Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Food and environmental risk assessment of herbicide-tolerant genetically modified maize NK603 for food uses, import and processing under Directive 2001/18 /EC (Notification C/ES/00/01)

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 authorised in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC. The request covers scope(s) relevant to the Gene Technology Act. The request does not cover GMOs that VKM already has conducted its final risk assessments on. However, the Agency and NFSA requests VKM to consider whether updates or other changes to earlier submitted assessments are necessary.

The herbicide-tolerant genetically modified maize NK603 from Monsanto (Unique Identifier MONØØ6Ø3-6) is approved under Directive 2001/18/EC as feed since 19 July 2004 (Commission Decision 2004/643/EC). Foods and food ingredients derived from NK603 was authorised under Novel Foods Regulation (EC) No 258/97 3 March 2005 (Commission Decision 2005/448/EC) (EC 2013).

Genetically modified maize NK603 has previously been risk assessed by the VKM Panel on Genetically Modified Organisms (GMOs), commissioned by the NFSA in connection with the national finalisation of the procedure of the notification in 2005 (VKM 2005a). NK603 has also been evaluated by the VKM GMO Panel as a component of several stacked GM maize events (VKM 2005b,c,d,e VKM 2007a,b, VKM 2008a,b, VKM 2009, VKM 2010, VKM 2011, VKM 2012a, VKM 2013a,b). 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 risk assessment of NK603. This updated assessment only covers health and environmental risks with regard to maize NK603 in food products.

The risk assessment of maize NK603 is based on information provided by the applicant in the notification C/EC/00/01, the applications EFSA/GMO/NL/2005/22 and EFSA/GMO/RX/NK603, and scientific comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment also considers other relevant peer-reviewed scientific literature.

The VKM GMO Panel has assessed maize NK603 with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed. The Norwegian Scientific Committee for Food Safety has also decided to take account of the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2011a), the environmental risk assessment of GM plants (EFSA 2010), selection of comparators for the risk assessment of GM plants (EFSA 2011b) and for the post-market environmental monitoring of GM plants (EFSA 2011c).

The scientific risk assessment of maize NK603 include molecular characterisation of the inserted DNA and expression of novel proteins, comparative assessment of agronomic and phenotypic characteristics, nutritional assessments, toxicology and allergenicity, unintended effects on plant fitness, potential for gene transfer, interactions between the GM plant and target and non-target organisms, effects on biogeochemical processes and evaluations of the post-market environmental plan.

It is emphasised that the VKM mandate does not include assessments of contribution to sustainable development, societal utility and ethical considerations, according to the Norwegian Gene Technology Act and Regulations relating to impact assessment pursuant to the Gene Technology Act. These

considerations are therefore not part of the risk assessment provided by the VKM Panel on Genetically Modified Organisms.

The genetically modified maize NK603 has been developed to provide tolerance to glyphosate by the introduction, via particle gun acceleration, of a gene coding for 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* sp. strain CP4 (CP4 EPSPS).

Molecular characterisation

NK603 was developed for tolerance to glyphosate by the introduction of the gene *cp4 epsps* from *Agrobacterium* sp. strain CP4. via a particle acceleration method. The molecular characterisation data indicate that only one copy of the tandem *cp4 epsps* cassette is integrated in the DNA of maize NK603, and that it is inherited as a dominant, single locus trait. Appropriate analyses of the integration site, inserted DNA sequence, flanking regions, and bioinformatics have been performed. No potential new ORFs with sequence similarities to known toxins or allergens were detected. The Chi square analyses of the segregation results for the glyphosate tolerance trait in the progeny are also consistent with a single active site of insertion. The VKM GMO Panel considers the molecular characterisation of maize NK603 as adequate.

Comparative assessment

Comparative analyses of data from field trials located at representative sites and environments in North America and Europe indicate that maize NK603 is compositionally, agronomically and phenotypically equivalent to conventional maize, with the exception of the glyphosate tolerance conferred by the CP4 EPSPS protein.

Food and feed risk assessment

Whole food feeding studies on rats have not indicated any adverse effects of maize NK603. Nutritional feeding studies on broilers, pigs, steers and cows indicate that NK603 is nutritionally equivalent to conventional maize. The CP4 EPSPS protein does not show resemblance to any known toxins or IgE allergens, nor has CP4 EPSPS been reported to cause IgE mediated allergic reactions.
An acute oral toxicity test in mice did not indicate toxic effects of purified *E. coli* produced CP4 EPSPS protein. However, such a test does not provide any additional information about possible adverse effects of maize NK603.

Based on current knowledge, the VKM GMO Panel concludes that maize NK603 is nutritionally equivalent to conventional maize varieties, and that it is unlikely that the CP4 EPSPS protein will introduce a toxic or allergenic potential in food derived from maize NK603 compared to conventional maize.

Environmental assessment

The authorisations of maize NK603 under Directive 2001/18/EC and the Novel Foods Regulation (EC) No 258/97 include import and processing of maize NK603 for food and feed uses. Considering the intended uses of maize NK603, excluding cultivation, the environmental risk assessment has been concerned with accidental release into the environment of viable grains during transportation and processing.

The available data indicate that NK603 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. 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, 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 is nutritionally equivalent to conventional maize varieties, and that it is unlikely that the CP4 EPSPS protein will introduce a toxic or allergenic potential in food derived from maize NK603 compared to conventional maize. The VKM GMO Panel likewise concludes that maize NK603, 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, C/ES/00/01, herbicide-tolerance, CP4 EPSPS, glyphosate, food risk assessment, environmental risk assessment, Directive 2001/18/EC, 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 i EU 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, herbicidtolerante maislinjen NK603 fra Monsanto (unik kode MONØØ6Ø3-6) ble i juli 2004 godkjent til bruk som all annen mais, unntatt som mat eller til dyrking under direktiv 2001/18/EF (Kommisjonsbeslutning 2004/643/EC). Maislinjen ble videre godkjent til bruk som, eller i næringsmidler under Novel Foods-forordningen (EF.) Nr. 258/97 om nye næringsmidler og næringsmiddelingredienser i mars 2005 (Kommisjonsbeslutning 2005/448/EC) (EC 2013). Linjen ble videre notifisert som eksisterende produkt under forordning 1829/2003/EF i 2004. Godkjenningen av NK603 gikk ut i april 2007, og Monsanto har søkt om fornyet godkjenning fram til 2017 (EFSAGMO-RX-NK603). Det er også søkt om godkjenning av NK603 for dyrking og frøavl under forordning 1829/2003/EF (søknad EFSA/GMO/NL/2005/22). I mai 2009 publiserte EFSA en felles risikovurdering for begge disse søknadene (EFSA 2009b). I tillegg foreligger det søknader om godkjenning av hybrider der en eller flere av foreldrelinjene inngår.

Den genmodifiserte maislinjen har tidligere vært vurdert av VKM med hensyn på mulige helseeffekter ved bruk som mat og fôr (VKM 2005a). Risikovurderingen ble utarbeidet på oppdrag fra Mattilsynet i forbindelse med vurdering av markedsadgang i Norge. I juni 2008 anbefalte Miljødirektoratet Miljøverndepartementet å godkjenne NK603 for omsetning som mat og fôr på det norske markedet. Saken ligger fortsatt til behandling i departementet. 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 miljø -risikovurdering av mais NK603. Denne oppdaterte risikovurderingen omfatter kun helse og miljø -risiko knyttet til bruk av mais NK603 som mat, ikke som fôr. VKMs faggruppe for GMO har også risikovurdert en rekke maishybrider der NK603 inngår som en av foreldrelinjene (2005b,c,d,e VKM 2007a,b, VKM 2008a,b, VKM 2009, VKM 2010, VKM 2011, VKM 2012a, VKM 2013a,b).

Risikovurderingen av den genmodifiserte maislinjen NK603 er basert på dokumentasjon gjort tilgjengelig på EFSAs nettside EFSA GMO Extranet, og relevante uavhengige vitenskapelige publikasjoner. 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 2006, 2010, 2011a,b,c) lagt til grunn for vurderingen.

Den vitenskapelige vurderingen omfatter transformeringsprosess og vektorkonstruksjon, karakterisering og nedarving av genkonstruksjoner, komparativ analyse av ernæringsmessig kvalitet, mineraler, kritiske toksiner, metabolitter, antinæringsstoffer, allergener og nye proteiner. Videre er agronomiske egenskaper, potensiale for utilsiktede effekter på fitness, genoverføring og effekter på ikke-målorganismer vurdert.

Det presiseres at VKMs mandat ikke omfatter vurderinger av etikk, bærekraft og samfunnsnytte, i henhold til kravene i den norske genteknologiloven og dens konsekvensutredningsforskrift. Disse aspektene blir derfor ikke vurdert av VKMs faggruppe for genmodifiserte organismer.

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.

Molekylær karakterisering
Mais NK603 ble utviklet for toleranse til glyfosat via introduksjon av genet *cp4 epsps* fra jordbakterien *Agrobacterium* sp. linje CP4. ved hjelp av en partikkelakselerasjonsmetode. Data fra den molekylære karakteriseringen indikerer at det kun er integrert ett eksemplar av ekspresjonskassetten med *cp4 epsps* - genet i genomet til mais NK603, og at genet og egenskapene er dominant og stabilt nedarvet. Passende bioinformatikk og sekvens -analyser er utført av integreringssete i plantens genom, og innsatt og flankerende DNA. Bioinformatikk- analysene har ikke avdekket potensielle nye åpne leserammer med sekvenslikhet til kjente toksiner eller allergener. Segresjonsanalyser for glyfosat-toleranse, ved hjelp av Chi-kvadrat-test, er i overenstemmelse med at det kun er integrert ett eksemplar av ekspresjonskassetten med *cp4 epsps* – genet i mais NK603. VKMs faggruppe for genmodifiserte organismer vurderer den molekylære karakteriseringen av mais NK603 som tilfredsstillende.

Komparative analyser
Feltforsøk i Nord-Amerika og Europa viser små eller ingen signifikante forskjeller mellom den transgene maislinjen NK603 og korresponderende, nær-isogene kontrollhybrider med hensyn på næringsmessige, morfologiske og agronomiske karakterer, med unntak av herbicidtoleranse. Resultatene viser ingen indikasjon på at det innsatte genet NK603 har medført utilsiktede endringer i egenskaper knyttet til vekst og utvikling hos maisplantene

Helserisiko
Fôringsstudier utført på rotter har ikke indikert helseskadelige effekter av mais NK603. Fôringsstudier utført på produksjonsdyrene: broiler, gris, storfe og melkekyr indikerer at mais NK603 er næringsmessig vesentlig lik konvensjonell mais. CP4 EPSPS – proteinet viser ingen likhet til kjente toksiner eller allergener, og er heller ikke rapportert å ha forårsaket IgE-medierte allergiske reaksjoner. I en akutt toksisitetsstudie utført på mus ble det ikke påvist toksiske effekter av renset *E.coli*-produsert CP4 EPSPS-protein. Denne typen studier anses derimot ikke å gi ytterligere informasjon om mulige helseskadelige egenskaper ved mais NK603.

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at mais NK603 er næringsmessig vesentlig lik konvensjonell mais, og at det er lite trolig at CP4 EPSPS proteinet vil introdusere et toksisk eller allergent potensiale i mat basert på mais NK603 sammenliknet med konvensjonelle maissorter.

Miljørisiko
Godkjenningene av genmodifisert mais NK603 omfatter import, prosessering og bruk som/i næringsmidler og fôrvarer, og omfatter ikke dyrking. Med bakgrunn i tiltenkt bruksområde er miljørisikovurderingen avgrenset til mulige effekter av utilsiktet frøspredning i forbindelse med transport og prosessering.

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 utkrysning med dyrkede sorter vurderes av GMO panelet til å være ubetydelig. Ved foreskreven bruk av maislinjen NK603 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 er næringsmessig vesentlig lik konvensjonell mais, og at det er lite trolig at CP4 EPSPS proteinet vil introdusere et toksisk eller allergent potensiale i mat basert på mais NK603 sammenliknet med konvensjonelle maissorter. Faggruppen finner at mais NK603, 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
CaMV	Cauliflower mosaic virus
Codex	Set by The Codex Alimentarius Commission (CAC), an intergovernmental body to implement the Joint FAO/WHO Food Standards Programme. Its principle objective is to protect the health of consumers and to facilitate the trade of food by setting international standards on foods (i.e. Codex Standards)
Cp4 epsps	Gene from *Agrobacterium* sp. strain CP4
CTP	Chloroplast transit peptide
DAP	Days after planting
DN	Norwegian Directorate for Nature Management (Direktoratet for naturforvalting)
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/Community
ECB	European maize borer, *Ostrinia nubilalis*
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 Practices

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 that a given gene occupies on a chromosome
LOD	Limit of detection
LOQ	Limit of quantitation
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 maize borer, *Sesamia nonagrioides*
mRNA	Messenger RNA
MT	Norwegian Food Safety Authority (Mattilsynet)
NDF	Neutral detergent fibre, measure of fibre used for animal feed analysis. NDF measures most of the structural components in plant cells (i.e. lignin, hemicellulose and cellulose), but not pectin
Northern blot	Northern blot is a technique used in molecular biology research to study gene expression by detection of RNA or isolated mRNA in a sample
NTO	Non-target organism
Nicosulfuron	Herbicide for maize that inhibits the activity of acetolactate synthase
Near-isogenic lines	Term used in genetics, defined as lines of genetic codes 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 the part of a reading frame that contains no stop codons
OSL	Overseason leaf
OSR	Overseason root
OSWP	Overseason whole plant
PCR	Polymerase chain reaction, a biochemical technology in molecular biology to amplify a single or a few copies of a piece of DNA
R0	Transformed parent
Rimsulfuron	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 detection of DNA sequences in DNA samples. Combines transfer of electrophoresis-separated DNA fragments to a filter membrane and 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*. The bacterium transfers this DNA fragment into the host 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.

TMDI	Theoretical maximum daily intake (TMDI)
TTC	Threshold of toxicological concern
TI	Trait integration
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, Kernels are filled with clear fluid and the embryo can be seen R3: Milk stage. Kernels are filled with a white, milky fluid. R4: Dough stage. Kernels are filled with a white paste R5: Dent stage. If the genotype is a dent type, the grains are dented R6: Physiological maturity Seedling growth (stages VE and V1); Vegetative growth (stages V2, V3... Vn); Flowering and fertilization (stages VT, R0, and R1); Grain filling and maturity (stages R2 to R6)
Western blot	Analytical technique used to detect specific proteins in the given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native proteins by 3-D structure or denatured proteins by the length of the polypeptide. The proteins are then transferred to a membrane where they are stained with antibodies specific to the target protein.
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

The European Commission has granted the following authorisation for maize NK603 (EC 2013):

- The Commission Decision of 19 July 2004 concerning the placing on the market, in accordance with Directive 2001/18/EC of the European Parliament and of the Council, of a maize product (*Zea mays* L. line NK603) genetically modified for glyphosate tolerance, to be used as any other maize, with the exception of cultivation and uses as or in food (Notification C/ES/00/01).
- The Commission Decision (2005/448/EC) of 3 March 2005, authorising the placing on the market of foods and food ingredients derived from genetically modified line maize NK603 as novel foods or novel food ingredients, under Regulation (EC) No 258/97.

An application for authorisation of maize NK603 for cultivation, food and feed uses under Regulation (EC) No 1829/2003 was submitted by Monsanto in October 2005 (EFSA/GMO/NL/2005/22). The application was submitted jointly with an application for renewal of the authorisation of existing feed materials and food and feed additives produced from maize NK603, notified as existing products under Regulation (EC) 1829/2003 (EFSA/GMO/RX/NK603). The EFSA GMO Panel assessed these two applications together, and published its scientific opinion in May 2009 (EFSA 2009b).

Maize NK603 has previously been assessed as food and feed by the VKM GMO Panel commissioned by the Norwegian Food Safety Authority in connection with the national finalisation of the procedure of the notification in 2005 (VKM 2005a). 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 and environmental risk assessment of NK603. NK603 has also been evaluated by the VKM GMO Panel as a component of several stacked GM maize events (VKM 2005b,c,d,e, VKM 2007a,b, VKM 2008a,b, VKM 2009, VKM 2010, VKM 2011, VKM 2012a, VKM 2013a,b). The 90 days public consultation of the market application of NK603 for cultivation (EFSA/GMO/NL/2005/22) was conducted before VKM's assignment from the Norwegian Environment Agency, and the VKM GMO Panel did not participate in the official hearing.

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. 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://mattilsynet.no/genmodifisering/dispensasjon_fra_godkjenningskrav_i_f_ocirc_rvareforskriften_73820

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 authorised in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC. The request covers scope(s) relevant to the Gene Technology Act.

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

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

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

The Norwegian Food Safety Authority

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

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 authorised in the European Union.

The assignment from NFSA includes food and feed risk 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

Genetically modified maize NK603 was modified 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.

Maize NK603 was evaluated with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed.

The Norwegian Scientific Committee for Food Safety has also decided to take account of the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2011a), the environmental risk assessment of GM plants (EFSA 2010), the selection of comparators for the risk assessment of GM plants (EFSA 2011b), and for the post-market environmental monitoring of GM plants (EFSA 2011c).

The risk assessment of the genetically modified maize NK603 is based on information provided by the applicant in the applications EFSA/GMO/UK/2004/05 and EFSA/GMO/UK/2005/17, and scientific opinions and comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment is also based on a review and assessment of relevant peer-reviewed scientific literature.

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

2 Molecular characterisation

2.1 Information related to the genetic modification

NK603 expresses tolerance to the broad-spectrum agricultural herbicide Roundup (containing the active ingredient glyphosate) by the expression of glyphosate-tolerant 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) enzymes derived from Agrobacterium sp., strain CP4 (CP4 EPSPS). 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. With the expression of the glyphosate-tolerant CP4 EPSPS enzymes in NK603, the continued function of the aromatic amino acid pathway is ensured in the crop, even in the presence of the herbicide.

2.1.1 Description of the methods used for the genetic modification

An agarose gel-isolated MluI restriction fragment of plasmid DNA, designated as PV-ZMGT32L, was introduced into embryogenic maize cells using the particle acceleration method (Klein et al. 1987; Gordon-Kamm et al. 1990). Description for the construction of the restriction fragment and its parent plasmid vector PV-ZMGT32 is presented in Fig. 1 and 2. Using the particle acceleration method, DNA was precipitated onto microscopic gold particles using calcium chloride and spermidine. A drop of the coated particles was then placed onto a plastic macrocarrier, which is accelerated at a high velocity through a barrel by the discharge of compressed helium gas. The macrocarrier hits a metal screen which stops the flight of the macrocarrier but allows continued flight of the DNA-coated particles. The particles penetrate the target plant cells, where the DNA is deposited and incorporated into the cell chromosome.

