Final health- and environmental risk assessment of genetically modified maize MON 88017 x MON 810

Scientific opinion on insect-resistant and herbicide-tolerant, genetically modified maize MON 88017 x MON 810 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (EFSA/GMO/CZ/2006/33)

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

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Acknowledgment

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 88017 x MON 810 from Monsanto (Unique Identifier DAS-MON 88017-3 x MON-ØØ81Ø-6) was approved under Regulation (EC) No 1829/2003 in the EU for food and feed uses, import and processing on 28th of July 2010 (Commission Decision 2010/429/EC).

Genetically modified maize MON 88017 x MON 810 has previously been risk assessed by the VKM Panel on Genetically Modified Organisms (GMO), commissioned by the Norwegian Food Safety Authority related to the EFSA public hearing of the application in 2007 (VKM 2007a). In addition, MON 88017 and MON 810 has been evaluated by the VKM GMO Panel as single events and as a component of several stacked GM maize events and Regulation (EC) 1829/2003 and Directive 2001/18/EC (VKM 2005a,b,c, VKM 2007b,c,d, VKM 2008, VKM 2009, VKM 2010 a,b,c, VKM 2012, VKM 2013, VKM 2016).

The food/feed and environmental risk assessment of the maize MON 88017 x MON 810 is based on information provided by the applicant in the application EFSA/GMO/CZ/2006/33 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 as relevant.

The VKM GMO Panel has evaluated MON 88017 x MON 810 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 88017 x MON 810 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 emphasized 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 88017 x MON 810 was produced by conventional crosses between inbred lines containing MON 88017 and MON 810 events to combine resistance to certain coleopteran and lepidopteran pests, and to confer tolerance towards glyphosate-containing herbicides.

Maize MON 88017 was developed to express a modified Cry3Bb1 insecticidal protein, derived from *Bacillus thuringiensis* subsp. *kumamotoensis*, which confers protection against coleopteran target pests belonging to the genus *Diabrotica* such as Western corn rootworm (*Diabrotica 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). Maize MON 810 expresses the Cry1Ab insecticidal protein, derived from *Bacillus thuringiensis* subsp. *kurstaki,* which confers protection against lepidopteran pests such as *Ostrinia nubilaris* and species belonging to the genus *Sesamia.*

Molecular characterisation

Southern and PCR analyses indicate that the recombinant inserts in the single maize events MON 88017 and MON 810 are retained in the stacked event MON 88017 x MON 810. Genetic stability of the inserts has previously been demonstrated in the single events. The levels of CP4 EPSPS, Cry3Bb1 and Cry1Ab 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 88017 x MON 810 satisfactory.

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Comparative assessment

The applicant has performed comparative analyses of data from field trials located at representative sites and environments in USA during the 2002 growing season. With the exception of small intermittent variations and the insect resistance and herbicide tolerance conferred by the CP4 EPSPS, Cry3Bb1 and Cry1Ab proteins, the results showed no biologically relevant differences between maize stack MON 88017 x MON 810 and its conventional counterpart. Based on the assessment of available data, the VKM GMO Panel concludes that maize MON 88017 x MON 810 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 on broilers indicates no adverse health effects of maize MON 88017 x MON 810, and shows that it is nutritionally equivalent to conventional maize varieties. The Cry3Bb1, Cry1Ab 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 88017 x MON 810 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry3Bb1, Cry1Ab and CP4 EPSPS proteins will cause toxic or IgE-mediated allergic reactions to food or feed based on maize MON 88017 x MON 810 compared to conventional maize.

Environmental risk assessment

Considering the intended uses of maize MON 88017 x MON 810, 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 88017 x MON 810.

Maize MON 88017 x MON 810 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 88017 x MON 810. 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 88017 x MON 810 is compositionally, nutritionally, agronomically and phenotypically equivalent to its conventional counterpart except for the new proteins. It is unlikely that the Cry3Bb1, Cry1Ab 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 x MON 810 compared to conventional maize varieties.

The VKM GMO Panel concludes that maize MON 88017 x MON 810, based on current knowledge, is comparable to conventional maize varieties concerning environmental risk in Norway with the intended usage.

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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 88017 x MON 810 fra Monsanto (unik kode DAS-MON 88017-3 x MON-ØØ81Ø-6) ble godkjent i EU til import, videreforedling og til bruk som mat og fôr under forordning 1829/2003, den 28. juli 2010 (Kommisjonsbeslutning 2010/429/EU).

Maislinjen har tidligere vært vurdert av VKMs faggruppe for genmodifiserte organismer med hensyn på mulig helserisiko i forbindelse med EFSAs offentlige høring av søknaden i 2007 (VKM 2007a). VKMs faggruppe for GMO har også risikovurdert foreldrelinjene MON 88017 og MON 810, og i maishybrider der MON 88017 x MON 810 inngår som en av foreldrelinjene (VKM 2005a,b,c, VKM 2007b,c,d, VKM 2008, VKM 2009, VKM 2010 a,b,c, VKM 2012, VKM 2013, 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, genoverføring og effekter på ikke-målorganismer vurdert.

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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.

Maishybriden MON 88017 x MON 810 er dannet ved konvensjonelle kryssinger mellom de to transgene maislinjene MON 88017 og MON 810. MON 88017 x MON 810 uttrykker Cry3Bb1-, Cry1Ab og CP4-EPSPS-proteinene, som er resultat av introduksjon av genene *cry3Bb1, cry1Ab* og *cp4-epsps* fra jordbakteriene *B. thuringiensis* subsp. *kumamotoensis, B. thuringiensis* subsp *kurstaki* 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 88017 og MON 810 er bevart i den kryssede maishybriden MON 88017 x MON 810. Genetisk stabilitet av de innsatte sekvensene har tidligere blitt vist for mais MON 88017 og MON 810. Nivåene av Cry3Bb1, Cry1Ab og CP4 EPSPS -protein målt i korn og vegetativt vev fra MON 88017 x MON 810, samsvarer med nivåene i de respektive foreldrelinjene. Fenotypiske analyser viser at egenskapene for insektsresistens og herbicidtoleranse er stabile også i MON 88017 x MON 810. VKMs faggruppe for GMO anser den molekylære karakteriseringen av mais MON 88017 x MON 810 som tilfredsstillende.

Komparative analyser

Søker har utført komparative analyser av data fra feltforsøk gjort ved representative dyrkningsområder i USA under vekstsesongen 2002. Med unntak av små spredte variasjoner, insekts-resistens og herbicidtoleransen mediert av Cry3Bb1-, Cry1Ab- og CP4 EPSPS-proteinene, viste resultatene ingen biologisk relevante forskjeller mellom maishybriden MON 88017 x MON 810 og konvensjonell kontroll.

Basert på gjennomgangen av tilgjengelige data konkluderer VKMs faggruppe for GMO at mais MON 88017 x MON 810 er vesentlig lik konvensjonell kontroll med hensyn til næringsstoffsammensetning og agronomiske og fenotypiske egenskaper, med unntak av de nye proteinene.

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Helserisiko

En fôringsstudie utført på broilere indikerer ikke helseskadelige effekter av mais MON 88017 x MON 810, og studien viser at den er ernæringsmessig lik konvensjonell mais. Proteinene Cry3Bb1, Cry1Ab 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 andre allergiske reaksjoner (virke som adjuvans).

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at mais MON 88017 x MON 810 er ernæringsmessig lik konvensjonell mais, og at det er lite sannsynlig at proteinene Cry3Bb1, Cry1Ab eller CP4 EPSPS vil føre til økt risiko for toksiske eller IgE-medierte allergiske reaksjoner fra mat eller fôr basert på mais MON 88017 x MON 810 sammenliknet med konvensjonelle maissorter.

Miljørisiko

Søknaden gjelder godkjenning av maishybrid MON 88017 x MON 810 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 88017 x MON 810 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 88017 x MON 810 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 Cry3Bb1, Cry1Ab eller CP4 EPSPS vil føre til økt risiko for toksiske eller IgE-medierte allergiske reaksjoner fra mat eller fôr basert på mais MON 88017 x MON 810 sammenliknet med konvensjonelle maissorter.

VKMs faggruppe for genmodifiserte organismer konkluderer at mais MON 88017 x MON 810, ut i fra dagens kunnskap og tiltenkt bruksområde, tilsvarer konvensjonell mais når det gjelder mulig miljørisiko i Norge.

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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.

Cry1Ab	Protein from *Bacillus thuringiensis* subsp. *kurstaki.* Provide protection against certain lepidopteran target pests.
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

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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.

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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.

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Background

On 3 January 2006, the European Food Safety Authority (EFSA) received from the Competent Authority of Czech Republic an application (Reference EFSA/GMO/CZ/2006/33) for authorisation of the insect-resistant and herbicide tolerant genetically modified (GM) maize MON 88017 x MON 810 (Unique Identifier DAS-MON 88017-3 x MON-ØØ81Ø-6), 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/CZ/2005/27 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 21 February 2007, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the 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 June 2007 (VKM 2007a). EFSA published its scientific opinion 2 Juky 2009 (EFSA 2009b), and maize MON 88017 x MON 810 was approved for food and feed uses, import and processing in 28 July 2010 (Commission Decision 2010/429/EC).

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Maize MON 88017 and maize MON 810 has also been evaluated by the VKM GMO Panel as single events and as a component of several stacked GM maize events and Regulation (EC) 1829/2003 and Directive 2001/18/EC (VKM 2005a,b,c, VKM 2007b,c,d, VKM 2008, VKM 2009, VKM 2010 a,b,c, VKM 2012, VKM 2013, VKM 2016).

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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

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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.

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Assessment

1 Introduction

The hybrid maize MON 88017 x MON 810 was produced by conventional crosses between inbred lines containing MON 88017 and MON 810 events to combine resistance to certain coleopteran and lepidopteran pests, and to confer tolerance towards glyphosate-containing herbicides.

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*). The mode of action of the Cry3Bb1 protein and other 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 septicema (ref. EFSA 2011d). None of the target pests for maize MON 88017 are present in the Norwegian agriculture.

