Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

VKM Report 2016: 12

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

Scientific opinion on insect-resistant and herbicide tolerant, genetically modified maize MON 88017 from Monsanto for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (EFSA/GMO/CZ/2005/27)

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

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2016: 12
Final health and environmental risk assessment of genetically modified maize MON 88017.

Opinion of the Panel on Genetically Modified Organisms of the Norwegian Scientific Committee for Food Safety
8.04.2016

ISBN: 978-82-8259-202-4

Norwegian Scientific Committee for Food Safety (VKM)
Po 4404 Nydalen
N – 0403 Oslo
Norway

Phone: +47 21 62 28 00
Email: vkm@vkm.no

www.vkm.no
www.english.vkm.no

Suggested citations: VKM (2016) Final health and environmental risk assessment of genetically modified maize MON 88017. Scientific opinion on insect-resistant and herbicide tolerant genetically modified maize MON 88017 from Monsanto for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (Application EFSA/GMO/CZ/2005/27). Opinion of the Panel on Genetically Modified Organisms of the Norwegian Scientific Committee for Food Safety, ISBN: 978-82-8259-202-4, Oslo, Norway.

Scientific opinion on insect-resistant and herbicide tolerant, genetically modified maize MON 88017 from Monsanto for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (Application EFSA/GMO/CZ/2005/27)

Authors preparing the draft opinion

Åshild Kristine Andreassen (Chair), Per Brandtzæg, Hilde-Gunn Sorteberg, Askild Holck, Olavi Junttila, Heidi Sjursen Konestabo, Richard Meadow, Kåre M. Nielsen, Rose Vikse, Anne-Marthe Jevnaker (VKM staff), Ville Erling Sipinen (VKM staff), and Merethe Aasmo Finne (VKM staff).

Assessed and approved

Åshild Kristine Andreassen (Chair), Per Brandtzæg, Hilde-Gunn Sorteberg, Askild Holck, Olavi Junttila, Heidi Sjursen Konestabo, Richard Meadow, Kåre M. Nielsen, Rose Vikse.

Acknowledgment

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

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

Competence of VKM experts

Experts 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

Table of Contents

Summary

In preparation for a legal implementation of 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 from Monsanto (Unique Identifier DAS-MON 88017-7) was approved in the EU under Regulation (EC) No 1829/2003 for food and feed uses, import and processing the 30th of October 2009 (Commission Decision 2009/814/EC).

Genetically modified maize MON 88017 has previously been risk assessed by the VKM Panel on Genetically Modified Organisms (GMO), commissioned by the Norwegian Food Safety Authority and the Norwegian Environment Agency related and to the EFSA public hearing of the applications EFSA/GMO/CZ/2005/27 and EFSA/GMO/CZ/2008/54 in 2007 and 2010 (VKM 2007a, 2010a). In addition, MON 88017 has been evaluated by the VKM GMO Panel as a component of several stacked GM maize events and Regulation (EC) 1829/2003 (VKM 2007b, VKM 2008, VKM 2009, VKM 2010b).

The food/feed and environmental risk assessment of the maize MON 88017 is based on information provided by the applicant in the applications EFSA/GMO/UK/2005/27 and EFSA/CZ/2008/CZ/2008/54, 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 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 2010a), 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 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, interactions between the GM plant and target and non-target organisms, effects on biogeochemical processes. It is emphasised that the VKM mandate does not include assessments of contribution to sustainable development, societal utility and ethical considerations, according to the Norwegian Gene Technology Act and Regulations relating to impact assessment pursuant to the Gene Technology Act. These considerations are therefore not part of the risk assessment provided by the VKM Panel on Genetically Modified Organisms.

Genetically modified maize MON 88017 expresses a 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).

Molecular characterisation

The molecular characterisation data has established that only one copy of the transgene is integrated in the maize genomic DNA. Appropriate analyses of the integration site including sequence determination of the inserted DNA and flanking regions and bioinformatics analysis have been performed. Bioinformatics analyses of junction regions have demonstrated the absence of any potential new ORFs coding for known toxins or allergens. The genetic stability of transformation event MON 88017 was demonstrated at the genomic level over multiple generations by Southern analysis. Segregation analysis shows that event MON 88017 is inherited as a dominant, single locus trait. The VKM GMO Panel considers the molecular characterisation of maize MON 88017 satisfactory.

Comparative assessment

Comparative analyses of maize MON 88017 and its conventional counterpart have been performed during field trials located at representative sites and environments in Europe and USA. A total of 12-16 different conventional maize varieties were included in the field trials and used as references. With the exception the insect resistance and herbicide tolerance conferred by the Cry3Bb1 and CP4 EPSPS proteins, no biologically relevant differences were found between maize MON 88017 and controls. Based on the assessment of available data, the VKM GMO Panel concludes that maize MON 88017 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart except for the new proteins.

Food and feed safety assessment

Whole food feeding studies on rats and broilers indicate no adverse health effects of maize MON 88017. These studies also show that maize MON 88017 is nutritionally equivalent to conventional maize. The Cry3Bb1 and CP4 EPSPS proteins do not show relevant sequence resemblance to other known toxins or IgE-allergens, nor have they been reported to cause IgE-mediated allergic reactions. However, some studies have indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

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

Environmental risk assessment

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

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

Overall conclusion

Based on current knowledge, the VKM GMO Panel concludes that maize MON 88017 is compositionally, nutritionally, agronomically and phenotypically equivalent to its conventional counterpart except for the new proteins. 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.

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

Keywords

Maize, *Zea mays* L., genetically modified maize MON 88017, EFSA/GMO/CZ/2005/27, insect-resistance, herbicide-tolerance, *cry3Bb1, cp4 epsps*, glyphosate, food/feed safety assessment, environmental risk assessment, Regulation (EC) No 1829/2003, Directive 2001/18.Maize.

Norsk sammendrag

I forbindelse med forberedelse til implementering av forordning 1829/2003 i norsk rett, er Vitenskapskomiteen for mattrygghet (VKM) bedt av Miljødirektoratet (tidligere Direktoratet for naturforvalting (DN)) 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 fra Monsanto (unik kode DAS-MON 88017-7) ble godkjent i EU til import, videreforedling og til bruk som mat og fôr under forordning 1829/2003 den 30. oktober 2009 (søknad EFSA/GMO/CZ/2005/27, Kommisjonsbeslutning 2009/814/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). En søknad om godkjenning av MON 88017 til dyrking (EFSA/GMO/CZ/2008/54), som var på offentlig høring høsten 2008, er også vurdert av faggruppen med hensyn på mulig miljørisiko (VKM 2010a). VKMs faggruppe for GMO har også risikovurdert en rekke maishybrider der MON 88017 inngår som en av foreldrelinjene (VKM 2007b, VKM 2008, VKM 2009a,b, VKM 2010b). Risikovurderingen av den genmodifiserte maislinjen er basert på uavhengige vitenskapelige publikasjoner og dokumentasjon som er gjort tilgjengelig på EFSAs nettside EFSA 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.

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.

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

Molekylær karakterisering

Adekvate analyser av det transgene DNA-innskuddet, dets integreringssete, antall integreringer og flankerende DNA-sekvenser i mais-genomet, har blitt utført. Resultatene viser at kun ett transgent innskudd er til stede i mais MON 88017. Homologisøk i databaser over kjente toksiner og allergener indikerer at genmodifiseringen ikke har ført til potensiell produksjon av skadelige proteiner eller polypeptider i mais MON 88017. Southern blot og segresjonsanalyser viser at de introduserte genene *cry3Bb1* og *cp4 epsps* er stabilt uttrykt og nedarvet over flere generasjoner, og i samsvar med de fenotypiske karakterene til mais MON 88017. VKMs faggruppe for genmodifiserte organismer vurderer den molekylære karakteriseringen av mais MON 88017 som tilfredsstillende.

Komparative analyser

Komparative analyser av mais MON 88017 og konvensjonell kontroll har blitt utført av søker i feltforsøk i representative områder for maisdyrking i USA og Europa. Totalt 12-16 forskjellige konvensjonelle maissorter var inkludert i de ulike feltforsøkene og brukt som referanse. Med unntak av insektsresistens og herbicidtoleranse mediert av Cry3Bb1 og CP4 EPSPS proteinene, viste resultatene ingen biologisk relevante forskjeller mellom mais MON 88017 og kontroll. Basert på vurderingen av tilgjengelige data, konkluderer VKMs faggruppe for GMO at mais MON 88017 er vesentlig lik konvensjonell kontroll med hensyn til næringsstoffsammensetning og agronomiske og fenotypiske egenskaper, med unntak av de nye proteinene.

Helserisiko

Fôringsstudier utført på rotter og broilere indikerer ikke helseskadelige effekter av mais MON 88017. Studiene viser også at MON 88017 er ernæringsmessig vesentlig lik konvensjonell mais. Proteinene Cry3Bb1 og CP4 EPSPS viser ingen relevante sekvenslikheter med andre kjente toksiner eller IgE-avhengige allergener, og er heller ikke rapportert å ha forårsaket IgE-medierte allergiske reaksjoner. Enkelte studier har derimot indikert at Cry-proteiner potensielt kan forsterke allergiske reaksjoner (fungere som adjuvans).

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

Miljørisiko

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

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

Samlet vurdering

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

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

Abbreviations and explanations

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

Cry3	A class of *Bacillus thuringiensis* crystal proteins with insecticidal activity against coleopteran species.
Cry3Bb1	Coding sequence for the Cry3Bb1 protein
Cry3Bb1	Protein with activity against coleopteran insects, produced by *B. thuringiensis* subsp. *kumamotoensi.*
CTP	Chloroplast transit peptide
DAP	Days after planting
DNA	Deoxyribonucleic acid
DT50	Time to 50% dissipation of a protein in soil
DT90	Time to 90% dissipation of a protein in soil
dw	Dry weight
dwt	Dry weight tissue
EC	European Commission
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	Environmental risk assessment
E-score	Expectation score
EU	European Union
fa	Fatty acid
FAO	Food and Agriculture Organisation
FIFRA	US EPA Federal Insecticide, Fungicide and Rodenticide Act
Fitness	Describes an individual's ability to reproduce successfully relative to that of other members of its population.
fw	Fresh weight
fwt	Fresh weight tissue

GAT	Glyphosate N-acetyltransferase
GLP	Good Laboratory Practice
Glyphosate	Broad-spectrum systemic herbicide
GM	Genetically Modified
GMO	Genetically Modified Organism
GMP	Genetically Modified Plant
H	Hybrid
ha	Hectare
ILSI	International Life Sciences Institute
IPM	Integrated Pest Management
IRM	Insect Resistance Management
Locus	The position/area that a given gene occupies on a chromosome
LOD	Limit of detection
LOQ	Limit of quantification
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization-Time Of Flight. A mass spectrometry method used for detection and characterisation of biomolecules, such as proteins, peptides, oligosaccharides and oligonucleotides, with molecular masses between 400 and 350,000 Da.
MCB	Mediterranean corn borer, *Sesamia nonagrioides*
mRNA	Messenger RNA
MT	Norwegian Food Safety Authority (Mattilsynet)
NDF	Neutral detergent fibre, measure of fibre used for animal feed analysis. NDF measures most of the structural components in plant cells (i.e. lignin, hemicellulose and cellulose), but not pectin.
Northern blot	Northern blot is a technique used to study gene expression by detection of RNA or mRNA separated in a gel according to size.

NTO	Non-target organism
Nicosulfuron	Herbicide for maize that inhibits the activity of acetolactate synthase
Near-isogenic lines	Term used in genetics/plant breeding, and defined genetic lines that are identical except for differences at a few specific locations or genetic loci.
OECD	Organisation for Economic Co-operation and Development
ORF	Open Reading Frame, in molecular genetics defined as a reading frame that can code for amino acids between two stop codons (without stop codons).
OSL	Over season leaf
OSR	Over season root
OSWP	Over season whole plant
PCR	Polymerase chain reaction, a technique to amplify DNA by copying it
R0	First transformed generation, parent
RNA	Ribonucleic acid
RP	Recurrent parent
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis. Technique to separate proteins according to their approximate size
SAS	Statistical Analysis System
SD	Standard deviation
Southern blot	Method used for transfer of electrophoresis-separated DNA fragments to a filter membrane and possible subsequent fragment detection by probe hybridisation
T-DNA	Transfer DNA, the transferred DNA of the tumour-inducing (Ti) plasmid of some species of bacteria such as *Agrobacterium tumefaciens* and *A. rhizogenes*, into plant's nuclear genome. The T-DNA is bordered by 25-base-pair repeats on each end. Transfer is initiated at the left border and terminated at the right border and requires the *vir* genes of the Ti plasmid.
TI	Trait integrated

TMDI	Theoretical Maximum Daily Intake
U.S. EPA	United States Environmental Protection Agency.
Maize growth stages	*Vegetative* VE: emergence from soil surface V1: collar of the first leaf is visible V2: collar of the second leaf is visible Vn: collar of the leaf number 'n' is visible VT: last branch of the tassel is completely visible *Reproductive* R0: Anthesis or male flowering. Pollen shed begins R1: Silks are visible R2: Blister stage. The kernels are filled with a clear nourishing endosperm fluid and the embryo can be seen R3: Milk stage. The kernels endosperm is milky white. R4: Dough stage. The kernels endosperm has developed to a white paste R5: Dent stage. If the genotype is a dent type, the grains are dented R6: Physiological maturity
Western blot	Technique used to transfer proteins separated by gel electrophoresis by 3-D structure or denatured proteins by the length of the polypeptide to a membrane, where they might be identified by antibody labelling.
WHO	World Health Organisation
ZM	*Zea maize* L.

Background

On 10 November 2005, the European Food Safety Authority (EFSA) received from the Competent Authority of Czech Republic an application (Reference EFSA/GMO/CZ/2005/27) for authorisation of the insect-resistant and herbicide tolerant genetically modified (GM) maize MON 88017 (Unique Identifier MON-88Ø17-3), submitted by Monsanto within the framework of Regulation (EC) No 1829/2003.

The scope of the application covers:

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

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

After receiving the application EFSA/GMO/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 11 January 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 April 2007 (VKM 2007a). EFSA published its scientific opinion 21 April 2009 (EFSA 2009b), and maize MON 88017 was approved for food and feed uses, import and processing in the EU on 30th of October 2009 (Commission Decision 2009/814/EC).

An application for authorisation of maize MON 88017 for cultivation in the EU was submitted by Monsanto in April 2008 (EFSA/GMO/CZ/2008/54). On 12 September 2008 EFSA declared

the application as valid, and made the valid application available to Member States and the European Commission. The VKM GMO Panel participated in the official hearing, and submitted a preliminary environmental risk assessment report in April 2010 (VKM 2010a). The EFSA GMO Panel adopted its scientific opinion on maize MON 88017 on 19th of October 2011 (EFSA 2011d).

In addition, MON 88017 has been evaluated by the VKM GMO Panel as a component of several stacked GM maize events under Regulation (EC) 1829/2003 (VKM 2007b, VKM 2008, VKM 2009, VKM 2010b).

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 authorisation process in Norway. The Agency is responsible for assessing environmental risks upon 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 upon the deliberate release of GMOs pursuant to the Gene Technology Act and the Food Safety Act. In addition, 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 regulation 1829/2003, the Norwegian Environment Agency, by letter dated 13 June 2012 (ref. 2008/4367/ART-BI-BRH), requests VKM, to conduct final environmental risk assessments for all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are authorised 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 Norwegian Environment Agency requests VKM 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, 2010a; EFSA, 2011d), and OECD guidelines will be useful tools in the preparation of the Norwegian risk assessments.

The risk assessments' primary geographical focus should be Norway, and the risk assessments should include the potential environmental risks of the product(s) related to any changes in agricultural practices. The assignment covers assessment of direct environmental impact of the intended use of pesticides with the GMO under Norwegian conditions, as well as changes to agronomy and possible long-term changes in the use of pesticides.

The Norwegian Food Safety Authority

In preparation for a legal implementation of regulation 1829/2003, the Norwegian Environment Agency has requested NFSA to give final opinions on all GMOs and products containing or consisting of GMOs that are authorised 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.

NFSA has therefore, by letter dated 13 February 2013 (ref. 2012/150202), requested VKM to carry out final scientific risk assessments of 39 GMOs and products containing or consisting of GMOs that are authorised in the European Union.

The assignment from NFSA includes food and feed safety assessments of GMOs 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 cultivars (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 and 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 NFSA. In addition, the changes related to herbicide residues of GMPs as a result of the application of plant-protection products fall outside the remit of the Norwegian VKM panels.

Assessment

1 Introduction

The genetically modified maize 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 (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.

Maize MON 88017 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 2010a), 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 environmental risk assessment of the genetically modified maize MON 88017 is based on information provided by the applicant in the applications EFSA/GMO/CZ/2005/27 and EFSA/GMO/CZ/2008/54, and scientific opinions and comments from EFSA and other member

states made available on the EFSA website GMO Extranet. The risk assessment is also based on a review and assessment of relevant peer-reviewed scientific literature.

It is emphasised that the VKM mandate does not include assessments of contribution to sustainable development, societal utility and ethical considerations, according to the Norwegian Gene Technology Act and Regulations relating to impact assessment pursuant to the Gene Technology Act. These considerations are therefore not part of the risk assessment provided by the VKM Panel on Genetically Modified Organisms.

2 Molecular characterisation

2.1 Information related to the genetic modification

MON 88017 was developed to express the genes *cry3Bb1* derived from *Bacillus thuringiensis* subsp. *kumamotoensis* and *cp4 epsps* derived from *Agrobacterium* sp. strain CP4. The genes encode the modified insecticidal protein Cry3Bb1, providing the plants protection against certain coleopteran insect pests, and the CP4 EPSPS protein which provides tolerance to glyphosate based herbicides.

2.1.1 Description of the methods used for the genetic modification

MON 88017 was produced by Agrobacterium-mediated transformation of immature embryos from the maize line A x Hi-II. *Agrobacterium tumefaciens* strain ABI, containing plasmid PV-ZMIR39, was the transformation vector.

Following an incubation period, the immature embryos were transferred to selection medium containing carbenicillin to eliminate Agrobacterium, and glyphosate to eliminate untransformed cells. The resulting transformed cells were then sub-cultured several times on a selection medium and regenerated into plants. Plants were screened for insect protection, glyphosate tolerance, and field performance.

2.1.2 Nature and source of vector used

PV-ZMIR39 (Figure 1) 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 position and orientations of the different elements present on the transformation vector are detailed on the plasmid map (Figure 1). The specific genetic elements and origins of the various components used to construct plasmid vector PV-ZMIR39 are provided in Table 1. The T-DNA contains two expression cassettes, one for CP4 EPSPS and one for Cry3Bb1.

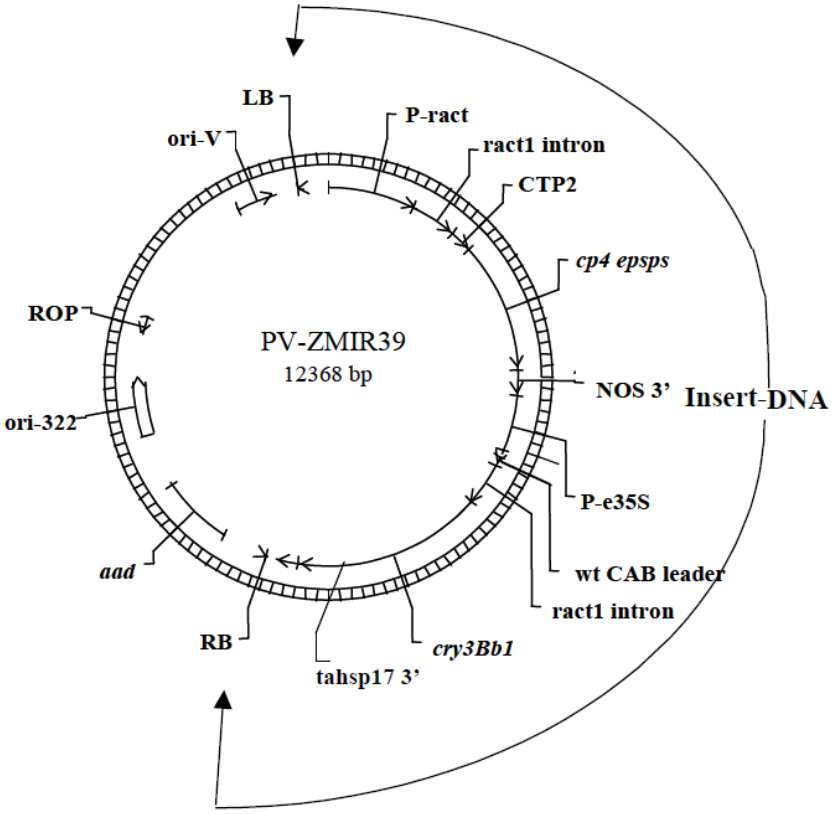
The *cp4 epsps* coding sequence derived from *Agrobacterium* sp. Strain CP4, a common soil-borne bacterium, has been sequenced and shown to encode a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids. In the plant gene expression cassette, the *cp4 epsps* coding sequence is 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 *cry3Bb1* sequence present in plasmid vector PV-ZMIR39. The *cry3Bb1* gene expression cassette consists of the *P-e35S* promoter, the wt *CAB* leader, and the intron from the *ract1* gene joined to the synthetic *cry3Bb1* coding sequence at the 5' end. Joined to the 3' end of the *cry3Bb1* coding sequence is the *tahsp17* 3'-sequence, which ends transcription and provides the signal for mRNA polyadenylation.