2.1.2 Nature and source of vector used

NK603 was generated using a particle acceleration transformation system and a gel-isolated MluI fragment, PV-ZMGT32L, containing a 5- enolpyruvylshikimate-3-phosphate synthase (*epsps*) gene from *Agrobacterium* sp. strain CP4 (CP4 EPSPS).

The plant expression plasmid vector, PV-ZMGT32, contains two adjacent plant gene expression cassettes each containing a single copy of the *cp4 epsps* gene (Fig.2). The vector also contains an *nptII* bacterial selectable marker gene encoding kanamycin resistance allowing selection of bacteria containing the plasmid, and an origin of replication (*ori*) necessary for replicating the plasmid in *E. coli*. The agarose gel-isolated MluI restriction fragment of plasmid vector, PV-ZMGT32L, utilised for transformation of NK603 contains only the *cp4 epsps* plant gene expression cassettes and does not contain the *nptII* selectable marker gene or origin of replication.

In both plant gene expression cassettes, the *cp4 epsps* gene is fused to chloroplast transit peptide (CTP) sequences based on sequences isolated from Arabidopsis thaliana EPSPS. The CTP targets the CP4 EPSPS protein to the chloroplast, the location of EPSPS in plants and the site of aromatic amino acid biosynthesis (Kishore & Shah 1988).

In the first gene cassette, the *ctp2-cp4 epsps* coding sequence is under the control of the 5' end of the rice actin 1 sequence (*ract1*) containing the promoter and first intron introduced upstream of the CTP sequence. The second cassette contains the *ctp2-cp4 epsps* sequence under the control of the enhanced CaMV 35S promoter (*e35S*). Located between the *e35S* promoter and the *cp4 epsps* sequence is the intron from the maize hsp70 (heat shock protein), present to increase the levels of gene transcription.

In each cassette, the *cp4 epsps* sequence is joined to the 0.3 kb nopaline synthase 3' non-translated sequence, *NOS 3'*, which provides the mRNA polyadenylation signal. An origin of replication sequence (*ori*) was present in the plasmid PV-ZMGT32 to allow for its replication in E. coli. Following the *ori* region is the sequence for the enzyme neomycin phosphotransferase type II (*nptII*). This enzyme confers resistance to certain aminoglycoside antibiotics (e.g., kanamycin and neomycin) and was used for selection of bacteria during the construction of the plasmid. The coding sequence for the *nptII* gene was derived from the prokaryotic transposon *Tn5* and is present under its own bacterial promoter. The resulting plasmid was designated PV-ZMGT32 (Fig. 2). The plasmid PV-ZMGT32 was amplified in *E. coli* and purified from bacterial lysates. The *cp4 epsps* gene expression linear DNA fragment was isolated from the plasmid prior to maize transformation experiments by digesting PV-ZMGT32 with the restriction enzyme MluI (Fig.1). The plasmid backbone (~2.6 kb) and the CP4 EPSPS expression cassettes (~6.7 kb) were separated by gel electrophoresis and the expression cassette fragment was electroeluted from a gel slice. The agarose gel-isolated MluI restriction fragment utilised in the transformation of NK603 was designated PV-ZMGT32L.

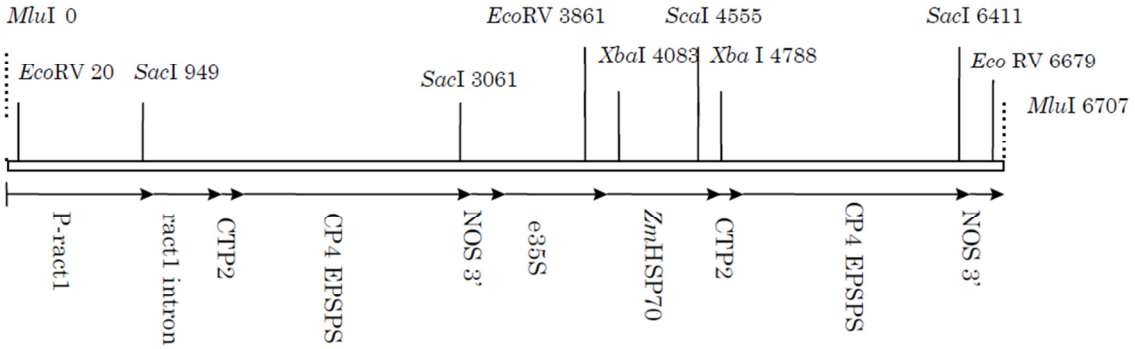

Figure1. Linear map of PV-ZMGT32L

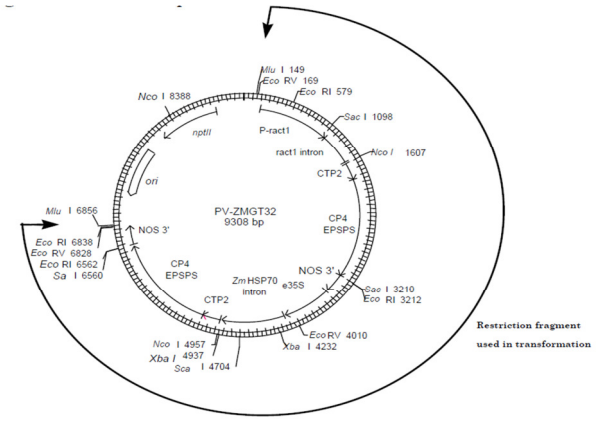

Figure2. Plasmid map of PV-ZMGT32

Table 1. Summary of the DNA components of the plasmid PV-ZMGT32

Genetic Element	Source	Size (kb)	Function
Genetic elements present in the *Mlu*I restriction fragment, designated PV-ZMGT32L, used for transformation:			
<u>*cp4 epsps* Gene Cassette (1)</u>			
P-ract1 / ract1 intron	*Oryza sativa*	1.4	5' region of the *rice actin 1* gene containing the promoter, transcription start site and first intron (McElroy *et al.*, 1990).
ctp2	*Arabidopsis thaliana*	0.2	DNA sequence for chloroplast transit peptide, isolated from *Arabidopsis thaliana* EPSPS, present to direct the CP4 EPSPS protein to the chloroplast, the site of aromatic amino acid synthesis (Klee *et al.*, 1987).
cp4 epsps	*Agrobacterium* sp. strain CP4	1.4	The DNA sequence for CP4 EPSPS, isolated from *Agrobacterium* sp. strain CP4 which imparts tolerance to glyphosate (Harrison *et al.*, 1993; Padgette *et al.*, 1996).
NOS 3'	*Agrobacterium tumefaciens*	0.3	A 3' nontranslated region of the nopaline synthase gene from *Agrobacterium tumefaciens* T-DNA which ends transcription and directs polyadenylation of the mRNA (Fraley, *et al.*, 1983).
<u>*cp4 epsps* Gene Cassette (2)</u>			
e35S	*Cauliflower mosaic virus*	0.6	The cauliflower mosaic virus (CaMV) promoter (Odell *et al.*, 1985) with the duplicated enhancer region (Kay *et al.*, 1985).
Zmhsp70	*Zea mays L.*	0.8	Intron from the maize *hsp70* gene (heat-shock protein) present to stabilize the level of gene transcription (Rochester *et al.*, 1986).
ctp2	*Arabidopsis thaliana*	0.2	DNA sequence for chloroplast transit peptide, isolated from *Arabidopsis thaliana* EPSPS, present to direct the CP4 EPSPS protein to the chloroplast, the site of aromatic amino acid synthesis (Klee *et al.*, 1987).
cp4 epsps	*Agrobacterium* sp. strain CP4	1.4	The DNA sequence for CP4 EPSPS, isolated from *Agrobacterium* sp. Strain CP4 which imparts tolerance to glyphosate (Harrison *et al.*, 1993; Padgette *et al.*, 1996).
NOS 3'	*Agrobacterium tumefaciens*	0.3	A 3' nontranslated region of the nopaline synthase gene from *Agrobacterium tumefaciens* T-DNA which ends transcription and directs polyadenylation of the mRNA (Fraley, *et al.*, 1983).
Genetic elements present in the PV-ZMGT32 plasmid backbone, but <u>not</u> present in the *Mlu*I restriction fragment (PV-ZGMT32L) used for transformation:			
ori	*Escherichia coli*	0.65	The origin of replication from the *E. coli* high copy plasmid pUC119 (Vieira and Messing, 1987).
nptII	*Transposon Tn5*	0.8	The gene for the enzyme neomycin phosphotransferase type II. This enzyme confers resistance to certain aminoglycoside antibiotics and thereby allows for selection of bacteria containing the plasmid (Beck *et al.*, 1982).

2.2 Information relating to the GM plant

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

NK603 was developed for tolerance to glyphosate by the introduction of a gene coding for glyphosate tolerant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* sp. strain CP4; (CP4 EPSPS). Particle acceleration was used to introduce a fragment of DNA isolated from the bacterial plasmid vector PV-ZMGT32.

2.2.2 Information on the sequences actually inserted or deleted

Molecular analysis was performed to characterise the inserted DNA in NK603. Genomic DNA was analysed using Southern blot analysis to determine the insert number (number of integration sites within the maize genome), the copy number (the number of integrated linear DNA fragments used for transformation within one insertion site), the integrity of the inserted promoters, coding regions, and polyadenylation sequences, and the presence or absence of the plasmid backbone sequence. Polymerase chain reaction (PCR) was performed to verify the sequences at the 5' and 3' ends of the insert. Further, PCR analysis and subsequent DNA sequencing of four overlapping products spanning the length of the insert in NK603 were undertaken to confirm the characterisation of the inserted DNA in NK603 (Kesterson et al. 2002a, unpublished Monsanto technical report).

The results showed that NK603 contains only one copy of the complete T-DNA, and that the DNA sequence of the insert is identical to the plasmid DNA sequence used for transformation. The genome of NK603 did not contain any detectable plasmid backbone DNA. Further, the insertion included an inversely linked 217 bp fragment of the enhancer region of the rice actin promoter at the 3' end. The 217 bp fragment did not contain the elements needed to act as a promoter and does not form part of any detectable transcription product. Adjacent to the 217 bp fragment of the rice actin promoter are 305 bp with homology to chloroplast DNA but without homology to known toxins or allergens. The results of the 3' and 5' end bioinformatic analyses, which were updated in 2008, demonstrated that in the unlikely event that any of the junction polypeptides were translated, they do not share sequence similarity or identity to known toxic or allergenic proteins. The results were supported by western blot analyses.

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

The number of integration sites of transgenic DNA in the maize genome was investigated using Southern blot analysis. DNA was extracted from young leaf tissue samples from NK603 and non-transgenic control line B73. NK603 and B73 genomic DNA were digested with the restriction enzyme StuI, which does not cleave within the DNA fragment used for transformation and would cut within the plant genomic DNA. This digestion generates a single fragment containing the inserted DNA and adjacent plant genomic DNA from NK603 if there is a single insertion in the maize genome. Non-transgenic genomic DNA spiked with plasmid PV-ZMGT32 was digested with both StuI and ScaI. Since StuI does not cleave within PV-ZMGT32, a second restriction enzyme, ScaI, was necessary to linearise the plasmid. The plasmid was linearised to facilitate its migration through the gel so that it could serve as an accurate size standard. This result suggested that NK603 contains one insertion of integrated DNA located within a 23 kb StuI restriction fragment. Due to the size of the StuI restriction fragment, it is possible for more than one hybridising band to be located within this fragment. However, the data support the conclusion of a single insert. When NK603 genomic DNA is digested with XbaI, a restriction enzyme that cleaves only once within the transformation cassette, two border

fragments are produced when probed with PV-ZMGT32. If there were more than one insert located within the 23 kb StuI fragment, more than two border fragments would be detected. Therefore, it is very likely that the genome of NK603 contains only one insert located within a 23 kb StuI restriction fragment.

The number of copies of DNA fragments used for transformation inserted into one locus was investigated. NK603 test DNA, non-transgenic control DNA, and non-transgenic control DNA spiked with plasmid PV-ZMGT32 DNA were digested with the restriction enzyme XbaI followed by Southern blotting. The presence of two hybridising bands indicated that NK603 contains only one copy of the transformation cassette at the locus of DNA integration.

The results support the assumption that the two inserted *cp4 epsps* gene cassettes are intact in NK603. Two nucleotide changes have occurred in the second of the two *cp4 epsps* encoding regions of the plant insert compared to the plasmid, one of which is silent and the other resulting in a single amino acid change in the expressed protein. In addition, a 217 bp fragment containing a portion of the enhancer region of the rice actin promoter is inversely linked to the 3' end of the inserted cp4 epsps gene cassettes (Fig. 3).

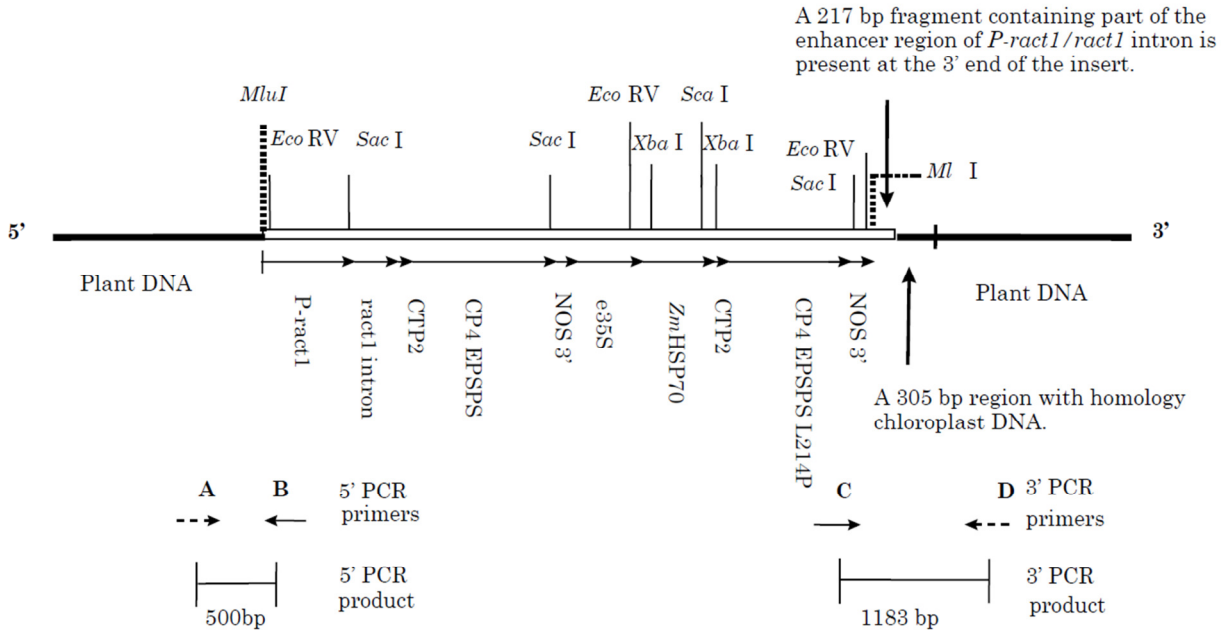

Figure 3. Schematic representation of the NK603 insert.

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

The structural organisation of the insert in NK603 was analysed by Southern blot analysis. Verification of the insert by DNA sequencing was conducted by Kesterson et al. (Kesterson et al. 2002a, unpublished Monsanto technical report). The results of the molecular characterisation established that NK603 contains a single DNA insert, containing one intact copy of the restriction fragment PV-ZMGT32L that was used for transformation. DNA sequencing of the insert showed that two nucleotide changes had occurred in the second of the two *cp4 epsps* coding regions of the plant

insert compared to the plasmid, one of which is silent and the other resulting in a single amino acid change in the expressed protein, which is referred to as CP4 EPSPS L214P. Both nucleotide changes have been present in NK603 since its initial transformation.

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

Not applicable.

2.2.2.4 Chromosomal location(s) of insert(s)

Segregation data for nine generations of NK603 progeny indicated the location and stability of the inserted DNA. Statistical analysis of the segregation data indicates that the insert in NK603 segregates according to standard Mendelian patterns, in agreement with a single insertion site in the nuclear genome.

2.3 Information on the expression of the insert

The levels of CP4 EPSPS and CP4 EPSPS L214P proteins in various tissues of NK603, produced during the 1999 growing season in the Europe and the 2002 growing season in the USA., were estimated using an enzyme-linked immunosorbent assay (ELISA).

In 1999, forage and grain tissues were produced in European field trials at four sites in France and Italy. Four replications were used at each of the four sites. The field trials were conducted using agronomic practices and field conditions typical of commercial maize cultivation in the EU. CP4 EPSPS protein levels were measured in maize forage and grain using a validated direct double antibody sandwich ELISA method. All protein values are expressed as micrograms (µg) of the specific protein per gram (g) of tissue on a fresh weight (fw) basis.

In maize forage, the mean CP4 EPSPS protein level from the four different field sites ranged from 44.2 – 60.9 µg/g fw. The overall mean CP4 EPSPS protein level in maize forage across all four sites was 48.6 µg/g fw. In maize grain, the mean CP4 EPSPS protein level ranged from 2.2 – 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. Control maize samples were below the Limit of Detection (LOD) for CP4 EPSPS protein. The values represent the sum of both CP4 EPSPS and CP4 EPSPS L214P, as the ELISA analytical method recognises both these proteins expressed in NK603. The levels of CP4 EPSPS in forage and grain are presented in Table 2.

Table 2. Summary of the CP4 EPSPS protein levels in tissue from NK603 plants from the European field trials.

Tissue type	Mean (µg/g fw)[1] (SD)	Range (µg/g fw)[2]
Forage [(a,c)]	46.6	43.6-60.9
	(8.3)	
Grain [(b,c)]	8.4	2.2-13.2
	(5.4)	

[1] The mean and standard deviation were calculated from the analyses of tissue samples from NK603 across four sites (n=16).
[2] Minimum and maximum values from the analyses of samples across four sites.
[a] Forage tissue: LOD=0.39 µg/g fw.
[b] Grain tissue: LOD=0.16 µg/g fw.
[c] Values for all non-transgenic control samples were below the LOD specific for that tissue type (n=16).