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 parental line MON 810 was developed to provide protection against certain lepidopteran insect larvae, including European corn borer (*Ostrinia nubilalis*) and species belonging to the genus *Sesamia*. None of these target pests are present in the Norwegian agriculture. Insect protection is achieved through expression in the plant of the insecticidal Cry protein Cry1Ab, derived from *Bacillus thuringiensis* ssp. *kurstaki*, a common soil bacterium.

The genetic modification in maize MON 88017 x MON 810 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 88017 x MON 810 (Unique Identifier DAS-MON 88017-3 x MON-ØØ81Ø-6) 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 88017 x MON 810 is based on information provided by the applicant in the application EFSA/GMO/CZ/2006/33 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 emphasied 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.

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2 Molecular characterisation

2.1 Evaluation of relevant scientific data

2.1.1 Method of production of maize MON 88017 x MON 810

The stacked maize MON 88017 x MON 810 was developed through conventional breeding by crossing the single maize events MON 88017 and MON 810. Maize MON 88017 x MON 810 combines the glyphosate tolerance and insect resistance of maize MON 88017 with the insect resistance of MON 810, conferred through the expression of the *cp4 epsps, cry3Bb1* and *cry1Ab* genes, respectively.

Expression of the Cry3Bb1 protein, derived from *Bacillus thuringiensis* subsp. *kumamotoensis.*, provides protection against certain coleopteran insect pests, including members of the corn rootworm (CRW) complex (Diabrotica spp.), which includes Western corn rootworm (*Diabrotica virgifera virgifera* LeConte), Northern corn rootworm (*Diabrotica barberi* Smith), and Southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber). Expression of the Cry1Ab protein, also derived from *Bacillus thuringiensis* subsp. *kurstaki*, provides protection from certain lepidopteran insect pests (including European Corn Borer (*Ostrinia nubilalis*) and *Sesamia* spp).

2.1.2 Summary of evaluation of the single events

2.1.2.1 *Maize MON 88017*

Genetically modified maize MON 88017 was developed to express 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 1) was used for the transformation of maize 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-borne 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,

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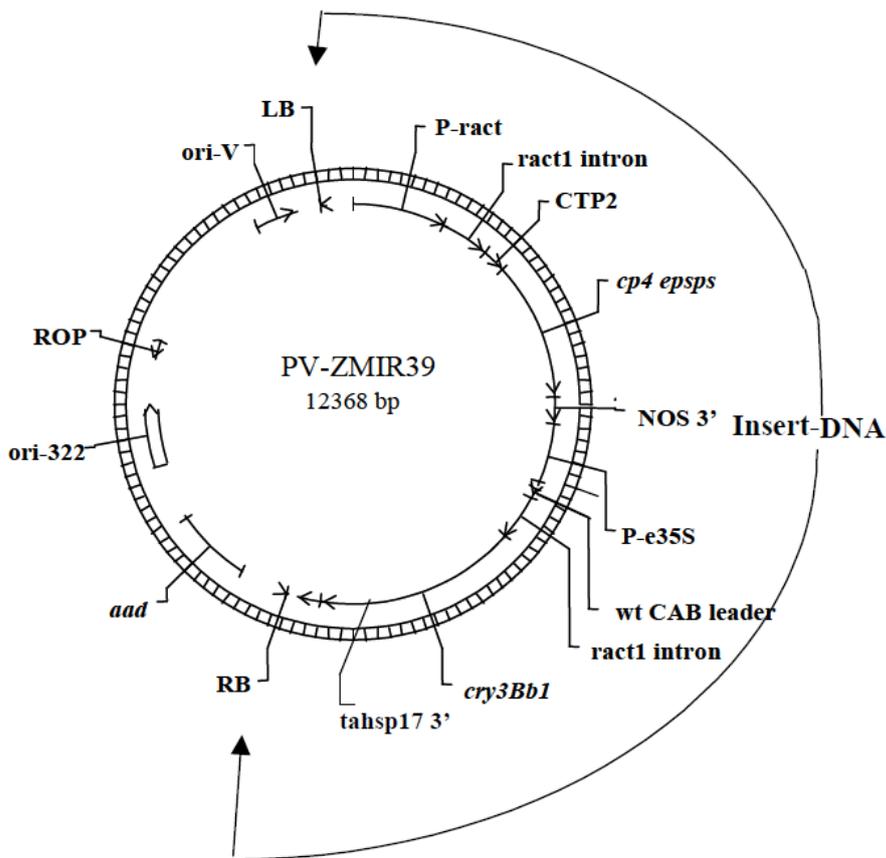
A circular map of the plasmid vector PV-ZMIR39 used in *Agrobacterium*-mediated transformation to create MON 88017. In this procedure, only the DNA present between the right and left borders (*i.e.*, RB and LB) was transferred into the host maize cells.

Figure 1. Map of the plasmid PV-ZMIR39

Southern analysis of genomic DNA digested with two different restriction enzymes (Sac I and Xba I) using 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 was confirmed by PCR amplification of seven overlapping regions of DNA that span the entire length of the insert. These PCR fragments were sequenced confirming the identity between the sequences inserted in MON 88017 and the corresponding sequences of the PV-ZMIR39 plasmid. Further, the absence of vector backbone sequences in MON 88017 plants was established by Southern analysis using two probes that cover 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 expression levels observed for both Cry3Bb1 and CP4 EPSPS proteins 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. The levels of Cry3Bb1 protein in tissue samples from the control substances were below the Cry3Bb1 assay LOQ or LOD for each tissue type, with one exception.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. The levels of CP4 EPSPS protein in tissue samples from the control substances were below the CP4 EPSPS assay LOQ or LOD for each tissue type, with one exception.

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 also in agreement with the published results of field trials conducted with MON 88017 in Germany between 2005-2007.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-fertilization and three generations of self-pollination.

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2.1.2.2 *Maize MON 810*

MON 810 produces the Cry1Ab insecticidal protein that protects the plant from feeding damage caused by certain lepidopteran insect pests, e.g. the European corn borer (ECB, *Ostrinia nubilalis*) and the Mediterranean Corn borer (MCB, *Sesamia nonagrioides*).

Maize event MON 810 was generated by particle acceleration technology using plasmids PV-ZMBK07 and PV-ZMGT10. The molecular characterisation of maize MON 810 shows that MON 810 contains a single insertion event which consists of elements derived from plasmid PV-ZMBK07. Data indicated that no other portion of plasmid PV-ZMBK07 DNA and no portion of plasmid PV-ZMGT10 were present in maize MON 810. This included the absence of the *nptII* gene. The organisation of the elements within the insert in maize MON 810 was confirmed by PCR. The insert was sequenced to further confirm the organisation of the elements within the insert.

The molecular characterisation of maize MON 810 shows that MON 810 contains a single insertion event which consists of elements derived from plasmid PV-ZMBK07, including the enhanced *35S* promoter, the maize *Hsp70* intron, and a *cry1Ab* coding sequence sufficient to encode an active insecticidal Cry1Ab protein. Additional experiments confirmed that the MON 810 insert contains a portion of the 3' end of the *e35S* promoter as well as a portion of the 5' end of the *cry1Ab* coding sequence. Data indicated that no other portion of plasmid PV-ZMBK07 DNA and no portion of plasmid PV-ZMGT10 were present in maize MON 810. This included the absence of the *nptII* gene. Probes that were derived from sequences spanning the *cry1Ab* expression unit in PV-ZMBK07, the plasmid backbone sequence that encompasses both PV-ZMBK07 and PV-ZMGT10 backbone, and elements from plasmid PV-ZMGT10, show that MON 810 contains part of the *e35S* promoter, the *Hsp70* intron, and part of the *cry1Ab* coding sequence, but does not contain the *nos* transcriptional sequence.

The organisation of the elements within the insert in maize MON 810 was confirmed by PCR. The insert was sequenced to further confirm the organisation of the elements within the insert. Sequence data indicate that the *e35S* promoter that regulates expression for the *cry1Ab* gene has been modified into a shorter promoter version *e35S*$^{MON\ 810}$ (307 bp at the 3' end of the 620 bp promoter), that the *Hsp*70 is intact and that 2448 bp of the *cry1Ab* coding sequence (corresponding to the 5' end of the 3470 bp gene) encompassing the insecticidal active tryptic core is present. A portion from the 3' ends of the *cry1Ab* gene as well the *nos* terminator has been deleted as the result of the integration process. The PCR was performed with a forward primer specific to the genomic DNA sequence flanking the 5' end of the insert paired with a reverse primer specific to the genomic DNA sequence flanking the 3' end of the insert. The amplified PCR product from the conventional counterpart was subjected to DNA sequence analysis. DNA sequence analyses performed on MON 810 determined the DNA sequence of the insert in MON 810, confirmed the predicted organisation of the genetic elements within the insert, determined the sequences flanking the insert, and examined the MON 810 insertion site.

Additional information submitted by the applicant confirmed the DNA sequences of the 5' and 3' DNA flanking regions originally provided. The applicant has also supplied additional sequence information. This revealed an additional 400 bp of maize DNA at the 3' flank and an additional 1000 bp of maize DNA at the 5' flank. A schematic representation of the insert is shown in figure 2.

Tissues of MON 810 plants were analysed for the three proteins, Cry1Ab, CP4 EPSPS, and GOX using ELISA. Tissue samples for analysis were collected from American and European field trials conducted in 1994 and 1995, respectively. Tissue samples for analysis were collected from six field trials conducted in the USA in 1994. Tissue samples from MON 810 for analysis of protein expression were collected from five field trials conducted within the major maize growing regions of France and Italy in 1995. Field trials were also conducted at two field sites in Italy and France in 1995 to produce leaf, forage and grain samples for expression analysis of MON 810 hybrids. Nguyen & Jehle (2007) conducted a quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in maize MON 810 plants (cultivar "Novelis") from two field trials in Germany.

The CP4 EPSPS and GOX proteins were not detected in any of the plant tissues of maize MON 810. This was expected since the molecular analysis of maize MON 810 established that the *cp4 epsps* and *gox* genes were not present in the nuclear genomic DNA.