The Left and Right Border regions of plasmid vector PV-ZMIR39 define the extent of the DNA that should be transferred into the plant genome. The Right Border is a 24 bp nucleotide sequence that was originally isolated from *A. tumefaciens* plasmid pTiT37. The Left Border is a 25 bp nucleotide sequence, contained within a 34 bp region, isolated from *A. tumefaciens* plasmid pTi5955.

The backbone region outside of the inserted DNA, which is not integrated into the maize genome during transformation, contains a bacterial selectable marker gene, *add*, which encodes an aminoglycoside-modifying enzyme that provides resistance to the action of the antibiotics spectinomycin and streptomycin, as well as two origins of replication necessary for replication and maintenance of the plasmid in bacteria. A detailed description of all elements in the bacterial backbone region is presented in Table 1.



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

Table 1. Summary of the genetic elements in PV-ZMIR39

Genetic element	Source	Location in plasmid (bp)	Function (Reference)
LB (Left Border)	Octopine Ti plasmid, pTi15955	12067-12090	Left border sequence essential for transfer of T-DNA from the octopine Ti plasmid, pTi15955 (Barker *et al.*, 1983)
Intervening sequence	*Agrobacterium*	12091-12364	Polylinker (Barker *et al.*, 1983)
Intervening sequence	Synthetic	12365-12	Polylinker
***cp4 epsps* gene cassette**			
P-ract1	Rice actin gene	13-946	Promoter (McElroy *et al.*, 1990)
ract1 intron	Rice actin gene	947-1407	Intron (McElroy *et al.*, 1991)
Intervening sequence	Synthetic	1408-1423	Polylinker
CTP2	*Arabidopsis thaliana*	1424-1651	DNA sequence coding for the N-teminal chloroplast transit peptide (Klee *et al.*, 1987)
cp4 epsps	*Agrobacterium sp.* strain CP4	1652-3019	DNA sequence coding for the native CP4 EPSPS protein (Padgette *et al.*, 1996).
Intervening sequence	Synthetic	3020-3031	Polylinker
NOS 3'	*Agrobacterium tumefaciens*	3032-3287	3' nontranslated region of the nopaline synthase (NOS) gene which terminates transcription and directs polyadenylation (Bevan *et al.*, 1983)
Intervening sequence	Synthetic	3288-3320	Polylinker
***MON 88017 cry3Bb1* gene cassette**			
P-e35S	Cauliflower mosaic virus	3321-3933	Promoter with the duplicated enhancer region (Kay *et al.*, 1987; Odell *et al.*, 1985)
Intervening sequence	Synthetic	3934-3957	Polylinker
wt CAB leader	Wheat	3958-4028	5' untranslated leader of the wheat chlorophyll a/b-binding protein (Lamppa *et al.*, 1985)
Intervening sequence	Synthetic	4029-4056	Polylinker
ract1 intron	Rice actin gene	4057-4517	Intron (McElroy *et al.*, 1991)
Intervening sequence	Synthetic	4518-4533	Polylinker
MON 88017 cry3Bb1	*Bacillus thuringiensis* subsp. *kumamotoensis*	4534-6495	DNA sequence coding for a synthetic variant of Cry3Bb1 protein (Romano, 2002).
Intervening sequence	Synthetic	6496-6510	Polylinker
tahsp17 3'	Wheat heat shock protein	6511-6744	3' nontranslated region of the DNA sequence coding for wheat 17.3 kDa heat-shock protein, which ends transcription and directs polyadenylation (McElwain and Spiker, 1989)
Intervening sequence	*E. coli* and synthetic	6745-6840	Polylinker (Depicker *et al.*, 1982)
RB (Right Border)	Nopaline Ti plasmid, pTiT37	6841-6865	Right border sequence essential for transfer of T-DNA from the nopaline Ti plasmid, pTiT37 (Depicker *et al.*, 1982)

Table 1 continued

Genetic element	Source	Location in plasmid (bp)	Function (Reference)
Backbone genetic elements for expression of the plasmid in *E. coli*			
Intervening sequence	*E. coli* and synthetic	6866-7350	Polylinker (Depicker *et al.*, 1982; Fling *et al.*, 1985; Sutcliffe, 1978)
aad	Bacterial transposon Tn7	7351-8139	Bacterial promoter and DNA sequence coding for an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase from the transposon Tn7 (Fling *et al.*, 1985) GenBank accession X03043
Intervening sequence	*E. coli*	8140-8681	Polylinker (Fling *et al.*, 1985; Sutcliffe, 1978)
ori-322	Plasmid pBR322 from *E. coli*	8682-9310	Origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1978)
Intervening sequence	Plasmid pBR322 from *E. coli*	9311-9727	Portion of the plasmid (Sutcliffe, 1978)
ROP	*E. coli*	9728-9919	DNA sequence coding for repressor of primer protein for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989)
Intervening sequence	Plasmid pBR322 from *E. coli*	9920-11182	Portion of the plasmid (Sutcliffe, 1978)
Intervening sequence	*E. coli* and synthetic	11183-11430	Plasmid DNA (Stalker *et al.*, 1981)
ori-V	*Agrobacterium*, plasmid RK2	11431-11824	Origin of replication for *Agrobacterium* (Stalker *et al.*, 1981)
Intervening sequence	*E. coli* and synthetic	11825-11910	Plasmid DNA (Stalker *et al.*, 1981)
Intervening sequence	*Agrobacterium*	11911-12066	DNA sequences (Barker *et al.*, 1983)

2.2 Information relating to the GM plant

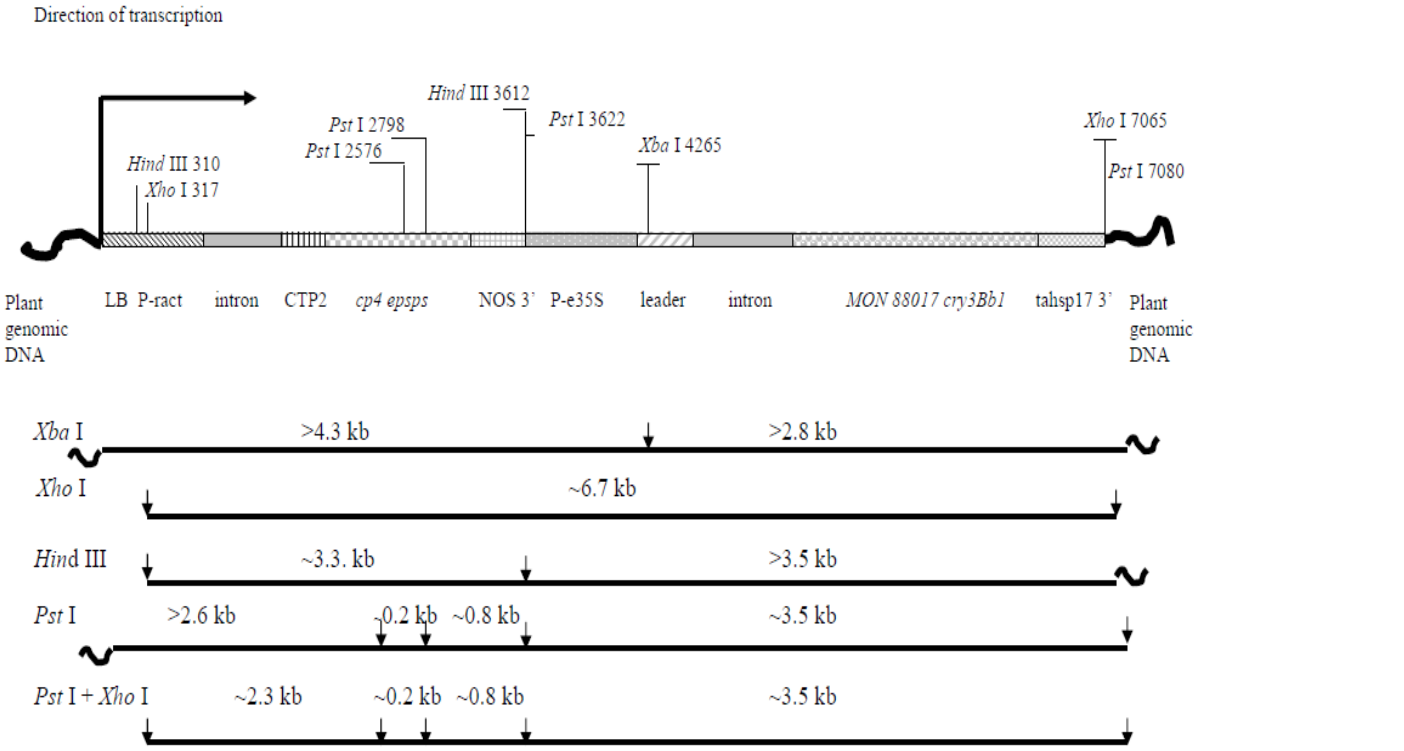
2.2.1 Description of the trait(s) and characteristics that have been introduced or modified

The modified Cry3Bb1 protein provides plants of maize MON 88017 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*). The CP4 EPSPS protein provides tolerance to glyphosate based broad spectrum herbicides.

2.2.2 Information on the sequences actually inserted or deleted

Southern analysis of genomic DNA digested with two different restriction enzymes (*SacI* and *XbaI*) 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. According to the applicant, the intactness of the two expression cassettes 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. A schematic presentation of the DNA inserted into the genome of MON 88017, including restriction enzyme sites and expected restriction fragments is provided in Figure 2 (*Sac*I does not cut within the PV-ZMIR39).

Further, the absence of vector backbone sequences in MON 88017 plants was established by Southern analysis with two probes that cover the entire vector backbone.



A linear map of the inserted DNA from transferred PV-ZMIR39 T-DNA is shown. Genetic elements are annotated. Positions of the restriction sites for enzymes used in the Southern blot analyses are included for reference. Arrows indicate sites of the restriction digest. Sizes of predicted restriction fragments, calculated from the size of the linear map are identified. MON 88017 contains one copy of the insert at a single integration locus.

Figure 2. Schematic presentation of the insert present in MON 88017

2.2.2.1 ***The size and copy number of all detectable inserts, both complete and partial***

The insert number was evaluated by digesting the MON 88017 and conventional maize genomic DNA with the restriction enzyme *ScaI*, which does not cleave within the plasmid PV-ZMIR39. The enzyme should release a restriction fragment containing the inserted DNA and adjacent plant genomic DNA. The number of restriction fragments detected indicates the number of inserts present. According to the applicant, MON 88017 genomic DNA digested with *ScaI* produced one band at approximately 13 kb. The result indicates that MON 88017 contains one insert located on an approximately 13 kb *ScaI* restriction fragment.

Plasmid PV-ZMIR39 DNA, previously digested with *EcoRI*, was mixed with control maize genomic DNA digested with *ScaI*, and then loaded on the gel to serve as a positive hybridization control. According to the applicant, plasmid PV-ZMIR39 DNA previously digested with *EcoRI*, mixed with control DNA digested with *ScaI*, produced bands of approximately 6.3 kb, 3.5 kb and 2.6 kb, correlating to the sizes of PV-ZMIR39 *EcoRI* fragments. The ~6.3 kb band produced a weaker signal. According to the applicant, a smaller portion of the target DNA sequence is present on this ~6.3 kb *EcoRI* restriction fragment in comparison to the ~3.5 kb and ~2.6 kb fragments.

The number of copies of the introduced DNA was determined by digesting the MON 88017 genomic DNA with *XbaI*, a restriction enzyme that cuts only once within PV-ZMIR39. According to the applicant, MON 88017 DNA digested with *XbaI* produced two unique bands at approximately 7.4 kb and 5.5 kb, representing two border fragments. In combination with the insert number analysis, these results indicate that MON 88017 contains one copy of the introduced DNA at a single locus of integration.

The sequences of the plant genome adjacent to the 3' and 5' sequences of the insert were analysed. 878 bp and 1000 bp flanking the insert at 3' and 5' ends, respectively, were amplified by PCR and sequenced. According to the applicant, these sequences showed homology to maize DNA. Following an updated analysis of the pre-insertion site in conventional maize the applicant concluded that a 26 bp fragment of genomic DNA at the target site was deleted and a 20 bp fragment was inserted. The insert lies 174 bp upstream of a region showing high sequence similarity to ESTs annotated as corresponding to putative purine permeases.

2.2.2.2 *The organisation of the inserted genetic material at the insertion site and methods used for characterisation*

Genomic DNA from MON 88017 was analysed by Southern blotting to determine the intactness of both inserts, and the presence or absence of plasmid backbone sequences. The organization of the elements within the insert in MON 88017 was further confirmed using PCR analysis and sequencing of the insert.

MON 88017 contains one copy of the introduced DNA at a single integration locus on an approximately 13 kb ScaI restriction fragment. No additional elements from the transformation vector PV-ZMIR39, linked or unlinked to intact cassettes, were detected in the genome of MON 88017. No backbone sequences from the transformation vector PV-ZMIR39 were detected in the tested generations. These data support the conclusion that only the two expected full-length proteins, MON 88017 Cry3Bb1 and CP4 EPSPS, are encoded by the insert in MON 88017.

2.2.2.3 *In the case of deletion(s), size and function of the deleted region(s)*

NA

2.2.2.4 *Chromosomal location(s) of insert(s)*

According to the applicant, the presence of MON 88017 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 the maize nuclear DNA.

2.3 Information on the expression of insert

Three field studies have been carried out in order to estimate the levels of newly expressed proteins Cry3Bb1 and CP4 EPSPS in various parts of maize MON 88017. MON 88017 was grown in field trials in USA in 2002 and Argentina in 2003/2004 (application EFSA/GMO/CZ/2005/27) and in Europe during the 2006 growing season (application EFSA/GMO/CZ/2008/54). Tissue samples were collected at various growth stages throughout the growing seasons and analysed for Cry3Bb1 and CP4 EPSPS protein levels using validated ELISA methods.

Application EFSA/GMO/CZ/2005/27

Samples for 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 Cry3Bb1 and CP4 EPSPS proteins were determined in several tissues collected from the test material MON 88017 (obtained from generation LH59xLH198BC_3F_3 (Figure 3) and the conventional maize hybrid H1200902, which has a genetic background similar to the test material. No information regarding the herbicide treatment of the plots is available in the

dossier. Over-season leaf, whole plant and roots at 4-6 different stages of development were collected from each replicated plot at all field sites. The field trial was also used to analyse the expression of the proteins in pollen, silk, forage, forage root, grain, stover and senescent roots.

The results obtained from the expression analysis have been summarized in Table 3 and 5. 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 (Table 3). 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) (Table 5). 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 (Table 5). 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 (data not shown) (Dudin & Jennings 2005).

Application EFSA/GMO/CZ/2008/54

Another field study was conducted during the 2006 growing season at seven locations in Europe: three locations in Germany and four locations in Spain. The field sites were selected to represent geographical regions in Europe where maize is grown commercially.

There were two test substances for this study, both were MON 88017 that were introgressed into different genetic backgrounds. The first was adapted to northern (Germany) European growing regions (DKC3945), and the second was adapted to the southern (Spain) European growing regions (DKC5143). According to the applicant, the seed used as test material was from a generation that was several breeding steps downstream from a branch point at the LH198BC0F2 generation of the MON 88017 breeding tree (Figure x). The control substances had background genetics similar to the test substances. Maize MON 88017 and the near-isogenic controls were planted in a three replicate, randomised, complete block field design at each test site. No information regarding the herbicide treatment of the plots is available in the dossier.

In this study, over season leaf, root, whole plant, forage root, senescent root, silk, pollen, and grain tissues were used for ELISA analysis. The over season samples (leaf, root, and whole plant) were collected four times at different growth stages: (1) V2 – V4 stage, (2) V6 – V8 stage, (3) V10 – V12 stage, and (4) pre-VT stage.

The results obtained from the expression analysis have been summarized in Table 4 and 6. 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 (Nguyen & Jehle 2009).The results also showed that the means and ranges of Cry3Bb1 and CP4 EPSPS proteins in maize MON 88017 grown in Europe were generally lower than those observed in samples collected from maize MON 88017 grown in 2002 in the USA.

Table 3. Levels of the Cry3Bb1 and CP4 EPSPS proteins (µg/g dry weight) in several tissues of maize MON 88017. Data from field trials in USA in the 2002 growing season.

Tissue type	Growth stages	Cry3Bb1 Protein Mean (SD) Range	CP4 EPSPS Protein Mean (SD) Range
Over-season leaf tissues	V2-V3	570 (170) 230-820	220 (30) 160-260
	V5	430 (58) 310-510	190 (26) 130-250
	V8	310 (45) 240-380	170 (37) 140-240
	V11-V17	260 (44) 190-340	150 (19) 120-170
Over-season whole plant tissues	V2-V3	500 (64) 410-590	-
	V5	380 (170) 150-600	-
	V8	310 (48) 230-380	-
	V11-V17	220 (23) 190-250	-

Over-season root tissues	V2-V3	370 (80) 240-510	150 (34) 110-220
	V5	250 (71) 190-420	110 (29) 74-160
	V8	210 (78) 150-410	100 (30) 62-160
	V11-V17	180 (37) 110-230	97 (18) 72-130
	R4-R6	130 (29) 98-170	70 (20) 47-110
	Senescent root (after harvest)	100 (19) 77-140	-

Table 4. Levels of the Cry3Bb1 and CP4 EPSPS proteins (µg/g dry weight) in several tissues of maize MON 88017. Data from field trials in EU in the 2006 growing season.

Tissue type	Growth stages	Cry3Bb1 protein Mean (SD) Range	CP4 EPSPS protein Mean (SD) Range
Over-season leaf tissues	V2-V4	300 (55) 220-400	190 (47) 120-300
	V6-V8	290 (69) 190-420	130 (34) 94-220
	V10-V12	200 (43) 140-270	120 (28) 75-180
	Prev-VT	200 (47) 120-330	140 (33) 81-200
Over –season whole plant tissues	V2-V4	250 (29) 210-290	160 (47) 82-230
	V6-V8	210 (62) 140-330	130 (36) 68-200
	V10-V12	NA	NA
Over-season root tissues	V2-V4	160 (53) 110-310	50 (15) 23-71

	V6-V8	140 (52) 67-270	37 (15) 18-69
	V10-V12	75 (13) 44-91	22 (4.2) 16-30
	Pre-VT	75 (18) 54-110	23 (5.4) 14-33
	R4-R6	30 (10) 11-46	16 (7.3) 5.3 -27
	Senescent root (after harvest)	22 (7.1) 12-38	14 (7.4) 6.0-30

Table 5. Levels of the Cry3Bb1 and CP4 EPSPS proteins in pollen, silk, forage, grain and stover tissues of maize MON 88017 (µg/g dry weight). Data from field trials in USA in the 2002 growing season.

Tissue type (growth stages)	Cry3Bb1 protein Mean (SD) Range	CP4 EPSPS protein Mean (SD) Range
Pollen (R1)	25 (4,2) 17-32	390 (85) 210-470
Silk (R1)	380 (65) 300-500	- -
Forage (R4-R6)	95 (19) 75-130	57 (7.6) 42-69
Grain (R6)	15 (3.6) 10-22	5.8 (0.97) 4.1-7.1
Stover (after grain harvest)	88 (13) 71-110	- -

Table 6. Levels of the Cry3Bb1 and CP4 EPSPS proteins in pollen, silk and grain tissues of maize MON 88017(µg/g dry weight). Data from field trials in EU in the 2006 growing season.

Tissue type (growth stages)	Cry3Bb1 protein Mean (SD) Range	CP4 EPSPS protein Mean (SD) Range
Silk (R1)	160 (28) 110-220	-
Pollen (R1)	13 (2.7) 10-19	280 (44) 160-330
Grain (R6)	8,7 (2,3) 5.8-15	3.9 (0.94) 2.4-5.5

2.3.1 Parts of the plant where the insert is expressed

Cry3Bb1 and CP4 EPSPS proteins were found to be expressed in leaf, root, pollen, silk, forage, forage root, grain, stover and senescent root at appropriate times of plant development. Grain and forage are the most relevant tissues for the food and feed safety assessment of MON 88017, while leaf, root, pollen, silk and stover are relevant tissues in terms of environmental risk assessment.

2.3.2 Potential fusion proteins

Bioinformatic analyses were performed to assess the potential for allergenicity, toxicity, or pharmacological activity of putative polypeptides encoded by the 5' and 3' inserted DNA-maize genomic DNA junctions. Sequences spanning the 5' maize genomic DNA-inserted DNA junction and the 3' inserted DNA-maize genomic DNA junction were translated from stop codon to stop codon in all six reading frames. Putative peptides/polypeptides from each reading frame were compared to databases that contained peptides/polypeptides, including allergens and toxins, using bioinformatic tools. Furthermore, no short (eight amino acid) polypeptide matches were shared between any of the putative polypeptides and proteins in the allergen database.

