Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Food/feed and environmental risk assessment of herbicide-tolerant and insect-resistant genetically modified maize NK603 x MON810 for import, processing, food and feed uses under Regulation (EC) No 1829/2003 (EFSA/GMO/UK/2004/01)

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

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

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Summary

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Scientific Committee for Food Safety (VKM) has been requested by the Norwegian Environment Agency (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 herbicide-tolerant and insect-resistant genetically modified maize NK603 x MON810 (Unique Identifier MON-ØØ6Ø3-6 x MON-ØØ81Ø2-6) from Monsanto Company is approved under Regulation (EC) No 1829/2003 for food and feed uses, import and processing since 24 October 2007 (Commission Decision 2007/701/EC).

VKM participated in the 90 days public consultation of the application for placing on the market of maize NK603 x MON810 for food and feed uses, import and processing (EFSA/GMO/ UK/2004/01) in 2005, and submitted a preliminary opinion in June 2005 (VKM 2005a). Maize NK603 x MON810 has also been assessed as food and feed by the VKM GMO Panel, commissioned by the Norwegian Environment Agency and the Norwegian Food Safety Authority in connection with the national finalisation of the application in 2008 (VKM 2008a). In addition, NK603 and MON810 has been evaluated by the VKM GMO Panel as single events and as a component of several stacked GM maize events (VKM 2005 a,b,c,d,e, VKM 2007 a,b,c,d,e, 2008b, 2009, 2010 a,b, 2011, 2012 a,b, 2013a,b,c,d,e,f,g,h,i,j). Due to the publication of new scientific literature and updated guidelines for risk assessment of genetically modified plants, the VKM GMO Panel has decided to deliver an updated food/feed and environmental risk assessment of maize NK603 x MON810.

The food/feed and environmental risk assessment of the maize NK603 x MON810 is based on information provided by the applicant in the applications EFSA/GMO/UK/2004/01 and EFSA/GMO/2005/26, 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 NK603 x MON810 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 NK603 x MON810 include molecular characterisation of the inserted DNA and expression of novel proteins, comparative assessment of agronomic and phenotypic characteristics, nutritional assessments, toxicology and allergenicity, unintended effects on plant fitness, potential for gene transfer, interactions between the GM plant and target and non-target organisms and effects on biogeochemical processes.

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.

Molecular characterisation

The stacked maize NK603 x MON810 was produced by conventional crossing of the single maize events NK603 and MON810. Southern blot and PCR analyses have shown that the recombinant inserts from the parental events are retained in the stacked event, and that their structures are intact. Genotypic stability of the inserts has previously been demonstrated for the single events. Protein measurements show comparable levels of CP4 EPSPS and Cry1Ab in forage and grain samples from maize NK603 x MON810 to those measured in maize NK603 and MON810, respectively.

The VKM GMO Panel considers the molecular characterisation of maize NK603 x MON810 and its parental events NK603 and MON810 satisfactory.

Comparative assessment

The applicant has performed comparative analyses of data from field trials located in maize growing regions of Europe and USA in 2000 and 2002. With the exception of small intermittent variations and the insect resistance and herbicide tolerance conferred by the Cry1Ab and CP4 EPSPS proteins, the results showed no biologically significant differences between maize stack NK603 x MON810 and conventional control. Based on the assessment of available data, the VKM GMO Panel concludes that maize NK603 x MON810 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, except for for the introduced characteristics, and that its composition fell within the normal ranges of variation observed among non-GM varieties.

Food and feed risk assessment

A whole food feeding study performed on broilers did not indicate any adverse health effects of maize NK603 x MON810, and shows that maize NK603 x MON810 is nutritionally equivalent to conventional maize. The CP4 EPSPS or Cry1Ab proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize NK603 x MON810 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1Ab or CP4-EPSPS proteins will introduce a toxic or allergenic potential in food or feed based on maize NK603 x MON810 compared to conventional maize.

Environmental risk assessment

Considering the intended uses of maize NK603 x MON810, 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 NK603 x MON810.

Maize NK603 x MON810 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 NK603 x MON810. 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 NK603 x MON810 is nutritionally equivalent to conventional maize varieties. It is unlikely that the CP4 EPSPS and Cry1Ab proteins will introduce a toxic or allergenic potential in food or feed based on maize NK603 x MON810 compared to conventional maize.

The VKM GMO Panel likewise concludes that maize NK603 x MON810, based on current knowledge, is comparable to conventional maize varieties concerning environmental risk in Norway with the intended usage.

Keywords

Maize, *Zea mays* L., genetically modified maize NK603 x MON810, EFSA/GMO/UK/2004/01, insect-resistance, herbicide-tolerance, Cry protein, *cry1Ab*, Cry1Ab, *cp4 epsps*, CP4 EPSPS, glyphosate, food and feed risk assessment, environmental risk assessment, Regulation (EC) No 1829/2003

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 genmodifiserte maishybriden NK603 x MON810 (Unik kode MON-ØØ6Ø3-6 x MON-ØØ81Ø2-6) fra Monsanto Company ble godkjent til import, videreforedling og bruk som mat og fôr under EU-forordning 1829/2003 24. oktober 2007 (Kommisjonsbeslutning 2007/701/EC).

Maishybrid NK603 x MON810 ble første gang vurdert av VKMs faggruppe for genmodifiserte organismer i 2005 (VKM 2005a). Den foreløpige risikovurderingen ble utført på oppdrag fra Mattilsynet i forbindelse med EFSAs høring av søknad EFSA/GMO/UK/2004/01, og inkluderte vurderinger av potensielle helseeffekter ved bruk av NK603 x MON810 som næringsmiddel og fôrvare. I forbindelse med vurdering av markedsadgang i Norge, utarbeidet VKM en endelig helse- og miljørisikovurdering av mais NK603 x MON810 i 2008 på oppdrag fra Mattilsynet og Miljødirektoratet (VKM 2008a). Foreldrelinjene NK603 og MON810 er også tidligere risikovurdert av VKM, både som enkelteventer og i en rekke andre hybrider (VKM 2005 a,b,c,d,e, VKM 2007 a,b,c,d,e, 2008b, 2009, 2010 a,b, 2011, 2012 a,b, 2013a,b,c,d,e,f,g,h,i,j). Etablering av nye, reviderte retningslinjer for helse- og miljørisikovurderinger av genmodifiserte planter og publisering av ny vitenskapelig litteratur har medført at VKM har valgt å utarbeide en ny, oppdatert helse- og miljørisikovurdering av mais NK603 x MON810.

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 (EFSA 2010a, 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, samt effekter på målorganismer, ikke-målorganismer og biogeokjemiske prosesser 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.

Mais NK603 x MON810 er fremkommet ved konvensjonelle kryssinger av de to genmodifiserte maislinjene NK603 x MON810.

Foreldrelinje NK603 uttrykker CP4-EPSPS-proteiner, som et resultat av introduksjon av *cp4-epsps*-genet fra jordbakterien *Agrobacterium tumefaciens*. Genet koder for enzymet 5-enolpyruvylsikimat-3-fosfatsyntetase, som omdanner fosfoenolpyruvat og sikimat-3-fosfat til 5-enolpyruvylsikimat-3-fosfat, en viktig metabolitt i syntesen av aromatiske aminosyrer. I motsetning til plantens enzym er det bakterielle enzymet også aktivt ved nærvær av N-fosfonometylglycin (glyfosat). De transgene plantene vil derfor tolerere høyere doser av herbicider med virkestoff glyfosat sammenlignet med konkurrerende ugras.

Foreldrelinje MON810 inneholder genet *cry1Ab* fra jordbakterien *Bacillus thuringiensis* ssp. *kurstaki* HD-1. Genet koder for et δ-endotoksin som gir resistens mot enkelte skadeinsekter i ordenen Lepidoptera, eksempelvis maispyralide (*Ostrinia nubilalis*), og enkelte arter i slekten *Sesamia*.

Molekylær karakterisering
Maishybriden NK603 x MON810 ble produsert ved konvensjonell krysning av de genmodifiserte maislinjene NK603 og MON810. Southern blot og PCR -analyser viser at de rekombinante innskuddene fra foreldrelinjene er bevart i hybriden, og at innskuddene er intakte. Genotypisk stabilitet av innskuddene har tidligere blitt vist for foreldrelinjene. Proteinmålinger har vist at det er sammenlignbare nivåer av CP4 EPSPS og Cry1Ab i prøver av vegetativt vev og korn fra NK603 x MON810, med nivåer funnet i tilsvarende prøver fra henholdsvis mais NK603 og MON810.

VKMs faggruppe for GMO anser den molekylære karakteriseringen av mais NK603 x MON810 som tilfredsstillende.

Komparative analyser
Søker har utført komparative analyser av mais NK603 x MON810 og tilhørende umodifisert kontroll («konvensjonell motpart») basert på feltforsøk i representative områder for maisdyrking Europa og USA i 2000 og 2002. Med unntak av enkelte små variasjoner viste studiene ingen biologisk relevante forskjeller mellom den genmodifiserte maishybriden NK603 x MON810 og umodifisert konvensjonell kontroll. Basert på en vurdering av tilgjengelige data, konkluderer VKMs faggruppe for GMO at mais NK603 x MON810 er ernæringsmessig, agronomisk og fenotypisk vesentlig lik dens konvensjonelle motpart, med unntak av de introduserte egenskapene. Variasjonsområdene for de undersøkte parameterne ligger innenfor det normale variasjonsområdet til konvensjonelle maissorter.

Helserisiko
I en fôringsstudie utført på broilere ble det vist at mais NK603 x MON810 ikke førte til negative helseeffekter blant dyrene, og at maisen var ernæringsmessig vesentlig lik konvensjonell mais. Proteinene Cry1Ab og CP4 EPSPS viser ingen likhetstrekk til andre kjente toksiner eller allergener, og er heller ikke rapporterte å ha forårsaket IgE-medierte allergiske reaksjoner. Enkelte studier har derimot indikert at noen typer Cry-proteiner kan forsterke andre allergiske reaksjoner, dvs. fungere som adjuvans. Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at mais NK603 x MON810 er ernæringsmessig vesentlig lik konvensjonell mais, og at det er lite trolig at proteinene Cry1Ab og CP4 EPSPS vil introdusere et toksisk eller allergent potensiale i mat eller fôr basert på mais NK603 x MON810 sammenliknet med konvensjonelle maissorter.

Miljørisiko
Med bakgrunn i tiltenkt bruksområde for søknaden 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 maishybriden NK603 x MON810 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 NK603 x MON810 er ernæringsmessig ekvivalent konvensjonell mais. Det er lite sannsynlig at proteinene Cry1Ab og CP4 EPSPS vil introdusere et toksisk eller allergent potensiale i mat eller fôr basert på mais NK603 x MON810 sammenliknet med konvensjonelle maissorter.

Likeledes, finner faggruppen at maishybrid NK603 x MON810, ut fra dagens kunnskap og omsøkt bruk, er sammenlignbar med 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
AMPA	Aminomethylphosphonic acid, one of the primary degradation products of glyphosate
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).
Cry	Any of several proteins that comprise the crystal found in spores of *Bacillus thuringiensis*. Activated by enzymes in the insects midgut, these proteins attack the cells lining the gut, and subsequently kill the insect.
Cry1Ab	Protein from *Bacillus thuringiensis* subsp. *Kurstaki.* Provide protection against certain lepidopteran target pests.
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*
mEPSPS	Modified 5-enolpyruvylshikimate-3-phosphate synthase
mRNA	Messenger RNA
MT	Norwegian Food Safety Authority (Mattilsynet)
NDF	Neutral detergent fibre, measure of fibre used for animal feed analysis. NDF measures most of the structural components in plant cells (i.e. lignin,

	hemicellulose and cellulose), but not pectin.
Northern blot	Northern blot is a technique used to study gene expression by detection of RNA or mRNA separated in a gel according to size.
NTO	Non-target organism
Nicosulfuron	Herbicide for maize that inhibits the activity of acetolactate synthase
Near-isogenic lines	Term used in genetics/plant breeding, and defined genetic lines that are identical except for differences at a few specific locations or genetic loci.
OECD	Organisation for Economic Co-operation and Development
ORF	Open Reading Frame, in molecular genetics defined as a reading frame that can code for amino acids between two stop codons (without stop codons).
OSL	Over season leaf
OSR	Over season root
OSWP	Over season whole plant
pat	*Phosphinothricin-Acetyl-Transferase* gene
PCR	Polymerase chain reaction, a technique to amplify DNA by copying it
PMI	Phosphomannose Isomerase enzyme. Metabolizes mannose and allows positive selection for recovery of transformed plants.
R0	First transformed generation, parent
Rimsulferon	Herbicide, inhibits acetolactate synthase
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.
ZM-HRA	A modified version of the native acetolactate synthase protein from maize. Confers tolerance to the ALS-inhibiting class of herbicides

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Background

On 10 June 2004, the European Food Safety Authority (EFSA) received from the Competent Authority of the United Kingdom an application (Reference EFSA/GMO/UK/2004/01) for authorisation of the herbicide-tolerant and insect-resistant genetically modified (GM) maize NK603 x MON810 (Unique Identifier MON-ØØ6Ø3-6 x MON-ØØ81Ø2-6), submitted by Monsanto Company 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/UK/2005/01 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 17 June 2005, 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 hearing, and submitted a preliminary opinion in June 2005 (VKM 2005). Maize NK603 and MON810 has also been evaluated by the VKM GMO Panel as single events and as a component of several stacked GM maize events (VKM 2005a,b, 2007a,b,c,d, 2008a,b, 2013a). EFSA published its scientific opinion 13 October 2005 (EFSA 2005), and maize stack NK603 x MON810 was approved for food, feed, import and processing as any other maize in 24 October 2007 (Commission Decision 2007/701/EC).

