Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Food/feed and environmental risk assessment of insect-resistant and herbicide-tolerant genetically modified maize 1507 x NK603 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (EFSA/GMO/UK/2004/05)

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

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Contributors

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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Assessed by

Panel on Genetically Modified Organisms
Åshild K. Andreassen (Chair), Per Brandtzæg, Hilde-Gunn Hoen-Sorteberg, Askild Holck, Olavi Junttila, Heidi Sjursen Konestabo, Richard Meadow, Kåre M. Nielsen, Rose Vikse

Scientific coordinators from the secretariat
Merethe Aasmo Finne, Anne-Marthe Jevnaker, Ville Erling Sipinen, Arne Mikalsen

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 insect-resistant and herbicide-tolerant genetically modified maize 1507 x NK603 from Pioneer Hi-Bred International, Inc. og Mycogen Seeds (Unique Identifier DAS-Ø15Ø7-1 x MONØØ6Ø3-6) is approved under Regulation (EC) No 1829/2003 for food and feed uses, import and processing since 24 October 2007 (Commission Decision 2007/703/EC).

Genetically modified maize 1507 x NK603 has previously been risk assessed by the VKM Panel on Genetically Modified Organisms (GMOs), commissioned by the Norwegian Food Safety Authority related to the EFSAs public hearing of the application EFSA/GMO/UK/2004/05 in 2005 (VKM 2005a). In addition, maize 1507 x NK603 has been assessed by the VKM GMO Panel commissioned by the Norwegian Environment Agency and NFSA in connection with the national finalisation of the procedure of the notification in 2008 (VKM 2008). 1507 x NK603 has also been evaluated by the VKM GMO Panel as single events and as a component of several stacked GM maize events (VKM 2004, VKM 2005b, VKM 2007a, VKM 2009, and VKM 2012a).

The food/feed and environmental risk assessment of the maize 1507 x NK603 is based on information made available on the EFSA website GMO Extranet, and relevant peer-reviewed scientific literature.

The VKM GMO Panel has evaluated 1507 x 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 2006, 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 1507 x NK603 includes molecular characterisation of the inserted DNA and expression of novel proteins, comparative assessment of agronomic and phenotypic characteristics, nutritional assessments, toxicology and allergenicity, unintended effects on plant fitness, potential for gene transfer, interactions between the GM plant and target and non-target organisms, and effects on biogeochemical processes.

It is 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 stack 1507 x NK603 was produced by conventional breeding between inbred lines of maize containing the 1507 and NK603 events. The hybrid was developed to provide protection against certain lepidopteran target pests, and to confer tolerance to glufosinate-ammonium and glyphosate herbicides.

Molecular characterisation

Southern blot and PCR analyses have indicated that the recombinant inserts in the parental maize events1507 and NK603 are retained in the stacked maize 1507 x NK603. Genetic stability of the inserts has previously been demonstrated in the parental events. Protein measurements show comparable levels of Cry1F, PAT and CP4 EPSPS proteins in the stacked maize 1507 x NK603 and the parental lines. Phenotypic analyses also indicated stability of the insect resistance and herbicide tolerance traits.

The VKM Panel on GMO considers the molecular characterisation of maize 1507 x NK603 and its parental events 1507 and NK603 as adequate.

Comparative assessment

Comparative analyses of the compositional, agronomic and phenotypic characteristics of maize stack 1507 x NK603 and near-isogenic comparators were performed during multiple field trials in Chile and Europe in 2002/2003. With the exception of small intermittent variations, the results show no indications of unwanted unintentional effects, and that maize stack 1507 x NK603 is compositionally, agronomically and phenotypically equivalent to its comparators, with the exception of the introduced insect resistance and herbicide tolerance traits.

Food and feed risk assessment

Whole food feeding studies on rats have not indicated any adverse effects of the parental maize lines 1507 and NK603. No rodent whole food feeding study has been performed on the stacked maize 1507 x NK603; the applicant has however provided a nutritional feeding study performed on broilers. No adverse effects were observed in the study. Bioinformatics analyses have not revealed expression of any known ORFs in the parental maize lines, and none of the newly expressed proteins show resemblance to any known toxins or IgE allergens. Nor have the newly expressed proteins been reported to cause IgE mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Acute and repeated dose toxicity tests in rodents have not indicated toxic effects of the Cry1F, PAT or CP4 EPSPS proteins. However, these tests do not provide any additional information about possible adverse effects of maize 1507 x NK603.

Based on current knowledge, the VKM GMO Panel concludes that the stacked maize 1507 x NK603 is nutritionally equivalent to conventional maize varieties, and that it is unlikely that the newly expressed proteins introduce a toxic or allergenic potential in food and feed derived from maize 1507 x NK603 compared to conventional maize.

Environmental risk

Considering the intended uses of maize 1507 x NK603, excluding cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize 1507 x NK603.

Maize 1507 x 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 1507 x NK603. Maize is the only

representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation. The risk of gene flow from occasional feral GM maize plants to conventional maize varieties is negligible. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered to be an issue.

Overall conclusion
The VKM GMO Panel has not identified toxic or altered nutritional properties in maize 1507 x NK603 or its processed products compared to conventional maize. Based on current knowledge, it is also unlikely that the Cry1F protein will increase the allergenic potential of food and feed derived from maize 1507 x NK603 compared to conventional maize varieties. The VKM GMO Panel likewise concludes that maize 1507 x 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 1507 x NK603, EFSA/GMO/UK/2004/05, insect-resistance, herbicide-tolerance, Cry proteins, *cry1F*, PAT, CP4 EPSPS, glufosinate-ammonium, glyphosate, food and feed risk assessment, environmental risk assessment, Regulation (EC) No 1829/2003

Norsk sammendrag

I forbindelse med forberedelse til implementering av EU-forordning 1829/2003 i norsk rett, er Vitenskapskomiteen for mattrygghet (VKM) bedt av Miljødirektoratet (tidligere Direktoratet for naturforvalting (DN)) og Mattilsynet om å utarbeide endelige helse- og miljørisikovurderinger av alle genmodifiserte organismer (GMOer) og avledete produkter som inneholder eller består av GMOer som er godkjent under forordning 1829/2003 eller direktiv 2001/18, og som er godkjent for ett eller flere bruksområder som omfattes av genteknologiloven. Miljødirektoratet og Mattilsynet har bedt VKM om endelige risikovurderinger for de EU-godkjente søknader hvor VKM ikke har avgitt endelige risikovurderinger. I tillegg er VKM bedt om å vurdere hvorvidt det er nødvendig med oppdatering eller annen endring av de endelige helse- og miljørisikovurderingene som VKM tidligere har levert.

Den insektresistente og herbicidtolerante maishybriden 1507 x NK603 (unik kode DAS-Ø15Ø7-1 x MONØØ6Ø3-6) fra Pioneer Hi-Bred International og Mycogen Seeds ble godkjent til import, videreforedling og til bruk som mat og fôr under EU-forordning 1829/2003 i 2007 (søknad EFSA/GMO/UK/2004/05, Kommisjonsbeslutning 2007/703/EC).

Maishybriden har tidligere vært vurdert av VKMs faggruppe for genmodifiserte organismer med hensyn på mulig helserisiko i forbindelse med EFSAs offentlige høring av søknaden i 2005 (VKM 2005a). På oppdrag fra Miljødirektoratet og Mattilsynet har maishybriden 1507 x NK603 også vært vurdert av VKM med hensyn på mulige helse- og miljøeffekter i forbindelse med vurdering av markedsadgang i Norge (VKM 2008). Foreldrelinjene 1507 og NK603 er også tidligere risikovurdert av VKM, både som enkelt-eventer og i en rekke andre hybrider (VKM 2004, VKM 2005b, VKM 2007a, VKM 2009 og VKM 2012a).

Risikovurderingen av den genmodifiserte maislinjen 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.

F_1-hybriden 1507 x NK603 er resultat av konvensjonelle kryssinger mellom de genmodifiserte maislinjene 1507 og NK603. Kryssingene er utført for å utvikle en maishybrid med resistens mot visse skadegjørere i sommerfuglordenen Lepidoptera, samt toleranse mot herbicider med virkestoff glufosinat-ammonium og glyfosat.

Foreldrelinjen 1507 har fått innsatt et *cry1F*-gen fra bakterien *Bacillus thuringiensis* var. *aizawai* og et *pat*-gen, som er isolert fra *Streptomyces viridochromogenes*. *Cry1F*-genet koder for et δ-endotoksin og gir resistens mot enkelte arter i sommerfuglordenen Lepidoptera, eksempelvis maispyralide (*Ostrinia nubilatis*) og nattflyarten *Sesamia nonagrioides*. *Pat*-genet koder for enzymet fosfinotricin acetyltransferase (PAT), som acetylerer og inaktiverer glufosinat-ammonium, virkestoffet i fosfinotricin-herbicider av typen Finale. Fosfinotricin er et ikke-selektivt kontaktherbicid som hemmer glutaminsyntetase. Enzymet deltar i assimilasjonen av nitrogen og katalyserer omdanning av glutamat og ammonium til aminosyren glutamin. Hemming av glutaminsyntetase fører til akkumulasjon av ammoniakk, og til celledød i planten. De transgene maisplantene vil derfor tolerere høyere doser av sprøytemiddelet glufosinat sammenlignet med konkurrerende ugras.