In the American field trial, the level of Cry1Ab protein ranged from 7.93-10.34 µg/g fresh weight (fw) in young leaf tissue; 3.65-4.65 µg/g fw in whole plant tissue; and 0.19-0.39 µg/g fw in harvested grain. The foliar expression of Cry1Ab protein remained high during the vegetative growth stages of the maize plant as measured in overseason leaf samples.

In the European field trials in 1994 and 1995, the level of Cry1Ab protein ranged from 7.59-9.39 µg/g fw in young leaf tissue; 4.21-9.23 µg/g fw in forage tissue; and 0.42-0.69 µg/g fw in harvested grain. The 1995 analysis confirmed that CP4 EPSPS and GOX proteins were not present in plant tissues of maize MON 810. With regard to Cry1Ab, the protein levels were similar for plants grown in the USA and European field trials over two consecutive generations. The level of Cry1Ab protein in progeny of MON 810 ranges from 8.20-10.51 µg/g fwt in young leaf tissue, 4.00-5.11 µg/g fwt in forage tissue, and 0.35-0.60 µg/g fwt in harvested grain. The Cry1Ab protein levels were similar for MON 810 plants derived from backcrosses to B73/Mo17 and commercial hybrids.

In the European field trials in 2001-2003, the highest Cry1Ab levels were detected in the leaves (5.5-6.4 µg/g fw) at BBCH83, whereas the lowest Cry1Ab contents were detected in the pollen (1-97 ng/g fw). Cry1Ab content of residual root stocks collected in the field nine months after harvest was 15-17 ng/g fw, equivalent to about one-hundredth of the fresh root. This large-scale monitoring of Cry1Ab expression in maize MON 810 showed a considerable variation in the expression levels of Cry1Ab between genotypes, plant tissues and growth stages

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The presence of MON 810 insert in the nuclear genome is best shown by the Chi square analysis of the segregation results. The Chi square analysis of the segregation pattern, according to Mendelian genetics, was consistent with a single site of insertion into maize nuclear DNA.
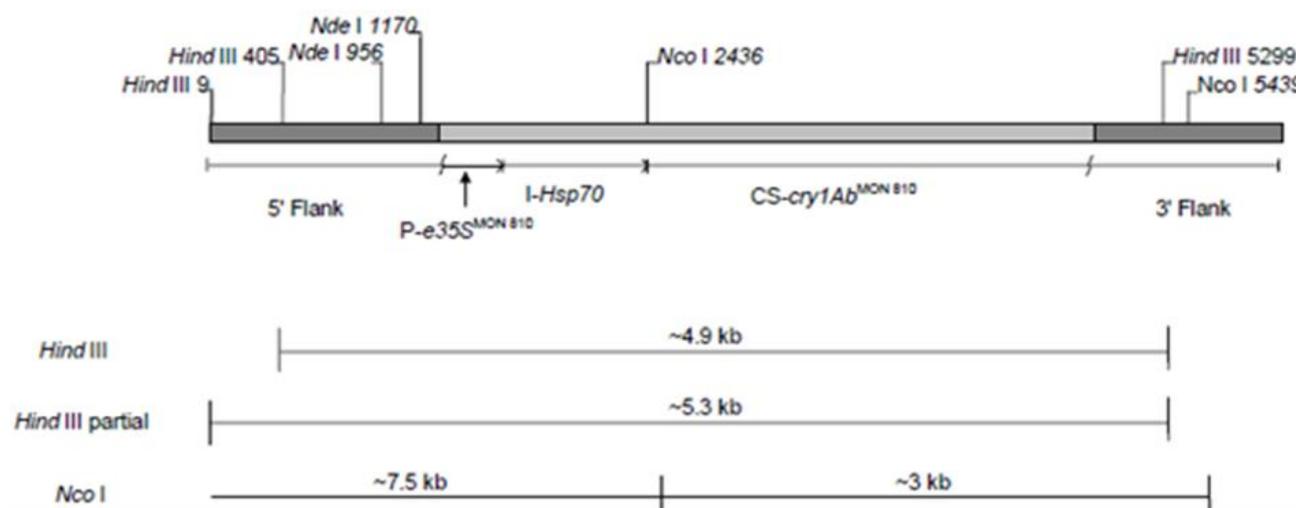
Figure 2. Schematic representation of the insert and flanking DNA in MON 810.

2.1.3 Transgene constructs in maize MON 88017 x MON 810

The MON 88017 x MON 810 maize was obtained by conventional crossing between two genetically modified maize events: MON 88017 and MON 810 maize. No new genetic modification was used for the development of the MON 88017 x MON 810 maize (figure 3).

A detailed molecular analysis was conducted to investigate the copy number, structure and organization of the inserts found in MON 88017 x MON 810 maize. The integrity of the individual inserts present in this maize was investigated using Southern analyses. This involved the use of DNA probes specific for the MON 88017 and MON 810 inserts and enzymatic digestions informative of the structure of both events, including the junctions with the host genomic DNA. The predicted DNA hybridisation patterns from each single event were retained in the MON 88017 x MON 810 hybrid. The results obtained from Southern Blot analyses indicate molecular equivalence, and identical copy number of the inserts present in MON 88017 x MON 810 maize to those present MON 88017 and MON 810 maize.

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2.1.3.1 *Information on the expression of insert*

A study was conducted to estimate the amount of CP4 EPSPS, Cry3Bb1 and Cry1Ab protein present in maize tissues collected from MON 88017 x MON 810 grown in three filed trails in the USA during the 2002 growing season (Bhakta et al 2003). These field sites were located within the major maize-growing region of the U.S.A. and provided a variety of environmental conditions. At each site, three replicated plots each containing MON 88017 × MON 810, control hybrids H1200902, MON 88017, and MON 810, were planted using a randomized complete block field design.

CP4 EPSPS, Cry3Bb1 and Cry1Ab protein levels were investigated in forage and grain. Levels of Cry3Bb1 protein were measured in young leaf, root, pollen and forage root, while levels of Cry1Ab protein were measured in leaf and pollen.

Enzyme-linked immunosorbent assay (ELISA) methods were used to validate each protein. All protein values are reported as micrograms (µg) of the specific protein per gram (g) of tissue on a fresh weight (fw) and a dry weight (dw) basis. Levels of proteins are summarised in Table 1-3 (Appendix). The CP4 EPSPS and Cry3Bb1 protein levels in MON 88017 x MON 810 were compared to MON 88017, whereas, the Cry1Ab protein levels in MON 88017 x MON 810 were compared to MON 810.

The mean level of the CP4 EPSPS protein was 4.3 µg/g dw (SD 1.6 µg/g dw) in MON 88017 x MON 810 grain samples, as compared to 5.8 µg/g dw (SD 0.97 µg/g dw) in grain from MON 88017. The mean level of the CP4 EPSPS protein was 51 µg/g dw (SD 9.2 µg/g dw) in MON 88017 x MON 810 forage samples, as compared to 57 µg/g dw (SD 7.6 µg/g dw) in forage from MON 88017.

The mean level of the Cry3Bb1 protein was 9.3 µg/g dw (SD 3.4 µg/g dw) in MON 88017 x MON 810 grain samples, compared to 15 µg/g dw (SD 3.6 µg/g dw) in grain from MON 88017. The mean level of the Cry3Bb1 protein was 100 µg/g dw (SD 23 µg/g dw) in MON 88017 x MON 810 forage samples, as compared to 95 µg/g dw (SD 19 µg/g dw) in forage from MON 88017.

The mean level of the Cry1Ab protein was 0.39 µg/g dw (SD 0.13 µg/g dw) in MON 88017 x MON 810 grain samples, as compared to 0.43 µg/g dw (SD 0.091 µg/g dw) in grain from MON 810. The mean level of the Cry1Ab protein was 14 µg/g dw (SD 2.1 µg/g dw) in MON 88017 x MON 810 forage samples, compared to 14 µg/g dw (SD 3.4 µg/g dw) in forage from MON 810.

Overall, the ranges across three sites for the CP4 EPSPS, Cry3Bb1 and Cry1Ab protein levels in MON 88017 x MON 810 were comparable to the corresponding ranges in MON 88017 and MON 810.

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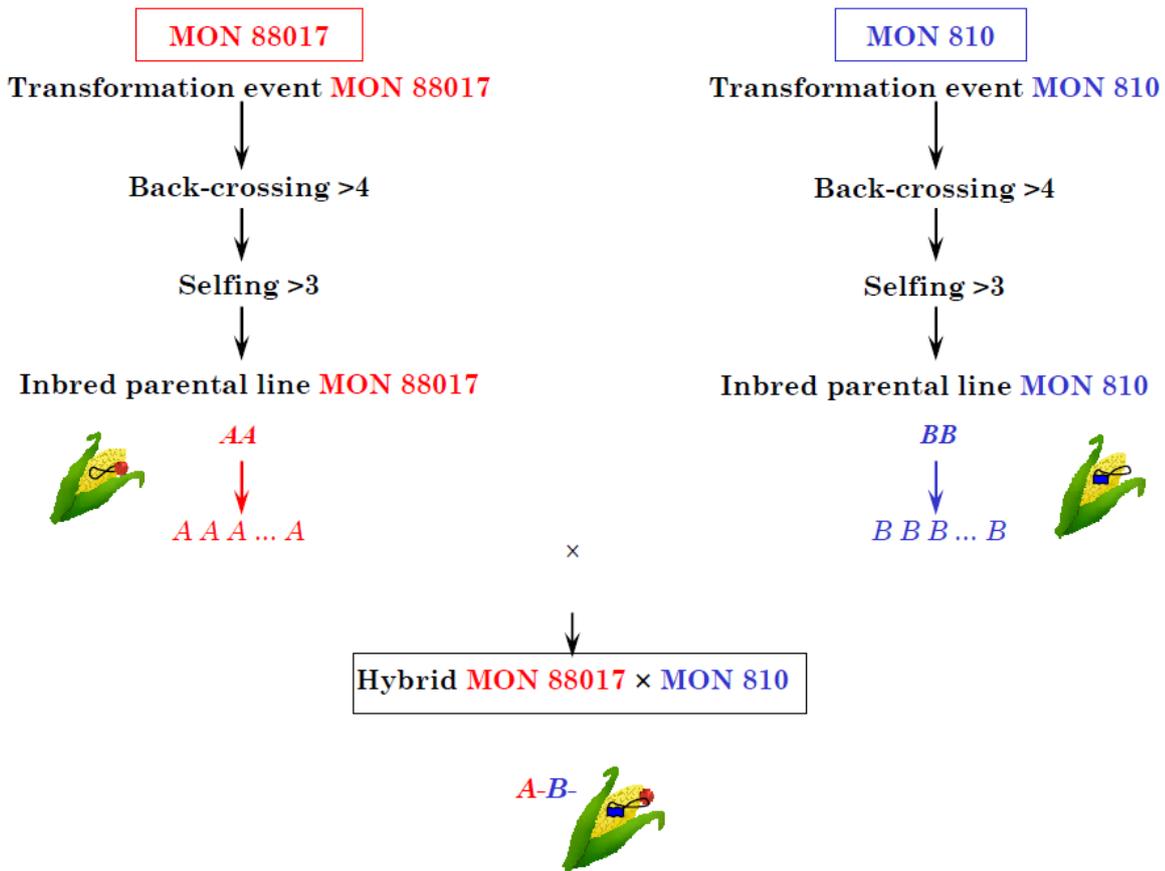
Figure 3. Traditional breeding strategy for MON 88017 x MON 810. A containing two expression cassettes/inserts, one that codes for the CP4 EPSPS and one that codes for MON 88017 Cry3Bb1. B containing one insert that codes for Cry1Ab.