An application for authorisation of maize NK603 x MON810 for cultivation in the EU was submitted by Monsanto in November 2005 (EFSA/GMO/NL/2005/26). EFSA declared the application as valid in January 2007, and held a public hearing the first quarter of 2007. The application was, however, withdrawn in August 2013. According to the notifier, the withdrawal was the result of a commercial decision to discontinue all EU investments in the development of agricultural biotechnology products.

Exemption of the authorisation requirements of 19 existing products in Norway

Through the Agreement of the European Economic Area (EEA), Norway is obliged to implement the EU regulations on GM food and feed (regulations 1829/2003, 1830/2003 et al). Until implementation of these regulations, Norway has a national legislation concerning processed GM food and feed products that are harmonised with the EU legislation. These national regulations entered into force 15 September 2005. For genetically modified feed and some categories of genetically modified food, no requirements of authorisation were required before this date. Such products that were lawfully placed on the Norwegian marked before the GM regulations entered into force, the so-called existing products, could be sold in a transitional period of three years when specific notifications were sent to the Norwegian Food Safety Authority. Within three years after 15. September 2005, applications for authorisation should be sent to the Authority before further marketing. Four fish feed producing companies have once a year since 2008, applied for an exemption of the authorisation requirements of 19 existing products, including maize NK603 and MON810. These 19 GM events are all authorised in the EU, and the Norwegian Food Safety Authority has granted exemption for a period of one year each time.

http://www.mattilsynet.no/planter_og_dyrking/genmodifisering/fire_virksomheter_har_faatt_dispensasjon_fra_kravet_om_godkjenning_av_genmodifisert_fiskefor.10951

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

Maize NK603 x MON810 has been obtained from traditional breeding methods between progeny (inbred lines) of the genetically modified maize lines NK603 and MON810.

The parental line NK603 was developed to provide tolerance to the broad spectrum herbicide glyphosate, the active ingredient in the proprietary product with the commercial name Roundup. Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an essential enzyme involved in aromatic amino acid synthesis in plants. Blocking the enzyme results in the breakdown of the synthesis of aromatic amino acids, ultimately leading to the death of the plant. In glyphosate-tolerant maize NK603, the herbicide tolerance trait is generated in the plants through the addition of a bacterial epsps gene derived from a common soil bacterium, *Agrobacterium* sp. strain CP4 (CP4 EPSPS). The enzyme produced from the CP4 EPSPS gene has a lower affinity for the herbicide compared with the maize enzyme, and thus confers glyphosate-tolerance to the whole plant.

The parental line MON810 was developed to provide protection against certain lepidopteran insect larvae, including European corn borer (*Ostrinia nubilalis*) and species belonging to the genus *Sesamia*. None of these target pests are present in the Norwegian agriculture. Insect protection is achieved through expression in the plant of the insecticidal Cry protein Cry1Ab, derived from *Bacillus thuringiensis* ssp. *kurstaki*, a common soil bacterium.

The genetic modification in maize NK603 x MON810 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 NK603 x MON810 (Unique Identifier MON-ØØ6Ø3-6 x MON-ØØ81Ø2-6) 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).

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 NK603 x MON810

The stacked maize NK603 x MON810 was developed through conventional breeding by crossing the single maize events NK603 and MON810. Maize NK603 x MON810 combines the glyphosate tolerance of maize NK603 with the insect resistance of MON810, conferred through the expression of the *cp4 epsps* and *cry1Ab* genes, respectively.

2.1.2 Summary of evaluation of the single events

2.1.2.1 Maize NK603

Maize event NK603 produces the glyphosate tolerant protein 5-enoylpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* sp. strain CP4 (CP4 EPSPS), an enzyme that protects the plant from broad spectrum herbicides such as Roundup.

Particle acceleration technology was used to develop NK603 by the introduction of two genes encoding the CP4 EPSPS protein to immature maize embryos from a proprietary maize line called AW x CW. The bacterial plasmid vector PV-ZMGT32 was used as source of the transgenes. Conventional breeding methods were used to backcross plants generated from the initial transformation into the final recurrent event.

PV-ZMGT32 contains two adjacent plant gene expression cassettes, each containing a single copy of the *cp4 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 subcellular 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 (PV-ZMGT32L) of the PV-ZMGT32 plasmid, which only contains the *cp*4 *epsps* plant gene expression cassettes, was used in the transformation of the maize embryos. The *npt*II gene as well as the *ori* is not present in the fragment PV-ZMGT32L.

The EPSPS enzyme catalyses 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 prevents the in-season use of this herbicide on the crop. In maize NK603 the glyphosate-tolerant CP4 EPSPS enzyme ensures continued function of the aromatic amino acid pathway even in the presence of Roundup.

The levels of CP4 EPSPS and CP4 EPSPS L214P proteins in various tissues of NK603, produced during the 1999 growing season in the EU and the 2002 growing season in the USA, were estimated with an enzyme-linked immunosorbent assay (ELISA). The expression of the *cp4 epsps* genes occurs

throughout the plant since the rice actin and CaMV e35S promoters have been shown to drive constitutive expression in genetically modified maize. 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, over season leaf (OSL) and over season root (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 were 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 NK603 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 NK603 forage across all four sites was 48.6 µg/g fw. In maize NK603 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, calculated mean dry weight (dw) value was reported as 12 µg/g. These values represent the sum of both CP4 EPSPS and CP4 EPSPS L214P, as the ELISA analytical method does not differentiate between the two proteins.

In 2002, test and control samples were produced in USA. field trials. CP4 EPSPS protein levels in the different tissue types were estimated with a validated 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 levels for forage and grain were in 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 USA. In these trials the CP4 EPSPS 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. PCR analysis and subsequent DNA sequencing of four overlapping products spanning the length of the insert in NK603 were performed to characterise the inserted DNA in NK603 (Kesterson et al., 2002a). Genomic DNA from the NK603 maize and control (B73) were digested with the restriction enzyme *StuI*. The result suggested that NK603 contains one integrated insertion of DNA located within a 23 kb *StuI* restriction fragment. The genome of NK603 does not contain any detectable plasmid backbone DNA or the *ori* and *nptII* coding sequences. PCR amplification and DNA sequencing was used for characterisation 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.

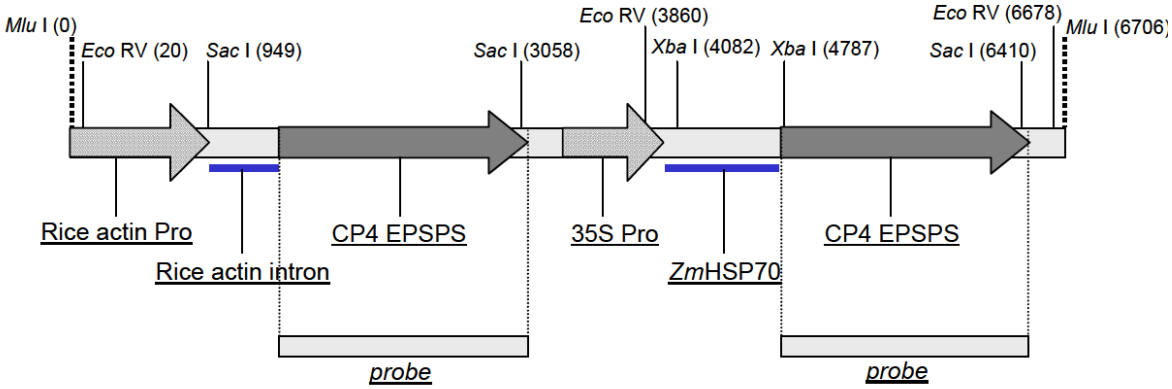

Figure 1. Restriction map of the various gene elements of the recombinant DNA fragment inserted in the genome of the maize strain NK603.

2.1.2.2 Maize MON810

MON810 produces the Cry1Ab insecticidal protein that protects the plant from feeding damage caused by certain lepidopteran insect pests, e.g. the European corn borer (ECB, *Ostrinia nubilalis*) and the Mediterranean Corn borer (MCB, *Sesamia nonagrioides*).

Maize event MON810 was generated by particle acceleration technology with the plasmids PV-ZMBK07 and PV-ZMGT10.

Plasmid PV-ZMBK07 contained the *CaMV35S* promoter with a duplicated enhancer region (*e35S*); an intron from the maize *Hsp70* (heat-shock protein) gene; the *cry1Ab* gene; *nos 3'* - a 3' non-translated region of the nopaline synthase gene (transcriptional termination; polyadenylation); a *lac* operon fragment (a partial *Escherichia coli lacI* coding sequence, the promoter *lac* and a partial coding sequence for β-D-galactosidase or *lacZ* protein from pUC119); *ori-pUC* (replication origin for pUC plasmids, originally derived from plasmid ColE1); and the *npt*II gene as a selectable marker.

Plasmid PV-ZMGT10 contained the *e35S* promoter; the *Hsp70* intron; transit peptides *CPT1* and *CPT2* (from *Arabidopsis thaliana*); the *CP4 epsps* gene (from *Agrobacterium* sp.) which allows for selection on glyphosate; and the *gox* gene (from *Ochrobactrum anthropi* sp.) which encodes a glyphosate metabolising enzyme, the *nos 3'* terminator, the *lacZ* region, *ori-pUC* and the *npt*II gene.

The molecular characterisation showed that the resulting maize event MON810 contained a single insertion that consisted of elements derived only from plasmid PV-ZMBK07, and that no portion of plasmid PV-ZMGT10 was present in the maize. These elements included the enhanced *e35S* promoter, the maize *Hsp70* intron, and the *cry1Ab* coding sequence.

Additional experiments (sequence data and PCR) showed that the *e35S* promoter, that regulates the expression of the *cry1Ab* gene, had been modified into a shorter version called $e35S^{MON810}$ (307 bp at the 3' end of the 620 bp promoter), that the *Hsp*70 intron was intact, and that only 2448 bp of the *cry1Ab* coding sequence (corresponding to the 5' end of the 3470 bp gene) encompassing the insecticidal active tryptic core was present. Parts of the 3' ends of the *cry1Ab* gene and the *nos* terminator had been deleted in the integration process.

The PCR was performed with a forward primer specific to the genomic DNA sequence flanking the 5' end of the insert paired with a reverse primer specific to the genomic DNA sequence flanking the 3' end of the insert (Figure 1). DNA sequence analyses performed on MON810 (and the conventional counterpart) determined the DNA sequence of the insert in MON810, the organisation of the genetic elements within the insert, flanking sequences, and examined the MON810 insertion site.

Additional sequence information has been submitted by the applicant that confirmed the DNA sequences of the 5' and 3' DNA flanking regions originally provided, as well as identifying an additional 400 bp of maize genomic DNA at the 3' flank and 1000 bp at the 5' flank.

Chi square analysis of segregation patterns showed that the insert in maize MON810 was inherited by subsequent generations in a Mendelian fashion consistent with a single site of insertion in the maize nuclear DNA.

Analysis of open reading frames (ORFs) indicated no new potential chimeric proteins showing homologies with potential toxins or allergens, confirming the original bioinformatic assessment. *In silico* analysis did reveal that the 3' genomic region corresponded to a gene putatively coding for the HECT-ubiquitin ligase protein.

A publication by Rosati et al. (2008) confirmed that the 3' genomic region corresponded to a gene putatively coding for the HECT E3 ubiquitin ligase. In addition, with RT-PCR they showed that this 3' region produced cDNA variants of different length. *In silico* translation of these transcripts identified 2 and 18 putative additional amino acids in different variants, all derived from the adjacent host genomic sequences, added to the truncated Cry1Ab protein. These putative recombinant proteins did not show homology with any known protein. Results of this analysis show that it is unlikely that endogenous ORFs that encode protein sequences have been disrupted by the insertion of T-DNA in MON810.

Tissues of MON810 plants were analysed for the three proteins, Cry1Ab, CP4 EPSPS, and GOX with ELISA. Tissue samples used in the analyses were collected during six field trials in USA in 1994, and five field trials conducted within the major maize growing regions of France and Italy in 1995. Additional field trials were also conducted at two sites in Italy and France in 1995 to produce leaf, forage and grain samples for expression analysis of MON810 hybrids. Nguyen & Jehle (2007) conducted a quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in maize MON810 plants (cultivar "Novelis") from two field trials in Germany.

The CP4 EPSPS and GOX proteins were not detected in any of the plant tissues of maize MON810. This was expected since the molecular analysis of maize MON810 established that the *cp4 epsps* and *gox* genes were not present in the nuclear genomic DNA.

