Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

VKM Report 2016: 17

Final health and environmental risk assessment of genetically modified maize MON 89034 x NK 603

Scientific opinion on insect-resistant and herbicide tolerant, genetically modified maize MON 89034 x NK 603 from Monsanto for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (EFSA/GMO/NL/2007/38)

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: 17
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15.04.2016

ISBN: 978-82-8259-207-9

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Suggested citations: VKM (2016) Final health and environmental risk assessment of genetically modified maize MON 89034 x NK 603. Scientific opinion on insect-resistant and herbicide tolerant genetically modified maize MON 89034 x NK 603 from Monsanto for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (Application EFSA/GMO/NL/2007/38). Opinion of the Panel on Genetically Modified Organisms of the Norwegian Scientific Committee for Food Safety, ISBN: 978-82-8259-207-9, Oslo, Norway.

Scientific opinion on insect-resistant and herbicide tolerant, genetically modified maize MON 89034 x NK 603 from Monsanto for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (Application EFSA/GMO/NL/2007/38)

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Acknowledgment

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed the Panel on Genetically Modified Organisms (GMO) to answer the request from the Norwegian Food Safety Authority and the Norwegian Environment Agency. Project leaders from the VKM secretariat have been 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.

Summary

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

The insect-resistant and glyphosate-tolerant genetically modified maize MON 89034 x NK 603 from Monsanto (Unique Identifier MON-89Ø34-3 × MON-ØØ6Ø3-6) was approved under Regulation (EC) No 1829/2003 in the EU for food and feed uses, import and processing on 28 July 2010 (Commission Decision 2010/420/EC).

Genetically modified maize MON 890314 x NK 603 has previously been risk assessed by the VKM Panel on Genetically Modified Organisms (GMO), commissioned by the Norwegian Food Safety Authority and the Norwegian Environment Agency related and to the EFSA public hearing of the applications EFSA/GMO/NL/2007/38 and EFSA/GMO/NL/2009/72 in 2007 and 2009/2010 (VKM 2008a, VKM 2010a). In addition, the parental lines MON 89034 and NK 603 have been evaluated by the VKM GMO Panel as single events and as a component of several stacked GM maize events (VKM 2005a,b,c,d,e, VKM 2007a,b, VKM 2008b,c,d, VKM 2009a,b, VKM 2010 a,b, VKM 2011, VKM 2012a,b, VKM 2013 a,b, VKM 2014).

The food/feed and environmental risk assessment of the maize MON 89034 x NK 603 is based on information provided by the applicant in the applications EFSA/GMO/NL/2007/38 EFSA/GMO/NL/2009/72 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 89034 x NK 603 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 89034 x NK 603 include molecular characterisation of the inserted DNA and expression of novel proteins, comparative assessment of agronomic and phenotypic characteristics, nutritional assessments, toxicology and allergenicity, unintended effects on plant fitness, potential for gene transfer, effects on biogeochemical processes and interactions between the GM plant and target and non-target organisms.

It is emphasized that the VKM mandate does not include assessments of contribution to sustainable development, societal utility and ethical considerations, according to the Norwegian Gene Technology Act and Regulations relating to impact assessment pursuant to the Gene Technology Act. These considerations are therefore not part of the risk assessment provided by the VKM Panel on Genetically Modified Organisms. Likewise, the VKM mandate does not include evaluations of herbicide residues in food and feed from genetically modified plants.

The hybrid maize MON 89034 x NK 603 has been produced by conventional crosses between inbred lines containing MON 89034 and NK 603 events to combine resistance to certain lepidopteran pests and to confer tolerance towards glyphosate-containing herbicides.

Maize MON 89034 was developed to provide protection against specific lepidopteran target pest, including *Ostrinia nubilalis, Spodoptera* spp. and *Agrotis ipsilon.* Protection is achieved through expression in the plant of two insecticidal Cry proteins, Cry1A.105 and Cry2Ab2, derived from *Bacillus thuringiensis* subsp. *aizawai* and *kurstaki.* Maize NK 603 has been developed to provide tolerance to glyphosate by the introduction, of a gene coding for 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* sp. strain CP4 (CP4 EPSPS).

Molecular characterisation

Southern and PCR analyses indicate that the recombinant inserts in the single maize events MON 89034 and NK 603 are retained in maize stack MON 89034 x NK603. Genetic stability of the inserts has previously been demonstrated in the parental lines MON 89034and NK603. The level of Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins in grain and forage from the stacked event are comparable to the levels in the corresponding single events. Phenotypic analyses also indicate stability of the insect resistance and herbicide tolerance traits of the stacked event. Based on current knowledge and the previous assessments of the parental maize events, the VKM GMO Panel considers the molecular characterisation of maize MON 89034 x NK 603 satisfactory.

Comparative assessment

The applicant has performed comparative analyses of data from field trials located at representative sites and environments in Argentina in 2004/2005 and Europe in 2007. With the exception of small intermittent variations and the insect resistance and herbicide tolerance conferred by the Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins, the results showed no biologically relevant differences between maize stack MON 89034 x NK 603 and conventional control. Based on the assessment of available data, the VKM GMO Panel concludes that maize MON 89034 x NK 603 is compositionally, agronomical and phenotypically equivalent to its conventional counterpart, except for the new proteins.

Food/feed safety assessment

A whole food feeding study on broilers has not indicated any adverse health effects of maize MON 89034 x NK 603, and shows that it is nutritionally equivalent to conventional maize varieties. The Cry1A.105, Cry2Ab2, and CP4 EPSPS proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE mediated allergic reactions. However, some studies have indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize MON 89034 x NK 603 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1A.105, Cry2Ab2, and CP4 EPSPS proteins will cause toxic or IgE-mediated allergic reactions to food or feed based on maize MON 89034 x NK 603 compared to conventional maize.

Environmental risk

Considering the intended uses of maize MON 89034 x NK603, excluding cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize MON 89034 x NK603.

Maize MON 89034 x NK 603 has no altered survival, multiplication or dissemination characteristics, and there are no indications of an increased likelihood of spread and establishment of feral maize plants in the case of accidental release into the environment of seeds from maize MON 89034 x NK603. Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation. The VKM GMO Panel considers the risk of gene flow from occasional feral GM maize plants to conventional maize varieties to be negligible in Norway. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered by the GMO Panel to be an issue.

Overall conclusion

Based on current knowledge, the VKM GMO Panel concludes that maize MON 89034 x NK 603 is compositionally, nutritionally, agronomically and phenotypically equivalent to its conventional counterpart except for the new proteins. It is unlikely that the Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins will cause an increased risk of toxic or IgE-mediated allergic reactions to food or feed based on maize MON 89034 x NK 603 compared to conventional maize varieties.

The VKM GMO Panel concludes that maize MON 89034 x NK603, based on current knowledge, is comparable to conventional maize varieties concerning environmental risk in Norway with the intended usage.

Keywords

VKM, Norwegian Scientific Committee for Food Safety, maize, *Zea mays* L., genetically modified maize MON 89034 x NK603, EFSA/GMO/NL/2007/38, insect-resistance, herbicide-tolerance, *cry1A.105, cry2Ab2, cp4 epsps*, glyphosate, food/feed safety assessment, environmental risk assessment, Regulation (EC) No 1829/2003, Directive 2001/18.

Norsk sammendrag

I forbindelse med forberedelse til implementering av EU-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 89034 x NK 603 fra Monsanto (unik kode MON-89Ø34-3 × MON-ØØ6Ø3-6) ble godkjent i EU til import, videreforedling og til bruk som mat og fôr under EU-forordning 1829/2003 28. juli 2010 (Kommisjonsbeslutning 2010/420/EU).

I forbindelse med EFSAs offentlige høring av søknadene EFSA/GMO/NL/2007/38 og EFSA/GMO/NL/2009/72 i 2007 og 2009/2010 vurderte VKMs faggruppe for genmodifiserte organismer maishybriden med hensyn på mulig helse- og miljørisiko (VKM 2008a, VKM 2010a). VKMs faggruppe for GMO har også risikovurdert foreldrelinjene MON 89034 og NK603 og en rekke maishybrider der MON 89017 x NK603 inngår som en av foreldrelinjene (VKM 2005a,b,c,d,e, VKM 2007a,b, VKM 2008,b,c,d, VKM 2009a,b, VKM 2010 a,b, VKM 2011, VKM 2012a,b, VKM 2013 a,b, VKM 2014).

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 EU-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 (2010a,b, 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. Vurderinger av mulige plantevernmiddelrester i den genmodifiserte planten som følge av endret sprøytemiddelbruk faller per i dag utenfor VKMs ansvarsområde og er derfor heller ikke vurdert.

F1-hybriden MON 89034 x NK 603 er resultat av konvensjonelle kryssinger mellom innavlede maislinjer med eventene MON 89034 og NK603. Kryssingene er utført for å utvikle en maishybrid med resistens mot visse skadegjørere i sommerfuglordenen Lepidoptera samt toleranse mot herbicider med virkestoff glyfosat.

Den genmodifiserte maislinjen MON 89034 er fremkommet ved *Agrobacterium*-mediert transformasjon av umodne maisceller. MON 89034-plantene har fått satt inn et rekombinant DNA-fragment med to genekspresjonskassetter, inneholdende genene *cry1A.105* og *cry2Ab2*. *Cry1A.105* er et syntetisk gen, som er sammensatt av sekvenser fra genene *cry1Ac*, *cry1Ab* og *cry1F* fra *Bacillus thuringiensis* subsp. *aizawai. Cry2Ab*-genet stammer fra *B. thuringiensis* subsp. *kurstaki*. *Cry1A.105*- og *cry2Ab2*-genene koder for δ-endotoksiner, som gir plantene resistens mot enkelte arter i ordenen Lepidoptera, eksempelvis europeisk maispyralide (*Ostrinia nubilalis*), *Spodoptera* spp. og stort jordfly (*Agrotis ipsilon*). *Cp4-epsps*-genet i NK 603 koder for enzymet 5-enolpyruvylsikimat-3-fosfatsyntetase, som omdanner fosfoenolpyruvat og sikimat-3-fosfat til 5-enolpyruvylsikimat-3-fosfat, en viktig metabolitt i syntesen av aromatiske aminosyrer. I motsetning til plantens enzym er det bakterielle enzymet også aktivt ved nærvær av N-fosfonometylglycin (glyfosat). De transgene plantene vil derfor tolerere høyere doser av herbicider med virkestoff glyfosat sammenlignet med konkurrerende ugras.

Molekylær karakterisering

Southern- og PCR- analyser viser at de rekombinante gensekvensene som ble satt inn i maislinjene MON 89034 og NK 603 er bevart i den kryssede maishybriden MON 89034 x NK6030. Genetisk stabilitet av de innsatte sekvensene har tidligere blitt vist for mais MON 89034 og NK603. Nivåene av Cry1A.105, Cry2Ab2 og CP4 EPSPS -protein målt i korn og vegetativt vev fra MON 8034 x NK603, samsvarer med nivåene i de respektive foreldrelinjene. Fenotypiske analyser viser at egenskapene for insektsresistens og herbicidtoleranse er stabile også i MON 89034 x NK603. VKMs faggruppe for GMO anser den molekylære karakteriseringen av mais MON 89034 x NK 603 som tilfredsstillende.

Komparative analyser

Søker har utført komparative analyser av data fra feltforsøk på representative dyrkningsområder i Argentina under i vekstsesongen 2004/2005 og Europa under vekstsesongen i 2007. Med unntak av små spredte variasjoner, insektsresistens og herbicidtoleranse mediert av Cry1A.105, Cry2Ab2 and CP4 EPSPS proteinene, viste resultatene ingen biologisk relevante forskjeller mellom maishybriden MON 89034 x NK 603 og konvensjonell kontroll.

Basert på gjennomgang av tilgjengelige data, konkluderer VKMs faggruppe for GMO at maishybriden MON 89034 x NK 603 vesentlig lik konvensjonelle kontroll med hensyn til næringsstoffsammensetning, og agronomiske og fenotypiske egenskaper, med unntak av de nye proteinene.

Helserisikovurdering

En fôringsstudie utført på broilere indikerer ikke helseskadelige effekter av mais MON 89034 x NK603, og studien viser at den er ernæringsmessig lik konvensjonell mais. Proteinene Cry1A.105, Cry2Ab2 og CP4 EPSPS viser ingen relevante sekvenslikheter med andre kjente toksiner eller IgE-avhengige allergener, og er heller ikke rapportert å ha forårsaket IgE-medierte allergiske reaksjoner. Enkelte studier har derimot indikert at Cry-proteiner potensielt kan forsterke andre allergiske reaksjoner (virke som adjuvans).