According to the applicant, no biologically relevant structural similarities to allergens, toxins, or pharmacologically active proteins were observed for any of the putative polypeptides. Furthermore, no short (eight amino acid) polypeptide matches were shared between any of the putative polypeptides and proteins in the allergen database.

2.4 Genetic stability of the insert and phenotypic stability of the GM plant

2.4.1 Genetic stability of the insert in maize MON 88017

In order to determine generational stability of the integrated DNA, a Southern blot analysis to determine the number of copies of integrated transgenes was performed on seven generations from the breeding tree of MON 88017. For this analysis, MON 88017 and control genomic DNA samples were digested with XbaI, which digests only once within PV-ZMIR39. Further, probes spanning the entire insert produced the expected bands in all cases demonstrating the stability of the integrated DNA. Six generations of MON 88017 (produced the same size bands as the previously characterised generation (~7.4 kb and ~5.5 kb). According to the applicant, these results demonstrate that the expected Southern fingerprint of the MON 88017 insert has been maintained across the branches of the breeding tree that were tested. Therefore, the stability of the integrated DNA in MON 88017 has been established over multiple generations.

2.4.2 Phenotypic stability of the GM plant

The phenotypic stability was determined following the segregation of the traits over seven generations of cross-fertilization and three generations of self-pollination (Figure 3). Results from chi square analysis of the Mendelian inheritance data are summarized in Table 7. With two exceptions, all $\chi 2$ values were less than the critical value of 3.84, thus indicating no significant differences between observed and expected frequency for the insect-resistant/glyphosate-tolerant phenotypes in eight breeding generations of MON 88017.

The results obtained for the LH198BC0F1 × LH59 generation were attributed to gamete selection caused by glyphosate application to plants of the previous (LH198BC0F1) generation (Walker et al. 2006). This was conducted to obtain non-segregating seed for purposes of field evaluation. Gamete selection caused by glyphosate application has been previously observed for glyphosate-tolerant maize NK603.

These results indicate that the inserted DNA and the traits are stable in MON 88017and confirm that MON 88017contains a single locus insertion.

Table 7. Comparison of expected and observed segregation frequencies for progeny and MON 88017 plants.

Generation	Observed		Expected		χ^2
	+	-	+	-	
LH198 BC_0F_1	21	14	17.5	17.5	1.03^{ns}
LH198 BC_0F_2	53	12	48.75	16.25	1.15^{ns}
LH198 BC_1F_1	21	9	15	15	4.03^{*}
LH198 BC_2F_1	10	15	12.5	12.5	0.64^{ns}
LH198 BC_3F_1	8	5	6.5	6.5	0.31^{ns}
LH198 BC_3F_2	21	3	18	6	1.39^{ns}
LH198 BC_0F_1 x LH59	29	0	14.5	14.5	27.03^{**}
LH59 BC_1F_1	7	5	6	6	0.08^{ns}
LH59 BC_2F_1	8	5	6.5	6.5	0.31^{ns}
LH59 BC_2F_2	35	13	36	12	0.03^{ns}

ns: not signficant at $p \leq 0.05$ (Chi square =3.84, 1df)

*: significant at $p \leq 0.05$ (Chi square=3.84, 1 df)

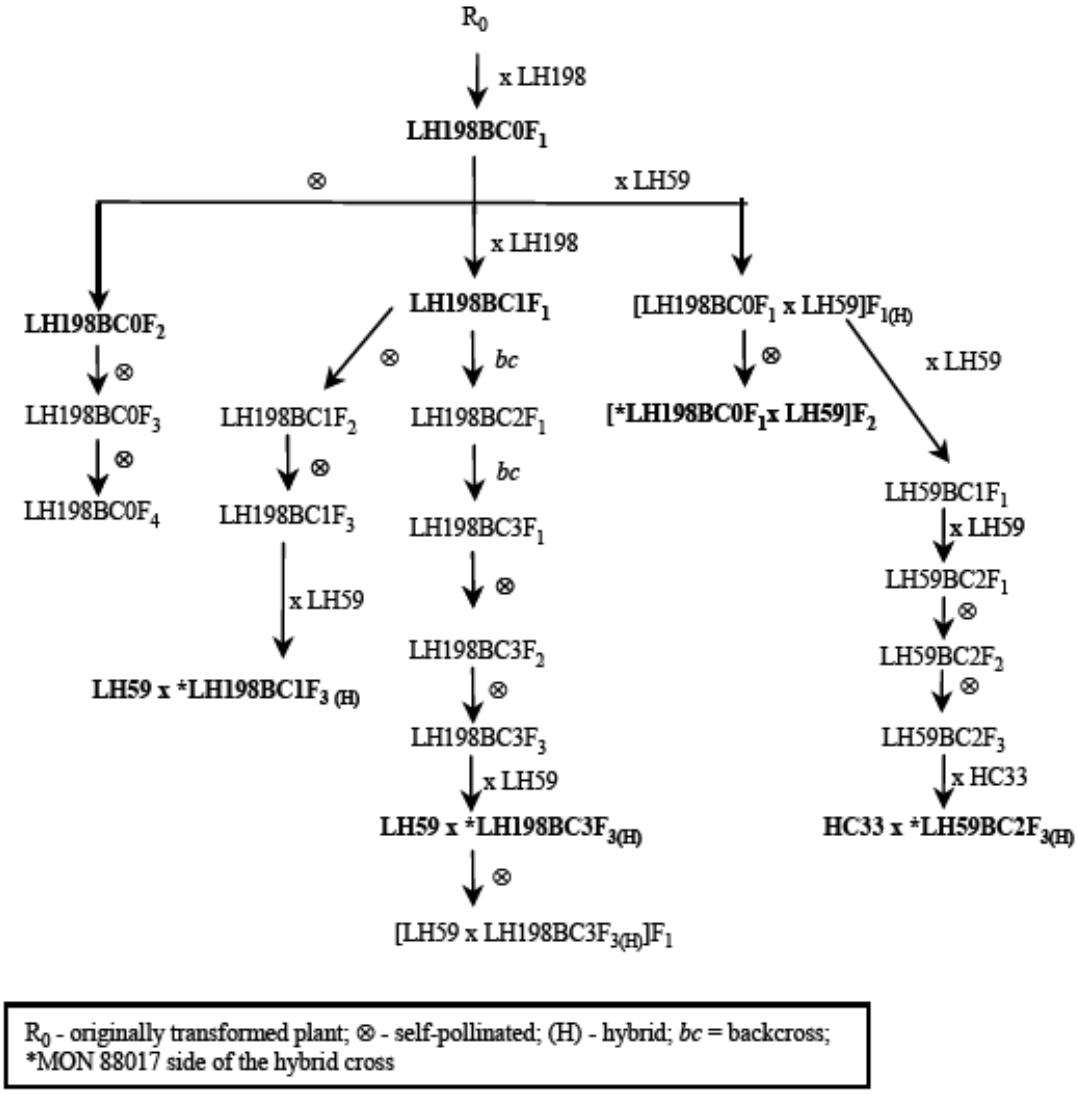


Figure 3. Breeding tree of MON 88017. The specific generations tested are indicated in bold.

2.5 Conclusion

The molecular characterisation data has established that only one copy of the transgene is integrated in the maize genomic DNA. Appropriate analyses of the integration site including sequence determination of the inserted DNA and flanking regions and bioinformatics analysis have been performed. Bioinformatics analyses of junction regions have demonstrated the absence of any potential new ORFs coding for known toxins or allergens. The genetic stability of transformation event MON 88017 was demonstrated at the genomic level over multiple generations by Southern analysis. Segregation analysis shows that event MON 88017 is inherited as a dominant, single locus trait. The VKM GMO Panel considers the molecular characterisation of maize MON 88017 satisfactory.

3 Comparative assessment

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

3.1.1 Experimental design & statistical analysis

Phenotypic evaluation of maize MON 88017 and production of materials for the comparative assessments has been 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. Results of the compositional analyses of MON 88017 from the 2002 and 2003/2004 field seasons were presented in the context of Application EFSA-GMO-CZ-2005-27. The results from the EU field trials performed in 2006 complement the previous results and are presented in the Application EFSA-GMO-CZ-2008-54.

Field trials USA (2001, 2002)

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 have a LH59 x LH198 genetic background (Figure 2) and were tested as hybrid pairs. The test hybrid was derived by initially self-pollinating a BC11F2 maize plant hemizygous for MON 88017 with a LH198 background to create homozygous MON 88017-positive and homozygous MON 88017-negative BC1F3 plants. The incomplete BC1F3 conversion of LH198 was subsequently crossed to LH59 to make the test hybrid for this study. The MON 88017-negative BC1F3 plants were crossed with LH59 to make the control hybrid for this study. In addition to maize MON 88017 and the non-GM counterpart, four commercially available maize hybrids were grown at each of the same field sites to provide a total of 12 different reference substances.

At each field site, the test, control and reference varieties were planted in a randomised complete block design with three or four replications at each site. All the plants were grown under normal agronomic field conditions for their respective geographic region. All test plots received an application of glyphosate according to label directions. Further details on the agronomical practices, the field experimental design, the materials and statistical methods used, can be found in the field production reports from Rosenbaum et al. (2003) and Pester & Woodrum (2003).

Data were collected for the following 14 phenotypic characteristics: seedling vigor, early stand count, days to 50% pollen shed, days to 50% silking, ear height, plant height, stay green, final stand count, dropped ears, stalk lodging, root lodging, test weight, grain moisture and yield. In addition, observational data on the presence of and differential response to biotic and abiotic stressors were collected. Phenotypic data were analysed using

Statistical Analysis Software (SAS). Means were calculated within-sites and across-sites for each characteristic, and MON 88017 maize and the control hybrid means were compared using analysis of variance (ANOVA) methods. Two types of analyses were conducted: within-site and across-site. Differences were considered significant at the 5% level of significance ($p<0.05$).

Field trials Argentina (2003-2004)

MON 88017 and the 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. At each field site, the test, control and reference seed were planted in a randomised 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 according to label instructions. Further details on the agronomical practices, the field experimental design, the materials and statistical methods used, can be found in the attached field production report (Marcinkiewicz, 2005).

Field trials EU (2006)

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. According to the applicant, these locations provided a range of environmental and agronomic conditions representative of European maize-growing regions were commercial production of MON 88017 is expected to be located.

In these field trials, the test hybrid MON 88017 was compared with conventional counterparts consisting of the varieties designed as DKC3945 and DKC5143. DKC3945 and DKC5143 have genetic backgrounds similar to the test substances with the exception of the insect-protection and glyphosate tolerant traits, and were adapted to northern and southern European growing regions, respectively.

Additionally, six different conventional maize hybrids were included as reference substances in the European test sites. Three reference substances were grown at each of the three northern field sites and three reference substances were acquired from breeding stations at five locations in the southern growing region. The reference substances were locally adapted hybrids with similar relative maturities as the test and control substances. Plants from the references were evaluated at each site.

Plots were established at each of the sites in a randomised complete block design with three replications. Each plot consisted of six rows spaced approximately 70 cm apart and approximately 6-10 m in length. Rows 1 and 2 were designated for the collection of samples for further analyses, while rows 4 and *5* were designated for the collection of phenotypic and ecological interaction data. Rows 3 and 6 were used as buffer rows. Roundup Ready maize which was subsequently de-tasseled was planted in the alleyways between plots and blocks and in a border surrounding the entire study area. All fields were isolated at least 200 m

from other maize. Once in the course of the growing season, all test plots were treated with glyphosate. Further agronomic practices used to prepare and maintain each study site were characteristic of the respective geographic region. Pesticides containing *Bt* were not applied to the study area at any site.

Phenotypic and agronomic characteristics related to dormancy and germination, emergence and vegetative growth, reproductive growth, seed retention and stress (i.e., disease, biotic and abiotic stress responses) were collected. In both field trials, seedling vigor, early stand count, number of days after planting to 50% pollen shed and 50% silking, stay green, ear height (Spanish sites only), ear length (German sites only), plant height, number of dropped ears, number of stalk and root lodged plants, final stand count, grain moisture and yield were assessed (Table 9).In addition, observational data on the presence of and differential response to biotic and abiotic stressors are presented by the applicant.

Separate analyses of variance were carried out on the data from Spain and Germany. Combined-site and individual by-site statistical analyses were conducted and evaluated on each measured phenotypic characteristic. The level of statistical significance was predetermined to be 5 % ($p<0.05$).

3.2 Compositional Analysis

Compositional analyses were conducted according to the OECD consensus document on compositional consideration for new varieties of maize (OECD, 2002) on key maize tissues produced from trials conducted in USA during the 2002 field season (Dossier ref. McCann, 2003a, b **CBI**), Argentina during the 2003-2004 field season (Dossier ref. McCann et al., 2005 **CBI**) and in Europe during the growth season of 2007 (ref. Drury et al. 2008 **CBI**, correspondence with EFSA).

USA field trials 2002

MON 88017 and the conventional control were grown at three replicated field sites in Iowa, Illinois and Nebraska, together with four commercially available maize references at each of the test sites, providing a total of 12 maize references.

MON 88017, the control and maize references were all planted in a randomised complete block design with three replicates per block. All plants were grown under normal agronomic field conditions for the respective geographical regions and all test plots received an application of glyphosate herbicide according to label directions. Forage samples were harvested at the late dough/early dent stage and grain samples were harvested at maturity.

Compositional analyses of the forage samples included proximates (protein, fat, ash and moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), minerals (calcium, phosphorous) and carbohydrates by calculation. Compositional analyses of the grain samples included proximates, ADF, NDF, total dietary fibre (TDF), amino acids, fatty acids (C8-C22), minerals (calcium, copper, iron, magnesium, manganese, phosphorous, potassium, sodium and zinc), vitamins (B1, B2, B6, E, niacin and folic acid), antinutrients (phytic acid and raffinose), secondary metabolites (furfural, ferulic

acid, and p-coumaric acid) and carbohydrates by calculation. In all, 68 analytical components of grain were measured, 15 of these were however below the limit of quantitation of the assay and were excluded from statistical analysis.

Statistical analyses of the compositional data were conducted using a mixed model analysis of variance method. Four sets of comparison were made based on data from each of the three field sites plus data from a combination of all three sites. Level of Statistical significance was set to $p<0.05$.

The results of the combined site comparisons, and statistically significant differences between MON 88017and control are presented in Tables 1 and 2 in Appendix (Tables 17 and 19 in Technical Dossier).

For 232 of the 248 comparisons made between MON 88017 and control, there were no statistically significant differences ($p<0.05$) in forage and grain. The 16 comparisons observed to be statistically different in grain included: 16:0 palmitic acid, 18:1 oleic acid, 18:3 linolenic acid, 20:0 arachidic acid, copper, methionine, moisture, niacin, and serine,

18:2 linoleic acid, and vitamin B1. Except for vitamin B1, none of the statistically significant differences were oserved in all four statistical groups. The values for vitamin B1 were similar to literature and historical values of vitamin B1 in maize grain. All test values were also within the 99% tolerance interval for the 16 comparisons observed to be statistically different between MON 88017 and the non-transgenic control. No statistically significant differences were found in forage.

Argentinean field trials 2003 – 2004

MON 88017 and control were grown at four replicated field sites across Argentina during the 2003-2004 field season, together with four commercially available maize references, providing a total of 16 maize references.

MON 88017, control and maize references were all planted in a randomised complete block design with three replicates per block. All plants were grown under normal agronomic field conditions for the respective geographical regions and all test plots received an application of glyphosate herbicide according to label directions. Forage samples were harvested at the late dough/early dent stage and grain samples were harvested at maturity.

Forage and grain samples were harvested from all plots and analysed for nutritional components. Compositional analyses of the forage samples included proximates (protein, fat, ash, and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), minerals (calcium and phosphorus), and carbohydrates by calculation. Compositional analyses of the grain samples included proximates (protein, fat, ash, and moisture), ADF, NDF, total dietary fiber (TDF), amino acids, fatty acids (C8-C22), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), vitamins (B, 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 measured. Of these evaluated components, 16 had more than 50% of the observations below the assay limit of quantitation (LOQ) and, were excluded from the statistical analysis. As a result only 61 of the components were assessed statistically (nine in forage and 52 in grain). Statistical analyses were conducted using a mixed model analysis of variance method. Five sets of analyses were made, four based on the data from each of the replicated field sites and the fifth based on data from the combination of all four field sites. Level of Statistical significance was set to $p<0.05$. The results of the combined site comparisons, and statistically significant differences between MON 88017 and the control are presented in Tables 3 and 4 in Appendix (Tables 18 and 20 in Technical Dossier).

For 257 of the 296 comparisons made between MON 88017 and the conventional control, there were no significant differences in forage and grain. Two comparisons were observed to be significantly different in forage: phosphorus and total fat (one comparison each). These differences were only observed at a single site.

The 37 comparisons observed to be significantly different in grain included alanine, copper, p-coumaric acid, cystine, ferulic acid, glutamic acid, glycine, iron, isoleucine, leucine, lysine, magnesium, methionine, moisture, total fat, zinc, 18:3 linolenic acid, 20:0 arachidic acid and 20:1 eicosenoic acid (one comparison each); aspartic acid, folic acid, proline, and vitamin B, (two comparisons each); 18:2 linoleic acid and 18:1 oleic acid (five comparisons each).

All test values observed to be significantly different ($p<0.05$) between MON 88017 and the conventional control were within the 99% tolerance interval, except isoleucine (which showed a difference in only one of the five comparisons). The test values for isoleucine were however within literature and historical ranges for maize grain.

European field trials 2006

Following the request from the Food/Feed Working Group in EFSA, the applicant has provided composition data of forage and grain from maize MON 88017 not treated with glyphosate herbicide.

Forage and grain were harvested from a total of six replicated field sites: three sites across Germany representing northern Europe and three sites across Spain representing southern Europe. The field design had two sets of maize event MON 88017 and conventional control maize: one set of similar genetic backgrounds adapted to northern Europe, and the second set of similar genetic backgrounds adapted to southern Europe. A total of 13 different maize references were cultivated across the two European growing regions.

Forage samples were analysed for proximates (protein, fat, ash, and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), minerals (calcium and phosphorus), and carbohydrates by calculation. Grain samples were analysed for proximates (protein, fat, ash, and moisture), ADF, NDF, total dietary fiber (TDF), amino acids, fatty acids (C8-C22), Vitamins (A, B1, B2, B6, E, niacin, and folic acid), anti-nutrients (phytic acid and raffinose), secondary metabolites (furfural, ferulic acid,and p-coumaric acid), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc) and carbohydrates by calculation.

A total of 78 different analytical components (nine in forage and 69 in grain) were measured. Of these components, 16 had more than 50% of the observations below the assay LOQ, and were excluded from the statistical analysis. As a result only 62 of the components were statistically assessed (nine in forage and 53 in grain).

Statistical analyses were conducted using a mixed model analysis of variance on eight sets of data: analyses of data from each of the three replicated field trials in each of the two growing regions, plus data from a combination of all three field trials across each of the two growing regions (a total of 496 statistical comparisons between MON 89034 and conventional control). Level of Statistical significance was set to $p<0.05$.

For the northern growing region – Germany, the combined site statistical analysis showed that only the three analytes potassium, vitamin B1 and raffinose were found to be

significantly different between MON 88017 and control. The individual site analysis showed that vitamin B was significantly different at two individual sites, and that six analytes were significantly different at only one of the individual sites. All means and range of values from the MON 88017 were within the range of values obtained from a 99% tolerance interval.

Likewise for the southern growing region – Spain, the combined site statistical analysis showed that only the four analytes methionine, iron, vitamin A, and vitamin B1, were found to be significantly different between MON 88017 and control. The individual site analysis showed that 12 analytes were observed to be significantly different at only one individual site. All means and range of values from the MON 89034 were within the range of values obtained from a 99% tolerance interval.

The results of the combined site comparisons, and statistically significant differences between MON 88017and the control are presented in Tables 5 and 6 in Appendix (Tables 1 and 2 in Drury et al. 2008 CBI, correspondence with EFSA)

3.3 Agronomic and phenotypic characters

Field trials USA (2001, 2002)

Analyses of variance across trial locations in the USA in 2001 and 2002 showed statistically significant differences between MON 88017 and the non-GM comparator for seedling vigour, with a higher average score for MON 88017 in the 2002 season (7.6 vs. 6.5, respectively) and a lower score (3.7 vs. 4.1) in 2001 ($p<0.05$) (Table 8). In the 2002 within-site analysis, seedling vigour was significantly greater for MON 88017 compared to the control at three of the ten sites and numerically greater at eight of the ten sites. The observed increase in seedling vigor was not manifested in other characteristics of growth, fitness, and reproduction. The differences detected for seedling vigor at the three sites contributed largely to the difference detected across sites and were not indicative necessarily of a trend in the data.

Except for the number of days to 50% pollen shed in 2002, no statistically significant differences between the transgenic maize and the comparator were detected for any of the other assessed phenotypic characteristics in the across location analyses ($p>0.05$). The number of days to 50% pollen shed was fewer for MON 88017 compared to the control (58.8 vs. 59.2 days, respectively). This difference was less than one day at most sites and it is not likely to be biologically meaningful with respect to plant weed potential.