In the American field trials, the level of Cry1Ab protein ranged from 7.93-10.34 µg/g fresh weight (fw) in young leaf tissue; 3.65-4.65 µg/g fw in whole plant tissue; and 0.19-0.39 µg/g fw in harvested grain (Table 2). The foliar expression of Cry1Ab protein remained high during the vegetative growth stages of the maize plant as measured in overseason leaf samples.

In the European field trials in 1994 and 1995, the level of Cry1Ab protein ranged from 7.59-9.39 µg/g fw in young leaf tissue; 4.21-9.23 µg/g fw in forage tissue; and 0.42-0.69 µg/g fw in harvested grain (Table 3). The 1995 analysis confirmed that CP4 EPSPS and GOX proteins were not present in plant tissues of maize MON810. With regard to Cry1Ab, the protein levels were similar for plants grown in the American and European field trials over two consecutive generations. The level of Cry1Ab protein in progeny of MON810 ranged from 8.20-10.51 µg/g fwt in young leaf tissue, 4.00-5.11 µg/g fwt in forage tissue, and 0.35-0.60 µg/g fwt in harvested grain. The Cry1Ab protein levels were similar for MON810 plants derived from backcrosses to B73/Mo17 and commercial hybrids.

During European field trials in 2001-2003, the highest Cry1Ab levels were detected in leaves (5.5-6.4 µg/g fw), whereas the lowest Cry1Ab contents were detected in pollen (1-97 ng/g fw). Cry1Ab content of residual root stocks collected in the field nine months after harvest was 15-17 ng/g fw, equivalent to about one-hundredth of the fresh root. This large-scale monitoring of Cry1Ab elevels in maize MON810 showed a considerable variation in the levels of Cry1Ab between genotypes, plant tissues and growth stages

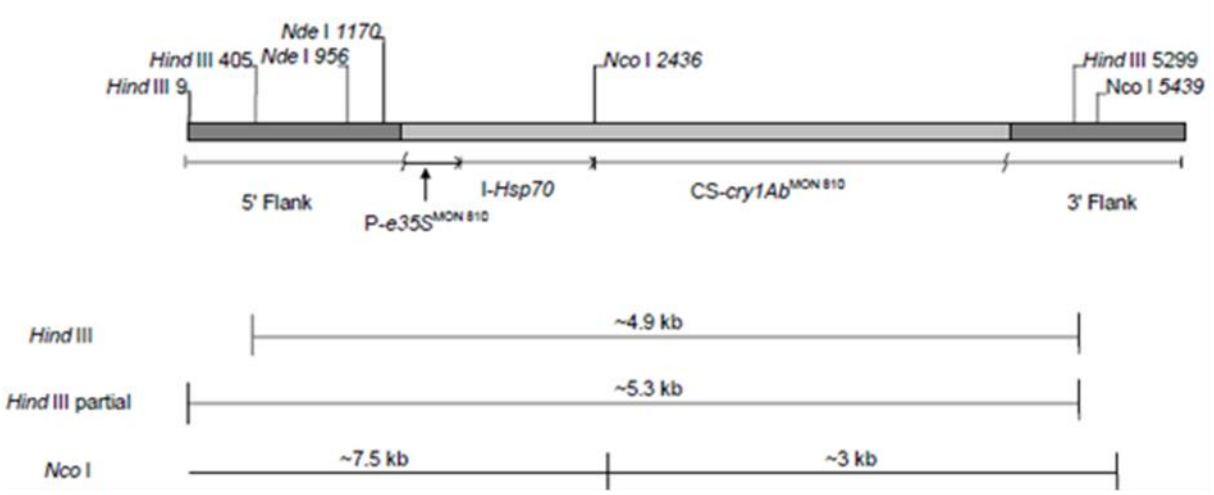

Figure 1. Schematic representation of the insert and flanking DNA in MON810.

2.1.3 Transgene constructs in NK603 x MON810 maize

Maize NK603 x MON810 was obtained by conventional crossing between the two genetically modified maize events NK603 and MON810. No new genetic modification was used in the development of NK603 x MON810 maize.

A detailed molecular analysis was conducted to investigate the copy number, structure and organisation of the inserts found in NK603 x MON810 maize.

To test for the presence of the NK603 insert, test and control DNA samples were digested with the restriction enzyme *Eco* RV. The restriction endonuclease *Eco* RV cleaves the plasmid PV-ZMGT32 into two restriction fragments of 3.8 kb and 2.8 kb that would be detected by the *ctp2-cp4 epsps* probe. No hybridisation was observed in either the negative control or MON810.

Plasmid PV-ZMGT32 produced the 3.8 kb and 2.8 kb bands that result from hybridisation of the *ctp2-cp4 epsps* probe to the linearised plasmid restriction fragments. Both NK603 DNA and NK603 x MON810 DNA produced the 3.8 kb and 2.8 kb banding patterns expected from the NK603 insert. Observation of these bands with this restriction enzyme and probe combination is consistent with results previously reported for the single event NK603.

To test for the presence of the insert from MON810, test and control DNA samples were digested with restriction enzymes *Nco*I/*Eco*RI. These enzymes cleave the plasmid PV-ZMBK07 into one restriction

fragment of 3.5 kb that would be detected by a probe composed of ~900 bp of the *cry1Ab* coding region. No hybridisation was observed in either the negative control or NK603.

Plasmid PV-ZMBK07 produced the expected 3.5 kb band that results from hybridisation of the *cry1Ab* probe to the linearised plasmid restriction fragment. Both MON810 DNA and NK603 x MON810 DNA produced the 3.1 kb banding pattern expected for the MON810 insert. Observation of this band with these restriction enzymes and probe combination is consistent with results previously reported for the single event MON810.

The results obtained from the Southern Blot analyses indicate molecular equivalence, and identical copy number of the inserts present in NK603 x MON810 maize to those present in NK603 and MON810 maize.

2.1.3.1 Information on the expression of insert

A study was conducted to estimate the levels of CP4 EPSPS (incl. CP4 EPSPS L214P) proteins and Cry1Ab protein present in maize tissues collected from NK603 x MON810 grown in French field trials at three sites in 2000. The levels of CP4 EPSPS and Cry1Ab proteins in NK603 x MON810 were established for forage and grain samples since these tissues are most relevant to food and feed product safety.

Protein values are reported as micrograms (µg) of the specific protein per gram (g) of tissue on a fresh weight (fw) basis.

The mean level of the CP4 EPSPS proteins across all sites in forage samples from NK603 x MON810 was 36.3 µg/g fw as compared to the mean level of 37.2 µg/g fw in forage samples from the single event NK603. The mean level of the CP4 EPSPS proteins across all sites in grain samples from NK603 x MON810 was 12.7 µg/g fw as compared to the mean level of 13.4 µg/g fw in grain samples from the single event NK603 (Table 1).

The levels of CP4 EPSPS in tissue samples from the non-transgenic control hybrid and MON810, which do not contain the *cp4 epsps* insert, were below the limit of detection (LOD) for each tissue type (0.38 µg/g fw in forage and 0.08 µg/g fw in grain).

The mean level of the Cry1Ab protein across all sites in forage samples from NK603 x MON810 was 6.06 µg/g fw as compared to the mean level of 6.40 µg/g fw in forage samples from the single event MON810. The mean level of the Cry1Ab protein across all sites in grain samples from NK603 x MON 810 was 0.73 µg/g fw as compared to the mean level of 0.72 µg/g fw in forage samples from the single event MON810 (Table 2). The levels of Cry1Ab protein in tissue samples from the non-transgenic control hybrid and NK603, which do not contain the *cry1Ab* insert, were below the limit of detection for each tissue type (0.27 µg/g fw in forage and 0.13 µg/g fw in grain).

Table 1. Summary of levels of CP4 EPSPS proteins in forage and grain samples collected from multi-site field trials in France in the year 2000

Test/Control Substance	Protein Levels (µg/g fw)[1] Mean (Std. Dev.)[2] Range[3] Forage[4]	Grain[5]
NK603 × MON 810	36.3 (16.7) 12.6 – 61.4	12.7 (6.8) 2.0 - 21.9
NK603	37.2 (25.8) 8.5 - 78.2	13.4 (4.4) 1.2 - 17.8
MON 810	< 0.38 (LOD)[6]	< 0.08 (LOD)[6]
Nontransgenic control	< 0.38 (LOD)[6]	< 0.08 (LOD)[6]

1 Protein levels are expressed as microgram (µg) of protein per gram (g) fresh weight of tissue (fw) and are corrected for method bias. The data is from three sites: L'Isle Jourdain (Site 1); Samatan (Site 2); and Labrihe (Site 3).

2 The mean and standard deviation were calculated from the analyses of four replicate samples from four plots across three field sites (n = 12).

3 Minimum and maximum values from the analyses of samples across three sites.

4 The samples were prepared from 4 plants from each plot in all replicates at all sites. Total n = (3 field sites × 4 replicates for each site) = 12 for each line

5 The samples were from 30 hand pollinated ears from each plot in all replicates from three sites. Total n = (3 field sites × 4 replicates for each site) = 12 for each line

6 The Limit of Detection (LOD) for the CP4 EPSPS assay is 0.38 µg/g fw in forage tissue and 0.08 µg/g fw in grain tissue.

Table 2. Summary of levels of Cry1Ab protein in forage and grain samples collected from multi-site field trials in France in the year 2000

Test/Control Substance	Protein Levels (µg/g fw)[1] Mean (Std. Dev.)[2] Range[3] Forage[4]	Grain[5]
NK603 × MON 810	6.06 (1.87) 2.76 - 8.80	0.73 (0.14) 0.53 - 0.98
NK603	< 0.27 (LOD)[6]	< 0.13 (LOD)[6]
MON 810	6.40 (2.62) 1.99 - 9.91	0.72 (0.21) 0.38 - 1.11
Nontransgenic control	< 0.27 (LOD)[6]	< 0.13 (LOD)[6]

1 Protein levels are expressed as microgram (µg) of protein per gram (g) fresh weight of tissue (fw). The data is from three sites: L'Isle Jourdain (Site 1); Samatan (Site 2); and Labrihe (Site 3).
2 The mean and standard deviation were calculated from the analyses of four replicate samples from four plots across three field sites (n = 12).
3 Minimum and maximum values from the analyses of samples across three sites.
4 The samples were prepared from 4 plants from each plot in all replicates at all sites. Total n = (3 field sites × 4 replicates for each site) = 12 for each line
5 The samples were from 30 hand pollinated ears from each plot in all replicates from three sites. Total n = (3 field sites × 4 replicates for each site) = 12 for each line
6 The Limit of Detection (LOD) for the Cry1Ab assay is 0.27 µg/g fw in forage tissue and 0.13µg/g fw in grain tissue.

2.1.3.2 Inheritance and genetic stability of inserted DNA

Genetic stability of the recombinant inserts has previously been demonstrated in the parental maize lines NK603 and MON810 (VKM 2013b, c). Comparative Southern blot analyses have shown that these inserts are retained in the stacked maize NK603 x MON810, and that their structures are intact. This is supported by protein measurements with ELISA that show comparable levels of the CP4 EPSPS and Cry1Ab proteins between the stacked and single maize events.

2.2 Conclusion

The stacked maize NK603 x MON810 was produced by conventional crossing of the single maize events NK603 and MON810. Southern blot and PCR analyses have shown that the recombinant inserts from the parental events are retained in the stacked event, and that their structures are intact. Genotypic stability of the inserts has previously been demonstrated for the single events. Protein measurements show comparable levels of CP4 EPSPS and Cry1Ab in forage and grain samples from maize NK603 x MON810 to those measured in maize NK603 and MON810, respectively.

The VKM GMO Panel considers the molecular characterisation of maize NK603 x MON810 and its parental events NK603 and MON810 satisfactory.

3 Comparative assessment

3.1 Summary of the previous evaluations of the single events

3.1.1 Maize NK603

Compositional analyses were performed on forage and grain samples collected from NK603 maize 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 NK603 and non-transgenic maize. The biological relevance of statistically significant differences was assessed by performing additional comparisons of the levels of the various compounds in maize NK603 and conventional non-GM maize lines grown in field trials conducted in 1994-1995 or 1998. In the latest risk assessment of maize NK603 the VKM GMO Panel concludes that maize NK603 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 2013a).

3.1.2 Maize MON810

The original field trials with maize MON810 were performed in major maize-growing areas of the USA during the 1994 growth season (6 field sites). In addition, European field trials with MON810 and MON810 hybrids and conventional control maize were performed in France and Italy during the 1995 field season (5 sites) and France in 1995 (4 sites). The non-GM maize control material was maize MON818 in all 1994 field trials and maize MON820 in the 1995 field trials. No consistent compositional differences were observed between maize MON810 and non-transgenic maize. In the latest risk assessment of maize MON810 the VKM GMO Panel concludes that maize MON810 is compositionally, agronomically and phenotypically equivalent to conventional maize varieties, except for the insect resistance conferred by the Cry1Ab protein (VKM 2013b).