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at mais MON 89034 x NK 603 er ernæringsmessig lik konvensjonell mais, og at det er lite sannsynlig at proteinene Cry1A.105, Cry2Ab2 og CP4 EPSPS vil føre til økt risiko for toksiske eller IgE-medierte allergiske reaksjoner fra mat eller fôr basert på mais MON 89034 x NK 603 sammenliknet med konvensjonelle maissorter.

Miljørisiko

Med bakgrunn i tiltenkt bruksområde er miljørisikovurderingen avgrenset til mulige effekter av utilsiktet frøspredning i forbindelse med transport og prosessering, samt indirekte eksponering gjennom gjødsel fra husdyr fôret med genmodifisert mais.

Det er ingen indikasjoner på økt sannsynlighet for spredning, etablering og invasjon av maislinjen i naturlige habitater eller andre arealer utenfor jordbruksområder som resultat av frøspill i forbindelse med transport og prosessering. Risiko for utkryssing med dyrkede sorter vurderes av GMO panelet til å være ubetydelig. Ved foreskreven bruk av maislinjen MON 89034 x NK 603 antas det ikke å være risiko for utilsiktede effekter på målorganismer, ikke-målorganismer eller på abiotisk miljø i Norge.

Samlet vurdering

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO, at mais MON 89034 x NK 603 er vesentlig lik konvensjonell kontroll med hensyn til næringsstoffsammensetning og ernæringsmessige, agronomiske og fenotypiske egenskaper, med unntak av de nye proteinene. Det lite sannsynlig at proteinene Cry1A.105, Cry2Ab2 og CP4 EPSPS vil føre til økt risiko for toksiske eller IgE-medierte allergiske reaksjoner fra mat eller fôr basert på mais MON 89034 x NK 603 sammenliknet med konvensjonelle maissorter.

VKMs faggruppe for genmodifiserte organismer konkluderer at mais MON 89034 x NK603, ut i fra dagens kunnskap og tiltenkt bruksområde, tilsvarer konvensjonell mais når det gjelder mulig miljørisiko i Norge.

Abbreviations and explanations

ALS	Acetolactate synthase, an enzyme that catalyses the first step in the synthesis of the branched-chain amino acids, valine, leucine,

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

Cry1A.105	Chimeric protein comprised of domains from the naturally occurring Cry1Ab, Cry1F, and Cry1Ac proteins of *Bacillus thuringiensis*
Cry2Ab2	A Cry2 class crystal protein from *Bacillus thuringiensis* subsp. *kurstaki*
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.

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Background

On 1st of February 2007, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands an application (Reference EFSA/GMO/NL/2007/38) for authorisation of the insect-resistant and herbicide tolerant genetically modified (GM) maize MON 89034 x NK 603 (Unique Identifier MON-89Ø34-3 × MON-ØØ6Ø3-6), submitted by Monsanto within the framework of Regulation (EC) No 1829/2003.

The scope of the application covers:

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

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

After receiving the application EFSA/GMO/NL/2007/38 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 24 August 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 2008 (VKM 2008a). EFSA published its scientific opinion 9 September 2009 (EFSA 2009a), and maize MON 89034 x NK 603 was approved for food and feed uses, import and processing in 28 July 2010 (Commission Decision 2010/420/EC).

An application for authorisation of maize MON 89034 x NK 603 for cultivation in the EU was submitted by Monsanto in June 2009 (EFSA/GMO/NL/2009/72). On 9 October 2009 EFSA

declared the application as valid, and made the valid application available to Member States and the European Commission. VKM participated in the 90 days public consultation, and submitted a preliminary environmental risk assessment report in April 2010 (VKM 2010b). On 21 August 2013 the application was, however, withdrawn by the applicant.

The parental lines MON 89034 and NK 603 have also been evaluated by the VKM GMO Panel as single events and as a component of several stacked GM maize events (VKM 2005a,b,c,d,e, VKM 2007a,b, VKM 2008,b,c,d, VKM 2009a,b, VKM 2010 a,b, VKM 2011, VKM 2012a,b, VKM 2013 a,b, VKM 2014).

Terms of reference

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

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

The Norwegian Environment Agency

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

The request covers scope(s) relevant to the Gene Technology Act. The request does not cover GMOs that the Committee already has conducted its final risk assessments on. However, the Norwegian Environment Agency requests the Committee to consider whether updates or other changes to earlier submitted assessments are necessary. The basis for evaluating the applicants' environmental risk assessments is embodied in the Act Relating to the Production and Use of Genetically Modified Organisms etc. (the Norwegian Gene Technology Act), Regulations relating to impact assessment pursuant to the Gene Technology Act, the Directive 2001/18/EC on the deliberate release of genetically modified organisms into the environment, Guidance note in Annex II of the Directive 2001/18 (2002/623/EC) and the Regulation 1829/2003/EC. In addition, the EFSA guidance documents on risk assessment of genetically modified plants and food and feed from the GM plants (EFSA 2010a, 2011a), and OECD guidelines will be useful tools in the preparation of the Norwegian risk assessments.

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

The Norwegian Food Safety Authority

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

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

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

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

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

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

Assessment

1 Introduction

The hybrid maize MON 89034 x NK 603 was produced by conventional crosses between inbred lines containing MON 89034 and NK 603 events to combine resistance to certain lepidopteran pests, and to confer tolerance towards glyphosate-containing herbicides.

The parental line MON 89034 was developed to provide protection against certain lepidopteran insect larvae, including European corn borer (*Ostrinia nubilalis*), fall armyworm (*Spodoptera* ssp.), black cutworm (*Agrotis ipsilon*) and corn earworm (*Helicoverpa zea*). Insect protection is achieved through expression in the plant of two insecticidal Cry proteins, Cry1A.105 and Cry2Ab2, derived from *Bacillus thuringiensis*, a common soil bacterium. Cry1A.105, encoded by the *cry1A.105* gene, is a chimeric protein made up of different functional domains derived from three wild-type Cry proteins from *B. thuringiensis* subspecies *kurstaki* and *aizawai*. The Cry2Ab2 protein is encoded by the *cry2Ab2* gene derived from *B. thuringiensis* subspecies *kurstaki*. The mode of action of the Cry proteins is to bind selectively to specific receptors on the epithelical surface of the midgut of larvae of susceptible insect species, leading to death of larvae through pore formation, cell burst and subsequently septicaemia (EFSA 2011d).

The parental line NK 603 has 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 NK603, a gene has been introduced that codes for the expression of the CP4 EPSPS protein, which is insensitive towards inhibition by glyphosate. This protein is similar to the native EPSPS found in wild-type plants, but it is not inactivated by glyphosate thus allowing the crop to be protected from the recommended dosages of glyphosate.

The genetic modification in maize MON 89034 x NK 603 is intended to improve agronomic performance only, and is not intended to influence the nutritional properties, the processing characteristics and the overall use of maize as a crop.

Maize stack MON 89034 x NK 603 (Unique Identifier MON-89Ø34-3 × MON-ØØ6Ø3-6) has been evaluated with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of

genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed.

The Norwegian Scientific Committee for Food Safety has also decided to take account of the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2011a), the environmental risk assessment of GM plants (EFSA 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 food/feed and environmental risk assessment of the genetically modified maize MON 89034 x NK 603 is based on information provided by the applicant in the applications EFSA/GMO/NL/2007/39 and EFSA/GMO/NL/2009/72 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 emphasized that the VKM mandate does not include assessments of contribution to sustainable development, societal utility and ethical considerations, according to the Norwegian Gene Technology Act and Regulations relating to impact assessment pursuant to the Gene Technology Act. These considerations are therefore not part of the risk assessment provided by the VKM Panel on Genetically Modified Organisms.

2 Molecular characterisation

2.1 Evaluation of relevant scientific data

2.1.1 Method of production of maize MON 89034 x NK 603

The stacked maize MON 89034 x NK 603 was developed through conventional breeding by crossing the single maize events MON 89034 and NK603. Maize MON 89034 x NK 603 combines the insect resistance of maize MON 89034 with the glyphosate tolerance of maize NK603, conferred through the expression of the *cry1A.105*, *cry2Ab2* and *cp4 epsps* genes, respectively.

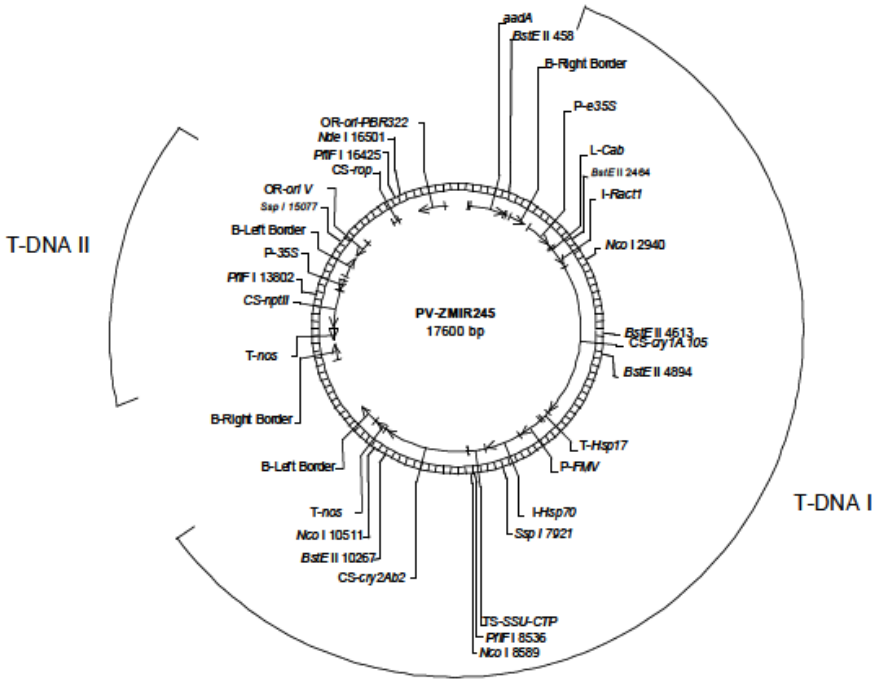
2.1.2 Summary of previous evaluation of the single events

2.1.2.1 Maize MON 89034

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

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

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



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

Figure 1. Map of the plasmid PV-ZMIR245

2.1.2.2 Maize NK 603

The maize line AW x CW, a proprietary maize cell culture, was transformed by acceleration to develop the NK 603 maize event. Conventional breeding methods were used to backcross plants generated from the initial transformation into a recurrent, desired inbred maize line with a genetic background of interest to the breeder.

NK 603 has been developed for tolerance to glyphosate by the introduction of two genes coding for glyphosate tolerant 5-enoylpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). Particle acceleration was used to introduce a

fragment DNA from the bacterial plasmid vector PV-ZMGT32. The plasmid vector contains two adjacent plant gene expression cassettes each containing a single copy of the *cp*4 *epsps* gene fused to chloroplast transit peptide (CTP) sequences based on sequences derived from *Arabidopsis thaliana* EPSPS. CTP targets the CP4 EPSPS protein to its natural sub cellular location in the chloroplast. In the first *ctp*2-*cp*4 *epsps* cassette the coding sequence is regulated by the rice actin promoter and a rice intron sequence introduced upstream of the CTP sequence. Expression of the second *ctp*2-*cp*4 *epsps* cassette is regulated by an enhanced 35S CaMV promoter and a maize intron derived from a gene encoding a heat shock protein. In each cassette the *cp*4 *epsps* sequence is linked to the nopaline synthase terminator (NOS 3') sequence from *Agrobacterium tumefaciens*. The vector also contains an *npt*II bacterial selectable marker gene (for kanamycin resistance; derived from the prokaryotic transposon *Tn*5) and an origin of replication (*ori*). A *Mlu*I restriction fragment of the PV-ZMGT32 plasmid vectoresignated PV-ZMGT32L was used for transformation and this fragment only contains the *cp*4 *epsps* plant gene expression cassettes. The *npt*II gene as well as the *ori* is not present in the fragment PV-ZMGT32L.

The EPSPS enzyme catalyzes the penultimate step of the shikimic acid pathway for the biosynthesis of aromatic amino acids, which is present in all green plants. Inhibition of this enzyme by glyphosate leads to a reduction of aromatic amino acids, interfering with plant growth, and ultimately leading to plant death. The herbicide Roundup has broad-spectrum weed control capabilities, but the sensitivity of traditional maize to glyphosate had prevented the in-season use of this herbicide in the crop. With the expression of the glyphosate-tolerant CP4 EPSPS enzymes in NK603, the continued function of the aromatic amino acid pathway is ensured in the crop, even in the presence of the herbicide.