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

Molekylær karakterisering
Maishybriden 1507 x NK603 er dannet ved konvensjonell kryssing av foreldrelinjene, mais 1507 og mais NK603. Spaltingsdata og PCR-analyser indikerer at de innsatte genkonstruksjonene i foreldrelinjene er stabilt nedarvet og bevart i maishybriden. Genetisk stabilitet av de innsatte genene har tidligere blitt vist i foreldrelinjene. Proteinmålinger viser at nivåene av Cry1F-, PAT- og CP4 EPSPS -proteinene i hybriden er sammenlignbare med nivåene i foreldrelinjene. Feltanalyser viser også tilsvarende egenskaper for insektsresistens og herbicidtoleranse. VKMs faggruppe for genmodifiserte organismer vurderer den molekylære karakteriseringen av maishybriden 1507 x NK603 og dens foreldrelinjer som tilfredsstillende.

Komparative analyser
Feltforsøk over en vekstsesong i henholdsvis Chile og Europa viser små eller ingen signifikante forskjeller mellom den transgene maishybriden 1507 x NK603 og korresponderende, nær-isogene kontrollhybrider med hensyn på næringsmessige, morfologiske og agronomiske karakterer, med unntak av insektsresistens og herbicidtoleranse. Resultatene viser ingen indikasjon på at de innsatte genene i 1507 x NK603 har medført utilsiktede endringer i egenskaper knyttet til vekst og utvikling hos maisplantene.

Helserisiko
Fôringsstudier utført på rotter med mais 1507 og mais NK603, har ikke indikert helseskadelige effekter av de to maislinjene. Tilsvarende rottestudie er ikke utført med den kryssede maisen 1507 x NK603, men det er utført en fôringsstudie på broilere. Fôringsstudien viste ingen forskjell på matinntak, vekst eller generell helse blant broilere som ble fôret med mais 1507 x NK603 sammenlignet med umodifisert mais. Bioinformatikk-analyser (databasesøk), har ikke avdekket uttrykk av kjente åpne leserammer i maislinjene 1507 og NK603, og det er ikke funnet likhetstrekk mellom Cry1F-, PAT- eller CP4 EPSPS- proteinet og kjente toksiner eller IgE-allergener. Det er heller ikke dokumentert at noen av proteinene kan utløse IgE-medierte allergiske reaksjoner. Enkelte studier har derimot indikert at noen typer Cry-proteiner potensielt kan forsterke andre allergiske reaksjoner (virke som adjuvans).

Eksponeringsstudier på gnagere med enkelt- eller repeterte doser av proteinene Cry1F, PAT eller CP4 EPSPS har ikke indikert toksiske effekter av proteinene. Denne typen studier gir derimot ingen tilleggsinformasjon om mulige helseskadelige egenskaper ved mais 1507 x NK603.

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at mais 1507 x NK603 er næringsmessig vesentlig lik konvensjonell mais, og at det er lite trolig at de nye proteinene vil introdusere et toksisk eller allergent potensiale i mat og fôr basert på mais 1507 x NK603 sammenliknet med konvensjonelle maissorter.

Miljørisiko
Søknaden gjelder godkjenning av maishybrid 1507 x NK603 for import, prosessering og til bruk i næringsmidler og fôrvarer, og omfatter ikke dyrking. Med bakgrunn i tiltenkt bruksområde er miljørisikovurderingen avgrenset til mulige effekter av utilsiktet frøspredning i forbindelse med transport og prosessering, samt indirekte eksponering gjennom gjødsel fra husdyr fôret med genmodifisert mais.

Det er ingen indikasjoner på økt sannsynlighet for spredning, etablering og invasjon av maislinjen i naturlige habitater eller andre arealer utenfor jordbruksområder som resultat av frøspill i forbindelse med transport og prosessering. Risiko for utkryssing med dyrkede sorter vurderes av GMO panelet til å være ubetydelig. Ved foreskreven bruk av maislinjen 1507 x NK603 antas det ikke å være risiko for utilsiktede effekter på målorganismer, ikke-målorganismer eller på abiotisk miljø i Norge.

Samlet vurdering
VKMs faggruppe for GMO har ikke identifisert toksiske eller endrede næringsmessige egenskaper ved mais 1507 x NK603 eller dens prosesserte produkter sammenliknet med konvensjonell mais. Ut i fra dagens kunnskap er det også lite trolig at Cry1F-proteinet vil øke det allergene potensialet til mat og fôr basert på mais 1507 x NK603 sammenliknet med konvensjonelle maissorter. Faggruppen finner at mais 1507 x NK603, ut i fra dagens kunnskap og omsøkt bruk, er sammenlignbar med konvensjonell mais når det gjelder mulig miljørisiko i Norge.

Abbreviations and explanations

ALS	Acetolactate synthase, an enzyme that catalyses the first step in the synthesis of the branched-chain amino acids, valine, leucine, and isoleucine
AMPA	Aminomethylphosphonic acid, one of the primary degradation products of glyphosate
ARMG	Antibiotic resistance marker gene
BC	Backcross. Backcross breeding in maize is extensively used to move a single trait of interest (e.g. disease resistance gene) from a donor line into the genome of a preferred or "elite" line without losing any part of the preferred lines existing genome. The plant with the gene of interest is the donor parent, while the elite line is the recurrent parent. BC_1, BC_2 etc. designates the backcross generation number.
BLAST	Basic Local Alignment Search Tool. Software that is used to compare nucleotide (BLASTn) or protein (BLASTp) sequences to sequence databases and calculate the statistical significance of matches, or to find potential translations of an unknown nucleotide sequence (BLASTx). BLAST can be used to understand functional and evolutionary relationships between sequences and help identify members of gene families.
bp	Basepair
Bt	*Bacillus thuringiensis*
CaMV	Cauliflower mosaic virus
Codex	Set by The Codex Alimentarius Commission (CAC), an intergovernmental body to implement the Joint FAO/WHO Food Standards Programme. Its principle objective is to protect the health of consumers and to facilitate the trade of food by setting international standards on foods (i.e. Codex Standards)
Cp4 epsps	Gene from *Agrobacterium* sp. strain CP4
Cry	Any of several proteins that comprise the crystal found in spores of *Bacillus thuringiensis*. Activated by enzymes in the insects midgut, these proteins attack the cells lining the gut, and subsequently kill the insect.
Cry1F	Cry1 class crystal protein from *Bacillus thuringiensis* var. *aizawai*
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 corn 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
Glufosinate-ammonium	Broad-spectrum systemic herbicide
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 corn borer, *Sesamia nonagrioides*
mRNA	Messenger RNA
MT	Norwegian Food Safety Authority (Mattilsynet)
NDF	Neutral detergent fibre, measure of fibre used for animal feed analysis. NDF measures most of the structural components in plant cells (i.e. lignin, hemicellulose and cellulose), but not pectin
Northern blot	Northern blot is a technique used 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
pat	Phosphinothricin-Acetyl-Transferase gene
PAT	Phosphinothricin-Acetyl-Transferase protein
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

On 1 October 2004, the European Food Safety Authority (EFSA) received from the Competent Authority of United Kingdom an application (Reference EFSA/GMO/UK/2004/05) for authorisation of the insect-resistant and herbicide tolerant genetically modified (GM) maize 1507 x NK603 (Unique Identifier DAS-Ø15Ø7-1 x MONØØ6Ø3-6), submitted by Pioneer Hi-Bred International, Inc. and Mycogen Seeds within the framework of Regulation (EC) No 1829/2003.

The scope of the application covers:

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

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

After receiving the application EFSA/GMO/UK/2004/05 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the EU- and EFTA Member States (MS) and the European Commission and made the summary of the dossier publicity available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of regulation (EC) No 1829/2003. On 1 April 2005, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the EC and consulted nominated risk assessment bodies of the MS, including the Competent Authorities within the meaning of Directive 2001/18/EC (EC 2001), following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1929/2003, to request their scientific opinion. Within three months following the date of validity, all MS could submit via the EFSA GMO Extranet to EFSA comments or questions on the valid application under assessment. The VKM GMO Panel assessed the application in connection with the EFSA official hearing, and submitted a preliminary opinion in June 2005 (VKM 2005a). EFSA published its scientific opinion 28 March 2006 (EFSA 2006), and maize stack 1507 x NK603 was approved for food and feed uses, import and processing in 24 October 2007 (Commission Decision 2007/703/EC).

An application for authorisation of maize 1507 x NK603 for cultivation in the EU was submitted by Pioneer Hi-Bred International, Inc. in June 2005 (EFSA/GMO/UK/2005/17). The 90 days public consultation of the application was conducted before VKM's assignment from the Norwegian Environment Agency, and the VKM GMO Panel did not participate in the official hearing. Pending the requested additional information from EFSA and the Spanish Competent Authority, the clock for the application was stopped by EFSA in July 2006. The clock for application EFSA/GMO/UK/2005/17 was restarted in January 2003.