In 2002, test and control samples were produced in USA field trials in Iowa, Missouri, Ohio and Nebraska. These field sites were located within major maize growing region of the USA and provided a variety of environmental conditions. At each site, three replicate plots containing NK603 and the non-transgenic control were planted using a randomised complete block design. Over season leaf (OSL), over season root (OSR), pollen forage, forage root and grain tissues were collected from each replicated plot at all field sites. CP4 EPSPS protein levels in the different tissue types were estimated using a validated direct double antibody sandwich ELISA method. All protein levels for all tissue types were calculated on a microgram (µg) per gram (g) fresh weight (fw) basis. Moisture content was determined for all tissue types, and all protein levels greater than the assay limit of quantitation (LOQ) were converted to a dry weight (dw) value. The control for this study was a traditional maize hybrid that provided a background matrix for the analytical evaluation of the CP4 EPSPS protein levels in the plant samples. On a dry weight basis, the mean CP4 EPSPS protein levels across four field sites for overseason leaf (OSL-1, OSL-3, OSL-4, and OSL-5) tissues were 410, 300, 430, and 400 µg/g dw, respectively. The mean CP4 EPSPS protein levels across four field sites for overseason root (OSR-1, OSR-3, OSR-4, and OSR-5) tissues were 160, 76, 100, and 99 µg/g dw, respectively. 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.

According to the applicant, the expression levels for forage and grain reported in Tables 2 and 3 are 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, previously reported in Monsanto's notification C/ES/00/01 under Directive 2001/18/EC. In these trials, CP4 EPSPS expression levels ranged from 18.0 to 31.2 µg/g fw for forage and from 6.9 to 15.6 µg/g fw for grain samples, respectively.

Table 3. Summary of the CP4 EPSPS protein levels in tissue from NK603 plants from the USA field trials.

Tissue type	Mean (µg/g fw) (SD)	Range (µg/g fw)	Mean (µg/g ww) (SD)	Range (µg/g dw)
OSL-1	60 (7.2)	49-73	410 (78)	310-560
OSL-3	63 (6.1)	54-76	300 (49)	220-400
OSL-4	96 (29)	71-160	430 (170)	290-890
OSL-5	113 (26)	72-150	400 (96)	280-560
OSR-1	21 (6.7)	13-31	160 (54)	86-250
OSR-3	13 (3.5)	5.8-19	76 (24)	37-120
OSR-4	15 (2.6)	11-20	100 (20)	71-140
OSR-5	17 (3.9)	11-25	99 (32)	60-170
Forage	32 (12)	15-52	100 (56)	32-200
Forage Root	23 (6.8)	12-33	140 (53)	75-220
Pollen	340 (85)	250-460	650 (150)	450-1000
Grain	12 (2.8)	7.5-16	14 (3.2)	8.5

2.3.1 Part of the plant where the insert is expressed

The expression of the CP4 EPSPS proteins occurs throughout the plant since the rice actin and CaMV e35S promoters have been shown to drive constitutive expression of the encoded protein in genetically modified maize.

2.3.2 Expression of potential fusion proteins

Bioinformatics analyses of junctions and flanking regions of the NK603 insert have been performed. DNA sequences were translated from stop codon to stop codon for all reading frames. According to the applicant, none of the encoded polypeptides shared sufficient sequence similarity to known toxins or allergens to indicate any health risk in case they were translated in maize NK603. (Silvanovich et al. 2000; Silvanovich et al. 2002; McCoy et al. 2002c, from unpublished Monsanto technical report).

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

2.4.1 Genetic stability of the insert in NK603

Southern blot analyses were undertaken to investigate the genetic stability of the inserted DNA in maize NK603. Genomic DNA extracted from leaf tissues of the F_1 generation (the progeny from a R0 back cross) and the fifth generation of back-crossing (BC5F1) of maize NK603 and both control

samples were appropriately cleaved, and probed with the full-length *ctp2-CP4 epsps* fragment. No significant differences in banding patterns were observed between DNA extracted from the F_1 generation and the BC5F1 generation of NK603, indicating stability of the inserted DNA in samples spanning five generations. This is consistent with a single site of integration into the genomic DNA of NK603.These results demonstrate that the integrated segment in maize NK603 is stable spanning at least five generations

2.4.2 Phenotypic stability of the GM plant

The inheritance of glyphosate tolerance in the progeny of the original transformant was studied in six generations of backcrossing with the commercial inbred maize line B73. Statistically analysed segregation data for the six generations, based on the frequency of observed versus expected numbers of progeny with tolerance to glyphosate, are presented in Table 4. All generations segregated as anticipated for a single insertion site, except the BC_2F_1 generation. As a possible explanation, the applicant states that the higher number of positive (containing the *cp4 epsps* gene) plants in the BC_2F_1 generation may be explained by gamete selection as a result of high application rates of glyphosate in the generation prior to the BC_2F_1 (i.e., BC_1F_1). Preferential selection for positive gametes has been documented in plants when selective agents such as herbicides have been applied (Sari-Gorla et al. 1994; Touraev et al. 1995). The glyphosate tolerance was studied in three additional generations of progeny, created by self-fertilisation of heterozygous glyphosate tolerant plants (Table 4). In these cases no significant differences were found from the expected 1:2:1 distribution for the homozygous tolerant, heterozygous tolerant and homozygous sensitive plants, respectively.

Table 4. Segregation data and analysis of progeny of NK603.

Generation	Observed			Expected			
	Positive	Negative	Segregating	Positive	Negative	Segregating	ChiSq
BC_0F_1	14	15		14.5	14.5		0.00^{ns}
BC_1F_1	32	23		27.5	27.5		1.16^{ns}
BC_2F_1	135	81		108.0	108.0		13.00**
BC_2F_2	86	26		84.0	28.0		0.12^{ns}
BC_2F_3	9	16	24	12.3	12.3	24.5	2.02#
BC_3F_1	44	45		44.5	44.5		0.00^{ns}
BC_4F_1	127	103		115.0	115.0		2.30^{ns}
BC_4F_3	12	5	17	8.5	8.5	17.0	2.88#
BC_5F_1	26	35		30.5	30.5		1.05^{ns}

** Significant at p=0.01 (chi square = 6.63, 1df)

2.5 Conclusion

NK603 was developed for tolerance to glyphosate by the introduction of the gene *cp4 epsps* from *Agrobacterium* sp. strain CP4. via a particle acceleration method. The molecular characterisation data indicate that only one copy of the tandem *cp4 epsps* cassette is integrated in the DNA of maize NK603, and that it is inherited as a dominant, single locus trait. Appropriate analyses of the integration site, inserted DNA sequence, flanking regions, and bioinformatics have been performed. No potential new ORFs with sequence similarities to known toxins or allergens were detected. The Chi square analyses of the segregation results for the glyphosate tolerance trait in the progeny are also consistent with a single active site of insertion. The VKM GMO Panel considers the molecular characterisation of maize NK603 as adequate.

3 Comparative assessment

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

3.1.1 Experimental design and statistical analysis

Compositional analyses were conducted for forage and grain samples collected from NK603 that was grown in field trials at multiple locations in the USA in 1998 and in the EU in 1999.

USA field trials

Compositional analyses were conducted on key maize tissues produced from 8 field trials in commercial maize-growing regions of the USA in 1998. Two replicated trials were performed in Illinois and Ohio and six non-replicated trials were performed in Iowa, Illinois, Indiana, and Kansas. Six genetically modified test lines, one of which was NK603, and the control line were planted at each site. According to the applicant, maize event NK603 in an LH82 inbred background was crossed with the non-transgenic inbred line, B73, to form the test hybrid. A hybrid formed from the cross of two related non-transgenic inbreds, LH82 and B73, both of which lacked the *cp4 epsps* gene, was used as the non GM-control. No conventional commercial reference varieties were included in the field trials and the comparative assessments. Comparisons with baseline data on commercial maize, compiled from publicly available literature, have been used in the comparisons with maize NK603 for consideration of natural variations.

According to the updated EFSA guidance on risk assessment of food and feed from genetically modified plants (EFSA 2011a), there should be at least three appropriate non-GM reference varieties of the crop that have a known history of safe use at each site. The test of equivalence is used to verify whether the agronomic, phenotypic and compositional characteristics of the GM plant fall within the normal range of natural variation. Such a range of natural variation is estimated from a set of non-GM reference varieties with a history of safe use (EFSA 2011b) and therefore allows comparisons of the GM plant with a similar food or feed produced without the help of genetic modification and for which there is a well-established history of safe use. These requirements were however not in place at the time of submission.

At the non-replicated field sites, there were two blocks (treated and untreated) separated by a minimum buffer of 30 m. The treated block contained six plots, one each for the six test lines, with a minimum separation distance of 13 m between plots, and the untreated block contained a single plot for the control line.

For the non-replicated sites, NK603 and its conventional counterpart were planted in a randomised complete block design with four blocks or replicates per site. Each block contained seven plots, one each for the seven lines, separated by a minimum distance of 3 m. To decrease inadvertent cross-pollination between the lines, buffer rows were planted between plots. Each plot was bordered by a single row of non-transgenic, commercial maize in order to limit edge effects.

The NK603 plots were treated with three applications of a Roundup herbicide during the growth season; at pre-emerge, at early post-emerge (V4-V6 stage) and at late post-emerge (V8-V30). The genetic purity of maize plants was maintained by bagging the tassels and ear shoots at anthesis and by self-pollinating selected plants by hand in the non-replicated sites and all plants in the replicated sites.

Forage was collected at the late dough/early dent stage, and grain was collected at normal kernel maturity. The forage and grain from two of the field sites were of poor quality, caused by weather and *Ustilago*-infestation and were therefore not used in the compositional analysis.

European field trials

Field trials were conducted in the EU in 1999 at four field sites located in France (3) and Italy (1). NK603 and the near-isogenic control were planted at all field sites. In addition to the test and control hybrid, a total of 19 different conventional, commercial hybrids (five per site with one hybrid planted at two sites) were planted as reference varieties. Due to space limitations at two of the sites in France, the test entry maize NK603 and the conventional counterpart were not planted in the same block, and therefore an incomplete block design was used for these two sites. Each plot was bordered by a single row of non-transgenic, commercial maize in order to limit edge effects. Prior to planting, each site prepared a proper seed bed according to local agronomic practices which could include tillage, fertility and pest managements practices. Each field location was scouted for agronomic and pest management needs including pest arthropods, diseases and weeds. Fertiliser, irrigation, agricultural chemicals and other management practices were applied as necessary.

All plants of the test events, control lines and reference hybrids were manually self-pollinated. The NK603 plots were treated with Roundup herbicide (containing 360 g/L glyphosate acid equivalent), with a single broadcast spray application at a rate of 3 L/ha, when a majority of the maize plants were at the 4-6 leaf stage (V4-V6 stage).

Statistical analysis

Analytes that had > 50 % (1998 data) or > 85 % (1999 data) of values at or below the LOD of the assay were excluded from statistical analyses. In 1998, statistical analysis was conducted on 51 components analysed for three sets of comparisons, these being each of the two replicated trials and a combination of trials at different field sites. In 1999, statistical analysis was conducted using a randomised complete block model analysis of variance for three further sets of comparisons, these being each of the two replicated trials and data from the combination of both trials. Therefore, a total of 153 comparisons between NK603 and the non-transgenic control line were made for each year. In these analyses, NK603 was compared to the non-transgenic control line LH82 × B73 to determine statistically significant differences at $p < 0.05$. Since a randomised complete block design was not possible for 1999 replicated trials at two of the sites in the EU, descriptive statistics including means, standard errors (S.E.) and the range of values were reported.

Compositional analysis data for the commercial reference lines were not included in the statistical analysis of variance. However, the range of the reference values was determined for each component.

For each analytical component, tolerance intervals were calculated which were expected to contain, with 95% confidence, 99% of the values expressed in the population of commercial references.

Because negative quantities are not possible, calculated lower tolerance bounds that were negative, were set to zero. A comparison of NK603 to the 99% tolerance interval for the commercial reference varieties was conducted to determine if the range of values for NK603 fell within the population of commercial maize

An additional statistical evaluation of the compositional analyses of NK603 across all four EU trial sites was conducted. This 'meta-analysis' compared the individual standardised differences of NK603 and its conventional control within each site and in a combination of all four field trial sites. The means and standard errors for each analyte at each site were taken from the tables in the original statistical report and were used to generate the data needed for input into the software, Comprehensive Meta Analysis™ (1999). These statistical analyses were conducted for five sets of comparisons: analyses for each of four replicated EU trials and for a combination of trials across all field sites. Therefore, a total of 255 comparisons were made: 51 comparisons for each of these five statistical analyses.

3.2 Compositional Analysis

USA trials (1998)

Compositional analyses were performed for forage and grain tissues collected from NK603 and a non-transgenic control line grown under field conditions at eight locations in the USA in 1998. The compounds analysed were selected based on OECD guidance. Grain samples were analysed for proximates (protein, fat, ash, and moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), amino acid, fatty acid, vitamin E, mineral (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), phytic acid and trypsin inhibitor content. Forage samples were analysed for proximates, ADF and NDF content. Carbohydrate values in forage and grain were estimated by calculation. The same methods were used for the analyses of proximates in forage and grain except for the analysis of fat. In all, 51 different components (7 in forage and 44 in grain) were evaluated as part of the compositional assessment of NK603 (Tables 1 and 2, Appendix). Statistical analysis of the field trial data revealed one significant difference in 1998, for stearic acid in grain. This difference was minor (NK603: 1.95% of total fatty acids; control: 1.86%) and was not observed in 1999. Fifteen components (sodium, 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, 16:1 palmitoleic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma linolenic, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid) are not listed in the tables since they had > 50 % of values below the LOD of the assay.

EU trials (1999)

Compositional analyses were performed for key maize tissues collected from NK603, the non-transgenic control and commercial reference hybrids grown under field conditions at four locations in the EU in 1999. Forage and grain samples were collected from all sites. The composition of maize NK603 and the non-GM control maize was compared with regard to 7 parameters in forage and 44 in grain (Tables 3 and 4, Appendix). The analysed parameters in grain were ash, carbohydrates, fibre, moisture, protein, total fat, amino- and fatty acids, minerals (Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn), vitamin E, phytic acid and trypsin inhibitor, whereas forage was analysed for proximates and neutral and acid detergent fibre. Tissue samples were also collected from a large number of commercially available non-transgenic reference hybrids that were grown at the same locations as the test and control hybrids. Compositional analyses were conducted to measure proximates (protein, fat, ash, carbohydrate, moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), amino acids, fatty acids, vitamin E, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), phytic acid and trypsin inhibitor content of grain; as well as proximates, ADF and NDF content of forage.

The biological relevance of the statistically significant differences was further assessed by performing additional comparisons of the level of these compounds in maize NK603 and conventional non-GM maize lines grown in field trials conducted in 1994-1995 or 1998. No conclusive differences requiring further studies were found. Fifteen components (sodium, 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, 16:1 palmitoleic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma linolenic, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid) are not listed in the tables since > 85 % of the observations were at or below the LOD of the assay.

Statistical evaluation showed that there were no statistically significant differences in 126 of the 153 comparisons made between NK603 and the non-transgenic control. Five of the statistically significant differences identified were assessed in more detail (phosphorus, leucine, zinc, protein and carbohydrate levels in grain). As differences were modest, not observed at other trials, and the levels were within the range identified in conventional maize varieties and reported in the literature, also these statistically significant differences were not considered biologically relevant. Of the 27 comparisons found to be statistically different for the E.U. trials, 5 % or approximately eight differences, were expected to be false positives based on chance alone. Differences that were observed for only one or two of these comparisons, and not consistently across all three comparisons, are unlikely to be of biological significance. The differences between the test product and the control expressed as percent of the control values ranged between 1.13 % and 22.93 %. Furthermore, the range of values for those compositional components associated with the small statistically significant differences were all found to fall within the 99 % tolerance interval for commercial reference varieties planted at the same EU sites in 1999. This indicates, with a confidence level of 95 %; that the levels of key nutrients and other biochemical components for NK603 were within the same population as expected for non-transgenic commercial reference maize used in the study.

An additional statistical meta-analysis was conducted. This evaluation compared the standardised individual differences of NK603 and its control within each site and in a combination of all four field trial sites. This analysis showed that there were no statistically significant differences in 212 of the 255 comparisons made between NK603 and the control. There were no statistically significant differences between NK603 and the control for forage. In grain, statistically significant differences ($p < 0.05$) between NK603 and control were found in 43 of the comparisons. In 39 of these 43 comparisons, the range of values for NK603 lay within the 99% tolerance interval of the population of commercial reference varieties planted at the same EU sites in 1999. This indicates (confidence level of 95%) that the levels of key nutrients and other biochemical components for NK603 were within the sample population expected for traditional maize. The four analytes with statistically significant differences and with values that were outside the 99% tolerance interval were: phosphorous and glycine, protein and carbohydrate. However, these differences were only observed in 1 - 4 of the comparisons and not consistently across all five comparisons, and are unlikely to be of biological significance.

Proximates, fibres, and minerals

The results from the USA and EU field trials demonstrate that the levels of proximate components (protein, ash, carbohydrate), fibres (ADF and NDF), and minerals (calcium, copper, iron, magnesium, manganese, phosphorus, and zinc) in forage and grain of NK603 were comparable to forage and grain of the non-transgenic control (Tables 5 and 6, Appendix). These values were also either within published literature ranges, within the tolerance interval determined for commercial varieties evaluated in the 1999 field trials, or within the range of historical conventional control values determined from previous studies. No measurable differences were observed for the content of fat or potassium in forage data from either 1998 or 1999 field trials or the grain data from the 1998 field trials. Even though the contents of fat and potassium in the grain of NK603 were statistically significantly different from those in the non-transgenic control in data from 1999 field trials, the range of values for both analytes of NK603 fell within the 99% tolerance interval for the commercial varieties grown at

the same field trials. These results show (confidence level of 95%) that the levels of fat and potassium in NK603 lay within the same population as those of non-transgenic, commercially available maize hybrids.

Amino acids
The content of the 18 amino acids measured in grain of maize NK603 was comparable to that in the grain of the non-transgenic control (Table 7, Appendix). The values were either within published literature ranges, within the 99% tolerance interval for commercial varieties evaluated in 1999 field trials, or within the range of historical conventional control values determined from previous studies. No statistically significant differences were observed in the content of the aromatic amino acids, phenylalanine, tyrosine, and tryptophan, between NK603 and the non-transgenic control in either 1998 or 1999 field trials. A majority of the amino acids in NK603 were comparable to the control in the 1999 field trials. However, small statistically significant differences (1.1-6.4%) were observed for alanine, arginine, glutamic acid, histidine, lysine, and methionine ($p < 0.05$). No differences were found for these amino acids in the 1998 field trials, and in all cases the range of values found for NK603 fell within the 99% tolerance interval for conventional commercial varieties grown in the same field trials. The results show that the levels of these amino acids were within the same population as those of non-transgenic, commercially available maize hybrids (confidence level of 95%).