The levels of CP4 EPSPS and CP4 EPSPS L214P proteins in various tissues of NK603, produced during the 1999 growing season in the E.U. and the 2002 growing season in the U.S.A. were estimated using an enzyme-linked immunosorbent assay (ELISA). The expression of the CP4 EPSPS proteins occurs throughout the plant since the rice actin and CaMV e35S promoters have been shown to drive constitutive expression of the encoded protein in genetically modified maiz. As forage and grain are the most relevant tissues for the safety assessment, protein levels in these tissues were estimated in both growing seasons. Additionally, protein levels in pollen, forage root, OSL and OSR were estimated in the 2002 growing season.

In 1999, forage and grain tissues were produced in European field trials at four sites. Four replications were used at each of the four sites. CP4 EPSPS protein levels were measured in maize forage and grain. All protein values are expressed as micrograms (µg) of the specific protein per gram (g) of tissue on a fresh weight (fw) basis. Control maize samples were below the Limit of Detection (LOD) for CP4 EPSPS protein. In maize NK 603 forage, the mean CP4 EPSPS protein levels from the four different field sites ranged from 43.6 µg/g fw to 60.9 µg/g fw. The overall mean CP4 EPSPS protein level in maize NK 603 forage across all four sites was 48.6 µg/g fw. In maize NK 603 grain, the mean CP4 EPSPS protein levels ranged from 2.2 µg/g fw to 13.2 µg/g fw. The overall mean CP4 EPSPS protein level in maize grain across all four sites was 8.4 µg/g fw. The values given represent the sum of both CP4

EPSPS and CP4 EPSPS L214P, as the ELISA analytical method recognizes both these proteins expressed in NK603.

In 2002, test and control samples were produced in U.S.A. field trials. CP4 EPSPS protein levels in the different tissue types were estimated using a validated direct double antibody sandwich ELISA method. On a dry weight basis, the mean CP4 EPSPS protein levels across four field sites for overseason leaf tissues were 300-430 µg/g dw. The mean CP4 EPSPS protein levels across four field sites for overseason root tissues were 76-160 µg/g dw. The mean CP4 EPSPS protein levels across four field sites for forage, forage root, pollen, and grain tissues were 100, 140, 650, and 14 µg/g dw, respectively. The expression levels for forage and grain general agreement with the CP4 EPSPS levels measured in forage and grain samples collected from six non-replicated and two replicated field trials conducted in 1998 in the U.S.A. In the U.S.A. trials from 1998, CP4 EPSPS expression levels ranged from 18.0 to 31.2 µg/g fw for forage and from 6.9 to 15.6 µg/g fw for grain samples, respectively.

Southern blot analysis was used to study the insert number, the copy number, integrity of the inserted promoters, coding regions, and polyadenylation sequences, and the presence or absence of the plasmid backbone sequence. Polymerase chain reaction (PCR) was performed to verify the sequences at the 5' and 3' ends of the insert. Further, PCR analysis and subsequent DNA sequencing of four overlapping products spanning the length of the insert in NK 603 were undertaken to characterize of the inserted DNA in NK 603 (Kesterson et al., 2002a). Genomic DNA from the NK 603 maize and control (B73) were digested with the restriction enzyme *StuI*. The result suggested that NK 603 contains one insertion of integrated DNA located within a 23 kb *StuI* restriction fragment. The genome of NK 603 does not contain any detectable plasmid backbone DNA including *ori* or the *nptII* coding sequence. PCR amplification and DNA sequencing was used for characterization of the insert and the sequences flanking the insert. The results indicate that these sequences are native to the maize genome. These data indicate that only the expected full-length CTP2-CP4 EPSPS and CTP2-CP4 EPSPS L214P proteins are encoded by the insert in NK603. The contents of genes and regulatory elements in the recombinant DNA fragment are outlined in Figure 1.

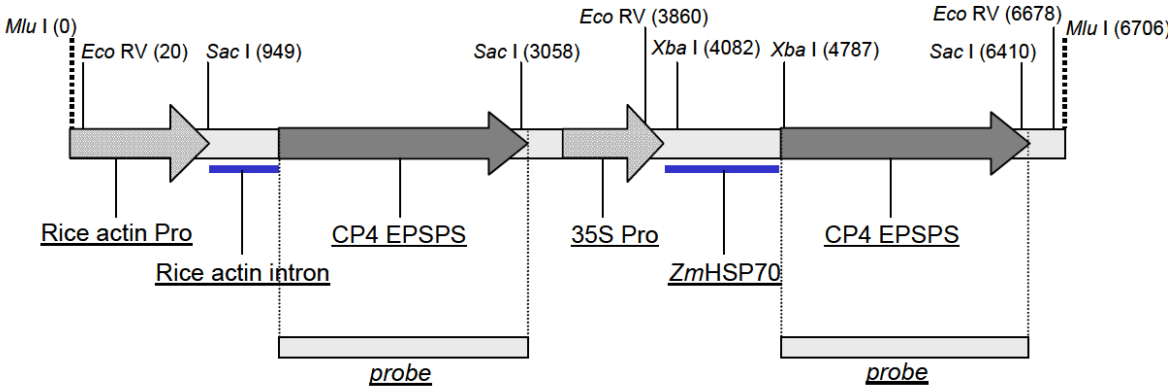


Figure 2. Restriction map of the various gene elements of the recombinant DNA fragment inserted in the genome of the maize strain NK603.

2.1.3 Transgene constructs in MON 89034 x NK 603 maize

The MON 89034 x NK 603 maize was obtained by conventional crossing between two genetically modified maize events: MON 89034 and NK 603 maize. No new genetic modification was used for the development of the MON 89034 x NK 603 maize.

A detailed molecular analysis was conducted to investigate the copy number, structure and organization of the inserts found in MON 89034 x NK 603 maize. The integrity of the individual inserts present in this maize was investigated using Southern analyses. This involved the use of DNA probes specific for the MON 89034 and NK 603 inserts and enzymatic digestions informative of the structure of both events, including the junctions with the host genomic DNA. The predicted DNA hybridisation patterns from each single event were retained in the MON 89034 x NK 603 hybrid. The results obtained from Southern Blot analyses indicate molecular equivalence, and identical copy number of the inserts present in MON 89034 x NK 603 maize to those present MON 89034 and NK603.

2.1.4 Information on the expression of the inserts

A study was conducted to estimate the amount of Cry1A.105, Cry2Ab2 and CP4 EPSPS protein present in maize tissues collected from MON 89034 x NK 603 grown in five filed trails in Argentina during the 2004 growing season (Hartmann et al 2006).

The trials were located in the provinces of Buenos Aires, Cordoba and Santa Fe, which represent the major maize growing region of Argentina and provide a variety of environmental conditions. At each site, three replicated plots of MON 89034 x NK 603, MON 89034 and NK603, as well as the conventional control, were planted using a randomized complete block field design.

Young leaf, young root, over season whole plant 3 (OSWP-3), forage, forage-root, pollen, and grain tissues were collected from each replicated plot at all field sites. The samples from young leaf (over season leaf; OSL-1) and young root (over season root; OSR-1) were collected at the V2 – V4 growth stage and the OSWP-3 samples were collected at the V10 – V12 growth stage.

ELISA methods were developed and validated for each protein. All protein levels for all ten tissues types were calculated on a microgram (µg) per gram (g) fresh weight (fwt) basis. Moisture content was then measured for all tissue types and all protein levels were converted and reported on a dry weight (dwt) basis. Levels of proteins are summarized in Table 4-6.

The mean Cry1A.105 protein levels in MON 89034 x NK 603 across all sites were 220 µg/g dwt in OSL-1, 66 µg/g dwt in OSR-1, 83 µg/g dwt in OSWP-3, 30 µg/g dwt in forage, 24 µg/g dwt in forage-root, 9.6 µg/g dwt in pollen and 3.1 µg/g dwt in grain.

The mean Cry2Ab2 protein levels in MON 89034 x NK 603 across all sites were 140 µg/g dwt in OSL-1, 37 µg/g dwt in OSR-1, 72 µg/g dwt in OSWP-3, 33 µg/g dwt in forage, 27 µg/g dwt in forage-root, 0.66 µg/g dwt in pollen and 1.2 µg/g dwt in grain.

The mean CP4 EPSPS protein levels in MON 89034 x NK 603 across all sites were 240 µg/g dwt in OSL-1, 78 µg/g dwt in OSR-1, 210 µg/g dwt in OSWP-3, 74 µg/g dwt in forage, 48 µg/g dwt in forage root, 390 µg/g dwt in pollen and 8.1 µg/g dwt in grain.

Overall, the ranges across all sites for the Cry1A.105, Cry2Ab2 and CP4 EPSPS protein levels in MON 89034 x NK 603 were comparable to the corresponding ranges in either MON 89034 or NK603.

Table 4. Summary of the level of the Cry1A.105 protein in maize tissues collected from MON 89034 x NK 603 and MON 89034 produced in field trails in Argentina conducted in 2004.

Tissue Type[1]	MON 89034 x NK603		MON 89034	
	Mean (SD)[2] Range[3] (µg/g fwt)[4]	Mean (SD) Range (µg/g dwt)[5]	Mean (SD) Range (µg/g fwt)	Mean (SD) Range (µg/g dwt)
OSL-1	31 (11) 11 – 50	220 (91) 62 – 350	35 (6.5) 24 – 50	240 (59) 140 – 330
OSR-1	7.9 (1.5) 5.3 – 10	66 (11) 48 – 83	7.0 (1.6) 4.3 – 9.8	57 (10) 35 – 78
OSWP-3	9.4 (2.2) 6.2 – 14	83 (25) 57 – 140	6.9 (4.0) 3.2 – 16	61 (35) 27 – 140
Forage	8.4 (2.5) 2.9 – 12	30 (8.7) 10 – 44	9.0 (2.1) 5.6 – 13	31 (6.6) 21 – 42
Forage-Root	4.3 (1.3) 3.0 – 7.9	24 (4.1) 19 – 34	5.2 (1.0) 4.2 – 7.2	29 (3.1) 23 – 34
Pollen	7.0 (1.2) 5.3 – 9.5	9.6 (1.4) 8.0 – 12	6.0 (0.52) 5.2 – 6.8	8.4 (0.69) 6.7 – 9.3
Grain	2.6 (0.36) 2.3 – 3.7	3.1 (0.41) 2.6 – 4.3	2.2 (0.44) 1.5 – 3.4	2.6 (0.52) 1.7 – 3.9

1. Tissues were collected at the following growth stages (Ritchie *et al.*, 1997):
   a. OSL-1 and OSR-1: V2 – V4
   b. OSWP-3: V10 – V12
   c. Forage: Early dent
   d. Forage-Root: Early dent
   e. Pollen: At pollination
   f. Grain: At physiological maturity
2. Mean and standard deviation were calculated across sites (n=15).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt) basis. The dry weight values were calculated by dividing the fwt by the dry weight conversion factors obtained from moisture analysis data.

Table 5. Summary of the level of the Cry2Ab2 protein in maize tissues collected from MON 89034 x NK 603 and MON 89034 produced in field trails in Argentina conducted in 2004.

Tissue Type[1]	MON 89034 × NK603		MON 89034	
	Mean (SD)[2] Range[3] (µg/g fwt)[4]	Mean (SD) Range (µg/g dwt)[5]	Mean (SD) Range (µg/g fwt)	Mean (SD) Range (µg/g dwt)
OSL-1	20 (6.9) 4.9 – 31	140 (48) 27 – 200	15 (3.8) 10 – 27	110 (31) 60 – 180
OSR-1	4.4 (2.0) 2.3 – 8.0	37 (18) 19 – 70	5.7 (1.5) 2.5 – 7.6	48 (11) 23 – 63
OSWP-1	7.1 (3.1) 2.9 – 16	72 (41) 27 – 200	8.0 (1.9) 4.5 – 11	80 (25) 41 – 140
Forage	9.3 (2.8) 4.7 – 15	33 (10) 17 – 53	12 (2.5) 8.5 – 17	40 (7.3) 30 – 54
Forage-Root	5.1 (2.4) 2.5 – 11	27 (9.6) 15 – 47	5.7 (1.9) 3.6 – 9.7	31 (6.0) 23 – 42
Pollen	0.48 (0.14) 0.36 – 0.86	0.66 (0.18) 0.46 – 1.1	0.43 (0.10) 0.25 – 0.65	0.60 (0.12) 0.38 – 0.83
Grain	1.0 (0.15) 0.80 – 1.3	1.2 (0.18) 0.92 – 1.5	0.85 (0.15) 0.60 – 1.2	1.0 (0.18) 0.69 – 1.4

1. Tissues were collected at the following growth stages (Ritchie *et al.*, 1997):
   a. OSL-1 and OSR-1: V2 – V4 c. Forage: Early dent e. Pollen: At pollination
   b. OSWP-3: V10 – V12 d. Forage-Root: Early dent f. Grain: At physiological maturity
2. Mean and standard deviation were calculated across sites (n=15, except OSL-1 line MON 89034, n=16).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt) basis. The dry weight values were calculated by dividing the fwt by the dry weight conversion factors obtained from moisture analysis data

Table 6. Summary of the level of the CP4 EPSPS protein in maize tissues collected from MON 89034 x NK 603 and NK 603 produced in field trails in Argentina conducted in 2004.