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2.1.3.2 *Inheritance and genetic stability of inserted DNA*

The genetic stability of the inserted DNA in events MON 88017 and MON 810 has previously been evaluated by the VKM GMO Panel (VKM 2007b, VKM2016, VKM 2013).

The Southern analyses presented by the applicant show that both parental events are present in the stacked event MON 88017 x MON 810, and that the structures of the inserts are retained. Protein expression levels, phenotypic characteristics and agronomic performance, along with the introduced insecticidal and herbicide tolerance traits, further confirm the integrity of the inserts in the stacked event MON 88017 x MON 810.

2.2 Conclusion

Southern and PCR analyses indicate that the recombinant inserts in the single maize events MON 88017 and MON 810 are retained in the stacked event MON 88017 x MON 810. Genetic stability of the inserts has previously been demonstrated in the single events. The levels of CP4 EPSPS, Cry3Bb1 and Cry1Ab 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 88017 x MON 810 satisfactory.

3 Comparative assessment

3.1 Summary of the previous evaluations of the single events

3.1.1 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 2006/2007 growing 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).

3.1.2 Maize MON 810

The original field trials with maize MON 810 were performed in major maize-growing areas of the USA during the 1994 growth season (6 field sites). In addition, European field trials with MON 810 and MON 810 hybrids and conventional control maize were performed in France and Italy during the 1995 field season (5 locations) and France in 1995 (4 field sites). The non-GM maize control material was maize MON818 in all 1994 field trials and maize MON820 in the 1995 field trials. No consistent compositional differences were observed between maize MON 810 and non-transgenic maize. In the updated risk assessment of maize MON 810 the VKM GMO Panel concludes that maize MON 810 is compositionally, agronomically and phenotypically equivalent to conventional maize varieties, except for the insect resistance conferred by the Cry1Ab protein (VKM 2013).

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

3.2.1 Experimental design & statistical analysis

Maize stack MON 88017 x MON 810 and the conventional control maize were grown at three replicated field sites in major maize-growing areas of the USA during the 2002 field season (Iowa, Illinois, Nebraska). Four commercially available maize hybrids were grown also at each of the same field sites to provide a total of 12 different reference varieties. At each field site, the test, control and reference seed were planted in a randomized complete block design with three replicates per block. All the plants were grown under normal agronomic field conditions for their respective geographic regions. All test plots received an application of Roundup® UltraMAX herbicide according to label directions.

Statistical analyses of the composition data were performed with the SAS7 statistical program. The maize compositional data for the test and control substances were statistically analyzed using a mixed model analysis of variance. The sites were analyzed separately and combined. For each compositional component, the forage and grain from the test substance was compared to the conventional control. A range of observed values from the reference substances were 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 commercial hybrids. Each tolerance interval estimate was based upon one observation per unique reference variety. Individual hybrids with multiple observations were summarized across sites to obtain a single estimate for inclusion in tolerance interval calculations. Because negative quantities are not possible, calculated negative lower tolerance bounds were set to zero.

3.3 Compositional Analysis

The composition of forage and grain produced by MON 88017 x MON 810 was compared to a non-transgenic control maize, as well as with other commercially available maize hybrids. Reference hybrids were grown in the same field locations and under the same conditions as the test and control. Where statistical differences occurred, the measured analyte was compared to a confidence interval developed from the reference hybrids. Differences were also compared to historical ranges and ranges reported in the literature.

The compounds selected for analysis in the compositional study were chosen on the basis of internationally accepted guidance provided by the OECD (OECD, 2002), in addition to other selected compounds. Forage samples were analyzed for proximates (protein, fat, ash, and moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), minerals (calcium,

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), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), vitamins (B1, B2, B6, E, niacin, and folic acid), anti-nutrients (phytic acid and raffinose), secondary metabolites (furfural, ferulic acid, and p-coumaric acid), and carbohydrates by calculation. In all, 77 different analytical components (nine in forage and 68 in grain) were analyzed.

Table 4 (Appendix) summarises results of the compositional analyses of MON 88017 x MON 810 for all sites combined, 2002 field season. Altogether a total of 248 statistical comparisons were made between MON 88017 x MON 810 and the non-transgenic control. Table 5 (Appendix) presents a summary of the statistically significant differences. Results of the forage and grain sample analysis showed that there were no statistically significant differences ($p < 0.05$) between MON 88017 x MON 810 and the conventional control for 216 of the 248 comparisons conducted. The 32 comparisons observed to be statistically different included calcium (one comparison) in forage and the following in grain: 16:1 palmitoleic acid, 18:1 oleic acid, 18:3 linolenic acid, glutamic acid, leucine, methionine, moisture, niacin, protein, vitamin B6, and vitamin B1 (one comparison each); 20:0 arachidic acid, alanine, ferulic acid, potassium, and vitamin B2 (two comparisons each); 18:2 linoleic acid, and copper (three comparisons each); and 20:1 eicosenoic acid (four comparisons). Five percent, or approximately 12 (0.05×248) comparisons, were expected to be statistically significant based upon chance alone.

The magnitude of the differences between the test and control values of the 32 comparisons observed to be statistically different ranged from 1.08% to 24.3%, with the greatest differences observed for copper (24.3%) in grain and calcium (20.2%) in forage. The 20:1 eicosenoic acid values were statistically lower in the test substance than the control substance in all four analyses (each individual site and the combination of all sites). However, the magnitude of these differences was small (6.6% to 8.7%). All test values were also within the 99% tolerance interval for the 32 comparisons observed to be statistically different between MON 88017 x MON 810 and the non-transgenic control.

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3.4 Agronomic and phenotypic characters

Field studies were conducted at four locations in the USA (Iowa, Missouri, Nebraska and Ohio) in the 2002 growing season to assess phenotypic and ecological characteristics of maize stack MON 88017 x MON 810 and its conventional counterpart (near-isogenic conventional maize). According to the applicant, the four test locations were selected to be representative of the range of environmental conditions under which the tested hybrid varieties would typically be grown. Sixteen conventional commercially available maize hybrids (four at each location) were included as reference substances to assess natural variation of plant characteristics between commercial maize varieties. Each of the agronomic trials was conducted as a randomized complete block design with three replications per location. Agricultural practices (pesticide and fertilizer applications) were typical for commercial maize production in the regions chosen for this study. The test plots were sprayed with the glyphosate-containing herbicide Roundup UltraMAX. In addition, the applicant planted a single, non-randomized replicated supplemental production with the test, control and reference hybrids.

Six developmental, agronomic and morphological characteristics were assessed at each location (Table 6, Appendix). In addition, grain weight and plant interactions with endemic insect, disease and abiotic stressors were observed throughout the growing season at all sites (data not shown).

Analyses of variance across trial locations showed no statistically significant differences between MON 88017 x MON 810 and the corresponding non-GM comparator (Table 7, Appendix). In the within-site analysis, various statistical differences between maize MON 88017 x MON 810 and the comparator were observed. However, each of these observed differences occurred only in one location and the values of the maize stack MON 88017 x MON 810 were within the natural range defined by the commercial reference varieties grown at the same locations. The data on biotic and abiotic stressors did not show any noticeable differences between test and control maize besides a difference in infestation of Western corn rootworm (WCR) at one location, which relates to the insect resistance trait introduced into MON 88017 and to the heterogeneous dispersal of WCR.

3.5 Conclusion

The applicant has performed comparative analyses of data from field trials located at representative sites and environments in USA during the 2002 growing season. With the exception of small intermittent variations and the insect resistance and herbicide tolerance conferred by the CP4 EPSPS, Cry3Bb1 and Cry1Ab proteins, the results showed no biologically relevant differences between maize stack MON 88017 x MON 810 and its conventional counterpart. Based on the assessment of available data, the VKM GMO Panel concludes that maize MON 88017 x MON 810 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 88017 and MON 810, have previously been evaluated by the VKM GMO Panel, and updated risk assessments were finalised in April 2016 and September 2013, respectively (VKM 2016, VKM 2013).

4.1 Summary of the previous evaluations of the single events

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.

Maize MON 810

Maize MON 810 has a long history of use, and has been evaluated extensively by the VKM GMO Panel. In the updated risk assessment (VKM 2013) it was concluded that MON 810 is nutritionally equivalent to conventional maize varieties and that it is unlikely that the Cry1Ab protein will introduce a toxic or allergenic potential in food or feed based on maize MON 810 compared to conventional maize.