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

3.2.1 Experimental design & statistical analysis

In the application EFSA/GMO/UK/2004/01 for food and feed uses, import and processing of maize NK603 x MON810 within the European Union, the applicant present compositional data from grain and forage samples collected during field trials in France in 2000. In addition, data derived from material obtained from field trials with the single events and the respective comparators were provided by the applicant.

Maize NK603 x MON810, non-transgenic control and five different reference hybrids were grown under field conditions in France in 2000 at three replicated sites: L'Isle Jourdain (Site 1), Samatan (Site 2) and Labrihe (Site 3). The five different non-transgenic commercial maize hybrids (LG2447, NAUDI, GLODUA, MONFORT and CUARTAL) were grown in replicated plots at the same field sites as NK603 x MON810. Additional data from six commercial reference hybrids grown in EU during the 1999 growing season were included for the construction of a statistically valid 99% tolerance interval for component concentrations in traditional maize. For each single-trait maize, compositional trials were placed at multiple locations in the field (replicated where possible), representing a diversity of environmental conditions, over different growing seasons.

The plants were grown under agricultural practices that are typical for maize production within these regions. At each location, the stacked maize NK603 x MON810, non-transgenic and single-trait controls, and the five commercial reference hybrids were planted in single plots randomly assigned within each of four replicated blocks. Within each replicate, the maize were blocked according to their glyphosate tolerance. Each row was clearly marked with its unique code for maize line identification. Where necessary, weed control was maintained by herbicide applications. The maintenance herbicides used were acetochlor, or atrazine and 2,4-D, when appropriate. For each site, the maintenance herbicide(s) was applied to all treatment groups and replicates. In addition, all plots containing glyphosate-tolerant maize were treated with a single application of MON 52276 herbicide (containing 360 g/L glyphosate acid-equivalent) at a dose rate of 3 L/ha.

All component values were converted from a fresh weight to a dry weight basis. Statistical analyses were conducted with a mixed model analysis of variance for four sets of comparisons for each component in forage and grain: analyses for each of the three replicated trials, and for a combination of all three trials. There were a total of 59 components evaluated (7 in forage and 52 in grain). Within each set of comparisons (an individual site or the combination of all sites), maize NK603 x MON 810 was compared with the non-transgenic control hybrid to determine statistically significant differences at $p < 0.05$. The five commercial reference lines were included in the analysis but were not included in individual statistical comparisons.

For each component a 99% tolerance interval was calculated which was expected to contain, with 95% confidence, 99% of the measured values expressed in the population of commercial maize lines. All available commercial hybrid reference data of EU origin (1999 and 2000) were used to develop population tolerance intervals. However, complete datasets for four components, folic acid, inositol and vitamins B1 and B2, were not available; therefore, tolerance intervals for these variables were not calculated. SAS-software was used to generate all summary statistics and other statistical analyses. Report tables present p-values as either $p<0.001$ or as the actual value.

3.3 Compositional Analysis

Analyses were conducted on grain from the NK603 x MON810, control and reference hybrids to measure proximates (protein, fat, ash, moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), amino acids, fatty acids, vitamin B1, vitamin B2, vitamin E, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), folic acid, phytic acid and trypsin inhibitor content. The amounts of the secondary metabolites, raffinose, inositol, 2-furaldehyde (furfural), ferulic acid and p-coumaric acid, were also determined in grain. In forage, the proximate, ADF and NDF contents were determined. In addition, the carbohydrate content of forage and grain was determined by calculation. Results are presented in table 1-3 in Appendix 1.

Results for 15 components, for which more than 50% of observations were at or below the limit of quantitation (LOQ) of the assay, were excluded from statistical analysis. These components were sodium, 2-furaldehyde, 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid and 20:4 arachidonic acid.

There were 39 out of 108 observations below the LOQ for 16:1 palmitoleic acid. One observation out of 108 observations was below the LOQ for 22:0 behenic acid. For raffinose, there were 12 out of 108 observations below the LOQ of the assay. To include these three analytes in the statistical analysis, a value equal to half the quantitation limit was assigned for these 52 data points.

Three "outlier" data points were identified in the data set and were excluded from the statistical analysis. They were 20:1 eicosenoic acid (Site 1, reference), 16:1 palmitoleic acid (Site 2, control), and 18:0 stearic acid (Site 2, control).

According to the applicant, there were a total of 59 components evaluated (7 in forage and 52 in grain), resulting in a total of 236 statistical comparisons made between the NK603 x MON810 maize and the non-transgenic control hybrid. There were 56 significant differences ($p<0.05$) out of the 236 comparisons. For 55 of these 56 significant differences, the range of the values for the test hybrid was either within the 99% tolerance interval or, in cases where a 99% tolerance interval was not available, within the range of commercial reference hybrids used in this study. On this basis, the applicant considered these 55 statistically significant differences to be of no biological significance.

The remaining statistically significant difference was observed for phosphorous content (% dw) in NK603 x MON810 grain from one site (Site 3) but not the other two sites. There was no statistically significant difference in phosphorus content when the data from the three sites were pooled. Furthermore, the phosphorous content in NK603 x MON810 grain from Site 3 was also outside the 99 % tolerance interval for the commercial references, but within the range reported in peer-reviewed literature. Further data for phosphorous content were provided by the applicant from the analyses of grain samples grown in bulk at two additional sites, in the USA: Monmouth (Illinois) during the 2000 field season and Carlyle (Illinois) during the 2001 field season. The phosphorus contents in NK603 x MON810 grain from these two sites (0.279 % dw in Monmouth, and 0.350 % dw in Carlyle) were equivalent to their controls at the sites (0.273 % dw and 0.352 % dw, respectively).

3.4 Agronomic and phenotypic characters

Field studies were conducted at four locations in the USA (Iowa, Missouri, Nebraska and Ohio) in the 2002 growing season to assess phenotypic and ecological characteristics of maize stack NK603 x MON810 and its conventional counterpart (near-isogenic conventional maize). Sixteen conventional maize hybrids (four at each location) were included as reference substances to assess natural variation of plant characteristics between commercial maize varieties.

According to the applicant, the four test locations were selected to be representative of the range of environmental conditions under which the tested hybrid varieties would typically be grown. Each of the agronomic trials was conducted as a randomised complete block design with three replications per location. Agricultural practices (pesticide and fertiliser applications) were typical for commercial maize production in the regions chosen for this study. No information regarding potential glyphosate treatment of the test plots are available.

Fourteen separate developmental, agronomic and morphological characteristics were assessed at each location (Table 4, Appendix 1). In addition, plant interactions with endemic insect, disease and abiotic stressors were observed throughout the growing season at all sites (Table 5, Appendix 1).

The statistical significance of the genotype effect (NK603 x MON810 vs. the near-isogenic control) was determined with a standard F-test at the 5% probability. For each phenotypic characteristic measured, minimum and maximum values (range) and a 99 % tolerance interval with 95 % confidence were determined based on the population of sixteen reference maize hybrids. At the Missouri site, significante injury occurred to maize plants in two plots due to herbicide drift at application. All data for these plots were excluded from statistical analysis.

Seedling vigour and stay green were evaluated and recorded in qualitative scores on a scale of 1-9, where 1 is a good rating and 9 is a bad rating. Early and final stand count, dropped ears and lodging

were recorded as number per plot. Flowering data were recorded as heat units accumulated from date of planting.

Analyses of variance across trial locations did not revealed statistically significant differences between maize NK603 x MON810 and the near-isogenic control hybrid for any of the 14 agronomic and phenotypic characters recorded ($p>0.05$) (Table 4, Appendix 1). A comparisation of insect, disease and abiotic stressors incidence between NK603 x MON810 and the conventional control are presented in Table 5, Appendix 1. No observable differences between NK603 x MON810 and the conventional control were demonstrated, with two exceptions noted at the Missouri location. According to the applicant, these differences were related to location within the study area and not a differential response among NK603 x MON810, the control or conventional maize hybrids.

3.5 Conclusion

The applicant has performed comparative analyses of data from field trials located at representative sites and environments in North America and Europe during the 2000 and 2002 growing seasons. With the exception of small intermittent variations and the insect resistance and herbicide tolerance conferred by the Cry1Ab and CP4 EPSPS proteins, the results showed no biologically significant differences between maize stack NK603 x MON810 and conventional control. Based on the assessment of available data, the VKM GMO Panel concludes that maize NK603 x MON810 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, except for the newly expressed proteins.

4 Food /feed safety assessment

Both single maize events, MON810 and NK603, have previously been evaluated by the VKM GMO Panel, and updated risk assessments were finalised 2013 (VKM 2013b,c)

4.1 Summary of the previous evaluations of the single events

4.1.1 NK603

In the latest safety assessment of maize NK603 the VKM GMO Panel concluded, based in part on data from whole food feeding studies on rats and broilers, that maize NK603 is nutritionally equivalent to conventional maize varieties. It is unlikely that the CP4-EPSPS protein will introduce a toxic or allergenic potential in food or feed based on maize NK603 compared to conventional maize (VKM 2013b).

4.1.2 MON810

Maize MON810 has a long history of use, and has been evaluated extensively by The VKM GMO Panel. In the latest risk assessment (VKM 2013c) it was concluded that MON810 is nutritionally equivalent to conventional maize varieties and that it is unlikely that the Cry1Ab protein will introduce a toxic or allergenic potential in food or feed based on maize MON810 compared to conventional maize.

With regard to animal studies with the whole product, feeding studies with maize MON810 grain with different target animals, such as rats (Hammond et al 2006), Atlantic salmon (Sanden et al. 2005; Sanden et al. 2006, Sagstad et al. 2007; Hemre et al. 2007; Bakke-McKellep et al. 2008; Froystad-Saugen et al. 2009; Sissener et al. 2010), dairy cows (Donkin et al. 2003), broiler (Taylor et al. 2003) and pigs (Walsh et al. 2012 a,b***),*** have all indicated nutritional equivalence between maize MON810 and its non-GM maize counterpart and to conventional maize.

In a study performed by Sissener et al. (Sissener et al. 2011a) it was suggested that the effects observed in Atlantic salmon fed maize MON810 probably could be related to the content of the mycotoxin deoxynivalenol (DON) in the MON810 ingredient (0.09 ppm). The Cry1Ab content was quantified in the maize MON810 ingredient and was between 110-130 ng/g (Sanden et al. 2005). Cry1Ab protein has not been detected in any of the investigated Atlantic salmon feeds (Sanden et al. 2005; Jørgensen 2012).

4.2 Product description and intended uses

The genetic modification in NK603 x MON810 field maize will not impact the existing production processes used for maize. All NK603 x MON810 maize products will be produced and processed for food, animal feed and industrial products in the same way as other commercial maize. The NK603 x MON810 field maize and all food, feed and processed products derived from NK603 x MON810 field maize are expected to replace a portion of similar products from commercial maize, with total consumption of maize products remaining unchanged.

4.3 Effects of processing

There are two basic methods employed in processing field maize grain, dry milling and wet milling. In dry milling, maize is separated into flour, maize-meal, grits and other products. Wet milling is the process by which maize is separated into starch, germ to produce oil and fiber, and gluten for animal feed. Maize NK603 x MON810 will be produced and processed in the same way as any field maize.

Food manufacturing of NK603 x MON810 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 proteins are denatured and DNA degraded. This also applies to the Cry1Ab and CP4 EPSPS proteins and their genes (Dien et al. 2002; Hammond & Jez 2011, Fernandes et al. 2013). Concentrations of these proteins will be below the limit of detection in wet-milled fractions, in maize chips and maize oil. In the study performed by Fernandes et al. (2013) it was shown that when baking the maize bread broa, containing 11 % of TC1507 and 20 % MON 810 maize flour, the baking process sheared DNA into fragments less than 1000 bp, typically around 200 bp. Transgenic proteins and DNA will probably be found in quantifiable amounts in unprocessed grain and all of the dry-milled fractions.

4.4 Toxicological assessment

In assessing the potential risks of GM foods, it is important to consider any possible adverse health effects that may arise from substances that are intentionally introduced or modified in food crops, as well as any possible unexpected adverse effects resulting from the genetic modification process itself (Chao & Krewski 2008).

4.4.1 Toxicological assessment of the newly expressed protein

The VKM GMO Panel has previously evaluated the proteins Cry1Ab and CP4 EPSPS in the risk assessments of the parental maize lines NK603 and MON810 (VKM 2013b,c).

4.4.2 Toxicological assessment of the whole GM food/feed

The applicant has not performed a 90-day subchronic feeding study on rats. The applicant has however performed a 42-day broiler feeding study with emphasis on nutritional properties of maize NK603 x MON810, which also considers health effects. The study is described in detail under section 4.6.2

4.5 Allergenicity assessment

The strategies used when assessing the potential allergenic risk focuses on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation, or to elicit allergic reactions in already sensitised individuals and whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (EFSA 2006, EFSA 2011a).

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

amino acids, IgE cross-reactivity between the novel protein and the allergen should be considered a possibility.

