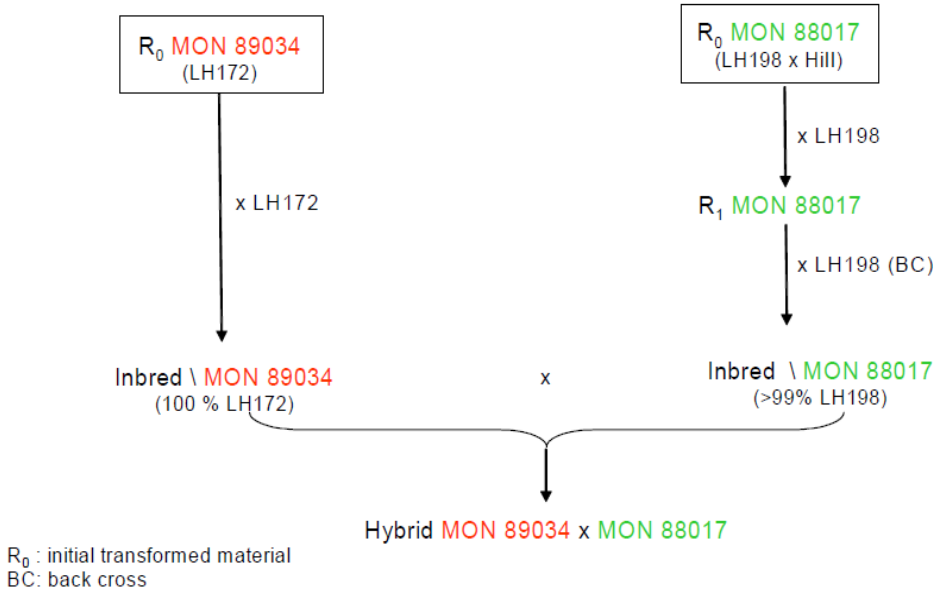


Figure 3. Traditional breeding strategies were applied to develop maize MON 89034 x MON 88017.

15 commercially available conventional maize hybrids were included in the study as reference lines to provide data for the development of a 99 % tolerance interval for each component analysed, three varieties at each location. Plots were established at each of the field sites in a randomised complete block design with three replications. Each plot consisted of two to six rows of maize spaced approximately 75 cm apart. All the maize lines at each of the field sites were grown under normal agronomic field conditions for their respective geographic regions, and all replicates at the same location underwent similar agronomic treatments. In the study report on the compositional analyses (Reynolds et al. 2006), it is not indicated whether MON 89034 x MON 88017 maize plots were treated with glyphosate or not.

Field trials in Europe (2007)

Compositional, phenotypic and agronomic data were collected from eight field locations in Europe, five in Spain and three in Germany in the 2007 growth season. According to the applicant, these locations provided a range of environmental and agronomic conditions representative of the northern and southern European maize growing regions where commercial production of MON 89034 x MON 88017 is expected. In these field trials genetically modified maize MON 89034 x MON 88017 was compared with a conventional counterpart having a comparable genetic background. Event MON 89034 x MON 88017 was introgressed into two different genetic backgrounds; DKC3945 adapted to northern (Germany) and DKC5143 adapted to southern (Spain) European growing regions. The control substances included in the field trials were conventional maize DKC3945 (Germany) and DKC5143 (Spain). DKC3945 and DKC5143 have genetic backgrounds similar to the test plants grown in Germany and Spain, respectively, except for the newly introduced traits. Relatedness between the controls and the test is shown in Figure 4.

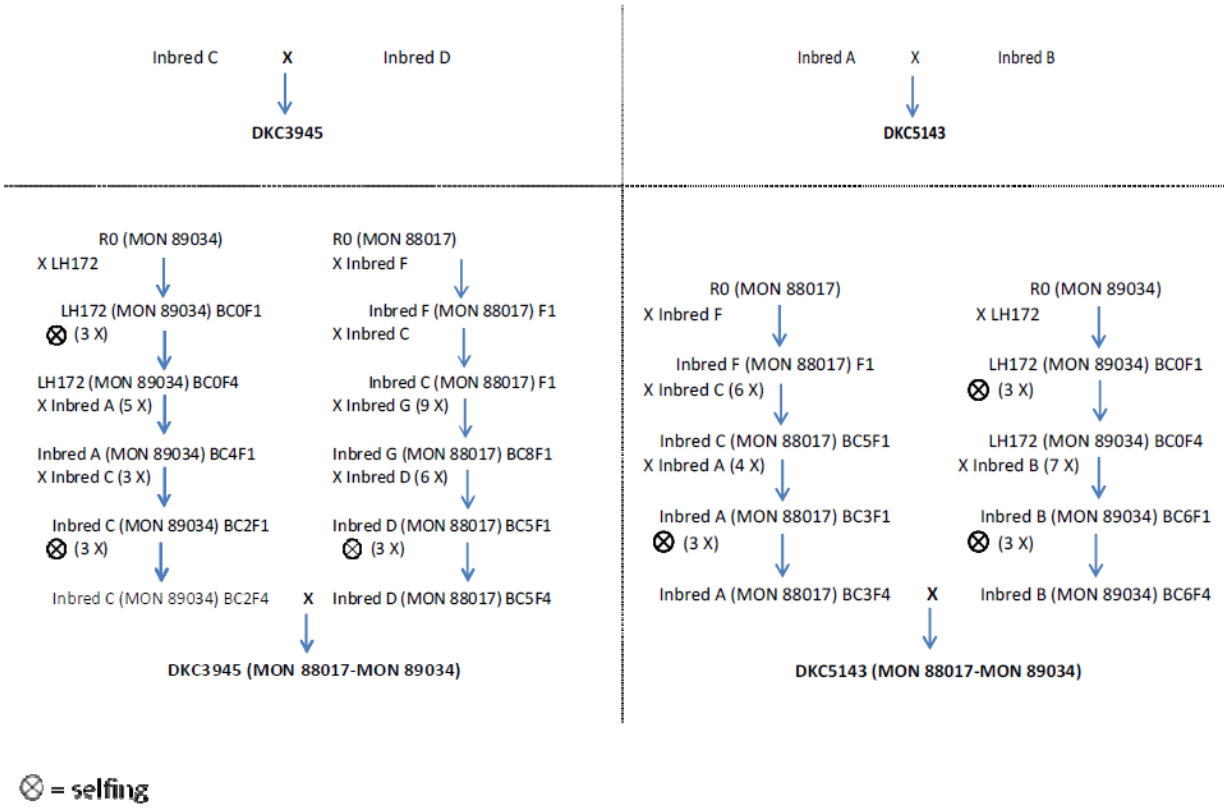


Figure 4. Relatedness between the controls (DKC 3945 (Germany) and DKC 5143 (Spain)) and the test (MON 89034 x MON 88017)

15 conventional, commercial available maize hybrids with similar relative maturities as the test and control substances were included in the comparative assessments to verify whether any differences observed between the GMO and its comparator fall within the range of natural variation. Six locally adapted hybrids were used in Germany and nine different locally adapted hybrids were grown in Spain. Plots were established at each site in a randomised complete block design with three replications. Each plot consisted of six rows spaced approximately 70 cm apart and approximately 6-10 m in length. Rows 4 and 5 were designated for phenotypic and ecological interaction data, while row 3 and 6 were used as buffer rows. Agronomic practices used to prepare and maintain each study were characteristic of the respective region. Maintenance pesticides were applied as needed at the field sites. Pesticides containing Bt were not applied to the study area at any site. None of the plots with maize stack MON 89034 x MON 88017 were treated with the target herbicide.

According to the EFSA Guidance for risk assessment of food and feed from GM plants (EFSA 2011a, the risk assessment of herbicide-tolerant GM plants, containing single or stacked events, the experimental design should include a comparison of three test materials: the GM plants exposed to the intended herbicide, the comparator treated with conventional herbicide management regimes and the GM plants treated with the same conventional herbicide management regimes. For comparative assessment, EFSA also explicitly advices to include

both GM plants exposed and not exposed to the intended herbicide in the trials in the guidance from 2006 (EFSA 2006).

Statistical analysis

US field trials

Statistical analysis was performed with a mixed model analysis of variance. Each individual analyte for MON 89034 x MON 88017 was compared to that of the conventional control, for the combination of all five sites, and for the indiviual site. The statistical significance was defined at the level of $p < 0.05$. Statistical evaluation of the composition data involved a comparison of the forage and grain form MON 89034 x MON 88017 to a conventional control maize. A total of 77 analytes were measured, of these 16 analytes more than 50% of the observations had a value below the LOQ of the assay, and they were therefore excluded from the statistical analysis. In total, 61 components were statistically assessed (nine in forage and 52 in grain). There were 366 statistical comparisions conducted between each test substance and the conventional control (61 comparisions in the combined site and 305 comparisions in the individual sites). The overall data set was then examined for evidence of biologically relevant changes. Data from each component obtained from the 15 uniqe conventional substances was used to calculate a 99% tolerance interval to include, with 95% confidence, 99% of the values contained in the population of conventional corn substances. When the testing identified statistically significant differences between the MON 89034 x MON 88017 and control, the test range was compared to the 99% tolerance interval in order to determine if the range was within the tolerance interval, and therefore considered to be within the normal variation of the conventional corn. A comparision with the data from the ILSI crop composition database was also performed.

European field trials

The six replicated sites were analyzed both separately and combined. For each component analysis, mean comparison tests of each test substance vs. the conventional control substance within each geographical region were conducted. Due to missing data, some mean comparison tests within individual sites would have been unavailable or based upon a single pair of test and control values. For those situations, mean comparison tests were not conducted. A range of observed values from the reference substances was determined for each analytical component. Additionally, the reference substances data were used to develop population tolerance intervals. For each compositional component, 99% tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of conventional references. Each tolerance interval estimate was based upon one observation per unique reference substance. Data were first summarised by substance within site and then by substance across sites. Because negative quantities are not possible, calculated negative lower tolerance bounds were set to zero.

Compositional analyses were conducted on a total of 78 different analytical components (nine in forage and 69 in grain). Of these components, 16 had more than 50% of the observations below the assay LOQ and were excluded from the statistical analysis. Statistical analyses of the remaining 62 components (nine in forage and 53 in grain) were statistically assessed with a mixed model analysis of variance. Statistical evaluation of the compositional data was based on analyses of data from each of the three replicated field sites in the two different growing regions, plus data from a combination of all three field sites across each of the two growing regions, referred to below as combined site. The overall dataset was evaluated for evidence of biologically relevant changes between each test substance, untreated with glyphosate, and the corresponding conventional control within each growing region (De Billot 2008).

Analytes for which the levels were not statistically different were deemed to be present at equivalent levels between MON 89034 × MON 88017 and the control. For those comparisons in which the test was statistically different from the control, the test range was compared to the 99% tolerance interval in order to determine if the test range was within the interval and therefore considered to be part of the population of the commercial maize.

3.3 Compositional Analysis

2004 USA field trials (Reynolds et al. 2006)

The VKM GMO Panel has previously evaluated the compositional data for the single events of MON 89034 and MON 88017 (VKM 2010b, VKM 2014), and concluded that they were compositionally and agronomical equivalent to their respective conventional counterparts, except for the newly introduced traits. The MON 88017 was treated and untreated with glyphosate (the target herbicide). Evaluation of maize MON 88017 treated with glyphosate, did moreover not affect its compositional characteristics compared to maize MON 88017 untreated with glyphosate.

Forage and grain samples from all plots were analysed for the components recommended by the OECD (OECD, 2002) and the results are presented in the Appendix (Table 1). Forage samples were analysed for proximates (proteins, fat, ash and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), minerals (calcium and phosphorus), and carbohydrates by calculation. Grain samples were analysed for proximates, ADF, NDF, total dietary fiber (TDF), amino acids, fatty acids (C8-C22), vitamins (B1, B2, B6, E, niacin, and folic acid), anti-nutrients (phytic acid and raffinose), secondary metabolites (furfural, ferulic acid, and p-coumaric acid), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and carbohydrates by calculation. A total of 77 analytes were measured, of these had 16 analytes more than 50% of the observations a value below the LOQ of the assay, and they were therefore excluded from the statistical analysis. In total, 61 components were statistically assessed (nine in forage and 52 in grain).

In the combined site analysis statistically significant MON 89034 and MON 88017 differences from the control were found for 25 analytes. For nine of these analytes, differences were found at more than one of the individual sites, while for the remaining 16 analytes statistical significant differences were found in only one of the individual sites. Moreover, statistical analyses for MON 89034 and MON 88017 from the five individual sites showed that 31 analytes were observed to be statistically different from the control in more than one of the individual sites and 31 analytes were observed to be statistically different from the control in only one of the individual sites. Some details of the observations are as follows: In the grain of MON 89034 x MON 88017, 18:0 stearic acid was found to be statistically different from the control in the combined site and also in four individual sites. Manganese was found to be statistical different from the control in the combined site and three of the five individual sites. Statistical differences for 18:1 oleic acid, carbohydrates, protein, and p-coumaric acid were observed in the combined site and in two individual sites. In additon to this, 15 amino acids (expressed as % dry weight) were found to be statistically different from the control in the combined site and one or two of the five individual sites.When a statistical analysis of amino acids expressed as per total amino acids was performed, only one statistically significant difference was observed. Statistical differences for 20:1 eicosaenoic acid, calcium, and ferulic acid were also observed in the combined site and one of the individual sites. Protein from the forage of the test substance was found to be statistically different from the control in the combined site and in one of the five individual sites. All values obtained from MON 89034 x MON 88017 were either within the 99% tolerance interval for the population of conventional reference substances and/or the range of values from the ILSI database, none of the differences were considered biologically relevant. For five analytes (10 comparisons) in MON 89034 x MON 88017 statistic significant differences from the control were seen at more than one individual site and not in the combined site. In the grain of MON 89034 x MON 88017, 18:2 linoleic acid, moisture, fat, vitamin B1 and B6 values were observed to be statistically different from the control at each of two individual field sites. Since the statistical differences were observed in only two of the five individual sites, and the mean and range of values from the test substances were all within the calculated 99% tolerance interval for the population of conventional reference, these differences were not considered to be biologically relevant. In summary, the statistical analyses showed that all of the 366 comparisions were either a) not statistically significantly different, b) significantly different ($p<0.05$) but the composition values were within the 99% tolerance interval of the population of conventional refenrence hybrids used in the study, or c) significantly different but the composition values were within the range of values from the ILSI database.