Field trials EU (2006)

Results for the combined-site phenotypic comparisons of MON 88017 to the conventional control from the European field trials are presented in Tables 9. In the combined-site analysis for Germany, two significant differences were found between maize MON 88017 and the conventional counterpart. MON 88017 plants were shorter than the control (254.2 cm vs. 262.9 cm) and had lower yield (10.3 vs. 11.4 tonnes/ha) compared with the conventional counterpart. However, these differences were not observed at all locations, and the mean values for both parameters fall within the range of values observed in the commercially available references.

In the by-site analysis for Germany, no differences were detected for early stand count, days to 50% pollen shed and silking, stay green, dropped ears, stalk and root lodging, and final stand count (Table 9). Five differences between MON 88017 and the control were found in the by-site analysis of sites in Germany. The differences were distributed among four of the thirteen phenotypic characteristics. The test was less vigorous than the control at site 1 (7.0 vs. 7.7 respectively) and at site 3 (6.0 vs. 6.7, respectively); ears from MON 88017 were longer than ears from the control at site 3 (27.8 vs. 26.7 cm, respectively); MON 88017 was shorter than the control at site 3 (222.7 vs. 230.1 cm, respectively); and yield was lower for MON 88017 compared to the control at site 3 (8.0 vs. 9.5 tonnes/ha, respectively). The differences detected for seedling vigor and ear length were not detected in the combined-site analysis (Table xx*).* Therefore, differences in seedling vigor and ear length were not indicative of a consistent trend in the data and are unlikely to be biologically meaningful in terms of increased weed potential of MON 88017 compared to the control (Section 3.8, step 2). Differences were detected for plant height and yield between MON 8801 7 and the control in both the by-site and combined-site analyses. However, mean values for MON 88017 for both assessments fall within the range of the references in the combined analysis. Furthermore, reduced plant height and lower yield does not represent a change in the plant that would confer an increase in weediness potential.

No statistically significant differences were detected for seedling vigor, early stand count, days to pollen shed and silking, stay green, ear length, dropped ears, stalk and root lodged plants, final stand count and grain moisture in the combined-site analysis for Germany*.*

In the combined-site analysis for Spain, no differences were detected for any of the assessed phenotypic plant characteristics (Table xx). One difference was detected between MON 88017 and the control in the by-site analysis (data not shown). MON 88017 had more plants in the final stand count than the control at one of the sites (102.3 vs. 95.0 plants/plot, respectively). The difference in final stand count was not detected in the combined-site analysis.

The information regarding the comparative analysis of agronomic and phenotypic data in the applications EFSA/GMO/CZ/2005/27 and EFSA/GMO/CZ/2008/54 has earlier been assessed by the VKM GMO Panel in the frame of EFSAs official hearing of the applications in 2007 and 2008 (VKM 2007a, 2010).

Table 8. Agronomic and phenotypic characteristics of maize MON 88017 and conventional control. Data from field trials in the USA in 2001 and 2002.

Characteristics	2001		2002	
	MON 88017	Control	MON 88017	Control
Seedling vigour	3.7*	4.1	7.6*	6.5
Early stand count (#/plot)	69.1	66.8	64.1	61.9
Days to 50% pollen shed	66.8	66.6	58.8*	59.2
Days to 50% silk emergence	66.9	66.9	59.5	59.8
Ear height (m)	1.16	1.15	1.03	1.05
Plant height (m)	2.26	2.23	2.09	2.10
Stay green	5.5	5.7	3.8	3.9
Final stand count (#/plot)	61.6	63.3	53.8	53.0
Dropped ears (#/plot)	0.5	0.7	0.5	0.4
Stalk lodged plants (#/plot)	5.4	4.1	2.5	3.4

Root lodged plants (#/plot)	0.7	0.6	3.9	4.1
Test weight (kg/l)	0.70	0.69	0.71	0.70
Grain moisture (%)	22.1	22	19.0	19.4
Yield (kg/ha)	9615	9282	9420	8993

*: significant at $p \leq 0.05$

Table 9. Agronomic and phenotypic characteristics of maize MON 88017 and conventional control. Data from field trials in the EU in 2006.

Characteristics	German Field Sites		Spanish Field Sites	
	MON 88017	**Control**	**MON 88017**	**Control**
Seedling vigour	6.4	6.8	6.1	6.3
Early stand count (#/plot)	74.8	77.7	100.9	99.9
Days to 50% pollen shed	65.8	64.8	70.8	70.8
Days to 50% silking	65.8	64.5	61.5	61.5
Stay green	1.9	1.8	0.1	0.0
Ear height (m)	-	-	1.04	1.07
Ear length (m)	0.26	0.26	-	-
Plant height (m)	2.54*	2.63	2.28	2.32
Dropped ears (#/plot)	1.0	0.8	0.0	0.0
Stalk lodged plants (#/plot)	0.2	0.0	0.1	0.3
Root lodged plants	0.0	0.0	0.0	0.0

(#/plot)				
Final stand count (#/plot)	74.5	77.3	99.8	97.9
Grain moisture (%)	29.0	30.3	19.5	19.8
Yield (kg/ha)	10.3*	11.4	9.8	10.7

*: significant at p≤0.05

3.4 Dormancy and germination

Seed dormancy is an important characteristic that is often associated with plants that are weeds (Anderson 1996). Dormancy mechanisms, including hard seed, vary with species and tend to include complex processes. For most crops, including maize, the number of hard seeds is negligible or nonexistent. Standardized germination assays are routinely used to measure the germination potential of maize seed (AOSA 1998).

Seed materials included MON 88017, one control, and three reference materials from each production location. The test and control materials were the positive (LH59 x MON 88017[+]/LH198BC3F3) test and negative (LH59 x MON 88017 [-] /LH198BC3F3) control isolines, respectively, having the same genetic background.Evaluations were conducted in temperature-controlled growth chambers using rolled towel tests to measure dormancy and germination characteristics. Four replicates of MON 88017, control, and three reference maize seeds were tested in seven growth chambers, each maintained in the dark under one of the following temperature regimes:

• Constant target temperature of approximately 5, 10, 20, or 30°C

• Alternating target temperatures of approximately 5/20, 10/20, or 20/30°C.

Temperatures <20°C were included to detect dormant seeds since most seeds would be expected to germinate at the optimal temperatures. The following five germination characteristics were evaluated: percent normal germinated seed, percent viable hard seed, percent abnormal germinated seed, percent viable firm swollen seed, and percent dead seed.

Out of 70 comparisons of germination characteristics made between MON 88017 and control maize, 61 were not statistically different at $p \leq 0.05$. No hard seed were observed and no differences in percent viable hard seed were detected between MON 88017 and the control from either location. Statistically significant differences observed between MON 88017 and the control maize were as follows: one each for percent normal germinated seed and percent dead seed, two for percent abnormal germinated seed, and five for percent viable firm swollen seed. These nine differences occurred in temperature regimes containing temperatures of ≤10°C. These temperature regimes were below the optimal germination conditions for maize (AOSA, 1998). Testing under suboptimal conditions can exacerbate small genetic differences. Isolated differences between MON 88017 and the control, with no concurrent trends across temperature regimes or production locations, are most likely due to random experimental error and not the result of altered germination characteristics of the seed. These results, and in particular, the lack of hard seed in MON 88017, indicate that the genetic modification process, the presence of the coding sequence, or the gene products did not alter dormancy mechanisms in the seed. Thus, it is concluded that there was no change in the pest potential of MON 88017 from increased dormancy through hard seed.

3.5 Conclusion

Comparative analyses of maize MON 88017 and its conventional counterpart have been performed during field trials located at representative sites and environments in Europe and USA. A total of 12-16 different conventional maize varieties were included in the field trials and used as references. With the exception the insect resistance and herbicide tolerance conferred by the Cry3Bb1 and CP4 EPSPS proteins, no biologically relevant differences were found between maize MON 88017 and controls. Based on the assessment of available data, the VKM GMO Panel concludes that maize MON 88017 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart except for the new proteins.

4 Food and feed safety assessment

4.1 Product description and intended uses

The genetic modification in MON 88017field maize will not impact the existing production processes used for maize. All MON 88017maize 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 88017field maize and all food, feed and processed products derived from MON 88017 field maize are expected to replace a portion of similar products from commercial maize, with total consumption of maize products remaining unchanged. The total anticipated Cry3Bb1 and CP4 EPSPS intake/extent of use of maize and all food, feed and processed products derived from maize will remain the same.

4.2 Effect of processing

Food manufacturing of MON 88017 field maize includes many harsh processing steps, e.g. cooking, heating, high pressures, pH treatments, physical shearing, extrusion at high temperatures etc. under which the majority of DNA and proteins are denatured, which also applies to the Cry3Bb1 and CP4 EPSPS proteins and c*ry3Bb1* and *cp4 epsps* genes (Dien et al 2002, Hammond & Jez 2011, Fernandes et al 2013). Baking of the maize bread broa containing 11 % of TC1500 and 20 % MON810 maize flour, showed that the baking process sheared the DNA into small fragments, less than 1000 bp (Fernandes et al 2013).

4.3 Toxicological assessment

In assessing the potential risks of GM foods, 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).

4.3.1 **Toxicity testing**

The potential toxicity of MON 88017 maize, expressing the Cry3Bb1 and CP4 EPSPS protein, has been assessed in toxicity studies in rodents and broiler chicken.

4.3.1.1 ***Acute toxicity testing***

Cry3Bb1 crystal protein in MON 88017 is a wild type variant isolated from *Bacillus thuringiensis* var. *kumamotoensis*. The base sequence of the *cry3Bb1* gene is modified to encode six amino acid substitutions with respect to the protein from the wild type Cry3Bb1 protein. The translated Cry3Bb1 protein has similar insecticidal activity as the protein expressed by the bacteria.

14-day acute oral toxicity study in mice with E. coli produced Cry3Bb1.

The potential toxicity of the Cry3Bb1 protein to humans and animals was examined in an acute oral toxicity study in mice (Kaempfe & Bonner, 2003).

It was not possible to isolate sufficient quantities of the Cry3Bb1 protein from MON 88017 maize for use as test material in the toxicity study, therefore the test material was produced in the laboratory using an *E. coli* large-scale fermentation system. The equivalence of the *E. coli*- and MON 88017 maize produced Cry3Bb1-protein was established using a range of methods, including MALDI-TOF mass spectrometry, N-terminal sequencing, immunoblotting, glycosylation analysis and insect bioassay.

This acute toxicity study was performed in general conformance with the US EPA Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity, December 2002 (US EPA 2002); the OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects, Subsection 401, February 24, 1987 (OECD 1987); and the EEC Part B: Methods for the Determination of Toxicity, B. 1, No L 383 AM/lb, December 29, 1992 (EEC 1992).

E. coli-produced Cry3Bb1 variant protein was administered by gavage to 10 male and 10 female CD-1 mice as two separate oral doses administered approximately 4 hours apart. The limited solubility of the E. coli produced MON 88017 Cry3Bb1 protein precluded its administration as a single dose. The target dose of 2442 mg/kg was based on the maximum attainable Cry3Bb1 concentration of the dosing solution (estimated at 37 mg/ml) and a total dose volume of 66.6 ml/kg body weight. The Cry3Bb1 protein was dissolved in sodium carbonatebicarbonate buffer. Analysis of the dosing solutions revealed that the concentration of Cry3Bb1 protein was 79% of target and, therefore, the achieved dose was actually 1930 mg/kg.

A separate control group of ten male and ten female animals received bovine serum albumin (BSA) at a dose of 1900 mg/kg. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals (NIH 1996). Study animals were observed for clinical signs twice on day 0, following each dose of test substance, then daily for 14 days. Clinical observations included, but were not limited to, changes in the skin and fur, eyes and mucous membranes, respiratory system, circulatory system, autonomic and central nervous system, including tremors and convulsions, changes in level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength and unusual behaviors. A general health/mortality check was performed twice daily.

Animals were weighed at the beginning of the study and then weekly thereafter. Food consumption was measured weekly. On day 14 of the study, the animals were killed and examined for gross necropsy and any abnormalities recorded.

No significant differences in food consumption were observed during the study. No animal deaths occurred during the course of the study and no significant clinical abnormalities were observed.

As no evidence of toxicity was observed in any of the animals, the acute oral LD50 of *E. coli*-produced Cry3Bb1 protein is greater than 1930 mg/kg body weight in the mouse.

The MON 88017 Cry3Bb1 protein is similar to MON863 Cry3Bb1 protein, differing by one of the 653 amino acid residues. The Cry3Bb1-protein has already been found safe to human health during the assessment of insect resistant maize (VKM 2005a,b, 2008, 2013) EFSA 2004, 2005, 2012, US EPA 2007).

A 9-day acute exposure of CP4 EPSPS protein in rodents

The CP4 EPSPS protein has been assessed by VKM when present in other genetically modified glyphosate tolerant crop varieties including soybean, cotton, rape seed and other lines of maize (see food and feed risk assessments of several maize e.g. NK603 x MON810; 1507 x NK603; MON 863 x NK603; MON 1445 x MON 531, GA21; and more).

Monsanto has conducted an acute toxicity study (Harrison et al 1996) in mice. Male and female CD-1 mice were dosed by gavage with the CP4-EPSPS protein produced in *E. coli*. Purity of the protein was > 90 %. This acute oral toxicity study was published in The Journal of Nutrition in 1996 by Harrison et al. A similar study has been published by Brooks in 2000.

The study was conducted in general compliance with the US EPA FIFRA GLP (40 CFR Part 160), EU-directive 88/320/EC) and acute oral toxicity guidelines of U.S. EPA and OECD (U.S. EPA Health Effects Test Guidelines. OPPTS 870.1100; Acute Oral Toxicity (US EPA 2002), OECD Guideline for Testing of Chemicals; Method No. 420: Acute Oral Toxicity-Fixed Dose Method; July 17, 1992 (OECD 2001)).

A total of 100 animals (50 males and 50 females) were used in the study, ranging from 5.5 weeks to 7 weeks of age. Test groups were randomised for weight and comprised 10 CD-1 mice of each sex per group.

The protein preparation containing the CP4 EPSPS was administered as a single dose by gavage to three groups of mice at dosages of 49, 154 and 572 mg/kg body weight respectively. The doses corresponded to 40, 100 and 400 mg/kg of CP4 EPSPS protein based on the level of purity of the protein and ELISA analyses of the dosing solutions. A control group received bovine serum albumin (BSA) at a dosage of 363 mg/kg in the same solution and delivery volume as the test substance. The second control group was administered vehicle solution only, 50 mM sodium bicarbonate.

At defined stages throughout the duration of the study, clinical observations were performed for mortality and signs of toxicity, and body weights and food consumption measured. Signs of toxicity included such occurrences as changes in the skin and fur, eyes and mucous membranes, respiratory, autonomic and central nervous systems as well as behavioural changes. At the termination of the study (day 8-9), animals were sacrificed, examined for gross pathology and numerous tissues were collected.

Tissues retained from the animals included aorta, adrenals, brain, colon, oesophagus, eyes, gall bladder, heart, kidneys, lung, liver, lymph nodes, muscle, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, seminal vesicles, skin, spinal cord, spleen, stomach, testes, thymus, uterus and bladder. Hollow organs were opened and examined.

The results of the study showed no statistically significant differences in group mean body weights, cumulative weight gains or food consumption in any of the groups treated with either BSA or the CP4 EPSPS protein, when compared with the carrier control group. The data were evaluated according to a decision-tree analysis procedure which, depending on the results of early statistical tests, determined further statistical analysis applied to detect group differences and analysis of trends. All animals survived to the scheduled termination of the study, and there were no clinical signs observed that could be related to the test material.

EHL decision-tree analysis (two-tailed): Terminal body weights were evaluated by decision-tree statistical analyses which, depending on the results of tests for normality (2) and homogeneity of variances [Bartlett's,Test (3)], utilised either parametric [Dunnett's Test (1) and Linear Regression (4)] or nonparametric [Kruskal-Wallis (5), Jonckheere's (6) antior Mann-Whitney (7) Tests] routines to detect differences and analysis of trends.

The acute oral LD_{50} of microbially-derived CP4 EPSPS protein in mice is greater than 572 mg/kg.

The VKM GMO panels agrees with the evaluation in the EFSA guideline that acute toxicity testing of newly expressed proteins is discouraged since this is of little additional value to the risk assessment for human and animal consumption of food and feed derived from GM plants (EFSA, 2011b).

4.3.1.2 ***Repeated dose toxicity testing***

Repeated dose 14-day oral toxicity study of CP4 EPSPS protein in rodents

Monsanto has performed a sub-chronic oral toxicity study of the CP4 EPSPS-protein in rats (Harrison et al 1996).

The study was performed in accordance with the principles of OECDs Good Laboratory practice of OECD (Organisation for Economic Cooperation and Development) and Principles of Good Laboratory Practice, 1992. Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles, March 1986 and the Japanese Ministry of Agriculture, Forestry and Fisheries: On Good Laboratory Practice Standards for Toxicological Studies on Agricultural Chemicals, Agricultural Production Bureau, 59 NohSan Notification Number 3850, August 10, 1984. Test guidelines: The study procedures mostly conform to OECD Guidelines for Testing of Chemicals, number 407 "Repeated Dose 28-day Oral Toxicity Study in Rodents", adopted by the Council on July 27, 1995).

According to the OECD guidelines for testing of chemicals (OECD 1995) the duration of exposure should normally be 28 days although a 14-day study may be appropriate under certain circumstances; justification for use of a 14-day exposure period should be provided. The duration of this repeated dose oral toxicity study was 14-days. No justification for using 14-days has been found in the dossier from the applicant.

The study comprised four groups of five male and five female Wistar rats in each group. The rats in group 1 received a standard diet without CP4 EPSPS protein, whereas rats in group 2, 3 and 4 received diets with the inclusion of CP4 EPSPS and/or soybean protein: group 1 (standard diet), group 2 (0.5 % CP4 EPSPS + 4.5 % soybean), group 3 (5 % CP4 EPSPS), group 4 (5 % soybean), for a period of 14 days.

The mean intake of CP4 EPSPS-protein in group 2 over the treatment period was 712 mg/kg body weight/day for males and 703 mg/kg body weight/day for females. In group 3 the mean intake of CP4 EPSPS-protein was 7965 mg/kg body weight/day for males and 7619 mg/kg body weight/day for females.

The results showed no unscheduled deaths or clinical signs. Food consumption and body weights were unaffected by treatment. No treatment-related changes were seen in haematology or urinalysis parameters. Organ weight data, macroscopical and microscopical findings did not distinguish treated groups from controls.

The only changes which might be attributed to treatment were observed in clinical biochemistry parameters. They consisted of a slightly lower glucose level in males of group 4, slightly higher total cholesterol and phospholipid levels in males of groups 2, 3 and 4 and slightly higher triglyceride levels in females of group 4 when compared with rats of group 1. Animals of group 4 received no CP4 EPSPS-protein but - with respect to the protein content - a diet most similar to that of groups 2 and 3. The changes mentioned above were considered to reflect differences in the dietary composition and not related to the CP4 EPSPS protein itself. Further, comparing the increased total cholesterol and phospholipid levels between group 3 (5 % CP4 EPSPS) and group 4 (5 % soybean) they were found to be within similar range, which may suggest a similar nutritional value of the proteins.

The repeated dose toxicity study in rats gave no indications for adverse effects attributable to the CP4 EPSPS protein up to the highest dose tested.

4.3.2 **Toxicological assessment of the whole GM food/feed**

90-day subchronic feeding studies in rats

The applicant has performed three 90-day feeding studies with the Sprague-Dawley –derived rat strain Crl:CD (SD)IGS BR in accordance to OECD 408 guidelines. The studies were conducted in compliance with the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice [C (97) 186/Final], November 26, 1997.

In the first feeding study (Kirkpatric 2005a, Monsanto study number WIL-50283) MON 88017 was formulated into rodent diets at 11 or 33% (w/w) levels. The diet containing 11 % MON 88017 maize was supplemented with 22 % of the conventional control maize LH59 x LH198. The control group of rats were fed 33 % LH59 x LH198 maize.

The three groups of Sprague-Dawley rats each consisted of 20 animals/gender/group. Male and female rats were approximately six weeks old at the start of the study. Body weight at the initiation of the study ranged from 153 grams to 206 grams for males and from 128 grams to 172 grams for females. Group mean body weights at study initiation were not statistically different by sex at the 5% probability level. Rats were housed individually and provided food and water *ad libitum* for a minimum of 90 days.

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily and detailed physical examinations were performed weekly. Individual body weights and food consumption were recorded weekly. Clinical pathology evaluations (hematology, serum chemistry, and urinalysis) were performed on the first 10 animals/sex/group at the scheduled necropsy (study week 13). Complete necropsies were conducted on all animals and selected organs were weighed at the scheduled necropsy. Selected tissues were examined microscopically from all animals fed diets containing 33% control and 33% MON 88017 maize grain.

Clinical observation results showed that all animals survived to the scheduled necropsy. There were no test substance-related clinical observations. Body weights, food consumption and clinical pathology parameters were unaffected by test substance administration. There were no test substance-related effects on organ weights. There were no test substance-related findings at the macroscopic or microscopic examinations.