Fatty acids
The content of the fatty acids in grain of NK603 was comparable to that observed in the grain of the non-transgenic control (Table 8, Appendix). These values were either within published literature ranges, within the 99% tolerance interval determined for commercial hybrids evaluated in 1999 field trials, or within the range of historical conventional control values determined from previous studies. Statistically significant differences between NK603 and the non-transgenic control were observed in the levels of 18:1 oleic acid, 16:0 palmitic acid, and 18:0 stearic acid for the 1998 field trials and 20:0 arachidic acid in the 1999 trials. In general, the magnitude of the differences was small (2.6-4.8 %), and in no case found to be significantly different in NK603 when compared to the control. The ranges of values found for these fatty acids were in all cases within the 99% tolerance interval for the commercial varieties grown in the 1999 field trials, demonstrating that NK603 was within the same population as conventional, commercially available maize hybrids.

Phytic acid, trypsin inhibitor, vitamin E and secondary metabolites
The content of phytic acid, trypsin inhibitor, and vitamin E in grain from NK603 was comparable with that observed in grain of the non-transgenic control (Table 9, Appendix). These values were also either within published literature ranges, within the 99% tolerance interval for the commercial varieties in the 1999 field trials, or within the range of historical conventional control values determined from previous studies. No measurable differences in the levels of these analytes between NK603 and the non-transgenic control were observed in the data from the 1998 or 1999 field trials.
The levels of 2-furaldehyde were below the limit of quantitation (<0.5 ppm of fresh weight) for all grain samples analysed from the 1998 field trials. The levels of ferulic acid, *p*-coumaric acid, and raffinose in grain from NK603 were comparable with levels in grain from the non-transgenic control No statistically significant differences were observed in the comparisons conducted for the 1998 field trials. These secondary metabolites were not analysed in the grain samples from the 1999 trials.

3.3 Agronomic and phenotypic characters

Field trials conducted at nine field locations in Germany and France between 2000 and 2002 were used for the comparative assessment of phenotypic and agronomic characteristics of maize NK603 varieties and their appropriate non-GM control maize varieties. The controls did not contain the NK603 insert but had similar background genetics to the respective test hybrids.According to the

applicant, the field sites provided a range of environmental and agronomic conditions representative of a major temperate region for maize production.

The German experiments conducted in 2000 and 2001 were designed as part of larger crop safety (selectivity) trials by addition of plots sown with traditional maize controls. Comparative characterisation of NK603 and conventional maize varieties was conducted using the data collected from the NK603 plots and the traditional comparator plots. The French trials conducted in 2002 were specifically designed to assess phenotypic and agronomic characteristics.

All trials were designed as randomised blocks with four replicates and minimum plot dimensions of 24 m² (3 m × 8 m). NK603 and controls were sown on the same day on dates typical for the region. Maize was planted using local planting methods and equipment (drill with precision plate, row width of 75 cm, sowing depth of 2.5 to 5 cm). After drilling, no glyphosate was applied for weed control in the test or control plots. However, a pre-emergence residual herbicide (e.g. atrazine, alachlor, or acetochlor) was applied over all plots in certain trials to ensure that the location was weed-free. The two central rows of the plots were harvested for yield assessment and pest and disease susceptibility ratings.

During the field trials in Germany and France, phenotypic and agronomic data related to plant growth and development, yield, plant morphology, plant health and pest susceptibility were collected. Evaluations of the plots were made at different stages during the development of the maize plants. Parameters evaluated in these trials included: growth and developmental characteristics (plant stand count, % male and female flowering, days to 50% pollen shed, days to 50% silk emergence), plant and ear morphology (leaf deformities, leaf colour, plant height, % ear deformations, ear height), plant health and vigour indicators and pest susceptibility (vigour, % lodging, susceptibility to insect attack, susceptibility to diseases, susceptibility to applied pesticides), and yield characteristics (% barren plants without an ear, fresh weight at harvest, grain or forage yield, and % moisture or dry matter at harvest).

Where replicated measurements were available for NK603 and control maize, means were calculated per trial site. Where appropriate, for each trial site, statistical comparisons were made between the means for NK603 and control maize plots at the significance level of $p < 0.05$ (Student-Newman-Keuls). For non-replicated measurements and descriptive observations no statistical analysis was performed.

Analyses of variance across trial locations did not indicate differences between NK603 and its conventional counterpart. Where statistically significant differences ($p < 0.05$) were detected, those differences were numerically small and did not show any consistent trend across trials or hybrids tested.

By-site comparisons between the test and control maize for the nine trial locations did not detect statistically significant differences in plant stand count at harvest, number of days from planting to 50% pollen shed, days from planting to 50% silk emergence, % male flowering, % female flowering, % leaf alterations, plant height, % ear deformation and % lodging. Visual inspections of leaf shape, susceptibility to insect attack and disease and susceptibility to damage from any pesticides applied at any location did not detect differences between the test entry and the control. No statistically significant differences were detected for any of the locations in the percentage moisture at harvest (grain or total plant), yield at 15% moisture level (for grain maize) or total plant biomass yield as dry matter (for forage maize).

For each of the five trials planted in Germany in 2000 and 2001, the growth and developmental characteristics, morphological parameters, plant health and vigour indicators, and yield characteristics measured were not statistically different between NK603 and traditional maize, with two exceptions. A significantly higher average ear height for the NK603 test hybrid (96 cm) was noted in one trial

compared to the traditional control (87 cm). In another trial, a significantly higher average fresh weight was noted at harvest for NK603 (86.73 kg/sampled area) compared to the control (80.88 kg/sampled area), which coincided with a numerical (non-significant) higher moisture level at harvest for NK603. These differences were not consistent across the trials, were small in the context of the natural biological variability known for maize, and are unlikely to be related to any ecologically or agronomically relevant characteristics of NK603 that may be different from the control.

For each of the four French trials planted in 2002, the growth and developmental characteristics, morphological parameters, plant health and vigour indicators, and yield characteristics measured were not significantly different between the NK603 test and traditional maize, with seven exceptions. In three out of four French trials, the early plant count (3-5 leaves stage of maize) prior to thinning was significantly higher for traditional maize than for NK603. According to the applicant, the lower germination of the NK603 seeds used in this trial is most likely due to differences in seed quality or differences in seed treatment.

At one French site, the percent of barren plants lacking an ear was slightly higher for NK603 (4%) than for the control (2%). At this site, the ear height at harvest was lower for NK603 (86.6 cm) than for the control (96.2 cm), but this difference was not consistent across the trials. In one French trial, the plant vigour at the 4-8 leaves stage was slightly higher for the NK603 test hybrid (9.0) than for the traditional control (8.3), but this difference was not seen at any of the other sites. These occasional differences were not consistent across sites and are not considered biologically meaningful in terms of pest potential or adverse plant growth and development. No observed differences or statistically significant differences were detected between NK603 and the control for numbers of European maize borer (*Ostrinia nubilalis*) or *Sesamia* sp. larvae in either the stalks or ears or damage done by these borers in any of the five German trials or in the four French trials, respectively. Plant damage was measured as total tunnel length in stalks or ears. At one French site, there was a significant difference between NK603 (56%) and control maize (76%) for the percent of ears damaged by borers, but this difference is thought to have occurred by chance given that the insect pressure at this location was rather high. NK603 does not confer a benefit against insect pests. No differences were noted in disease susceptibility between NK603 and its control hybrid.

3.4 Conclusion

Comparative analyses of data from field trials located at representative sites and environments in North America and Europe indicate that maize NK603 is compositionally, agronomically and phenotypically equivalent to conventional maize, with the exception of the glyphosate tolerance conferred by the CP4 EPSPS protein.

4 Food /feed safety assessment

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

4.1 Effects of processing

Food manufacturing includes many harsh processing steps, e.g. cooking, heating, high pressures, pH treatments, physical shearing, extrusion at high temperatures etc. under which the majority proteins are denatured, which should also apply to the CP4 EPSPS protein.

4.3 Toxicological assessment

4.3.1 Toxicological assessment of the newly expressed proteins

In maize NK603, the only expressed protein product from the inserted gene cassettes is the CP4 EPSPS protein, and the sequence variant CP4 EPSPS L214P, which differs from CP4 EPSPS by one amino acid.

4.3.1.1 Acute oral toxicity testing

*Acute oral exposure of **CP4 EPSPS** protein*
Monsanto has performed an acute toxicity study in mice. Male and female CD-1 mice were dosed by gavage with the CP4-EPSPS protein produced in *E. coli*, purity of the protein was >90 % (Harrison et al. 1996).

The study was conducted in general compliance with the EPA FIFRA GLP (40 CFR Part 160), EU-directive 88/320/EC) and acute oral toxicity guidelines of U.S. EPA and OECD (U.S. EPA Health Effects Test Guidelines. OPPTS 870.1100; Acute Oral Toxicity (August 1998), OECD Guideline for Testing of Chemicals; Method No. 420: Acute Oral Toxicity-Fixed Dose Method; July 17, 1992).

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

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

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

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

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

4.3.1.2 Repeated dose toxicity testing

Not available

4.3.2 Toxicological assessment of the whole GM food/feed

4.3.2.1 Subchronic (90-day) feeding study on rats

A study was undertaken to compare the responses of Sprague Dawley rats (from Charles River Laboratory) when fed either a diet containing grain from maize NK603, or one of several control diets containing either grain from the non-transformed parental variety or from one of a series of non-transformed commercial maize hybrids designated as reference controls (Hammond et al 2004).The study design was adapted from OECD Guideline No. 408 (1981) and the study was conducted in general compliance with OECD Good Laboratory Practice (GLP) guidelines at the Metabolism and Safety Evaluation-Newstead, toxicology laboratory.

Young animals (6 weeks of age, 20 rats/gender/diet group) were assigned to one of the following diets for a period of 13 weeks: a). 11% or 33% (wt/wt) NK603 maize; b). 11% or 33% (wt/wt) parental control maize; or c). 33% (wt/wt) reference control maize grain (six commercial hybrids tested).

There were a total number of 10 diet groups involving 400 rats in the study. In the diets composed of 11% test grain (NK603 or parental line), the formulated diet was supplemented with 22% maize grain from a non-transformed commercial hybrid to bring the total maize content in these groups to the standard 33% used in this experiment. The grain samples and diets were analysed for nutrient composition and pesticide and mycotoxin residues. According to the applicant, the experimental diets showed that they met the specifications for Certified Rodent LabDiet 5002 established by PMI. Levels of heavy metals, aflatoxins, chlorinated hydrocarbons, organophosphate insecticides, and glyphosate were all below detection limits. For chlordane, the Covance Laboratories' limit of detection was higher (250 ppb) than the maximum specified concentration of 50 ppb. All diets were balanced for similar fat and protein content.

Certified rodent diet was administered during week 1 of the study to establish baseline food consumption data for each animal and was followed by administration of the test and control diets from weeks 2 to 14. Food consumption was determined daily for days 1, 2, 3 and during days 4-7 for each of the first two weeks of the study. Following week 2, food consumption was measured weekly. All animals were observed twice daily for morbidity and moribundity. Averaged consumption of feed per animal during the study was ~ 21 grams/kg body weight/day, corresponding to ~ 2.3 and ~ 6.9 grams/kg body weight/day of maize NK603 in the 11% and 33% groups, respectively. Body weight was recorded at weekly intervals. After 5 and 14 weeks, blood and urine were collected from 10 animals/gender/group for blood chemistry, haematology and qualitative and quantitative urine analyses. Coagulation parameters were determined at the terminal blood collection only. After 14 weeks, all animals were sacrificed and necropsied. Specified tissues were collected according to the protocol and organs were weighed. Selected tissues were examined microscopically.

Observations and results:
There were two mortalities during the study, one from the high dose NK603 male group (at day 82) and the second from a reference control male group (at day 86). Neither death was considered to be diet or treatment related. There were no other adverse clinical reactions observed during the course of the study.

The results of the herbicide analysis show that the glyphosate residue in the test grain was 0.09 ppm, slightly above the analytical detection limit of 0.05 ppm (according to FAO / WHO 2006, MRL for Glyphosate in maize is 5 mg/Kg = 5 ppm). The parent and reference lines were not assayed for glyphosate. The growth of male and female rats fed NK603 maize grain was comparable to that of rats fed grain from the parental control and reference control groups. Body weight gain and food consumption were comparable across all groups. At necropsy, no gross lesions were observed that were considered to be test article related. The findings observed were randomly distributed among all groups and were the type commonly observed in rats of this age and strain.

Organ weights were similar across test and control groups and gross pathology findings were unremarkable in test groups and comparable to control groups. The majority of clinical pathology parameters (chemistry, haematology, coagulation, urinalysis) were similar across all groups with only a few exceptions. A closer examination of the few statistically significant differences in clinical parameters demonstrated that these were artefacts of various statistical calculations and were not considered biologically meaningful as they were either not dose related, or the values were within the range of the reference controls. Microscopic examination of tissues showed no differences between rats fed diets containing 33% NK603 maize grain compared to those fed diets with 33% non-transformed grain.

In summary, the rats fed grain composed of maize line NK603 responded similarly to the animals fed parental control and reference control grain diets.

4.3.2.2 Chronic (2-year) feeding study on rats

A two year feeding study performed by Séralini and co-workers (Séralini et al. 2012), was undertaken to compare the response of laboratory rats (Sprague Dawley from Harlan laboratory, Gannat, France) when fed either a diet containing grain from glyphosate-tolerant maize line NK603 or control diets containing grain from the closest isogenic non-transgenic maize control or tap water supplemented with Roundup herbicide (R).

In the study, 100 male and 100 female animals were randomly assigned into 10 equivalent groups. For each sex, one control group had access to water and standard diet from the closest isogenic non-transgenic maize control; six groups were fed with 11, 22 and 33% of NK603 maize either treated or not with Roundup. The final three groups were fed with the control diet and had access to water supplemented with respectively $1.1x10^{-8}$ % of Roundup (0.1 ppb of R or 50 ng/L of glyphosate, the contaminating level of some regular tap waters), 0.09% of R (400 mg/kg, US MRL of glyphosate in some GM feed) and 0.5% of R (2.25 g/L, half of the minimal agricultural working dilution). Animals were monitored twice weekly for measurements of food and water consumption, sample collections, individual body weights, palpation of animals and recordings of any clinical signs e.g. tumours etc.

Observations and results:
According to the study the inclusion of NK603 in the animal feed and/or the use of Roundup herbicide either on maize crops or added in drinking water, led to several severe pathologies among the animals, including an increased mortality rate, higher rate of tumour development, kidney nephropathies and hormone disruptions etc.

The study by Séralinis group has however been thoroughly investigated by regulatory authorities in several countries (e.g. Belgium, Denmark, France, Germany, Italy and the Netherlands) as well as

EFSA and The Norwegian Scientific Committees Panel on GMOs (VKM 2012b), and deemed to be of such poor scientific quality that the data from the study cannot possibly support the stated findings.

4.4 Allergenicity assessment

Most food allergies are mediated by IgE and are characteristic of type-I reactions.
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 and structural similarity to known human IgE-allergens using an array of bioinformatics tools. Sequence homology searches comparing the structure of novel proteins to known IgE-allergens in a database are conducted using various algorithms such as FASTA to predict overall structural similarities. According to FAO/WHO (2001) in cases where a novel protein and a known IgE-allergen have more than 35% identity over a segment of 80 or greater amino acids, IgE cross-reactivity between the novel protein and the allergen should be considered a possibility.

4.4.1 Assessment of allergenicity of the newly expressed protein

The applicant has performed a weight-of-evidence approach (Metcalfe et al. 1996; FAO/WHO 2001; Codex 2003) for an overall assessment of the IgE allergenic potential of the CP4 EPSPS (and CP4 EPSPS L214P) protein which includes:

- 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

The donor organism *Agrobacterium* sp. strain CP4 is a common soil bacteria and has no history of causing allergy. 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; Silvanovich et al. 2002; McCoy et al. 2002c, from unpublished Monsanto technical report).

The CP4 EPSPS protein is considered heat labile (Hammond et al 2011), it is not glycosylated and is readily digested in simulated digestive fluids (Astwood et al 2001; Leach et al 2002a; Leach et al 2002b, from unpublished Monsanto technical report).

The information listed above indicates that the CP4 EPSPS protein in maize NK603 lacks IgE allergenic potential with regard to human and animal health. However, it does not cover allergic reactions that are not IgE mediated, e.g. some gluten-sensitive enteropathies or other enteropathies that are not IgE-mediated.

4.4.2 Assessment of the allergenicity of the whole GM plant

Allergenicity of maize NK603 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 or the parental events, with the exception of the introduced trait, no increased allergenicity is anticipated for maize NK603. Moreover, maize is not considered a common allergenic food.

4.4.3 Assessment of the allergenicity of proteins from the GM plant

The issue of a potentially increased allergenicity of maize NK603 does not appear relevant to the Panel since maize is not considered a common allergenic food. Food allergies to maize are of low frequency and mainly occur in populations of specific geographic areas. There is no reason to expect that the use of maize NK603 will significantly increase the intake and exposure to maize. A possible over-expression of any endogenous protein, which is not known to be allergenic, would be unlikely to alter the overall allergenicity of the whole plant or the allergy risk for consumers

4.4.4 Adjuvanticity

According to the EFSA guidance document for risk assessment of food and feed from GM plants (EFSA 2011b), adjuvants are substances that, when co-administered with an antigen increase the immune response to the antigen and therefore might increase the allergic response. In cases when known functional aspects of the newly expressed protein or structural similarity to known strong adjuvants may indicate possible adjuvant activity, the possible role of these proteins as adjuvants should be considered. As for allergens, interactions with other constituents of the food matrix and/or processing may alter the structure and bioavailability of an adjuvant and thus modify its biological activity. No such associations have been reported for CP4 EPSPS.

4.5 Nutritional assessment of GM food/feed

4.5.1 Intake information/exposure assessment

Maize is the most produced food staple in the world. However, net import of maize staple, e.g. flour, starch and mixed products, in Norway in 2007 was only 7600 tons, corresponding to 4.4 g dry weight/person/day, or an estimated daily energy intake of 0.6 % for adults (Vikse 2009, unpublished). The production of maize porridge for children in 2007 was about 37.5 tons, corresponding to a daily intake of 1.7 g/day, or an estimated daily energy intake of 0.6 % for a 6 month-old child (Vikse 2009, unpublished). In comparison the daily intake in Europe is 8.8 g dry weight/person/day. The expression levels of the CP4 EPSPS proteins in grain from maize NK603 ranged from 8.5 - 18 µg/g dw in the USA field trials, and 2.6-15.5 µg/g dw in the European field trials.
Based on these numbers, and that all foods from maize were derived from maize NK603 grain, the estimated maximum daily intake for an average Norwegian adult of CP4 EPSPS proteins is calculated to be 79.2 µg on a dry weight basis (based on maximum level from US field trials). These levels are several orders of magnitude below levels shown to have no effect in laboratory toxicology tests. 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).