2007 European field trials (Drury et al. 2008)

Compositional analyses of the forage samples included proximates (protein, fat, ash, and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), minerals (calcium and phosphorus), and carbohydrates by calculation. Compositional analyses of the grain samples included proximates (protein, fat, ash, and moisture), ADF, NDF, total dietary fiber (TDF), amino acids, fatty acids (C8-C22), vitamins (A, B1, B2, B6, E, niacin, and folic acid), anti-nutrients (phytic acid and raffinose), secondary metabolites (furfural, ferulic acid, and p-

coumaric acid), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and carbohydrates by calculation.

In total, there were 496 statistical comparisons conducted between each test substance (where sufficient data was available) and the conventional controls (6 individual sites (three in the north and three in the south) plus two across site combinations × 62 components assessed). Statistical analyses showed that 94% (232) of the 248 comparisons made between MON 89034 × MON 88017 and the conventional control from the northern European region and 96% (239) of the 248 comparisons made between MON 89034 x MON 88017 and the conventional control from the southern European region were not statistically significantly different ($p > 0.05$).

Table 2 and Table 3 in Appendix summarise results of the compositional analyses of MON 89034 x MON 88017 for all sites combined in the Northern and Southern regions, respectively. Analysis site by site can be found in Drury et al. (2008).

Northern Region

Statistical analyses for MON 89034 x MON 88017 from the combined sites showed statistically significant differences for one analyte in forage (ash) and five analytes in grain (calcium, iron, moisture, vitamin B1, and raffinose). From the individual northern site analysis, vitamin B1 was also significantly different in two of the individual sites and eight analytes were observed to be statistically different from the control in only one of the individual northern sites (Appendix Table 4). All means and range of values from the test substance, with the exception of the maximum range of values for calcium, were within the range of values obtained from the 99% tolerance interval. The magnitude of difference between the maximum test value for calcium and the maximum value obtained from the tolerance interval established from the conventional reference substances grown alongside the test and control substances in this study was very small: 2.1%.

Southern Region

There were no statistical differences noted from the combined site analyses. From the individual southern site analysis, nine analytes were observed to be statistically different from the control at only one site (Appendix Table 5). All means and range of values from the test substance were within the range of values obtained from the 99% tolerance interval. The differences noted above are not considered to be biologically meaningful from a food/feed safety or nutritional perspective, thus it is concluded that the forage and grain from MON 89034 x MON 88017 are compositionally equivalent to conventional maize forage and grain.

3.4 Agronomic and phenotypic characters

Field trials USA (Sammons et al. 2006)

During the North American field trials in 2004, the following agronomic and phenotypic parameters were measured and statistically analysed: early stand count, seedling vigour, days to 50 % silking, days to 50 % pollen shed, plant height, ear height, stay green, dropped ears, stalk and root lodging, final stand count, grain moisture, test weight and yield. For each phenotypic characteristic measures, minimum and maximum values (range) and a 99 % tolerance interval, with 95 % confidence, were determined based on the population of the 15 reference varieties, three at each of five sites. Results of the statistical comparisons of the phenotypic characteristics of maize stack MON 89034 x MON 88017 to the control for the combined sites are presented in Appendix (Table 6). Analyses of variance across trial locations showed statistically significant differences between maize MON 89034 x MON 88017 and the corresponding non-GM comparator for the parameters "number of days to 50 % silking", stalk lodging and grain yield ($p<0.05$). The number of days from planting until date when ~50% of the plants have multiple silks present was greater for MON 89034 x MON 88017 compared to the control (59.1 vs. 58.5 days). Likewise, the number of stalk lodged plants per plot was lower for MON 89034 x MON 88017 compared to the control (1.0 vs. 3.4), probably a result of protection against feeding damage caused by lepidopteran pests, and the grain yield was higher for the test line compared to the control (13941.2 vs. 12692.1 kg/ha) in the across-site analysis. The mean values for the three parameters were within the reference range and 99% tolerance interval of the reference varieties. No differences in general appearance of the plants or any other phenotypic differences that could indicate unintended effects of the genetic modification were found.

Field trials Europe (De Billot 2009)

During the European field trials in 2007, the following agronomic and phenotypic parameters were measured and statistically analysed: early stand count, seedling vigour, days to 50 % silking, days to 50 % pollen shed, plant height, ear height, ear/kernel rot, stay green, dropped ears, stalk and root lodging, final stand count, stalk rot and yield. Separate combined site analyses were conducted within Germany and Spain. For each phenotypic characteristic measured, minimum and maximum values (range) were determined from the references across the sites, for each country. Results of the statistical comparisons of the phenotypic characteristics of MON 89034 x MON 88017 to the control for the combined sites are presented in Appendix (Table 7). In the combined-site analysis for Germany, no statistically significant differences were detected for any of the parameters measured $p>0.05$). In the combined-site analysis for Spain for MON 89034 x MON 88017 and the corresponding non-GM comparator, significant differences were detected for two of the 14 characteristics observed. Ear height was lower for the test line MON 89034 x MON 88017 compared to the conventional counterpart (91.3 vs. 97.6, respectively ($p<0.05$). The mean value for the maize stack MON 89034 x MON 88017 across the Spanish sites was, however,

within the range of values observed for commercially available reference varieties included in the field trial. Significantly fewer stalk lodged plants were also detected in the MON 89034 x MON 88017 plots (0.0 vs. 0.5, respectively), probably as a result of the insect resistant traits introduced in the GM maize stack.

3.5 Conclusion

Comparative analyses of maize MON 89034 x MON 88017 and its conventional counterpart have been performed by the applicant during field trials located at representative sites and environments in USA during 2004, and in Europe in 2007. Several different conventional maize varieties were included in the field trials and used as references. With the exception of small variations, and the insect resistance and herbicide tolerance conferred by the Cry3Bb1, Cry1A105, Cry2Ab2, and CP4 EPSPS proteins, the results from these studies showed no biologically relevant differences between the maize stack MON 89034 x MON 88017 and its conventional counterpart.

Based on the assessment of available data, the VKM GMO Panel concludes that maize MON 89034 x MON 88017 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, except for the new proteins.

4 Food and feed safety assessment

Both single maize events, MON 89034 and MON 88017, have previously been evaluated by the VKM GMO Panel, and updated risk assessments were finalised in 2014 and 2016, respectively (VKM 2014, VKM 2016).

4.1 Summary of the previous evaluations of the single events

Maize MON 89034

In the updated risk assessment of maize MON 89034 the VKM GMO Panel concluded, based in part on data from whole food feeding studies on rats, feedlot cattle and broilers, that maize MON 89034 is nutritionally equivalent to conventional maize varieties, and, that it is unlikely that the Cry1A.105 and Cry2Ab2 proteins will introduce a toxic or allergenic potential in food or feed based on maize MON 89034 compared to conventional maize varieties.

Maize MON 88017

In the updated risk assessment of maize MON 88017 the VKM GMO Panel concluded, based in part on data from whole food feeding studies on rats and broilers, that maize MON 88017 is nutritionally equivalent to conventional maize varieties, and, that it is unlikely that the Cry3Bb1 and CP4 EPSPS proteins will cause an increased risk of toxic or IgE-mediated allergic reactions to food or feed based on maize MON 88017 compared to conventional maize varieties.

4.2 Product description and intended uses

The genetic modification in MON 89034 x MON 88017 maize will not impact the existing production processes used for maize. All MON 89034 x MON 88017 maize products will be produced and processed for use in food, animal feed and industrial products in the same way as other commercial maize. The MON 89034 x MON 88017 maize and all food, feed and processed products derived from MON 89034 x MON 88017 maize are expected to replace a portion of similar products from commercial maize, with total consumption of maize products remaining unchanged. The total anticipated Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS intake/extent of use of maize and all food, feed and processed products derived from maize will remain the same.

4.3 Effects of processing

Food manufacturing of MON 89034 x MON 88017 field maize includes many harsh processing steps, e.g. cooking, heating, high pressures, pH treatments, physical shearing, extrusion at high temperatures etc. under which the majority of DNA and proteins are denatured, which

also applies to the Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS proteins and *cry1A.105, cry2Ab2,* c*ry3Bb1* and *cp4 epsps* genes (Dien et al. 2002, Hammond & Jez 2011, Fernandes et al 2013). Baking of the maize bread broa containing 11% of TC1500 and 20% MON810 maize flour, showed that the baking process sheared the DNA into small fragments, less than 1000 bp (Fernandes et al 2013).

4.4 Toxicological assessment

In assessing the potential risks of GM food and feed it is important to consider both adverse health effects that may arise from substances that are intentionally introduced or modified in food and feed crops, and adverse effects that may be produced unexpectedly as a result of the genetic modification process (Chao & Krewski 2008).

4.4.1 Toxicological assessment of the newly expressed protein

The VKM GMO Panel has previously evaluated the proteins Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS proteins in the risk assessments of the parental maize lines MON 89034 and MON 88017 (VKM 2007a, VKM 2014).

4.4.2 Toxicological assessment of the whole GM food/feed

The applicant has not performed a 90-day subchronic feeding study on rats. The applicant has however performed a 42-day broiler feeding study with emphasis on nutritional properties of maize MON 89034 x MON 88017, which also considers health effects of maize MON 89034 x MON 88017. The study is described in detail under section 4.6.2.

4.5 Allergenicity assessment

The strategies used when assessing the potential allergenic risk focuses 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 individuals 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 (EFSA 2010). Most of the major food and respiratory IgE-allergens have been identified and cloned, and their protein sequences incorporated into various databases. As a result, novel proteins can be routinely screened for amino acid sequence homology with, and structural similarity to, known human IgE-allergens with an array of bioinformatic tools. Sequence homology searches comparing the structure of novel proteins to known IgE-allergens in a database are conducted with various algorithms such as FASTA to predict overall structural similarities. According to FAO/WHO (2001) in cases where a novel protein and a known IgE-allergen have more than 35% identity over a segment of 80 or greater amino acids, IgE cross-reactivity between the novel protein and the allergen should be considered a possibility.

4.5.1 Assessment of IgE mediated allergenicity of the newly expressed protein

The applicant has performed a weight-of-evidence approach (FAO/WHO, 2001; Codex, 2003) for an overall assessment of the IgE allergenic potential of the Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS proteins. These assessments have previously been described by the applicant for the parental maize events MON 89034 and MON 88017 and include:

- assessing the allergenicity potential of the source of the genes
- homology searches with known protein allergens
- susceptibility to *in vitro* simulated digestion and thermolability
- evaluation of protein glycosylation
- assessment of protein exposure

The protein assessments were based on the following aspects:

Cry1A.105:

i) The *cry1A.105* coding sequence comes from *Bacillus thuringiensis*. The Cry1A.105 protein is chimeric, with an overall amino acid sequence identity to the Cry1Ac, Cry1Ab and Cry1F proteins of 93.6, 90.0 and 76.7 %, respectively. These proteins are not considered common food allergens (US EPA 2010).

ii) The produced Cry1A.105 protein in maize event MON 89034 is a single polypeptide. Comparison of all folds of Cry1Ac, Cry1Ab and Cry1F showed that Cry1Ab and Cry1A.105 have essentially the same main chain structure, and that Cry1Ac differs slightly in its main chain structure from the other two in domain

III. Thus, comparison of the modeled crystal structures of the Cry1A.105, Cry1Ab, and Cry1Ac with that of the experimental Cry1Aa X-ray crystal structure demonstrated high structure similarity between the four proteins (US EPA 2010).

iii) Immunoblot and glycosylation analysis of Cry1A.105 derived from recombinant *E.coli* and from extracts of leaf material from transgenic MON 89034 maize, indicate that post-translational glycosylation of Cry1A.105 protein has not occurred (US EPA 2010).

iv) A comparison of amino acid sequence with known allergens indicated no homology between Cry1A.105 and known allergens at the level of 8 contiguous amino acids (US EPA 2010).

v) The Cry1A.105 protein is rapidly degraded by simulated gastric fluids *in vitro*. Digestability of the Cry1A.105 protein in simulated intestinal fluid assay showed that 99.5 % of the full-length protein was digested within 5 minutes (Kapadia & Rice 2005, US EPA 2010).

Cry2Ab2:

i) The Cry2Ab2 protein is isolated from *Bacillus thuringiensis* strain EG7699. The protein is not considered a common food allergen (US EPA 2010).

ii) The produced Cry2Ab2 protein in maize event MON 89034 is a single polypeptide with similar sequence identity to the wild type with a peptide mass of 61 kDa. The plant-produced protein sample had an additional immunoreactive band migrating at approximately 50 kDa; N-terminal amino acid analysis of this protein indicated that it is a truncated Cry2Ab2 protein with its N-terminus starting at amino acid 145 (MON 89034 dossier).

iii) Immunoblot and glycosylation analysis of Cry2Ab2 derived from recombinant *E.coli* and from extracts of leaf material from transgenic MON 89034 maize, indicate that post-translational glycosylation of Cry2Ab2 protein has not occurred (US EPA 2010).

iv) A comparison of amino acid sequence to known allergens indicated no homology between Cry2Ab2 and known allergens at the level of 8 contiguous amino acids (US EPA 2010).

v) The Cry2Ab2 protein is rapidly degraded by simulated gastric and intestinal fluids in vitro (Kapadia and Rice 2006, US EPA 2010).

vi) At 4°C, 25°C, and 37° C there was little or no effect on Cry2Ab2 bioactivity, while at 65°C there was some reduction in the bioactivity. At 95°C Cry2Ab2 protein was completely inactivated (US EPA 2010).