Tissue Type[1]	MON 89034 x NK603		NK603	
	Mean (SD)[2] Range[3] (µg/g fwt)[4]	Mean (SD) Range (µg/g dwt)[5]	Mean (SD) Range (µg/g fwt)	Mean (SD) Range (µg/g dwt)
OSL-1	34 (7.0) 18 – 44	240 (66) 100 – 340	31 (2.9) 28 – 38	220 (38) 160 – 290
OSR-1	9.4 (3.1) 6.1 – 18	78 (22) 52 – 140	12 (4.5) 5.3 – 19	97 (31) 44 – 150
OSWP-1	21 (4.3) 14 – 30	210 (47) 130 – 290	20 (3.5) 13 – 27	190 (49) 110 – 270
Forage	21 (4.3) 16 – 31	74 (13) 54 – 100	18 (4.5) 11 – 28	63 (14) 40 – 87
Forage-Root	8.6 (2.2) 5.9 – 13	48 (9.7) 36 – 70	8.5 (3.3) 4.1 – 17	47 (14) 24 – 72
Pollen	280 (99) 140 – 450	390 (140) 180 – 610	230 (53) 150 – 340	320 (69) 220 – 440
Grain	7.0 (1.4) 5.0 – 9.7	8.1 (1.6) 5.8 – 11	5.8 (1.6) 3.7 – 8.2	6.7 (1.8) 4.3 – 9.5

1. Tissues were collected at the following growth stages (Ritchie *et al.*, 1997):
   a. OSL-1 and OSR-1: V2 – V4 c. Forage: Early dent e. Pollen: At pollination
   b. OSWP-3: V10 – V12 d. Forage-Root: Early dent f. Grain: At physiological maturity
2. Mean and standard deviation were calculated across sites (n=15).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt) basis. The dry weight values were calculated by dividing the fwt by the dry weight conversion factors obtained from moisture analysis data

2.1.5 Inheritance and genetic stability of inserted DNA

The genetic stability of the inserted DNA in events MON 89034 and NK 603 was evaluated previously (VKM 2005c, VKM 2012a, VKM 2013a, VKM 2013b, VKM 2014). The Southern data showed that both events are present and the structure of each insert is retained. Furthermore, each of the traits has been conserved in this maize. Furthermore, protein expression levels, phenotypic characteristics and agronomic performance, indicate that the integrity of the inserts inherited from the single events is preserved in maize stack MON 89034 x NK 603.

2.2 Conclusion

Southern and PCR analyses indicate that the recombinant inserts in the single maize events MON 89034 and NK 603 are retained in maize stack MON 89034 x NK603. Genetic stability of the inserts has previously been demonstrated in the parental lines MON 89034 and NK603. The level of Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins in grain and forage from the stacked event are comparable to the levels in the corresponding single events. Phenotypic analyses also indicate stability of the insect resistance and herbicide tolerance traits of the stacked event. Based on current knowledge and the previous assessments of the parental maize events, the VKM GMO Panel considers the molecular characterisation of maize MON 89034 x NK 603 satisfactory.

3 Comparative assessment

3.1 Summary of the previous evaluations of the single events

3.1.1 Maize MON 89034

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

3.1.2 Maize NK 603

Compositional analyses were conducted for forage and grain samples collected from NK 603 that was grown in field trials at multiple locations in the USA in 1998 and in the EU in 1999. No consistent compositional differences were observed between maize NK 603 and non-transgenic maize. However, the biological relevance of statistically significant differences was assessed by performing additional comparisons of the level of the various compounds in maize NK 603 and conventional non-GM maize lines grown in field trials conducted in 1994-1995 or 1998. In the latest risk assessment of maize NK 603 the VKM GMO Panel concludes that maize NK 603 is compositionally, agronomically and phenotypically equivalent to conventional maize varieties, except for the presence of the herbicide tolerance trait conferred by the CP4 EPSPS protein (VKM 2013b).

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

3.2.1. Experimental design & statistical 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 Argentina during the 2004-2005 field season (Drury et al. 2006). The composition of forage and grain produced by MON 89034 x NK 603 was compared to conventional control maize with similar genetic background, as well as with other commercially available maize hybrids included in the study, to provide data for the development of a 99% tolerance interval. These plants were grown at five field sites located in the major maize-growing areas of Argentina, in a randomized complete block design with three replicates per block.

Compositional analyses were conducted on a total of 77 different analytical components (nine in forage and 68 in grain). Of these components, 16 had more than 50% of the observations below the assay LOQ and were excluded from the statistical analysis. Statistical analyses of the remaining 61 components (nine in forage and 52 in grain) were conducted for comparison of MON 89034 x NK 603 with the control maize, using a mixed model analysis of variance. Each individual analyte for MON 89034 x NK 603 was compared to that of the conventional control, for the combination of all five sites (the combined-site) and for each individual site. The statistical significance was defined at the level of $p < 0.05$. The overall data set was examined for evidence of biologically relevant changes. Analytes for which the levels were not statistically different were deemed to be present at equivalent levels between MON 89034 x NK 603 and the control. For those comparisons in which the test was statistically different from the control, the test range was compared to the 99% tolerance interval and to the ILSI Crop Composition Data base ranges, in order to determine if the test range was within the interval and therefore considered to be part of the population of the commercial maize.

3.3 Compositional Analysis

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

In total, 366 statistical comparisons were made between the MON 89034 x NK 603 test and the conventional control hybrids (61 comparisons in the combined site and 305 comparisons in the individual sites). Analyses using data from the combined sites indicated that there were no statistical differences in the levels of 90% of the analytes (55 of the 61). Table 1 in appendix summarizes results of the compositional analyses of MON 89034 × NK 603 for all sites combined. Analyses using data from the five single sites indicated that there were no statistically significant differences in the levels of 93% of the analytes (283 of the 305 comparisons made between MON 89034 × NK 603 and the control). Analysis site by site can be found in (Drury et al., 2006).

Combined-site analysis

For the combined-site analyses, six statistically significant differences ($p<0.05$) between MON 89034 x NK 603 and the control maize were observed for nutrients in maize forage and grain, which included 18:0 stearic acid, ADF, vitamin B2 and total fat, in grain, and ash and total fat, in forage. The differences observed are generally small (4.66 - 19.08%), and the mean levels and ranges of MON 89034 x NK 603 were all well within the 99% tolerance intervals for commercial maize. Furthermore, the mean levels and ranges of nutrients for MON 89034 x NK 603 were within the range of values obtained from the International Life Sciences Institute Crop Composition Database, as well as within published literature ranges. Additionally, three of these analytes (ADF in grain and ash and total fat, in forage), were found to be statistically different from the control in the combined site, but not in the individual sites. For the other three analytes, statistical differences were only observed in up to three of the five individual sites.

Individual sites analysis

The reproducibility and trends at the five individual sites were also examined, and comparisons made to conventional maize hybrids using the 99% tolerance intervals. Of the 21 statistical differences observed in nutrients of the individual site analyses, 14 analytes (in grain: vitamin B2, ash, moisture, copper, phosphorous, potassium, threonine, 16:0 palmitic acid, 18:1 oleic acid, 18:2 linoleic acid and 20:0 arachidic, in forage, moisture, protein and calcium) were observed only at one site (Table 2 in appendix). In addition, one difference was observed in the individual site analysis for the secondary metabolite p-coumaric acid. Of the remaining seven differences in nutrients observed at more than one site, there were no analyte that were consistently and statistically different across five or four sites. In addition, there were no analyte that showed statistically significant differences in two or three sites (i.e., total fat and 18:0 stearic acid in grain, respectively) that had not been previously observed to be different in the combined-site analysis. Statistically significant differences were observed in as many as two sites for only one analyte (NDF in forage), which was previously not found to be different in the combined-site analysis.

According to the applicant, the statistical analyses showed that all of the 366 comparisons made between the test and the control maize were either: a) not statistically significantly different, b) significantly different ($p<0.05$) but the composition values for the test were within the calculated 99% tolerance interval for the population of conventional reference hybrids used in this study, or c) significantly different but the composition values for the test were within the range of values obtained from the ILSI Crop Composition Database and, therefore, not considered biologically relevant.

3.4 Agronomic and phenotypic characters

Field trials Argentina 2004/2005

The applicant provided information on agronomic performance, phenotypic characteristics and natural ecological interaction of maize MON 89034 x NK 603 and its non-GM counterpart. The data were obtained from field trials performed at five sites in Argentina the growth season 2004/2005. At each trial site three conventional maize varieties were fully randomized with the test materials.

Phenotypic characteristics investigated included seedling vigour, early stand count, 50% pollen shed and silking, staygreen, ear heights, plant heights, dropped ears, stalk lodging, root lodging, final stand count, grain moisture, test weight and yield. The seedling vigour could not be appropriately analysed with statistical methods due to lack of variability between trial sites.

The only character that differed between the tested stacked genetically modified maize and the non-GM counterpart when the data was pooled across all five sites was 50% pollen shed which on average was 58 days (range 54.7-63.3 days) in MON 89034 NK 603 and 57 days (range 54.7-60.7) in the non-GM counterpart (Table 7). At the individual site analysis a difference was noted at two of the five trial sites. The mean value was however within the reference range for the 15 conventional maize varieties included in the field trials (range 54.0-68.7 days). The VKM GMO Panel interpreted the observed statistical difference in 50% pollen shed as biologically irrelevant.

Table 7. Combined sites analysis: phenotypic characteristics of maize MON 89034 x NK 603 compared to the conventional counterpart. Field trials conducted in Argentina in the 2004/2005 growth season.

Phenotypic Characteristic (units)	Means		References			
			Range[1]		99% Tolerance interval[2]	
	Test	Control	Min.	Max.	LL	UL
Early stand count (plants/2 rows)	60	62	59.3	69.0	52.9	77.6
Seedling vigor (0 – 9)[3]	7.2 [3]	7.5	4.0	9.0	1.1	9.0
Days to 50% pollen shed	58*	57	54.0	68.7	45.7	73.9
Days to 50% silking	58	57	54.0	69.7	46.2	74.9
Stay green rating (0 – 9)	3.3	3.3	0.0	7.7	0.0	9.0
Ear height (cm)	85	82	75.7	129.3	42.5	148.7
Plant height (cm)	187	186	168.3	257.2	125.1	284.8
Dropped ears (ears/2 rows)[4]	0	0	0.0	0.0	N/A	N/A
Stalk lodged plants (plants/2 rows)	1.8	2.0	0.0	4.3	0.0	7.7
Root lodged plants (plants/2 rows)	5.9	5.1	0.0	22.7	0.0	27.2
Final stand count (plants/2 rows)	59	61	58.7	65.7	56.0	70.1
Grain moisture (%)	24.8	24.9	18.9	39.7	3.7	56.4
Test weight (kg/l)	0.69	0.69	0.65	0.82	0.47	0.94
Yield (kg/ha)	9628.9	8869.4	5856.4	12014.2	2812.1	15058.5

* Indicates a statistically significant difference between MON 89034 × NK603 and the control at $p \leq 0.05$
[1] Reference range = Minimum and maximum mean values among the references
[2] 99% tolerance interval with 95% confidence. LL = lower limit; UL = upper limit, N/A=not applicable
[3] No combined site statistical analysis of variance was conducted for seedling vigor due to lack of variability in the data at three of five sites
[4] No statistical analysis was conducted for dropped ears due to a lack of variability in the data

Field trials Europe 2007

Phenotypic and agronomic data were collected from eight field locations in Europe, five in Spain and three in Germany, during the 2007 growing season. According to the applicant, these locations provided a range of environmental and agronomic conditions representative of the northern and southern European maize growing regions where commercial production of MON 89034 x NK 603 is expected. In these field trials genetically modified maize MON 89034 x NK 603 was compared with a conventional counterpart having a comparable genetic background. Event MON 89034 x NK 603 was introgressed into two different genetic backgrounds; DKC3945 adapted to northern (Germany) and DKC5143 adapted to southern (Spain) European growing regions. The control substances included in the field trials were conventional maize DKC3945 (Germany) and DKC5143 (Spain). DKC3945 and DKC5143 have genetic backgrounds similar to the test plants grown in Germany and Spain, respectively, except for the insect-protection trait.