Scientific opinions on the parental lines of the stack 1507 x NK603 have previously been submitted by the VKM GMO Panel (VKM 2004, 2005a). In addition, maize 1507 and NK603 have been evaluated

by the VKM GMO Panel as a component of other stacked GM maize events under Directive 2001/18/EC and Regulation (EC) 1829/2003 (VKM 2007a, VKM 2009, and VKM 2012a).

Terms of reference

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

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

The Norwegian Environment Agency

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

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

The basis for evaluating the applicants' environmental risk assessments is embodied in the Act Relating to the Production and Use of Genetically Modified Organisms etc. (the Norwegian Gene Technology Act), Regulations relating to impact assessment pursuant to the Gene Technology Act, the Directive 2001/18/EC on the deliberate release of genetically modified organisms into the environment, Guidance note in Annex II of the Directive 2001/18 (2002/623/EC) and the Regulation 1829/2003/EC. In addition, the EFSA guidance documents on risk assessment of genetically modified plants and food and feed from the GM plants (EFSA 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 authorized in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC within the Authority's sectoral responsibility. The request covers scope(s) relevant to the Gene Technology Act.

The Norwegian Food Safety Authority has therefore, by letter dated 13 February 2013 (ref. 2012/150202), requested the Norwegian Scientific Committee for Food Safety (VKM) to carry out

final scientific risk assessments of 39 GMOs and products containing or consisting of GMOs that are authorized in the European Union.

The assignment from NFSA includes food and feed 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

Maize 1507 x NK603 was produced through traditional breeding methods between progeny (inbred lines) of the genetically modified maize lines 1507 and NK603.

The parental line maize 1507 was developed to provide protection against certain lepidopteran target pests (such as the European corn borer (ECB), *Ostrinia nubilalis,* and some species belonging to the genus *Sesamia,* and in particular the Mediterranean corn borer (MCB), *Sesamia nonagrioides*) by the introduction of a part of a *Bacillus thuringiensis* (*Bt*) gene encoding the insecticidal Cry1F protein. Maize 1507 also express the phosphinothricin-N-acetyltransferase (PAT) protein from *Streptomyces viridochromogenes*, which confers tolerance to the herbicidal active substance glufosinate-ammonium.

The parental line NK603 is tolerant to glyphosate-based herbicides due to the expression of the *CP4 epsps* gene from *Agrobacterium* sp. strain CP4 (CP4 EPSPS and CP4 EPSPS L214P, a variant of CP4 EPSPS containing a proline residue at position 214 instead of leucine).

None of the target pests for maize 1507 are present in the Norwegian agriculture. The PAT protein expressed in maize 1507 has been used as selectable markers to facilitate the selection process of transformed plant cells and is not intended for weed management purposes.

Maize stack 1507 x NK603 has been evaluated with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed.

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

The environmental risk assessment of the genetically modified maize 1507 x 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 Evaluation of relevant scientific data

2.1.1 Method of production of maize 1507 x NK603

Conventional breeding methods were used to develop the insect-resistant and herbicide-tolerant maize 1507 x NK603. The two inserts present in maize 1507 x NK603 are derived from the parental events: 1507 and NK603 and combines resistance to certain lepidopteran pests, and tolerance to glufosinate-ammonium and glyphosate based herbicides.

2.1.2 Summary of evaluation of the single events

2.1.2.1 Maize 1507

Maize 1507 was developed to provide protection against certain lepidopteran target pests (such as the European corn borer, *Ostrinia nubilalis,* and species belonging to the genus *Sesamia*) by the introduction of a part of a *Bacillus thuringiensis* gene encoding the insecticidal Cry1F protein. The bacteria produce the intracellular crystal protein which has entomopathogenic effect.
The base sequence of the *cry1F* gene is modified to improve expression in maize, while the amino acid sequence of the translated Cry1F protein remains identical to the protein expressed by the bacteria. The expression of *cry1F* is regulated by the maize promoter *ubi*ZM1. Termination of expression is controlled by the terminator *mas1* from *Agrobacterium tumefaciens.*

Maize 1507 also expresses the phosphinothricin-N-acetyltransferase (PAT) protein from *Streptomyces viridochromogenes*, which confers tolerance to the herbicidal active substance glufosinate-ammonium.

Maize 1507 was developed through particle acceleration. The intended insert in 1507 maize consisted of a linear DNA fragment, containing the *cry*1F and *pat* coding sequences together with the necessary regulatory components. Transformation of 1507 resulted in the stable insertion of the PHP8999 plasmid region PHI8999A. No additional DNA sequences were used in the introduction of the respective inserts into 1507 maize.

Levels of Cry1F and PAT proteins were measured by enzyme linked immunosorbent assay (ELISA), in various plant tissues at different developmental stages in five field studies in the USA during the growth season of 2006. Three samples were collected from each field. Cry1F was detected in leaves, pollen, female flowers, stalks, seeds and in whole plants. The expression of the protein varied amongst the different plant tissues and developmental stages. Average concentration in pollen was 20.0 µg/g dw (maximum of 29.3 µg/g dw), whereas the concentrations varied between 1.2 - 3.1 µg/g dw, in seeds and 1.0 - 6.6 µg/g dw in whole plants. The levels of Cry1F were independent of cultivation conditions and herbicide treatment. With the exception of leaves and extracts from whole plants, the levels of PAT protein were below the detection limit.

Western blot and detection with polyclonal antibodies showed that both the Cry1F and PAT proteins had the expected molecular weights. Cry1F exists as a doublet of 65 kb and 68 kb, respectively. This is explained by plant proteases that cleave off an N-terminal fragment, since trypsin treatment of Cry1F also yields a protein of 65 kb. There are no indications of fusion proteins.

A detailed study was performed to detect open reading frames. Five ORFs were detected: ORF1, ORF2, ORF3, ORF4 and ORF25PolyA. ORF25PolyA is part of the CaMV 35S promoter and

terminator. ORF4 lies within ORF25PolyA. ORF1 and 2 are parts of the 1507 transcript and originate from the maize genome. These ORFs were also detected in unmodified maize, but do not share homology to described sequences in the maize genome, and do not contain regulatory elements that can lead to transcription. ORF3 and ORF4 are located at the border of, and inside the inserted fragment in maize 1507, respectively. No transcripts of ORF3 were detected by Northern blot or RT-PCR. Neither did analyses of ORF4 with Northern blot or RT-PCR indicate that ORF4 is capable of transcription even though it resides within ORF25PolyA.

Southern blot and sequence analysis have demonstrated that an almost full length copy of the 1507 DNA fragment (6186 bp out of 6235 bp) was inserted into the maize genome. An approx. 11 kb long DNA fragment of the maize genome wherein the 1507 fragment resides has been sequenced. This sequence contains both genes, the respective regulatory elements of the 1507 DNA fragment, and an additional six non-functional DNA fragments from the 6235 bp 1507 fragment. The six DNA fragments are located either at the 5' or 3' end of the 6186 bp 1507 fragment. The contents of genes and regulatory elements in the recombinant DNA fragment are outlined in Figure 1.

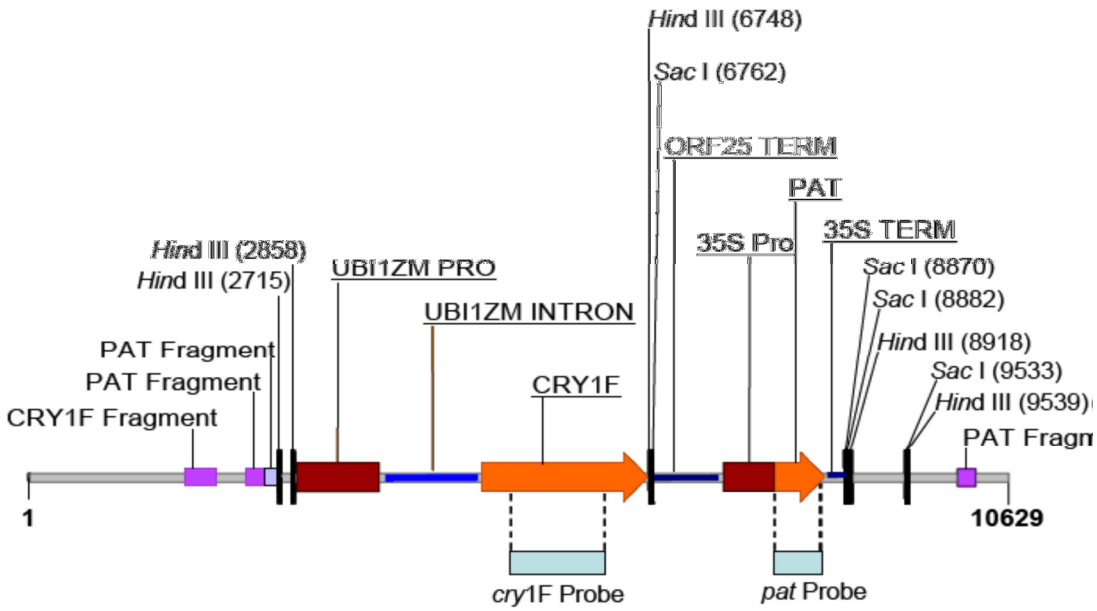

Figure 1. Restriction map of the various gene elements of the recombinant DNA fragment inserted in the genome of the maize strain 1507.