Cry3Bb1 and CP4 EPSPS

i) The sources of the transgene genes are *Bacillus thuringiensis* var. *kumamotoensis* (*cry3Bb1*-gene) and *Streptomyces. viridochromogenes* (*CP4 EPSPS*-gene). These bacteria have no history of causing allergy.

ii) Cry proteins as microbial pesticides have a history of safe use (US EPA 2005, 2007, 2010), and there have been no indications of Cry proteins originating from *Bacillus thuringiensis* exhibiting harmful effects on human or animal health (US EPA 2005 a,b, 2007, 2010a,b).

iii) The CP4 EPSPS protein has been subjected to previous safety assessments for genetically modified plants and found to have no IgE-inducing allergenic potential (Herouet et al 2005, US EPA 1995)

iv) The CP4 EPSPS protein has no homology to known toxins or IgE-allergenic proteins (Hérouet et al. 2005).

v) The microbially produced Cry3Bb1 and CP4 EPSPS proteins were rapidly degraded in simulated gastric fluids *in vitro*. No degradation assay in gastrointestinal fluids has been performed by the applicant (Monsanto technical dossier).

vi) CP4 EPSPS and Cry3Bb1 do not resemble any characteristics of known IgE-allergens, and no significant homologies between the amino acid sequences of the CP4 EPSPS and Cry3Bb1 proteins and IgE-allergenic proteins have been found (Fard et al, 2013, Herouet et al, 2005, Kim et al, 2010, Randhawa et al 2011, Meyer, 1999, US EPA, 2007).

vii) The CP4 EPSPS and Cry3Bb1 proteins are not glycosylated (Herouet et al, 2005, Raybould et al, 2013, US EPA, 2007)

viii) Cry3Bb1 and CP4 EPSPS are considered heat labile (Herouet et al, 2005, US EPA, 2007)

The information listed above indicates that the newly expressed proteins in maize event MON 89034 x MON 88017 lack IgE allergenic potential with regard to human and animal health. However, it does not cover possible allergic reactions (e.g. enteropathies) that are not IgE mediated.

4.5.2 Assessment of the IgE mediated allergenicity of the whole GM plant

Allergenicity of maize event MON 89034 x MON 88017 could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, e.g. through qualitative or quantitative modifications of the expression of endogenous proteins. However, given that no biologically relevant agronomic or compositional changes have been identified in maize MON 89034 x MON 88017 with the exception of the introduced traits, no increased allergenicity is anticipated for maize MON 89034 x MON 88017. Moreover, maize is not considered a common allergenic food.

4.5.3 Assessment of the IgE mediated allergenicity of proteins from the GM plant

It is the opinion of the VKM GMO Panel that a possible over-expression of any endogenous protein, which is not known to be allergenic, in maize MON 89034 x MON 88017 would be unlikely to alter the overall allergenicity of the whole plant or the allergy risk for consumers.

4.5.4 Adjuvanticity

According to the EFSA Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed (EFSA 2010) adjuvants are substances that, when co-administered with an antigen increase the immune response to the antigen and therefore might increase the allergic response. In cases when known functional aspects of the newly expressed protein or structural similarity to known strong adjuvants may indicate possible adjuvant activity, the possible role of these proteins as adjuvants should be considered. As for allergens, interactions with other constituents of the food matrix and/or processing may alter the structure and bioavailability of an adjuvant and thus modify its biological activity.

Only two of the ~ 10 Cry proteins that are currently used in genetically modified plants, Cry1Ab and Cry1Ac, have been studied experimentally regarding adjuvant effects. To the knowledge of the VKM GMO Panel, adjuvant effects have not been investigated for the other Cry proteins normally used in GM plants, or other groups of Cry proteins.

Studies with immunological mapping of the systemic and mucosal immune responses to Cry1Ac have shown that mice produce both systemic IgM and IgG and secretory IgA following intraperitonal (i.p.), intragastric (i.g.) or intranasal (i.n.) immunisation, and that the adjuvant effects of Cry1Ac is comparable to that of cholera toxin (Guerrero et al. 2004; Vazquez-Padron et al., 1999a, b; 2000a, b; Moreno-Fierros et al., 2003). It is uncertain whether this applies to the same extent to other Cry proteins. A possible immunogenicity and adjuvanticity of Cry proteins has been considered by EFSA and VKM (EFSA 2009, VKM 2012).

"Bystander sensitisation"

"Bystander sensitisation" can occur when an adjuvant in food, or an immune response against a food antigen, results in an increased permeability of the intestinal epithelium for other components in food. Traditionally it was assumed that the epithelial cells of the intestine were permanently "glued together" by the so-called "tight junctions". Studies have however shown that these complex protein structures are dynamic and that they can be opened up by different stimuli.

Both *in vitro* and *in vivo* experiments have demonstrated that when an IgG response which can result in a complement activation (among other) is not balanced by an IgA response, the epithelial barrier may become leaky, allowing unwanted proteins to enter the body (bystander-penetration) and possibly lead to allergic sensitisation (Brandtzaeg & Tolo 1977; Lim & Rowley 1982).

Additional information can be found in the report by VKM on Cry-proteins and adjuvanticity: "Health risk assessment of the adjuvant effects of Cry proteins from genetically modified plants used in food and fodder" (VKM 2012).

4.6 Nutritional assessment of GM food and feed

Compositional analyses of maize MON 89034 x MON 88017 indicate nutritional equivalence to the non-GM control maize with a comparable genetic background as well as 15 other tested conventional maize varieties. The nutritional equivalence between MON 89034 x MON 88017 maize and non-GM control maize has been further shown by the results of a poultry feeding study, described in 4.6.2.

4.6.1 Intake information/exposure assessment

Net import of maize staple, e.g. flour, starch and mixed products, in Norway in 2007 was 7600 tons, corresponding to 4.4 g dry weight/person/day or an estimated daily energy intake for adults to be 0.6 % (Vikse 2009, unpublished). The estimated median daily intake of sweet maize is 3.25 g/day, with a 97.5 % percentile of 17.5 g/day. The production of maize porridge for children in 2007 was about 37.5 tons, corresponding to a daily intake of 1.7 g/day or an estimated daily energy intake to be 0.6 % for a 6 month child (Vikse 2009, unpublished).

Since most foods and foodstuffs from maize are derived from field maize grains, an estimated maximum daily intake for a Norwegian adult of Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS proteins from maize MON 89034 x MON 88017 is calculated to be 33 µg, 8.4 µg, 42.7 µg, and 20.7 µg respectively, based on intake of maize staple (4.4 g/person/day) and the maximum protein levels in grain at physiological maturity, reported in Tables 1-4 (molecular characterisation, section 2.1.3.1). The corresponding numbers for children (6 month, intake of maize staple is 1.7 g/person/day) are 12.8 µg, 3.2 µg, 16.5 µg, and 8 µg, respectively.

The estimated maximum daily intake for a Norwegian adult of Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS proteins from sweet maize is calculated to be 113.8 µg, 28 µg, 145.3, and 71.8 µg, respectively, based on a daily intake of 17.5 g fresh sweet maize/day (97.5 % percentile) and maximum fresh weight values in Tables 1-4. These levels are far below the levels shown to have no effect in laboratory toxicology testing. Also, these levels are considerably below the proposed threshold of toxicological concern (TTC) level of 1800 µg/person/day (Class 1, oral exposure) for chemicals considered to have a low potential for toxicity based on metabolism and mechanistic data (Vermeire et al. 2010). Transgenic proteins produced by genetically modified plants are generally considered non-toxic to humans.

The VKM GMO Panel notes that production animals e.g. pigs and poultry often are fed diets with a substantial inclusion of unprocessed maize grain, and that the exposure to transgenic proteins from maize MON 89034 x MON 88017 may be higher for these animals.

This dietary exposure assessment is very conservative as it assumes that all maize consumed comes from maize MON 89034 x MON 88017 and that the transgenic proteins are not denatured by processing.

4.6.2 Nutritional assessment of feed derived from the GM plant

The applicant has performed a 42-day broiler feeding study to confirm the nutritional equivalence of the MON 89034 x MON 88017 with the conventional non-transgenic maize H1325023 (identical to LH198 x LH172) and four non-GM commercial maize (Asgrow RX690, Asgrow RX772, DKC60-15, DKC57-01) Taylor et al 2007). The non-transgenic maize H1325023 has a genetic background representative of MON 88017, but is not genetically modified and does not express either the Cry1A.105, Cry2Ab2, Cry3Bb1 or CP4 EPSPS proteins.

Samples of maize grain lots were analysed for mycotoxins, pesticide, and nutrient analyses. These analyses were conducted prior to the start of the study (Taylor et al 2007). These analyses were performed in order to verify whether pesticide and mycotoxin levels were below levels of concern for feeding studies, and also to obtain individual nutrient analysis information for use in formulating diets for each test, control, and commercial feed material.

Mycotoxin and pesticide levels in corn grain from both MON 89034 and MON 89034 × MON 88017 and their respective control and conventional references mixed into the diets were below the limits of concern for broiler performance. Aflatoxins were not detectable, and levels of fumonisins in the grain ranged from not detectable to 10 ppm. All pesticide values were below the assay limits of detection reported: organophosphates (0.050 ppm), organonitrogens (0.500 ppm), organochlorinates (0.200 ppm), and N-methylcarbamates (0.100 ppm).

A total of 600 birds (720 at start of the study) of commercial strain of Ross x Ross 308, were randomly distributed into 60 pens at one day of age. At start of the study (day one) each pen contained 12 broilers (6 males/6 females). Birds were identified by a wingband indicating animal number. Birds which were smaller than other birds, and/or showing signs of leg problems, or other abnormal conditions were removed first. If a pen had less than the required number of birds, then extra birds from another pen in the same treatment were relocated to bring the count in each pen to 10 birds. If additional birds still needed to be removed, they were selected arbitrarily (i.e. the first bird within reach). Removed birds were killed by cervical dislocation. All removed birds were weighed and recorded.

The in-life portion of the study meets the US EPA Good Laboratory Practice (GLP) requirements for 21 CFR Part 58. Portions of the study conducted by Monsanto meet the EPA GLP requirements for 40 CFR Part 160.

According to the OECD guidelines of animal feedstuffs derived from genetically modified plants (OECD 2003) broilers are useful for comparative growth studies. Because of their

rapid weight gain, broilers are particularly sensitive to any change in nutrient supply or the presence of toxic elements in their feed and are particularly useful for this purpose.

The test, control and reference substance diet mixtures were fed continuously for 42-days. Broilers were fed starter feed on trial days 0-21 (55 % maize), and grower/finisher feed on trial days 22-42 (59 %). Analyses of the starter and grower/finisher diets were conducted in compliance with US EPA Good Laboratory Practice standards (40 CFR Part 160). The grains were also analysed for the presence of transgenic DNA with PCR analysis. The analyses confirmed the presence of the Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS proteins in the diets containing MON 89034 x MON 88017. These proteins were not detected in control substances.

Pens were set up as a randomised complete block experimental design with 6 diets (treatments) with 10 numbers of pens per treatment, and with 10 birds per pen for a total of 600 birds (300 males and 300 females). The GLM and Mixed procedures in Release 9.1.3 of the Statistical Analysis System (SAS) version 8.2 were used in analysing each experiment.

Statistics were conducted on performance, carcass yield, and meat quality parameters: starting and final live weights, feed intake, feed conversion, adjusted feed conversion, chill weight, percent chill weight (chill weight/live weight), breast weight, percent breast weight (breast weight/chill weight), wing weight, percent wing weight (wing weight/chill weight), thigh weight, percent thigh weight (thigh weight/chill weight), drum weight, percent drum weight (drum weight/chill weight), fat pad weight, percent fat pad (fat pad/live weight), moisture, protein, and fat in breast and thigh meat. The statistical analysis was carried out with SAS®12, a linear mixed model procedure (SAS Institute, Cary, NC). Each measurement was statistically analysed by two different procedures. The first method was a two-factor analysis of variance under a randomised complete block structure. The two factors were diet and sex. The main effects of diet and sex along with the diet-by-sex interaction were tested. If the interaction was not significant ($P \geq 0.15$) then the comparison of the diets was done with the main effect for diets, i.e., diet means will be averaged over sex. If the interaction was significant ($p < 0.15$) then the diet comparisons were done, separately for each sex at a 5% level of significance. The second analysis was performed to compare the response of broilers fed the MON 89034 × MON 88017 diet to the response of the population of the control and reference grain containing diets to determine whether the responses obtained from broilers fed diets containing MON 89034 × MON 88017 were consistent with the responses of broilers fed diets containing the other maize sources. Mean separation procedures were performed with the protected LSD (Least Significant Difference) method with a 0.05 significant level in SAS.

There are five diets specified in model (1), which was identified as "treat" in the SAS program. The results of these analyses for Average Bird Weight, Feed Intake, Feed Conversion, and Adjusted Feed Conversion on day 42 are summarised in tables in Davis 2006 (data not shown).

Body weight, daily weight gain (gram/bird/day), feed conversion, and survival data were analysed to determine statistical differences between maize grain diets. No statistically significant clinical findings of health were observed during the studied period. Consistent with historical data and study type, a low incidence of mortality occurred among all study groups. Mortality was recorded daily between trial days 0-42. There were no statistical differences in mean percent mortality among any of the six treatments. All survival rates were consistently high. Individual body weight was recorded on days 0 and 42. There were no statistically significant differences in mean body weight on trial day 0 among any of the six treatments, and no statistical significant differences in mean daily weight gain among any of the six treatments.

The animals were analysed post-mortem for carcass characteristics, including the weights of the carcass and various carcass parts, as well as the composition of the meat of thighs and breast (fat, moisture, protein). Following a request from the EFSA GMO Panel the applicant has performed a direct comparison of the test and control broilers for each observed parameter. No statistically significant differences for the tested parameters were observed between the group fed maize MON 89034 x MON 88017 and its conventional counterpart, apart from a minor but statistically significant difference in relative (%) breast weights for which female broilers fed maize MON 89034 x MON 88017 showed a higher value than animals fed the control diet. Additional, small statistically significant differences were observed in thigh protein in females and breast moisture in males. The difference in relative breast weight was not observed in absolute breast weights. In the absence of any other treatment-related effects on performance, it is considered that these statistically significant differences are of no biological relevance.

4.7 Conclusion

A whole food feeding study performed on broilers indicates no adverse health effects of maize MON 89034 x MON 88017, and shows that it is nutritionally equivalent to conventional maize varieties. The Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS proteins do not show relevant sequence resemblance to other known toxins or IgE-allergens, nor have they been reported to cause IgE-mediated allergic reactions. However, some studies have indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize MON 89034 x MON 88017 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS proteins will cause toxic or IgE-mediated allergic reactions to food or feed derived from maize MON 89034 x MON 88017 compared to conventional maize.