4.5.2 Nutritional assessment of feed derived from the GM plant

Feeding study in Broiler Chickens

Rapidly growing broiler chickens are sensitive to changes in nutrient quality in diets, and therefore serve as a useful model species to evaluate the wholesomeness of protein/amino acid sources. Several nutritional performance studies using diets containing 50-63% grain of non- or glyphosate-sprayed maize NK603 or conventional herbicide-treated non-GM maize have been carried out with rapidly growing broiler chickens, which reach full size within approximately six weeks.

Taylor et al (2003) conducted a study consisting of two 42-day experiments comparing the nutritional value of the glyphosate-tolerant maize event NK603 (experiment 1) and the combined insect resistant and glyphosate tolerant stacked maize event MON810 × NK603 (experiment 2), to their respective non-transgenic controls and to commercial reference maize varieties, when fed to growing broilers. Analyses of the diets showed that mycotoxin levels were low and herbicide residues were below the maximum levels stipulated by the EU legislation on plant protection products (according to FAO / WHO 2006, MRL for Glyphosate in maize is 5 mg/Kg). For each experiment, a randomised complete block design was used with eight dietary treatments in each of five replicated blocks of pens (eight pens for males and eight pens for females per block.

Final live weights and feed conversion were not different ($P > 0.05$) across all treatments in both experiments. In experiment 1, broilers fed diets containing Roundup Ready maize had similar feed conversion adjusted for mortalities to those fed the non-transgenic control and one of the commercial maize diets. Chill weights and thigh, drum, and wing weights were not affected by diets. Differences ($P < 0.05$) were noted for breast meat and fat pad weights across treatments. In experiment 2, the adjusted feed conversion and carcass parameters were not affected by diets. Differences ($P < 0.05$) were noted only for protein content of breast meat. Differences observed in both experiments were consistent with natural variability. Broilers in general performed consistently and had similar carcass yields and meat compositions when fed diets containing maize NK603 or MON810 × NK603 as compared with their respective non-transgenic control and commercial diets; supporting similar feeding values among diets.

A study performed by George B. and co-workers (George B. et al 2001) compared the broiler performance and processing parameters of rapidly growing broiler chickens raised on a diet containing either maize NK603, the non-transformed parental maize line (B73HTxLH82), or one of five commercially available reference maize lines, over approximately 43 days. The broilers, commercial strain (Ross x Ross 508), were one day of age at the beginning of the study, and were separated by gender and randomly assigned to treatments. For each treatment group, there were 100 birds (50 males and 50 females) in 10 pens (10 birds/pen), giving a total of 700 birds. During the course of the study, the birds were examined twice daily for general health, and any abnormal health symptoms were recorded.

Any birds sacrificed were weighed, and any deaths were necropsied to determine the possible cause of death. As much as possible, environmental conditions simulated commercial conditions for raising broilers to market weight (around 2 kg) in approximately 43 days.

From days 1-20, chickens were fed a starter diet containing approximately 55% w/w maize (crude protein ranging from 20.7% –21.9%). From days 20-42, chickens were fed a grower/finisher diet containing approximately 60% w/w maize. These dietary maize concentrations are within the range used by commercial poultry growers in the United States. No growth promotants or other medications were added to the test diets which were provided ad libitum.

The results of the broiler feeding study show that there were no differences in parameters tested between birds fed a diet containing maize NK603 and the non-transformed parental line (B73HT x LH82). In addition, when individual treatment comparisons were made, broiler chickens in general performed and had similar carcass yield and meat composition independent of the diets. The results support the conclusion that there are no differences between the non-transformed control and maize NK603 in terms of the ability to provide adequate nutrition to rapidly growing broiler chickens.

Dela Cruz et al (2012): This study consist of four 42-day experiments comparing the nutritional value of a commercial maize (experiment 1), the combined insect resistant and glyphosate tolerant stacked maize event MON 89034 × NK603 (experiment 2), insect resistant maize MON 89034 (experiment 3) and maize NK603 (experiment 4). A total of 300, day-old straight-run chicks (Cobb 500) were group-brooded. After 7 days of brooding, 280 broiler chicks of almost similar body weight (127-128 g) and health condition were randomly selected and distributed to 28 cages. Four treatments were randomly assigned to the caged birds following a completely randomised design (CRD). Each treatment was replicated 7 times with 10 birds per cage and each cage represented a replicate. Birds fed diets with any of the GM maize: insect resistant, glyphosate tolerant or combined traits – elicited similar growth and efficiency on feed utilisation. However, birds fed diets with the commercial maize performed better than those fed diets with GM maize.

According to the authors maize MON89034 x NK603, maize MON89034 and maize NK603 are statistically equivalent in proximate composition but lower in crude protein compared to commercial maize.
The carcass yield and organoleptic characteristics of cooked broiler meat, except for tenderness, were likewise similar in all groups. Results indicate that the feeding value of maize MON89034 x NK603 was equivalent to any of the single trait GM maize, though slightly lower compared to the commercial maize.

Feeding study in grower and finisher pigs

Fischer et al (2002): This study compares growth performance and carcass quality measurements in growing-finishing pigs provided diets containing maize NK603, the non-transformed control maize, or two commercial reference sources of non-genetically modified maize.

The experiment used 72 animals of each gender with an initial body weight of 22.6 ± 0.03 kg. Animals were allotted to treatments randomly such that both genders received all four maize hybrids. The animals were sacrificed when the average body weight had reached 116 kg. The nutrient composition of the maize was similar across all lines used in the study in terms of crude protein and total digestible nutrients. Maize was incorporated into the diets at 68.1% (grower1), 74.2% (grower2), 78.1% (finisher1) and 81.8% (finisher2), along with de-hulled soybean meal.

Ultrasound measurements of back fat and loin area were taken on the final day of the experiment. Carcass quality measurements were made 24 hours post-mortem. Most parameters measured, including average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (ADG/ADFI), were not affected by diet but showed an expected difference between the males and females. Loin muscle quality and composition (protein, fat and water percentages) were similar among diets and between genders.

No differences were observed between the test and control/reference maize lines used in this study in terms of growth performance and carcass measurements in growing-finishing pigs.

Hyun et al (2004): Two studies were conducted at two locations to evaluate growth performance and carcass characteristics of growing-finishing pigs fed diets containing either maize NK603, a non-transgenic genetically similar control maize (RX670), or two conventional sources of non-transgenic maize (RX740 and DK647). A randomised complete block design (three and four blocks in Studies 1

and 2, respectively) with a 2 × 4 factorial arrangement of treatments (two genders and four maize lines) was used. Study 1 used 72 barrows and 72 gilts (housed in single-gender groups of six; six pens per dietary treatment) with initial and final BW of approximately 22 and 116 kg, respectively. Study 2 used 80 barrows and 80 gilts (housed in single-gender groups of five; eight pens per dietary treatment) with initial and final BW of approximately 30 and 120 kg, respectively. Pigs were housed in a modified open-front building in Study 1 and in an environmentally controlled finishing building in Study 2. The test maize were included at a fixed proportion of the diet in both studies. Animals had ad libitum access to feed and water. Pigs were slaughtered using standard procedures and carcass measurements were taken. In Study 1, overall ADG, ADFI (as-fed basis), and gain:feed (G:F) were not affected ($P > 0.05$) by maize line. In Study 2, there was no effect of maize line on overall ADFI (as-fed basis) or G:F ratio. In addition, overall ADG of barrows fed the four maize lines did not differ ($P > 0.05$); however, overall ADG of gilts fed maize DK647 was greater ($P < 0.05$) than that of pigs fed the other maize lines. There was no effect ($P > 0.05$) of maize line on carcass yield or fatness measurements in either study. Differences between barrows and gilts for growth and carcass traits were generally similar for both studies and in line with previous research. Overall, these results indicate that maize NK603 gives equivalent animal performance to conventional maize for growing pigs.

Feeding study on Angus-continental cross steers

Three experiments were conducted to compare the feeding value of genetically modified maize GA21 and NK603 with non-transgenic hybrids (Erickson et al 2003). Four treatments included two separate reference hybrids (REF), the near-isogenic control hybrid (CON), and the genetically modified maize (Roundup Ready maize, RR: GA21 or NK603), resulting in two pre-planned comparisons of CON vs. RR and RR vs. the average of REF.

In Exp. 1 (GA21), 175 steers (BW = 427 kg) were fed in 25 pens with seven pens per maize hybrid, except control (CON), which contained four pens due to limited quantities of that hybrid.

In Exp. 2 (NK603), 196 steers (BW = 420 kg) were fed in 28 pens with seven pens per maize.

In Exp. 3 (NK603), 200 steers were fed in 20 pens, with a similar treatment design to Exp. 2 and five pens per maize.

All experiments were conducted as completely randomised designs and utilised maize produced at University of Illinois (Exp. 1 and 2) and University of Nebraska (Exp. 3) research farms under identity-preserved protocols.

In all experiments, dry matter intake (DMI), average daily gain (ADG), and feed efficiency were similar for GM-maize (GA21 or NK603) and the reference hybrids ($P > 0.30$). In experiments 1 and 2 the GM-maize did not affect growth performance of steers compared to control maize ($P > 0.25$). In experiment 3, NK603 did not affect ADG or DMI compared to control ($P > 0.15$), nor feed efficiency ($P = 0.08$). No differences were observed between GM-maize and control, or GM-maize and reference hybrids for carcass weight, the longissimus dorsi area, or marbling scores. Fat depth was higher in the animals in experiment 3 compared to experiment 1 and 2, however these differences were attributed to natural variation. The results indicate that both maize GA21 and NK603 are nutritionally similar to conventional maize when fed to finishing feedlot cattle.

Feeding study on lactating cows

Sixteen multiparous Holstein cows averaging 74 d in milk were used in a replicated 4 × 4 Latin square to compare the effects on animal performance of feeding whole plant silage and grain from maize NK603, a non-transgenic control hybrid, and two commercial non-transgenic hybrids (DK647 and RX740), (Ipharraguerre et al 2003).

The grain and silage from the four maize hybrids were produced using the same procedures and under similar agronomic conditions at the University of Illinois. On a dry matter (DM) basis, diets contained 30% maize silage and 27.34% maize grain produced either from maize NK603, a non-transgenic control, or commercial hybrids. Apart from the DM content of silages, the chemical composition of both grain and silage produced from the four maize hybrids were substantially equivalent.

Feeding diets that contained maize NK603 and DK647 hybrids tended to decrease DM intake (DMI) compared with the control non-transgenic and RX740. The intakes of crude protein (CP), acid and neutral detergent fibre, and non-fibre carbohydrates were not different for cows fed maize NK603 and control diets. The RX740 diet resulted in the highest intakes of fibre and CP, whereas the DK647 diet resulted in the lowest intake of CP. These differences in nutrient intake arose from small variations in both the DMI and the chemical composition of feed ingredients and experimental diets. Production of milk and 3.5% fat corrected milk; milk fat, CP, and true protein percentage and yield; milk urea N; milk total solids percentage and yield; and somatic cell count were not affected by treatments. These data indicate that maize NK603 does not affect lactating dairy cows differently than conventional maize.

Grant et al. (2003): Two studies were conducted to evaluate the effect of maize NK603 and maize MON863 on feed intake and milk production compared with a non-transgenic hybrid and two reference hybrids.

In Experiment 1, 16 multiparous Holstein cows were assigned to one of four treatments in replicated 4 x 4 Latin squares with 28-d periods. Diets contained 40%, on a dry matter (DM) basis, of either: 1) maize NK603 silage (GT), 2) non-transgenic control maize silage, or 3) two non-transgenic reference hybrids which are commercially available. Each diet also contained 23% maize grain from the same hybrid that supplied the silage. At ensiling, rapid drying conditions prevailed and NK603 was the last to be harvested which resulted in greater DM content at similar physiological maturity. The 4% fat-corrected milk (FCM) yield and DMI were reduced for cows fed the NK603 diet due to the higher DM content of the NK603 silage (37.1 vs. 33.2 kg/d and 4.05 vs. 3.61% of BW, respectively). There was no effect of NK603 diet on milk composition or efficiency of 4% FCM production that averaged 1.43 kg/kg of DM intake for all diets.

In Experiment 2, 16 multiparous Holstein cows were assigned to one of four treatments in replicated 4 x 4 Latin squares with 21-d periods. Diets contained 26.7% (DM basis) maize grain from either: 1) maize MON863, 2) non-transgenic control maize hybrid, or 3) the same two non-transgenic reference hybrids used in Experiment 1. The 4% FCM yield (34.8 kg/d) and DM intake (4.06% of BW) were unaffected by diet. Efficiency of FCM production (average 1.32 kg/kg of DMI) was not affected by diet. The two studies indicate that neither maize NK603 nor maize MON863 affected performance of lactating dairy cows differently than conventional maize hybrids.

4.6 Conclusion

Whole food feeding studies on rats have not indicated any adverse effects of maize NK603. Nutritional feeding studies on broilers, pigs, steers and cows indicate that NK603 is nutritionally equivalent to conventional maize. The CP4 EPSPS protein does not show resemblance to any known toxins or IgE allergens, nor has CP4 EPSPS been reported to cause IgE mediated allergic reactions.
An acute oral toxicity test in mice did not indicate toxic effects of purified *E. coli* produced CP4 EPSPS protein. However, such a test does not provide any additional information about possible adverse effects of maize NK603.

Based on current knowledge, the VKM GMO Panel concludes that maize NK603 is nutritionally equivalent to conventional maize varieties, and that it is unlikely that the CP4 EPSPS protein will introduce a toxic or allergenic potential in food derived from maize NK603 compared to conventional maize.

5 Environmental risk assessment

5.1 Potential unintended effects on plant fitness due to the genetic modification

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

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

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

It is considered very unlikely that the establishment, spread and survival of maize NK603 would be increased due to the 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. It is considered very unlikely that maize NK603 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 relative to its conventional counterpart. A series of field trials with maize NK603 were carried out across nine locations in France and Germany between 2000 and 2002. Information on phenotypic (e.g. crop

physiology, morphology, development) and agronomic characteristics was provided to assess the agronomic performance of maize NK603 in comparison with its conventional counterpart (see section 3.1). Field data from the European trials did not indicate differences between maize NK603 and the corresponding conventional counterpart for the growth, developmental and morphological characteristics, agronomic parameters, plant vigour, plant health characteristics, susceptibility to pests and diseases. Where statistical differences ($p<0.05$) were detected, those differences were numerically small and did not show any consistent trend across trials or hybrid tested. Moreover, the results were within normal variability expected for maize.

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, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of maize NK603 are unchanged, glyphosate tolerance is 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 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. 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; Nielsen 2003; De Vries & Wackernagel 2002, reviewed in EFSA 2004, 2009a; 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 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 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 gene from NK603 to soil bacteria, no novel property would be introduced into or expressed in the soil microbial communities; as sequence-similar 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 (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 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 Potential interactions between the GM plant and target organisms

Considering the intended uses of maize NK603, excluding cultivation, and the absence of target organisms, potential interactions of the GM plant with target organisms were not considered an issue by the VKM GMO Panel.

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

Considering the intended uses of maize NK603, excluding cultivation, potential interactions of the GM maize with non-target organisms were not considered an issue by the VKM GMO Panel.

5.5 Potential interactions with the abiotic environment and biochemical cycles

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

5.6 Post-market environmental monitoring

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

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

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

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

5.7 Conclusion

The authorisations of maize NK603 under Directive 2001/18/EC and the Novel Foods Regulation (EC) No 258/97 include import and processing of maize NK603 for food and feed uses. Considering the intended uses of maize NK603, excluding cultivation, the environmental risk assessment has been 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

The data provided suggest that maize NK603 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. 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 concludes that 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 Data gaps

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.

7 Conclusions

Molecular characterisation
NK603 was developed for tolerance to glyphosate by the introduction of the gene *cp4 epsps* from *Agrobacterium* sp. strain CP4. via a particle acceleration method.
The molecular characterisation data indicate that only one copy of the tandem *cp4 epsps* cassette is integrated in the DNA of maize NK603, and that it is inherited as a dominant, single locus trait. Appropriate analyses of the integration site, inserted DNA sequence, flanking regions, and bioinformatics have been performed. No potential new ORFs with sequence similarities to known toxins or allergens were detected. The Chi square analyses of the segregation results for the glyphosate tolerance trait in the progeny are also consistent with a single active site of insertion. The VKM GMO Panel considers the molecular characterisation of maize NK603 as adequate.

Comparative assessment
Comparative analyses of data from field trials located at representative sites and environments in North America and Europe indicate that maize NK603 is compositionally, agronomically and phenotypically equivalent to conventional maize, with the exception of the glyphosate tolerance conferred by the CP4 EPSPS protein.

Food and feed risk assessment
Whole food feeding studies on rats have not indicated any adverse effects of maize NK603. Nutritional feeding studies on broilers, pigs, steers and cows indicate that NK603 is nutritionally equivalent to conventional maize. The CP4 EPSPS protein does not show resemblance to any known toxins or IgE allergens, nor has CP4 EPSPS been reported to cause IgE mediated allergic reactions.
An acute oral toxicity test in mice did not indicate toxic effects of purified *E. coli* produced CP4 EPSPS protein. However, such a test does not provide any additional information about possible adverse effects of maize NK603.

Based on current knowledge, the VKM GMO Panel concludes that maize NK603 is nutritionally equivalent to conventional maize varieties, and that it is unlikely that the CP4 EPSPS protein will introduce a toxic or allergenic potential in food derived from maize NK603 compared to conventional maize.

Environmental assessment
The authorisations of maize NK603 under Directive 2001/18/EC and the Novel Foods Regulation (EC) No 258/97 include import and processing of maize NK603 for food and feed uses. Considering the intended uses of maize NK603, excluding cultivation, the environmental risk assessment has been 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

The data provided suggest that maize NK603 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. 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 concludes that 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.

Overall conclusion

Based on current knowledge, the VKM GMO Panel concludes that maize NK603 is nutritionally equivalent to conventional maize varieties, and that it is unlikely that the CP4 EPSPS protein will introduce a toxic or allergenic potential in food derived from maize NK603 compared to conventional maize. The VKM GMO Panel likewise concludes that maize NK603, based on current knowledge, is comparable to conventional maize varieties concerning environmental risk in Norway with the intended usage.