4.5.1 Assessment of IgE mediated allergenicity of the newly expressed protein

The applicant has performed a weight-of-evidence approach (Metcalfe et al. 1996; FAO/WHO 2001; Codex 2003; König et al. 2004; Poulsen 2004) for an overall assessment of the IgE allergenic potential of the Cry1Ab and CP4 EPSPS (and CP4 EPSPS L214P) proteins. These assessments have previously been reported by the applicant for the single maize events NK603 (Notification C/ES/00/01, EFSA-GMO-NL-2005-22, EFSA-GMO-RX-NK603) and MON810 (Notification C/F/12/12/02, EFSA-GMO-RX-MON8101a), and include the following:

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

i) The donor organisms of the *cry1Ab* and *cp4 epsps* genes are the common soil bacteria *Agrobacterium* sp. strain CP4 and *Bacillus thuringiensis* subsp. *kurstaki*, respectively. None of these bacteria have a known history of causing allergies.

ii) Cry proteins as microbial pesticides have a history of safe use and there have been no indications of Cry proteins originating from *Bacillus thuringiensis* exhibiting harmful effects on human or animal health (US EPA 2001, 2005, 2010).

iii) 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 (Silvanovich et al. 2000 & 2002; McCoy et al. 2002c, McClain & Silvanovich 2007, from unpublished Monsanto technical reports).

iv) Likewise, the Cry1Ab protein, and Cry-proteins in general, do not show significant amino acid sequence similarities to other known protein toxins, and do not share immunologically relevant sequence similarities to known IgE allergens (McCoy and Silvanovich 2004; McClai and Silvanovic 2007. From unpublished Monsanto technical reports). The Cry1Ab protein found in MON810 and stacked events is not considered to be allergenic by the EFSA GMO Panel (EFSA 2009a).

v) The Cry1Ab and CP4 EPSPS proteins are both considered heat labile (Hammond et al 2011), they are not glycosylated and and are readily digested in simulated digestive fluids (Astwood et al 2001; Leach et al 2002a; Leach et al 2002b; Ream 1994. From unpublished Monsanto technical reports).

vi) There are independent reports indicating no, or very low risk, for allergenicity of maize MON810. Nakajima et al. (2007) monitored the occurrence of IgE antibodies specific to the Cry1Ab protein expressed in maize MON810 in food allergic patients of the Japanese population. IgE levels were within background levels in sera of all the 44 participating patients. When sera from maize allergic patients were tested against extracts of non-GM maize and maize MON810, similar staining patterns were found for both types of maize. Thus, no significant level of IgE antibodies specific to the Cry1Ab protein could be found in the studied food-allergic patients.

vii) Batista et al. (2005) performed skin prick tests with extracts of maize MON810 on children with food and inhalant allergy and individuals with asthma-rhinitis. None of the individuals tested reacted differently to the MON810 and the non-transgenic maize samples. Similarly, when IgE of sera from food allergic patients were blotted to the transgenic Cry1Ab protein expressed in maize MON810, none of the tested samples contained detectable levels of IgE antibodies against the tested protein.

The information listed above indicates that the CP4 EPSPS and Cry1Ab proteins in the parent maize lines NK603 and MON810, and the stacked event NK603 x MON810, lack IgE allergenic potential with regard to human health. However, it does not cover possible allergic reactions (e.g. enteropathies) that are not IgE mediated.

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

Allergenicity of the maize NK603 x MON810 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 NK603 x MON810 with the exception of the introduced traits, no increased allergenicity is anticipated for maize NK603 x MON810. Moreover, maize is generally not considered a common allergenic food.

4.5.3 Assessment of the allergenicity of proteins from the GM plant

It is the opinion of the VKM GMO Panel that a possible over-expression of any endogenous protein, which is not known to be allergenic, in maize NK603 x MON810 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 potential role of these proteins as adjuvants should be considered. As for allergens, interactions with other constituents of the food matrix and/or processing may alter the structure and bioavailability of an adjuvant and thus modify its biological activity.

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

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

“Bystander sensitisation”

"Bystander sensitisation" can occur when an adjuvant in food, or an immune response against a food antigen, results in 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/feed

Compositional analyses of maize NK603 x MON810 indicate nutritional equivalence to the non-GM control maize with a comparable genetic background and to the published range of values in the literature. Spraying with glyphosate herbicides did not affect the nutritional composition of the maize. The nutritional equivalence has been further supported by a poultry feeding study where broiler chickens were fed over a 42-day period with diets containing grain from herbicide (glyphosate) treated NK603 x MON810 maize. The study is described in section 4.6.2.

4.6.1 Intake information/exposure assessment

In Norway, the net import of maize staple (e.g. flour, starch and mixed products) was 7600 tons in 2007, corresponding to 4.4 g dry weight/person/day, or an estimated daily energy intake of 0.6 % from maize staple for adults (Vikse 2009, unpublished). In comparison the daily intake in Europe is 8.8 g dry weight/person/day. The production of maize porridge for children in Norway in 2007 was approximately 37.5 tons, corresponding to 1.7 g dry weight/child/day, or an estimated daily energy intake of 0.6 % from maize staple for a 6 month old child. The estimated median daily intake of sweet maize in Norway is 3.25 g/day, with a 97.5 percentile of 17.5 g/day.

Based on these numbers, the estimated mean daily intakes of CP4 EPSPS protein from maize staple by adults and 6 month old children in Norway would be ~ 53 and ~ 20 µg/day, respectively, (based on the reported mean dry weight (12µg/g) value of CP4 EPSPS in NK603 grain from European trials in 1999). The corresponding daily intakes of Cry1Ab would be far less, given the much lower levels of Cry1Ab found in both MON810 and NK603 x MON810 maize.

The estimated mean intake levels mentioned above are several orders of magnitude below the levels shown to have no effect in laboratory toxicology testing. Also, these levels are considerably below the proposed threshold of toxicological concern (TTC) level of 1800 µg/person/day (Class 1, oral exposure) for chemicals considered to have a low potential for toxicity based on metabolism and mechanistic data (Vermeire et al. 2010). Transgenic proteins produced by genetically modified plants are generally considered non-toxic to humans.

This dietary exposure assessment is very conservative, as it assumes that all consumed maize consists of maize NK603 x MON810 and that protein levels are not reduced by processing.

The VKM GMO Panel notes that farm (production) animals e.g. pigs and poultry often are fed diets with a substantial inclusion of unprocessed maize grain, and that the exposure to transgenic proteins from maize NK603 x MON810 may be higher for these animals.

4.6.2 Nutritional assessment of feed derived from the GM plant

Feeding studies with maize NK603 x MON810

A 42-day feeding study on broiler chickens

Nutritional equivalence of NK603 x MON810 to non-GM maize has been shown in a 42-day feeding study with broiler chickens. The study was completed in November 2000. In the study, grain from NK603 × MON810 maize, the non-transgenic control line (RX 730) and six commercially available maize hybrids were used. Analyses for potential contamination and for nutritional composition were performed. NK603 x MON810 was formulated into representative poultry diets. The six commercially available maize hybrids (grown in 2000) were obtained from US growers: SC1096, SC1087, and SC1140 (Fayette County, Ohio); As-grow 740, Pioneer 34B23, and DEKALB 626 (Clinton County, Illinois). Mycotoxin and pesticide screens of the grain used in these experiments were conducted prior to initiation of each experiment to verify that levels were below the limits of concern for broiler performance. A total of 800 Ross-Cobb day-old broiler chicks were distributed on eight treatment groups within a randomised block design. The diets were formulated to National Research Council (NRC) requirements and contained between 50 and 65% of grain from either NK603 x MON810, a non-transgenic control, or six commercially available maize hybrids. Animal performance was evaluated by measuring live weight at day 0 and day 42, total feed intake, feed efficiency and a comprehensive set of carcass measurements. All measurements recorded, including chick mortality, were similar across diets containing maize grain from NK603 x MON810 maize, control and the six commercial hybrids. The study showed no diet related biologically significant differences among the chickens, when comparing results of the measured parameters for NK603 x MON810 maize, control or the six commercial hybrids (Taylor et al. 2003).

4.7 Conclusion

A whole food feeding study performed on broilers did not indicate any adverse health effects of maize NK603 x MON810, and shows that maize NK603 x MON810 is nutritionally equivalent to conventional maize. The CP4 EPSPS or Cry1Ab proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize NK603 x MON810 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1Ab or CP4-EPSPS proteins will introduce a toxic or allergenic potential in food or feed based on maize NK603 x MON810 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 by seed produced predominantly by cross-pollination (OECD 2003). In contrast to weedy plants, maize has a pistillate inflorescence (ear) with a cob enclosed with husks. Due to the structure of the cob, the seeds remain on the cob after ripening and natural dissemination of the kernels rarely occurs.

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

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

It is considered very unlikely that the establishment, spread and survival of maize NK603 x MON810 would be increased due to the insect resistance and herbicide tolerance traits. The herbicide tolerant trait can only be regarded as providing a selective advantage for the GM maize plant where and when glyphosate based herbicides are applied. Similarly insect resistance against certain coleopteran pests provides a potential advantage in cultivation of NK603 x MON810 under infestation conditions. It is considered very unlikely that maize NK603 x MON810 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 NK603 x MON810 relative to its conventional counterpart. A series of field trials with maize NK603 x MON810 were carried out across three locations in France in 2000 (compositional assessment) and four locations in the USA in 2002 (phenotypic and agronomic assessment) (application EFSA/GMO/UK/2004/01). Information on phenotypic (e.g. crop physiology, morphology, development) and agronomic (e.g. grain yield) characteristics was provided to assess the agronomic performance of maize in comparison with its conventional counterpart (see section 3.4). Data from the field trials shows no statistical significant differences for the parameters assessed.

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 NK603 x MON810, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of maize NK603 x MON810 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 NK603 x MON810 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 NK603 x MON810. This means that micro-organisms in the digestive tract in humans and animals (both domesticated animals and other animals feeding on fresh or decaying plant material from the transgenic maize line) may be exposed to transgenic DNA.

Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation with which maize can hybridise and form backcross progeny (Eastham & Sweet 2002; OECD 2003). Vertical gene transfer in maize therefore depends on cross-pollination with other conventional or organic maize varieties. All maize varieties which are cultivated in Europe can interbreed. In addition, unintended admixture/adventitious presences of genetically modified material/transgenes in seeds represent a possible way for gene flow between different production systems.

5.2.1 Plant to micro-organisms gene transfer

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

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 NK603 x MON810 to unrelated species such as bacteria.

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

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

In conclusion, the VKM GMO Panel consider it is unlikely that the introduced gene from maize NK603 x MON810 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 *cp4 epsps* and *cry1Ab* genes from NK603 x MON810 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 NK603 x MON810 (excluding cultivation) and the physical characteristics of maize seeds, possible pathways of gene dispersal are grain spillage and dispersal of pollen from potential transgenic maize plants originating from accidental grain spillage during transport and/or processing.

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

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

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

As maize NK603 x MON810 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

The genetically modified maize MON810 has been developed to provide protection against certain lepidopteran target pests, such as the European corn borer (ECB, *Ostrinia nubilalis*), and some species belonging to the genus *Sesamia*. The insect resistence is achieved through expression of the truncated *cry1Ab* gene derived from *Bacillus thuringiensis* subsp. *kurstaki,* a common soil bacterium.

The European corn borer is widely distributed in Europe covering the Iberian Peninsula, Czech Republic and Slovakia, southwest of France, northern Italy and the southern regions of Germany and Poland. The Mediterranean corn borer is present in the Mediterranean region (Andreadis 2011). There are ten reports of *O. nubilalis* in Norway, restricted to the counties of Vestfold, Telemark, Aust-Agder

and Vest Agder. *Sesamia* spp. has not been reported in Norway. There are no reports of *O. nubilalis* attaining pest status in Norway, and the Plant Clinic (Planteklinikken) at Bioforsk has never received samples of this pest or plant material damaged by this pest (K. Ørstad pers. com.). Consequently, there are no insecticides authorised or previous applications for registrations of insecticides against this herbivore in Norway.

Considering the intended uses of maize NK603 x MON810, 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 Cry1Ab protein is likely to be extremely low and of no ecological relevance.

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

Considering the intended uses of maize stack NK603 x MON810, 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). 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 Cry1Ab protein from GM plants in soil (Icoz & Stotzky 2008). Data supplied by the applicant indicate that a limited amount of the Cry1Ab protein enters the environment due to expression in the grains (mean value of 0.5 µg/g d.w). In addition, the data show that at least 99% of microbially produced Cry1Ab protein was rapidly degraded in simulated gastric fluid.

In conclusion, the VKM GMO Panel considers that the exposure of potentially non-target organisms to the Cry1Ab protein is likely to be very low and of no ecological relevance.

5.5 Potential interactions with the abiotic environment and biochemical cycles

Considering the intended uses of maize NK603 x MON810, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with the abiotic environment and biogeochemical cycles were not considered an issue by the VKM GMO Panel.

5.6 Conclusion

Considering the intended uses of maize NK603 x MON810, 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 NK603 x MON810.

Maize NK603 x MON810 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 NK603 x MON810. 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 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 NK603 x MON810 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 monitoring plan provided by the applicant is in line with the intended uses of maize NK603 x MON810.