2.1.2.2 Maize NK603

Maize line AW x CW, used in the initial transformation, is a proprietary maize cell culture, which was transformed using particle acceleration technology to develop the NK603 maize event. Embryonic maize cells of AW x CW were, therefore, the initial recipient of the introduced DNA. Conventional breeding methods were used to backcross plants generated from the initial transformation into a recurrent, desired inbred maize line with a genetic background of interest to the breeder.

NK603 was developed to tolerate glyphosate through the introduction of a gene encoding the glyphosate tolerant 5-enoylpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* sp. strain CP4, (CP4 EPSPS). The introduced DNA fragment was isolated from the bacterial plasmid vector PV-ZMGT32. The plasmid vector contains two adjacent plant gene expression cassettes, each containing a single copy of the *cp*4 *epsps* gene fused to chloroplast transit peptide (CTP) sequences based on sequences derived from *Arabidopsis thaliana* EPSPS. CTP targets the CP4 EPSPS protein to its natural sub cellular location in the chloroplast. In the first *ctp*2-*cp*4 *epsps* cassette the coding sequence is regulated by the rice actin promoter and a rice intron sequence introduced upstream of the CTP sequence. Expression of the second *ctp*2-*cp*4 *epsps* cassette is regulated by an enhanced 35S CaMV promoter and a maize intron derived from a gene encoding a heat shock protein. In each cassette the *cp*4 *epsps* sequence is linked to the nopaline synthase terminator (NOS 3') sequence from *Agrobacterium tumefaciens*. The vector also contains an *npt*II bacterial selectable marker gene (for kanamycin resistance; derived from the prokaryotic transposon *Tn*5) and an origin of replication (*ori*). A *Mlu*I restriction fragment of the PV-ZMGT32 plasmid vector designated PV-ZMGT32L was used for transformation; this fragment only contains the *cp*4 *epsps* plant gene expression cassettes. The *npt*II gene, as well as the *ori* is not present in the fragment PV-ZMGT32L.

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

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

In 1999, forage and grain tissues were produced in European field trials at four sites. Four replications were used at each of the four sites. CP4 EPSPS protein levels were measured in maize forage and grain. All protein values are expressed as micrograms (µg) of the specific protein per gram (g) of tissue on a fresh weight (fw) basis. Control maize samples were below the Limit of Detection (LOD) for CP4 EPSPS protein. In maize NK603 forage, the mean CP4 EPSPS protein levels from the four different field sites ranged from 43.6 µg/g fw to 60.9 µg/g fw. The overall mean CP4 EPSPS protein level in maize NK603 forage across all four sites was 48.6 µg/g fw. In maize NK603 grain, the mean CP4 EPSPS protein levels ranged from 2.2 µg/g fw to 13.2 µg/g fw. The overall mean CP4 EPSPS protein level in maize grain across all four sites was 8.4 µg/g fw. The values given represent the sum

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

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

Southern blot analysis was used to determine the insert number, the copy number, integrity of the inserted promoters, coding regions, and polyadenylation sequences, and the presence or absence of the plasmid backbone sequence. Polymerase chain reaction (PCR) was performed to investigate the sequences at the 5' and 3' ends of the insert. PCR analysis and subsequent DNA sequencing of four overlapping products spanning the length of the insert in NK603 were undertaken to determine the characterisation of the inserted DNA in NK603. Genomic DNA from the NK603 maize and control (B73) were digested with the restriction enzyme StuI. The result suggested that NK603 contains one insertion of integrated DNA located within a 23 kb StuI restriction fragment. The genome of NK603 does not contain any detectable plasmid backbone DNA including *ori* or the *nptII* coding sequence. PCR amplification and DNA sequencing supported the characterisation of the insert and showed that the sequences flanking the insert are native to the maize genome. These data suggest that only the expected full-length CTP2-CP4 EPSPS and CTP2-CP4 EPSPS L214P proteins are encoded by the insert in NK603. The contents of genes and regulatory elements in the recombinant DNA fragment are outlined in Figure 2.

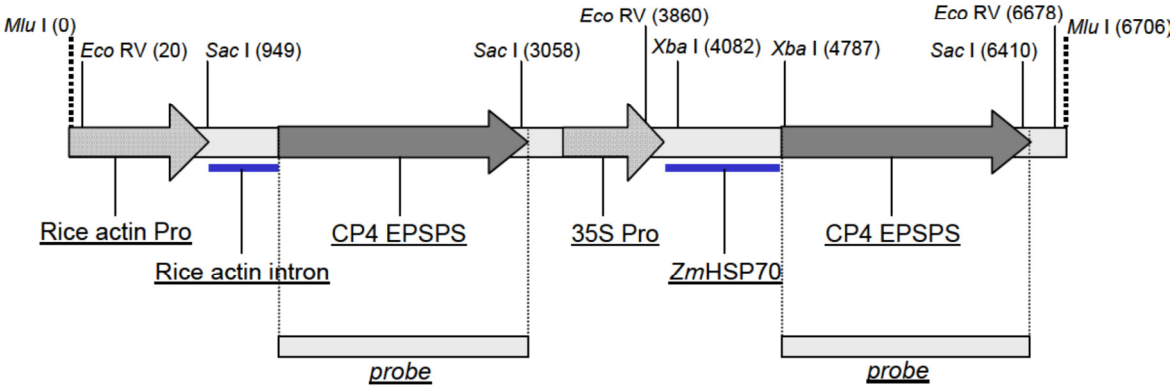

Figure 2. Restriction map of the various gene elements of the recombinant DNA fragment inserted in the genome of the maize strain NK603.

2.1.3 Transgene constructs in maize 1507 x NK603

A detailed molecular analysis was conducted to investigate if the copy number, structure and organisation of the inserts found in maize 1507 x NK603 were equivalent to that of the parental lines 1507 and NK603. Genomic DNA was extracted from leaves harvested from maize 1507, NK603 and 1507 x NK603. The DNA samples were analysed by Southern blot using different restriction enzymes and genetic probes specific for the 1507 or NK603 maize inserts.

DNA from four individual plants of maize 1507 x NK603 and six individual 1507 plants were digested with the restriction enzyme *Hind* III and subjected to Southern blot analysis with the *cry*1F and *pat* gene probes. The results showed the same number and size of bands in 1507 x NK603 and 1507 maize, which indicated molecular equivalence and equal copy numbers of the inserts.

Additional comparisons were made between 1507 x NK603 and 1507 and NK603 maize. DNA samples were prepared from twelve maize plants corresponding to four individual plants from each of the maize events. Two restriction enzymes, *Eco*R V and *Sac* I, were selected for the digestion of the DNA samples. Three genetic probes were used for this characterisation: the 35S promoter probe, which is common to both 1507 and NK603 maize; the *cry*1F gene probe; and, the *pat* gene probe.

Additional Southern blot analyses were carried out with the *cry*1F probe on 1507 and 1507 x NK603 maize DNA following digestion with the enzymes *Sac* I, *Eco*R V and *Nco* I, either individually or in combination (double restriction) with *Nco*I. According to the applicant, the results provided further support to demonstrate the structure of the 1507 maize insert and the absence of any secondary insertion sites in the genome of 1507 or 1507 x NK603 maize.

Southern blot analyses were also carried out with a DNA probe containing the coding region of the *cp4 epsps* gene, referred to as the *cp4 epsps* probe. DNA extracts were prepared from 44 individual 1507 x NK603 maize plants. These samples were analysed with the *cp4 epsps* probe after digestion with the restriction enzyme *EcoR* V. According to the applicant all of the 1507 x NK603 maize plants showed the same hybridisation pattern as NK603 maize plants, while no bands were observed with the *cp4 epsps* probe on samples from 1507 maize plants.

The results obtained from the Southern blot analyses indicate molecular equivalence and equal copy numbers of the inserts present in 1507 x NK603 maize to those present in the parental lines 1507 and NK603 maize.

2.1.4 Information on the expression of the inserts

Two field studies were carried out in order to estimate the level of expression of CRY1F, PAT and CP4 EPSPS proteins in forage and grain obtained from maize 1507 x NK603. One study was conducted at five field sites in Europe in 2003 (EFSA-GMO-UK-2005-17). Protein levels of CRY1F, PAT and CP4 EPSPS in grain from maize 1507 x NK603 was characterised using a specific Enzyme Linked Immunosorbent Assay (ELISA) developed for each protein. The forage and grain samples were taken from plots that were sprayed with either i) glyphosate herbicide; ii) with glufosinate-ammonium herbicide, or iii) with glyphosate followed by glufosinate-ammonium herbicides. The results obtained from the expression analysis are summarised in Table 1. The levels of the proteins CRY1F, PAT and CP4 EPSPS in forage and grain from maize 1507 x NK603 were comparable regardless of herbicide treatments.
The second study was conducted at six field sites in Chile in 2002-2003 (EFSA-GMO-UK-2004-05). Sampling and measurements of protein levels of CRY1F, PAT and CP4 EPSPS in grain from maize 1507 x NK603 were performed using the same herbicide treatments and ELISA as described above.