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Appendix

Table 1. Fibre and proximate content of forage from USA field trials (1998)

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Commercial[e] (Range)	Reported[f] (Range)
Ash (% dw)	3.81 ± 0.46 (2.36 - 6.80)	4.02 ± 0.46 (2.46 - 6.28)	-0.21 ± 0.18 (-0.99 - 0.51)	0.249	-0.56, 0.15	(2.03 - 7.49)	(2.9 - 5.1)
Carbohydrates (% dw)	86.71 ± 0.76 (82.68 - 90.32)	87.11 ± 0.76 (83.71 - 90.03)	-0.40 ± 0.43 (-2.41 - 2.72)	0.363	-1.30, 0.50	(81.5 - 88.9)	(84.6 - 89.1)
ADF (% dw)	25.72 ± 1.30 (17.01 - 33.52)	24.84 ± 1.30 (19.53 - 31.83)	0.89 ± 0.88 (-4.15 - 8.05)	0.321	-0.89, 2.66	(17.6 - 34.5)	(21.4 - 29.2)
NDF (% dw)	42.09 ± 1.77 (36.39 - 49.03)	42.45 ± 1.77 (35.44 - 53.24)	-0.35 ± 1.21 (-4.21 - 10.56)	0.774	-2.87, 2.17	(29.6 - 50.7)	(39.9 - 46.6)
Moisture (% fw)	67.02 ± 1.91 (60.30 - 75.00)	66.24 ± 1.91 (61.00 - 73.70)	0.78 ± 0.58 (-2.30 - 5.20)	0.223	-0.63, 2.19	(47.0 - 78.8)	(68.7 - 73.5)
Protein (% dw)	7.14 ± 0.44 (5.57 - 8.98)	6.80 ± 0.44 (5.49 - 8.69)	0.34 ± 0.32 (-1.61 - 2.20)	0.292	-0.31, 0.99	(4.9 - 11.0)	(4.8 - 8.4)
Total fat (% dw)	2.36 ± 0.29 (0.69 - 3.64)	2.17 ± 0.29 (0.61 - 3.42)	0.20 ± 0.18 (-0.77 - 1.53)	0.299	-0.20, 0.59	(0.79 - 3.64)	(1.4 - 2.1)

[a]ADF = acid detergent fiber; NDF = neutral detergent fiber; dw = dry wt.; fw = fresh wt.
[b]The mean of all values.
[c]S.E. = standard error of the mean.
[d]C.I. = confidence interval.
[e]The range of sample values across commercial lines grown in 1998 (Sidhu *et al.*, 1999).
[f]Range for two control lines analyzed in Monsanto Company trials conducted in 1994 and 1995 (Sanders *et al.*, 1996b; 1997a).

Table 2. Amino acid, fatty acid, fibre, mineral, proximate, phytic acid, trypsin inhibitor and vitamin E content of grain from USA field trials (1998)

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Amino acids (% of total)								
Alanine	7.93 ± 0.064 (7.78 - 8.22)	7.89 ± 0.064 (7.65 - 8.17)	0.036 ± 0.036 (-0.13 - 0.28)	0.351	-0.046, 0.12	(7.1 - 8.2)	(6.4-9.9)	(7.3-8.8)
Arginine	4.16 ± 0.10 (3.79 - 4.49)	4.24 ± 0.10 (3.90 - 4.63)	-0.076 ± 0.081 (-0.46 - 0.27)	0.371	-0.25, 0.10	(4.0 - 5.5)	(2.9-5.9)	(3.6-5.0)
Aspartic acid	6.45 ± 0.035 (6.29 - 6.62)	6.40 ± 0.035 (6.18 - 6.56)	0.057 ± 0.040 (-0.17 - 0.19)	0.159	-0.023, 0.14	(6.3 - 7.4)	(5.8-7.2)	(6.3-7.5)
Cystine	2.00 ± 0.065 (1.69 - 2.27)	2.00 ± 0.065 (1.63 - 2.22)	0.0037 ± 0.058 (-0.38 - 0.52)	0.948	-0.12, 0.12	(1.8 - 2.9)	(1.2-1.6)	(1.8-2.7)
Glutamic acid	19.84 ± 0.16 (19.16 - 20.47)	19.81 ± 0.16 (19.19 - 20.41)	0.037 ± 0.12 (-0.44 - 0.54)	0.768	-0.22, 0.30	(17.4 - 20.1)	(12.4-19.6)	(19.5-22.8)
Glycine	3.49 ± 0.073 (3.22 - 3.74)	3.51 ± 0.073 (3.22 - 3.86)	-0.024 ± 0.056 (-0.35 - 0.24)	0.682	-0.15, 0.10	(3.4 - 4.6)	(2.6-4.7)	(3.2-4.2)
Histidine	2.72 ± 0.043 (2.45 - 2.81)	2.74 ± 0.043 (2.56 - 2.88)	-0.018 ± 0.024 (-0.13 - 0.10)	0.477	-0.071, 0.036	(2.6 - 3.4)	(2.0-2.8)	(2.8-3.3)
Isoleucine	3.87 ± 0.037 (3.59 - 4.06)	3.80 ± 0.037 (3.65 - 3.93)	0.065 ± 0.034 (-0.060 - 0.19)	0.071	-0.0063, 0.14	(3.0 - 4.1)	(2.6-4.0)	(3.2-4.3)
Leucine	14.20 ± 0.19 (13.63 - 14.79)	14.07 ± 0.19 (13.59 - 14.60)	0.12 ± 0.14 (-0.52 - 0.99)	0.399	-0.18, 0.42	(11.3 - 14.4)	(7.8-15.2)	(12.6-15.8)

Table 2. Continued

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Lysine	2.69 ± 0.078 (2.42 - 2.96)	2.67 ± 0.078 (2.35 - 3.00)	0.024 ± 0.066 (-0.36 - 0.30)	0.727	-0.12, 0.17	(2.6 - 3.9)	(2.0-3.8)	(2.6-3.5)
Methionine	1.94 ± 0.053 (1.76 - 2.16)	2.03 ± 0.053 (1.74 - 2.21)	-0.097 ± 0.061 (-0.41 - 0.42)	0.125	-0.22, 0.029	(1.6 - 2.9)	(1.0-2.1)	(1.3-2.6)
Phenylalanine	5.32 ± 0.047 (5.18 - 5.52)	5.24 ± 0.047 (5.09 - 5.36)	0.075 ± 0.035 (-0.10 - 0.21)	0.052	-0.00082, 0.15	(4.7 - 5.5)	(2.9-5.7)	(5.0-6.1)
Proline	8.88 ± 0.078 (8.44 - 9.10)	8.96 ± 0.078 (8.59 - 9.26)	-0.076 ± 0.049 (-0.35 - 0.25)	0.129	-0.17, 0.023	(8.0 - 9.9)	(6.6-10.3)	(8.7-10.1)
Serine	4.87 ± 0.043 (4.72 - 5.09)	4.86 ± 0.043 (4.68 - 4.99)	0.010 ± 0.049 (-0.18 - 0.25)	0.839	-0.091, 0.11	(3.5 - 5.5)	(4.2-5.5)	(4.9-6.0)
Threonine	3.37 ± 0.026 (3.26 - 3.46)	3.33 ± 0.026 (3.19 - 3.50)	0.036 ± 0.030 (-0.16 - 0.14)	0.246	-0.026, 0.098	(3.1 - 4.0)	(2.9-3.9)	(3.3-4.2)
Tryptophan	0.53 ± 0.013 (0.44 - 0.58)	0.54 ± 0.013 (0.48 - 0.60)	-0.015 ± 0.014 (-0.11 - 0.072)	0.274	-0.044, 0.014	(0.4 - 0.8)	(0.5-1.2)	(0.4-1.0)
Tyrosine	3.02 ± 0.14 (2.36 - 3.73)	3.25 ± 0.14 (2.43 - 3.64)	-0.23 ± 0.17 (-1.12 - 0.42)	0.195	-0.58, 0.12	(2.1 - 4.0)	(2.9-4.7)	(3.7-4.3)
Valine	4.74 ± 0.032 (4.59 - 4.85)	4.71 ± 0.032 (4.62 - 4.94)	0.031 ± 0.040 (-0.094 - 0.16)	0.450	-0.052, 0.11	(3.9 - 5.5)	(2.1-5.2)	(4.2-5.3)

Table 2. Continued

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Fatty acids (% of total)								
16:0 palmitic acid	9.13 ± 0.083 (8.67 - 9.57)	8.89 ± 0.083 (8.41 - 9.44)	0.24 ± 0.054 (-0.068 - 0.64)	<0.001	0.12, 0.35	(8.8 - 13.8)	(7-19)	(9.9-12.0)
18:0 stearic acid	1.92 ± 0.039 (1.80 - 2.06)	1.83 ± 0.039 (1.67 - 1.98)	0.094 ± 0.025 (-0.066 - 0.19)	0.001	0.041, 0.15	(1.4 - 2.6)	(1-3)	(1.4-2.2)
18:1 oleic acid	22.40 ± 0.24 (21.37 - 23.12)	23.08 ± 0.24 (22.15 - 24.14)	-0.68 ± 0.23 (-2.27 - 0.46)	0.007	-1.15, -0.20	(20.7 - 37.7)	(20-46)	(20.6-27.5)
18:2 linoleic acid	64.62 ± 0.28 (63.79 - 65.80)	64.26 ± 0.28 (63.07 - 65.65)	0.35 ± 0.25 (-1.23 - 2.23)	0.172	-0.17, 0.87	(48.0 - 66.1)	(35-70)	(55.9-66.1)
18:3 linolenic acid	1.11 ± 0.011 (1.07 - 1.17)	1.11 ± 0.011 (1.07 - 1.20)	0.00027 ± 0.014 (-0.13 - 0.060)	0.985	-0.031, 0.032	(0.9 - 1.5)	(0.8-2)	(0.8-1.1)
20:0 arachidic acid	0.36 ± 0.0083 (0.34 - 0.39)	0.37 ± 0.0083 (0.33 - 0.40)	-0.0029 ± 0.0041 (-0.019 - 0.016)	0.489	-0.012, 0.0058	(0.3 - 0.6)	(0.1-2)	(0.3-0.5)
20:1 eicosenoic acid	0.29 ± 0.0072 (0.28 - 0.32)	0.30 ± 0.0072 (0.27 - 0.34)	-0.013 ± 0.0069 (-0.038 - 0.019)	0.066	-0.028, 0.00098	(0.2 - 0.4)	(na)	(0.2-0.3)
22:0 behenic acid	0.16 ± 0.0048 (0.14 - 0.19)	0.16 ± 0.0048 (0.14 - 0.19)	-0.0019 ± 0.0033 (-0.010 - 0.011)	0.564	-0.0085, 0.0047	(0.08 - 0.3)	(na)	(0.1-0.3)

Table 2. Continued

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Minerals								
Calcium (%)	0.0047 ± 0.00026 (0.0037 - 0.0056)	0.0046 ± 0.00026 (0.0033 - 0.0058)	0.00017 ± 0.00016 (-0.00050 -0.00091)	0.286	-0.00016, 0.00050	(0.003 - 0.009)	(0.01-0.1)	(0.003-0.006)
Copper (mg/kg dw)	1.79 ± 0.11 (1.19 - 2.37)	1.90 ± 0.11 (1.50 - 2.33)	-0.11 ± 0.13 (-0.63 - 0.36)	0.399	-0.38, 0.16	(0.9 - 2.8)	(0.9-10)	(na)
Iron (mg/kg dw)	22.71 ± 0.88 (19.08 - 25.94)	22.95 ± 0.88 (18.77 - 26.62)	-0.24 ± 0.49 (-4.42 - 2.18)	0.627	-1.21, 0.74	(11 - 49)	(1-100)	(na)
Magnesium (%)	0.12 ± 0.0023 (0.11 - 0.13)	0.12 ± 0.0023 (0.11 - 0.13)	0.00028 ± 0.0022 (-0.016 - 0.010)	0.901	-0.0046, 0.0052	(0.08 - 0.2)	(0.09-1.0)	(na)
Manganese (mg/kg dw)	6.47 ± 0.54 (4.64 - 9.63)	6.55 ± 0.54 (4.96 - 8.83)	-0.081 ± 0.27 (-0.88 - 1.34)	0.768	-0.65, 0.48	(2.6 - 7.8)	(0.7-54)	(na)
Phosphorus (%)	0.36 ± 0.0053 (0.32 - 0.39)	0.36 ± 0.0053 (0.32 - 0.39)	-0.0033 ± 0.0059 (-0.042 - 0.025)	0.584	-0.016, 0.0093	(0.24 - 0.43)	(0.26-0.75)	(0.31-0.36)
Potassium (%)	0.36 ± 0.0068 (0.35 - 0.39)	0.36 ± 0.0068 (0.34 - 0.41)	-0.0018 ± 0.0068 (-0.039 - 0.022)	0.791	-0.016, 0.012	(0.29 - 0.53)	(0.32-0.72)	(na)
Zinc (mg/kg dw)	28.35 ± 1.42 (20.23 - 33.17)	28.72 ± 1.42 (23.47 - 33.26)	-0.37 ± 0.64 (-4.95 - 4.14)	0.566	-1.66, 0.92	(15 - 33)	(12-30)	(na)

Table 2. Continued

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Fiber and Proximates								
Ash (% dw)	1.45 ± 0.035 (1.28 - 1.62)	1.49 ± 0.035 (1.32 - 1.75)	-0.044 ± 0.043 (-0.29 - 0.21)	0.326	-0.14, 0.048	(0.8 - 1.8)	(1.1-3.9)	(1.2-1.8)
Carbohydrates (% dw)	82.76 ± 0.51 (80.71 - 84.33)	82.29 ± 0.51 (80.23 - 83.70)	0.47 ± 0.29 (-1.60 - 2.01)	0.117	-0.13, 1.08	(83.1 - 89.6)	(na)	(na)
ADF (% dw)	3.72 ± 0.22 (3.14 - 5.17)	3.60 ± 0.22 (2.79 - 4.28)	0.12 ± 0.20 (-0.71 - 1.48)	0.578	-0.32, 0.55	(2.3 - 5.7)	(3.3 - 4.3)	(3.1 - 5.3)
NDF (% dw)	10.06 ± 0.74 (7.89 - 12.53)	10.00 ± 0.74 (8.25 - 15.42)	0.057 ± 0.76 (-3.72 - 2.89)	0.940	-1.47, 1.59	(8.2 - 16.1)	(8.3-11.9)	(9.6 - 15.3)
Moisture (% fw)	11.13 ± 0.51 (9.01 - 13.30)	11.78 ± 0.51 (8.56 - 14.80)	-0.66 ± 0.35 (-2.60 - 2.54)	0.079	-1.40, 0.088	(6.1 - 15.6)	(7-23)	(9.4 - 15.8)
Total fat (%)	3.61 ± 0.12 (2.92 - 3.94)	3.67 ± 0.12 (2.88 - 4.13)	-0.058 ± 0.091 (-0.69 - 0.90)	0.524	-0.24, 0.12	(1.7 - 4.3)	(3.1-5.7, 2.9-6.1)	(2.4-4.2)
Protein (% dw)	12.20 ± 0.59 (10.30 - 14.77)	12.60 ± 0.59 (11.02 - 14.84)	-0.40 ± 0.30 (-1.62 - 1.42)	0.192	-1.03, 0.22	(6.7 - 13.4)	(6.0 - 12.0, 9.7 - 16.1)	(9.0 - 13.6)

Table 2. Continued

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Miscellaneous								
Phytic Acid (%)	0.97 ± 0.032 (0.70 - 1.06)	1.00 ± 0.032 (0.81 - 1.21)	-0.029 ± 0.040 (-0.29 - 0.18)	0.481	-0.12, 0.059	(0.5 - 1.3)	(to 0.9%)	(na)
Trypsin Inhibitor (TIU/mg dw)	3.16 ± 0.30 (2.34 - 5.08)	2.67 ± 0.30 (1.39 - 5.14)	0.49 ± 0.34 (-2.15 - 2.84)	0.149	-0.18, 1.17	(3.40 - 7.18)	(na)	(na)
Vitamin E (mg/g dw)	0.0088 ± 0.00039 (0.0070 - 0.010)	0.0090 ± 0.00039 (0.0064 - 0.011)	-0.00015 ± 0.00028 (-0.0024 - 0.0013)	0.602	-0.00075, 0.00046	(0.006 - 0.022)	(0.017-0.047)	(0.008-0.012)

[a]ADF = acid detergent fiber; NDF = neutral detergent fiber; dw = dry wt.; fw = fresh wt; TIU = trypsin inhibitor units.
[b]The mean of all values.
[c]S.E. = standard error of the mean.
[d]C.I. = confidence interval.
[e]Comm. = commercial. The range of sample values for commercial lines grown in 1998 (Sidhu *et al*., 1999).
[f]Lit. = literature. For amino and fatty acids, Watson, 1982; for all other components, Watson, 1987; protein and fat second values from Jugenheimer, 1976.
[g]Rpt. = reported. For amino and fatty acids, range for five control lines analysed in Monsanto trials conducted between 1993 and 1995 (Sanders and Patzer, 1995; Sanders *et al*., 1996a,b; 1997a,b).
[h]For ash, moisture and total fat, range for five control lines analysed in Monsanto trials conducted between 1993 and 1995 (Sanders and Patzer, 1995; Sanders *et al*., 1996a,b; 1997a,b).
[i]For ADF and NDF, range for three control lines analysed in Monsanto trials conducted between 1994 and 1995 (Sanders *et al*., 1996b; 1997a,b).
[j]For calcium and phosphorus, range for three control lines analysed in Monsanto trials conducted between 1993 and 1995 (Sanders and Patzer, 1995; Sanders *et al*., 1996a; 1997b).