According to the applicant administration of MON 88017 to rats for 90 consecutive days at concentrations up to and including 33% in the diet had no adverse effects on the growth or health of Sprague Dawley (Crl:CD(SD)IGS BR) rats.

In the second 90-day feeding study (Kirkpatric 2005b, Monsanto study number WIL-50284) six different reference maize control varieties (Moews 3260, N.K. (Northrup King) N60-N2,

SC 1091, SC 1122, DK477 and LH295 x LH224) that had different genetic backgrounds and were grown in different geographies, were separately presented *ad libitum* in the diet for a minimum of 13 consecutive weeks to six groups (Groups 1-6) of Crl:CD(SD)IGS BR rats.

Variables measured in these groups can be considered to approximate the normal range of biological responses for the larger population of control rats.

Dietary incorporation levels of the maize kernels in the diets were approximately 33% (w/w) for each of the six groups. Each group consisted of 20 male rats and 20 female rats. Homogeneity of the diet formulations was not determined because an appropriate methodology to quantify the concentration of transgenic or non-transgenic maize grain in diets was not available. Nutritional components were analysed from samples of each diet and evaluated for possible environmental contaminants.

These analytes included proximates: moisture, protein, fat, ash, and crude fiber; minerals: calcium and phosphorus; heavy metals: arsenic, cadmium, lead, mercury, and selenium; aflatoxins; and a pesticide screen. Additionally, samples of each diet were shipped from Purina TestDiet to Monsanto Company, St. Louis, Missouri, for verification of identity.

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed approximately weekly. Individual body weights and food consumption were recorded approximately weekly. Clinical pathology evaluations (hematology, serum chemistry, and urinalysis) were performed on 10 animals/sex/group at scheduled necropsy (study week 13). Complete necropsies were conducted on all animals, and selected organs were weighed at scheduled necropsy. Tissues were saved, but only the liver and kidneys were examined microscopically.

All animals except one male from one of the six control groups fed different reference maize varieties survived to the scheduled necropsy. The rat (fed reference maize SC 1122) died on study day 51 of unknown causes.

The third 13-week feeding study in rats using grains of maize MON 88017 as a component of the diet has been presented by Healy et al (2008). The study design was according to OECD Guideline No. 408 (1998) and was conducted in general compliance with OECD Good Laboratory Practice (GLP) guidelines (OECD, 1997). Groups of 20 male and 20 female rats (Crl:CD(SD) IGS BR) were fed diets containing 11% or 33% (w/w) grains from maize MON 88017 treated with glyphosate. The control group comprising 20 male and 20 female Crl:CD(SD) IGS BR rats fed 33% (w/w) grains from the near isogenic non-GM control maize. Additionally, six diets containing grain from different conventional (non-biotechnology-derived) reference maize (name not given) were formulated, each at 33% (w/w) levels of one of six reference grains. All diets were nutritionally balanced and conformed to PMI specifications for Certified LabDiet 5002.

All animals were observed twice daily for mortality and moribundity and once daily for overt signs of toxicity; physical examinations were given weekly. Individual weights were obtained one day prior to group allocation and weekly thereafter. Individual food consumption was determined weekly. Animals continued on test, control, and reference diets for a minimum of 90 days.

Results of the study showed no toxicologically significant differences between treatment groups. At the end of the experiment clinical pathological evaluation was performed including haematology, serum chemistry and urine analysis. In addition a complete necropsy was carried out including weight of organs, macroscopic examinations and histopathology. Observations included nutritional variables, clinical and neurobehavioral signs, ophthalmology, clinical pathology (haematology, clinical chemistry, coagulation, and urinalysis), organ weights, and gross and microscopic pathology. Tissues collected were: Aorta, adrenals, bone marrow smear, brain, epididymides, esophagus, eyes (with optic nerve), heart, intestine (ileum, jejunum, duodenum, colon, cecum), kidneys, larynx, lesions or abnormal masses, liver, lungs (with mainstem bronchi), lymph nodes (mandibular and mesenteric), nasal cavity, ovaries (with oviducts), pancreas, peripheral nerve (sciatic), pharynx, pituitary, prostate, rectum, salivary glands (mandibular), seminal vesicles, skeletal muscle (rectus femoris), skin (with mammary tissue), spinal cord (three levels), spleen, sternum with marrow, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus (corpus and cervix), and vagina. Following collection, tissues were placed directly into 10% neutral buffered formalin for fixation.

Selected tissues (adrenal glands, brain, epididymides, stomach, duodenum, jejunum, ileum, colon, rectum, heart, kidneys, liver, lymph nodes (mesenteric), ovaries with oviducts, pancreas, spleen, testes, thymus, and thyroid (with parathyroids, if present), gross lesions) representing the major organs/systems from all animals fed 33% w/w MON 88017 and control grain were processed, embedded in paraffin, sectioned (4 – 8 µm), stained with hematoxylin and eosin using standard histological methods, and examined by a board-certified veterinary pathologist using light microscopy.

Statistical analysis

Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test-group treated with MON 88017 to the control group by sex. These are presented in the text and tables as statistically significantly different at "$p < 0.01$" or "$p < 0.05$." Each group mean is presented with the standard deviation (SD) and the number of animals (N) used to calculate the mean. Body weight, body weight change, food consumption, clinical pathology and organ weight data were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistically significant ($p < 0.05$) intergroup variance, Dunnett's test was used to compare the test-article treated groups to the control group.

Clinical pathology values for white blood cell types that occur at a low incidence (i.e., monocytes, eosinophils and basophils) and histopathology data were not subjected to statistical analyses.

No toxicologically significant diet-related differences were observed among the groups fed with any of the different diets with respect to clinical signs of toxicity, ophthalmological observations, neurobehavioral assessments, clinical pathology (clinical chemistry, coagulation, or urinalysis parameters), organ weights, and gross or microscopic pathology.

For haematology parameters a statistically significantly higher mean absolute neutrophil count was observed only in females fed 33% maize MON 88017 compared with the control group. There was no difference in the relative neutrophil count between rats fed 33% maize MON 88017 and the control group. Since the higher mean absolute neutrophil count was within the range of normal variation and there were no differences in related parameters the observed difference was considered by the authors as an incidental finding.

There were no significant differences ($p < 0.05$) in serum chemistry and urinalysis values when control and test-article treated groups were compared.

Organ weights were not adversely affected by administration of MON 88017. There were no significant differences ($p < 0.05$) in absolute or organ to body and organ to brain weight ratios when the control and test-article treated groups were compared.

At necropsy, no gross (data not shown) or microscopic lesions were observed that were considered to relate to the feed containing MON 88017.

The few findings that were observed were randomly distributed among all groups, including controls, and were of the type commonly observed in rats of this age and strain.

4.4 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 2010b).

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

4.4.1 **Assessment of 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 and CP4 EPSPS proteins, which includes:

- 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

These assessments have previously been described by the applicant for Cry3Bb1 and CP4 EPSPS, and were based on the following aspects:

Cry3Bb1 and CP4 EPSPS

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

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

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

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

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

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

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

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

The information listed above indicates that the newly expressed proteins in MON 88017 maize 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.

4.4.2 Assessment of the allergenicity of the whole GM plant

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

4.4.3 Assessment of the 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 would be unlikely to alter the overall allergenicity of the whole plant or the allergy risk for consumers.

4.4.4 Adjuvanticity

According to the EFSA Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed (EFSA 2010b) 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).

4.5 Nutritional assessment of GM food/feed

Compositional analyses of maize MON 88017 indicate nutritional equivalence to the non-GM control maize with comparable genetic background and to the published range of values in the literature. The poultry feeding study has shown nutritional equivalence between MON 88017 maize and non-GM control maize as described in 4.5.2.

4.5.1 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).

FG3 has calculated a Theoretical Maximum Daily Intake (TMDI) for acute dietary consumption of Cry3Bb1 protein in maize and maize products. The mean level of Cry3Bb1 in grain was 15 µg/g tissue dry weight. The TMDI is 66 µg of Cry3Bb1 protein per adults per day, and 26 µg per child per day.

These exposure estimates are based on the mean Cry3Bb1 protein expression levels reported for MON 88017 maize grain (6.9 µg Cry3Bb1 per g grain dry weight). These results indicate that there is reasonable certainty that there will be no toxic or any other adverse effects associated with consumption of MON 88017 maize food products.

Based on these numbers, and that all foods from maize are derived from maize MON 88017 grain, the estimated maximum daily intake for this level is several orders of magnitude 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).

This dietary exposure assessment is very conservative, as it assumes that all consumed maize consists of MON 88017 and that protein levels are not reduced by processing.

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 therefore may be higher for these animals.

4.5.2 Nutritional assessment of feed derived from the GM plant

42-day feeding study on broilers

A 42-day broiler feeding study was conducted to confirm the nutritional equivalence of the MON 88017 with conventional non-transgenic maize LH59 x LH198 and four non-GM commercial maize (Asgrow RX708, Pioneer 34B23, Burrus789, Burrus582, Burrus569) (Monsanto study number: MN-03-3, Taylor et al 2005). The non-transgenic maize LH59 x LH198 has a genetic background representative of MON 88017, but is not genetically modified and does not express either the Cry3Bb1 or CP4 EPSPS proteins.

A total of 350 broiler of each sex, commercial strain of Ross x Ross 508, were randomly distributed into 35 pens of each sex at one day of age. Each pen contained 10 broilers. Pens were set up as a randomised complete block experimental design with 7 diets (treatments) in each of 5 replicated blocks of pens. Each block contained 14 pens (one for each diet and sex combination), with 10 birds per pen for a total of 700 birds (350 males and 350 females). The GLM and Mixed procedures in Release 9.1 of the Statistical Analysis System (SAS) version 8.2 were used in analyzing each experiment. According to the OECD guidelines of animal feedstuffs derived from genetically modified plants (OECD 2003) broiler are useful for comparative growth studies. Because of their rapid weight gain, broiler are particularly sensitive to any change in nutrient supply or the presence of toxic elements in their feed and are particularly useful for this purpose.

The test, control and reference substance diet mixtures were fed continuously for 42-days. Broilers were fed starter feed on trial days 0-21 (54 % maize), and grower/finisher feed on trial days 22-42 (59 %). 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 Cry3Bb1 and CP4 EPSPS proteins in the diets containing MON 88017. These proteins were not detected in control substances.

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

Each measurement was statistically analysed by two different procedures. The first method was a two-factor analysis of variance under a randomised complete block structure. The two factors were diet and sex. The main effects of diet and sex along with the diet-by-sex interaction were tested. If the interaction was not significant ($P \geq 0.15$) then the comparison of the diets was done using the main effect for diets, i.e., diet means will be averaged over sex. If the interaction were significant then the diet comparisons were done, separately for each sex. Mean separation procedures were performed using the protected LSD (Least Significant Difference) method with 0.05 significant level in SAS.

There are five diets specified in model (1), which was identified as "treat" in the SAS program. The results of these analyses for Average Bird Weight, Feed Intake, Feed Conversion, and Adjusted Feed Conversion on day 42 are summarized tables in Taylor 2005 (not shown).

Body weight, daily weight gain (gram per bird per 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. Consistent with historical data and study type, a low incidence of mortality occurred among all study groups. The results showed that at day 0, start of feeding, there was no overall statistically significant difference in the mean body weight of broiler in the different treatment groups. On trial day 42 there were no statistically significant differences in mean body weight among any of the six treatments. There were no statistically significant differences in mean feed conversion corrected for body weight among any of the six treatments, and for cumulative unadjusted and adjusted feed conversion ratios at any time. Mortality was recorded daily between trial days 0-42. There were no statistical differences in mean percent mortality among any of the six treatments. All survival rates were consistently high. Individual body weight was recorded on days 0 and 42. There were no statistically significant differences in mean body weight on trial day 0 among the any of the six treatments, and no statistical significant differences in mean daily weight gain among any of the six treatments. At the 5% level of significance, no differences were noted for males or females for live weight (day 42), final live body weight, and chill weight. In one case where a diet-by-gender interaction was noted at the 15% level of significance, a difference at the 5% level of significance was noted for the thigh weight (kg) of males only. Male broilers fed diets containing MON 88017 had similar ($P>0.0.5$) thigh weight as males fed diets containing the conventional control and commercial references Asgrow RX708, Pioneer 34B23 and Burrus 789 but greater ($P <0.05$) thigh weight than the commercial references Burrus 569 and Burrus 582. However, this result is not likely to be of biological significance since some differences at the 5% level of significance were also noted among the commercial references, and pairwise comparisons between diets (combined gender) showed no differences ($P > 0.05$). There were no statistically significant differences in mean feed conversion corrected for body weight among any of the six treatments.

The results of the broiler feeding study show that there were no differences in parameters tested between broilers fed a diet containing MON 88017 or LH59 x LH198 and diets

containing maize grain from non-transgenic control line or standard diets containing three of the non-GM commercial maize. The results show that maize MON 88017 and LH59 x LH198 are equivalent to three non-GM commercial maize in the ability to provide adequate nutrition to rapidly growing broiler.

4.6 Conclusion

Whole food feeding studies on rats and broilers indicate no adverse health effects of maize MON 88017. These studies also show that maize MON 88017 is nutritionally equivalent to conventional maize. The Cry3Bb1 and CP4 EPSPS proteins do not show relevant sequence resemblance to other known toxins or IgE-allergens, nor have they been reported to cause IgE-mediated allergic reactions. However, some studies have indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

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

5 Environmental risk assessment

5.1 Unintended effects on plant fitness due to the genetic modification

Maize (*Zea mays* L.) is an annual plant and member of the grass family Poacea. The species, originating from Central America, is highly domesticated and generally unable to survive in the environment without management intervention (Eastham & Sweet 2002). Maize propagates entirely by seed produced predominantly by cross-pollination (OECD 2003). In contrast to weedy plants, maize has a pistillate inflorescence (ear) with a cob enclosed with husks. Due to the structure of the cob, the seeds remain on the cob after ripening and natural dissemination of the kernels rarely occurs.

The survival of maize in Europe is limited by a combination of absence of a dormancy phase resulting in a short persistence, high temperature requirements for germination, low frost tolerance, low competitiveness and susceptibility to plant pathogens, herbivores and climatic conditions (van de Wiel et al. 2011). Maize plants cannot survive temperatures below 0ºC for more than 6 to 8 hours after the growing point is above ground (OECD 2003), and in Norway and most of Europe, maize kernels and seedlings do not survive the winter cold (Gruber et al. 2008). Observations made on cobs, cob fragments or isolated grains shed in the field during harvesting indicate that grains may survive and overwinter in some regions in Europe, resulting in volunteers in subsequent crops. The occurrence of maize volunteers has been reported in Spain and other European regions (e.g. Gruber et al. 2008). However, maize volunteers have been shown to grow weakly and flower synchronously with the maize crop (Palaudelmás et al. 2009). Cross-pollination values recorded were extremely variable among volunteers, most probably due to the loss of hybrid vigour and uniformity. Overall cross-pollination to adjacent plants was estimated as being low.

Despite cultivation in many countries for centuries, seed-mediated establishment and survival of maize outside cultivation or on disturbed land in Europe is rare (BEETLE Report 2009). Maize plants occasionally grow in uncultivated fields and by roadsides. However the species is incapable of sustained reproduction outside agricultural areas in Europe and is non-invasive of natural habitats (Eastham & Sweet 2002; Devos et al. 2009). There are no native or introduced sexually cross-compatible species in the European flora with which maize can hybridise and form backcross progeny (Eastham & Sweet 2002; OECD 2003). The only recipient plants that can be cross-fertilised by maize are other cultivated maize cultivars.

It is considered very unlikely that the establishment, spread and survival of maize MON 88017 would be increased due to the insect resistance and herbicide tolerance traits. The herbicide tolerant trait can only be regarded as providing a selective advantage for the GM maize plant where and when glyphosate-based herbicides are applied. Similarly insect resistance against certain coleopteran pests provides a potential advantage in cultivation of MON 88017 under infestation conditions. It is considered very unlikely that maize MON

88017 plants or their progeny will differ from conventional maize cultivars in their ability to survive as volunteers until subsequent seasons, or to establish feral populations under European environmental conditions.

Field trials carried out by the applicant do not indicate altered fitness of maize MON 88017 relative to its conventional counterpart. A series of field trials with maize MON 88017 were carried out across several locations in the USA in 2001 and 2002, and in Argentina in the 2003/2004 growing season (application EFSA/GMO/UK/2005/21). In addition, agronomic observations performed in field trials in the EU in 2006 (Spain and Germany) have been provided by the applicant in application EFSA/GMO/CZ/2008/54. Information on phenotypic (e.g. crop physiology, morphology, development) and agronomic characteristics was provided to assess the agronomic performance of maize MON 88017 in comparison with its conventional counterpart (see section 3.3). 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, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of maize MON 88017 are unchanged, insect resistance and glyphosate tolerance are not likely to provide a selective advantage outside of cultivation in Europe. The VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects based on establishment and survival of maize MON 88017 will not differ from that of conventional maize varieties.

5.2 Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or vertical gene flow via pollen or seed dispersal. Exposure of microorganisms to transgenic DNA occurs during decomposition of plant material remaining in the field after harvest or comes from pollen deposited on cultivated areas or the field margins. Transgenic DNA is also a component of a variety of food and feed products derived from maize MON 88017. This means that micro-organisms in the digestive tract in humans and animals (both domesticated animals and other animals feeding on fresh or decaying plant material from the transgenic maize line) may be exposed to transgenic DNA.

Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation with which maize can hybridise and form backcross progeny (Eastham & Sweet 2002; OECD 2003). Vertical gene transfer in maize therefore depends on cross-pollination with other conventional or organic maize varieties. All maize varieties which are cultivated in Europe can interbreed. In addition,

unintended admixture/adventitious presences of genetically modified material/transgenes in seeds represent a possible way for gene flow between different production systems.

5.2.1 Plant to micro-organisms gene transfer

Experimental studies have shown that gene transfer from transgenic plants to bacteria rarely occurs under natural conditions and that such transfer depends on the presence of DNA sequence similarity between the DNA of the transgenic plant and the DNA of the bacterial recipient (Nielsen et al. 2000; De Vries & Wackernagel 2002, reviewed in EFSA 2004, 2009a; Bensasson et al. 2004; VKM 2005c).

Based on established scientific knowledge of the barriers for gene transfer between unrelated species and the experimental research on horizontal transfer of genetic material from plants to microorganisms, there is today little evidence pointing to a likelihood of random transfer of the transgenes present in maize MON 88017 to unrelated species such as bacteria.

It is however pointed out that there are limitations in the methodology used in these experimental studies (Nielsen & Townsend 2004). Experimental studies of limited scale should be interpreted with caution given the scale differences between what can be experimental investigation and commercial plant cultivation.

Experiments have been performed to study the stability and uptake of DNA from the intestinal tract in mice after M13 DNA was administered orally. The DNA introduced was detected in stool samples up to seven hours after feeding. Small amounts (<0.1%) could be traced in the blood vessels for a period of maximum 24 hours, and M13 DNA was found in the liver and spleen for up to 24 hours (Schubbert et al. 1994). By oral intake of genetically modified soybean it has been shown that DNA is more stable in the intestine of persons with colostomy compared to a control group (Netherwood et al. 2004). No GM DNA was detected in the faeces from the control group. Rizzi et al. (2012) provides an extensive review of the fate of feed-derived DNA in the gastrointestinal system of mammals.

In conclusion, the VKM GMO Panel consider it is unlikely that the introduced gene from maize MON 88017 will transfer and establish in the genome of microorganisms in the environment or in the intestinal tract of humans or animals. In the rare, but theoretically possible case of transfer of the *cry*, pat and CP4 EPSPS genes from MON 88017 to soil bacteria, no novel property would be introduced into or expressed in the soil microbial communities; as these genes are already present in other bacteria in soil. Therefore, no positive selective advantage that would not have been conferred by natural gene transfer between bacteria is expected.

5.2.2 **Plant to plant gene flow**

Considering the intended uses of maize MON 88017 (excluding cultivation) and the physical characteristics of maize seeds, possible pathways of gene dispersal are grain spillage and dispersal of pollen from potential transgenic maize plants originating from accidental grain spillage during transport and/or processing.

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

Survival of maize plants outside cultivation in Europe is mainly limited by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens, herbivores and frost. As for any other maize cultivars, GM maize plants would only survive in subsequent seasons in warmer regions of Europe and are not likely to establish feral populations under European environmental conditions. In Norway, maize plants from seed spillage occasionally grow on tips, waste ground and along roadsides (Lid & Lid 2005).

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

As maize MON

88017 has no altered survival, multiplication or dissemination characteristics, the VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this GM maize in Norway will not differ from that of conventional maize varieties. The likelihood of cross-pollination between cultivated maize and the occasional feral maize plants resulting from grain spillage is considered extremely low.