7 Data gaps

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

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

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

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

- **Herbicide residue levels**
  Herbicide residue levels on plants with engineered resistance to one or two broad spectrum herbicides could entail higher levels of herbicide residue cocktails compared to plants produced by conventional farming practices. Since it is difficult to predict the toxicity of cocktails from the toxicity of the single components, there is uncertainty related to risk of confounding effects such as additive or synergistic effects between the residues in herbicide resistant plants. The transgene technology used can possibly lead to different metabolic products of the applied herbicides from what is expected from conventional usage. The risk assessment of herbicides should take into account plants with altered metabolism. At present the changes related to herbicide residues of genetically modified plants as a result of the application of plant-protection products fall outside the remit of the Norwegian VKM panels.

8 Conclusions

Molecular characterisation
The stacked maize NK603 x MON810 was produced by conventional crossing of the single maize events NK603 and MON810. Southern blot and PCR analyses have shown that the recombinant inserts from the parental events are retained in the stacked event, and that their structures are intact. Genotypic stability of the inserts has previously been demonstrated for the single events. Protein measurements show comparable levels of CP4 EPSPS and Cry1Ab in forage and grain samples from maize NK603 x MON810 to those measured in maize NK603 and MON810, respectively.

The VKM GMO Panel considers the molecular characterisation of maize NK603 x MON810 and its parental events NK603 and MON810 satisfactory.

Comparative assessment
The applicant has performed comparative analyses of data from field trials located in maize growing regions of Europe and USA in 2000 and 2002. With the exception of small intermittent variations and the insect resistance and herbicide tolerance conferred by the Cry1Ab and CP4 EPSPS proteins, the results showed no biologically significant differences between maize stack NK603 x MON810 and conventional control. Based on the assessment of available data, the VKM GMO Panel concludes that maize NK603 x MON810 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, except for for the introduced characteristics, and that its composition fell within the normal ranges of variation observed among non-GM varieties.

Food and feed risk assessment
A whole food feeding study performed on broilers did not indicate any adverse health effects of maize NK603 x MON810, and shows that maize NK603 x MON810 is nutritionally equivalent to conventional maize. The CP4 EPSPS or Cry1Ab proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize NK603 x MON810 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1Ab or CP4-EPSPS proteins will introduce a toxic or allergenic potential in food or feed based on maize NK603 x MON810 compared to conventional maize.

Environmental risk assessment
Considering the intended uses of maize NK603 x MON810, 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 NK603 x MON810.

Maize NK603 x MON810 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 NK603 x MON810. 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 NK603 x MON810 is nutritionally equivalent to conventional maize varieties. It is unlikely that the CP4 EPSPS and Cry1Ab proteins will introduce a toxic or allergenic potential in food or feed based on maize NK603 x MON810 compared to conventional maize.

The VKM GMO Panel likewise concludes that maize NK603 x MON810, based on current knowledge, is comparable to conventional maize varieties concerning environmental risk in Norway with the intended usage.

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Appendix 1.

Table 1. Summary of statistically significant results of the comparison of component levels of NK603xMON810 maize to non-transgenic control hybrid and comercial reference hybrids

Location	Component	Mean NK603 × MON 810	Mean Control	Mean Diff. (NK603 × MON 810 minus Control) % of Control	Signif. (p-Value)	NK603 × MON 810 (Range)	Commercial [Tolerance Int.][a]
Forage							
Site 1	Fat, total (% dw)	2.18	2.73	-20.11	0.037	(1.88 - 2.37)	[0.66,4.49]
Site 2	Ash (% DW)	4.33	3.53	22.64	0.007	(3.81 - 5.12)	[1.30,7.03]
	Carbohydrates (% dw)	87.65	89.03	-1.55	0.025	(86.81 - 88.42)	[79.16,92.40]
Combined Sites	Ash (% dw)	4.03	3.52	14.50	0.022	(2.91 - 5.12)	[1.30,7.03]
Grain							
Site 1	Alanine (% Total AA)	7.66	7.47	2.54	<0.001	(7.47 - 7.77)	[6.73,8.46]
	Arginine (% Total AA)	4.29	4.63	-7.55	0.017	(4.14 - 4.55)	[3.33,5.67]
	Cystine (% Total AA)	1.99	2.16	-7.87	0.020	(1.79 - 2.09)	[1.25,3.23]
	Glutamic acid (% Total AA)	19.75	19.30	2.38	0.008	(19.33 - 19.96)	[15.97,21.95]
	Glycine (% Total AA)	3.43	3.68	-7.06	0.004	(3.33 - 3.53)	[2.38,5.34]
	Histidine (% Total AA)	2.94	3.04	-3.25	0.012	(2.87 - 3.00)	[2.04,3.91]
	Isoleucine (% Total AA)	3.85	3.75	2.94	0.009	(3.79 - 3.91)	[3.21,4.09]
	Leucine (% Total AA)	13.57	13.04	4.14	0.013	(13.07 - 13.98)	[9.39,16.13]
	Lysine (% Total AA)	2.85	3.14	-8.92	0.036	(2.71 - 3.01)	[1.94,4.35]
	Phenylalanine (% Total AA)	5.25	5.10	2.94	<0.001	(5.14 - 5.34)	[4.14,5.87]
	18:1 Oleic (% Total FA)	21.59	23.20	-6.94	0.026	(20.68 - 22.01)	[18.11,31.92]
	18:3 Linolenic (% Total FA)	1.09	1.00	9.62	0.010	(1.06 - 1.14)	[0.69,1.53]
	Vitamin B2 (µg/g dw)	0.92	1.00	-8.27	0.012	(0.84 - 0.99)	(0.70-1.21)[b]
	p-Coumaric Acid (% dw)	0.021	0.012	75.02	<0.001	(0.018 - 0.025)	[0,0.075]

(continued)

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

[b]The range of values for commercial reference hybrids found in Sites 1-3 for this study. The 99% Tolerance Interval was not calculated for these analytes due to limited data.

Table 1. Cont.

Location	Component	Mean NK603 × MON 810	Mean Control	Mean Diff. (NK603 × MON 810 minus Control) % of Control	Signif. (p-Value)	NK603 × MON 810 (Range)	Commercial [Tolerance Int.][a]
Grain							
Site 2	Threonine (% Total AA)	3.11	3.38	-8.00	0.049	(2.91 - 3.34)	[2.82,4.01]
	16:0 Palmitic (% Total FA)	10.36	9.81	5.61	0.047	(10.27 - 10.46)	[7.11,13.88]
	18:1 Oleic (% Total FA)	22.33	24.89	-10.29	<0.001	(21.17 - 23.26)	[18.11,31.92]
	18:2 Linoleic (% Total FA)	63.54	61.39	3.50	0.033	(62.39 - 64.91)	[55.98,65.66]
	Acid Detergent Fiber (% dw)	3.74	2.95	27.14	0.049	(2.97 - 4.84)	[2.24,5.25]
	Calcium (% dw)	0.0042	0.0047	-10.93	0.022	(0.0040 - 0.0043)	[0.0018,0.0093]
	Vitamin E (mg/g dw)	0.012	0.014	-15.37	0.011	(0.011 - 0.013)	[0,0.024]
	Inositol (µg/g dw)	1511.15	1319.16	14.55	0.008	(1398.60 - 1800.72)	(1116.12-2288.33)[b]
Site 3	Arginine (% Total AA)	4.21	4.51	-6.65	0.042	(3.73 - 4.49)	[3.33,5.67]
	Glutamic acid (% Total AA)	19.59	19.07	2.73	0.039	(19.01 - 20.35)	[15.97,21.95]
	16:0 Palmitic (% Total FA)	10.54	9.91	6.36	0.015	(10.44 - 10.69)	[7.11,13.88]
	16:1 Palmitoleic (% Total FA)	0.16	0.066	139.91	0.002	(0.15 - 0.16)	[0.00081,0.21]
	18:1 Oleic (% Total FA)	20.83	21.81	-4.54	0.024	(20.70 - 20.96)	[18.11,31.92]
	18:3 Linolenic (% Total FA)	1.14	0.99	14.11	0.005	(1.06 - 1.21)	[0.69,1.53]
	20:1 Eicosenoic (% Total FA)	0.26	0.30	-13.47	0.007	(0.24 - 0.27)	[0.18,0.40]
	Neutral Detergent Fiber (% dw)	15.21	12.11	25.51	0.046	(11.54 - 18.91)	[4.02,19.77]
	Copper (mg/kg dw)	2.01	1.78	12.34	0.033	(1.87 - 2.15)	[0,3.69]
	Iron (mg/kg dw)	28.56	25.13	13.65	0.001	(25.96 - 30.97)	[4.13,36.90]
	Magnesium (% dw)	0.12	0.11	14.66	0.026	(0.11 - 0.14)	[0.074,0.16]
	Phosphorus (% dw)	0.34	0.31	9.90	0.009	(0.33 - 0.38)	[0.27,0.37]
	Potassium (% dw)	0.42	0.39	8.47	0.004	(0.42 - 0.43)	[0.23,0.50]
	Carbohydrates (% dw)	83.46	84.07	-0.73	0.019	(83.24 - 83.60)	[79.23,92.35]
	Protein (% dw)	11.87	11.11	6.84	<0.001	(11.66 - 12.05)	[3.27,15.87]
	Phytic Acid (% dw)	0.68	0.57	17.50	0.043	(0.61 - 0.76)	[0.43,0.92]

(continued)

[a]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.
[b]The range of values for commercial reference hybrids found in Sites 1-3 for this study. The 99% Tolerance Interval was not calculated for these analytes due to limited data.

Table 1. Cont.

Location	Component	Mean NK603 × MON 810	Mean Control	Mean Diff. (NK603 × MON 810 minus Control) % of Control	Signif. (p-Value)	NK603 × MON 810 (Range)	Commercial [Tolerance Int.][a]
Grain							
Combined Sites	Alanine (% Total AA)	7.66	7.50	2.13	<0.001	(7.25 - 8.22)	[6.73,8.46]
	Arginine (% Total AA)	4.39	4.66	-5.80	0.010	(3.73 - 5.02)	[3.33,5.67]
	Cystine (% Total AA)	2.13	2.24	-4.91	0.008	(1.79 - 2.54)	[1.25,3.23]
	Glutamic acid (% Total AA)	19.42	19.01	2.16	0.009	(18.30 - 20.35)	[15.97,21.95]
	Glycine (% Total AA)	3.58	3.77	-5.04	0.006	(3.27 - 4.14)	[2.38,5.34]
	Histidine (% Total AA)	3.01	3.11	-3.21	0.001	(2.81 - 3.34)	[2.04,3.91]
	Leucine (% Total AA)	13.20	12.82	2.96	0.008	(11.87 - 14.06)	[9.39,16.13]
	Phenylalanine (% Total AA)	5.10	5.01	1.82	0.011	(4.75 - 5.34)	[4.14,5.87]
	16:0 Palmitic (% Total FA)	10.44	9.91	5.35	0.008	(10.20 - 10.80)	[7.11,13.88]
	16:1 Palmitoleic (% Total FA)	0.16	0.12	34.47	0.033	(0.14 - 0.19)	[0.00081,0.21]
	18:1 Oleic (% Total FA)	21.58	23.27	-7.22	0.004	(20.68 - 23.26)	[18.11,31.92]
	18:3 Linolenic (% Total FA)	1.11	1.01	9.77	<0.001	(1.05 - 1.21)	[0.69,1.53]
	Neutral Detergent Fiber (% dw)	14.07	12.28	14.57	0.035	(9.44 - 18.91)	[4.02,19.77]
	Protein (% dw)	11.04	10.37	6.46	0.042	(8.23 - 12.05)	[3.27,15.87]

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

Tabel 2. Statistical summary of combined site corn forage fiber and proximate content for NK603xMON810 vs. non-transgenic control

Analytical Component	Comparator	Mean ± S.E. (Range)	Difference (NK603 × MON 810 minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Fiber						
Acid Detergent Fiber (% dw	NK603 × MON 810	22.93 ± 1.28 (19.15 - 27.34)				(16.13 - 29.69) [13.77,30.79]
	Control	22.40 ± 1.28 (20.15 - 25.29)	0.53 ± 1.60 (-3.64 - 4.87)	-2.86,3.92	0.744	
Neutral Detergent Fiber (% dw)	NK603 × MON 810	38.67 ± 1.45 (33.86 - 42.42)				(20.29 - 52.02) [25.68,47.27]
	Control	36.14 ± 1.45 (22.84 - 44.19)	2.52 ± 1.89 (-5.36 - 15.38)	-1.49,6.53	0.201	
Proximate						
Ash (% dw)	NK603 × MON 810	4.03 ± 0.21 (2.91 - 5.12)				(2.11 - 7.09) [1.30,7.03]
	Control	3.52 ± 0.21 (2.99 - 4.19)	0.51 ± 0.20 (-0.13 - 2.03)	0.080,0.94	0.022	
Carbohydrates (% dw)	NK603 × MON 810	85.94 ± 1.23 (82.15 - 88.42)				(79.47 - 89.73) [79.16,92.40]
	Control	86.44 ± 1.23 (83.87 - 89.50)	-0.50 ± 0.64 (-2.55 - 1.97)	-1.84,0.84	0.442	

(continued)

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

Tabel 2. Cont.