With regard to animal studies with the whole product, feeding studies with maize MON 810 grain with different target animals, such as rats (Hammond et al 2006), Atlantic salmon (Sanden et al. 2005, Sanden et al. 2006, Sagstad et al. 2007; Hemre et al. 2007; Bakke-McKellep et al. 2008, Froystad-Saugen et al. 2009, Sissener et al. 2010;), dairy cows (Donkin et al. 2003), broiler (Taylor et al. 2003) and pigs (Buzoiau et al. 2012, Walsh et al. 2012 a,b), have all indicated nutritional equivalence between maize MON 810 and its non-GM maize counterpart and to conventional maize.

In a study performed by Sissener et al. (2011a) it was suggested that the effects observed in Atlantic salmon fed maize MON 810 probably could be related to the content of the mycotoxin Deoxynivalenol (DON) in the MON 810 ingredient (0.09 ppm). The Cry1Ab content was quantified in the maize MON 810 ingredient and was between 110-130 ng/g (Sanden et al. 2005). Cry1Ab protein has not been detected in any of the investigated Atlantic salmon feeds (Sanden et al. 2005, Jørgensen 2012).

4.2 Product description and intended uses

The genetic modification in MON 88017 x MON 810 field maize will not impact the existing production processes used for maize. All MON 88017 x MON 810 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 88017 x MON 810 field maize and all food, feed and processed products derived from MON 88017 x MON 810 field maize are expected to replace a portion of similar products from commercial maize, with total consumption of maize products remaining unchanged.

4.3 Effect of processing

Food manufacturing of MON 88017 x MON 810 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 Cry1Ab, Cry3Bb1 and CP4 EPSPS proteins and *cry1Ab,* 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% MON 810 maize flour, showed that the baking process sheared the DNA into small fragments, less than 1000 bp (Fernandes et al. 2013). It is emphasized that maize used for animal feed are often exposed to less harsh processing steps as compared to food manufacturing.

4.4 Toxicological assessment

In assessing the potential risks of GM foods and feed, it is important to consider both adverse health effects that may arise from substances that are intentionally introduced or modified in food 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 Cry1Ab, Cry3Bb1 and CP4 EPSPS in the risk assessments of the parental maize lines MON 88017 x MON 810 (VKM 2016, VKM 2013).

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 88017 x MON 810, which also considers health effects. 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 using an array of bioinformatic tools. Sequence homology searches comparing the structure of novel proteins to known IgE-allergens in a database are conducted using 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 Cry3Bb1, CP4 EPSPS and Cry1Ab proteins. These assessments have previously been described by the applicant for the parental maize events MON 88017 and MON 810 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:

Cry3Bb1, Cry1Ab and CP4 EPSPS

i) The sources of the transgene genes are *Bacillus thuringiensis* var. *kumamotoensis* (*cry3Bb1*-gene), *Bacillus thuringiensis* subsp. *kurstaki* (*cry1Ab*-gene) and *Streptomyces. viridochromogenes* (*cpr epsps* gene). These bacteria are not known to cause allergies.

ii) Cry proteins as microbial pesticides has a history of safe use (US EPA 2005, 2007, 2010), and there have been no indications of Cry proteins originating from

Bacillus thuringiensis having harmful effects on the health of humans and animals (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 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 MON 88017).

vi)   Likewise, the Cry1Ab protein is rapidly degraded in simulated gastric fluids *in vitro*, and expected to be degraded when digested (Monsanto technical dossier MON 810).

vii)   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).

viii)   CP4 EPSPS, Cry3Bb1, and Cry1Ab do not resemble any characteristics of known IgE-allergens, and no significant homologies between the amino acid sequences of the CP4 EPSPS Cry3Bb1, and Cry1Ab 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, Monsanto Technical Reports in Annex 3.5 - updated toxicity and allergenicity data - EFSA-GMO-RX-MON 810)

ix)   The CP4 EPSPS, Cry3Bb1, and Cry1Ab proteins are not glycosylated (Herouet et al. 2005, Raybould et al, 2013, US EPA 2007, US EPA 2010c)

x)   Cry3Bb1, Cry1Ab and CP4 EPSPS are considered heat labile (Herouet et al. 2005; US EPA 2007, Hammond et al. 2013, Review)

The information listed above indicates that the newly expressed proteins in maize MON 88017 x MON 810 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 the maize MON 88017 x MON 810 could be increased as an unintended effect after random insertion of the transgene in the genome of the recipient, e.g. through qualitative or quantitative modifications of endogenous protein expression. However, given that no biologically relevant agronomic or compositional changes have been identified in maize MON 88017 x MON 810 with the exception of the introduced traits, no increased allergenicity is anticipated for maize MON 88017 x MON10. Moreover, maize is not considered to be 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 88017 x MON 810 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 (CT) (Guerrero et al. 2004; Vazquez-Padron et al., 1999a, b; 2000; 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/feed

Compositional analyses of maize MON 88017 x MON 810 indicate nutritional equivalence to the non-GM control maize with comparable genetic background and to the published range of values in the literature. The nutritional equivalence between MON 88017 x MON 810 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). 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 Cry3Bb1, Cry1Ab and CP4 EPSPS proteins from maize MON 88017 x MON 810 is calculated to be 57.2 µg, 2.8 µg, and 27.3 µg, respectively, based on intake of maize staple (4.4 g/person/day) and the maximum protein levels in grain at physiological maturity, shown in Tables 1, 2 and 3 (Appendix). The corresponding numbers for children (6 month, intake of maize staple is 1.7 g/person/day) are 22.1 µg, 1.1µg and 10.5 µg for the Cry3Bb1, Cry1Ab and CP4 EPSPS respectively.

The estimated maximum daily intake for a Norwegian adult of Cry3Bb1, Cry1Ab and CP4 EPSPS proteins from sweet maize is calculated to be 210 µg, 9.5 µg and 96.3 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, and 3 (Appendix). 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 farm (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 88017 x MON 810 may be higher for these animals.

This dietary exposure assessment is very conservative as it assumes that all maize consumed comes from maize MON 88017 x MON 810 and that the transgenic proteins are not denatured by processing.

4.6.2 **Nutritional assessment of feed derived from the GM plant**

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 applicant has performed a 42-day broiler (commercial strain Ross x Ross 508) feeding study to compare the nutritional performance of maize MON 88017 x MON 810 (as well as maize MON 88017) with the conventional non-transgenic maize LH59 x LH198 (control) and five non-GM commercial maize varieties (Asgrow RX708, Pioneer 34B23, Burrus789, Burrus582, Burrus569) (Taylor *et al* 2005a,b). The non-transgenic maize LH59 x LH198 has a genetic background representative of MON 88017 x MON 810. There were eight treatment groups (one diet pr. maize) with 120 broilers randomly distributed and placed in pens in each group (960 broilers total): 5 pens of males (12 broilers/pen) and 5 pens of females (12 broilers/pen). On day 8 all birds within a pen were counted and the number of birds per pen was adjusted to 10. All birds removed on day 8 were healthy and they were selected arbitrarily (i.e. the first bird within reach).

The test (MON 88017 or MON 88017 x MON 810), control and referencediet mixtures were fed continuously for 42-days. Broilers were fed starter feed on trial days 1-21 (~54% maize), and grower/finisher feed on trial days 21-42 (~60%). Analyses of the starter and grower/finisher diets were conducted in compliance with EPA Good Laboratory Practice standards (40 CFR Part 160). The analyses confirmed the presence of the Cry1Ab, Cry3Bb1 and CP4 EPSPS proteins in the diets containing MON 88017 x MON 810. These proteins were not detected in control substances. Samples of maize grain lots were analysed for mycotoxins, pesticide, and nutrients prior to the start of the study. All measured levels of mycotoxins and pesticides were below the limits of concern for broiler performance. Statistical analyses were performed on starting and final live weights, feed consumption, feed conversion, adjusted feed conversion, carcass chill weight, percentage chill weight (chill weight/live weight), breast weight, percentage breast weight (breast weight/chill weight), wing weight, percentage wing weight (wing weight/chill weight), thigh weight, percentage

thigh weight (thigh weight/chill weight), drum weight, percentage drum weight (drum weight/chill weight), fat pad weight, percentage fat pad (fat pad/live weight), as well as moisture, protein, and fat values for breast and thigh meat. All percentage values were calculated by dividing the response variable by chill or live weight as appropriate. Statistical analysis (ANOVA) was carried out using a linear mixed model procedure of SAS software (SAS Institute Inc., 2000).

All performance parameters of broilers fed diets containing MON 88017 × MON 810 were similar to those fed the conventional control and commercial maize. No differences were observed in live weight at day 0, live weight at day 42, total feed intake, and feed conversion across all treatments. Body weight, daily weight gain (gram/bird/day) 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. A low incidence of mortality occurred among all study groups, which is consistent with historical data and type of study.

Comparison of the broilers fed MON 88017 × MON 810 diet to those receiving the other diets, showed no differences in performance parameters, carcass yields, and meat quality parameters of thigh moisture, protein, and fat and breast protein and moisture. Statistically significant differences were noted for breast fat when comparing the different diet groups. However, no differences were observed in the pairwise comparisons between treatment diets for the breast meat measurements. According to the applicant all of the observed statistically significant differences in the study were similar to values reported in the literature, and that maize MON 88017 x MON 810 is nutritionally equivalent to conventional maize varieties.

4.7 Conclusion

A whole food feeding study on broilers indicates no adverse health effects of maize MON 88017 x MON 810, and shows that it is nutritionally equivalent to conventional maize varieties. The Cry3Bb1, Cry1Ab 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 88017 x MON 810 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry3Bb1, Cry1Ab and CP4 EPSPS proteins will cause toxic or IgE-mediated allergic reactions to food or feed based on maize MON 88017 x MON 810 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 88017 x MON 810 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 pests provides a potential advantage in cultivation of MON 88017 x MON 810 under infestation conditions. It is considered very unlikely that maize MON 88017 x MON 810 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 88017 x MON 810 relative to its conventional counterpart. A series of field trials with maize MON 88017 x MON 810 were carried out by the applicant across four locations in the USA in 2002. Information on phenotypic (e.g. crop physiology, morphology, development) and agronomic characteristics was provided to assess the agronomic performance of maize MON 88017 x MON 810 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 88017 x MON 810, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of maize MON 88017 x MON 810 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 88017 x MON 810 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 88017 x MON 810. 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 88017 x MON 810 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 88017 x MON 810 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 88017 x MON 810 (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 88017 x MON 810 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 88017 was transformed to express the *cry3Bb1* gene from *Bacillus thuringiensis* subsp. *kumamotoensis.* The insecticidal toxin conferring 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)

The genetically modified maize MON 810 has been developed to provide protection against certain lepidopteran target pests, such as the European corn borer (ECB, *Ostrinia nubilalis*), and some species belonging to the genus *Sesamia.* The insect resistence is achieved through expression of the truncated *cry1Ab* gene derived from *Bacillus thuringiensis* subsp. *kurstaki,* a common soil bacterium.