5 Environmental risk assessment

5.1 Unintended effects on plant fitness due to the genetic modification

Maize (*Zea mays* L.) is an annual plant and member of the grass family Poacea. The species, originating from Central America, is highly domesticated and generally unable to survive in the environment without management intervention (Eastham & Sweet 2002). Maize propagates entirely by seed produced predominantly by cross-pollination (OECD 2003). In contrast to weedy plants, maize has a pistillate inflorescence (ear) with a cob enclosed with husks. Due to the structure of the cob, the seeds remain on the cob after ripening and natural dissemination of the kernels rarely occurs. The survival of maize in Europe is limited by a combination of absence of a dormancy phase resulting in a short persistence, high temperature requirements for germination, low frost tolerance, low competitiveness and susceptibility to plant pathogens, herbivores and climatic conditions (van de Wiel et al. 2011). Maize plants cannot survive temperatures below 0ºC for more than 6 to 8 hours after the growing point is above ground (OECD 2003), and in Norway and most of Europe, maize kernels and seedlings do not survive the winter cold (Gruber et al. 2008). Observations made on cobs, cob fragments or isolated grains shed in the field during harvesting indicate that grains may survive and overwinter in some regions in Europe, resulting in volunteers in subsequent crops. The occurrence of maize volunteers has been reported in Spain and other European regions (e.g. Gruber et al. 2008). However, maize volunteers have been shown to grow weakly and flower synchronously with the maize crop (Palaudelmás et al. 2009). Cross-pollination values recorded were extremely variable among volunteers, most probably due to the loss of hybrid vigour and uniformity. Overall cross-pollination to adjacent plants was estimated as being low. Despite cultivation in many countries for centuries, seed-mediated establishment and survival of maize outside cultivation or on disturbed land in Europe is rare (BEETLE Report 2009). Maize plants occasionally grow in uncultivated fields and by roadsides. However the species is incapable of sustained reproduction outside agricultural areas in Europe and is non-invasive of natural habitats (Eastham & Sweet 2002; Devos et al. 2009). There are no native or introduced sexually cross-compatible species in the European flora with which maize can hybridise and form backcross progeny (Eastham & Sweet 2002; OECD 2003). The only recipient plants that can be cross-fertilised by maize are other cultivated maize cultivars. It is considered very unlikely that the establishment, spread and survival of maize MON 89034 x MON 88017 would be increased due to the insect resistance and herbicide tolerance traits. The herbicide tolerant trait can only be regarded as providing a selective advantage for the GM maize plant where and when glyphosate-based herbicides are applied. Similarly insect resistance against certain coleopteran and lepidopteran pests provides a potential advantage in cultivation of MON 89034 x MON 88017 under infestation conditions. It is considered very unlikely that maize MON 89034 x MON 88017 plants or their progeny will differ from conventional maize cultivars in their ability to survive as volunteers

until subsequent seasons, or to establish feral populations under European environmental conditions.

Field trials carried out by the applicant do not indicate altered fitness of maize MON 89034 x MON 88017 relative to its conventional counterpart. A series of field trials with maize MON 89034 x MON 88017 were carried out by the applicant across five locations in the USA in 2004 and eight locations in Europe in 2007. Information on phenotypic (e.g. crop physiology, morphology, development) and agronomic characteristics was provided to assess the agronomic performance of maize MON 89034 x MON 88017 in comparison with its conventional counterpart and commercial reference varieties (see section 3.4). Data from the field trials shows some statistical significant differences at individual field sites. These differences were however small in magnitude and were not consistently observed over locations. The VKM GMO Panel is of the opinion that the observed differences are not biologically relevant and do not raise any environmental safety concern. In addition to the data presented by the applicant, the VKM GMO Panel is not aware of any scientific reports indicative of increased establishment or spread of maize MON 89034 x MON 88017, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of maize MON 89034 x MON 88017 are unchanged, insect resistance and glyphosate tolerance are not likely to provide a selective advantage outside of cultivation in Europe. The VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects based on establishment and survival of maize MON 89034 x MON 88017 will not differ from that of conventional maize varieties.

5.2 Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or vertical gene flow via pollen or seed dispersal. Exposure of microorganisms to transgenic DNA occurs during decomposition of plant material remaining in the field after harvest or comes from pollen deposited on cultivated areas or the field margins. Transgenic DNA is also a component of a variety of food and feed products derived from maize MON 89034 x MON 88017. This means that micro-organisms in the digestive tract in humans and animals (both domesticated animals and other animals feeding on fresh or decaying plant material from the transgenic maize line) may be exposed to transgenic DNA. Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation with which maize can hybridise and form backcross progeny (Eastham & Sweet 2002; OECD 2003). Vertical gene transfer in maize therefore depends on cross-pollination with other conventional or organic maize varieties. All maize varieties which are cultivated in Europe can interbreed. In addition, unintended admixture/adventitious presences of genetically modified material/transgenes in seeds represent a possible way for gene flow between different production systems.

5.2.1 Plant to micro-organisms gene transfer

Experimental studies have shown that gene transfer from transgenic plants to bacteria rarely occurs under natural conditions and that such transfer depends on the presence of DNA sequence similarity between the DNA of the transgenic plant and the DNA of the bacterial recipient (Nielsen et al. 2000; De Vries & Wackernagel 2002, reviewed in EFSA 2004, 2009a; Bensasson et al. 2004; VKM 2005c). Based on established scientific knowledge of the barriers for gene transfer between unrelated species and the experimental research on horizontal transfer of genetic material from plants to microorganisms, there is today little evidence pointing to a likelihood of random transfer of the transgenes present in maize MON 89034 x MON 88017 to unrelated species such as bacteria. It is however pointed out that there are limitations in the methodology used in these experimental studies (Nielsen & Townsend 2004). Experimental studies of limited scale should be interpreted with caution given the scale differences between what can be experimental investigation and commercial plant cultivation. Experiments have been performed to study the stability and uptake of DNA from the intestinal tract in mice after M13 DNA was administered orally. The DNA introduced was detected in stool samples up to seven hours after feeding. Small amounts (<0.1%) could be traced in the blood vessels for a period of maximum 24 hours, and M13 DNA was found in the liver and spleen for up to 24 hours (Schubbert et al. 1994). By oral intake of genetically modified soybean it has been shown that DNA is more stable in the intestine of persons with colostomy compared to a control group (Netherwood et al. 2004). No GM DNA was detected in the faeces from the control group. Rizzi et al. (2012) provides an extensive review of the fate of feed-derived DNA in the gastrointestinal system of mammals. In conclusion, the VKM GMO Panel consider it is unlikely that the introduced gene from maize MON 88017 will transfer and establish in the genome of microorganisms in the environment or in the intestinal tract of humans or animals. In the rare, but theoretically possible case of transfer of the *cry* and cp4 epsps genes from MON 89034 x MON 88017 to soil bacteria, no novel property would be introduced into or expressed in the soil microbial communities; as these genes are already present in other bacteria in soil. Therefore, no positive selective advantage that would not have been conferred by natural gene transfer between bacteria is expected.

5.2.2 Plant to plant gene flow

Considering the intended uses of maize MON 89034 x MON 88017 (excluding cultivation) and the physical characteristics of maize seeds, possible pathways of gene dispersal are grain spillage and dispersal of pollen from potential transgenic maize plants originating from accidental grain spillage during transport and/or processing. The extent of cross-pollination to other maize cultivars will mainly depend on the scale of accidental release during transportation and processing, and on successful establishment and subsequent flowering of the maize plant. For maize, any vertical gene transfer is limited to other varieties of *Zea mays* plants as populations of sexually compatible wild relatives of maize are not known in Europe (OECD 2003). 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. As for any other maize cultivars, GM maize plants would only survive in subsequent seasons in warmer regions of Europe and are not likely to establish feral populations under European environmental conditions. In Norway, maize plants from seed spillage occasionally grow on tips, waste ground and along roadsides (Lid & Lid 2005). The flowering of occasional feral GM maize plants origination from accidental release during transportation and processing is however 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 (Palaudelmás et al. 2009). As maize MON 89034 x MON 88017 has no altered survival, multiplication or dissemination characteristics, the VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this GM maize in Norway will not differ from that of conventional maize varieties. The likelihood of cross-pollination between cultivated maize and the occasional feral maize plants resulting from grain spillage is considered extremely low.

5.3 Interactions between the GM plant and target organisms

Maize MON 89034 is a second generation genetically modified insect resistant maize, and was developed to provide protection against a variety of target pests of the order Lepidoptera. Protection is achieved through expression in the plant of two insecticidal Cry proteins, Cry1A.105 and Cry2Ab2, derived from *Bacillus thuringiensis*, a common soil bacterium. Cry1A.105, encoded by the *cry1A.105* gene, is a chimeric protein made up of different functional domains derived from three wild-type Cry proteins from *B. thuringiensis* subspecies *kurstaki* and *aizawai*. The Cry2Ab2 protein is encoded by the *cry2Ab2* gene derived from *B. thuringiensis* subspecies *kurstaki.* Two Lepidoptera pests are primarily targeted by MON 89034; *Ostrinia nubilalis* (European corn borer, ECB) and *Sesamia nonagrioides* (Mediterranean corn borer, MCB). According to the applicant, the Cry1A.105 protein also provides increased activity against fall armyworm (*Spodoptera* spp.) and black cutworm (*Agrotis ipsilon*) compared to Cry1Ab. Further, the Cry2Ab2 toxin provides improved control over Cry1Ab products from damage caused by corn earworm (*Helicoverpa zea*). The European corn borer is widely distributed in Europe covering the Iberian Peninsula, Czech Republic and Slovakia, southwest of France, northern Italy and the southern regions of Germany and Poland. The Mediterranean corn borer is present in the Mediterranean region (Andreadis 2011). There are ten reports of *O. nubilalis* in Norway, restricted to the counties of Vestfold, Telemark, Aust-Agder and Vest Agder. *Sesamia* spp., *Spodoptera frugiperda* or *H. zea* have not been reported in Norway. There are no reports of *O. nubilalis* attaining pest status in Norway, and the Plant Clinic (Planteklinikken) at Bioforsk has never received samples of this pest or plant material damaged by this pest (K. Ørstad pers. com.). Consequently, there are no insecticides authorised or previous applications for registrations of insecticides against this herbivore in Norway. Aphids are the only pests reported on maize in Norway. Studies have shown that aphids are not affected by the Cry1Ab protein (Bourguet et al. 2002). Under the development of Bt maize expressing Cry1Ab, the noctuid *A. ipsilon*

was tested as a target, but there was little or no effect (Pilcher et al. 1997). This species is occasionally a pest in root crops in Norway and it is conceivable that it could become a pest of maize. Maize MON 88017 was transformed to express the *cry3Bb1* gene from *Bacillus thuringiensis* subsp. *kumamotoensis.* The insecticidal toxin confers resistance to coleopteran insect pests belonging to the genus *Diabrotica,* such as larvae of western corn rootworm (WCR; *D. virgifera virgifera*), Northern corn rootworm (NCR; *D. barberi*), Southern corn rootworm (SWR; *D. undecimpunctata howardi*). At present, the Western corn rootworm is the only species from the corn rootworm complex present in Europe. The species has been introduced to Europe from the USA, where it is endemic (Miller et al. 2005, ref. EFSA 2011d). The larval stages of this beetle can cause significant damages to maize roots, leading to reduction of plant growth, deficiencies in nutrient and water uptake, lodging, increased susceptibility to water stress and reduced grain yield. *D. virgifera virgifera* was first detected in Serbia in 1992, but has since spread across the continent, resulting in well-established populations in approximately 19 European countries (EC 2012). Western corn rootworm is considered a serious threat to agriculture in the EU, where this pest species is expected to expand further (Wesseler & Fall 2010). There have been no reports of *D. virgifera virgifera* in Norway (http://www.faunaeur.org/distribution.php). Considering the intended uses of maize MON 89034 x MON 88017, excluding cultivation, the environmental exposure is limited to exposure through manure and faeces from the gastrointestinal tract mainly of animals fed on the GM maize as well as to the accidental release into the environment of GM seeds during transportation and processing and subsequently to potential occurrence of sporadic feral plants. Thus the level of exposure of target organisms to the Cry3Bb1 and Cry1Ab protein is likely to be extremely low and of no ecological relevance.

5.4 Interactions between the GM plant and non-target organisms (NTOs)

Considering the intended uses of maize MON 89034 x MON 88017, excluding cultivation, the environmental risk assessment is concerned with accidental release of GM maize viable grains into the environment during transportation and processing, and exposure through manure and faeces from the gastrointestinal tracts of animals fed the GM maize. Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only very low amounts would remain intact to pass out in faeces (e.g. Lutz et al. 2005; Guertler et al. 2008; Paul et al. 2010). There would subsequently, be further degradation of the Cry proteins in the manure and faeces due to microbial processes. In addition, there will be further degradation of Cry proteins in soil, reducing the possibility for the exposure of potentially sensitive non-target organisms. Although Cry proteins bind rapidly on clays and humic substances in the soil and thereby reducing their availability to microorganisms for degradation, there is little evidence for the accumulation of Cry proteins from GM plants in soil (Icoz & Stotzky 2008). Data supplied by the applicant indicate that a limited amount of the Cry1A.105, Cry2Ab2 and Cry3Bb1 protein enters the environment due to the expression in the grains (mean values of 5.6, 1.3 and 4.1 µg/g dwt, respectively). Data have been submitted that demonstrate that the Cry1A.105, Cry2Ab2 and Cry3Bb1 protein is rapidly

degraded by gastric fluid *in vitro*.

In conclusion, the VKM GMO Panel considers that the exposure of potentially non-target organisms to the Cry1A.105, Cry2Ab2 and Cry3Bb1 protein is likely to be very low and of no biological relevance.

5.5 Potential interactions with the abiotic environment and biochemical cycles

Considering the intended uses of maize MON 89034 x MON 88017, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with the abiotic environment and biogeochemical cycles were not considered an issue by the VKM GMO Panel.

5.6 Conclusion

Considering the intended uses of maize MON 89034 x MON 88017, excluding cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize MON 89034 x MON 88017.