15 conventional, commercial available maize hybrids with similar relative maturities as the test and control substances were included in the comparative assessments to verify whether any differences observed between the GMO and its comparator fall within the range of natural variation. Six locally adapted hybrids were used in Germany and nine different locally adapted hybrids were grown in Spain.

Plots were established at each site in a randomised complete block design with three replications. Each plot consisted of six rows spaced approximately 70 cm apart and approximately 6-10 m in length. Rows 4 and 5 were designated for phenotypic and ecological interaction data, while row 3 and 6 were used as buffer rows. Agronomic practices used to prepare and maintain each study were characteristic of the respective region.

Pesticides containing Bt were not applied to the study area at any site. The applicant has, however, not provided information regarding spraying regime in general.

The following plant phenotypic and agronomic characteristics were assessed: seedling vigour, early stand count, number of days after planting to 50% pollen shed and 50% silking, stay green, ear height ,plant height, number of dropped ears, number of stalk and root lodged plants, final stand count, grain moisture, and yield (Table 8).

In addition, the applicant has presented observational data from studies of plant environmental interactions several times during the growing seasons. The purpose of these evaluations was to assess whether plant response to abiotic and biotic stressors were altered compared to control maize. The

Plots at each site were qualitatively evaluated for plant response to abiotic stressors (e.g. drought, frost, wind, flood damage, nutrient deficiency, etc), disease damage and arthropod damage at four growth stages.

Results from the combined-site phenotypic comparisons of MON 89034 x NK 603 to the control for the European field trials are presented in Table 8. Minimum and maximum mean values (reference range) observed among 15 commercially available reference maize hybrids provide benchmark values common to maize for each characteristics.

In the combined-site analysis for Spain for MON 89034 × NK 603 and the control, no differences were detected for 13 out of 14 characteristics: seedling vigor, early stand count, days to 50% pollen shed and silking, stay green, ear height, plant height, dropped ears, root lodging, final stand count, ear/kernel rot, stalk rot and grain yield (Table 8). There were fewer stalk lodged plants in MON 89034 × NK 603 plots than in the control plots (0.0 vs 0.5, respectively). Less stalk lodging does not represent a change in the plant that would confer an increase in weediness potential. It is likely that the change in stalk lodging is a direct agronomic consequence of the presence of lepidopteran protection trait present in MON 89034 × NK603.

In the combined-site analysis for Germany, no statistical differences were detected between MON 89034 × NK 603 and its control for all the parameters measured (Table 8).

No overall differences were observed across sites between MON 89034 x NK 603 and the control in their susceptibility or tolerance to the ecological stressors assessed.

Table 8. Combined sites analysis: phenotypic characteristics of MON 89034 x NK 603 (test) compared to the control – European field trials conducted in 2007 (Germany and Spain)

Characteristic	German Field Sites				Spanish Field Sites			
	MON 89034 × NK603	Control	References Range[1]		MON 89034 × NK603	Control	References Range[1]	
			Min.	Max.			Min.	Max.
Seedling vigour	5.7	5.8	4.7	7.3	2	2.1	1.0	3.0
Early stand count (#/plot)	93	93.4	75.7	100.0	78.9	79.1	43.2	79.7
Days to 50% pollen shed	71	71.4	66.0	73.3	81.5	81.8	75.0	91.0
Days to 50% silking	70.1	70.3	65.0	73.3	76.8	77	69.0	88.0
Stay green	5.8	5.3	2.8	6.3	9	9	8.7	9.0
Ear height (cm)	81.1	84.7	63.1	118.3	98.1	97.6	83.0	126.2
Plant height (cm)	195	203.6	177.9	233.7	194.9	196.2	165.0	226.2
Dropped ears (#/plot)	0.0†	0	0.0	0.0	2.5	1.9	0.0	13.3
Stalk lodged plants (#/plot)	0.0†	0	0.0	0.0	0.0*	0.5	0.0	0.3
Root lodged plants (#/plot)	0.0†	0	0.0	0.0	0.1	0.3	0.0	0.3
Final stand count (#/plot)	76.6	76.4	69.2	76.4	77.7	76.9	41.7	80.3
Ear/Kernel rot	0.0†	0.0†	0.0	0.0	0.0†	0.0†	0.0	0.0
Stalk rot	0.0†	0.0†	0.0	0.0	0.0†	0.0†	0.0	0.0
Yield (t/ha)	6.1	6.4	5.1	9.3	10.4	10.2	5.7	11.7

Note: No statistical differences were detected between the test substances and the control ($p<0.05$).
† Data not analyzed due to lack of variation.

3.5 Conclusion

The applicant has performed comparative analyses of data from field trials located at representative sites and environments in Argentina in 2004/2005 and Europe in 2007. With the exception of small intermittent variations and the insect resistance and herbicide tolerance conferred by the Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins, the results showed no biologically relevant differences between maize stack MON 89034 x NK 603 and conventional control. Based on the assessment of available data, the VKM GMO Panel concludes that maize MON 89034 x NK 603 is compositionally, agronomical and phenotypically equivalent to its conventional counterpart, except for the new proteins.

4 Food and feed safety assessment

Both single maize events, MON 89034 and NK603, have previously been evaluated by the VKM GMO Panel, and updated risk assessments were finalised in June 2014 and October 2013, respectively (VKM 2014, VKM 2013).

4.1 Summary of the previous evaluations of the single events

Maize MON 89034

In the latest risk assessment of maize MON 89034 the VKM GMO Panel concluded, based in part on data from whole food feeding studies on rats, feedlot cattle and broilers, that the maize is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1A.105 and Cry2Ab2 proteins will introduce a toxic or allergenic potential in food or feed based on maize MON 89034 compared to conventional maize varieties (VKM 2014).

Maize NK 603

In the latest risk assessment of maize NK 603 the VKM GMO Panel concluded, based in part on data from whole food feeding studies on rats and broilers, that maize NK 603 is nutritionally equivalent to conventional maize varieties. It is unlikely that the CP4-EPSPS proteins will introduce a toxic or allergenic potential in food or feed based on maize NK 603 compared to conventional maize (VKM 2013).

4.2 Product description and intended uses

The genetic modification in MON 89034 x NK 603 maize will not impact the existing production processes used for maize. All MON 89034 x NK 603 maize products will be produced and processed for use in food, animal feed and industrial products in the same way as other commercial maize. The MON 89034 x NK 603 maize and all food, feed and processed products derived from MON 89034 x NK 603 maize are expected to replace a portion of similar products from commercial maize, with total consumption of maize products remaining unchanged.

The total anticipated Cry1A.105, Cry2Ab2 and CP4 EPSPS (incl. CP4 EPSPS L214P) intake/extent of use of maize and all food, feed and processed products derived from maize will remain the same.

4.3 Effects of processing

Food manufacturing of MON 89034 x NK 603 maize includes many harsh processing steps, e.g. cooking, heating, high pressures, pH treatments, physical shearing, extrusion at high temperatures etc. under which the majority of DNA and proteins are denatured, which also applies to the Cry1A.105, Cry2Ab2, and CP4 EPSPS proteins and *cry1A.105, cry2Ab2,* and *cp4 epsps* genes (Dien et al 2002, Hammond & Jez 2011, Fernandes et al 2013). Baking of the maize bread broa containing 11% of TC1500 and 20% MON810 maize flour, showed that the baking process sheared the DNA into small fragments, less than 1000 bp (Fernandes et al 2013).

4.4 Toxicological assessment

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

4.4.1 Toxicological assessment of the newly expressed protein

The VKM GMO Panel has previously evaluated the proteins Cry1A.105, Cry2Ab2, and CP4 EPSPS proteins in the risk assessments of the parental maize lines MON 89034 and NK 603 (VKM 2014, VKM 2013).

4.4.2 Toxicological assessment of the whole GM food/feed

The applicant has not performed a 90-day subchronic feeding study on rats with maize MON 89034 x NK 603. The applicant has however performed a 42-day broiler feeding study with emphasis on nutritional properties of maize MON 89034 x NK 603, which also considers health effects of maize MON 89034 x NK 603. The study is described in detail under section 4.6.2.

4.5 Allergenicity assessment

The strategies used when assessing the potential allergenic risk focuses on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation, or to elicit allergic reactions in already sensitised individuals and whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (EFSA 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 and structural similarity to, known human IgE-allergens with an array of bioinformatic tools. Sequence homology searches comparing the structure of novel proteins to known IgE-allergens in a database are conducted with various algorithms such as FASTA to predict overall structural similarities. According to FAO/WHO (2001) in cases where a novel protein and a known IgE-allergen have more than 35% identity over a segment of 80 or greater amino acids, IgE cross-reactivity between the novel protein and the allergen should be considered a possibility.

4.5.1 Assessment of IgE mediated allergenicity of the newly expressed protein

The applicant has performed a weight-of-evidence approach (FAO/WHO, 2001; Codex, 2003) for an overall assessment of the IgE allergenic potential of the Cry1A.105, Cry2Ab2, and CP4 EPSPS proteins. These assessments have previously been described by the applicant for the parental maize events MON 89034 and NK 603 and include:

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

The protein assessments were based on the following aspects:

Cry1A.105:

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

ii) The produced Cry1A.105 protein in maize event MON 89034 is a single polypeptide. Comparison of all folds of Cry1Ac, Cry1Ab and Cry1F showed that Cry1Ab and Cry1A.105 have essentially the same main chain structure, and that Cry1Ac differs slightly in its main chain structure from the other two in domain III. Thus, comparison of the modeled crystal structures of the Cry1A.105, Cry1Ab, and Cry1Ac with that of the experimental Cry1Aa X-ray crystal structure demonstrated high structure similarity between the four proteins (US EPA 2010).

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

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

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

Cry2Ab2:

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

ii) The produced Cry2Ab2 protein in maize event MON 89034 is a single polypeptide with similar sequence identity to the wild type with a peptide mass of 61 kDa.

The plant-produced protein sample had an additional immunoreactive band migrating at approximately 50 kDa; N-terminal amino acid analysis of this protein indicated that it is a truncated Cry2Ab2 protein with its N-terminus starting at amino acid 145 (MON 89034 dossier).

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

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

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

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

CP4 EPSPS

i) The source of the transgene is *Streptomyces viridochromogenes*. These bacteria have no history of causing allergy.

ii) 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)

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

iv) The microbially produced CP4 EPSPS protein was rapidly degraded in simulated gastric fluids *in vitro*. No degradation assay in gastrointestinal fluids has been performed by the applicant (Monsanto technical dossier).

v) CP4 EPSPS does not resemble any characteristics of known IgE-allergens, and no significant homologies between the amino acid sequences of the CP4 EPSPS protein and IgE-allergenic proteins have been found (Fard et al, 2013, Herouet et al, 2005, US EPA, 2007).

vi) The CP4 EPSPS protein is not glycosylated (Herouet et al, 2005, Raybould et al, 2013, US EPA, 2007)

vii) CP4 EPSPS is considered heat labile (Herouet et al, 2005, US EPA 2007)

The information listed above indicates that the newly expressed proteins in maize event MON 89034 x NK 603 lack IgE allergenic potential with regard to human and animal health. However, it does not cover possible allergic reactions (e.g. enteropathies) that are not IgE mediated.

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

Allergenicity of maize event MON 89034 x NK 603 could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, e.g. through qualitative or quantitative modifications of the expression of endogenous proteins. However, given that no biologically relevant agronomic or compositional changes have been identified in maize MON 89034 x NK 603 with the exception of the introduced traits, no increased allergenicity is anticipated for maize MON 89034 x NK 603. Moreover, maize is not considered a common allergenic food.

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

It is the opinion of the VKM GMO Panel that a possible over-expression of any endogenous protein, which is not known to be allergenic, in maize MON 89034 x NK 603 would be unlikely to alter the overall allergenicity of the whole plant or the allergy risk for consumers.

4.5.4 Adjuvanticity

According to the EFSA Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed (EFSA 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 (Guerrero et al. 2004; Vazquez-Padron et al., 1999a, b; 2000a, b; Moreno-Fierros et al., 2003). It is uncertain whether this applies to the same extent to other Cry proteins. A possible immunogenicity and adjuvanticity of Cry proteins has been considered by EFSA and VKM (VKM 2012c).