The results obtained from the analysis are summarised in Table 2. Levels of the proteins CRY1F, PAT and CP4 EPSPS in grain from maize 1507 x NK603 were comparable regardless of the herbicide treatment.

CRY1F
In the European study, the level of CRY1F protein in maize 1507 x NK603 ranged from 5.19 - 10.2 µg/g dw in forage, and 0.71 - 2.7 µg/g dw in grain.
The level of CRY1F protein in grain from the field study in Chile ranged from 0.53 - 2.43 µg/g dw.

The levels of CRY1F protein in maize 1507 ranged from 1.0 to 6.9 µg/g dw in whole plant extracts, and 1.2 to 3.1 µg/g dw in grain.

PAT
In the European study, the level of PAT protein in maize 1507 x NK603 ranged from 0.48 to 2.66 µg/g dw in forage, and below the lower limit of quantification (0.075 µg/g dw) in grain.
The level of PAT in grain from the field study in Chile was below the lower limit of quantitation (0.075 µg/g dw).

Levels of PAT protein in maize 1507, ranged from below the limit of detection (LOD) to 38.0 pg/µg total extractable protein (i.e. approx. 0.16 ng PAT/mg dw) in whole plant extracts, and from below the LOD to 136.8 pg/µg total extractable protein (i.e. approx. 11.8 ng PAT/mg dw) in leaf extracts. Levels of PAT protein in grain were also below the lower limit of quantitation of the assay.

CP4 EPSPS
In the European study, the level of CP4 EPSPS protein in maize 1507 x NK603 ranged from 49.8 - 162 µg/g dw in forage, and 3.76 – 12.6 µg/g dw in grain.

In Chile the level of CP4 EPSPS protein in grain from 1507 x NK603 maize ranged from 3.80 - 11.10 µg/g dw (3.30 - 9.65 µg/g fw).

Levels of CP4 EPSPS protein in grain from maize NK603, ranged from 6.9 to 15.6 µg/g fw.

Table 1. Expression of CRY1F, PAT and CP4 EPSPS proteins in forage and grain from maize 1507 x NK603 plants sprayed with i) glyphosate; ii) glufosinate-ammonium, and; iii) with glyphosate followed by glufosinate-ammonium (Europe growth season 2003).

Hybrid	Mean[1] protein expression level (µg/g tissue dw)	Standard Deviation	Min/max Range (µg/g tissue dw)	Number of samples
Cry1F Protein				
1507 x NK603 + glyphosate Forage Grain	 7.89 1.55	 1.58 0.47	 5.74–10.2 0.71-2.6	 5 25
1507 x NK603 +GA[2] Forage Grain	 7.28 1.71	 1.08 0.34	 6.11-8.3 1.04-2.59	 5 25
1507 x NK603 + glyphosate+ GA Forage Grain	 8.06 1.76	 1.80 0.54	 5.19-9.95 0.74-2.70	 5 25
PAT Protein				
1507 x NK603 + glyphosate Forage Grain	 1.40 ND[3]	 0.62 ND	 0.70-2.05 ND	 5 25
1507 x NK603 +GA[2] Forage Grain	 1.53 ND	 0.89 ND	 0.55-2.66 ND	 5 25
1507 x NK603 + glyphosate+ GA Forage Grain	 1.17 ND	 0.70 ND	 0.48-2.27 ND	 5 25
CP4 EPSPS Protein				
1507 x NK603 + glyphosate Forage Grain	 96.2 8.55	 17.6 2.18	 66.7-111 4.27-11.6	 5 25
1507 x NK603 +GA[2] Forage Grain	 92.7 7.32	 17.5 2.10	 74.7-114 3.76-11.7	 5 25
1507 x NK603 + glyphosate+ GA Forage Grain	 104 8.25	 42.3 2.00	 49.8-162 5.05-12.6	 5 25

[1] Values are means across all six field sites
[2] GA: Plots treated with glufosinate-ammonium
[3] ND: below the lower limit of quantification (LLOQ for PAT protein was 0.075 µg/g d.w.)

Table 2. Expression of CRY1F, PAT and CP4 EPSPS proteins in grain from maize 1507 x NK603 plants sprayed with i) glyphosate; ii) glufosinate-ammonium; and iii) with glyphosate followed by glufosinate-ammonium (Chile growth season 2002/2003)

Hybrid	Mean[1] protein expression level (µg/g tissue dw)	Standard Deviation	Min/max Range (µg/g tissue dw)	Number of samples
Cry1F Protein				
1507 x NK603 + glyphosate	1.37	0.29	0.94-1.98	30
1507 x NK603 + GA[2]	1.57	0.34	0.98-2.43	30
1507 x NK603 + glyphosate + GA	1.42	0.34	0.53-2.17	30
PAT Protein				
1507 x NK603 + glyphosate	ND[3]	ND	ND	30
1507 x NK603 + GA2	ND	ND	ND	30
1507 x NK603 + glyphosate + GA	ND	ND	ND	30
CP4 EPSPS Protein				
1507 x NK603 + glyphosate	8.25	1.42	5.62-11.10	30
1507 x NK603 + GA2	6.66	1.06	3.83-8.68	30
1507 x NK603 + glyphosate + GA	6.62	1.42	3.80-9.52	30

[1] Values are means across all six field sites
[2] GA: Plots treated with glufosinate-ammonium
[3] ND: below the lower limit of quantification (LLOQ for PAT protein was 0.075 µg/g d.w.)

Parts of the plant where the insert is expressed

Maize 1507 x NK603 expresses the proteins CRY1F, PAT and CP4 EPSPS throughout the different parts of the plant. In the field studies, the proteins CRY1F and CP4 EPSPS were expressed at comparable levels regardless of the herbicide treatment in forage and grain samples from maize 1507 x NK603. Expression of the PAT protein in maize 1507 x NK603 grain was below the lower limit of quantitation of the assay, which was 0.075 µg/g grain dry weight.

Potential fusion proteins

Southern blot analyses performed on maize 1507 x NK603 have indicated molecular equivalence and equal copy numbers between the inserts found in maize 1507 x NK603 and those present in the single events 1507 and NK603. According to these findings it is unlikely that maize 1507 x NK603 expresses potential fusion proteins.

2.1.4 Inheritance and genetic stability of inserted DNA

According to data from the applicant the parental maize lines 1507 and NK603 have both incorporated a single DNA insert containing a single copy of their respective DNA fragments, and that these are located at different loci in the maize genome. Interaction of the two transgene inserts is therefore expected to be minimal during conventional breeding / development of the stacked event 1507 x NK603.
Southern blot analyses, protein expression levels, phenotypic characteristics and agronomic performance, indicate that the integrity of the inserts inherited from the single events is preserved in maize 1507 x NK603.

2.2 Conclusion

Southern blot and PCR analyses have indicated that the recombinant inserts in the parental maize events1507 and NK603 are retained in the stacked maize 1507 x NK603. Genetic stability of the inserts has previously been demonstrated in the parental events. Protein measurements show comparable levels of Cry1F, PAT and CP4 EPSPS proteins in the stacked maize 1507 x NK603 and the parental lines. Phenotypic analyses also indicated stability of the insect resistance and herbicide tolerance traits.

The VKM Panel on GMO considers the molecular characterisation of maize 1507 x NK603 and its parental events 1507 and NK603 as adequate.

3 Comparative assessment

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

3.1.1 Experimental design & statistical analysis

Application EFSA/GMO/UK/2004/05
In the application EFSA/GMO/UK/2004/05 for food and feed uses, import and processing of maize 1507 x NK603 within the European Union, the applicant presents compositional data from seed and forage material collected from field trials in Chile during the 2002/2003 growth season. In addition, data derived from material obtained from field trials with the single events and the respective comparators were provided by the applicant.

The field trials were performed at six separate sites in commercial maize-growing regions of Chile. These trials compared the composition of maize 1507 x NK603 with a conventional counterpart, a non-GM maize hybrid with a genetic background similar to the maize stack 1507 x NK603. The VKM GMO Panel considers the choice of comparator as appropriate.
In this application, comparisons with baseline data on commercial maize, compiled from publicly available literature, have been used in the comparisons with maize 1507 x NK603 for consideration of natural variations. No conventional commercial reference varieties were included in the field trials and the comparative assessments.

At each trial site, maize 1507 x NK603 and the conventional counterpart were planted following a randomised complete block design containing four blocks with test and control entries planted in 2-row plots located randomly within each block. 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 maintenance operations were performed uniformly across the entire study area.

Three of the blocks were used in the comparative assessment and the additional block was used for obtaining samples for protein expression analysis. 1507 x NK603 maize grown for compositional analysis either received two applications of glyphosate, two applications of glufosinate-ammonium, or one application of glyphosate followed by one application of glufosinate-ammonium. 1507 x NK603 maize grown for agronomic analysis received one application of glyphosate followed by one application of glufosinate-ammonium.

Statistical analysis of agronomic characteristics and nutrient composition data was conducted using SAS/STAT software to generate analysis of variance (ANOVA), means and standard deviations.