Maize MON 89034 x MON 88017 has no altered survival, multiplication or dissemination characteristics, and there are no indications of an increased likelihood of spread and establishment of feral maize plants in the case of accidental release into the environment of seeds from maize MON 89034 x MON 88017. Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation. The VKM GMO Panel considers the risk of gene flow from occasional feral GM maize plants to conventional maize varieties to be negligible in Norway. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered by the GMO Panel to be an issue.

6 Post-market environmental monitoring

Directive 2001/18/EC introduces an obligation for applicants to implement monitoring plans, in order to trace and identify any direct or indirect, immediate, delayed or unanticipated effects on human health or the environment of GMOs as or in products after they have been placed on the market. Monitoring plans should be designed according to Annex VII of the Directive. According to Annex VII, the objectives of an environmental monitoring plan 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 (ERA) 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. Post-market environmental monitoring is composed of case-specific monitoring and general surveillance (EFSA 2011c). Case-specific monitoring is not obligatory, but may be required to verify assumptions and conclusions of the ERA, whereas general surveillance is mandatory, in order to take account for general or unspecific scientific uncertainty and any unanticipated adverse effects associated with the release and management of a GM plant. Due to different objectives between case-specific monitoring and general surveillance, their underlying concepts differ. Case-specific monitoring should enable the determination of whether and to what extent adverse effects anticipated in the environmental risk assessment occur during the commercial use of a GM plant, and thus to relate observed changes to specific risks. It is triggered by scientific uncertainty that was identified in the ERA. The objective of general surveillance is to identify unanticipated adverse effects of the GM plant or its use on human health and the environment that were not predicted or specifically identified during the ERA. In contrast to case-specific monitoring, the general status of the environment that is associated with the use of the GM plant is monitored without any preconceived hypothesis, in order to detect any possible effects that were not anticipated in the ERA, or that are long-term or cumulative.

No specific environmental impact of genetically modified maize MON 89034 x MON 88017 was indicated by the environmental risk assessment and thus no case specific monitoring is required. The VKM GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicant is in line with the intended uses of maize MON 89034 x MON 88017 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects.

7 Conclusions

Molecular characterisation

Southern and PCR analyses indicate that the recombinant inserts in the single maize events MON 89034 and MON 88017 are retained in the stacked event MON 89034 x MON 88017. Genetic stability of the inserts has previously been demonstrated in the single events. The levels of Cry1A.105, Cry2Ab2, CP4 EPSPS and Cry3Bb1 proteins in grain and forage from the stacked event are comparable to the levels in the corresponding single events. Phenotypic analyses also indicate stability of the insect resistance and herbicide tolerance traits of the stacked event.

Based on current knowledge and the previous assessments of the parental maize events, the VKM GMO Panel considers the molecular characterisation of maize MON 89034 x MON 88017 satisfactory.

Comparative assessment

Comparative analyses of maize MON 89034 x MON 88017 and its conventional counterpart have been performed by the applicant during field trials located at representative sites and environments in USA during 2004, and in Europe in 2007. Several different conventional maize varieties were included in the field trials and used as references. With the exception of small variations, and the insect resistance and herbicide tolerance conferred by the Cry3Bb1, Cry1A105, Cry2Ab2, and CP4 EPSPS proteins, the results from these studies showed no biologically relevant differences between the maize stack MON 89034 x MON 88017 and its conventional counterpart.

Based on the assessment of available data, the VKM GMO Panel concludes that maize MON 89034 x MON 88017 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, except for the new proteins.

Food and feed safety assessment

A whole food feeding study performed on broilers indicates no adverse health effects of maize MON 89034 x MON 88017, and shows that it is nutritionally equivalent to conventional maize varieties. The Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS proteins do not show relevant sequence resemblance to other known toxins or IgE-allergens, nor have they been reported to cause IgE-mediated allergic reactions. However, some studies have indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize MON 89034 x MON 88017 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS proteins will cause toxic or IgE-mediated allergic reactions to food or feed derived from maize MON 89034 x MON 88017 compared to conventional maize.

Environmental risk assessment

Considering the intended uses of maize MON 89034 x MON 88017, excluding cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize MON 89034 x MON 88017.

Maize MON 89034 x MON 88017 has no altered survival, multiplication or dissemination characteristics, and there are no indications of an increased likelihood of spread and establishment of feral maize plants in the case of accidental release into the environment of seeds from maize MON 89034 x MON 88017. Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation. The VKM GMO Panel considers the risk of gene flow from occasional feral GM maize plants to conventional maize varieties to be negligible in Norway. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered by the GMO Panel to be an issue.

Overall conclusion

Based on current knowledge, the VKM GMO Panel concludes that maize MON 89034 x MON 88017 is compositionally, nutritionally, agronomically and phenotypically equivalent to its conventional counterpart except for the new proteins. It is unlikely that the Cry1A.105, Cry2Ab2, CryBb1 and CP4 EPSPS proteins will cause an increased risk of toxic or IgE-mediated allergic reactions to food or feed based on maize MON 89034 x MON 88017 compared to conventional maize varieties.

The VKM GMO Panel concludes that maize MON 89034 x MON 88017, based on current knowledge, is comparable to conventional maize varieties concerning environmental risk in Norway with the intended usage.

8 Data gaps

Adjuvanticity

There are many knowledge gaps related to assessment of adjuvants. Most of the immunologic adjuvant experiments have been performed with Cry1Ac. Whether the other Cry proteins have similar adjuvant properties is unknown.

The quantities of Cry proteins in genetically modified maize and soya are marginal compared with the amounts of other adjuvants that are natural components of food. However, the extent to which these naturally occurring adjuvants and Cry proteins contribute to the development of allergies is largely unknown. Determination of their importance is hampered by the lack of validated methods for measuring adjuvant effects.

The possibility that Cry proteins might increase the permeability of the intestinal epithelium and thereby lead to "bystander" sensitization to strong allergens in the diet of genetically susceptible individuals cannot be completely excluded. This possibility could be explored in a relevant animal model.

One element of uncertainty in exposure assessment is the lack of knowledge concerning exposure via the respiratory tract and the skin, and also the lack of quantitative understanding of the relationship between the extent of exposure to an adjuvant and its effects in terms of development of allergies.

Herbicide residue levels

Herbicide tolerant (HT) crops permit the use of broad-spectrum herbicides such as glyphosate, as an in-crop selective herbicide to control a wide range of broadleaf and grass weeds without sustaining crop injury. This weed management strategy enables post-emergence spraying of established weeds and gives growers more flexibility to choose spraying times in comparison with the pre-emergence treatments of conventional crops.

As the broad-spectrum herbicides are sprayed on the plant canopy and spraying often takes place later in the growing season than is the case with selective herbicides associated with conventional crops, the residue and metabolite levels of herbicides in plants with tolerance to glyphosate could be higher compared to plants produced by conventional farming practices. There are however limited amounts of data available on pesticide residues in HT crops.

More research is needed to elucidate whether the genetic modifications used to make a plant tolerant against certain herbicide(s) may influence the metabolism of this or other plant protection products, and whether possible changes in the spectrum of metabolites may result in altered toxicological properties.

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Appendix

Table 1. Compositional analysis og maize forage and grain collected from MON 89034 x MON 88917 compared to control and commercial varieties – 2004 UAS field trils – All sites combined

	MON 89034 × MON 88017		Control		Commercials[1]		
Tissue/Component (Units)[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Forage							
Fibre (% dw)							
ADF	28.92	23.37-36.19	27.26	19.93-35.59	[16.76,43.76]	18.3-41.0[b] ; 17.5-38.3[a]	16.13-47.39
NDF	40.51	31.67-47.81	37.60	31.44-43.96	[25.94,55.67]	26.4-54.5[b]; 27.9-54.8[a]	20.29-63.71
Proximates							
Ash (% dw)	3.71	2.19-5.00	3.90	2.59-5.10	[1.93,6.31]	2.43-9.64[a]; 2-6.6[b]	1.527-9.638
Carbohydrates (% dw)	85.75	83.97-88.82	86.69	84.36-89.57	[83.05,90.74]	83.2-91.6[b]; 76.5-87.3[a]	76.4-92.1
Total fat (% dw)	2.15	0.98-3.98	1.71	0.77-2.91	[0,4.54]	0.35-3.62[b] ; 1.42-4.57[a]	0.296-4.570
Moisture (% fw)	72.29	65.90-75.60	71.53	65.90-76.80	[57.62,86.45]	56.5-80.4[a] ; 55.3-75.3[b]	49.1-81.3
Protein (% dw)	8.39*	7.27-9.63	7.70	6.06-8.87	[4.78,10.38]	4.98-11.56[a]	3.14-11.57
Minerals (% dw)							
Calcium	0.19	0.12-0.27	0.19	0.13-0.28	[0.016,0.38]	0.0969-0.3184[b]	0.0714-0.5768
Phosphorus	0.22	0.17-0.29	0.21	0.15-0.25	[0.071,0.32]	0.1367-0.2914[b]	0.0936-0.3704

* significant difference at 5% level when compared with the control

1 15 commercial maize hybrids.

2 dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre, TDF = total dietary fibre, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

3 The mean of 15 replicate values

4 Tolerance Interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

5 Literature range references: [a] Ridley *et al.* (2002); [b] Sidhu *et al.* (2000); [c] Jugenheimer (1976); [d] Watson (1987); [e] Watson (1982); [f] Classen *et al.* (1990); [g] Dowd and Vega (1996); [h] Choi *et al.* (1999).

6 ILSI range is from ILSI Crop Composition Database, 2006.

Table 1. – cont.

	MON 89034 × MON 88017		Control		Commercials[1]		
Tissue/Component[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain							
Amino acids (% dw)							(% dw)
Alanine	0.83*	0.71-1.01	0.78	0.67-0.89	[0.48,1.08]	N/A	0.439-1.393
Arginine	0.49*	0.45-0.58	0.47	0.41-0.51	[0.33,0.56]	N/A	0.119-0.639
Aspartic acid	0.72*	0.64-0.87	0.67	0.60-0.76	[0.43,0.90]	N/A	0.335-1.208
Cystine	0.24*	0.21-0.27	0.23	0.21-0.25	[0.18,0.27]	N/A	0.125-0.514
Glutamic acid	2.13*	1.83-2.63	1.99	1.70-2.26	[1.25,2.75]	N/A	0.965-3.536
Glycine	0.40*	0.37-0.45	0.38	0.36-0.41	[0.28,0.46]	N/A	0.184-0.539
Histidine	0.32*	0.30-0.38	0.31	0.28-0.34	[0.22,0.38]	N/A	0.137-0.434
Isoleucine	0.39*	0.33-0.48	0.36	0.30-0.42	[0.23,0.51]	N/A	0.179-0.692
Leucine	1.42*	1.19-1.81	1.32	1.08-1.55	[0.77,1.92]	N/A	0.642-2.492
Lysine	0.33	0.30-0.39	0.32	0.29-0.36	[0.20,0.40]	N/A	0.172-0.668
Methionine	0.23	0.21-0.26	0.22	0.20-0.24	[0.14,0.25]	N/A	0.124-0.468
Phenylalanine	0.55*	0.47-0.69	0.52	0.43-0.60	[0.32,0.73]	N/A	0.244-0.930
Proline	0.99*	0.89-1.15	0.93	0.83-1.01	[0.68,1.21]	N/A	0.462-1.632
Serine	0.55*	0.49-0.66	0.52	0.46-0.60	[0.34,0.71]	N/A	0.235-0.769
Threonine	0.35*	0.32-0.42	0.33	0.29-0.36	[0.24,0.41]	N/A	0.224-0.666
Tryptophan	0.057	0.050-0.064	0.056	0.045-0.063	[0.032,0.072]	N/A	0.0271-0.215
Tyrosine	0.40*	0.32-0.48	0.36	0.24-0.42	[0.17,0.52]	N/A	0.103-0.642
Valine	0.52*	0.46-0.62	0.49	0.43-0.55	[0.35,0.62]	N/A	0.266-0.855

* significant difference at 5% level when compared with the control

1 15 commercial maize hybrids.

2 dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre, TDF = total dietary fibre, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

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6 ILSI range is from ILSI Crop Composition Database, 2006.

Table 1. – cont.

Tissue/Component[2]	MON 89034 × MON 88017 Mean[3]	MON 89034 × MON 88017 Range	Control Mean[3]	Control Range	Commercials[1] 99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain – continued							
Fatty acids (% of total fa)						(% total fat)	
16:0 palmitic acid	9.15	8.91-9.36	9.12	8.91-9.34	[6.12,15.67]	7-19[e]	7.94-20.71
16:1 palmitoleic acid	0.13	0.11-0.14	0.12	0.048-0.14	[0.0,0.28]	1[e]	0.095-0.447
18:0 stearic acid	1.91*	1.84-1.98	1.82	1.76-1.87	[0.86,2.98]	1-3[e]	1.02-3.40
18:1 oleic acid	24.30*	22.48-25.60	24.84	23.62-26.66	[7.51,46.46]	20-46[e]	17.4-40.2
18:2 linoleic acid	62.51	61.16-64.65	62.07	60.51-63.41	[39.41,76.74]	35-70[e]	36.2-66.5
18:3 linolenic acid	1.20	1.09-1.36	1.22	1.15-1.43	[0.63,1.77]	0.8-2[e]	0.57-2.25
20:0 arachidic acid	0.38	0.36-0.41	0.38	0.36-0.40	[0.23,0.54]	0.1-2[e]	0.279-0.965
20:1 eicosenoic acid	0.27*	0.25-0.29	0.28	0.25-0.29	[0.15,0.39]	N/A	0.170-1.917
22:0 behenic acid	0.14	0.12-0.17	0.15	0.13-0.18	[0.081,0.23]	N/A	0.110-0.349
Fibre (% dw)							
ADF	5.45	4.54-7.38	5.27	4.17-7.00	[2.77,7.56]	3.3-4.3[d]; 2.46-11.34[a,b]	1.82-11.34
NDF	10.06	8.64-12.04	9.75	8.48-11.75	[5.93,13.63]	8.3-11.9[d]; 7.58-15.91[b]	5.59-22.64
TDF	15.37	13.37-18.92	14.67	12.82-17.62	[9.20,20.27]	10.99-11.41[h]	8.82-35.31

* significant difference at 5% level when compared with the control

1 15 commercial maize hybrids.