Analytical Component	Control	Mean ± S.E. (Range)	Difference (NK603 × MON 810 minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Proximate						
Fat, total (% dw)	NK603 × MON 810	2.58 ± 0.21 (1.78 - 3.35)				(1.31 - 4.12) [0.66,4.49]
	Control	2.74 ± 0.21 (2.35 - 3.28)	-0.17 ± 0.19 (-0.89 - 0.64)	-0.56,0.23	0.385	
Moisture (% fw)	NK603 × MON 810	69.02 ± 3.00 (64.00 - 75.20)				(56.40 - 76.00) [50.06,83.63]
	Control	67.87 ± 3.00 (62.20 - 73.10)	1.15 ± 1.09 (-3.20 - 4.80)	-1.16,3.46	0.307	
Protein (% dw)	NK603 × MON 810	7.45 ± 1.08 (4.43 - 10.82)				(3.14 - 11.06) [0.18,14.77]
	Control	7.30 ± 1.08 (4.52 - 9.49)	0.15 ± 0.51 (-1.83 - 1.65)	-0.94,1.25	0.768	

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

Table 3. Statistical summary of combined site corn grain amino acids, fatty acids, fiber, minerals, vitamins, anti-nutrients and secondary metabolite content for NK603xMON810 maize vs. non-transgenic control

Analytical Component	Line	Mean ± S.E. (Range)	Difference (NK603 × MON 810 minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% Total AA)						
Alanine (% Total AA)	NK603 × MON 810	7.66 ± 0.095 (7.25 - 8.22)				(7.06 - 8.15) [6.73,8.46]
	Control	7.50 ± 0.095 (7.25 - 7.84)	0.16 ± 0.042 (-0.13 - 0.48)	0.078,0.24	<0.001	
Arginine (% Total AA)	NK603 × MON 810	4.39 ± 0.18 (3.73 - 5.02)				(3.67 - 5.54) [3.33,5.67]
	Control	4.66 ± 0.18 (4.45 - 4.96)	-0.27 ± 0.093 (-0.73 - 0.20)	-0.47,-0.075	0.010	
Aspartic acid (% Total AA)	NK603 × MON 810	6.56 ± 0.068 (6.41 - 6.81)				(6.22 - 7.53) [5.71,7.57]
	Control	6.59 ± 0.068 (6.44 - 6.84)	-0.031 ± 0.088 (-0.43 - 0.24)	-0.22,0.16	0.730	
Cystine (% Total AA)	NK603 × MON 810	2.13 ± 0.076 (1.79 - 2.54)				(1.87 - 2.80) [1.25,3.23]
	Control	2.24 ± 0.076 (2.01 - 2.53)	-0.11 ± 0.041 (-0.39 - 0.081)	-0.19,-0.029	0.008	

(continued)

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

Table 3. Cont.

Analytical Component	Line	Mean ± S.E. (Range)	Difference (NK603 × MON 810 minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% Total AA)						
Glutamic acid (% Total AA)	NK603 × MON 810	19.42 ± 0.28 (18.30 - 20.35)				(17.37 - 20.18) [15.97,21.95]
	Control	19.01 ± 0.28 (18.13 - 19.44)	0.41 ± 0.14 (-0.59 - 1.31)	0.12,0.70	0.009	
Glycine (% Total AA)	NK603 × MON 810	3.58 ± 0.14 (3.27 - 4.14)				(3.29 - 4.81) [2.38,5.34]
	Control	3.77 ± 0.14 (3.54 - 4.08)	-0.19 ± 0.065 (-0.56 - 0.26)	-0.32,-0.056	0.006	
Histidine (% Total AA)	NK603 × MON 810	3.01 ± 0.058 (2.81 - 3.34)				(2.63 - 3.54) [2.04,3.91]
	Control	3.11 ± 0.058 (2.97 - 3.32)	-0.10 ± 0.032 (-0.23 - 0.18)	-0.17,-0.041	0.001	
Isoleucine (% Total AA)	NK603 × MON 810	3.75 ± 0.050 (3.53 - 3.91)				(3.36 - 3.90) [3.21,4.09]
	Control	3.72 ± 0.050 (3.60 - 3.79)	0.030 ± 0.027 (-0.093 - 0.15)	-0.023,0.083	0.267	

(continued)

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

Table 3. Cont.

Analytical Component	Line	Mean ± S.E. (Range)	Difference (NK603 × MON 810 minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% Total AA)						
Leucine (% Total AA)	NK603 × MON 810	13.20 ± 0.30 (11.87 - 14.06)				(10.71 - 14.36) [9.39,16.13]
	Control	12.82 ± 0.30 (12.11 - 13.20)	0.38 ± 0.14 (-0.49 - 1.15)	0.099,0.65	0.008	
Lysine (% Total AA)	NK603 × MON 810	3.05 ± 0.12 (2.71 - 3.45)				(2.51 - 3.94) [1.94,4.35]
	Control	3.24 ± 0.12 (3.12 - 3.41)	-0.19 ± 0.095 (-0.65 - 0.30)	-0.39,0.011	0.062	
Methionine (% Total AA)	NK603 × MON 810	1.87 ± 0.096 (1.43 - 2.16)				(1.69 - 2.82) [1.23,2.99]
	Control	1.85 ± 0.096 (1.62 - 2.05)	0.027 ± 0.062 (-0.25 - 0.29)	-0.10,0.16	0.664	
Phenylalanine (% Total AA)	NK603 × MON 810	5.10 ± 0.081 (4.75 - 5.34)				(4.39 - 5.41) [4.14,5.87]
	Control	5.01 ± 0.081 (4.83 - 5.18)	0.091 ± 0.034 (-0.12 - 0.33)	0.022,0.16	0.011	

(continued)

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

Table 3. Cont.

Analytical Component	Line	Mean ± S.E. (Range)	Difference (NK603 × MON 810 minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% Total AA)						
Proline (% Total AA)	NK603 × MON 810	9.55 ± 0.13 (9.10 - 10.16)				(8.53 - 10.43) [8.10,10.74]
	Control	9.44 ± 0.13 (9.14 - 9.73)	0.10 ± 0.15 (-0.24 - 0.59)	-0.21,0.42	0.492	
Serine (% Total AA)	NK603 × MON 810	4.85 ± 0.085 (4.56 - 5.18)				(4.40 - 5.48) [4.18,5.44]
	Control	4.86 ± 0.085 (4.70 - 5.09)	-0.016 ± 0.066 (-0.31 - 0.46)	-0.16,0.12	0.809	
Threonine (% Total AA)	NK603 × MON 810	3.13 ± 0.065 (2.91 - 3.34)				(2.97 - 3.80) [2.82,4.01]
	Control	3.24 ± 0.065 (3.07 - 3.61)	-0.12 ± 0.061 (-0.43 - 0.11)	-0.24,0.0077	0.065	
Tryptophan (% Total AA)	NK603 × MON 810	0.57 ± 0.032 (0.49 - 0.66)				(0.48 - 0.78) [0.33,0.89]
	Control	0.59 ± 0.032 (0.49 - 0.71)	-0.019 ± 0.014 (-0.13 - 0.066)	-0.048,0.0092	0.182	

(continued)

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

Table 3. Cont.

Analytical Component	Line	Mean ± S.E. (Range)	Difference (NK603 × MON 810 minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% Total AA)						
Tyrosine (% Total AA)	NK603 × MON 810	3.28 ± 0.12 (2.24 - 3.55)				(2.12 - 3.78) [2.52,4.18]
	Control	3.41 ± 0.12 (2.56 - 3.61)	-0.13 ± 0.16 (-1.18 - 0.91)	-0.46,0.21	0.430	
Valine (% Total AA)	NK603 × MON 810	4.91 ± 0.036 (4.76 - 5.06)				(4.59 - 5.31) [4.43,5.46]
	Control	4.93 ± 0.036 (4.81 - 5.02)	-0.019 ± 0.044 (-0.19 - 0.21)	-0.11,0.073	0.663	

(continued)

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

Table 3. Cont.

Analytical Component	Line	Mean ± S.E. (Range)	Difference (NK603 × MON 810 minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	NK603 × MON 810	10.44 ± 0.15 (10.20 - 10.80)				(8.94 - 12.38) [7.11,13.88]
	Control	9.91 ± 0.15 (9.70 - 10.56)	0.53 ± 0.18 (-0.17 - 0.86)	0.15,0.90	0.008	
16:1 Palmitoleic (% Total FA)	NK603 × MON 810	0.16 ± 0.015 (0.14 - 0.19)				(0.050 - 0.25) [0.00081,0.21]
	Control	0.12 ± 0.015 (0.050 - 0.16)	0.041 ± 0.018 (-0.020 - 0.11)	0.0036,0.078	0.033	
18:0 Stearic (% Total FA)	NK603 × MON 810	1.59 ± 0.084 (1.32 - 1.81)				(1.29 - 2.41) [0.84,2.41]
	Control	1.70 ± 0.085 (1.42 - 1.96)	-0.10 ± 0.064 (-0.27 - 0.072)	-0.24,0.032	0.127	
18:1 Oleic (% Total FA)	NK603 × MON 810	21.58 ± 0.69 (20.68 - 23.26)				(20.99 - 30.49) [18.11,31.92]
	Control	23.27 ± 0.69 (21.63 - 25.38)	-1.68 ± 0.52 (-4.21 - -0.51)	-2.78,-0.58	0.004	

(continued)

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

Table 3. Cont.

Analytical Component	Line	Mean ± S.E. (Range)	Difference (NK603 × MON 810 minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Fatty Acid (% Total FA)						
18:2 Linoleic (% Total FA)	NK603 × MON 810	64.34 ± 0.74 (62.39 - 65.52)				(54.45 - 64.68) [55.98,65.66]
	Control	63.19 ± 0.75 (60.99 - 65.17)	1.15 ± 0.61 (-0.22 - 3.92)	-0.14,2.43	0.076	
18:3 Linolenic (% Total FA)	NK603 × MON 810	1.11 ± 0.019 (1.05 - 1.21)				(0.84 - 1.34) [0.69,1.53]
	Control	1.01 ± 0.019 (0.94 - 1.08)	0.099 ± 0.025 (0.027 - 0.24)	0.049,0.15	<0.001	
20:0 Arachidic (% Total FA)	NK603 × MON 810	0.35 ± 0.0094 (0.33 - 0.37)				(0.32 - 0.45) [0.27,0.47]
	Control	0.36 ± 0.0096 (0.33 - 0.39)	-0.010 ± 0.011 (-0.036 - 0.018)	-0.034,0.014	0.385	
20:1 Eicosenoic (% Total FA)	NK603 × MON 810	0.26 ± 0.030 (0.24 - 0.27)				(0.21 - 0.54) [0.18,0.40]
	Control	0.29 ± 0.031 (0.28 - 0.31)	-0.039 ± 0.040 (-0.065 - -0.019)	-0.13,0.052	0.359	

(continued)

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

Table 3. Cont.

Analytical Component	Line	Mean ± S.E. (Range)	Difference (NK603 × MON 810 minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Fatty Acid (% Total FA)						
22:0 Behenic (% Total FA)	NK603 × MON 810	0.17 ± 0.0072 (0.14 - 0.20)				(0.098 - 0.29) [0.10,0.24]
	Control	0.16 ± 0.0074 (0.14 - 0.19)	0.0074 ± 0.0087 (-0.0098 - 0.039)	-0.011,0.026	0.406	
Fiber						
Acid Detergent Fiber (% dw)	NK603 × MON 810	3.59 ± 0.26 (2.60 - 4.84)				(2.51 - 5.59) [2.24,5.25]
	Control	3.35 ± 0.26 (2.51 - 4.00)	0.24 ± 0.37 (-0.89 - 1.27)	-0.53,1.01	0.519	
Neutral Detergent Fiber (% dw)	NK603 × MON 810	14.07 ± 0.56 (9.44 - 18.91)				(8.72 - 17.75) [4.02,19.77]
	Control	12.28 ± 0.56 (10.37 - 15.79)	1.79 ± 0.77 (-4.16 - 5.80)	0.14,3.45	0.035	
Mineral						
Calcium (% dw)	NK603 × MON 810	0.0052 ± 0.00041 (0.0040 - 0.0072)				(0.0030 - 0.0083) [0.0018,0.0093]
	Control	0.0052 ± 0.00041 (0.0044 - 0.0070)	0.00003 ± 0.00031 (-0.00071 - 0.0017)	-0.00062,0.00069	0.915	

(continued)

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

Table 3. Cont.