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. has 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.

Considering the intended uses of maize MON 88017 x MON 810, 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 88017 x MON 810, 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 2009).

Data supplied by the applicant indicate that a limited amount of the Cry3Bb1 and Cry1Ab1 protein enters the environment due to the expression in the grains (mean values of 0.39 and 9.3 µg/g dwt, respectively). Data have been submitted that demonstrate that the Cry3Bb1 and Cry1Ab protein is rapidly degraded by gastric fluid *in vitro*.

Results from Icoz & Stotzcy (2008) indicate that Cry3Bb1 protein released in root exudates and from decaying plant residues of Bt corn, does not persist in soil and is degraded rapidly, suggesting that it probably poses little ecological or environmental risk. The persistence of the protein in soil amended with biomass of Bt corn (event MON863) was dependent on the type and amount of clay mineral present and on the pH of the soils. In general, the Cry3Bb1 protein persisted in the C, 3K, and 6K soils for ca. 40 days, whereas it persisted in the 3M and 6M soils for only 21 days, regardless of the amount of Bt biomass added.Cry3Bb1 protein was detected in rhizosphere soil unamended with Bt corn biomass (i.e., only released in root exudates) for only 14 days.

In conclusion, the VKM GMO Panel considers that the exposure of potentially non-target organisms to the 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 88017 x MON 810, 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.

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5.6 Conclusion

Considering the intended uses of maize MON 88017 x MON 810, 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 88017 x MON 810.

Maize MON 88017 x MON 810 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 88017 x MON 810. 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 88017 x MON 810 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 88017 x MON 810 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 88017 and MON 810 are retained in the stacked event MON 88017 x MON 810. Genetic stability of the inserts has previously been demonstrated in the single events. The levels of CP4 EPSPS, Cry3Bb1 and Cry1Ab 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 88017 x MON 810 satisfactory.

Comparative assessment

The applicant has performed comparative analyses of data from field trials located at representative sites and environments in USA during the 2002 growing season. With the exception of small intermittent variations and the insect resistance and herbicide tolerance conferred by the CP4 EPSPS, Cry3Bb1 and Cry1Ab proteins, the results showed no biologically relevant differences between maize stack MON 88017 x MON 810 and its conventional counterpart. Based on the assessment of available data, the VKM GMO Panel concludes that maize MON 88017 x MON 810 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 on broilers indicates no adverse health effects of maize MON 88017 x MON 810, and shows that it is nutritionally equivalent to conventional maize varieties. The Cry3Bb1, Cry1Ab 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 88017 x MON 810 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry3Bb1, Cry1Ab and CP4 EPSPS proteins will cause toxic or IgE-mediated allergic reactions to food or feed based on maize MON 88017 x MON 810 compared to conventional maize.

Environmental risk

Considering the intended uses of maize MON 88017 x MON 810, 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 88017 x MON 810.

Maize MON 88017 x MON 810 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 88017 x MON 810. 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 88017 x MON 810 is compositionally, nutritionally, agronomically and phenotypically equivalent to its conventional counterpart except for the new proteins. It is unlikely that the Cry3Bb1, Cry1Ab 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.

The VKM GMO Panel concludes that maize MON 88017 x MON 810, 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 using 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 residue levels on plants with engineered **r**esistance to one or two broad spectrum herbicides could entail higher levels of herbicide residue cocktails compared to plants produced by conventional farming practice.

Since it is difficult to predict the toxicity of cocktails from the toxicity of the single components, there is uncertainty related to risk of confounding effects such as additive or synergistic effects between the residues in herbicide resistant plants.

The transgene technology used can possibly lead to different metabolic products of the applied herbicides from what is expected from conventional usage. The risk assessment of herbicides should take into account plants with altered metabolism.

At present the changes related to herbicide residues of stacked plants as a result of the application of plant-protection products fall outside the remit of the Norwegian VKM Panels.

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Appendix I

Table 1. Summary of the level of the CP4 EPSPS protein in maize tissues collected from MON 88017 x MON 810 and MON 88017 produced in field trails in USA conducted in 2002

Tissue Type[2]	MON 88017 x MON 810		MON 88017[3]	
	Mean (SD) Range (μg/g fw)[4]	Mean (SD) Range (μg/g dw)[5]	Mean (SD) Range μg/g fw	Mean (SD) Range μg/g dw
Grain	3.8 (1.5)[6] 1.9 – 5.5[7]	4.3 (1.6) 2.2 – 6.2	5.1 (0.89) 3.7 – 6.3	5.8 (0.97) 4.1 – 7.1
Forage	15 (2.8) 11 – 21	51 (9.2) 38 – 70	16 (2.1) 12 – 19	57 (7.6) 42 – 69

1. The levels of CP4 EPSPS protein in tissue samples from the control hybrid H1200902 were below the LOQ for grain tissue (0.28 μg/g fw). The levels of CP4 EPSPS protein in tissue samples from the control hybrid H1200902 were below the LOD for forage tissue (0.18 μg/g fw).
2. Tissues were collected at the following growth stages:
    a. Grain: R6 (physiological maturity)
    b. Forage: early dent (R4 - R6)
3. The analyses of MON 88017 tissue samples were conducted at the same time and are reported in a separate study (Bhakta *et al.*, 2003a).
4. Protein levels are expressed as microgram (μg) of protein per gram (g) of tissue on a fresh weight (fw) basis and are corrected for method bias.
5. Protein levels are expressed as μg/g on a dry weight (dw) basis. The dry weight values were calculated by dividing the fw values by the dry weight conversion factors obtained from moisture analysis data.
6. The mean and standard deviation were calculated across sites (n=9).
7. Minimum and maximum values were determined for each tissue type across sites.

Table 2. Summary of the level of the Cry3Bb1 protein in maize tissues collected from MON 88017 x MON 810 and MON 88017 produced in field trails in USA conducted in 2002.

Tissue Type[2]	MON 88017 x MON 810		MON 88017[3]	
	Mean (SD) Range (µg/g fw)[4]	Mean (SD) Range (µg/g dw)[5]	Mean (SD) Range µg/g fw	Mean (SD) Range µg/g dw
OSR-1	37 (16)[6] 8.8 – 56[7]	350 (150) 88 – 560	39 (8.1) 24 – 51	370 (80) 240 – 510
OSL-1	90 (18) 65 – 120	670 (130) 550 – 920	76 (23) 28 – 110	570 (170) 230 – 820
Pollen	16 (3.5) 0.020[8] – 19	27 (5.7) N/A[9] – 34	14 (2.5) 11 – 20	25 (4.2) 17 – 32
Grain	8.2 (3.0) 3.3 – 12	9.3 (3.4) 3.9 – 13	13 (3.1) 8.7 – 19	15 (3.6) 10 – 22
Forage	29 (6.8) 20 – 43	100 (23) 71 – 150	27 (5.5) 22 – 39	95 (19) 75 – 130
Forage Root	21 (2.3) 16 – 24	140 (29) 89 – 180	21 (3.1) 17 – 27	130 (29) 98 – 170

1. The levels of MON 88017 Cry3Bb1 protein in tissue samples from the control hybrid H1200902 were below the LOQ for OSL-1, grain, forage, and forage root tissues (0.044, 0.051, 0.047, and 0.040 µg/g fw, respectively) and below the LOD for OSR-1 and pollen tissues (0.032 and 0.020 µg/g fw, respectively).
2. Tissues were collected at the following growth stages:
   a. OSR-1: V2 - V3
   b. OSL-1: V2 – V3
   c. Pollen: R1
   d. Forage: early dent (R4 - R6)
   e. Grain: R6 (physiological maturity)
   f. Forage Root: early dent (R4 - R6)
3. The analyses of MON 88017 tissue samples were conducted at the same time and are reported in a separate study (Bhakta *et al.*, 2003a).
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fw) basis and are corrected for method bias.
5. Protein levels are expressed as µg/g on a dry weight (dw) basis. The dry weight values were calculated by dividing the fw by the dry weight conversion factors obtained from moisture analysis data.
6. The mean and standard deviation were calculated across sites (n=9).
7. Minimum and maximum values were determined for each tissue type across sites.
8. The level of Cry3Bb1 for one of the nine replicates was below the LOD (0.020 µg/g fw).
9. Protein levels ≤ LOD on a fw basis are not reported on a dw basis.

Table 3. Summary of the level of the Cry1Ab protein in maize tissues collected from MON 88017 x MON 810 and MON 810 produced in field trails in USA conducted in 2002

| Tissue Type[2] | MON 88017 x MON 810 | | MON 810 | |
	Mean (SD) Range (µg/g fw)[4]	Mean (SD) Range (µg/g dw)[5]	Mean (SD) Range µg/g fw	Mean (SD) Range µg/g dw
OSL-1	15 (2.5)[5] 10 – 18[6]	110 (17) 85 – 140	14 (1.4) 11 – 16	100 (12) 89 – 130
Pollen	0.090[7] N/A	N/A N/A	0.090[7] N/A	N/A N/A
Grain	0.34 (0.11) 0.13 – 0.54	0.39 (0.13) 0.16 – 0.63	0.38 (0.078) 0.24 – 0.48	0.43 (0.091) 0.27 – 0.54
Forage	3.9 (0.65) 3.2 – 5.0	14 (2.1) 11 – 17	4.2 (1.0) 2.3 – 5.5	14 (3.4) 8.4 – 19

1. The levels of Cry1Ab protein in tissue samples from the control hybrid H1200902 were below the LOD for OSL-1, pollen, forage and grain tissues (1.1, 0.090, 0.26, and 0.13 µg/g fw, respectively).
2. Tissues were collected at the following growth stages:
   a. OSL-1: V2 – V3
   b. Pollen: R1
   c. Grain: R6 (physiological maturity)
   d. Forage: early dent (R4 - R6)
3. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fw) basis and are corrected for method bias.
4. Protein levels are expressed as µg/g on a dry weight (dw) basis. The dry weight values were calculated by dividing the fw values by the dry weight conversion factors obtained from moisture analysis data.
5. The mean and standard deviation were calculated across sites (n=9).
6. Minimum and maximum values were determined for each tissue type across sites.
The level of Cry1Ab for all samples from all sites was below the LOD (0.090 µg/g fw).