Two separate statistical analyses were carried out on the composition data. For the first analysis, the data from all replicates and all locations were combined and analysed. Least-square means and standard deviation were calculated for the data across all six locations and statistically significant differences were identified using a *t*-test at a 5% level of significance.

For the second statistical analysis, the results obtained were evaluated on a per location basis using data from the 3 replicates of each maize entry at each location. The least-square means and standard deviation for each location and maize entry were calculated, and statistically significant differences were identified using a *t*-test at a 5% level of significance.

Application EFSA/GMO/UK/2005/17
In the application for food and feed uses, import and processing and cultivation of maize 1507 x NK603 within the European Union, the applicant presents compositional data from grain and forage material collected in field trials in Europe during the 2003 growth season. In addition, agronomic data derived from material obtained from field trials with the single events and the respective comparators were provided by the applicant.

The field study was conducted at five locations in (three sites in Spain and two sites in Bulgaria). Each location included a randomised complete block design containing four blocks (or replicates). Three of these blocks were used in the comparative assessment and the additional block was used for obtaining samples for protein expression analysis. Each block contained the maize 1507 x NK603 and a non-modified, near isogenic control hybrid for comparative purposes. No conventional commercial reference varieties were included in the field trials or the comparative assessments.

Plots of 1507 x NK603 maize received either two sequential applications of glyphosate, two sequential applications of glufosinate-ammonium, or an application of glyphosate followed by an application of glufosinate-ammonium herbicide. Plots untreated with the target herbicides were not included in the field study.

Statistical analysis of agronomic characteristics and nutrient composition data was conducted using SAS/STAT software to generate analysis of variance (ANOVA), means and standard deviations. Two separate statistical analyses were carried out on the composition data. For the first analysis, the data from all replicates and all locations were combined and analysed by a linear mixed model. Least-square means and standard error of the means were calculated for the data across locations.

For the second statistical analysis, the obtained results were evaluated on a per location basis using data from the 3 replicates of each maize entry at each location. The least-square means and standard error of the means for each location and maize entries were calculated. Statistically significant differences, both for the across location analysis and the individual location analysis, were identified at a 5% level of significance.

In addition to the statistical analysis as described above, composition data were also analysed taking into account the multiple comparisons. The false discovery rate (FDR) method was used to account for the numerous comparisons and to minimise the number of differences being declared to be significant due to chance alone.

Composition data from commercial maize hybrids as well as publicly available literature references have been used as the baseline in the comparisons with maize 1507 x NK603. Composition data obtained for 1507 x NK603 forage and grain were also analysed taking into account tolerance intervals expected to contain, with 95% confidence, 99% of the values expressed in a population of commercial maize hybrids. Furthermore, a comparative assessment with non-GM control maize of comparable genetic background was carried out. Any statistically significant differences in nutrient composition between maize 1507 x NK603 and non-GM control maize were further investigated.

3.2 Compositional Analysis

3.2.1 Application EFSA/GMO/UK/2004/05 (Chile)

The nutritional analysis was undertaken on a broad range of compounds in grain from maize 1507 x NK603 in accordance with OECD guidelines for assessment of GM maize (OECD, 2002). The objective of this study was to determine if 1507 x NK603 maize treated with glyphosate followed by glufosinate-ammonium herbicides was equivalent to non-GM control maize with a comparable genetic background.
Forage samples from 1507 x NK603 maize and non-GM control maize were collected and analysed for proximates (crude protein, crude fat, ash, carbohydrates, crude fibre, acid detergent fibre and neutral detergent fibre) and minerals (calcium and phosphorous). Grain samples from 1507 x NK603 maize and non-GM control maize were collected and analysed for nutrient composition, including: proximates (crude protein, crude fat, ash, carbohydrates, crude fibre, acid detergent fibre and neutral detergent fibre), fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acids), amino acids (methionine, cystine, lysine, tryptophan, threonine, isoleucine, histidine, valine, leucine, arginine, phenylalanine, glycine, alanine, aspartic acid, glutamic acid, proline, serine, and tyrosine), minerals (phosphorus, calcium, copper, iron, magnesium, manganese, potassium, sodium, zinc) vitamins (beta-carotene, vitamin B1, vitamin B2, folic acid, and vitamin E [alpha tocopherol isomer]), secondary metabolites (inositol, furfural, p-coumaric acid and ferulic acid), and anti-nutrients (phytic acid, raffinose and trypsin inhibitor).
In accordance with OECD guidelines (OECD, 2002) substantial equivalence was evaluated by comparing mean nutrient composition values of 1507 x NK603 maize treated with glyphosate followed by glufosinateammonium herbicide to non-GM maize, and mean nutrient composition values of the 1507 x NK603 maize entry to nutrient ranges available in the published literature. Statistical analyses were conducted with data combined across all six locations as well as on a per location basis using data from the 3 replicates at each of the individual locations.

With the exception of intermittent minor, but statistically different variations, the compositional analyses show comparable levels for nutrient, anti-nutrient, mineral and vitamin composition between the stacked event and control lines within and across the different herbicide treatments. Detailed

results of the compositional analyses in grain and forage from the field trials in Chile are summarised in Tables 1 – 24 (Appendix).

3.2.2 Application EFSA/GMO/UK2005/17 (Europe)

The nutritional analysis was undertaken on a broad range of compounds in forage and grain from 1507 x NK603 maize in accordance with OECD guidelines for assessment of GM maize (OECD, 2002). Forage samples from 1507 x NK603 maize and non-GM control maize with a comparable genetic background were collected and analysed for proximates (crude protein, crude fat, ash, carbohydrates, crude fibre, acid detergent fibre and neutral detergent fibre) and minerals (calcium and phosphorous). Grain samples from 1507 x NK603 maize and non-GM control maize were collected and analysed for nutrient composition, including: proximates (crude protein, crude fat, ash, carbohydrates, crude fibre, acid detergent fibre and neutral detergent fibre), fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acids), amino acids (methionine, cystine, lysine, tryptophan, threonine, isoleucine, histidine, valine, leucine, arginine, phenylalanine, glycine, alanine, aspartic acid, glutamic acid, proline, serine, and tyrosine), minerals (phosphorus, calcium, copper, iron, magnesium, manganese, potassium, sodium, zinc) vitamins (beta-carotene, vitamin B1, vitamin B2, folic acid, and vitamin E [alpha tocopherol isomer]), secondary metabolites (inositol, furfural, p-coumaric acid and ferulic acid), and anti-nutrients (phytic acid, raffinose and trypsin inhibitor).

In accordance with OECD guidelines (OECD, 2002) substantial equivalence was evaluated by comparing mean nutrient composition values of each 1507 x NK603 maize entry to non-GM maize and mean nutrient composition values of each 1507 x NK603 maize entry to nutrient ranges available in the published literature. Statistical analyses were conducted with data combined across all five locations as well as on a per location basis using data from the 3 replicates at each of the individual locations.

With the exception of intermittent minor statistically different variations, the compositional analyses show comparable levels for nutrient, anti-nutrient, mineral and vitamin composition between the stacked event and control lines within and across the different herbicide treatments. Detailed results of the compositional analyses in grain and forage from the field trials in Europe are summarised in Tables 25 – 51 (Appendix).

3.3 Agronomic and phenotypic characters

During the field trials in Chile 2002/2003, phenotypic and agronomic data related to dormancy and germination, emergence and vegetative growth, reproductive growth, seed retention, and stress (i.e., disease and biotic stress responses) were collected. The following characters were recorded over the course of the growing season: early population/germination, seeding vigour, time to silking (growing degree units to 50 % silking), time to pollen shed (growing degree units to 50% pollen shed), stay green, plant height, ear height, number of stalk and root lodged plants, final stand count, pollen shape and colour, disease incidence and insect damage (Table 52, Appendix). Yield/grain yield was not measured in these trials.

Analyses of variance across trial locations indicated no statistically significant differences between maize 1507 x NK603 (treated with glyphosate and glufosinate ammonium) and the corresponding conventional counterpart for any of the agronomic characteristics measured ($p<0.05$) (Table 52, Appendix). Similarly, no unexpected changes in pollen production, seed production, seed viability and germination were observed for maize stack 1507 x NK603 when compared with the conventional counterpart.

In 2003, corresponding agronomic and phenotypic characters were also measured for maize 1507 x NK603 and the non-GM control maize during the field trials in Europe. Analyses of variance across trial locations showed statistically significant differences between maize 1507 x NK603 and the comparator for the characteristics growing degree units to 50 % pollen shed and 50 % silking, ear height and final population ($p<0.05$) (Table 53, Appendix). On average, maize 1507 x NK603 plants had a higher number of accumulated heat units before 50 % of the plants were shedding pollen and silking (860 vs. 833 GDU and 877 vs. 854 GDU, respectively) compared with the conventional counterpart. The transgenic hybrid also had significantly lower ear height (73 vs. 81 cm) and lower number of viable plants per plot remaining at maturity (44 vs. 46 plants). Significant differences for these parameters were observed at 1-2 of the individual field trial sites (Table 54, Appendix). No statistically significant differences between maize 1507 x NK603 and the comparator were detected for any of the other assessed phenotypic characteristics in the across location analysis ($p>0.05$).