5.3 Interactions between the GM plant and target organisms

Maize MON 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)

Considering the intended uses of maize MON 88017, excluding cultivation, the environmental exposure is limited to exposure through manure and faeces from the gastrointestinal tract mainly of animals fed on the GM maize as well as to the accidental release into the environment of GM seeds during transportation and processing and subsequently to potential occurrence of sporadic feral plants. Thus the level of exposure of target organisms to Cry3Bb1 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, 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 protein enters the environment due to the expression in the grains (mean value of 8.7 and 15 µg/g dwt in the EU and USA field trials, respectively (range 5.8-22 µg/g dwt). Data have been submitted that demonstrate that the Cry3Bb1 protein is rapidly degraded by gastric fluid *in vitro*. In a solution of simulated gastric fluid, complete degradation of detectable Cry3Bb1 protein occurred within 30 seconds (EPA 2007). Insect bioassay indicated that the protein loss insecticidal activity within 2 minutes of incubation in SFG.

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, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with the abiotic environment and biogeochemical cycles were not considered an issue by the VKM GMO Panel.

5.6 Conclusion

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

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

6 Post-market environmental monitoring

Directive 2001/18/EC introduces an obligation for applicants to implement monitoring plans, in order to trace and identify any direct or indirect, immediate, delayed or unanticipated effects on human health or the environment of GMOs as or in products after they have been placed on the market. Monitoring plans should be designed according to Annex VII of the Directive. According to Annex VII, the objectives of an environmental monitoring plan are 1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO or its use in the environmental risk assessment (ERA) are correct, and (2) to identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the environmental risk assessment.

Post-market environmental monitoring is composed of case-specific monitoring and general surveillance (EFSA 2011c). Case-specific monitoring is not obligatory, but may be required to verify assumptions and conclusions of the ERA, whereas general surveillance is mandatory, in order to take account for general or unspecific scientific uncertainty and any unanticipated adverse effects associated with the release and management of a GM plant. Due to different objectives between case-specific monitoring and general surveillance, their underlying concepts differ. Case-specific monitoring should enable the determination of whether and to what extent adverse effects anticipated in the environmental risk assessment occur during the commercial use of a GM plant, and thus to relate observed changes to specific risks. It is triggered by scientific uncertainty that was identified in the ERA.

The objective of general surveillance is to identify unanticipated adverse effects of the GM plant or its use on human health and the environment that were not predicted or specifically identified during the ERA. In contrast to case-specific monitoring, the general status of the environment that is associated with the use of the GM plant is monitored without any preconceived hypothesis, in order to detect any possible effects that were not anticipated in the ERA, or that are long-term or cumulative.

No specific environmental impact of genetically modified maize MON 88017 was indicated by the environmental risk assessment and thus no case specific monitoring is required. The VKM GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicant is in line with the intended uses of maize NK603 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects.

7 Conclusion

Molecular characterisation

The molecular characterisation data has established that only one copy of the transgene is integrated in the maize genomic DNA. Appropriate analyses of the integration site including sequence determination of the inserted DNA and flanking regions and bioinformatics analysis have been performed. Bioinformatics analyses of junction regions have demonstrated the absence of any potential new ORFs coding for known toxins or allergens. The genetic stability of transformation event MON 88017 was demonstrated at the genomic level over multiple generations by Southern analysis. Segregation analysis shows that event MON 88017 is inherited as a dominant, single locus trait. The VKM GMO Panel considers the molecular characterisation of maize MON 88017 satisfactory.

Comparative assessment

Comparative analyses of maize MON 88017 and its conventional counterpart have been performed during field trials located at representative sites and environments in Europe and USA. A total of 12-16 different conventional maize varieties were included in the field trials and used as references. With the exception the insect resistance and herbicide tolerance conferred by the Cry3Bb1 and CP4 EPSPS proteins, no biologically relevant differences were found between maize MON 88017 and controls. Based on the assessment of available data, the VKM GMO Panel concludes that maize MON 88017 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart except for the new proteins.

Food and feed safety assessment

Whole food feeding studies on rats and broilers indicate no adverse health effects of maize MON 88017. These studies also show that maize MON 88017 is nutritionally equivalent to conventional maize. The Cry3Bb1 and CP4 EPSPS proteins do not show relevant sequence resemblance to other known toxins or IgE-allergens, nor have they been reported to cause IgE-mediated allergic reactions. However, some studies have indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

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

Environmental risk assessment

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

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

Overall conclusion

Based on current knowledge, the VKM GMO Panel concludes that maize MON 88017 is compositionally, nutritionally, agronomically and phenotypically equivalent to its conventional counterpart except for the new proteins. 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.

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

8 Data gaps

Adjuvanticity

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

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

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

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

Herbicide residue levels

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

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

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

9 References

Anderson, W.P. (1996) Weed ecology. Weed Science Principles and Applications.

AOSA. (1998) Rules for testing seeds. Association of Official Seed Analysists.

BEETLE report (2009). Long term effects of genetically modified (GM) crops on health and the environment (including biodiversity): prioritization of potential risks and delimitation of uncertainties. German Federal Office of Consumer Protection and Food Safety, BLaU-Umweltstudien and Genius GmbH. http://ec.europa.eu/environment/biotechnology/pdf/beetle_report.pdf

Bensasson D, Boore JL, Nielsen KM (2004) Genes without frontiers. Heredity 92: 483-489

Brandtzaeg P, Tolo K (1977) Mucosal penetrability enhanced by serum-derived antibodies. Nature 266: 262-263.

Brooks, K.J. (2000) CP4 EPSPS microbial protein (FL): Acute toxicity study in CD-1 mice. Study number 991249. Unpublished technical report. Dow Agrosciences LLC.

Chao E, Krewski D (2008) A risk-based classification scheme for genetically modified foods I: Conceptual development. Regul. Toxicol. Pharmacol. 52: 208–222.

Codex (2003) "Codex principles and guidelines on foods derived from biotechnology." Codex Alimentarius Comission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organisation: Rome.

de Vries J, Wackernagel W (2002) Integration of foreign DNA during natural transformation of Acinetobacter sp. by homology-facilitated illegitimate recombination. The Proceedings of the National Academy of Sciences USA 99: 2094-2099

Devos Y, Demont M, Dillen K, Reheul D, Kaiser M, Sanvido O (2009) The coexistence of genetically modified (GM) and non-GM crops in the European Union. Agronomy for Sustainable Development 29: 11-30

Dien BS, Bothast RJ, Iten LB, Barrios L, Eckhoff SR (2002) Fate of Bt Protein and Influence of Maize Hybrid on Ethanol Production. Cereal Chem 79(4): 582-585.

Dill (2005) Dill, G.M., CaJacob, C.A. & Padgette, S.R. (2008). Glyphosate-resistant crops: adoption, use and future considerations. *Pest Management Science,* **64**, 326-331.

Dudin, Y.A. and Jennings, J.C. (2005) Assessment of CP4 EPSPS and Cry3Bb1 protein levels in corn grain from MON 88017 produced in 2003-2004 Argentina field trials. Monsanto Technical Report, MSL19781 and Individual data Dudin and Jennings 2005.

Drury (2008) Drury SM, Riordan SG, Miller KD, Sorbert R (2008) Compositional analyses of corn forage and grain from MON 88017 untreated with Roundup® herbicide produced at multiple locations in Germany and Spain in 2007. Monsanto Technical Report RAR-08-361

Duke, S.O. and Powles S.B. (2008) Glyphosate: a one-in-a-century herbicide. Pest Management Scinece, 64, 319-325.

Eastham K, Sweet J (2002) Genetically modified organisms (GMO): The significance of gene flow through pollen transfer. Environmental issue report. No 28. European Environment Agency (EEA), Copenhagen. http://reports.eea.eu.int/environmental_issue_report_2002_28/en

EEC (1992) European Union; Annex to Council Directive 92/69/EEC, Part B, Methods B.3, B.4, B.6, B.9, B.28, B.30 and B.32. Official Journal No. L383A, 29.12.92.

EFSA (2004) Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. The EFSA Journal, 48, 1-18. http://www.efsa.europa.eu/en/science/gmo/gmo_opinions/384.html.

EFSA (2004) Opinion of the Scientific Panel on genetically modified organisms on a request from the Commission related to the notification (Reference C/NL/00/10) for the placing on the market of insect-tolerant genetically modified maize MON88017, for import and processing, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds(Reference EFSA-Q-2004-011). The EFSA Journal 1-18.

EFSA (2005) Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/ES/01/01) for the placing on the market of insect-tolerant genetically modified maize MON88017, for import, feed and industrial processing and cultivation, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds (Question No EFSA-Q-2004-072).The EFSA Journal 181, 1-33.

EFSA (2006) Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. ISBN: 92-9199-019-1. European Food Safety Authority, Parma, Italy. 100 p.

http://www.efsa.europa.eu/en/science/gmo/gmo_guidance/660.html

EFSA (2009a) Use of antibiotic resistance genes as marker genes in genetically modified plants. Scientific Opinion of the Panel on Genetically Modified Organisms (GMO) and the Panel on Biological Hazards (BIOHAZ). The EFSA Journal 1034: 1-82

http://www.efsa.europa.eu/cs/BlobServer/Statement/gmo_biohaz_st_ej1108_ConsolidatedARG_en.pdf?ssbinary=true

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

http://www.efsa.europa.eu/en/efsajournal/doc/1075.pdf

EFSA (2010a) Guidance on the environmental risk assessment of genetically modified plants. Scientific option from the EFSA Panel on Genetically Modified Organisms (GMO). The EFSA Journal 8 (11):1-111

http://www.efsa.europa.eu/en/efsajournal/doc/1879.pdf

EFSA (2010b) Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010; 8(7):1700. [168 pp.]. doi:10.2903/j.efsa.2010.1700.

EFSA (2011a) Guidance for risk assessment of food and feed from genetically modified plants. The EFSA Journal 9(5): 2150.
http://www.efsa.europa.eu/en/efsajournal/doc/2150.pdf

EFSA (2011b) EFSA Panel on Genetically Modified Organisms (GMO). Scientific Opinion on Guidance on selection of comparators for the risk assessment of genetically modified plants and derived food and feed. EFSA Journal 9(5):2149

EFSA (2011c) Guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. The EFSA Journal 9(8):2316

EFSA (2011d) Scientific Opinion on application (EFSA-GMO-CZ-2008-54) for placing on the market of genetically modified insect resistant and herbicide tolerant maize MON 88017 for cultivation under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 9(11): 2428 http://www.efsa.europa.eu/en/search/doc/2428.pdf

EFSA (2012) Scientific Opinion updating the risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize MON88017. The EFSA Journal 2012; 10(10):2933. [46 pp.] doi:10.2903/j.efsa.2012.2933.

EPA (2007) Biopesticides registration action document. *Bacillus thuringiensis* Cry3Bb1 Corn. US Environmental Agency.

FAO/WHO (2001). "Evaluation of allergenicity of genetically modified foods. Report of a joint Food and Agriculture Organization/World Health Organization (FAO/WHO) expert consultation on allergenicity of foods derived from biotechnology." Joint Food

and Agriculture Organization/World Health Organization (FAO/WHO) expert consultation on allergenicity of foods derived from biotechnology, Rome, Italy., Food and Agriculture Organization of the United Nations.

Fard NA, Minuchehr Z, Mousawi A. (2013) Allergenicity study of genetically modified herbicide resistant crops (Bioinformatics Assessment). Bull. Env. Pharmacol. Life Sci. 2 (3): 24-32.

Fernandes TJR, Oliveria MBPP, Mafra I (2013) Tracing transgenic maize as affected by breadmaking process and raw material for the production of a traditional maize bread, broa. Food Chemistry 138(1); 687-692.

Gruber (2008) Gruber S, Colbach N, Barbottin A, Pekrun C (2008) Post-harvest gene escape and approaches for minimizing it. Cab reviews: Perspective in Agriculture, Veterinary Science, Nutrition and Natural Resources, 3, No. 015, 17 pp

Guertler (2008) Guertler P, Lutz B, Kuehn R, Meyer HHD, Einspanier R, Killermann B, Albrecht C (2008) Fate of recombinant DNA and Cry1Ab protein after ingestion and dispersal of genetically modified maize in comparison to rapeseed by fallow deer (Dama dama). European Journal of Wildlife Research 54: 36–43

Guerrero (2004) Guerrero GG, Dean DH, Moreno-Fierros L (2004) Structural implication of the induced immune response by Bacillus thuringiensis Cry proteins: role of the N-terminal region. Molecular Immunology 41 (2004) 1177–1183

Hammond RG, Jez JM (2011) Impact of food processing on the safety assessment for proteins introduced into biotechnology-derived soybean and corn crops. Food Chem. Toxicol. 49;711-721.

Harrison, L.A., Bailey, M.R., Naylor, M.W., Ream, J.E., Hammond, B.G., Nida, D.L., Burnette, B.L., Nickson, T.E., Mitsky, T.A., Taylor, M.L., Fuchs, R.L. and Padgette, S.R. (1996) The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3- phosphate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. J.Nutr., 126, 728-740.

Healy C, Hammond B, Kirkpatrick J (2008) Results of a 13-week safety assurance study with rats fed grain from corn rootworm-protected, glyphosate-tolerant MON 88017 corn. Food Chem. Toxicol. 46;2517-2524.

Hérouet C, Esdaile DJ, Mallyon BA, Debruyne E, Schulz A, Currier T, Hendrickx K, van der Klis R-J, Rouan D (2005) Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the CP4 EPSPS and bar sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants. Regul Toxicol Pharmacol 41; 134–149.

Kaempfe TA, Bonner HKS (2003) An acute oral toxicity study in mice with *E.coli*- produced Cry3Bb1.pvzmir39 protein. Monsanto Technical Report, MSL 18711.

Kim J-H, Seo Y-J, Kim J-Y, Han Y-S, Lee K-S, et al. (2010) Allergenicity assessment of Cry proteins in insect-resistant genetically modified maize Bt11, MON810, and MON863. Food Sci Biotechnol 18; 1273–1278.

Kirkpatrick, J.B. (2005a) A 90-day feeding study in rats with MON 88017 corn grain. WIL-50283.

Kirkpatrick, J.B. (2005b) A 90-day feeding study in rats with six different reference corn varieties. WIL-50284.

Kuiper HA, Kleter GA, Noteborn HP, Kok EJ (2001) Assessment of the food safety issues related to genetically modifed foods. Plant J 27(6); 503-528.

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

Icoz

Lid & Lid (2005) Lid J, Lid DT (2005) Norsk flora. Det Norske Samlaget, Oslo. 7. utgave. ISBN: 82-521-6029-8. 1230s.

Lim PL, Rowley D (1982) The effect of antibody on the intestinal absorption of macromolecules and on the intestinal permeability in adult mice. Int. Archs. Allergy Appl. Immun. 68; 41-46.

Lutz B, Wiedermann S, Einspanier R, Mayer J, Albrecht C (2005) Degradation of Cry1Ab protein from genetically modified maize in the bovine gastrointestinal tract. Journal of Agricultural and Food Chemistry 53: 1453–1456

Marcinkiewicz, B. (2005) Field production of tissues and grain from Coleopteranresistant and glyphosate tolerant corn MON 88017 in Argentina during 2003-2004, Monsanto Technical Report, MSL 19194

McCann (2003a) Evaluation of the Composition of Forage and Grain Collected from MON 88017 and MON 88017 x MON 810 Corn Grown in 2002 U.S.Field Trials

McCann (2003b) Evaluation of the composition of Forage and Grain Collected from MON 88017 Corn Grown in 2002 U.S. Field Trials

McCann (2005) Evaluation of the Composition of Corn Forage and Grain Collected from MON 88017 Grown in 2003-2004 Argentina Field Trials

McClintock JT, Schaffer CR, Sjoblad RD (1995) A comparative review of the mammalian toxicity of *Bacillus thuringiensis*-based pesticides. Pestic Sci 45;95-105.

Meyer T (1999) Comparison of amino acid sequence similarity of Cry1F and PAT proteins to known allergen proteins. Pioneer Hi-Bred International, Inc.

Miller, N., Estoup, A., Toepfer, S., Bourguet, D., Lapchin, L., Derridj, S., Kim, K. S., Reynaud, P., Furlan, L. and Guillemaud, T. (2005) Multiple Transatlantic Introductions of the Western Corn Rootworm, Science, 310, 992.

Moreno-Fierros L, Ruiz-Medina EJ, Esquivel R. et al. (2003) Intranasal Cry1Ac protoxin is an effective mucosal and systemic carrier and adjuvant of Streptococcus pneumoniae polysaccharides in mice. Scand. J. of Immunol 57: 45-55.

Netherwood T, Martín-Orúe SM, O'Donnell AG, Gockling S, Graham J, Mathers JC, Gilbert HJ (2004) Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. Nature Biotechnology 22: 204-209

Nguyen HT, Jehle JA (2009) Expression of the Cry3Bb1 in transgenic corn MON 88017. Journal of Agricultural and Food Chemistry 57: 9990-9996

Nielsen KM, van Elsas JD, Smalla K. (2000) Transformation of Acinetobacter sp. 13(pFG4deltanptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. Applied Environmental Microbiology 66: 1237-42

Nielsen KM, Townsend J P (2004) Monitoring and modeling horizontal gene transfer. Nature Biotechnology 22(9):1110-1114

NIH (1996) Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

Noteborn HPJM, Kuiper HA (1995) Safety evaluation of transgenic tomatoes expressing Bt endotoxin. In: Applications of the principles of substantial equivalence to the safety evaluation of foods or food components from plants derived by modern biotechnology. World Health Organization, pp. 51-61.

OECD (1987) OECD (1987) The OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects, Subsection 401, February 24, 1987; http://www.oecd.org/env/ehs/testing/45122762.pdf. Information: Following the OECD Council decision, the test 401 'Acute Oral Toxicity' was deleted on 17th December 2002.

OECD (1995) Guideline for the testing of chemicals. Repeated Dose 28-day Oral Toxicity Study in Rodents. Organization for Economic Co-operation and Development. Paris (France).

OECD (1997) Principles of Good Laboratory Practice (C(97) 186/Final), 26 November 1997.

OECD (2001) OECD Guidelines for the Testing of Chemicals, Section 4. Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure.

OECD (2003a) Considerations for the safety assessment of animal feedstuffs derived from genetically modified plants. Series on the Safety of Novel Foods and Feeds, No. 9.ENV/JM/MONO(2003)10.

OECD (2003b) Consensus Document on the biology of *Zea mays* subsp. *Mays* (Maize). Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO, No. 27, 1-9

Palaudelmás M, Peñas G, Melé E, Serra J, Salvia J, Pla, M-, Nadal A, Messeguer J (2009) Effect of volunteers on maize gene flow. Transgenic Research 18: 583-594

Paul V, Guertler P, Wiedemann S, Meyer HH (2010) Degradation of Cry1Ab protein from genetically modified maize (MON810) in relation to total dietary feed proteins in dairy cow digestion. Transgenic Research 19: 863-689

Pester, T. A. and Woodrum, C. L. (2003) Phenotypic and ecological observations of MON 88017 corn in U.S. field trials during 2002 for an assessment of equivalence and weed potential, Monsanto Technical Report, MSL 18944

Randhawa GJ, Singh M, Grover M (2011) Bioinformatic analysis for allergenicity assessment of *Bacillus thuringiensis* Cry proteins expressed in insect-resistant food crops. Food Chem Toxicol 49:356-362.

Raybould A, Kilby P, Graser G. (2013) Characterising microbial protein test substances and establishing their equivalence with plant-produced proteins for use in risk assessments of transgenic crops. Transgenic Res 22:445–460. DOI 10.1007/s11248-012-9658-3.

Rizzi AN, Raddadi C, Sorlini L, Nordgård L, Nielsen KM, Daffonchio D (2012) The stability and degradation of dietary DNA in the gastrointestinal tract of mammals - implications for horizontal gene transfer and the biosafety of GMOs. Crit. Rev. Food Science Nutr. 52:142-161

Rosenbaum, E.W. and Horak, M.J. (2003) Dormancy and germination evaluation of MON 88017 corn for and assessment of equivalence and weed potential using seed produced at two locations in 2002. Monsanto Technical Report, MSL 18800.

Schubbert GW, Lettmann C, Doerfler W (1994) Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. Molecular & general Genetics 242: 495-504

Taylor ML, Hyun Y, Hartnell GF, Nemeth MA, Karunanandaa K, George B, Glenn KC (2005) Comparison of broiler performance when fed diets containing MON 88017, MON

88017 x MON 810, control, or commercial corn. Poultry Science 83 (Suppl. 1), 322. (Abstract W39).

US EPA (1995a) Plant pesticide inert ingredient phosphinothricin acetyltransferase (CP4 EPSPS) and the genetic material necessary for its production (plasmid vector pCIBP3064) in corn; tolerance exemption. Fed. Reg., 60, 158, pp. 42450-42453.

US EPA (1995b) Plant pesticide Bacillus thuringiensis CryIIIA delta-endotoxin and the genetic material necessary for its production; tolerance exemption. Fed. Reg. PP3F4273/R2132; FRL-4953-2.

US EPA (1996) *Bacillus thuringiensis* CryIA(b) delta-endotoxin and the genetic material necessary for its production in all plants; exemption from requirement of a tolerance. Fed. Reg., 61, 150, pp. 40340-40343.