"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 2012c).

4.6 Nutritional assessment of GM food and feed

Compositional analyses of maize MON 89034 x NK 603 indicate nutritional equivalence to the non-GM control maize with a comparable genetic background (LH198 × LH172) as well as 15 other tested conventional maize varieties. The nutritional equivalence between MON 89034 x NK 603 maize and non-GM control maize has been further shown by the results of a poultry feeding study, described in 4.6.2.

4.6.1 Intake information/exposure assessment

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

Since most foods and foodstuffs from maize are derived from field maize grains, an estimated maximum daily intake for a Norwegian adult of Cry1A.105, Cry2Ab2, and CP4 EPSPS proteins from maize MON 89034 x NK 603 is calculated to be 18.9 µg, 6.6 µg, and 48.4 µg respectively, based on intake of maize staple (4.4 g/person/day) and the maximum protein levels in grain at physiological maturity, reported in Tables 4-6 (molecular characterisation, section 2.1.3.1). The corresponding numbers for children (6 month, intake of maize staple is 1.7 g/person/day) are 7.3 µg, 2.6 µg, and 18.7 µg, respectively.

The estimated maximum daily intake for a Norwegian adult of Cry1A.105, Cry2Ab2, and CP4 EPSPS proteins from sweet maize is calculated to be 64.8 µg, 22.8 µg, and 169.8 µg, respectively, based on a daily intake of 17.5 g fresh sweet maize/day (97.5 % percentile) and maximum fresh weight values in Tables 4-6. These levels are far below the levels shown to have no effect in laboratory toxicology testing. Also, these levels are considerably below the proposed threshold of toxicological concern (TTC) level of 1800 µg/person/day (Class 1, oral exposure) for chemicals considered to have a low potential for toxicity based on metabolism and mechanistic data (Vermeire et al., 2010). Transgenic proteins produced by genetically modified plants are generally considered non-toxic to humans.

The VKM GMO Panel notes that farm (production) animals e.g. pigs and poultry often are fed diets with a substantial inclusion of unprocessed maize grain, and that the exposure to transgenic proteins from maize MON 89034 x NK 603 may be higher for these animals.

This dietary exposure assessment is very conservative as it assumes that all maize consumed comes from maize MON 89034 x NK 603 and that the transgenic proteins are not denatured by processing.

4.6.2 Nutritional assessment of feed derived from the GM plant

The applicant has provided a 42-day broiler feeding study performed according to generally accepted guidelines (ILSI, 2003). The study was conducted to confirm the nutritional equivalence of maize MON 89034 x NK 603 with the non-transgenic control maize H1325023 (identical to LH198 x LH172). Six groups of chicken received other conventional non-transgenic maize varieties (Pioneer 32B33, Garst 8371, Midland 7B15, NK N72-J5, Nc + 5411, DKC61-50) (Davis 2006: Monsanto study number: 06-01-50-01. Taylor et al 2007). The non-transgenic maize H1325023 has a genetic background representative of MON 89034 x NK603, but is not genetically modified and does not express either the Cry1A.105, Cry2Ab2, CP4 EPSPS or CP4 EPSPS L214P proteins.

A total of 800 birds (960 at start of the study), commercial strain of Ross x Ross 308, were distributed into 80 pens at one day of age. Treatments were assigned to pens using a randomised complete block design. At start of the study (day one) each pen contained 12 broilers (6 males/6 females). Birds were identified by a wingband indicating animal number. Birds which were smaller than other birds, and/or showing signs of leg problems, crooked beak, swollen eyes or other abnormal conditions were removed first. If a pen had less than the required number of birds, then extra birds from another pen in the same treatment were relocated to bring the count in each pen to 10 birds. If additional birds still needed to be removed, they were selected arbitrarily (i.e. the first bird within reach). Removed birds were killed by cervical dislocation. All removed birds were weighed and recorded.The in-life portion of the study meets the US EPA Good Laboratory Practice (GLP) requirements for Title 21 CFR (Code of Federal Regulations) Part 58. Portions of the study conducted by Monsanto meet the US EPA GLP requirements for Title 40 CFR Part 160.

According to the OECD guidelines of animal feedstuffs derived from genetically modified plants (OECD 2003a) broiler chicks are useful for comparative growth studies. Because of their rapid weight gain, broiler chicks 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.

Samples of maize grain lots were analysed priot to the start of the study for mycotoxins, pesticide, and nutrient analyses. 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. However, analyses of transgenic DNA in the diets have not been documented in this broiler feeding study.

The test, control and reference substance diets mixtures were fed continuously for 42-days. Broilers were fed isocaloric starter feed on trial days 0-21 (56-58 % maize), and isocaloric grower/finisher feed on trial days 22-42 (59.5 %). Analyses of the starter and grower/finisher diets were conducted in compliance with US EPA Good Laboratory Practice standards (Title 40 CFR Part 160).

Pens were set up as a randomised complete block experimental design with 8 diets (treatments) in each of 5 replicated blocks of pens. Each block contained 16 pens (one for each diet and sex combination), with 10 birds/pen for a total of 800 birds (400 males and 400 females). Statistical Analysis System (SAS) version 8.2 were used in analysing each experiment.

Statistics were conducted on performance, carcass yield, and meat quality parameters: starting and final live weights, feed intake, feed conversion, adjusted feed conversion, chill weight, percent chill weight (chill weight/live weight), breast weight, percent breast weight (breast weight/chill weight), wing weight, percent wing weight (wing weight/chill weight), thigh weight, percent thigh weight (thigh weight/chill weight), drum weight, percent drum weight (drum weight/chill weight), fat pad weight, percent fat pad (fat pad/live weight), moisture, protein, and fat in breast and thigh meat. The statistical analysis was carried out using SAS®12, a linear mixed model procedure (SAS Institute, Cary, NC).

Each measurement was statistically analysed by two different procedures. The first method was a two-factor analysis of variance with 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 was significant ($p < 0.15$) then the diet comparisons were done, separately for each sex at a 5% level of significance.

The second analysis was performed to compare the response of broilers fed the MON 89034 × NK 603 diet with the control group and six reference groups. Analyses were averaged over sex unless there was a significant diet-by-sex interaction, in which case analyses were conducted by sex. Mean separation procedures were performed using the protected LSD (Least Significant Difference) method with $p<0.05$ as significance level in SAS.

Body weight, daily weight gain (gram/bird/day), feed conversion, and survival data were analysed to determine statistical differences between maize grain diets. The test facility, pens and birds were observed at least twice daily for general flock condition, lighting, water, feed, ventilation and unanticipated events. No statistically significant clinical findings of health were observed during the studied period. Consistent with historical data and study type, a low incidence of mortality occurred among all study groups. Mortality was recorded daily between trial days 0-42. The mortality rate was comparable after all treatments, being around 1% in the group receiving maize MON 89034 x NK603, which is a very low death rate for broiler chickens in feeding studies. The mortality was randomly distributed in treatment groups without any relationship to treatment. There were no statistical differences in mean percent mortality among any of the six treatments.

Pens were weighted by block. Birds were individually weighed immediately prior to slaughter for processing. Following a request from the EFSA GMO Panel the applicant has provided data on body weight gain for each sex separately.

The direct comparison of the test and control was made for each parameter using the Estimate statement in the SAS® PROC GLM procedure. There were 4 statistically significant differences ($p < 0.05$) between the test and control out of 58 tests.

The significant results were for female bird weight on day 0, female pen weight on day 0, average male wing weight on day 42 and average male thigh weight on day 42. Since bird weight Day 0 data was derived directly from Pen Weight Day 0 (divided by the same number of starting birds) and p-values for both variables are identical, there were 3 statistically significant differences out of 56 unique tests. In the original analysis across gender, only one measurement (Average Wing Weight) showed a significant treatment by gender interaction. These four measured differences were not considered biologically meaningful in the context of the many measures tested in the analysis.

Statistically, four significant differences out of 58 tests (6.9%), or three significant differences out of 56 unique tests (5.4%) are close to the expected false positive rate at the 5% significance level. All animals were analysed post-mortem for carcass characteristics, including the weights of the carcass and various carcass parts, as well as the composition of the meat of thighs and breast (fat, moisture, protein). Carcass measurements included chilled weight (kg and % of live weight) and weights of fat pad, breast, wing, drum and thigh parts (each expressed as kg and % of chilled weight). There were no differences ($P > 0.05$) between broilers fed diets containing MON 89034 × NK 603 or control maize. No differences ($P > 0.05$) among any of the diets were observed for the percentage of moisture, protein, or fat in thigh and breast meat samples collected at processing. Comparisons of the MON 89034 × NK 603 diet to the population of the control and six reference diets showed no difference ($P > 0.05$) in any performance, carcass, or meat quality parameter measured, except marginally increased wing and thigh weights in males (7 and 14g, respectively). These differences were not considered biologically meaningful.

The results of the study show that maize MON 89034 x NK 603 is nutritionally equivalent to its conventional counterpart and commercial non-GM maize varieties.

4.7 Conclusion

A whole food feeding study on broilers has not indicated any adverse health effects of maize MON 89034 x NK 603, and shows that it is nutritionally equivalent to conventional maize varieties. The Cry1A.105, Cry2Ab2, and CP4 EPSPS proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE mediated allergic reactions. However, some studies have indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize MON 89034 x NK 603 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1A.105, Cry2Ab2, and CP4 EPSPS proteins will cause toxic or IgE-mediated allergic reactions to food or feed based on maize MON 89034 x NK 603 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 2003b). 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 2003b), 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 2003b). The only recipient plants that can be cross-fertilised by maize are other cultivated maize cultivars.

It is considered very unlikely that the establishment, spread and survival of maize MON 89034 x NK 603 would be increased due to the insect resistance and herbicide tolerance traits. The herbicide tolerant trait can only be regarded as providing a selective advantage for the GM maize plant where and when glyphosate-based herbicides are applied. Similarly insect resistance against certain coleopteran and lepidopteran pests provides a potential advantage in cultivation of MON 89034 x NK 603 under infestation conditions. It is considered very unlikely that maize MON 89034 x NK 603 plants or their progeny will differ from conventional maize cultivars in their ability to survive as volunteers until subsequent seasons, or to establish feral populations under European environmental conditions.

Field trials carried out by the applicant do not indicate altered fitness of maize MON 89034 x NK 603 relative to its conventional counterpart. A series of field trials with maize MON 89034 x NK 603 were carried out by the applicant across five locations in Argentina in 2004/2005 and eight locations in Europe in 2007. Information on phenotypic (e.g. crop physiology, morphology, development) and agronomic characteristics was provided to assess the agronomic performance of maize MON 89034 x NK 603 in comparison with its conventional counterpart and commercial reference varieties (see section 3.4). Data from the field trials shows some statistical significant differences at individual field sites. These differences were however small in magnitude and were not consistently observed over locations. The VKM GMO Panel is of the opinion that the observed differences are not biologically relevant and do not raise any environmental safety concern.

In addition to the data presented by the applicant, the VKM GMO Panel is not aware of any scientific reports indicative of increased establishment or spread of maize MON 89034 x NK603, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of maize MON 89034 x NK 603 are unchanged, insect resistance and glyphosate tolerance are not likely to provide a selective advantage outside of cultivation in Europe. The VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects based on establishment and survival of maize MON 89034 x NK 603 will not differ from that of conventional maize varieties.

5.2 Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or vertical gene flow via pollen or seed dispersal. Exposure of microorganisms to transgenic DNA occurs during decomposition of plant material remaining in the field after harvest or comes from pollen deposited on cultivated areas or the field margins. Transgenic DNA is also a component of a variety of food and feed products derived from maize MON 89034 x NK603. 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 2003b). Vertical gene transfer in maize therefore depends on cross-pollination with other conventional or organic maize varieties. All maize varieties which are cultivated in Europe can interbreed. In addition, unintended admixture/adventitious presences of genetically modified material/transgenes in seeds represent a possible way for gene flow between different production systems.

5.2.1 Plant to micro-organisms gene transfer

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

Based on established scientific knowledge of the barriers for gene transfer between unrelated species and the experimental research on horizontal transfer of genetic material from plants to microorganisms, there is today little evidence pointing to a likelihood of random transfer of the transgenes present in maize MON 89034 x NK 603 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 genes from maize MON 89034 x NK603 will transfer and establish in the genome of microorganisms in the environment or in the intestinal tract of humans or animals. In the rare, but theoretically possible case of transfer of the *cry* and cp4 epsps genes from MON 89034 x NK 603 to soil bacteria, no novel property would be introduced into or expressed in the soil microbial communities; as these genes are already present in other bacteria in soil. Therefore, no positive selective advantage that would not have been conferred by natural gene transfer between bacteria is expected.