2 dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre, TDF = total dietary fibre, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

3 The mean of 15 replicate values

4 Tolerance Interval : with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

5 Literature range references: [a] Ridley *et al.* (2002); [b] Sidhu *et al.* (2000); [c] Jugenheimer (1976); [d] Watson (1987); [e] Watson (1982); [f] Classen *et al.* (1990); [g] Dowd and Vega (1996); [h] Choi *et al.* (1999).

6 ILSI range is from ILSI Crop Composition Database, 2006.

Table 1. – cont.

Tissue/Component[2]	MON 89034 x MON 88017 Mean[3]	MON 89034 x MON 88017 Range	Control Mean[3]	Control Range	Commercials[1] 99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain – continued							
Minerals							
Calcium (% dw)	0.0051*	0.0041-0.0063	0.0049	0.0040-0.0059	[0.0016,0.0059]	0.01-0.1[d]	0.00127-0.02084
Copper (mg/kg dw)	1.84	1.24-5.50	2.07	1.26-4.54	[0,4.20]	0.9-10[d]	0.73-18.50
Iron (mg/kg dw)	21.70	19.70-27.47	22.20	19.03-28.26	[8.88,34.51]	1-100[d]	10.42-49.07
Magnesium (% dw)	0.12	0.10-0.14	0.12	0.11-0.14	[0.075,0.17]	0.09-1[d]	0.0594-0.194
Manganese (mg/kg dw)	7.27*	6.77-8.07	6.51	5.57-8.00	[3.17,9.99]	0.7-54[d]	1.69-14.30
Phosphorus (% dw)	0.33	0.28-0.36	0.33	0.29-0.36	[0.18,0.45]	0.26-0.75[d]	0.147-0.533
Potassium (% dw)	0.36	0.33-0.39	0.36	0.34-0.40	[0.26,0.46]	0.32-0.72[d]	0.181-0.603
Zinc (mg/kg dw)	22.56	20.35-26.53	21.91	18.81-26.04	[7.16,38.55]	12-30[d]	6.5-37.2
Proximates (% dw)							
Ash	1.41	1.26-1.54	1.39	1.28-1.51	[0.74,1.96]	1.1-3.9[d]; 0.89-6.28[b]	0.616-6.282
Carbohydrates	84.38*	82.75-85.30	84.96	83.58-86.22	[81.08,88.80]	77.4-87.2[b]; 82.2-88.1[a]	77.4-89.5
Total fat	3.22	2.87-3.51	3.29	3.05-3.75	[2.20,4.55]	3.1-5.7[d]; 2.48-4.81[b]	1.742-5.823
Moisture	9.37	7.52-12.20	9.50	7.86-13.10	[0.45,19.52]	7-23[d]; 8.18-26.2[b]	6.1-40.5
Protein	11.00*	9.99-12.73	10.36	9.22-11.52	[7.54,13.13]	6-12[d]; 9.7-16.1[c]	6.15-17.26

* significant difference at 5% level when compared with the control

1 15 commercial maize hybrids.

2 dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre, TDF = total dietary fibre, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

3 The mean of 15 replicate values

4 Tolerance Interval : with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

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6 ILSI range is from ILSI Crop Composition Database, 2006.

Table 1. – cont.

Tissue/Component[2]	MON 89034 × MON 88017 Mean[3]	MON 89034 × MON 88017 Range	Control Mean[3]	Control Range	Commercials[1] 99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain – continued							
Vitamin (mg/kg dw)							
Folic acid	0.38	0.31-0.59	0.36	0.23-0.53	[0.012,0.69]	0.3[d]	0.147-1.464
Niacin	29.34	23.44-34.80	29.59	24.93-35.75	[6.97,37.83]	9.3-70[d]	10.37-46.94
Vitamin B1	2.62	1.86-3.56	2.94	2.39-3.36	[0.37,6.35]	3-8.6[e]	1.26-40.00
Vitamin B2	1.43	1.17-1.67	1.42	1.16-1.61	[0.91,2.30]	0.25-5.6[e]	0.50-2.36
Vitamin B6	6.32	4.92-7.27	6.26	5.37-6.80	[3.12,9.30]	5.3[d]; 9.6[e]	3.68-11.32
Vitamin E	6.93	2.70-10.19	6.63	2.72-9.02	[0,20.49]	3-12.1[e]; 17-47[d]	1.5-68.7
Antinutrient (% dw)							
Phytic acid	0.73	0.50-0.90	0.73	0.56-0.88	[0.21,1.22]	0.48-1.12[a]	0.111-1.570
Secondary metabolite (µg/g dw)							
Ferulic acid	2257.81*	1766.71-2751.00	2148.05	1878.66-2669.85	[1136.69,2806.24]	113-1194[f]; 3000[g]	291.9-3885.8
p-coumaric acid	201.66*	164.79-233.59	183.96	167.76-210.13	[0,378.57]	22-75[f]	53.4-576.2

* significant difference at 5% level when compared with the control

1 15 commercial maize hybrids.

2 dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre, TDF = total dietary fibre, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

3 The mean of 15 replicate values

4 Tolerance Interval : with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

5 Literature range references: [a] Ridley *et al.* (2002); [b] Sidhu *et al.* (2000); [c] Jugenheimer (1976); [d] Watson (1987); [e] Watson (1982); [f] Classen *et al.* (1990); [g] Dowd and Vega (1996); [h] Choi *et al.* (1999).

6 ILSI range is from ILSI Crop Composition Database, 2006.

Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw.

Table 2. Compositional analysis of maize forage and grain collected from MON 89034 x MON 88017 compared to control and commercial varieties – European field trials (Northern Region) conducted in 2007 – All sites combined

	MON 89034 × MON 88017		Control		Commercials[1]		
Tissue/Component (Units)[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Forage							
Fibre (% dw)							
ADF	22.58	18.86-29.33	23.02	19.85-28.96	16.38-30.00	18.3-41.0[b] ; 17.5-38.3[a]	16.13-47.39
NDF	33.78	24.74-40.19	34.91	27.67-42.35	21.46-49.82	26.4-54.5[b]; 27.9-54.8[a]	20.29-63.71
Proximates							
Ash (% dw)	3.32*	2.57-4.70	3.99	3.24-4.52	2.40-4.52	2.43-9.64[a]; 2-6.6[b]	1.527-9.638
Carbohydrates (% dw)	87.59	86.45-90.49	87.07	84.54-90.18	85.67-89.26	83.2-91.6[b]; 76.5-87.3[a]	76.4-92.1
Total fat (% dw)	1.94	1.51-2.28	1.91	1.07-2.51	1.01-3.32	0.35-3.62[b] ; 1.42-4.57[a]	0.296-4.570
Moisture (% fw)	70.31	67.20-73.70	71.69	67.70-74.80	54.20-74.10	56.5-80.4[a] ; 55.3-75.3[b]	49.1-81.3
Protein (% dw)	7.12	5.36-8.29	7.04	5.05-8.76	4.79-8.91	4.98-11.56[a]	3.14-11.57
Minerals (% dw)							
Calcium	0.2	0.14-0.26	0.22	0.12-0.28	0.11-0.30	0.0969-0.3184[b]	0.0714-0.5768
Phosphorus	0.2	0.16-0.28	0.2	0.16-0.25	0.16-0.29	0.1367-0.2914[b]	0.0936-0.3704

* significant difference at 5% level when compared with the control

[1] 13 commercial maize hybrids.

[2] dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre,

[3] The mean of seven and eight replicate values for respectively MON 89034 × MON 88017 and control (Respectively two and one replicate out of 9 of the test and control material did not meet the acceptance criteria for TCR characterization (<3.05 unintended traits))

[4] Tolerance Interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

[5] Literature range references: [a] (Ridley *et al.*, 2002); [b] (Sidhu *et al.*, 2000); [c] (Jugenheimer, 1976); [d] (Watson, 2003); [e] amino acid values reported as percent of total protein and fatty acid values reported as percent of total fat (Watson, 1982); [f] (Classen *et al.*, 1990); [g] (Dowd and Vega, 1996)

[6] ILSI range is from ILSI Crop Composition Database, 2006.

Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw.

Table 2. – cont.

	MON 89034 × MON 88017		Control		Commercials[1]		
Tissue/Component (Units)[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain							
Amino acids (% dw)							
Alanine	0.79	0.72-0.85	0.78	0.66-0.83	0.59-0.87	N/A	0.439-1.393
Arginine	0.44	0.35-0.49	0.45	0.41-0.47	0.36-0.48	N/A	0.119-0.639
Aspartic acid	0.69	0.63-0.74	0.71	0.65-0.75	0.52-0.80	N/A	0.335-1.208
Cystine	0.21	0.19-0.22	0.2	0.19-0.21	0.19-0.24	N/A	0.125-0.514
Glutamic acid	1.92	1.72-2.05	1.9	1.60-2.04	1.48-2.24	N/A	0.965-3.536
Glycine	0.35	0.33-0.37	0.35	0.33-0.36	0.31-0.41	N/A	0.184-0.539
Histidine	0.27	0.24-0.29	0.27	0.25-0.28	0.24-0.33	N/A	0.137-0.434
Isoleucine	0.37	0.33-0.40	0.37	0.33-0.39	0.29-0.42	N/A	0.179-0.692
Leucine	1.25	1.14-1.32	1.24	1.04-1.31	0.97-1.53	N/A	0.642-2.492
Lysine	0.31	0.28-0.33	0.31	0.30-0.32	0.25-0.31	N/A	0.172-0.668
Methionine	0.19	0.18-0.20	0.19	0.17-0.20	0.17-0.22	N/A	0.124-0.468
Phenylalanine	0.52	0.47-0.57	0.51	0.44-0.53	0.41-0.61	N/A	0.244-0.930
Proline	0.85	0.78-0.92	0.85	0.76-0.90	0.64-1.09	N/A	0.462-1.632
Serine	0.47	0.40-0.54	0.46	0.39-0.51	0.36-0.55	N/A	0.235-0.769
Threonine	0.35	0.32-0.39	0.35	0.31-0.37	0.28-0.39	N/A	0.224-0.666
Tryptophan	0.052	0.047-0.056	0.051	0.046-0.056	0.038-0.061	N/A	0.0271-0.215
Tyrosine	0.32	0.16-0.39	0.32	0.27-0.36	0.15-0.39	N/A	0.103-0.642
Valine	0.49	0.44-0.53	0.49	0.44-0.50	0.40-0.56	N/A	0.266-0.855

* significant difference at 5% level when compared with the control

[1] 13 commercial maize hybrids.

[2] dw = dry weight

[3] The mean of seven and eight replicate values for respectively MON 89034 × MON 88017 and control (Respectively two and one replicate out of 9 of the test and control material did not meet the acceptance criteria for TCR characterization (<3.05 unintended traits))

[4] Tolerance Interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

[5] Literature range references: [a] (Ridley *et al.*, 2002); [b] (Sidhu *et al.*, 2000); [c] (Jugenheimer, 1976); [d] (Watson, 2003); [e] amino acid values reported as percent of total protein and fatty acid values reported as percent of total fat (Watson, 1982); [f] (Classen *et al.*, 1990); [g] (Dowd and Vega, 1996)

[6] ILSI range is from ILSI Crop Composition Database, 2006.

Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw.

Table 2. – cont.

Tissue/Component[2]	MON 89034 × MON 88017 Mean[3]	MON 89034 × MON 88017 Range	Control Mean[3]	Control Range	Commercials[1] 99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain – continued							
Fatty acids (% dw)							
16:0 palmitic acid	0.44	0.41-0.47	0.43	0.39-0.48	0.30-0.50	7-19[e] (% of total fa)	7.94-20.71 (% of total fa)
18:0 stearic acid	0.043	0.037-0.049	0.043	0.036-0.048	0.036-0.081	1-3[e] (% of total fa)	1.02-3.40 (% of total fa)
18:1 oleic acid	0.73	0.68-0.77	0.69	0.62-0.76	0.58-1.34	20-46[e] (% of total fa)	17.4-40.2 (% of total fa)
18:2 linoleic acid	1.85	1.72-1.94	1.84	1.66-1.98	1.58-2.39	35-70[e] (% of total fa)	36.2-66.5 (% of total fa)
18:3 linolenic acid	0.054	0.048-0.057	0.052	0.046-0.055	0.030-0.062	0.8-2[e] (% of total fa)	0.57-2.25 (% of total fa)
20:0 arachidic acid	0.012	0.010-0.014	0.012	0.011-0.012	0.0095-0.017	0.1-2[e] (% of total fa)	0.279-0.965 (% of total fa)
20:1 eicosenoic acid	0.0077	0.0069-0.0081	0.0075	0.0070-0.0081	0.0065-0.012	N/A	0.170-1.917 (% of total fa)
22:0 behenic acid	0.006	0.0048-0.0077	0.0056	0.0047-0.0067	0.0022-0.0069	N/A	0.110-0.349 (% of total fa)
Fibre (% dw)							
ADF	3.36	2.97-3.74	3.37	2.84-4.50	2.86-4.72	3.3-4.3[d]; 2.46-11.34[a, b]	1.82-11.34
NDF	11.05	9.72-13.06	9.98	9.08-10.91	6.87-12.64	8.3-11.9[d]; 7.58-15.91[b]	5.59-22.64
TDF	13.59	13.06-14.37	13.6	12.90-15.64	9.89-16.01	10.99-11.41[h]	8.82-35.31

* significant difference at 5% level when compared with the control

[1] 13 commercial maize hybrids.

[2] dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre, TDF = total dietary fibre.

[3] The mean of seven and eight replicate values for respectively MON 89034 × MON 88017 and control (Respectively two and one replicate out of 9 of the test and control material did not meet the acceptance criteria for TCR characterization (<3.05 unintended traits))

[4] Tolerance Interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

[5] Literature range references: [a] (Ridley *et al.*, 2002); [b] (Sidhu *et al.*, 2000); [c] (Jugenheimer, 1976); [d] (Watson, 2003); [e] amino acid values reported as percent of total protein and fatty acid values reported as percent of total fat (Watson, 1982); [f] (Classen *et al.*, 1990); [g] (Dowd and Vega, 1996)

[6] ILSI range is from ILSI Crop Composition Database, 2006.

Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw.

Table 2. – cont.

Tissue/Component (Units)[2]	MON 89034 × MON 88017 Mean[3]	MON 89034 × MON 88017 Range	Control Mean[3]	Control Range	Commercials[1] 99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain - continued							
Minerals (mg/kg dw)							
Calcium	72.01*	55.66-98.18	63.3	56.46-71.27	31.50-67.20	0.01-0.1[d] (% dw)	0.00127-0.02084 (% dw)
Copper	1.36	1.06-1.70	1.13	1.00-1.30	1.03-2.07	0.9-10[d]	0.73-18.50
Iron	20.06*	16.21-25.06	18.27	16.15-20.32	15.46-23.22	1-100[d]	10.42-49.07
Magnesium	1428.18	1234.43-1612.73	1403.39	1256.75-1478.47	1134.59-1439.73	0.09-1[d] (% dw)	0.0594-0.194 (% dw)
Manganese	6.11	5.53-7.22	6.31	5.50-7.27	5.50-10.01	0.7-54[d]	1.69-14.30
Phosphorus	3747.39	3172.71-4075.41	3733.58	3252.12-4019.55	2826.82-4029.02	0.26-0.75[d] (% dw)	0.147-0.533 (% dw)
Potassium	4909.71	3995.67-5350.33	4987.44	4233.27-5453.56	3203.56-5237.02	0.32-0.72[d] (% dw)	0.181-0.603 (% dw)
Zinc	19.8	15.97-22.56	18.73	15.70-23.43	17.41-24.77	12-30[d]	6.5-37.2
Proximates (% dw)							
Ash	1.94	1.76-2.16	1.9	1.58-2.11	0.98-1.97	1.1-3.9[d] ; 0.89-6.28[b]	0.616-6.282
Carbohydrates	84.35	83.90-85.57	84.72	83.65-85.82	83.25-87.49	77.4-87.2[b] ; 82.2-88.1[a]	77.4-89.5
Total fat	3.42	3.28-3.58	3.36	3.06-3.71	2.97-4.65	3.1-5.7[d] ; 2.48-4.81[b]	1.742-5.823
Moisture	8.24*	7.31-9.41	7.48	5.11-9.50	6.87-10.70	7-23[d] ; 8.18-26.2[b]	6.1-40.5
Protein	10.32	9.43-10.56	10.04	8.95-10.93	7.93-11.19	6-12[d]; 9.7-16.1[c]	6.15-17.26

* significant difference at 5% level when compared with the control

[1] 13 commercial maize hybrids.

[2] dw = dry weight

[3] The mean of seven and eight replicate values for respectively MON 89034 × MON 88017 and control (Respectively two and one replicate out of 9 of the test and control material did not meet the acceptance criteria for TCR characterization (<3.05 unintended traits))

[4] Tolerance Interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

[5] Literature range references: [a] (Ridley *et al.*, 2002); [b] (Sidhu *et al.*, 2000); [c] (Jugenheimer, 1976); [d] (Watson, 2003); [e] amino acid values reported as percent of total protein and fatty acid values reported as percent of total fat (Watson, 1982); [f] (Classen *et al.*, 1990); [g] (Dowd and Vega, 1996)

[6] ILSI range is from ILSI Crop Composition Database, 2006.

Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw.

Table 2. – cont.

Tissue/Component[2]	MON 89034 × MON 88017		Control		Commercials[1]		
	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain – continued							
Vitamin (mg/kg dw)							
Folic acid	0.6	0.50-0.71	0.62	0.45-0.74	0.19 - 0.77	0.3[d]	0.147-1.464
Niacin	16.99	14.57-20.49	17.06	14.12-21.73	14.78 - 26.86	9.3-70[d]	10.37-46.94
Vitamin A	0.42	0.38-0.45	0.41	0.34-0.46	0.39-1.16	n/a	0.19-46.81
Vitamin B1	2.32*	1.73-2.76	3.31	2.89-3.65	2.62 - 4.87	3-8.6[e]	1.26-40.00
Vitamin B2	2.26	1.54-3.32	2.85	2.07-3.87	1.29 - 3.85	0.25-5.6[e]	0.50-2.36
Vitamin B6	4.8	3.62-5.27	4.98	3.97-7.66	3.22 - 8.35	5.3[d]; 9.6[e]	3.68-11.32
Vitamin E	5.86	2.70-13.29	5.34	2.63-14.57	2.74 - 17.03	3-12.1[e]; 17-47[d]	1.5-68.7
Antinutrient (% dw)							
Phytic acid	0.86	0.81-0.92	0.88	0.71-1.00	0.60 - 0.98	0.48-1.12[a]	0.111-1.570
Raffinose	0.14*	0.027-0.34	0.1	0.027-0.26	0.027 - 0.29	-	0.020-0.320
Secondary metabolite (µg/g dw)							
Ferulic acid	2288.98	1780.13-2658.82	2375.8	2182.78-2638.38	1263.98 - 2443.86	113-1194[f]; 3000[g]	291.9-3885.8
p-coumaric acid	268.66	229.56-339.98	254.22	206.55-309.61	122.21 - 304.86	22-75[f]	53.4-576.2

* significant difference at 5% level when compared with the control

[1] 13 commercial maize hybrids.

[2] dw = dry weight, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

[3] The mean of seven and eight replicate values for respectively MON 89034 × MON 88017 and control (Respectively two and one replicate out of 9 of the test and control material did not meet the acceptance criteria for TCR characterization (<3.05 unintended traits))

[4] Tolerance Interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

[5] Literature range references: [a] (Ridley *et al.*, 2002); [b] (Sidhu *et al.*, 2000); [c] (Jugenheimer, 1976); [d] (Watson, 2003); [e] (Watson, 1982); [f] (Classen *et al.*, 1990); [g] (Dowd and Vega, 1996); [h] (Choi *et al.*, 1999).

[6] ILSI range is from ILSI Crop Composition Database, 2006.

Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw.

Table 3. Compositional analysis of maize forage and grain collected from MON 89034 x MON 88017 compared to control and commercial varieties – European filed trials (Southern Region) conducted in 2007 – All sites combined

	MON 89034 × MON 88017		Control		Commercials[1]		
Tissue/Component (Units)[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Forage							
Fibre (% dw)							
ADF	20.34	15.05-27.29	21.04	18.84-27.24	16.38-30.00	18.3-41.0[b] ; 17.5-38.3[a]	16.13-47.39
NDF	30.17	21.16-38.28	33.2	25.42-52.30	21.46-49.82	26.4-54.5[b]; 27.9-54.8[a]	20.29-63.71
Proximates							
Ash (% dw)	3.44	2.91-3.87	3.67	2.64-4.91	2.40-4.52	2.43-9.64[a]; 2-6.6[b]	1.527-9.638
Carbohydrates (% dw)	87.21	85.67-88.44	87.46	86.22-88.91	85.67-89.26	83.2-91.6[b]; 76.5-87.3[a]	76.4-92.1
Fat (% dw)	2.44	1.83-2.99	2.11	0.88-2.85	1.01-3.32	0.35-3.62[b] ; 1.42-4.57[a]	0.296-4.570
Moisture (% fw)	65.55	62.20-69.80	66.17	61.40-71.70	54.20-74.10	56.5-80.4[a] ; 55.3-75.3[b]	49.1-81.3
Protein (% dw)	6.91	6.08-8.04	6.71	5.51-7.99	4.79-8.91	4.98-11.56 [a]	3.14-11.57
Minerals							
Calcium (% dw)	0.23	0.18-0.26	0.25	0.20-0.30	0.11-0.30	0.097-0.318[b]	0.0714-0.5768
Phosphorus (% dw)	0.2	0.15-0.27	0.2	0.16-0.26	0.16-0.29	0.137-0.291[b]	0.0936-0.3704

1 13 commercial maize hybrids.

2 dw = dry weight, ADF = acid detergent fiber, NDF = neutral detergent fiber,

3 The mean of six and eight replicate values for respectively MON 89034 × MON 88017 and control (Respectively three and one replicate out of 9 of the test and control material did not meet the acceptance criteria for TCR characterization (<3.05 unintended traits))

4 Tolerance Interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

5 Literature range references: [a] (Ridley *et al.*, 2002); [b] (Sidhu *et al.*, 2000); [c] (Jugenheimer, 1976); [d] (Watson, 2003); [e] (Watson, 1982); [f] (Classen *et al.*, 1990); [g] (Dowd and Vega, 1996); [h] (Choi *et al.*, 1999).

6 ILSI range is from ILSI Crop Composition Database, 2006.

Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw.

Table 3. – cont.

	MON 89034 × MON 88017		Control		Commercials[1]		
Tissue/Component[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain							
Amino acids (% dw)							
Alanine	0.67	0.53-0.79	0.66	0.58-0.79	0.59-0.87	N/A	0.439-1.393
Arginine	0.39	0.33-0.42	0.4	0.36-0.43	0.36-0.48	N/A	0.119-0.639
Aspartic acid	0.59	0.47-0.69	0.59	0.53-0.70	0.52-0.80	N/A	0.335-1.208
Cystine	0.21	0.20-0.22	0.2	0.18-0.21	0.19-0.24	N/A	0.125-0.514
Glutamic acid	1.71	1.34-2.03	1.7	1.45-2.05	1.48-2.24	N/A	0.965-3.536
Glycine	0.33	0.28-0.35	0.33	0.31-0.35	0.31-0.41	N/A	0.184-0.539
Histidine	0.26	0.21-0.28	0.26	0.24-0.28	0.24-0.33	N/A	0.137-0.434
Isoleucine	0.32	0.24-0.39	0.31	0.27-0.39	0.29-0.42	N/A	0.179-0.692
Leucine	1.13	0.87-1.37	1.13	0.94-1.38	0.97-1.53	N/A	0.642-2.492
Lysine	0.26	0.21-0.28	0.26	0.24-0.29	0.25-0.31	N/A	0.172-0.668
Methionine	0.2	0.19-0.22	0.19	0.17-0.21	0.17-0.22	N/A	0.124-0.468
Phenylalanine	0.46	0.35-0.55	0.47	0.40-0.55	0.41-0.61	N/A	0.244-0.930
Proline	0.82	0.66-0.91	0.81	0.71-0.90	0.64-1.09	N/A	0.462-1.632
Serine	0.43	0.35-0.50	0.44	0.39-0.51	0.36-0.55	N/A	0.235-0.769
Threonine	0.32	0.26-0.37	0.32	0.30-0.36	0.28-0.39	N/A	0.224-0.666
Tryptophan	0.047	0.043-0.052	0.047	0.045-0.049	0.038-0.061	N/A	0.0271-0.215
Tyrosine	0.25	0.20-0.36	0.27	0.18-0.37	0.15-0.39	N/A	0.103-0.642
Valine	0.43	0.34-0.50	0.42	0.37-0.50	0.40-0.56	N/A	0.266-0.855

[1] 13 commercial maize hybrids.

[2] dw = dry weight

[3] The mean of six and eight replicate values for respectively MON 89034 × MON 88017 and control (Respectively three and one replicate out of 9 of the test and control material did not meet the acceptance criteria for TCR characterization (<3.05 unintended traits))

[4] Tolerance Interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

[5] Literature range references: [a] (Ridley *et al.*, 2002); [b] (Sidhu *et al.*, 2000); [c] (Jugenheimer, 1976); [d] (Watson, 2003); [e] (Watson, 1982); [f] (Classen *et al.*, 1990); [g] (Dowd and Vega, 1996); [h] (Choi *et al.*, 1999).

[6] ILSI range is from ILSI Crop Composition Database, 2006.

Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw.

Table 3. – cont.

Tissue/Component[2]	MON 89034 × MON 88017 Mean[3]	MON 89034 × MON 88017 Range	Control Mean[3]	Control Range	Commercials[1] 99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain – continued							
Fatty acids (% dw)							
16:0 palmitic acid	0.43	0.39-0.49	0.41	0.39-0.44	0.30-0.50	7-19[e](% of total fa)	7.94-20.71 (% of total fa)
18:0 stearic acid	0.051	0.043-0.063	0.051	0.043-0.058	0.036-0.081	1-3[e] (% of total fa)	1.02-3.40 (% of total fa)
18:1 oleic acid	0.88	0.69-1.13	0.86	0.73-0.95	0.58-1.34	20-46[e] (% of total fa)	17.4-40.2 (% of total fa)
18:2 linoleic acid	1.77	1.61-2.06	1.74	1.62-1.89	1.58-2.39	35-70[e] (% of total fa)	36.2-66.5 (% of total fa)
18:3 linolenic acid	0.04	0.038-0.045	0.039	0.037-0.040	0.030-0.062	0.8-2[e]; 0.71-1.50 [a] (% of total fa)	0.57-2.25 (% of total fa)
20:0 arachidic acid	0.012	0.010-0.014	0.012	0.011-0.013	0.0095-0.017	0.1-2[e] (% of total fa)	0.279-0.965 (% of total fa)
20:1 eicosenoic acid	0.0094	0.0077-0.012	0.0093	0.0083-0.010	0.0065-0.012	N/A	0.170-1.917 (% of total fa)
22:0 behenic acid	0.0053	0.0022-0.0066	0.0051	0.0046-0.0056	0.0022-0.0069	N/A	0.110-0.349 (% of total fa)
Fibre (% dw)							
ADF	3.42	3.00-4.14	3.43	2.88-3.92	2.86-4.72	3.3-4.3[d]; 2.46-11.34[a, b]	1.82-11.34
NDF	8.23	7.42-9.67	9.27	6.99-14.75	6.87-12.64	8.3-11.9[d]; 7.58-15.91[b]	5.59-22.64
TDF	11.89	10.84-13.10	11.63	9.73-13.63	9.89-16.01	10.99-11.41[h]	8.82-35.31

1 13 commercial maize hybrids.