US EPA (2002) Health Effects Test Guidelines OPPTS 870.1100 Acute Oral Toxicity. EPA 712–C–02–190. December 2002.

http://ntp.niehs.nih.gov/iccvam/SuppDocs/FedDocs/EPA/EPA_870r_1100.pdf

US EPA (2005a) Biopesticides Registration Action Document Bacillus thuringiensis Cry1F Corn, http://www.epa.gov/opp00001/biopesticides/ingredients/tech_docs/brad_006481.pdf

US EPA (2005b) Bacillus thuringiensis Cry34Ab1 and Cry35Ab1 proteins and the genetic material necessary for their production (plasmid insert PHP 17662) in Event DAS-59122-7 corn (006490) Fact Sheet. Registered: August 31, 2005, Registration Numbers: 68467-5, 29964-4. http://www.epa.gov/oppbppd1/biopesticides/ingredients_keep/factsheets/factsheet_006490.htm

US EPA (2007) Cry3Bb1 Corn Biopesticide Registration Action Document. U.S. Environmental Protection Agency Office of Pesticide Programs, Biopesticides and Pollution Prevention Division.

US EPA (2010a) Biopesticides registration action document. mCry3A and Cry1F Bacillus thuringiensis (Bt) Corn Plant-Incorporated Protectants.

http://www.epa.gov/pesticides/biopesticides/pips/cry1f-cry1ab-brad.pdf

US EPA (2010b) Bacillus thuringiensis Cry34Ab1 and Cry35Ab1 Proteins and the Genetic Material Necessary for Their Production (PHP17662 T-DNA) in Event DAS-59122-7 Corn.

van de Wiel CCM, van den Brink L, Bus CB, Riemens MM, Lotz LAP (2011) Crop volunteers and climatic change. Effects of future climatic change on the occurrence of maize, sugar beet and potato in the Netherlands. COGEM report (CGM 2011-11)

Vázquez RI, Moreno-Fierros L, Neri-Bazán L, De La Riva GA, López-Revilla R (1999a) Bacillus thuringiensis Cry1Ac protoxin is a potent systemic and mucosal adjuvant. Scand J Immunol. 49(6):578-84

Vazquez-Padron, RI, Moreno-Fierros L, Neri-Bazan L, De La Riva, GA & Lopez-Revilla R (1999b) Intragastric and intraperitoneal administration of Cry1Ac protoxin from *Bacillus thuringiensis* induces systemic and mucosal antibody responses in mice. Life Sciences 64(21):1897-1912.

Vermeire, T., van de Bovenkamp, M., de Bruin, Y.B., Delmaar, C., van Ehgelen, J., Escher, S., Marquart, H., Meijsyer, T (2010). Exposure based waiving under REACH. Regul. Toxicol. Pharmacol. 58 (3), 408–420.

Vikse (2009) Betydning av mais i kostholdet i Norge. VKM report.

VKM (2007a) Foreløpig helserisikovurdering av genmodifiserte åkermais MON 88017 (EFSA/GMO/CZ/2005/27) fra Monsanto. Uttalelse fra Faggruppe for genmodifiserte organismer 11.4.2007 (07/306-endelig). Vitenskapskomiteen for mattrygghet, Oslo, Norge. http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&Content_6504=6187:2031914::0:6271:10:::0:0

VKM (2007b) Foreløpig helserisikovurdering av genmodifisert åkermais MON 88017 x MON810 (EFSA/GMO/CZ/2006/33) fra Monsanto. Uttalelse fra Faggruppe for genmodifiserte organismer 4.6.2007 (07/309-endelig). Vitenskapskomiteen for mattrygghet, Oslo, Norge.

http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&Content_6504=6187:2031904::0:6271:10:::0:0

VKM (2008) Foreløpig helse- og miljørisikovurdering av genmodifisert MON 89034 x MON 88017 (EFSA/GMO/NL/2007/39) fra Monsanto. Uttalelse fra Faggruppe for genmodifiserte organismer 9.5.2008 (07/323-endelig). Vitenskapskomiteen for mattrygghet, Oslo, Norge.

http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&Content_6504=6187:2031846::0:6271:10:::0:0

VKM (2009) Foreløpig helse- og miljørisikovurdering av genmodifisert mais MON 89034 x 1507 x MON 88017 x 59122 (EFSA/GMO/CZ/2008/62). Uttalelse fra Faggruppe for genmodifiserte organismer 25.9.2009 (09/307). Vitenskapskomiteen for mattrygghet, Oslo, Norge.

http://www.vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&6563=6566:3&Content_6504=6187:1685656::0:6566:21:::0:0

VKM (2010a) Foreløpig miljørisikovurdering av genmodifisert mais MON 88017 (EFSA/GMO/CZ/2008/54). Uttalelse fra Faggruppe for genmodifiserte organismer 7.4.2010 (08/335). Vitenskapskomiteen for mattrygghet, Oslo, Norge.

http://www.vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&6563=6566:2&Content_6504=6187:1770354::0:6566:14:::0:0

VKM (2010b) Foreløpig miljørisikovurdering av genmodifisert maishybrid MON 88017 x MON 88017 (EFSA/GMO/BE/2009/71). Uttalelse fra Faggruppe for genmodifiserte organismer 1.4.2010 (10/304). Vitenskapskomiteen for mattrygghet, Oslo, Norge.

http://www.vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&6563=6566:3&Content_6504=6187:1758627::0:6566:16:::0:0

VKM (2012) Helserisikovurdering av Cry-proteiner adjuvanseffekt. Uttalelse fra Faggruppe for genmodifiserte organismer 25.4.2012 -11/313/3-endelig. Vitenskapskomiteen for mattrygghet, Oslo, Norge.

http://www.english.vkm.no/eway/default.aspx?pid=278&trg=Content_6444&Main_6359=6582:0:31,2564&Content_6444=6393:1986886::0:6596:1:::0:0

Walker DR, Walker AK, Wood ED, Bonet Talavera ME, Fernandez FE et al. (2006) Gametic Selection by Glyphosate in Soybean Plants Hemizygous for the CP4 EPSPS Transgene. Crop Science 46: 30-35

Wesseler J, Fall EH (2010) Potential damage costs of *Diabrotica virgifera virgifera* infestion in Europe- the 'no control'. Journal of Applied Entomology 134: 385-394

Appendix

Table 1. (Table 17 in Technical Dossier) Compositional analysis of Forage and Grain, MON 88017 vs control and commercial maize varieties, USA field trials 2002 – all sites combined

	MON 88017		Control		Commercials[1]		
Tissue/Component (Units)[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	Historical range[6]
Forage							
Fibre (% dw)							
ADF	26.54	24.29-29.97	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	37.34	33.44-45.05	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)	3.99	3.30-5.53	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)	86.19	83.54-87.88	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)	1.61	0.80-3.13	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)	70.86	68.50-72.70	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)	8.20	7.44-8.97	7.82	6.79-8.54	[4.17, 11.81]	4.98-11.56[a]	3.86-11.0
Minerals (% dw)							
Calcium	0.22	0.19-0.26	0.23	0.18-0.31	[0.11, 0.32]	0.0969-0.3184[b]	0.0866-0.2754
Phosphorus	0.25	0.21-0.30	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	7.55	7.29-7.70	7.55	7.34-7.79	[6.66, 8.49]	6.4-9.9[e] (% tot. prot.)	7.06-8.19
Arginine	4.42	4.10-4.74	4.29	4.01-4.63	[3.34, 5.67]	2.9-5.9[e] (% tot. prot.)	3.49-5.48
Aspartic acid	6.22	6.09-6.34	6.25	6.04-6.45	[5.77, 7.16]	5.8-7.2[e] (% tot. prot.)	5.97-7.36
Cystine	2.14	1.93-2.26	2.15	1.93-2.30	[1.46, 2.89]	1.2-1.6[e] (% tot. prot.)	1.61-2.94
Glutamic acid	20.40	19.80-20.87	20.44	19.91-20.84	[18.01, 22.15]	12.4-19.6[e] (% tot. prot.)	17.3-20.4
Glycine	3.45	3.32-3.62	3.45	3.18-3.61	[2.81, 4.54]	2.6-4.7[e] (% tot. prot.)	3.22-4.91
Histidine	2.99	2.90-3.10	2.95	2.83-3.14	[2.16, 3.60]	2.0-2.8[e] (% tot. prot.)	2.46-3.35
Isoleucine	3.59	3.43-3.71	3.57	3.45-3.76	[3.30, 3.84]	2.6-4.0[e] (% tot. prot.)	2.95-4.08
Leucine	13.28	12.69-13.62	13.31	12.76-14.11	[10.72, 15.18]	7.8-15.2[e] (% tot. prot.)	11.2-14.6
Lysine	2.69	2.42-2.87	2.66	2.49-2.82	[2.06, 3.73]	2.0-3.8[e] (% tot. prot.)	2.35-4.18
Methionine	1.98	1.85-2.05	2.01	1.83-2.20	[1.37, 2.60]	1.0-2.1[e] (% tot. prot.)	1.61-2.89
Phenylalanine	5.18	4.97-5.31	5.14	5.01-5.32	[4.57, 5.71]	2.9-5.7[e] (% tot. prot.)	4.6-5.76
Proline	9.39	9.02-9.69	9.34	8.85-9.80	[7.60, 10.37]	6.6-10.3[e] (% tot. prot.)	8.03-9.9
Serine	4.83	4.65-5.04	4.91	4.63-5.13	[4.60, 5.43]	4.2-5.5[e] (% tot. prot.)	3.45-5.63
Threonine	3.22	3.10-3.38	3.25	3.06-3.37	[2.89, 3.84]	2.9-3.9[e] (% tot. prot.)	2.87-4.01
Tryptophan	0.54	0.48-0.60	0.55	0.41-0.68	[0.36, 0.77]	0.5-1.2[e] (% tot. prot.)	0.39-1.04
Tyrosine	3.35	2.35-3.66	3.43	2.58-3.66	[2.62, 4.26]	2.9-4.7[e] (% tot. prot.)	1.93-4.32
Valine	4.79	4.60-4.92	4.74	4.60-4.94	[4.22, 5.27]	2.1-5.2[e] (% tot. prot.)	3.94-5.46

Table 1. Continued – Grain - Fatty acids, Fibre, Minerals, Proximates, USA field trials 2002

	MON 88017		Control		Commercials[1]		
Tissue/Component[2]	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	10.24	10.07-10.52	11.27	10.10-14.57	[6.51, 16.50]	7-19[e] (% total fat)	8.41-12.5
16:1 palmitoleic acid	0.18	0.16-0.21	0.18	0.16-0.22	[0.0017, 0.28]	1[e] (% total fat)	0.05-0.18
18:0 stearic acid	2.01	1.80-2.19	2.07	1.76-2.23	[1.41, 2.53]	1-3[e] (% total fat)	1.33-2.61
18:1 oleic acid	22.74	22.20-23.53	22.87	21.43-23.51	[9.25, 44.14]	20-46[e] (% total fat)	20.1-37.7
18:2 linoleic acid	62.85*	61.86-63.72	61.52	59.10-63.18	[41.22, 74.09]	35-70[e] (% total fat)	48.0-66.1
18:3 linolenic acid	1.21	1.15-1.26	1.32	1.19-1.77	[0.42, 1.95]	0.8-2[e] (% total fat)	0.74-1.45
20:0 arachidic acid	0.37*	0.35-0.39	0.38	0.35-0.41	[0.31, 0.49]	0.1-2[e] (% total fat)	0.31-0.56
20:1 eicosenoic acid	0.24	0.23-0.26	0.25	0.24-0.26	[0.18, 0.40]	-	0.15-0.44
22:0 behenic acid	0.15	0.14-0.16	0.15	0.14-0.17	[0.071, 0.25]	-	0.075-0.3
Fibre (% dw)							
ADF	3.77	3.31-4.40	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	12.44	10.99-13.58	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	16.24	13.57-18.64	15.40	13.18-17.84	[5.72, 27.10]	10.99-11.41[b]	-
Minerals							
Calcium (% dw)	0.0054	0.0047-0.0060	0.0058	0.0049-0.0069	[0.0017, 0.0062]	0.01-0.1[d]	0.0024-0.0089
Copper (mg/kg dw)	1.73	1.48-2.05	1.99	1.64-2.63	[0.17, 3.00]	0.9-10[d]	0.98-3.43
Iron (mg/kg dw)	21.51	20.07-22.92	21.84	20.31-23.93	[12.60, 31.26]	1-100[d]	10.4-30.7
Magnesium (% dw)	0.14	0.13-0.15	0.14	0.13-0.16	[0.088, 0.16]	0.09-1[d]	0.082-0.16
Manganese (mg/kg dw)	9.72	9.01-10.76	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)	0.41	0.39-0.44	0.42	0.38-0.47	[0.27, 0.48]	0.32-0.72[d]	0.29-0.53
Zinc (mg/kg dw)	24.53	22.31-27.27	24.92	22.02-27.18	[13.42, 31.37]	12-30[d]	14.1-37.2
Proximates (% dw)							
Ash	1.54	1.31-1.68	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	82.32	81.61-83.39	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	3.64	3.44-3.96	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	11.10	9.03-13.20	11.60	9.73-14.20	[4.67, 17.56]	7-23[d] ; 8.18-26.2[b]	6.07-24.7
Protein	12.51	11.63-13.00	12.28	11.22-13.82	[6.20, 15.35]	6-12[d]; 9.7-16.1[c]	6.15-14.8

Table 1. Continued – Grain - Vitamins, Antinutrients, and secondary metabolites, USA field trials 2002

Tissue/Component[2]	MON 88017 Mean[3]	MON 88017 Range	Control Mean[3]	Control Range	Commercials[1] 99% T.I.[4]	Literature range[5]	Historical range[6]
Grain – continued							
Vitamin (mg/kg dw)							
Folic acid	0.48	0.38-0.60	0.48	0.42-0.59	[0.12, 0.77]	0.3[d]	0.33-0.75 (µg/g dw)
Niacin	20.94	17.04-24.14	21.75	19.08-23.92	[3.19, 34.49]	9.3-70[d]	-
Vitamin B1	2.47*	2.30-2.69	3.24	2.99-3.60	[1.96, 4.38]	3-8.6[e]	0.2-0.33 (mg/100 g dw)
Vitamin B2	1.10	0.98-1.22	1.13	0.99-1.33	[0.67, 1.51]	0.25-5.6[e]	0.83-1.74 (µg/g dw)
Vitamin B6	7.16	6.57-8.06	7.10	5.65-8.54	[4.29, 7.84]	5.3[d]; 9.6[e]	-
Vitamin E	14.15	6.08-16.93	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	0.95	0.83-1.05	0.89	0.72-1.03	[0.28, 1.12]	0.48-1.12[a]	0.42-1.37
Raffinose	0.17	0.14-0.20	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	2175.34	1986.75-2275.48	2121.05	1927.55-2339.71	[1415.19, 3173.90]	113-1194[f]; 3000[g]	0.17-0.28 (% dw)
p-coumaric acid	169.26	148.45-215.25	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 B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = 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 2. (Table 19 in Technical Dossier) Summary of statistically significant differences for the compositional comparison of MON 88017 to control maize – USA field trials 2002

Tissue/Site/ Component (Units)[a]	Mean MON 88017	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 88017 (Range)	99% Tolerance Interval[b]
Grain						
IA						
16:0 palmitic (% total fa)	10.16	12.94	-21.50	0.029	(10.11-10.23)	[6.51, 16.50]
18:2 linoleic (% total fa)	63.25	60.41	4.70	0.017	(62.73-63.72)	[41.22, 74.09]
18:3 linolenic (% total fa)	1.25	1.57	-20.26	0.036	(1.24-1.26)	[0.42, 1.95]
Methionine (% total aa)	2.02	2.16	-6.39	<0.001	(1.96-2.05)	[1.37, 2.60]
Moisture (% fw)	9.38	9.93	-5.54	0.034	(9.03-9.70)	[4.67, 17.56]
Vitamin B_1 (mg/kg dw)	2.54	3.07	-17.37	<0.001	(2.42-2.65)	[1.96, 4.38]
IL						
18:1 oleic (% total fa)	22.53	23.29	-3.26	<0.001	(22.50-22.56)	[9.25, 44.14]
18:2 linoleic (% total fa)	63.11	62.15	1.55	0.003	(62.84-63.29)	[41.22, 74.09]
Niacin (mg/kg dw)	21.10	22.52	-6.30	0.014	(20.39-21.52)	[3.19, 34.49]
Vitamin B_1 (mg/kg dw)	2.30	3.10	-25.63	<0.001	(2.30-2.30)	[1.96, 4.38]
NE						
Copper (mg/kg dw)	1.57	2.21	-28.80	0.023	(1.48-1.68)	[0.17, 3.00]
Serine (% total aa)	4.80	4.97	-3.37	0.042	(4.80-4.81)	[4.60, 5.43]
Vitamin B_1 (mg/kg dw)	2.58	3.56	-27.53	<0.001	(2.47-2.69)	[1.96, 4.38]
Combination of all sites						
18:2 linoleic (% total fa)	62.85	61.52	2.17	0.038	(61.86-63.72)	[41.22, 74.09]
20:0 arachidic (% total fa)	0.37	0.38	-2.24	0.012	(0.35-0.39)	[0.31, 0.49]
Vitamin B_1 (mg/kg dw)	2.47	3.24	-23.72	<0.001	(2.30-2.69)	[1.96, 4.38]

[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 3. (Table 18 in Technical Dossier) Compositional analysis of Forage and Grain, MON 88017 vs Control and commercial maize varieties, Argentinian field trials 2003 – 2004 – all sites combined

	MON 88017		Control		Commercials[1]		
Tissue/Component (Units)[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	Historical range[6]
Forage							
Fibre (% dw)							
ADF	27.60	24.48-32.06	25.91	23.06-29.10	[19.68,33.41]	18.3-41.0[b] ; 17.5-38.3[a] ; 16.1-41.9[j]	19.4-32.1
NDF	38.45	32.80-43.51	39.25	36.93-45.33	[26.28,50.79]	26.4-54.5[b]; 27.9-54.8[a]; 20.3-63.7[j]	22.8-53.9
Proximates							
Ash (% dw)	5.13	4.69-5.75	5.22	4.60-6.94	[3.54,6.73]	2.43-9.64[a]; 2-6.6[b]	2.46-8.23
Carbohydrates (% dw)	86.44	84.95-88.60	86.43	85.08-87.42	[80.91,89.90]	83.2-91.6[b]; 76.5-87.3[a]	80.6-90.0
Fat (% dw)	1.39	0.64-2.29	1.91	1.02-2.36	[0.073,3.71]	0.35-3.62[b] ; 1.42-4.57[a]	0.19-4.02
Moisture (% fw)	69.02	67.60-70.80	69.30	65.70-71.40	[56.80,81.85]	56.5-80.4[a] ; 55.3-75.3[b]	60.4-77.0
Protein (% dw)	7.04	5.90-7.68	6.44	5.78-7.25	[4.38,10.73]	4.98-11.56[a]; 3.14-11.6[j]	4.52-11.0
Minerals							
Calcium (% dw)	0.14	0.12-0.17	0.13	0.11-0.14	[0.071,0.26]	0.097-0.318[b]; 0.097-0.324[j]	0.13-0.31
Phosphorus (% dw)	0.18	0.16-0.22	0.17	0.15-0.19	[0.036,0.39]	0.137-0.291[b]; 0.118-0.323[j]	0.16-0.30
Grain							
Amino acids (% of total aa)							
Alanine	7.66*	7.37-7.85	7.57	7.28-7.87	[6.90,8.25]	6.4-9.9[e]	6.26-8.23
Arginine	4.49	4.04-5.12	4.67	4.24-5.09	[3.47,5.41]	2.9-5.9[e]	2.90-5.33
Aspartic acid	6.71	6.50-6.91	6.83	6.63-7.02	[5.88,7.28]	5.8-7.2[e]	5.67-6.99
Cystine	1.97	1.77-2.17	1.99	1.82-2.24	[1.32,2.75]	1.2-1.6[e]; 1.63-2.62[b]	1.61-3.43
Glutamic acid	19.53*	18.73-20.01	19.24	18.61-19.90	[18.10,21.32]	12.4-19.6[e]; 18.61-20.26[i]	18.1-20.9
Glycine	3.79*	3.55-4.06	3.92	3.74-4.18	[2.99,4.38]	2.6-4.7[e]	3.18-4.37
Histidine	2.83	2.70-3.05	2.86	2.76-3.05	[2.12,3.83]	2.0-2.8[e]; 2.72-3.21[i]	2.45-3.32
Isoleucine	3.59*	3.42-3.86	3.53	3.34-3.64	[3.19,3.79]	2.6-4.0[e]	3.18-3.93
Leucine	13.12*	12.49-13.59	12.78	12.18-13.30	[11.47,14.93]	7.8-15.2[e]	11.9-14.6
Lysine	3.13*	2.90-3.38	3.26	3.05-3.62	[2.37,3.57]	2.0-3.8[e]	2.35-3.95
Methionine	1.98	1.75-2.16	1.95	1.71-2.08	[1.42,2.50]	1.0-2.1[e]; 1.89-2.58[i]	1.62-2.58
Phenylalanine	5.25	5.15-5.31	5.19	5.02-5.35	[4.65,5.69]	2.9-5.7[e]	4.39-5.36
Proline	8.72*	8.45-8.95	8.58	8.45-8.78	[7.89,10.04]	6.6-10.3[e]; 8.60-10.56[i]	8.59-12.1
Serine	5.26	5.02-5.51	5.30	5.02-5.74	[4.57,5.86]	4.2-5.5[e]; 2.87-5.63[b]	4.20-5.44
Threonine	3.36	2.95-3.53	3.39	2.93-36.0	[2.64,3.97]	2.9-3.9[e]; 2.61-3.89[b]	2.87-5.42
Tryptophan	0.59	0.48-0.70	0.61	0.49-0.68	[0.43,0.73]	0.5-1.2[e]; 0.41-1.04[b]	0.41-0.83
Tyrosine	3.29	2.31-3.85	3.60	2.45-3.93	[2.25,4.62]	2.9-4.7[e]; 1.93-3.82[i]	1.66-3.97
Valine	4.73	4.52-4.97	4.72	4.56-4.84	[4.23,5.19]	2.1-5.2[e]; 3.93-5.40[b]	4.36-5.16