Table 3. Fibre and proximate content of forage from EU field trials (1999)

Component[a]	NK603	Control	Difference (NK603 minus Control)			Comm. Range[e]	Historical[g]
	Mean[b] ± S.E.[c] (Range)	Mean[b] ± S.E.[c] (Range)	Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	[95% T.I.[f] Lower, Upper]	Range
Ash (% dw)	4.38 ± 0.75 (2.82 - 6.44)	4.44 ± 0.76 (3.35 - 5.80)	-0.064 ± 0.40 (-1.89 - 1.52)	0.875	-0.94,0.81	(2.43 - 9.64) [0,12.47]	2.9 - 5.1
Carbohydrates (% dw)	83.67 ± 1.52 (80.43 - 87.53)	83.65 ± 1.53 (80.64 - 85.52)	0.016 ± 1.12 (-3.57 - 2.50)	0.991	-14.26,14.30	(76.50 - 87.29) [75.55,91.37]	84.6 - 89.1
ADF (% dw)	23.53 ± 1.47 (19.27 - 26.13)	22.07 ± 1.50 (19.39 - 26.90)	1.46 ± 1.03 (-3.02 - 6.37)	0.180	-0.78,3.71	(17.54 - 38.31) [9.80,44.43]	21.4 - 29.2
NDF (% dw)	37.34 ± 1.63 (31.77 - 44.35)	37.75 ± 1.68 (34.85 - 41.86)	-0.41 ± 1.43 (-3.84 - 7.19)	0.785	-3.98,3.16	(27.93 - 54.75) [20.77,61.87]	39.9 - 46.6
Moisture (% fw)	67.53 ± 4.16 (61.60 - 75.20)	66.30 ± 4.17 (60.40 - 72.60)	1.23 ± 1.21 (-2.50 - 8.30)	0.495	-14.14,16.60	(56.50 - 80.40) [45.40,96.42]	68.7 - 73.5
Protein (% dw)	8.71 ± 1.12 (6.37 - 10.79)	8.86 ± 1.12 (7.03 - 10.96)	-0.15 ± 0.52 (-1.81 - 1.52)	0.825	-6.73,6.43	(4.98 - 11.56) [4.02,12.46]	4.8 - 8.4
Total fat (% dw)	3.24 ± 0.47 (2.06 - 4.49)	3.05 ± 0.47 (2.09 - 4.02)	0.19 ± 0.47 (-0.49 - 1.63)	0.758	-5.78,6.16	(1.42 - 4.57) [0.84,4.80]	1.4 - 2.1

[a] ADF = acid detergent fiber; NDF = neutral detergent fiber; dw = dry wt.; fw = fresh wt.
[b] The mean of eight replicate values.
[c] S.E. = standard error of the mean.
[d] C.I. = confidence interval.
[e] The range of sample values for commercial lines grown at the same E.U. sites in 1999.
[f] T. I. = tolerance interval, specified to contain 99% of the commercial line population, negative limits set to zero.
[g] Range for control lines analyzed in Monsanto Company trials conducted in 1994 and 1995 (Sanders *et al.*, 1996b; 1997a).

Table 4. Amino Acid, Fatty Acid, Fibre, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain from EU field trials (1999)

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower,Upper)	Comm. Range[e] [95% T.I.[f] Lower, Upper]	Lit.[g] Range	Hist.[h] Range
Amino acids (% of total)								
Alanine	8.04 ± 0.029 (7.87 - 8.18)	7.95 ± 0.031 (7.88 - 8.05)	0.084 ± 0.033 (-0.039 - 0.22)	0.042	0.0040,0.16	(7.38 - 8.13) [7.20,8.35]	6.4-9.9	7.2-8.8
Arginine	4.00 ± 0.062 (3.74 - 4.27)	4.27 ± 0.067 (4.09 - 4.36)	-0.26 ± 0.082 (-0.55 - 0.032)	0.019	-0.46,-0.061	(3.77 - 4.98) [3.45,5.03]	2.9-5.9	3.5-5.0
Aspartic acid	6.45 ± 0.090 (6.27 - 6.96)	6.28 ± 0.092 (6.18 - 6.37)	0.17 ± 0.12 (-0.035 - 0.64)	0.302	-0.39,0.74	(6.02 - 7.51) [5.53,7.61]	5.8-7.2	6.3-7.5
Cystine	1.82 ± 0.062 (1.66 - 1.98)	1.92 ± 0.065 (1.61 - 2.09)	-0.10 ± 0.064 (-0.35 - 0.22)	0.143	-0.24,0.039	(1.68 - 2.51) [1.56,2.43]	1.2-1.6	1.8-2.7
Glutamic acid	19.93 ± 0.43 (18.98 - 20.62)	19.40 ± 0.43 (18.69 - 19.92)	0.54 ± 0.17 (-0.043 - 1.39)	0.009	0.16,0.91	(18.38 - 20.08) [18.03,20.76]	12.4-19.6	18.6-22.8
Glycine	3.44 ± 0.094 (3.23 - 3.64)	3.60 ± 0.095 (3.44 - 3.77)	-0.16 ± 0.057 (-0.31 - 0.044)	0.216	-0.88,0.56	(3.27 - 4.01) [3.06,4.15]	2.6-4.7	3.2-4.2
Histidine	2.65 ± 0.029 (2.56 - 2.74)	2.77 ± 0.030 (2.69 - 2.85)	-0.12 ± 0.027 (-0.22 - -0.027)	0.003	-0.18,-0.056	(2.58 - 3.15) [2.34,3.36]	2.0-2.8	2.8-3.4
Isoleucine	3.77 ± 0.048 (3.54 - 3.97)	3.76 ± 0.050 (3.61 - 3.85)	0.0047 ± 0.050 (-0.17 - 0.16)	0.927	-0.11,0.12	(3.34 - 3.85) [3.35,3.97]	2.6-4.0	3.2-4.3
Leucine	14.02 ± 0.28 (13.38 - 14.71)	13.69 ± 0.28 (13.27 - 13.96)	0.33 ± 0.22 (-0.40 - 0.76)	0.379	-2.51,3.17	(12.18 - 14.34) [11.73,14.76]	7.8-15.2	12.0-15.8

Table 4. Continued

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower,Upper)	Comm. Range[e] [95% T.I.[f] Lower, Upper]	Lit.[g] Range	Hist.[h] Range
Lysine	2.71 ± 0.14 (2.37 - 3.03)	2.83 ± 0.14 (2.56 - 3.20)	-0.12 ± 0.036 (-0.26 - 0.012)	0.015	-0.21,-0.031	(2.58 - 3.67) [2.22,3.68]	2.0-3.8	2.6-3.5
Methionine	1.77 ± 0.033 (1.66 - 1.85)	1.89 ± 0.035 (1.67 - 2.06)	-0.12 ± 0.049 (-0.32 - 0.12)	0.031	-0.22,-0.012	(1.49 - 2.32) [1.39,2.49]	1.0-2.1	1.3-2.6
Phenylalanine	5.28 ± 0.065 (5.13 - 5.46)	5.25 ± 0.065 (5.20 - 5.29)	0.034 ± 0.092 (-0.11 - 0.23)	0.748	-0.37,0.43	(4.85 - 5.54) [4.59,5.61]	2.9-5.7	4.9-6.1
Proline	9.33 ± 0.17 (8.89 - 9.71)	9.16 ± 0.17 (8.83 - 9.31)	0.17 ± 0.094 (-0.13 - 0.40)	0.317	-1.03,1.37	(8.74 - 9.91) [8.61,10.09]	6.6-10.3	8.7-10.1
Serine	4.84 ± 0.11 (4.47 - 5.17)	4.90 ± 0.11 (4.82 - 5.09)	-0.061 ± 0.15 (-0.45 - 0.25)	0.724	-0.73,0.60	(4.41 - 5.22) [4.36,5.19]	4.2-5.5	4.9-6.0
Threonine	3.31 ± 0.045 (3.14 - 3.57)	3.29 ± 0.047 (3.15 - 3.50)	0.018 ± 0.049 (-0.15 - 0.22)	0.763	-0.34,0.38	(3.24 - 3.66) [3.14,3.69]	2.9-3.9	3.3-4.2
Tryptophan	0.58 ± 0.028 (0.49 - 0.64)	0.62 ± 0.028 (0.57 - 0.69)	-0.036 ± 0.018 (-0.13 - 0.027)	0.090	-0.080,0.0075	(0.49 - 0.79) [0.45,0.76]	0.5-1.2	0.4-1.0
Tyrosine	3.24 ± 0.16 (2.11 - 3.65)	3.52 ± 0.18 (2.69 - 3.69)	-0.28 ± 0.23 (-1.53 - 0.37)	0.261	-0.82,0.26	(2.32 - 3.90) [3.00,4.03]	2.9-4.7	3.7-4.3
Valine	4.81 ± 0.085 (4.55 - 5.00)	4.90 ± 0.086 (4.74 - 5.04)	-0.094 ± 0.082 (-0.30 - 0.021)	0.455	-1.13,0.95	(4.65 - 5.29) [4.64,5.38]	2.1-5.2	4.2-5.3

Table 4. Continued

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower,Upper)	Comm. Range[e] [95% T.I.[f] Lower, Upper]	Lit.[g] Range	Hist.[h] Range
Fatty acids (% of total)								
16:0 palmitic acid	8.90 ± 0.24 (8.47 - 9.36)	9.00 ± 0.24 (8.89 - 9.13)	-0.11 ± 0.31 (-0.66 - 0.32)	0.787	-4.03,3.82	(9.12 - 12.62) [7.35,14.72]	7-19	9.9-12.0
18:0 stearic acid	1.73 ± 0.091 (1.59 - 1.88)	1.74 ± 0.091 (1.67 - 1.81)	-0.010 ± 0.061 (-0.083 - 0.10)	0.892	-0.78,0.76	(1.19 - 2.02) [1.02,2.27]	1-3	1.4-2.2
18:1 oleic acid	23.80 ± 0.68 (22.82 - 24.95)	24.20 ± 0.68 (23.52 - 25.56)	-0.40 ± 0.39 (-1.16 - 1.22)	0.494	-5.40,4.60	(20.21 - 34.64) [12.65,39.86]	20-46	20.6-27.5
18:2 linoleic acid	63.73 ± 1.05 (61.94 - 65.25)	63.15 ± 1.05 (61.63 - 64.04)	0.58 ± 0.76 (-1.65 - 1.66)	0.582	-9.03,10.19	(49.72 - 65.98) [44.59,73.50]	35-70	55.9-66.1
18:3 linolenic acid	1.02 ± 0.020 (0.97 - 1.05)	1.09 ± 0.020 (1.05 - 1.12)	-0.065 ± 0.023 (-0.13 - -0.012)	0.215	-0.36,0.23	(0.71 - 1.50) [0.54,1.72]	0.8-2	0.8-1.1
20:0 arachidic acid	0.36 ± 0.012 (0.34 - 0.39)	0.35 ± 0.012 (0.33 - 0.37)	0.010 ± 0.0024 (0.0010 - 0.019)	0.004	0.0043,0.016	(0.31 - 0.74) [0.17,0.64]	0.1-2	0.3-0.5
20:1 eicosenoic acid	0.30 ± 0.012 (0.28 - 0.34)	0.29 ± 0.012 (0.28 - 0.31)	0.013 ± 0.0077 (-0.0034 - 0.042)	0.339	-0.084,0.11	(0.26 - 0.40) [0.21,0.42]	na	0.2-0.3
22:0 behenic acid	0.16 ± 0.015 (0.12 - 0.20)	0.18 ± 0.016 (0.15 - 0.19)	-0.017 ± 0.0091 (-0.034 - 0.0085)	0.318	-0.13,0.099	(0.073 - 0.22) [0.093,0.24]	na	0.1-0.3

Table 4. Continued

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower,Upper)	Comm. Range[e] [95% T.I.[f] Lower, Upper]	Lit.[g] Range	Hist.[h] Range
Minerals								
Calcium (% dw)	0.0053 ± 0.00012 (0.0050 - 0.0058)	0.0053 ± 0.00013 (0.0050 - 0.0058)	-0.00005 ± 0.00015 (-0.00059 - 0.00059)	0.764	-0.00037,0.00028	(0.0039 - 0.0076) [0.0028,0.0082]	0.01-0.1	0.003-0.006
Copper (mg/kg dw)	1.89 ± 0.032 (1.77 - 1.99)	1.83 ± 0.034 (1.69 - 1.97)	0.054 ± 0.046 (-0.15 - 0.27)	0.265	-0.046,0.15	(1.16 - 2.78) [0.45,3.16]	0.9-10	na
Iron (mg/kg dw)	22.73 ± 3.07 (17.43 - 26.91)	21.81 ± 3.08 (18.52 - 25.87)	0.92 ± 0.50 (-1.32 - 2.63)	0.105	-0.25,2.10	(15.42 - 29.34) [10.60,33.63]	1-100	na
Magnesium (% dw)	0.12 ± 0.0076 (0.096 - 0.13)	0.11 ± 0.0076 (0.10 - 0.12)	0.0062 ± 0.0033 (-0.0063 - 0.013)	0.308	-0.035,0.048	(0.089 - 0.15) [0.079,0.16]	0.09-1.0	na
Manganese (mg/kg dw)	6.73 ± 0.83 (5.18 - 7.90)	6.42 ± 0.83 (5.63 - 7.32)	0.31 ± 0.26 (-0.59 - 0.92)	0.440	-2.96,3.58	(3.86 - 10.47) [2.50,12.03]	0.7-54	na
Phosphorus (% dw)	0.36 ± 0.016 (0.31 - 0.39)	0.35 ± 0.016 (0.32 - 0.37)	0.010 ± 0.0097 (-0.033 - 0.035)	0.479	-0.11,0.13	(0.27 - 0.39) [0.27,0.42]	0.26-0.75	0.288-0.363
Potassium (% dw)	0.36 ± 0.0046 (0.34 - 0.38)	0.38 ± 0.0049 (0.36 - 0.39)	-0.021 ± 0.0068 (-0.047 - 0.010)	0.008	-0.035,-0.0062	(0.32 - 0.45) [0.31,0.45]	0.32-0.72	na
Zinc (mg/kg dw)	23.78 ± 5.63 (15.95 - 31.45)	23.21 ± 5.63 (17.87 - 29.88)	0.56 ± 0.76 (-1.93 - 1.73)	0.594	-9.11,10.24	(13.51 - 27.98) [9.89,31.52]	12-30	na

Table 4. Continued

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower,Upper)	Comm. Range[e] [95% T.I.[f] Lower, Upper]	Lit.[g] Range	Hist.[h] Range
Fiber and Proximates								
Ash (% dw)	1.38 ± 0.046 (1.23 - 1.65)	1.34 ± 0.049 (1.25 - 1.50)	0.042 ± 0.067 (-0.25 - 0.40)	0.543	-0.10,0.19	(1.02 - 1.94) [0.77,2.22]	1.1-3.9	1.2-1.8
Carbohydrates (% dw)	82.39 ± 1.31 (80.49 - 84.57)	83.73 ± 1.31 (81.93 - 84.92)	-1.34 ± 0.48 (-2.66 - -0.21)	0.218	-7.39,4.72	(82.18 - 88.14) [79.38,88.91]	na	81.7-86.3
ADF (% dw)	3.21 ± 0.21 (2.63 - 3.87)	3.03 ± 0.21 (2.30 - 3.68)	0.18 ± 0.11 (-0.24 - 0.59)	0.161	-0.095,0.45	(2.46 - 6.33) [1.96,4.71]	3.3 - 4.3	3.1 - 5.3
NDF (% dw)	10.08 ± 0.69 (8.50 - 12.00)	10.57 ± 0.70 (9.35 - 11.63)	-0.49 ± 0.52 (-2.07 - 2.12)	0.362	-1.63,0.64	(8.45 - 14.75) [7.26,14.64]	8.3-11.9	9.6 - 15.3
Moisture (% fw)	7.62 ± 0.10 (7.34 - 7.82)	7.81 ± 0.11 (7.55 - 8.28)	-0.18 ± 0.10 (-0.68 - 0.16)	0.101	-0.41,0.042	(7.43 - 9.94) [7.06,9.53]	7-23	9.4 - 15.8
Total fat (% dw)	4.16 ± 0.078 (3.87 - 4.48)	3.60 ± 0.083 (3.24 - 3.84)	0.57 ± 0.092 (0.20 - 0.82)	<0.001	0.34,0.79	(2.57 - 4.95) [1.55,5.75]	3.1-5.7, 2.9-6.1	2.4-4.2
Protein (% dw)	12.07 ± 1.34 (10.23 - 13.92)	11.34 ± 1.34 (10.13 - 13.05)	0.72 ± 0.50 (-0.071 - 1.75)	0.385	-5.64,7.09	(7.77 - 12.99) [6.84,14.57]	6.0 - 12.0 6.0 - 12.0	9.0 - 13.6

Table 4. Continued

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower,Upper)	Comm. Range[e] [95% T.I.[f] Lower, Upper]	Lit.[g] Range	Hist.[h] Range
Miscellaneous								
Phytic Acid (% dw)	0.79 ± 0.036 (0.51 - 0.89)	0.70 ± 0.038 (0.55 - 0.77)	0.087 ± 0.052 (-0.26 - 0.20)	0.120	-0.026,0.20	(0.48 - 1.12) [0.32,1.18]	to 0.9%	na
Trypsin Inhibitor (TIU/mg dw)	1.56 ± 0.56 (0.54 - 2.57)	1.15 ± 0.57 (0.54 - 2.38)	0.41 ± 0.64 (-0.52 - 2.03)	0.635	-7.74,8.57	(0.54 - 4.13) [0,3.63]	na	na
Vitamin E (mg/g dw)	0.0062 ± 0.0011 (0.0046 - 0.0080)	0.0070 ± 0.0011 (0.0050 - 0.014)	-0.00083 ± 0.0010 (-0.0059 - 0.0030)	0.433	-0.0032,0.0015	(0.0027 - 0.015) [0,0.021]	0.017-0.047	0.008-0.015

[a] ADF = acid detergent fiber; NDF = neutral detergent fiber; dw = dry wt.; fw = fresh wt; TIU = trypsin inhibitor units.
[b] The mean of eight replicate values.
[c] S.E. = standard error of the mean.
[d] C.I. = confidence interval.
[e] Comm. = commercial. The range of sample values for commercial lines grown at the same E.U. sites in 1999.
[f] T.I. = tolerance interval, specified to contain 99% of the commercial line population, negative limits set to zero.
[g] Lit. = literature. For amino and fatty acids, Watson, 1982; for all other components, Watson, 1987; protein and fat second values from Jugenheimer, 1976.
[h] Hist. = historical. Range for control lines analyzed in Monsanto trials conducted between 1993 and 1995 (Sanders and Patzer, 1995; Sanders *et al.*, 1996a,b; 1997a,b,c).