5.2.2 Plant to plant gene flow

Considering the intended uses of maize MON 89034 x NK 603 (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 2003b).

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

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

As maize MON 89034 x NK603 has no altered survival, multiplication or dissemination characteristics, the VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this GM maize in Norway will not differ from that of conventional maize varieties. The likelihood of cross-pollination between cultivated maize and the occasional feral maize plants resulting from grain spillage is considered extremely low.

5.3 Interactions between the GM plant and target organisms

Maize MON 89034 is a second generation genetically modified insect resistant maize, and was developed to provide protection against a variety of target pests of the order Lepidoptera. Protection is achieved through expression in the plant of two insecticidal Cry proteins, Cry1A.105 and Cry2Ab2, derived from *Bacillus thuringiensis*, a common soil bacterium. Cry1A.105, encoded by the *cry1A.105* gene, is a chimeric protein made up of different functional domains derived from three wild-type Cry proteins from *B. thuringiensis* subspecies *kurstaki* and *aizawai*. The Cry2Ab2 protein is encoded by the *cry2Ab2* gene derived from *B. thuringiensis* subspecies *kurstaki.*

Two Lepidoptera pests are primarily targeted by MON 89034; *Ostrinia nubilalis* (European corn borer, ECB) and *Sesamia nonagrioides* (Mediterranean corn borer, MCB). According to the applicant, the Cry1A.105 protein also provides increased activity against fall armyworm (*Spodoptera* spp.) and black cutworm (*Agrotis ipsilon*) compared to Cry1Ab. Further, the Cry2Ab2 toxin provides improved control over Cry1Ab products from damage caused by corn earworm (*Helicoverpa zea*).

The European corn borer is widely distributed in Europe covering the Iberian Peninsula, Czech Republic and Slovakia, southwest of France, northern Italy and the southern regions of Germany and Poland. The Mediterranean corn borer is present in the Mediterranean region (Andreadis 2011). There are ten reports of *O. nubilalis* in Norway, restricted to the counties of Vestfold, Telemark, Aust-Agder and Vest Agder. *Sesamia* spp., *Spodoptera frugiperda* or *H. zea* have not been reported in Norway. There are no reports of *O. nubilalis* attaining pest status in Norway, and the Plant Clinic (Planteklinikken) at Bioforsk has never received samples of this pest or plant material damaged by this pest (K. Ørstad pers. com.). Consequently, there are no insecticides authorised or previous applications for registrations of insecticides against this herbivore in Norway.

Aphids are the only pests reported on maize in Norway. Studies have shown that aphids are not affected by the Cry1Ab protein (Bourguet et al. 2002). Under the development of Bt maize expressing Cry1Ab, the noctuid *A. ipsilon* was tested as a target, but there was little or no effect (Pilcher et al. 1997). This species is occasionally a pest in root crops in Norway and it is conceivable that it could become a pest of maize.

Considering the intended uses of maize MON 89034 x NK603, excluding cultivation, the environmental exposure is limited to exposure through manure and faeces from the gastrointestinal tract mainly of animals fed on the GM maize as well as to the accidental release into the environment of GM seeds during transportation and processing and subsequently to potential occurrence of sporadic feral plants. Thus the level of exposure of target organisms to the Cry proteins is likely to be extremely low and of no ecological relevance.

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

Considering the intended uses of maize MON 89034 x NK603, excluding cultivation, the environmental risk assessment is concerned with accidental release of GM maize viable grains into the environment during transportation and processing, and exposure through manure and faeces from the gastrointestinal tracts of animals fed the GM maize.

Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only very low amounts would remain intact to pass out in faeces (e.g. Lutz et al. 2005; Guertler et al. 2008; Paul et al. 2010). There would subsequently, be further degradation of the Cry proteins in the manure and faeces due to microbial processes. In addition, there will be further degradation of Cry proteins in soil, reducing the possibility for the exposure of potentially sensitive non-target organisms. Although Cry proteins bind rapidly on clays and humic substances in the soil and thereby reducing their availability to microorganisms for degradation, there is little evidence for the accumulation of Cry proteins from GM plants in soil (Icoz & Stotzky 2008).

Data supplied by the applicant indicate that a limited amount of the Cry1A.105 and Cry2Ab2 and protein enters the environment due to the expression in the grains (mean values of 3.1 and 1.2 µg/g dwt, respectively). Data have been submitted that demonstrate that the Cry1A.105 and Cry2Ab2 and protein is rapidly degraded by gastric fluid *in vitro*.

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

5.5 Potential interactions with the abiotic environment and biochemical cycles

Considering the intended uses of maize MON 89034 x NK603, 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 Post-market environmental monitoring

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

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

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

preconceived hypothesis, in order to detect any possible effects that were not anticipated in the ERA, or that are long-term or cumulative.

No specific environmental impact of genetically modified maize MON 89034 x NK 603 was indicated by the environmental risk assessment and thus no case specific monitoring is required. The VKM GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicant is in line with the intended uses of maize MON 89034 x NK 603 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects.

5.7 Conclusion

Considering the intended uses of maize MON 89034 x NK603, excluding cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize MON 89034 x NK603.

Maize MON 89034 x NK 603 has no altered survival, multiplication or dissemination characteristics, and there are no indications of an increased likelihood of spread and establishment of feral maize plants in the case of accidental release into the environment of seeds from maize MON 89034 x NK603. Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation. The risk of gene flow from occasional feral GM maize plants to conventional maize varieties is negligible. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered to be an issue.

6 Conclusions

Molecular characterisation

Southern and PCR analyses indicate that the recombinant inserts in the single maize events MON 89034 and NK 603 are retained in maize stack MON 89034 x NK603. Genetic stability of the inserts has previously been demonstrated in the parental lines MON 89034 and NK603. The level of Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins in grain and forage from the stacked event are comparable to the levels in the corresponding single events. Phenotypic analyses also indicate stability of the insect resistance and herbicide tolerance traits of the stacked event. Based on current knowledge and the previous assessments of the parental maize events, the VKM GMO Panel considers the molecular characterisation of maize MON 89034 x NK 603 satisfactory.

Comparative assessment

The applicant has performed comparative analyses of data from field trials located at representative sites and environments in Argentina in 2004/2005 and Europe in 2007. With the exception of small intermittent variations and the insect resistance and herbicide tolerance conferred by the Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins, the results showed no biologically relevant differences between maize stack MON 89034 x NK 603 and conventional control. Based on the assessment of available data, the VKM GMO Panel concludes that maize MON 89034 x NK 603 is compositionally, agronomical and phenotypically equivalent to its conventional counterpart, except for the new proteins.

Food and feed safety assessment

A whole food feeding study on broilers has not indicated any adverse health effects of maize MON 89034 x NK 603, and shows that it is nutritionally equivalent to conventional maize varieties. The Cry1A.105, Cry2Ab2, and CP4 EPSPS proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE mediated allergic reactions. However, some studies have indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize MON 89034 x NK 603 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1A.105, Cry2Ab2, and CP4 EPSPS proteins will cause toxic or IgE-mediated allergic reactions to food or feed based on maize MON 89034 x NK 603 compared to conventional maize.

Environmental risk assessment

Considering the intended uses of maize MON 89034 x NK603, excluding cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize MON 89034 x NK603.

Maize MON 89034 x NK 603 has no altered survival, multiplication or dissemination characteristics, and there are no indications of an increased likelihood of spread and establishment of feral maize plants in the case of accidental release into the environment of seeds from maize MON 89034 x NK603. Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation. The VKM GMO Panel considers the risk of gene flow from occasional feral GM maize plants to conventional maize varieties to be negligible in Norway. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered by the GMO Panel to be an issue.

Overall conclusion

Based on current knowledge, the VKM GMO Panel concludes that maize MON 89034 x NK 603 is compositionally, nutritionally, agronomically and phenotypically equivalent to its conventional counterpart except for the new proteins. It is unlikely that the Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins will cause an increased risk of toxic or IgE-mediated allergic reactions to food or feed based on maize MON 89034 x NK 603 compared to conventional maize varieties.

The VKM GMO Panel concludes that maize MON 89034 x NK603, based on current knowledge, is comparable to conventional maize varieties concerning environmental risk in Norway with the intended usage.

7 Data gaps

Adjuvanticity

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

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

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

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

Herbicide residue levels

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

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

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

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Appendix

Table 1. Compositional analysis of MON 89034 x NK 603 compared to control and commercial varieties 2004/2005 Argentinean filed trials – all sites combined.

	MON 89034 × NK603		Control		Commercials[1]		
Tissue/Component (Units)[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Forage							
Fibre (% dw)							
ADF	27.10	22.59-31.20	25.72	20.27-30.52	[17.39,38.71]	18.3-41.0[b] ; 17.5-38.3[a]	16.13-47.39
NDF	39.03	31.09-47.67	36.33	29.19-46.82	[23.84,55.56]	26.4-54.5[b]; 27.9-54.8[a]	20.29-63.71
Proximates							
Ash (% dw)	5.51*	4.10-7.48	4.98	3.76-5.87	[2.22,8.69]	2.43-9.64[a]; 2-6.6[b]	1.527-9.638
Carbohydrates (% dw)	84.50	82.65-88.38	84.87	82.83-88.30	[79.06,89.42]	83.2-91.6[b]; 76.5-87.3[a]	76.4-92.1
Total fat (% dw)	1.98*	0.99-3.19	2.45	1.46-3.13	[0,5.13]	0.35-3.62[b] ; 1.42-4.57[a]	0.296-4.570
Moisture (% fw)	71.28	68.00-77.50	70.13	65.90-74.10	[56.88,84.19]	56.5-80.4[a] ; 55.3-75.3[b]	49.1-81.3
Protein (% dw)	8.00	5.07-9.15	7.70	4.32-8.70	[3.90,12.06]	4.98-11.56[a]	3.14-11.57
Minerals (% dw)							
Calcium	0.15	0.13-0.18	0.14	0.11-0.16	[0,0.32]	0.0969-0.3184[b]	0.0714-0.5768
Phosphorus	0.26	0.15-0.36	0.23	0.14-0.29	[0,0.56]	0.1367-0.2914[b]	0.0936-0.3704

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

1 Fifteen commercial maize hybrids.

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

3 The mean of 15 replicate values

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

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

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

Table 1. Cont.

Tissue/Component (Units)[2]	MON 89034 × NK603 Mean[3]	MON 89034 × NK603 Range	Control Mean[3]	Control Range	Commercials[1] 99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain							
Amino acids (% dw)							
Alanine	0.75	0.68-0.86	0.75	0.69-0.85	[0.54,1.06]	N/A	0.439-1.393
Arginine	0.45	0.37-0.49	0.46	0.39-0.49	[0.33,0.58]	N/A	0.119-0.639
Aspartic acid	0.66	0.60-0.72	0.67	0.61-0.71	[0.50,0.89]	N/A	0.335-1.208
Cystine	0.23	0.21-0.25	0.23	0.21-0.25	[0.18,0.28]	N/A	0.125-0.514
Glutamic acid	1.88	1.71-2.19	1.89	1.75-2.14	[1.34,2.73]	N/A	0.965-3.536
Glycine	0.38	0.35-0.39	0.38	0.35-0.40	[0.30,0.47]	N/A	0.184-0.539
Histidine	0.28	0.26-0.30	0.29	0.27-0.30	[0.20,0.40]	N/A	0.137-0.434
Isoleucine	0.34	0.31-0.38	0.35	0.32-0.38	[0.26,0.47]	N/A	0.179-0.692
Leucine	1.26	1.12-1.50	1.27	1.14-1.47	[0.85,1.89]	N/A	0.642-2.492
Lysine	0.30	0.27-0.32	0.31	0.29-0.34	[0.23,0.37]	N/A	0.172-0.668
Methionine	0.22	0.19-0.25	0.22	0.20-0.25	[0.13,0.28]	N/A	0.124-0.468
Phenylalanine	0.52	0.47-0.59	0.52	0.48-0.59	[0.38,0.73]	N/A	0.244-0.930
Proline	0.88	0.80-0.97	0.89	0.82-0.98	[0.66,1.26]	N/A	0.462-1.632
Serine	0.51	0.46-0.57	0.51	0.46-0.56	[0.36,0.72]	N/A	0.235-0.769
Threonine	0.35	0.32-0.39	0.36	0.32-0.39	[0.28,0.47]	N/A	0.224-0.666
Tryptophan	0.064	0.059-0.071	0.065	0.061-0.070	[0.050,0.075]	N/A	0.0271-0.215
Tyrosine	0.33	0.21-0.39	0.35	0.21-0.41	[0.23,0.48]	N/A	0.103-0.642
Valine	0.47	0.44-0.52	0.47	0.45-0.51	[0.37,0.63]	N/A	0.266-0.855

1 Fifteen commercial maize hybrids.

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

3 The mean of 15 replicate values

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

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

Table 1. Cont.