Table 3. Continued – Grain – Fatty acids, Fibre, Minerals, Proximates, Argentinian field trials 2003 – 2004

Tissue/Component[2]	MON 88017		Control		Commercials[1]	Literature range[5]	Historical range[6]
	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]		
Grain – continued							
Fatty acids (% of total fa)							
16:0 palmitic acid	11.52	11.31-11.79	11.71	11.35-12.58	[7.03,14.36]	7-19[e]; 8.5-17.5[j]	8.15-21.5
16:1 palmitoleic acid	0.10	0.051-0.17	0.10	0.055-0.15	[0.028,0.21]	1[e]; 0.10-0.33[j]	0.05-0.22
18:0 stearic acid	2.05	2.00-2.11	2.07	1.95-2.20	[0.91,2.95]	1-3[e]; 1-2.76[j]	1.33-3.49
18:1 oleic acid	26.61*	25.72-27.74	32.12	30.50-33.97	[8.21,45.14]	20-46[e]; 18.6-40.1[j]	21.0-37.8
18:2 linoleic acid	57.69*	56.22-58.80	51.97	49.67-53.98	[40.78,76.51]	35-70[e]; 43.1-65.6[j]	37.4-65.7
18:3 linolenic acid	1.12*	1.08-1.16	1.08	0.92-1.14	[0.52,1.60]	0.8-2[e]; 0.71-1.50[a]; 0.39-0.42[j]	0.59-1.77
20:0 arachidic acid	0.43	0.41-0.44	0.45	0.42-0.49	[0.21,0.61]	0.1-2[e]; 0.28-0.72[j]	0.32-1.00
20:1 eicosenoic acid	0.30*	0.29-0.31	0.33	0.30-0.40	[0.13,0.47]	0.19-0.45[b]; 0.17-1.92[j]	0.19-0.43
22:0 behenic acid	0.17	0.15-0.19	0.17	0.15-0.20	[0.10,0.24]	0.073-0.22[a]; 0.13-0.24[b]; 0.11-0.35[j]	0.09-0.34
Fibre (% dw)							
ADF	4.69	3.28-6.58	4.84	3.66-5.84	[2.46,7.89]	3.3-4.3[d]; 2.46-11.34[a, b]; 1.82-11.3[j]	2.3-5.89
NDF	12.40	8.90-14.30	12.79	9.08-16.08	[7.39,17.08]	8.3-11.9[d]; 7.58-15.91[b, i]; 5.6-22.6[j]	8.25-18.1
TDF	18.18	12.80-23.31	18.01	14.22-21.97	[6.24,32.83]	10.99-11.41[b]; 11.8-25.6[j]	8.85-18.6
Minerals							
Calcium (% dw)	0.0043	0.0039-0.0048	0.0045	0.0040-0.0054	[0.0022,0.0072]	0.01-0.1[d]; 0.002-0.021[j]	0.0024-0.0089
Copper (mg/kg dw)	1.76	1.34-2.38	1.71	1.38-2.14	[0.33,3.13]	0.9-10[d]; 0.73-5.01[j]	1.20-3.22
Iron (mg/kg dw)	20.28*	17.87-22.97	19.08	16.40-23.90	[9.95,34.09]	1-100[d]; 10.4-49.1[j]	15.9-32.2
Magnesium (% dw)	0.12*	0.10-0.14	0.11	0.098-0.13	[0.086,0.16]	0.09-1[d]; 0.079-0.161[j]	0.08-0.16
Manganese (mg/kg dw)	7.77	6.74-10.10	7.48	6.03-10.58	[2.17,12.62]	0.7-54[d]; 2.61-11.3[j]	3.72-10.4
Phosphorus (% dw)	0.32	0.28-0.37	0.31	0.26-0.38	[0.25,0.43]	0.26-0.75[d]; 0.21-0.43[j]	0.24-0.46
Potassium (% dw)	0.39	0.35-0.47	0.39	0.36-0.48	[0.23,0.56]	0.32-0.72[d]; 0.27-0.53[j]	0.32-0.55
Zinc (mg/kg dw)	18.58	12.61-22.50	17.87	13.24-21.98	[5.64,36.71]	12-30[d]; 6.5-37.2[j]	17.9-33.3
Proximates (% dw)							
Ash (% dw)	1.51	1.14-2.05	1.46	1.19-1.82	[0.90,2.32]	1.1-3.9[d]; 0.89-6.28[b]; 0.62-6.28[j]	0.89-2.24
Carbohydrates	84.62	82.99-86.14	85.28	83.35-87.18	[80.54,87.24]	77.4-87.2[b]; 82.2-88.1[a]; 77.4-89.5[j]	79.8-87.8
Fat (% dw)	3.34	2.83-3.86	3.53	3.23-3.97	[1.82,5.17]	3.1-5.7[d]; 2.48-4.81[b]; 1.74-5.56[j]	2.40-5.82
Moisture (% fw)	12.23	11.40-12.80	12.42	11.70-13.40	[10.40,13.89]	7-23[d]; 8.18-26.2[b]; 6.1-26[j]	6.88-15.3
Protein (% dw)	10.52	9.27-11.45	9.73	7.88-11.78	[8.03,13.99]	6-12[d]; 9.7-16.1[c]; 6.15-15[j]	7.31-14.8

Table 3. Continued – Grain – Vitamins, Antinutrients, Secondary metabolites, Argentinian field trials 2003 – 2004

Tissue/Component[2]	MON 88017 Mean[3]	MON 88017 Range	Control Mean[3]	Control Range	Commercials[1] 99% T.I.[4]	Literature range[5]	Historical range[6]
Grain – continued							
Vitamin (mg/kg dw)							
Folic acid	0.77*	0.54-0.86	0.68	0.42-0.84	[0.48,0.99]	0.3[d]; 0.15-1.21[j]	0.33-1.46
Niacin (mg/kg dw)	22.26	20.30-23.80	21.33	19.59-24.13	[10.15,37.38]	9.3-70[d]; 14.1-36.3[j]	10.4-46.9
Vitamin B1 (mg/kg dw)	3.53	2.72-4.79	4.17	3.11-4.58	[2.16,6.13]	3-8.6[e]; 2.3-3.3[i]; 1.3-8.5[j]	2.00-12.6
Vitamin B2 (mg/kg dw)	1.35	1.24-1.68	1.28	1.03-1.38	[0.85,1.82]	0.25-5.6[e]; 0.7-1.93[j]	0.49-1.90
Vitamin B6 (mg/kg dw)	5.72	5.12-6.58	5.88	4.85-6.66	[3.83,9.30]	5.3[d]; 9.6[e]; 4.6-7.3[j]	4.75-11.0
Vitamin E (mg/kg dw)	19.26	16.84-33.03	17.63	1.71-21.48	[0,26.50]	3-12.1[e]; 17-47[d]; 1.5-68.7[j]	1.74-17.8
Antinutrient (% dw)							
Phytic acid	0.68	0.62-0.78	0.70	0.44-0.91	[0.24,1.12]	0.42-1.37[i]; 0.29-1.29[j]	0.06-1.47
Secondary metabolite (µg/g dw)							
Ferulic acid	1841.12	1642.13-2018.14	1782.07	1628.70-1895.57	[820.14,3209.76]	113-1194[f]; 3000[g]; 1340-3725[j]	936-2877
p-coumaric acid (µg/g dw)	157.44	133.79-182.44	176.95	150.96-210.59	[45.63,370.04]	22-75[f]; 160-260[g]; 90.7-576[j]	110-435

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

1 16 commercial maize hybrids.

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

3 The mean of 12 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, 2003; [e] amino acid values reported as percent of total protein and fatty acid values reported as percent of total fat (Watson, 1982); [f] Classen *et al.*, 1990; [g] Dowd and Vega, 1996; [h] Choi *et al.*, 1999; [i] George *et al.*, 2004b; [j] Ridley *et al.*, 2004b

6 Historical range from control samples (in some cases including commercial hybrid values) analyzed in previous Monsanto Company studies (George and Claussen, 2004; George *et al.*, 2003a; George *et al.*, 2004a; George *et al.*, 2003b; McCann, 2003; McCann *et al.*, 2001; Ridley *et al.*, 2002a; Ridley *et al.*, 2000; Ridely *et al.*, 2003; Ridley *et al.*, 2002b; Ridley *et al.*, 2004a; Sidhu and Ledesma, 2002)

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

Table 4. (Table 20 in Technical Dossier) Summary of statistical differences for the compositional comparison of MON 88017 to control maize – Argentinean field trials 2003 - 2004

Tissue/Site/ Component (Units)[a]	Mean MON 88017	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 88017 (Range)	99% Tolerance Interval[b]
Forage						
BA-2						
Phosphorus (% dw)	0.20	0.17	13.62	0.40	(0.19-0.22)	[0.036, 0.39]
CB						
Total fat (%dw)	1.23	2.11	-41.55	0.014	(1.18-1.31)	[0.073, 3.71]
Grain						
BA-1						
18:1 oleic (% total fa)	26.64	31.69	-15.94	0.027	(26.49-26.85)	[8.21, 45.14]
18:2 linoleic (% total fa)	57.78	52.22	10.66	0.044	(57.54-58.00)	[40.78, 76.51]
20:0 arachidic (% total fa)	0.43	0.48	-9.93	0.039	(0.43-0.43)	[0.21, 0.61]
Aspartic acid (% total aa)	6.63	6.70	-1.05	0.028	(6.58-6.66)	[5.88, 7.28]
Proline (% total aa)	8.85	8.66	2.22	0.008	(8.71-8.95)	[7.89, 10.04]
Vitamin B_1 (mg/kg dw)	2.79	4.25	-34.39	0.021	(2.72-2.83)	[2.16, 6.13]
BA-2						
18:1 oleic (% total fa)	25.82	30.71	-15.91	<0.001	(25.72-25.98)	[8.21, 45.14]
18:2 linoleic (% total fa)	58.56	53.52	9.41	<0.001	(58.35-58.80)	[40.78, 76.51]
Zinc (mg/kg dw)	21.64	20.33	6.45	0.041	(20.18-22.50)	[5.64, 36.71]
Cystine (% total aa)	1.87	2.01	-6.72	0.013	(1.80-2.00)	[1.32, 2.75]
Methionine (% total aa)	1.89	2.04	-7.39	0.029	(1.81-1.98)	[1.42, 2.50]
Ferulic acid (µg/g dw)	1875.64	1759.15	6.62	0.031	(1824.40-1909.09)	[820.14, 3209.76]
p-Coumaric acid (µg/g dw)	150.48	175.50	-14.25	0.028	(133.79-182.44)	[45.63, 370.04]

Table 4. Continued - Argentinean field trials 2003 - 2004

Tissue/Site/ Component (Units)[a]	Mean MON 88017	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 88017 (Range)	99% Tolerance Interval[b]
Grain						
CB						
18:1 oleic (% total fa)	27.17	32.76	-17.05	0.004	(26.75-27.74)	[8.21, 45.14]
18:2 linoleic (% total fa)	56.92	51.52	10.47	0.006	(56.22-57.50)	[40.78, 76.51]
Copper (mg/kg dw)	2.08	1.77	17.56	0.009	(2.01-2.13)	[0.33, 3.13]
Folic acid (mg/kg dw)	0.84	0.65	28.40	0.034	(0.81-0.86)	[0.48, 0.99]
Vitamin B_1 (mg/kg dw)	3.74	3.57	4.72	0.037	(3.21-4.12)	[2.16, 6.13]
Total fat (% dw)	3.41	3.59	-4.77	0.018	(3.36-3.52)	[1.82, 5.17]
SF						
18:1 oleic (% total fa)	26.82	33.34	-19.56	0.009	(26.52-27.04)	[8.21, 45.14]
18:2 linoleic (% total fa)	57.51	50.60	13.66	0.017	(57.34-57.68)	[40.78, 76.51]
Aspartic acid (% total aa)	6.67	6.96	-4.25	0.031	(6.50-6.83)	[5.88, 7.28]
Moisture (% fw)	12.63	12.00	5.28	0.048	(12.50-12.80)	[10.40, 13.89]
Combination of all sites						
18:1 oleic (% total fa)	26.61	32.12	-17.15	<0.001	(25.72-27.74)	[8.21, 45.14]
18:2 linoleic (% total fa)	57.69	51.97	11.02	<0.001	(56.22-58.80)	[40.78, 76.51]
18:3 linolenic (% total fa)	1.12	1.08	3.90	0.038	(1.08-1.16)	[0.52, 1.60]
20:1 eicosenoic (% total fa)	0.30	0.33	-8.25	0.034	(0.29-0.31)	[0.13, 0.47]
Alanine (% total aa)	7.66	7.57	1.09	0.032	(7.37-7.85)	[6.90, 8.25]
Glutamic acid (% total aa)	19.53	19.24	1.47	0.025	(18.73-20.01)	[18.10, 21.32]
Glycine (% total aa)	3.79	3.92	-3.10	<0.001	(3.55-4.06)	[2.99, 4.38]

Table 4. Continued - Argentinean field trials 2003 – 2004

Tissue/Site/ Component (Units)[a]	Mean MON 88017	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 88017 (Range)	99% Tolerance Interval[b]
Grain						
Combination of all sites						
Isoleucine (% total aa)	3.59	3.53	1.86	0.047	(3.42-3.86)	[3.19, 3.79]
Leucine (% total aa)	13.12	12.78	2.67	0.005	(12.49-13.59)	[11.47, 14.93]
Lysine (% total aa)	3.13	3.26	-3.87	0.003	(2.90-3.38)	[2.37, 3.57]
Proline (% total aa)	8.72	8.58	1.55	0.046	(8.45-8.95)	[7.89, 10.04]
Iron (mg/kg dw)	20.28	19.08	6.31	0.012	(17.87-22.97)	[9.95, 34.09]
Magnesium (% dw)	0.12	0.11	5.25	0.035	(0.10-0.14)	[0.086, 0.16]
Folic acid (mg/kg dw)	0.77	0.68	12.66	0.034	(0.54-0.86)	[0.48, 0.99]

[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: BA-1: Buenos Aires ; BA-2: Buenos Aires (different site from 1) ; CB: Cordoba ; SF: Santa Fe

Table 5. (Table 1 in Drury et al 2008) Summary of differences (p<0.05) for the comparison of maize component levels for northern regional test (MON88017) vs. conventional control (DKC3945) and commercial reference substances (European field trials).

Component (Units)[1]	Site	Test MON 88017 Mean	Control Mean	Mean Difference (Test minus Control) Mean Difference (% of Control)	Mean Difference (Test minus Control) Signif. (p-Value)	Test Range	Commercial Tolerance Interval[2]
Statistical Differences Observed in Combined Site Analyses							
Grain Mineral							
Potassium (mg/kg DW)	Combined Site	4520.46	4987.44	-9.36	0.002	[2693.02 - 5251.71]	[1468.52, 7007.49]
Grain Vitamin							
Vitamin B1 (mg/kg DW)	Combined Site	2.46	3.31	-25.65	<0.001	[2.16 - 2.76]	[1.06, 5.64]
Grain Antinutrient							
Raffinose (% DW)	Combined Site	0.14	0.10	40.96	0.030	[0.027 - 0.31]	[0, 0.39]
Statistical Differences Observed in More Than One Individual Site							
Grain Vitamin							
Vitamin B1 (mg/kg DW)	Site 4	2.65	3.60	-26.31	<0.001	[2.54 - 2.76]	[1.06, 5.64]
	Site 6	2.42	3.09	-21.83	<0.001	[2.16 - 2.50]	[1.06, 5.64]
Statistical Differences Observed in One Individual Site Only							
Grain Fiber							
Acid Detergent Fiber (% DW)	Site 6	3.30	3.92	-15.84	0.018	[3.22 - 3.36]	[1.75, 5.75]
Neutral Detergent Fiber (% DW)	Site 4	12.33	9.80	25.83	0.012	[11.83 - 13.21]	[4.67, 15.61]
Grain Mineral							
Copper (mg/kg DW)	Site 4	1.36	1.21	12.25	0.046	[1.30 - 1.43]	[0.42, 2.67]
Grain Proximate							
Total Fat (% DW)	Site 4	3.23	3.47	-6.77	0.047	[3.21 - 3.25]	[1.68, 5.61]

Table 5. Cont. (Table 1 in Drury et al 2008)

Component (Units)[1]	Site	Test MON 88017 Mean	Control Mean	Mean Difference (Test minus Control) Mean Difference (% of Control)	Mean Difference (Test minus Control) Signif. (p-Value)	Test Range	Commercial Tolerance Interval[2]
Statistical Differences Observed in One Individual Site Only							
Grain Vitamin							
Folic Acid (mg/kg DW)	Site 4	0.55	0.72	-22.95	0.039	[0.53 - 0.58]	[0, 1.23]
Vitamin E (mg/kg DW)	Site 4	6.89	2.76	149.59	0.043	[5.58 - 8.17]	[0, 26.03]

[1]DW = dry weight.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits were set to zero.

Table 6. (Table 2 in Drury et al 2008) Summary of differences (p<0.05) for the comparison of maize component levels for southern regional test (MON88017) vs. conventional control (DKC3945) and commercial reference substances (European field trials).

Component (Units)[1]	Site	Test MON 88017 Mean	Control Mean	Mean Difference (Test minus Control)		Test Range	Commercial Tolerance Interval[2]
				Mean Difference (% of Control)	Signif. (p-Value)		
Statistical Differences Observed in Combined Site Analyses							
Grain Amino Acid (% DW)							
Methionine (% DW)	Combined Site	0.21	0.19	6.36	0.017	[0.20 - 0.23]	[0.11, 0.27]
Grain Mineral							
Iron (mg/kg DW)	Combined Site	19.17	17.27	10.99	0.021	[14.10 - 25.00]	[11.99, 27.51]
Grain Vitamin							
Vitamin A (mg/kg DW)	Combined Site	0.64	0.57	13.29	0.024	[0.54 - 0.80]	[0, 1.58]
Vitamin B1 (mg/kg DW)	Combined Site	3.16	3.56	-11.22	0.002	[3.00 - 3.46]	[1.06, 5.64]
Statistical Differences Observed in One Individual Site Only							
Forage Fiber							
Acid Detergent Fiber (% DW)	Site 11	16.90	20.38	-17.05	0.039	[17.15 - 17.88]	[4.69, 37.24]
Neutral Detergent Fiber (% DW)	Site 10	37.05	30.15	22.90	0.043	[33.02 - 42.73]	[6.40, 59.86]
Grain Amino Acid (% DW)							
Methionine (% DW)	Site 10	0.22	0.20	10.24	0.036	[0.20 - 0.23]	[0.11, 0.27]
Grain Mineral							
Calcium (mg/kg DW)	Site 10	58.79	67.30	-12.64	0.025	[55.57 - 61.64]	[5.05, 96.16]
Zinc (mg/kg DW)	Site 11	21.22	19.34	9.73	0.036	[20.62 - 21.81]	[12.79, 29.65]

Table 6. Cont. (Table 2 in Drury et al 2008)

Component (Units)[1]	Site	Test MON 88017 Mean	Control Mean	Mean Difference (Test minus Control)		Test Range	Commercial Tolerance Interval[2]
				Mean Difference (% of Control)	Signif. (p-Value)		
Statistical Differences Observed in One Individual Site Only							
Grain Proximate							
Carbohydrates (% DW)	Site 10	84.74	85.48	-0.86	0.011	[84.24 - 85.72]	[79.63, 90.55]
Moisture (% FW)	Site 13	9.73	10.80	-9.86	0.022	[9.25 - 9.92]	[4.65, 13.98]
Grain Vitamin							
Niacin (mg/kg DW)	Site 13	21.40	17.80	20.27	0.010	[20.72 - 21.76]	[7.41, 33.20]
Vitamin A (mg/kg DW)	Site 10	0.68	0.58	18.11	0.014	[0.63 - 0.73]	[0, 1.58]
Vitamin B1 (mg/kg DW)	Site 10	3.10	3.77	-17.78	0.003	[3.08 - 3.13]	[1.06, 5.64]
Vitamin B2 (mg/kg DW)	Site 13	2.62	1.66	57.68	0.017	[2.58 - 2.67]	[0, 4.94]
Vitamin E (mg/kg DW)	Site 13	6.98	2.54	175.29	0.003	[7.19 - 7.29]	[0, 26.03]

[1]DW = dry weight; FW = fresh weight.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits were set to zero.