The VKM GMO Panel is of the opinion that the observed differences are not biologically relevant.

3.4 Conclusion

Comparative analyses of the compositional, agronomic and phenotypic characteristics of maize stack 1507 x NK603 and near-isogenic comparators were performed during multiple field trials in Chile and Europe in 2002/2003. With the exception of small intermittent variations, the results show no indications of unwanted unintentional effects, and that maize stack 1507 x NK603 is compositionally, agronomically and phenotypically equivalent to its comparators, with the exception of the introduced insect resistance and herbicide tolerance traits.

4 Food and feed risk assessment

4.1 Product description and intended uses

The genetic modification in maize 1507 x NK603 will not impact the existing production processes used for maize. All 1507 x NK603 maize products will be produced and processed for use in food, animal feed and industrial products in the same way as other commercial maize. Maize 1507 x NK603 and all food, feed and processed products derived from maize 1507 x NK603 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, feed and processed products derived from maize will remain the same.

4.2 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 Cry1F, PAT and CP4 EPSPS proteins (Hammond et al 2011).

4.3 Toxicological assessment

4.3.1 Toxicological assessment of the newly expressed proteins

4.3.1.1 Acute oral toxicity testing

*Acute intravenous exposure of **PAT** protein in rodents*

Bayer Crop Sciences has performed an acute toxicity study of the PAT-protein in rats by a single intravenous administration (Hèrouet et al. 2005). The study was performed in accordance with the principles of Good Laboratory practice (Organisation for Economic Cooperation and Development) Principles of Good Laboratory Practice, 1997, European Commission Directive 1999/1 I/EC, 1999, French decree n°98-1312, regarding Good Laboratory Practice, December 31, 1998, - E.P.A. (Environmental Protection Agency) • 40 CFR part 160 Federal Insecticide, Fungicide and Rodenticide Act (F1FRA): Good Laboratory Practice Standards: Final Rule, August 17, 1989, and Good Laboratory Practice Standards for Toxicology studies on Agricultural Chemicals, Ministry of Agriculture, Forestry and Fisheries (M.A.F.F.), notification 12 NohSan n°8628, (December 06 2000).

The objective of this study was to assess the acute intravenous toxicity in OF1 mice of the PAT (phosphoacetyl transferase) protein (>95% purity), a protein encoded by the *bar* gene. In addition, the acute intravenous toxicity of aprotinin (negative control) and melittin (positive control) were also compared. Groups of 5 female OF1 mice were administered either PAT protein, aprotinin or melittin in physiological saline at dose levels of 1 and 10 mg/kg body weight.

All animals were observed for clinical signs daily for fifteen days whilst their body weights were measured weekly. No clinical signs were noted in PAT protein-treated animals or in control groups throughout the study period. The body weight evolution was unaffected by the treatment with either PAT protein at 1 and 10 mg/kg or control substances up to Day 15. At termination of the study period, animals were subjected to a necropsy including macroscopic examination. No treatment-related macroscopic abnormalities were detected in animals treated with either PAT protein at 1 and 10 mg/kg or control substances. The positive control (melittin), at 10 mg/kg, induced 100% mortality. Animals treated at 1 mg/kg of melittin and negative control animals treated with aprotinin at 1 and 10 mg/kg showed no visible signs of systemic toxicity.

In another study, PAT Microbial Protein (FL), which was 84% pure, was evaluated for acute oral toxicity (Brooks 2000). Five male and five female CD-1 mice received 6000 mg/kg of the test material (containing approximately 5000 mg/kg PAT) as a 25% w/v suspension in aqueous 0,5% methylcellulose. Because the volume of the test material in methylcellulose exceeded 2 ml/100g body weight, the test material suspension was administrated as two fractional gavage doses, given approximately one hour apart. Parameters evaluated during the two-week observation period included body weights and detailed clinical observation. All animals were examined for gross pathological changes. All mice survived to the end of the two-week observation period. There were no treatment-related clinical observations. All mice except one female gained weight over the duration of the study. There were no gross pathological lesions found in any of the animals. Under the condition of this study, the acute oral LD_{50} of PAT Microbial (FL) in male and female CD-1 mice was greater than 6000 mg/kg.

*Acute oral exposure of **Cry1F** protein in rodents*

The potential toxicity of the Cry1F protein to humans and animals was examined in an study where Cry1F protein was evaluated for acute toxicity in mice (Kuhn, 1998). The test substance, Cry1F *B.thuringiensis* subsp. *aizawai* Delta-toksin, was evaluated for its acute oral toxicity potential in albino mice when administrated as a gavage dose at a level of 5050 mg/kg to males and females. The test substance was administrated as a 15% w/v concentration in 2% w/v aqueous carboxymethyl cellulose. No mortality occurred during the study. There were no clinical signs of toxicity exhibited at any time

throughout the study. There was no significant effect on body weight gain. The gross necropsy conducted at termination of the study revealed no observable abnormalities. The acute oral LD_{50}, as indicated by the data, was determined to be greater than 5050 mg/kg. The relatively high dose tested did not give rise to any toxicity and therefore the acute LD50 for Cry1F protein could not be determined other than to be estimated as higher than 5050 mg Cry1F per kg body weight.

*Acute oral exposure of **CP4 EPSPS** protein*

Monsanto has conducted an acute toxicity study (MSL-13077, 1993) 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).

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

The protein preparation containing the CP4 EPSPS was administered as a single dose by gavage to three groups of mice at dosages of 49, 154 and 572 mg/kg body weight respectively. The doses corresponded to 40, 100 and 400 mg/kg of CP4 EPSPS protein based on the level of purity of the protein and ELISA analyses of the dosing solutions. A control group received bovine serum albumin (BSA) at a dosage of 363 mg/kg in the same solution and delivery volume as the test substance. The second control group was administered 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 measured. Signs of toxicity included such occurrences as changes in the skin and fur, eyes and mucous membranes, respiratory, autonomic and central nervous systems as well as behavioural changes. At the termination of the study (day 8-9), animals were sacrificed, examined for gross pathology and numerous tissues were collected.

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

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

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

4.3.1.2 Repeated dose toxicity testing

*Repeated dose 14-day oral toxicity study of **PAT** protein in rodents*
Bayer Crop Sciences has performed a sub-chronic oral toxicity study of the PAT-protein in rats (Pfister et al. 1996).
The study was performed in accordance with the principles of OECDs Good Laboratory practice of OECD (Organisation for Economic Cooperation and Development) and Principles of Good Laboratory Practice, 1992. Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles, March 1986 and the Japanese Ministry of Agriculture, Forestry and Fisheries: On Good Laboratory Practice Standards for Toxicological Studies on Agricultural Chemicals, Agricultural Production Bureau, 59 NohSan Notification Number 3850, August 10, 1984. Test guidelines: The study procedures mostly conform to OECD Guidelines for Testing of Chemicals, number 407 "Repeated Dose 28-day Oral Toxicity Study in Rodents", adopted by the Council on July 27, 1995).

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

The study comprised four groups of five male and five female Wistar rats in each group. The rats in group 1 received a standard diet without PAT protein, whereas rats in group 2, 3 and 4 received diets with the inclusion of PAT and/or soybean protein: group 1 (standard diet), group 2 (0.5 % PAT + 4.5 % soybean), group 3 (5 % PAT), group 4 (5 % soybean), for a period of 14 days.
The mean intake of PAT-protein in group 2 over the treatment period was 712 mg/kg body weight/day for males and 703 mg/kg body weight/day for females. In group 3 the mean intake of PAT-protein was 7965 mg/kg body weight/day for males and 7619 mg/kg body weight/day for females.

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

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

4.3.2 Toxicological assessment of the whole GM food/feed

*Feeding study on poultry / Broiler chickens with the hybrid maize event **1507 x NK603***
A poultry feeding study was conducted over a 42-day period with diets containing grain from maize 1507 x NK603. The 1507 x NK603 maize grains used in the study were produced from plants that received either i) two sequential treatments with glufosinate-ammonium herbicide, ii) two sequential treatments with glyphosate herbicide or iii) treatments of glyphosate followed by glufosinate-ammonium herbicide. For comparison, diets containing grain from a non- GM maize with a

comparable genetic background and from three types of commercial maize (33P66, 33J56 and 33R77) were also fed to the chickens. Poultry studies are considered to be very useful because they utilise a fast growing organism e.g. broiler chickens, that consume a high percentage of maize in the diet, and that are very sensitive to potentially toxic effects of dietary components (OECD, 2003a). The chickens were observed for overall health, behavioural changes and/or evidence of toxicity. Body weights and feed weights were measured every 7 days. The body weight parameters evaluated at the end of the 42-day study included carcass yield, thighs, breasts, wings, legs, abdominal fat, kidneys, and whole liver. Mortality, body weight gain and feed conversion were also compared. The results of the study indicated not adverse effects of maize 1507 x NK603 regardless of herbicide treatment, and nutritional equivalence of maize 1507 x NK603 to the non-GM maize and the commercial maize varieties used.