2 dw = dry weight, ADF = acid detergent fiber, NDF = neutral detergent fiber, TDF = total dietary fiber

3 The mean of six and eight replicate values for respectively MON 89034 × MON 88017 and control (Respectively three and one replicate out of 9 of the test and control material did not meet the acceptance criteria for TCR characterization (<3.05 unintended traits))

4 Tolerance Interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

5 Literature range references: [a] (Ridley *et al.*, 2002); [b] (Sidhu *et al.*, 2000); [c] (Jugenheimer, 1976); [d] (Watson, 2003); [e] (Watson, 1982); [f] (Classen *et al.*, 1990); [g] (Dowd and Vega, 1996); [h] (Choi *et al.*, 1999).

6 ILSI range is from ILSI Crop Composition Database, 2006.

Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw.

Table 3. – cont.

	MON 89034 × MON 88017		Control		Commercials[1]		
Tissue/Component[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain – continued							
Minerals (mg/kg dw)							
Calcium	55.48	48.67-61.47	57.24	48.04-68.71	31.50-67.20	0.01-0.1[d] (% dw)	0.00127-0.02084 (% dw)
Copper	1.43	1.22-1.64	1.43	1.15-1.60	1.03-2.07	0.9-10[d]	0.73-18.50
Iron	18.88	15.87-22.51	17.27	13.23-20.68	15.46-23.22	1-100[d]	10.42-49.07
Magnesium	1294.23	1134.24-1469.93	1226.24	1076.06-1425.62	1134.59-1439.73	0.09-1[d] (% dw)	0.0594-0.194 (% dw)
Manganese	6.45	5.53-7.73	6.11	5.30-7.26	5.50-10.01	0.7-54[d]	1.69-14.30
Phosphorus	3167.24	2499.72-3708.24	3010.01	2522.42-3504.19	2826.82-4029.02	0.26-0.75[d] (% dw)	0.147-0.533 (% dw)
Potassium	3730.6	3446.76-4175.95	3643.35	3206.28-4643.17	3203.56-5237.02	0.32-0.72[d] (% dw)	0.181-0.603 (% dw)
Zinc	20.69	18.44-21.71	19.4	17.15-21.94	17.41-24.77	12-30[d]	6.5-37.2
Proximates (% dw)							
Ash (% dw)	1.55	1.27-1.83	1.45	1.14-1.75	0.98-1.97	1.1-3.9[d] ; 0.89-6.28[b]	0.616-6.282
Carbohydrates (% dw)	85.85	84.86-87.44	86.31	84.90-87.58	83.25-87.49	77.4-87.2[b] ; 82.2-88.1[a]	77.4-89.5
Fat (% dw)	3.5	3.16-4.18	3.37	3.18-3.66	2.97-4.65	3.1-5.7[d] ; 2.48-4.81[b]	1.742-5.823
Moisture (% fw)	9.93	8.91-10.80	10.01	8.11-10.80	6.87-10.70	7-23[d] ; 8.18-26.2[b]	6.1-40.5
Protein (% dw)	9.08	8.08-10.26	8.88	7.73-10.18	7.93-11.19	6-12[d]; 9.7-16.1[c]	6.15-17.26

[1] 13 commercial maize hybrids.

[2] dw = dry weight, ADF = acid detergent fiber, NDF = neutral detergent fiber, TDF = total dietary fiber, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

[3] The mean of six and eight replicate values for respectively MON 89034 × MON 88017 and control (Respectively three and one replicate out of 9 of the test and control material did not meet the acceptance criteria for TCR characterization (<3.05 unintended traits))

[4] Tolerance Interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

[5] Literature range references: [a] (Ridley *et al.*, 2002); [b] (Sidhu *et al.*, 2000); [c] (Jugenheimer, 1976); [d] (Watson, 2003); [e] (Watson, 1982); [f] (Classen *et al.*, 1990); [g] (Dowd and Vega, 1996); [h] (Choi *et al.*, 1999).

[6] ILSI range is from ILSI Crop Composition Database, 2006.

Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw.

Table 3. – cont.

Tissue/Component[2]	MON 89034 × MON 88017 Mean[3]	MON 89034 × MON 88017 Range	Control Mean[3]	Control Range	Commercials[1] 99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain – continued							
Vitamin (mg/kg dw)							
Folic acid	0.3	0.26-0.35	0.34	0.23-0.77	0.19-0.77	0.3[d]	0.147-1.464
Niacin	18.63	15.81-20.74	18.98	15.98-22.01	14.78-26.86	9.3-70[d]	10.37-46.94
Vitamin A	0.61	0.47-0.77	0.57	0.51-0.62	0.39-1.16	n/a	0.19-46.81
Vitamin B1	3.38	3.10-3.81	3.56	3.24-3.92	2.62-4.87	3-8.6[e]	1.26-40.00
Vitamin B2	2	1.36-2.66	1.94	1.08-3.34	1.29-3.85	0.25-5.6[e]	0.50-2.36
Vitamin B6	4.04	3.33-4.72	4.07	3.36-4.65	3.22-8.35	5.3[d]; 9.6[e]	3.68-11.32
Vitamin E	5.48	2.74-7.06	4.31	2.72-7.17	2.74-17.03	3-12.1[e]; 17-47[d]	1.5-68.7
Antinutrient (% dw)							
Phytic acid	0.83	0.64-1.02	0.82	0.73-0.88	0.60-0.98	0.48-1.12[a]	0.111-1.570
Raffinose	0.10	0.025-0.15	0.094	0.027-0.15	0.027 - 0.29	-	0.020-0.320
Secondary metabolite (µg/g dw)							
Ferulic acid	1831	1670.38-1960.14	1861.53	1586.59-2149.82	1263.98-2443.86	113-1194[f]; 3000[g]	291.9-3885.8
p-coumaric acid (µg/g dw)	138.46	120.67-162.48	148.6	119.55-183.95	122.21-304.86	22-75[f]	53.4-576.2

1 13 commercial maize hybrids.

2 dw = dry weight, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

3 The mean of six and eight replicate values for respectively MON 89034 × MON 88017 and control (Respectively three and one replicate out of 9 of the test and control material did not meet the acceptance criteria for TCR characterization (<3.05 unintended traits))

4 Tolerance Interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

5 Literature range references: [a] (Ridley *et al.*, 2002); [b] (Sidhu *et al.*, 2000); [c] (Jugenheimer, 1976); [d] (Watson, 2003); [e] (Watson, 1982); [f] (Classen *et al.*, 1990); [g] (Dowd and Vega, 1996); [h] (Choi *et al.*, 1999).

6 ILSI range is from ILSI Crop Composition Database, 2006.

Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw.

Table 4. Summary of the statistical differences for the compositional comparison of maize forage and grain collected from MON 89034 x MON 88017 to control maize – European filed trials (Northern region) conducted in 2007

Tissue/Site/ Component (Units)[a]	Mean MON 89034 × MON 88017	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 89034 × MON 88017 (Range)	99% Tolerance Interval[b]
Forage						
Combined Site						
Ash (% dw)	3.32	3.99	-16.85	0.033	[2.57-4.70]	[1.18, 6.01]
Site 8						
Calcium (% dw)	0.19	0.24	-22.03	0.011	[0.18-0.20]	[0.017, 0.39]
Grain						
Combined Site						
Calcium (mg/kg dw)	72.01	63.3	13.75	0.005	[55.66-98.18]	[5.05, 96.16]
Iron (mg/kg dw)	20.06	18.27	9.76	0.03	[16.21-25.06]	[11.99, 27.51]
Moisture (% FW)	8.24	7.48	10.21	0.047	[7.31-9.41]	[4.65, 13.98]
Vitamin B1 (mg/kg dw)	2.32	3.31	-29.87	<0.001	[1.73-2.76]	[1.06, 5.64]
Raffinose (% dw)	0.14	0.1	40.97	0.03	[0.027-0.34]	[0, 0.39]
Site 6						
Vitamin B1 (mg/kg dw)	1.95	3.09	-36.92	<0.001	[1.73-2.18]	[1.06, 5.64]
18:1 Oleic acid (% dw)	0.72	0.63	14.09	0.004	[0.71-0.75]	[0.14, 1.71]
ADF(% dw)	3.13	3.92	-20.11	0.004	[2.97-3.26]	[1.75, 5.75]
Calcium (mg/kg dw)	83.47	65.56	27.32	0.031	[76.06-98.18]	[5.05, 96.16]
Site 8						
Vitamin B1 (mg/kg dw)	2.31	3.22	-28.4	<0.001	[2.17-2.38]	[1.06, 5.64]
Moisture (% fw)	7.58	5.91	28.18	0.006	[7.34-7.75]	[4.65, 13.98]
Protein (% dw)	10.5	10.05	4.47	0.017	[10.47-10.53]	[5.59, 13.87]
Folic Acid (mg/kg dw)	0.62	0.51	21.9	0.008	[0.60-0.64]	[0, 1.23]
Raffinose (% dw)	0.31	0.22	37.12	0.005	[0.29-0.34]	[0, 0.39]

[a] dw=dry weight; fw=fresh weight; ADF = acid detergent fibre.

[b] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 5. Summary of the statistical differences for the compositional comparison of maize forage and grain collected from MON 89034 x MON 88017 to contr5ol maize – European field trials (Southern Region) conducted in 2007

Tissue/Site/ Component (Units)[a]	Mean MON 89034 × MON 88017	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 89034 × MON 88017 (Range)	99% Tolerance Interval[b]
Forage						
Site 10						
NDF(% dw)	37.9	30.15	25.72	0.044	[37.75 - 38.28]	[6.40, 59.86]
Site 13						
Total Fat (% dw)	2.61	1.5	74.27	0.047	[2.17 - 2.99]	[0.32, 4.40]
Grain						
Site 10						
Vitamin B1 (mg/kg dw)	3.32	3.77	-11.78	0.047	[3.18 - 3.56]	[1.06, 5.64]
Site 13						
TDF(% dw)	12.08	10.28	17.55	0.006	[11.41 - 13.10]	[4.38, 22.29]
Iron (mg/kg dw)	16.11	14.29	12.73	0.028	[15.87 - 16.50]	[11.99, 27.51]
Potassium (mg/kg dw)	3517.78	3263.08	7.81	0.03	[3446.76 - 3654.08]	[1468.52, 7007.49]
Ash (% dw)	1.37	1.21	13.25	0.047	[1.27 - 1.43]	[0.53, 2.57]
Moisture (% fw)	9.79	10.8	-9.29	0.021	[9.19 - 10.50]	[4.65, 13.98]
Vitamin E (mg/kg dw)	6.63	2.54	161.32	0.003	[6.39 - 6.80]	[0, 26.03]

[a] dw=dry weight; fw=fresh weight; NDF = neutral detergent fibre; TDF = total dietary fibre

[b] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 6. Phenotypic characteristics of MON 89034 x MON 88017 (test) compared to the conventional control. Field trials in the USA in the 2004 growth season

Phenotypic Characteristic (units)	MON 89034 x MON 88017	Control	References			
			Range[1]		99% Tolerance	
			Min.	Max.	LL	UL
Seedling vigor	7.0	7.0	5.0	9.0	1.8	9.0
Early stand count (#/plot)	63.9	62.8	58.3	69.7	53.0	77.0
Days to 50% pollen shed	60.3	59.8	55.3	70.0	44.6	78.4
Days to 50% silking	59.1*	58.5	52.0	69.7	39.8	79.9
Stay green	6.5	5.9	4.7	7.7	2.3	9.0
Ear height (cm)	106.68	105.66	95.25	138.68	67.56	163.57
Plant height (cm)	213.36	214.88	219.20	274.06	184.91	308.86
Dropped ears (#/plot)	0.0	0.1	0.0	0.5	0.0	0.7
Stalk lodged plants (#/plot)	1.0*	3.4	0.0	6.0	0.0	9.7
Root lodged plants (#/plot)	1.3	0.6	0.0	27.0	0.0	41.4
Final stand count (#/plot)	60.5	59.1	53.3	66.3	48.6	74.1
Grain moisture (%)	21.6	20.0	12.6	30.0	0.2	40.6
Test weight (kg/l)	0.69	0.71	0.64	0.74	0.57	0.84
Yield (kg/ha)	13941.2*	12692.1	8875.7	17814.1	3935.7	22892.2

* Indicates a statistically significant difference between the test and control at $p \leq 0.05$.

[1] Reference range = Minimum and maximum mean observed values among the fifteen references.

[2] 99% tolerance interval with 95% confidence. LL = lower limit; UL = upper limit

Table 7. Combined sites analysis: phenotypic characteristics of MON 89034 x MON 88017 compared to the conventional control. European field trials conducted in 2007 (Germany and Spain)

Characteristics	German field sites				Spanish field sites			
			Reference Range				References Range	
	MON 89034 x MON 88017	Control	Min.	Max.	MON 89034 x MON 88017	Control	Min.	Max.
Seedling vigour (V2-V4) (0-9)	5.7	5.8	4.7	7.3	1.9	2.1	1.0	3.0
Early stand count (V2-V4) (#/plot)	95.4	93.4	75.7	100.0	76.8	79.1	43.2	79.7
Days to 50 % pollen shed	72.1	71.4	66.0	73.3	81.6	81.8	75.0	91.0
Days to 50 % silking	71.2	70.3	65.0	73.3	76.8	77	69.0	88.0
Stay green (0-9)	5.9	5.3	2.8	6.3	9	9	8.7	9.0
Ear height (cm)[1]	87.1	84.7	63.1	118.3	91.3*	97.6	83.0	126.2
Plant height (cm)	201	203.6	177.9	233.7	193.7	196.2	165.0	226.2
Dropped ears (#/plot)	0.0[1]	0.0	0.0	0.0	2.1	1.9	0.0	13.3
Stalk lodged plants(#/plot)	0.0[1]	0.0	0.0	0.0	0.0*	0.5	0.0	0.3
Root lodged plants (#/plot)	0.0[1]	0.0	0.0	0.0	0.0	0.3	0.0	0.3
Final stand count (#/plot)	75	76.4	69.2	76.4	75.4	76.9	41.7	80.3
Ear/kernel rot (0-9)	0.0[1]	0.0[1]	0.0	0.0	0.0[1]	0.0[1]	0.0	0.0
Stalk rot (0-9)	0.0[1]	0.0[1]	0.0	0.0	0.0[1]	0.0[1]	0.0	0.0
Yields (t/ha)	5.8	6.4	5.1	9.3	10.8	10.2	5.7	11.7

[1] Data not analysed due to lack of variation