	MON 89034 × NK603		Control		Commercials[1]		
Tissue/Component[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain – continued							
Fatty acids (% of total fa)						(% total fat)	
16:0 palmitic acid	9.02	8.82-9.29	8.96	8.80-9.19	[7.54,13.55]	7-19[e]	7.94-20.71
16:1 palmitoleic acid	0.13	0.11-0.15	0.13	0.061-0.16	[0.0029,0.23]	1[e]	0.095-0.447
18:0 stearic acid	1.88*	1.81-1.97	1.79	1.73-1.87	[0.63,3.01]	1-3[e]	1.02-3.40
18:1 oleic acid	24.00	23.04-25.11	24.32	23.22-25.02	[8.77,43.80]	20-46[e]	17.4-40.2
18:2 linoleic acid	62.97	61.72-64.15	62.77	61.83-64.02	[41.30,77.09]	35-70[e]	36.2-66.5
18:3 linolenic acid	1.21	1.12-1.26	1.21	1.18-1.27	[0.63,1.66]	0.8-2[e]	0.57-2.25
20:0 arachidic acid	0.37	0.36-0.39	0.37	0.35-0.47	[0.15,0.66]	0.1-2[e]	0.279-0.965
20:1 eicosenoic acid	0.29	0.27-0.33	0.30	0.28-0.37	[0.14,0.48]	N/A	0.170-1.917
22:0 behenic acid	0.14	0.13-0.15	0.15	0.13-0.31	[0.059,0.30]	N/A	0.110-0.349
Fibre (% dw)							
ADF	5.12*	3.72-6.20	5.66	4.68-6.84	[2.74,9.13]	3.3-4.3[d]; 2.46-11.34[a,b]	1.82-11.34
NDF	10.01	8.85-11.24	10.85	9.22-12.34	[6.21,16.18]	8.3-11.9[d]; 7.58-15.91[b]	5.59-22.64
TDF	14.62	12.24-17.37	15.48	12.40-18.19	[7.95,25.13]	10.99-11.41[h]	8.82-35.31

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

1 Fifteen commercial maize hybrids.

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

3 The mean of 15 replicate values

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

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

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

Table 1. Cont.

	MON 89034 × NK603		Control		Commercials[1]		
Tissue/Component[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain – continued							
Minerals							
Calcium (% dw)	0.0061	0.0047-0.0072	0.0061	0.0051-0.0070	[0.0019,0.0079]	0.01-0.1[d]	0.00127-0.02084
Copper (mg/kg dw)	1.92	1.48-3.20	4.16	1.44-18.36	[0,8.23]	0.9-10[d]	0.73-18.50
Iron (mg/kg dw)	18.50	16.28-21.23	19.39	16.63-27.75	[12.48,29.03]	1-100[d]	10.42-49.07
Magnesium (% dw)	0.12	0.11-0.13	0.12	0.11-0.14	[0.078,0.18]	0.09-1[d]	0.0594-0.194
Manganese (mg/kg dw)	6.58	6.01-7.23	6.28	5.33-6.88	[2.33,11.13]	0.7-54[d]	1.69-14.30
Phosphorus (% dw)	0.34	0.28-0.39	0.34	0.28-0.40	[0.20,0.48]	0.26-0.75[d]	0.147-0.533
Potassium (% dw)	0.38	033-0.43	0.39	031-0.47	[0.21,0.58]	0.32-0.72[d]	0.181-0.603
Zinc (mg/kg dw)	20.28	16.59-25.11	20.80	16.86-23.64	[7.91,33.26]	12-30[d]	6.5-37.2
Proximates (% dw)							
Ash	1.43	1.17-1.69	1.41	1.20-1.61	[0.64,2.18]	1.1-3.9[d] ; 0.89-6.28[b]	0.616-6.282
Carbohydrates	85.31	84.37-86.12	85.42	84.53-86.22	[81.06,88.33]	77.4-87.2[b] ; 82.2-88.1[a]	77.4-89.5
Total fat	3.47*	3.11-3.72	3.22	2.96-3.40	[1.94,5.07]	3.1-5.7[d] ; 2.48-4.81[b]	1.742-5.823
Moisture	13.69	12.40-14.40	13.51	12.90-14.10	[11.40,15.35]	7-23[d] ; 8.18-26.2[b]	6.1-40.5
Protein	9.80	8.85-10.99	9.94	9.29-10.92	[7.56,13.21]	6-12[d]; 9.7-16.1[c]	6.15-17.26

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

1 Fifteen commercial maize hybrids.

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

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Table 1. Cont.

	MON 89034 × NK603		Control		Commercials[1]		
Tissue/Component[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain – continued							
Vitamin (mg/kg dw)							
Folic acid	0.63	0.27-0.91	0.56	0.27-0.92	[0,1.74]	0.3[d]	0.147-1.464
Niacin	33.88	28.39-39.63	34.18	27.13-39.47	[0,56.72]	9.3-70[d]	10.37-46.94
Vitamin B1	3.30	2.68-3.81	3.28	2.76-3.94	[1.92,5.79]	3-8.6[e]	1.26-40.00
Vitamin B2	1.79*	1.44-1.99	1.94	1.65-2.16	[1.19,3.11]	0.25-5.6[e]	0.50-2.36
Vitamin B6	5.97	4.93-7.86	6.55	5.02-10.83	[2.34,10.88]	5.3[d]; 9.6[e]	3.68-11.32
Antinutrient (% dw)							
Phytic acid	0.83	0.55-1.10	0.78	0.53-1.03	[0.39,1.12]	0.48-1.12[a]	0.111-1.570
Raffinose	0.066	0.029-0.075	0.062	0.029-0.076	[0,0.32]	0.08-0.30[e]	0.020-0.320
Secondary metabolite (µg/g dw)							
Ferulic acid	1882.07	1566.13-2146.17	1759.10	1471.61-2034.48	[552.46,3057.71]	113-1194[f]; 3000[g]	291.9-38885.8
p-coumaric acid	156.19	125.58-205.28	146.00	118.66-174.57	[0,326.22]	22-75[f]	53.4-576.2

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

1 Fifteen commercial maize hybrids.

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

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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. Summary of the statistical differences for the compositional comparison of MON 89034 x NK 603 to control maize – 2004-2005 Argentinian filed trials.

Tissue/Site/ Component (Units)[1]	Mean MON 89034 × NK603	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 89034 × NK603 (Range)	99% Tolerance Interval[2]
Statistical differences observed in combined site analyses						
Proximate						
Grain Total Fat (% DW)	3.47	3.22	7.58	0.022	(3.11-3.72)	[1.94,5.07]
Forage Ash (% DW)	5.51	4.98	10.74	0.019	(4.10-7.48)	[2.22,8.69]
Forage Total Fat (% DW)	1.98	2.45	-19.08	0.046	(0.99-3.19)	[0,5.13]
Fatty Acid						
Grain 18:0 Stearic (% total fa)	1.88	1.79	4.66	<0.001	(1.81-1.97)	[0.63,3.01]
Fiber						
Grain ADF (% DW)	5.12	5.66	-9.62	0.044	(3.72-6.20)	[2.74,9.13]
Vitamin						
Grain Vitamin B2 (mg/kg dw)	1.79	1.94	-7.53	0.011	(1.44-1.99)	[1.19,3.11]
Statistical differences observed in more than one site						
Fiber						
Site B1 Forage NDF (% DW)	41.07	33.28	23.41	0.008	(40.15-41.56)	[23.84,55.56]
Site CB Forage NDF (% DW)	43.49	35.17	23.66	0.025	(39.77-47.67)	[23.84,55.56]
Fatty Acid						
Site B2 Grain 18:0 Stearic (% Total FA)	1.89	1.82	3.79	0.049	(1.86-1.91)	[0.63,3.01]
Site B3 Grain 18:0 Stearic (% Total FA)	1.92	1.81	6.15	<0.001	(1.88-1.97)	[0.63,3.01]
Site SF Grain 18:0 Stearic (% Total FA)	1.85	1.75	5.97	0.003	(1.81-1.88)	[0.63,3.01]
Proximate						
Site CB Grain Total Fat (% DW)	3.53	3.21	9.72	0.010	(3.34-3.72)	[1.94,5.07]
Site SF Grain Total Fat (% DW)	3.58	3.02	18.36	0.016	(3.44-3.68)	[1.94,5.07]

[1]dw=dry weight; fw=fresh weight; fa=fatty acids. Combined site = analyses of the combined data from each of the five replicated field trials
[2] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Site codes: Site B1- Pergamino, Buenos Aires; Site B2-Tacuari, Buenos Aires; Site B3-Gahan, Buenos Aires; Site CB-Marcos Juarez, Córdoba; Site SF-Uranga, Santa Fe

Table 2. Cont.

Tissue/Site/ Component (Units)[1]	Mean MON 89034 × NK603	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 89034 × NK603 (Range)	99% Tolerance Interval[2]
Statistical differences observed in one site only						
Proximate						
Site B2 Grain Ash (% DW)	1.21	1.38	-12.05	0.024	[1.17 - 1.25]	[0.64, 2.18]
Site B2 Grain Moisture (% FW)	13.90	13.63	1.96	0.044	[13.80 - 14.00]	[11.40, 15.35]
Site B1 Forage Moisture (% dw)°	75.43	68.30	10.44	0.032	[74.10 - 77.50]	[56.88, 84.19]
Site B2 Forage Protein (% DW)	8.66	7.90	9.58	0.012	[8.16 - 9.15]	[3.90, 12.06]
Mineral						
Site B2 Grain Copper (mg/kg DW)	2.03	3.99	-49.01	0.038	[1.91 - 2.24]	[0, 8.23]
Site B2 Grain Phosphorus (% DW)	0.31	0.32	-3.45	0.047	[0.30 - 0.32]	[0.20, 0.48]
Site SF Grain Potassium (% DW)	0.39	0.44	-11.56	0.017	[0.37 - 0.40]	[0.21, 0.58]
Site CB Forage Calcium (% DW)	0.16	0.13	23.47	0.040	[0.14 - 0.18]	[0, 0.32]
Amino Acid						
Site SF Grain Threonine (% DW)	0.34	0.36	-6.06	0.045	[0.33 - 0.35]	[0.28, 0.47]

[1] dw=dry weight; fw=fresh weight; fa=fatty acids. Combined site = analyses of the combined data from each of the five replicated field trials

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

Site codes: Site B1- Pergamino, Buenos Aires; Site B2-Tacuari, Buenos Aires; Site B3-Gahan, Buenos Aires; Site CB-Marcos Juarez, Córdoba; Site SF-Uranga, Santa Fe

Table 2. Cont.

Tissue/Site/ Component (Units)[1]	Mean MON 89034 × NK603	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 89034 × NK603 (Range)	99% Tolerance Interval[2]
Statistical differences observed in one site only - continued						
Fatty acid						
Site B1 Grain 16:0 Palmitic (% Total FA)	8.84	9.06	-2.50	0.046	[8.82 - 8.86]	[7.54, 13.55]
Site B2 Grain 18:1 Oleic (% Total FA)	24.09	24.79	-2.85	0.001	[23.69 - 24.32]	[8.77, 43.80]
Site B2 Grain 18:2 Linoleic (% Total FA)	62.95	62.32	1.02	0.021	[62.60 - 63.40]	[41.30, 77.09]
Site SF Grain 20:0 Arachidic (% Total FA)	0.38	0.36	4.16	0.031	[0.37 - 0.38]	[0.15, 0.66]
Vitamin						
Site B1 Grain Vitamin B2 (mg/kg DW)	1.78	2.02	-12.06	0.028	[1.76 - 1.80]	[1.19, 3.11]
Secondary metabolite						
Site B1 Grain p-Coumaric Acid (µg/g DW)	157.31	132.04	19.14	0.018	[146.65 - 172.85]	[0, 326.22]

[1] dw=dry weight; fw=fresh weight; fa=fatty acids. Combined site = analyses of the combined data from each of the five replicated field trials
[2] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Site codes: Site B1- Pergamino, Buenos Aires; Site B2-Tacuari, Buenos Aires; Site B3-Gahan, Buenos Aires; Site CB-Marcos Juarez, Córdoba; Site SF-Uranga, Santa Fe