Table 4. Compositional analysis of MON 88017 x MON 810 compared to control and commercial varieties 2002 USA field trials.

Tissue/Component (Units)[2]	MON 88017 x MON !10 Mean[3]	Range	Control Mean[3]	Range	Commercials[1] 99% T.I.[4]	Literature range[5]	Historical range[6]
Forage							
Fibre (% dw)							
ADF	24.51	23.30-26.33	25.45	23.34-28.13	[13.95, 38.96]	18.3-41.0[b] ; 17.5-38.3[a]	17.6-36.7
NDF	36.60	32.58-44.41	38.33	35.86-41.18	[23.80, 54.73]	26.4-54.5[b]; 27.9-54.8[a]	29.6-55.2
Proximates							
Ash (% dw)	4.10	3.18-4.88	4.04	3.59-4.67	[0.72, 7.42]	2.43-9.64[a]; 2-6.6[b]	2.03-8.23
Carbohydrates (% dw)	85.91	83.22-87.75	86.48	84.43-87.71	[78.70, 93.43]	83.2-91.6[b]; 76.5-87.3[a]	80.6-90.8
Total fat (% dw)	1.77	0.87-2.97	1.65	0.83-2.97	[0.80, 2.95]	0.35-3.62[b]; 1.42-4.57[a]	0.61-4.02
Moisture (% fw)	70.47	68.70-72.10	70.66	69.10-72.70	[59.37, 80.83]	56.5-80.4[a] ; 55.3-75.3[b]	42-78.8
Protein (% dw)	8.22	7.06-8.93	7.82	6.79-8.54	[4.17, 11.81]	4.98-11.56[a]	3.86-11.0
Minerals (% dw)							
Calcium	0.21	0.18-0.24	0.23	0.18-0.31	[0.11, 0.32]	0.0969-0.3184[b]	0.0866-0.2754
Phosphorus	0.26	0.22-0.30	0.25	0.20-0.30	[0.095, 0.38]	0.1367-0.2914[b]	0.1602-0.2914
Grain							
Amino acids (% of total aa)							
Alanine	7.69*	7.53-7.94	7.55	7.34-7.79	[6.66, 8.49]	6.4-9.9[e] (% tot. prot.)	7.06-8.19
Arginine	4.28	3.95-4.55	4.29	4.01-4.63	[3.34, 5.67]	2.9-5.9[e] (% tot. prot.)	3.49-5.48
Aspartic acid	6.14	6.05-6.24	6.25	6.04-6.45	[5.77, 7.16]	5.8-7.2[e] (% tot. prot.)	5.97-7.36
Cystine	2.15	1.99-2.30	2.15	1.93-2.30	[1.46, 2.89]	1.2-1.6[e] (% tot. prot.)	1.61-2.94
Glutamic acid	20.61	20.29-21.12	20.44	19.91-20.84	[18.01, 22.15]	12.4-19.6[e] (% tot. prot.)	17.3-20.4
Glycine	3.42	3.32-3.56	3.45	3.18-3.61	[2.81, 4.54]	2.6-4.7[e] (% tot. prot.)	3.22-4.91
Histidine	2.96	2.89-3.06	2.95	2.83-3.14	[2.16, 3.60]	2.0-2.8[e] (% tot. prot.)	2.46-3.35
Isoleucine	3.55	3.41-3.73	3.57	3.45-3.76	[3.30, 3.84]	2.6-4.0[e] (% tot. prot.)	2.95-4.08
Leucine	13.49	13.12-13.96	13.31	12.76-14.11	[10.72, 15.18]	7.8-15.2[e] (% tot. prot.)	11.2-14.6
Lysine	2.62	2.50-2.79	2.66	2.49-2.82	[2.06, 3.73]	2.0-3.8[e] (% tot. prot.)	2.35-4.18
Methionine	2.08	2.00-2.22	2.01	1.83-2.20	[1.37, 2.60]	1.0-2.1[e] (% tot. prot.)	1.61-2.89
Phenylalanine	5.12	4.93-5.37	5.14	5.01-5.32	[4.57, 5.71]	2.9-5.7[e] (% tot. prot.)	4.6-5.76
Proline	9.23	9.04-9.48	9.34	8.85-9.80	[7.60, 10.37]	6.6-10.3[e] (% tot. prot.)	8.03-9.9
Serine	4.84	4.61-5.00	4.91	4.63-5.13	[4.60, 5.43]	4.2-5.5[e] (% tot. prot.)	3.45-5.63
Threonine	3.23	2.98-3.41	3.25	3.06-3.37	[2.89, 3.84]	2.9-3.9[e] (% tot. prot.)	2.87-4.01
Tryptophan	0.55	0.49-0.60	0.55	0.41-0.68	[0.36, 0.77]	0.5-1.2[e] (% tot. prot.)	0.39-1.04
Tyrosine	3.22	2.24-3.71	3.43	2.58-3.66	[2.62, 4.26]	2.9-4.7[e] (% tot. prot.)	1.93-4.32
Valine	4.82	4.63-5.07	4.74	4.60-4.94	[4.22, 5.27]	2.1-5.2[e] (% tot. prot.)	3.94-5.46

Table 4. Cont.

| Tissue/Component[2] | MON 88017 x MON 810 | | Control | | Commercials[1] | | |
	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	Historical range[6]
Grain – continued							
Fatty acids (% of total fa)							
16:0 palmitic acid	10.68	10.49-10.89	11.27	10.14-14.57	[6.51, 16.50]	7-19[e] (% total fat)	8.41-12.5
16:1 palmitoleic acid	0.19	0.17-0.21	0.18	0.16-0.22	[0.0017, 0.28]	1[e] (% total fat)	0.05-0.18
18:0 stearic acid	2.03	1.88-2.26	2.07	1.76-2.23	[1.41, 2.53]	1-3[e] (% total fat)	1.33-2.61
18:1 oleic acid	22.33	21.92-22.82	22.87	21.43-23.51	[9.25, 44.14]	20-46[e] (% total fat)	20.1-37.7
18:2 linoleic acid	62.78*	62.06-63.48	61.52	59.10-63.18	[41.22, 74.09]	35-70[e] (% total fat)	48.0-66.1
18:3 linolenic acid	1.22	1.17-1.29	1.32	1.19-1.77	[0.42, 1.95]	0.8-2[e] (% total fat)	0.74-1.45
20:0 arachidic acid	0.39*	0.37-0.41	0.38	0.35-0.41	[0.31, 0.49]	0.1-2[e] (% total fat)	0.31-0.56
20:1 eicosenoic acid	0.23*	0.21-0.25	0.25	0.24-0.26	[0.18, 0.40]	-	0.15-0.44
22:0 behenic acid	0.15	0.13-0.16	0.15	0.14-0.17	[0.071, 0.25]	-	0.075-0.3
Fibre (% dw)							
ADF	3.80	3.28-4.36	3.54	2.97-4.69	[1.89, 5.23]	3.3-4.3[d]; 2.46-11.34[a, b]	2.3-9.33
NDF	11.98	10.65-13.54	11.87	10.38-14.29	[3.51, 21.65]	8.3-11.9[d]; 7.58-15.91[b]	6.88-18.1
TDF	15.71	12.91-17.90	15.40	13.18-17.84	[5.72, 27.10]	10.99-11.41[h]	-
Minerals							
Calcium (% dw)	0.0054	00046-0.0063	0.0058	0.0049-0.0069	[0.0017, 0.0062]	0.01-0.1[d]	0.0024-0.0089
Copper (mg/kg dw)	1.68*	1.54-1.84	1.99	1.64-2.63	[0.17, 3.00]	0.9-10[d]	0.98-3.43
Iron (mg/kg dw)	22.06	21.08-22.85	21.84	20.31-23.93	[12.60, 31.26]	1-100[d]	10.4-30.7
Magnesium (% dw)	0.14	0.13-0.14	0.14	0.13-0.16	[0.088, 0.16]	0.09-1[d]	0.082-0.16
Manganese (mg/kg dw)	9.02	8.02-10.07	9.37	7.55-10.44	[2.45, 10.60]	0.7-54[d]	3.2-9.89
Phosphorus (% dw)	0.39	0.37-0.41	0.39	0.36-0.43	[0.24, 0.44]	0.26-0.75[d]	0.24-0.46
Potassium (% dw)	0.39*	0.37-0.41	0.42	0.38-0.47	[0.27, 0.48]	0.32-0.72[d]	0.29-0.53
Zinc (mg/kg dw)	24.04	22.76-25.48	24.92	22.02-27.18	[13.42, 31.37]	12-30[d]	14.1-37.2
Proximates (% dw)							
Ash	1.55	1.19-1.86	1.59	1.23-1.97	[0.94, 1.73]	1.1-3.9[d]; 0.89-6.28[b]	0.81-3.09
Carbohydrates	81.86	80.61-82.57	82.33	80.67-83.62	[79.39, 89.67]	77.4-87.2[b]; 82.2-88.1[a]	79.8-89.6
Total fat	3.88	3.60-4.12	3.79	3.53-4.36	[0.74, 6.01]	3.1-5.7[d]; 2.48-4.81[b]	1.74-4.83
Moisture	10.95	9.48-12.70	11.60	9.73-14.20	[4.67, 17.56]	7-23[d]; 8.18-26.2[b]	6.07-24.7
Protein	12.72	12.10-13.75	12.28	11.22-13.82	[6.20, 15.35]	6-12[d]; 9.7-16.1[c]	6.15-14.8

Table 4. Cont.