Table ix. Statistical Summary of Combined Site Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for NK603 × MON 810 Maize vs. Non-Transgenic Control - continued

Analytical Component	Line	Mean ± S.E. (Range)	Difference (NK603 × MON 810 minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Mineral						
Copper (mg/kg dw)	NK603 × MON 810	1.82 ± 0.095 (1.48 - 2.15)				(0.85 - 3.54) [0,3.69]
	Control	1.68 ± 0.095 (1.53 - 1.91)	0.14 ± 0.11 (-0.18 - 0.47)	-0.081,0.36	0.198	
Iron (mg/kg dw)	NK603 × MON 810	25.06 ± 1.34 (19.70 - 30.97)				(10.58 - 28.04) [4.13,36.90]
	Control	24.27 ± 1.34 (20.28 - 30.65)	0.79 ± 1.03 (-7.60 - 6.00)	-1.40,2.97	0.455	
Magnesium (% dw)	NK603 × MON 810	0.12 ± 0.0044 (0.11 - 0.14)				(0.085 - 0.15) [0.074,0.16]
	Control	0.11 ± 0.0044 (0.11 - 0.12)	0.0079 ± 0.0046 (-0.017 - 0.037)	-0.0017,0.018	0.100	
Manganese (mg/kg dw)	NK603 × MON 810	5.38 ± 0.48 (3.92 - 6.07)				(3.67 - 9.39) [0.82,11.04]
	Control	4.98 ± 0.48 (3.72 - 6.10)	0.41 ± 0.28 (-1.30 - 1.75)	-0.20,1.01	0.171	

(continued)

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

Table 3. Cont.

Analytical Component	Line	Mean ± S.E. (Range)	Difference (NK603 × MON 810 minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Mineral						
Phosphorus (% dw)	NK603 × MON 810	0.34 ± 0.017 (0.31 - 0.38)				(0.25 - 0.38) [0.27,0.37]
	Control	0.32 ± 0.017 (0.31 - 0.34)	0.014 ± 0.017 (-0.034 - 0.062)	-0.021,0.050	0.397	
Potassium (% dw)	NK603 × MON 810	0.39 ± 0.011 (0.36 - 0.43)				(0.29 - 0.47) [0.23,0.50]
	Control	0.38 ± 0.011 (0.36 - 0.40)	0.014 ± 0.015 (-0.030 - 0.047)	-0.018,0.047	0.360	
Zinc (mg/kg dw)	NK603 × MON 810	24.88 ± 1.49 (19.12 - 29.26)				(16.67 - 31.38) [7.52,38.15]
	Control	24.98 ± 1.49 (20.79 - 28.85)	-0.11 ± 1.33 (-4.81 - 5.65)	-2.94,2.72	0.935	
Proximate						
Ash (% dw)	NK603 × MON 810	1.50 ± 0.081 (1.26 - 1.74)				(1.07 - 2.25) [1.06,1.69]
	Control	1.50 ± 0.081 (1.23 - 2.24)	-0.00095 ± 0.11 (-0.70 - 0.42)	-0.23,0.23	0.993	

(continued)

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

Table 3. Cont.

Analytical Component	Line	Mean ± S.E. (Range)	Difference (NK603 × MON 810 minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Proximate						
Carbohydrates (% dw)	NK603 × MON 810	84.23 ± 0.51 (83.24 - 87.01)				(82.85 – 89.50) [79.23,92.35]
	Control	84.73 ± 0.51 (82.73 - 86.68)	-0.50 ± 0.30 (-2.94 - 2.02)	-1.14,0.14	0.116	
Fat, total (% dw)	NK603 × MON 810	3.23 ± 0.088 (2.73 - 3.72)				(2.35 - 4.12) [1.65,4.90]
	Control	3.40 ± 0.088 (3.17 - 3.93)	-0.17 ± 0.12 (-0.60 - 0.54)	-0.42,0.086	0.178	
Moisture (% fw)	NK603 × MON 810	14.28 ± 0.43 (12.50 - 16.70)				(7.02 - 13.70) [0.34,18.55]
	Control	13.72 ± 0.43 (12.30 - 15.10)	0.57 ± 0.40 (-0.80 - 2.30)	-0.28,1.41	0.173	
Protein (% dw)	NK603 × MON 810	11.04 ± 0.55 (8.23 - 12.05)				(6.15 - 11.75) [3.27,15.87]
	Control	10.37 ± 0.55 (8.33 - 12.10)	0.67 ± 0.30 (-1.57 - 3.22)	0.027,1.31	0.042	

(continued)

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

Table 3. Cont.

Analytical Component	Line	Mean ± S.E. (Range)	Difference (NK603 × MON 810 minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Vitamin						
Folic Acid (µg/g dw)	NK603 × MON 810	0.54 ± 0.051 (0.32 - 0.75)				(0.23 - 0.72) [--,--][2]
	Control	0.50 ± 0.051 (0.33 - 0.73)	0.037 ± 0.059 (-0.36 - 0.31)	-0.087,0.16	0.534	
Vitamin B1 (mg/100g dw)	NK603 × MON 810	0.28 ± 0.012 (0.23 - 0.34)				(0.13 - 0.33) [--,--][2]
	Control	0.27 ± 0.012 (0.20 - 0.32)	0.010 ± 0.013 (-0.057 - 0.079)	-0.016,0.037	0.425	
Vitamin B2 (µg/g dw)	NK603 × MON 810	1.06 ± 0.059 (0.84 - 1.25)				(0.70 - 1.21) [--,--][2]
	Control	1.04 ± 0.059 (0.83 - 1.27)	0.016 ± 0.047 (-0.19 - 0.27)	-0.083,0.11	0.738	
Vitamin E (mg/g dw)	NK603 × MON810	0.011 ± 0.00098 (0.0059 - 0.013)				(0.0036 - 0.017) [0,0.024]
	Control	0.012 ± 0.00098 (0.0074 - 0.014)	-0.0011 ± 0.00067 (-0.0034 - 0.0021)	-0.0025,0.00036	0.127	

(continued)

[1]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.
[2]Tolerance Intervals were not calculated due to insufficient data.

Table 3. Cont.

Analytical Component	Line	Mean ± S.E. (Range)	Difference (NK603 × MON 810 minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Antinutrient						
Phytic Acid (% dw)	NK603 × MON 810	0.75 ± 0.073 (0.49 - 1.10)				(0.36 - 1.10) [0.43,0.92]
	Control	0.70 ± 0.073 (0.48 - 0.84)	0.051 ± 0.049 (-0.27 - 0.33)	-0.052,0.15	0.312	
Trypsin Inhibitor (TIU/mg dw)	NK603 × MON 810	2.65 ± 0.16 (2.21 - 3.14)				(1.33 - 4.64) [0.85,4.08]
	Control	2.60 ± 0.16 (2.21 - 3.18)	0.056 ± 0.16 (-0.68 - 0.54)	-0.29,0.41	0.737	

(continued)

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

Table 3. Cont.

Analytical Component	Line	Mean ± S.E. (Range)	Difference (NK603 × MON 810 minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Secondary Metabolite						
Ferulic Acid (% dw)	NK603 × MON 810	0.25 ± 0.0087 (0.21 - 0.30)				(0.18 - 0.37) [0.079,0.42]
	Control	0.24 ± 0.0087 (0.21 - 0.28)	0.017 ± 0.011 (-0.042 - 0.056)	-0.0062,0.040	0.141	
Inositol (µg/g dw)	NK603 × MON 810	1449.76 ± 81.69 (1169.59 - 1800.72)				(1116.12 - 2288.33) [--,--][2]
	Control	1418.74 ± 81.69 (1273.15 - 1712.33)	31.02 ± 103.72 (-542.74 - 398.85)	-190.69,252.73	0.769	
Raffinose (% dw)	NK603 × MON 810	0.073 ± 0.016 (0.029 - 0.16)				(0.028 - 0.19) [0,0.22]
	Control	0.059 ± 0.016 (0.029 - 0.16)	0.014 ± 0.020 (-0.081 - 0.081)	-0.028,0.057	0.481	
p-Coumaric Acid (% dw)	NK603 × MON 810	0.020 ± 0.0024 (0.016 - 0.025)				(0.012 - 0.058) [0,0.075]
	Control	0.016 ± 0.0024 (0.011 - 0.020)	0.0033 ± 0.0025 (-0.0029 - 0.0098)	-0.0021,0.0087	0.210	

[1]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.
[2]Tolerance Intervals were not calculated due to insufficient data.

Table 4. Phenotypic and agronomic data from maize stack NK603 x MON810 and conventional control. Data from field trials at four locations in USA (2002 growing season)

Phenotypic characteristic (units)	LSMeans[2]		99% Tolerance interval[3]		Reference range[4]	
	NK603 × MON 810	Control	LL	UL	Min	Max
Seedling vigor	3	2	1	6	2	5
Early stand count (#/plot)	52	55	41	63	42	69
Days to 50% pollen shed	59	59	49	67	55	66
Days to 50% silking	60	59	52	66	55	66
Stay green	6	6	2	9	3	7
Ear height [6] (cm)	120.1	113.3	51.1	159.8	77.7	133.6
Plant height [6] (cm)	214.6	213.1	157.0	260.4	169.2	232.7
Dropped ears (#/plot)	0	1	0	5	0	7
Stalk lodged plants (#/plot)	1	0	0	11	0	11
Root lodged plants (#/plot)	4	1	0	10	0	11
Final stand count (#/plot)	50	53	36	65	42	63
Grain moisture (%)	18.8	18.0	7.9	28.5	14.6	25.5
Test weight [6] (kg/bu)	24.8	24.9	17.4	32.2	20.2	26.8
Yield [5,6] (MT/ha) - at 15.5% moisture level	9.69	9.07	0.00	19.97	3.68	18.37

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

[1] Data were collected from rows 4 and 5 of each plot.

[2] Least square means of three replicates at each of four sites. Data were rounded to the number of decimal places reported by Field Cooperators.

[3] 99% Tolerance interval with 95% confidence calculated from three replications (except Lewis Hybrids 4830 and Lewis Hybrids 5942 with two replications) of 16 (four references x four sites) commercially available, conventional corn reference substances. LL = lower limit; UL = upper limit. Negative lower limits adjusted to zero. Lower limit of seedling vigor adjusted upward to nearest practical value based on rating scale. Data rounded to number of decimal places reported by Field Cooperators. IA reference substances: Asgrow RX 708, DeKalb 579, Garst 8464 IT, Garst 8590 IT; MO reference substances: DKC60-15, DKC66-50, Lewis Hybrids 4830, Lewis Hybrids 5942; NE reference substances: Mycogen 7474, Mycogen 6431, Northrup King N60-N2, Northrup King N67-H6; OH reference substances: Crow 4908, Crow 504, Seed Consultants SC 1122, and Seed Consultants SC 1118.

[4] Minimum and maximum values from three replications (except Lewis Hybrids 4830 and Lewis Hybrids 5942 with two replications) of 16 (four references x four sites) commercially available, conventional corn reference substances. Min = minimum; Max = maximum. Data rounded to number of decimal places reported by Field Cooperators. IA reference substances: Asgrow RX 708, DeKalb 579, Garst 8464 IT, Garst 8590 IT; MO reference substances: DKC60-15, DKC66-50, Lewis Hybrids 4830, Lewis Hybrids 5942; NE reference substances: Mycogen 7474, Mycogen 6431, Northrup King N60-N2, Northrup King N67-H6; OH reference substances: Crow 4908, Crow 504, Seed Consultants SC 1122, and Seed Consultants SC 1118.

[5] Yield = ([100-grain moisture (%)][12 (in)][43560 ft^2][plot weight (lb)])/([100-15.5 (%)][1 (ft)][1 (a)][# rows][row width (in)][row length (ft)]). Plot weight (lb) not shown.

[6] Data converted into SI units

Table 5. Insect, disease and abiotic stressors incidence coparisation of maize stack NK603 x MON810 to the conventional control. Data from field trials at four locations in USA (2002 growing season)

Site	Observation date (dd/mm/yy)	Stressor or symptom	Level of stressor [1]	Plot differences [2]
IA	06/20/02	None	-	-
	07/17/02	None	-	-
	08/07/02	None	-	-
	08/16/02	None	-	-
	09/10/02	None	-	-
	10/04/02	None	-	-
MO	07/05/02	Heat and drought	Slight	Yes[3]
	07/19/02	Cutworm and armyworm	Slight	Yes[4]
	08/23/02	None	-	-
	09/26/02	None	-	-
NE	06/26/02	Drought	Moderate	No
	07/30/02	Drought	Moderate	No
	07/30/02	Heat	Moderate	No
	08/22/02	Drought	Slight	No
	09/20/02	Drought	Slight	No
OH	06/10/02	None	-	-
	07/01/02	None	-	-
	07/31/02	Heat	Slight	No
	08/22/02	Heat	Slight	No
	08/22/02	Dry weather	Slight	No
	09/16/02	Heat	Moderate	No
	09/16/02	Drought	Moderate	No
	10/08/02	Anthracnose	Slight	No

[1]Level of stressor: none, slight, moderate or severe.

[2]Yes = plants in at least one plot showed an unequal stressor incidence level relative to other plots; No = plants in all plots showed an equal stressor incidence level among all plots.

[3]At the MO location, plants in the north end of the study area showed a higher stressor incidence level than plants in other parts of the study area. Observational differences were related to location within the study area and not a differential response among NK603 × MON 810, the control, or conventional maize hybrids.

[4]At the MO location, plants in one plot had the highest stressor incidence level from cutworm and armyworm. Observational differences were related to location within the study area and not a differential response among NK603 × MON 810, the control, or conventional maize hybrids.

Note: Data were collected from rows 4 and 5 of each plot.