Table 5. Summarised data from USA and EU field trials – fibre, proximates and mineral content of Grain (Ridley et al. 2002)

	1998[a]		1999[b]				
component[c]	NK603 mean (range)[h]	control[d] mean (range)[h]	NK603 mean (range)[h]	control[d] mean (range)[h]	commercial hybrids[e] tolerance interval[f] (range)[h]	lit. (range)	historical[g] (range)[h]
protein	12.20 (10.30–14.77)	12.60 (11.02–14.84)	12.07 (10.23–13.92)	11.34 (10.13–13.05)	6.84, 14.57 (7.77–12.99)	(6.0–12.0)[j] (9.7–16.1)[j]	(9.0–13.6)
total fat	3.61 (2.92–3.94)	3.67 (2.88–4.13)	4.16[k] (3.87–4.48)	3.60 (3.24–3.84)	1.55, 5.75 (2.57–4.95)	(3.1–5.7)[j] (2.9–6.1)[j]	(2.4–4.2)
ash	1.45 (1.28–1.62)	1.49 (1.32–1.75)	1.38 (1.23–1.65)	1.34 (1.25–1.50)	0.77, 2.22 (1.02–1.94)	(1.1–3.9)[j]	(1.2–1.8)
ADF[l]	3.72 (3.14–5.17)	3.60 (2.79–4.28)	3.21 (2.63–3.87)	3.03 (2.30–3.68)	1.96, 4.71 (2.46–6.33)	(3.3–4.3)[j]	(3.1–5.3)
NDF[l]	10.06 (7.89–12.53)	10.00 (8.25–15.42)	10.08 (8.50–12.00)	10.57 (9.35–11.63)	7.26, 14.64 (8.45–14.75)	(8.3–11.9)[j]	(9.6–15.3)
carbohydrates	82.76 (80.71–84.33)	82.29 (80.23–83.70)	82.39 (80.49–84.57)	83.73 (81.93–84.92)	79.38, 88.91 (82.18–88.14)	not reported in this form	(81.7–86.3)
moisture	11.13 (9.01–13.30)	11.78 (8.56–14.80)	7.62 (7.34–7.82)	7.81 (7.55–8.28)	7.06, 9.53 (7.43–9.94)	(7–23)[j]	(9.4–15.8)
calcium	0.0047 (0.0037–0.0056)	0.0046 (0.0033–0.0058)	0.0053 (0.0050–0.0058)	0.0053 (0.0050–0.0058)	0.0028, 0.0082 (0.0039–0.0076)	(0.01–0.1)[j]	(0.003–0.006)
copper	1.79 (1.19–2.37)	1.90 (1.50–2.33)	1.89 (1.77–1.99)	1.83 (1.69–1.97)	0.45, 3.16 (1.16–2.78)	(0.9–10)[j]	na[m]
iron	22.71 (19.08–25.94)	22.95 (18.77–26.62)	22.73 (17.43–26.91)	21.81 (18.52–25.87)	10.60, 33.63 (15.42–29.34)	(1–100)[j]	na
magnesium	0.12 (0.11–0.13)	0.12 (0.11–0.13)	0.12 (0.096–0.13)	0.11 (0.10–0.12)	0.079, 0.16 (0.089–0.15)	(0.09–1.0)[j]	na
manganese	6.47 (4.64–9.63)	6.55 (4.96–8.83)	6.73 (5.18–7.90)	6.42 (5.63–7.32)	2.50, 12.03 (3.86–10.47)	(0.7–54)[j]	na
phosphorus	0.36 (0.32–0.39)	0.36 (0.32–0.39)	0.36 (0.31–0.39)	0.35 (0.32–0.37)	0.27, 0.42 (0.27–0.39)	(0.26–0.75)[j]	(0.288–0.363)
potassium	0.36 (0.35–0.39)	0.36 (0.34–0.41)	0.36[k] (0.34–0.38)	0.38 (0.36–0.39)	0.31, 0.45 (0.32–0.45)	(0.32–0.72)[j]	na
zinc	28.35 (20.23–33.17)	28.72 (23.47–33.26)	23.78 (15.95–31.45)	23.21 (17.87–29.88)	9.89, 31.52 (13.51–27.98)	(12–30)[j]	na

[a] Data from five nonreplicated U.S. sites and two replicated U.S. sites; NK603 grain harvested from plants treated with Roundup Ultra herbicide. [b] Data from two replicated EU sites; NK603 grain harvested from plants treated with Roundup (MON 52276) herbicide. [c] Percent dry weight of sample, except moisture as percent fresh weight and copper, iron, manganese, and zinc as mg/kg of dry weight. [d] Nontransgenic control. [e] Commercial hybrids; local hybrids planted at each EU site. [f] Tolerance interval is specified to contain 99% of the commercial line population, negative limits set to zero. [g] Range for nontransgenic control lines planted in Monsanto Co. field trials conducted in 1993 and 1995. [h] Range denotes the lowest and highest individual values across sites. [i] Watson (*55*). [j] Jugenheimer (*56*). [k] Statistically significantly different from the control at the 5% level ($p < 0.05$). [l] ADF, acid detergent fiber; NDF, neutral detergent fiber. [m] na = not available.

Table 6. Summarised data from USA and EU field trials - fibre and proximate content of forage (Ridley et al. 2002)

	1998[a]		1999[b]			
component[c]	NK603 mean (range)[h]	control[d] mean (range)[h]	NK603 mean (range)[h]	control[d] mean (range)[h]	commercial hybrids[e] tolerance interval[f] (range)[h]	historical[g] (range)
protein	7.14 (5.57–8.98)	6.80 (5.49–8.69)	8.71 (6.37–10.79)	8.86 (7.03–10.96)	4.02, 12.46 (4.98–11.56)	(4.8–8.4)
ash	3.81 (2.36–6.80)	4.02 (2.46–6.28)	4.38 (2.82–6.44)	4.44 (3.35–5.80)	0, 12.47 (2.43–9.64)	(2.9–5.1)
ADF[i]	25.72 (17.01–33.52)	24.84 (19.53–31.83)	23.53 (19.27–26.13)	22.07 (19.39–26.90)	9.80, 44.43 (17.54–38.31)	(21.4–29.2)
NDF[i]	42.09 (36.39–49.03)	42.45 (35.44–53.24)	37.34 (31.77–44.35)	37.75 (34.85–41.86)	20.77, 61.87 (27.93–54.75)	(39.9–46.6)
total fat	2.36 (0.69–3.64)	2.17 (0.61–3.42)	3.24 (2.06–4.49)	3.05 (2.09–4.02)	0.84, 4.80 (1.42–4.57)	(1.4–2.1)
carbohydrates	86.71 (82.68–90.32)	87.11 (83.71–90.03)	83.67 (80.43–87.53)	83.65 (80.64–85.52)	75.55, 91.37 (76.50–87.29)	(84.6–89.1)
moisture	67.02 (60.30–75.00)	66.24 (61.00–73.70)	67.53 (61.60–75.20)	66.30 (60.40–72.60)	45.40, 96.42 (56.50–80.40)	(68.7–73.5)

[a] Data from five nonreplicated U.S. sites and two replicated U.S. sites; NK603 forage harvested from plants treated with Roundup Ultra herbicide. [b] Data from two replicated EU sites; NK603 forage harvested from plants treated with Roundup (MON 52276) herbicide. [c] Percent dry weight of sample, except for moisture. [d] Nontransgenic control. [e] Commercial hybrids; local hybrids planted at each site. [f] Tolerance interval is specified to contain 99% of the commercial line population, negative limits set to zero. [g] Range for nontransgenic control lines planted in Monsanto Co. field trials conducted in 1994 and 1995. [h] Range denotes the lowest and highest individual values across sites. [i] ADF, acid detergent fiber; NDF, neutral detergent fiber.

Table 7. Summarised data from USA and EU field trials – amino acids in Grain (Ridley et al. 2002)

	1998[b]		1999[c]				
amino acid[a]	NK603 mean (range)[i]	control[d] mean (range)[i]	NK603 mean (range)[i]	control[d] mean (range)[i]	commercial hybrids[e] tolerance interval[f] (range)[i]	lit.[g] (range)	historical[h] (range)[i]
alanine	7.93 (7.78–8.22)	7.89 (7.65–8.17)	8.04[j] (7.87–8.18)	7.95 (7.88–8.05)	7.20, 8.35 (7.38–8.13)	(6.4–9.9)	(7.2–8.8)
arginine	4.16 (3.79–4.49)	4.24 (3.90–4.63)	4.00[j] (3.74–4.27)	4.27 (4.09–4.36)	3.45, 5.03 (3.77–4.98)	(2.9–5.9)	(3.5–5.0)
aspartic acid	6.45 (6.29–6.62)	6.40 (6.18–6.56)	6.45 (6.27–6.96)	6.28 (6.18–6.37)	5.53, 7.61 (6.02–7.51)	(5.8–7.2)	(6.3–7.5)
cysteine/cystine	2.00 (1.69–2.27)	2.00 (1.63–2.22)	1.82 (1.66–1.98)	1.92 (1.61–2.09)	1.56, 2.43 (1.68–2.51)	(1.2–1.6)	(1.8–2.7)
glutamic acid	19.84 (19.16–20.47)	19.81 (19.19–20.41)	19.93[j] (18.98–20.62)	19.40 (18.69–19.92)	18.03, 20.76 (18.38–20.08)	(12.4–19.6)	(18.6–22.8)
glycine	3.49 (3.22–3.74)	3.51 (3.22–3.86)	3.44 (3.23–3.64)	3.60 (3.44–3.77)	3.06, 4.15 (3.27–4.01)	(2.6–4.7)	(3.2–4.2)
histidine	2.72 (2.45–2.81)	2.74 (2.56–2.88)	2.65[j] (2.56–2.74)	2.77 (2.69–2.85)	2.34, 3.36 (2.58–3.15)	(2.0–2.8)	(2.8–3.4)
isoleucine	3.87 (3.59–4.06)	3.80 (3.65–3.93)	3.77 (3.54–3.97)	3.76 (3.61–3.85)	3.35, 3.97 (3.34–3.85)	(2.6–4.0)	(3.2–4.3)
leucine	14.20 (13.63–14.79)	14.07 (13.59–14.60)	14.02 (13.38–14.71)	13.69 (13.27–13.96)	11.73, 14.76 (12.18–14.34)	(7.8–15.2)	(12.0–15.8)
lysine	2.69 (2.42–2.96)	2.67 (2.35–3.00)	2.71[j] (2.37–3.03)	2.83 (2.56–3.20)	2.22, 3.68 (2.58–3.67)	(2.0–3.8)	(2.6–3.5)
methionine	1.94 (1.76–2.16)	2.03 (1.74–2.21)	1.77[j] (1.66–1.85)	1.89 (1.67–2.06)	1.39, 2.49 (1.49–2.32)	(1.0–2.1)	(1.3–2.6)
phenylalanine	5.32 (5.18–5.52)	5.24 (5.09–5.36)	5.28 (5.13–5.46)	5.25 (5.20–5.29)	4.59, 5.61 (4.85–5.54)	(2.9–5.7)	(4.9–6.1)
proline	8.88 (8.44–9.10)	8.96 (8.59–9.26)	9.33 (8.89–9.71)	9.16 (8.83–9.31)	8.61, 10.09 (8.74–9.91)	(6.6–10.3)	(8.7–10.1)
serine	4.87 (4.72–5.09)	4.86 (4.68–4.99)	4.84 (4.47–5.17)	4.90 (4.82–5.09)	4.36, 5.19 (4.41–5.22)	(4.2–5.5)	(4.9–6.0)
threonine	3.37 (3.26–3.46)	3.33 (3.19–3.50)	3.31 (3.14–3.57)	3.29 (3.15–3.50)	3.14, 3.69 (3.24–3.66)	(2.9–3.9)	(3.3–4.2)
tryptophan	0.53 (0.44–0.58)	0.54 (0.48–0.60)	0.58 (0.49–0.64)	0.62 (0.57–0.69)	0.45, 0.76 (0.49–0.79)	(0.5–1.2)	(0.4–1.0)
tyrosine	3.02 (2.36–3.73)	3.25 (2.43–3.64)	3.24 (2.11–3.65)	3.52 (2.69–3.69)	3.00, 4.03 (2.32–3.90)	(2.9–4.7)	(3.7–4.3)
valine	4.74 (4.59–4.85)	4.71 (4.62–4.94)	4.81 (4.55–5.00)	4.90 (4.74–5.04)	4.64, 5.38 (4.65–5.29)	(2.1–5.2)	(4.2–5.3)

[a] Values expressed as percent of total amino acids for statistical comparisons. [b] Data from five nonreplicated U.S. sites and two replicated U.S. sites; NK603 grain harvested from plants treated with Roundup Ultra herbicide. [c] Data from two replicated EU sites; NK603 grain harvested from plants treated with Roundup (MON 52276) herbicide. [d] Nontransgenic control. [e] Commercial hybrids; local hybrids planted at each EU site. [f] Tolerance interval is specified to contain 99% of the commercial line population, negative limits set to zero. [g] Watson (*57*). Values are percent of total protein. [h] Range for nontransgenic control lines planted in Monsanto Co. field trials conducted between 1993 and 1995; values are percent of total protein. [i] Range denotes the lowest and highest individual values across sites. [j] Value statistically significantly different than the control at the 5% level ($p < 0.05$).

Table 8. Summarised data from USA and EU field trials – content of fatty acids in grain Grain (Ridley et al. 2002)

	1998[b]		1999[c]				
fatty acid[a]	NK603 mean (range)[i]	control[d] mean (range)[i]	NK603 mean (range)[i]	control[d] mean (range)[i]	commercial hybrids[e] tolerance interval[f] (range)[i]	lit.[g] (range)	historical[g] (range)[h]
arachidic (20:0)	0.36 (0.34–0.39)	0.37 (0.33–0.40)	0.36[j] (0.34–0.39)	0.35 (0.33–0.37)	0.17, 0.64 (0.31–0.74)	(0.1–2)	(0.3–0.5)
behenic (22:0)	0.16 (0.14–0.19)	0.16 (0.14–0.19)	0.16 (0.12–0.20)	0.18 (0.15–0.19)	0.093, 0.24 (0.073–0.22)	(not reported)	(0.1–0.3)
eicosenoic (20:1)	0.29 (0.28–0.32)	0.30 (0.27–0.34)	0.30 (0.28–0.34)	0.29 (0.28–0.31)	0.21, 0.42 (0.26–0.40)	(not reported)	(0.2–0.3)
linoleic (18:2)	64.62 (63.79–65.80)	64.26 (63.07–65.65)	63.73 (61.94–65.25)	63.15 (61.63–64.04)	44.59, 73.50 (49.72–65.98)	(35–70)	(55.9–66.1)
linolenic (18:3)	1.11 (1.07–1.17)	1.11 (1.07–1.20)	1.02 (0.97–1.05)	1.09 (1.05–1.12)	0.54, 1.72 (0.71–1.50)	(0.8–2)	(0.8–1.1)
oleic (18:1)	22.40[j] (21.37–23.12)	23.08 (22.15–24.14)	23.80 (22.82–24.95)	24.20 (23.52–25.56)	12.65, 39.86 (20.21–34.64)	(20–46)	(20.6–27.5)
palmitic (16:0)	9.13[j] (8.67–9.57)	8.89 (8.41–9.44)	8.90 (8.47–9.36)	9.00 (8.89–9.13)	7.35, 14.72 (9.12–12.62)	(7–19)	(9.9–12.0)
stearic (18:0)	1.92[j] (1.80–2.06)	1.83 (1.67–1.98)	1.73 (1.59–1.88)	1.74 (1.67–1.81)	1.02, 2.27 (1.19–2.02)	(1–3)	(1.4–2.2)

[a] Value of fatty acids expressed as % of total fatty acid. The method included the analysis of the following fatty acids, which were not detected in the majority of samples analyzed: caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), myristoleic acid (14:1), pentadecanoic acid (15:0), pentadecenoic acid (15:1), palmitoleic acid (16:1), heptadecanoic acid (17:0), heptadecenoic acid (17:1), gamma linolenic (18:3), eicosadienoic acid (20:2), eicosatrienoic acid (20:3), and arachidonic acid (20:4). [b] Data from five nonreplicated U.S. sites and two replicated U.S. sites; NK603 grain harvested from plants treated with Roundup Ultra herbicide. [c] Data from two replicated EU sites; NK603 grain harvested from plants treated with Roundup (MON 52276) herbicide. [d] Nontransgenic control. [e] Commercial hybrids; local hybrids planted at each EU site. [f] Tolerance interval is specified to contain 99% of the commercial line population, negative limits set to zero. [g] Watson (*57*). Values expressed as % of total fat except for palmitic acid (16:1), which is expressed as % of triglyceride fatty acids. [h] Range for nontransgenic control lines planted in Monsanto Co. field trials conducted between 1993 and 1995; values are expressed as % of total fatty acids. [i] Range denotes the lowest and highest individual values across sites. [j] Statistically significantly different from the control at the 5% level ($p < 0.05$).

Table 9. Summarised data from USA and EU field trials – content of phytic acid, trypsin inhibitor and vitamin E in grain (Ridley et al. 2002)

	1998[a]		1999[b]				
component	NK603 mean (range)[h]	control[c] mean (range)[h]	NK603 mean (range)[h]	control[c] mean (range)[h]	commercial hybrids[d] tolerance interval[e] (range)[h]	lit.[f] (range)	historical[g] (range)
phytic acid (% dw)	0.97 (0.70–1.06)	1.00 (0.81–1.21)	0.79 (0.51–0.89)	0.70 (0.55–0.77)	0.32, 1.18 (0.48–1.12)	to 0.9%	na[i]
trypsin inhibitor (TIU/mg dw)	3.16 (2.34–5.08)	2.67 (1.39–5.14)	1.56 (0.54–2.57)	1.15 (0.54–2.38)	0, 3.63 (0.54–4.13)	na	na
vitamin E (mg/g of dw)	0.0088 (0.0070–0.010)	0.0090 (0.0064–0.011)	0.0062 (0.0046–0.0080)	0.0070 (0.0050–0.014)	0, 0.021 (0.0027–0.015)	(0.017–0.047)	(0.008–0.015)
ferulic acid (% dw)	0.20 (0.15–0.25)	0.20 (0.17–0.23)	na	na	na	na	(0.17–0.27)[j]
p-coumaric acid (% dw)	0.016 (0.012–0.022)	0.015 (0.012–0.020)	na	na	na	na	(0.011–0.030)[j]
raffinose (% dw)	0.13 (0.098–0.20)	0.13 (0.082–0.21)	na	na	na	na	(0.053–0.16)[j]

[a] Data from five nonreplicated U.S. sites and two replicated U.S. sites; NK603 grain harvested from plants treated with Roundup Ultra herbicide. [b] Data from two replicated EU sites; NK603 grain harvested from plants treated with Roundup (MON 52276) herbicide. [c] Nontransgenic control. [d] Commercial hybrids; local hybrids planted at each EU site. [e] Tolerance interval is specified to contain 99% of the commercial line population, negative limits set to zero. [f] Watson (*50*). [g] Range for nontransgenic control hybrids planted in Monsanto Co. field trials conducted between 1993 and 1995. [h] Range denotes the lowest and highest individual values across sites for each line. [i] na, not available. [j] Range for 13 commercial varieties planted in Monsanto Co. field trials or purchased from growers in 1998.