Tissue/Component[2]	MON 88017 x MON 810		Control		Commercials[1]	Literature range[5]	Historical range[6]
	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]		
Grain – continued							
Vitamin (mg/kg dw)							
Folic acid	0.43	0.37-0.50	0.48	0.42-0.59	[0.12, 0.77]	0.3[d]	0.33-0.75 (µg/g dw)
Niacin	21.06	16.84-24.07	21.75	19.08-23.92	[3.19, 34.49]	9.3-70[d]	-
Vitamin B1	3.09	2.71-3.37	3.24	2.99-3.60	[1.96, 4.38]	3-8.6[e]	0.2-0.33 (mg/100 g dw)
Vitamin B2	1.02*	0.85-1.17	1.13	0.99-1.33	[0.67, 1.51]	0.25-5.6[e]	0.83-1.74 (µg/g dw)
Vitamin B6	6.89	6.11-7.94	7.10	5.65-8.54	[4.29, 7.84]	5.3[d]; 9.6[e]	-
Vitamin E	13.42	5.52-16.48	14.07	1.74-17.77	[0, 29.69]	3-12.1[e]; 17-47[d]	0.005-0.037 (mg/g dw)
Antinutrient (% dw)							
Phytic acid	0.85	0.50-1.00	0.89	0.72-1.03	[0.28, 1.12]	0.48-1.12[a]	0.42-1.37
Raffinose	0.18	0.15-0.22	0.17	0.14-0.23	[0, 0.32]	0.08-0.30[e]	0.027-0.21
Secondary metabolite (µg/g dw)							
Ferulic acid	2306.33*	2098.98-2491.54	2121.05	1927.55-2339.71	[1415.19, 3173.90]	113-1194[f]; 3000[g]	0.17-0.28 (% dw)
p-coumaric acid	143.16	121.14-158.62	154.83	141.41-173.24	[43.13, 384.34]	22-75[f]	0.011-0.028 (% dw)

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

[1] 12 commercial maize hybrids.
[2] dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre, TDF = total dietary fibre, Vitamin B1= Thiamine, Vitamine B2 = Riboflavin, Vitamin B6 = Pyridoxine
[3] The mean of nine 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., 2002c; [b] Sidhu et al., 2000a; [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] Historical range from control samples (in some cases including commercial hybrid values) analyzed in previous Monsanto Company studies (George et al., 2003b; McCann et al., 2001; Ridley et al., 2002a; Ridely et al., 2003; Ridley et al., 2001a; Ridley et al., 2001b; Ridley et al., 2002c; Sidhu, 1999; Sidhu et al., 2000a; Sidhu et al., 1999; Sidhu and Lee, 1999; Sidhu et al., 2000b).

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 5. Summary of the statistical differences for the compositional comparison of MON 88017 x MON 810 to control maize - 2002 USA field trials.

Tissue/Site/ Component (Units)[a]	Mean MON 88017 x MON 810	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 88017- MON 810 (Range)	99% Tolerance Interval[b]
Forage						
IA						
Calcium	0.21	0.27	-20.17	0.026	(0.19-0.24)	[0.11,0.32]
Grain						
IA						
18:2 linoleic (% total fa)	63.23	60.41	4.68	0.017	(63.07-63.48)	[41.22,74.09]
18:3 linolenic (% total fa)	1.26	1.57	-19.84	0.038	(1.24-1.29)	[0.42,1.95]
20:0 arachidic (% total fa)	0.38	0.36	5.00	0.003	(0.37-0.38)	[0.31,0.49]
20:1 eicosenic (% total fa)	0.22	0.24	-8.72	<0.001	(0.21-0.22)	[0.18,0.40]
Alanine (% total aa)	7.58	7.36	3.01	0.001	(7.53-7.63)	[6.66,8.49]
Copper (mg/kg dw)	1.58	1.95	-18.90	0.009	(1.54-1.62)	[0.17,3.00]
Glutamic acid (% total aa)	20.36	20.02	1.71	0.018	(20.29-20.44)	[18.01,22.15]
Leucine (% total aa)	13.16	12.91	1.94	0.037	(13.12-13.24)	[10.72,15.18]
Protein (% dw)	12.26	11.51	6.54	0.048	(12.10-12.42)	[6.20,15.35]
Vitamin B6 (mg/kg dw)	6.49	7.56	-14.18	0.023	(6.11-7.01)	[4.29,7.84]
IL						
18:1 oleic (%total fa)	22.34	23.29	-4.09	<0.001	(22.26-22.6)	[9.25,44.14]
18:2 linoleic (% total fa)	62.82	62.15	1.08	0.019	(62.39-63.08)	[41.22,74.09]
20:1 eicosenic (% total fa)	0.23	0.25	-6.61	0.044	(0.22-0.24)	[0.18,0.40]
Ferulic acid (μg/g dw)	2363.76	2140.23	10.44	0.023	(2217.14-2491.54)	[1415.19,3173.90]
Moisture (% fw)	12.17	13.87	-12.26	0.004	(11.30-12.70)	[4.67,17.56]
Niacin (mg/kg dw)	21.06	22.52	-6.50	0.012	(20.73-21.53)	[3.19,34.49]
Potassium (% dw)	0.37	0.41	-9.06	0.005	(0.37-0.38)	[0.24,0.48]

Table 5. Cont.

Tissue/Site/ Component (Units)[a]	Mean MON 88017 x MON 810	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 88017-MON 810 (Range)	99% Tolerance Interval[b]
Grain						
NE						
16:1 palmitoleic (% total fa)	0.20	0.17	12.52	0.007	(0.19-0.20)	[0.0017,0.28]
20:1 eicosenic (% total fa)	0.23	0.25	-8.37	0.046	(0.23-0.25)	[0.18,0.40]
Copper (mg/kg dw)	1.67	2.21	-24.29	0.043	(1.56-1.74)	[0.17,3.00]
Methionine (% total aa)	2.06	1.88	9.56	0.022	(2.03-2.12)	[1.37,2.60]
Vitamin B_1 (mg/kg dw)	3.22	3.56	-9.54	0.016	(3.14-3.37)	[1.96,4.38]
Vitamin B_2 (mg/kg dw)	1.03	1.24	-16.65	0.031	(0.85-1.17)	[0.67,1.51]
Combination of all sites						
18:2 linoleic (% total fa)	62.78	61.52	2.06	0.046	(62.06-63.48)	[41.22,74.09]
20:0 arachidic (% total fa)	0.39	0.38	2.81	0.002	(0.37-0.41)	[0.31,0.49]
20:1 eicosenic (% total fa)	0.23	0.25	-7.90	<0.001	(0.21-0.25)	[0.18,0.40]
Alanine (% total aa)	7.69	7.55	1.82	0.012	(7.53-7.94)	[6.66,8.49]
Copper (mg/kg dw)	1.68	1.99	-15.73	0.031	(1.54-1.84)	[0.17,3.00]
Ferulic acid (μg/g dw)	2306.33	2121.05	8.74	<0.001	(2098.98-2491.54)	[1415.19,3173.90]
Potassium (% dw)	0.39	0.42	-6.79	0.022	(0.37-0.41)	[0.27,0.48]
Vitamin B_2 (mg/kg dw)	1.02	1.13	-9.62	0.026	(0.85-1.17)	[0.67,1.51]

[a] dw=dry weight; fw=fresh weight; aa=amino acids; fa=fatty acids
[b] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.
Site codes : IA: Iowa ; IL : Illinois ; NE : Nebraska

Table 6. Phenotypic characteristics measures at each test site

Characteristic	Evaluation Timing	Evaluation Description
Seedling vigor	Stage V2-V3	Rated on a 1-9 scale, where 1-3 = excellent vigor, 4-6 = average vigor and 7-9 = poor vigor
Early stand count	Stage V2-V3	Number of emerged plants per plot (tillers excluded)
Days to 50% pollen shed	Pollen shed	Days from planting until 50% of plants had begun to shed pollen
Days to 50% silking	Silking	Days from planting until 50% of the plants had silks exposed
Stay green	Stage R6 (Maturity)	Rated on a 1-9 scale, where 1 = 100% of leaves are green, 5 = all leaves above the ear are green, and 9 = 100% of the leaves are brown (dead)
Plant height	At the conclusion of hand pollination	Distance (cm) from the soil surface at the base of the plant to the flag leaf collar (or attachment) (average from five representative plants per plot)

Table 7. Comparison of phenotypic characteristics of maize stack MON 88017 x MON 810 to the conventional control- across site analysis.

	Least Square Means[1]		99% Tolerance Interval[2]		Reference Range[3]	
	MON 88017 x MON 810	Control	Lower Limit	Upper Limit	Minimum	Maximum
Phenotypic characteristic	51.0	52.7	27.0	79.1	37.5	61.3
Early stand count (no./plot)	77.8	76.2	40.8	110.2	63.7	91.2
Plant height (cm)	15.75	16.26	0.0	32.26	6.86	20.32
Stay green[4]	63.6	64.7	53.4	72.8	59.3	67.0
Days to 50% pollen shed	63.8	64.6	53.9	71.8	58.3	66.0
Days to 50% silk	2.0	2.1	0.8	3.5	2.0	3.0
Seedling vigor[5]	51.0	52.7	27.0	79.1	37.5	61.3

* Indicates a statistically significant difference between the test and control (p≤ 0.05) - None detected.

[1] Least square means of three replicates, across site analysis.

[2] 99% Tolerance interval with 95% confidence calculated from three replications of 16 (four references x four sites) commercially available, conventional maize reference substances. Negative lower limits adjusted to zero. Lower limit of seedling vigor adjusted upward to nearest practical value based on rating scale.

[3] Minimum and maximum values from three replications of 16 (four references x four sites) commercially available, conventional maize reference substances.

[4] Stay green ratings: rated on a 1-9 scale, where 1 = 100% of leaves are green, 5 = all leaves above the ear are green, and 9 = 100% of the leaves are brown (dead).

[5] Seedling vigor ratings: rated on a 1-9 scale, where 1-3 = excellent vigor, 4-6 = average vigor and 7-9 = poor vigor.