1507 maize
A 90-day feeding study was performed on Sprague-Dawley rats in accordance to OECD 408 guidelines with control and test (11 and 33% 1507 maize in feed) diets (MacKenzie et al. 2007). Results of the study showed no toxicologically significant differences between treatment groups. Observations included nutritional performance variables, clinical and neurobehavioral signs, ophthalmology, clinical pathology (haematology, clinical chemistry, coagulation, and urinalysis), organ weights, and gross and microscopic pathology.

NK603 maize
Likewise, a published 90-day study on Sprague–Dawley rats conducted with diets prepared with NK603 (11 and 33% inclusion in feed), resulted in no consistent differences in the measured clinical, biochemical and histological parameters, except for slightly elevated levels of average corpuscular volume and average corpuscular haemoglobin in female rats administered the high dose (33%), equalling 7 grams/kg body weight/day of maize NK603 (Hammond et al. 2004).

According to a two year feeding study performed by Séralini and co-workers (Séralini et al. 2012), 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 with, and structural similarity to, known human IgE-allergens using an array of bioinformatic tools. Sequence homology searches comparing the structure

of novel proteins to known IgE-allergens in a database are conducted using various algorithms such as FASTA to predict overall structural similarities. According to FAO/WHO (2001) in cases where a novel protein and a known IgE-allergen have more than 35% identity over a segment of 80 or greater amino acids, IgE cross-reactivity between the novel protein and the allergen should be considered a possibility.

4.4.1 Assessment of allergenicity of the newly expressed proteins

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 Cry1F, PAT and CP4 EPSPS proteins, 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
- assessment of protein exposure

These assessments have previously been described by the applicant for the single maize events 1507 (EFSA-GMO-NL-2004-02, EFSA-GMO-RX-1507) and NK603 (Notification C/ES/00/01, EFSA-GMO-NL-2005-22, EFSA-GMO-RX-NK603), and were based on the following aspects:

i) The source of the genes inserted in maize 1507 and NK603: *B. thuringiensis* (*cry*1F), *S. viridochromogenes* (*pat*), and *Agrobacterium* sp. strain CP4 (*cp*4 *epsps*) have no history of causing allergy.
ii) History of safe use of Cry proteins as microbial pesticides; no indications of Cry proteins originating from *Bacillus thuringiensis* having harmful effects on the health of humans or animals.
iii) The Cry1F protein does not show significant amino acid sequence similarity to known protein toxins, and do not share immunologically relevant sequence similarity to known allergens.
iv) The Cry1F protein is rapidly degraded, as shown by SDS-PAGE, under simulated gastric fluids.
v) The Cry1F protein is considered heat labile, since biological activity of Cry1F is lost after exposure to 75 °C for 30 minutes.
vi) The Cry1F protein is not glycosylated.
vii) The PAT protein has been the subject of previous safety assessments of genetically modified plants and found to have no potential for allergenicity.
viii) The PAT protein lacks homology to known toxins or IgE-allergenic proteins.
ix) Rapid degradation of the PAT protein in simulated gastric fluids.
x) 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.
xi) The CP4 EPSPS protein is readily degraded in simulated digestive fluids and is not glycosylated.
xii) CP4 EPSPS is considered as heat labile.

The information listed above indicates that the newly expressed proteins in 1507 x NK603 maize lack 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 the maize 1507 x 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 1507 x Nk603 or the parental events 1507 and NK603 with the exception of the introduced trait, no increased allergenicity is anticipated for maize 1507 x 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 potential increased allergenicity of 1507 x NK603 maize 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 1507 x 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 2011a), adjuvants are substances that, when co-administered with an antigen increase the immune response to the antigen and therefore might increase the allergic response. In cases when known functional aspects of the newly expressed protein or structural similarity to known strong adjuvants may indicate possible adjuvant activity, the possible role of these proteins as adjuvants should be considered. As for allergens, interactions with other constituents of the food matrix and/or processing may alter the structure and bioavailability of an adjuvant and thus modify its biological activity.

Only two of the 10 Cry proteins that are currently used in genetically modified plants, Cry1Ab and Cry1Ac, have been studied experimentally regarding adjuvant effects. To the knowledge of the VKM GMO Panel, adjuvant effects have not been investigated for the other 8 Cry proteins used in GM plants, or for other groups of Cry proteins. Immunological mapping of the systemic and mucosal immune responses to Cry1Ac have shown that mice produce both systemic IgM and IgG and secretory IgA following intraperitonal and intragastric immunisation. In a mouse study by Vazquez et al., the adjuvant effect of Cry1Ac was found to be as strong as the effect of cholera toxin (CT) (Vazquez et al. 1999). The adjuvant effect of CT is thus a relevant basis for comparison in a risk assessment of Cry1Ac. It is uncertain whether this applies to the same extent to other Cry proteins.

“Bystander sensitisation”

"Bystander sensitisation” can occur when an adjuvant in food, or an immune response against a food antigen, results in an increased permeability of the intestinal epithelium for other components in food. Previously it was assumed that the epithelial cells of the intestine were permanently "glued together" by the so-called "tight junctions". More recent knowledge shows that these complex protein structures are dynamic and can be opened up by different stimuli.

Both *in vitro* and *in vivo* experiments have demonstrated that when an IgG response which can result in a complement activation (among other) is not balanced by an IgA response, the epithelial barrier can be opened and unwanted proteins are able to enter the body (bystander-penetration) and lead to allergic sensitisation (Brandtzaeg P, Tolo K 1977; Lim PL, Rowley D1982).

Additional information can be found in the report by VKM on Cry-proteins and adjuvanticity: "Health risk assessment of the adjuvant effects of Cry proteins from genetically modified plants used in food and fodder" (VKM 2012c)

4.5 Nutritional assessment of GM food/feed

The compositional analyses of 1507 x NK603 maize indicate nutritional equivalence to the non-GM comparators and to the range of values published in the literature. Spraying with glufosinate-ammonium or glyphosate herbicides did not affect the nutrient composition of the maize.
The nutritional equivalence is further supported by the poultry feeding study where broiler chickens were fed over a 42-day period with diets containing grain from herbicide treated 1507 x NK603 maize

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 for adults of 0.6 % (Vikse 2009). 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 child (Vikse 2009). In comparison the daily intake in Europe is 8.8 g dry weight/person/day. Maize is also a common feed ingredient and some farm animals such as poultry and pigs are fed diets composed of up to 80% maize.

The expression levels of the CRY1F and CP4 EPSPS proteins in grain from 1507 x NK603 maize ranged from 0.71 – 2.7 µg/g dw (Europe) and 3.76 – 12.6 µg/g dw (Europe) respectively. Expression of the PAT protein in 1507 x NK603 grain was below the limit of detection (0.075 µg/g grain dw).

Based on these numbers, and that all foods from maize are derived from maize 1507 x NK603 grain, the estimated maximum daily intake for an average Norwegian adult of Cry1F and CP4 EPSPS proteins is calculated as 11.9 µg and 55.4 µg, respectively, on a dry weight basis. These levels are several orders of magnitude below the levels shown to have no effect in laboratory toxicology testing. Also, these levels are considerably below the proposed threshold of toxicological concern (TTC) level of 1800 µg/person/day (Class 1, oral exposure) for chemicals considered to have a low potential for toxicity based on metabolism and mechanistic data (Vermeire et al., 2010). Farm animals such as poultry and pigs could on the other hand reach a daily intake of Cry1F and CP4 EPSPS proteins beyond the TTC level.

This dietary exposure assessment is conservative. It assumes that all maize consumed consists of 1507 x NK603 maize and that protein levels are not reduced by processing.

4.6 Conclusion

Whole food feeding studies on rats have not indicated any adverse effects of the parental maize lines 1507 and NK603. No rodent whole food feeding study has been performed on the stacked maize 1507 x NK603; the applicant has however provided a nutritional feeding study performed on broilers. No

adverse effects were observed in the study. Bioinformatics analyses have not revealed expression of any known ORFs in the parental maize lines, and none of the newly expressed proteins show resemblance to any known toxins or IgE allergens. Nor have the newly expressed proteins been reported to cause IgE mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Acute and repeated dose toxicity tests in rodents have not indicated toxic effects of the Cry1F, PAT or CP4 EPSPS proteins. However, these tests do not provide any additional information about possible adverse effects of maize 1507 x NK603.

Based on current knowledge, the VKM GMO Panel concludes that the stacked maize 1507 x NK603 is nutritionally equivalent to conventional maize varieties, and that it is unlikely that the newly expressed proteins introduce a toxic or allergenic potential in food and feed derived from maize 1507 x NK603 compared to conventional maize.

5 Environmental risk assessment

5.1 Unintended effects on plant fitness due to the genetic modification

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

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

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

It is considered very unlikely that the establishment, spread and survival of maize 1507 x NK603 would be increased due to the insect resistance and herbicide tolerance traits. The herbicide tolerant

trait can only be regarded as providing a selective advantage for the GM maize plant where and when glufosinate ammonium-based herbicides are applied. Glufosinate ammonium-containing herbicides have been withdrawn from the Norwegian market since 2008, and the substance will be phased out in the EU in 2017 for reasons of reproductive toxicity. Similarly insect resistance against certain lepidopteran and coleopteran pests provides a potential advantage in cultivation of 1507 x NK603 under infestation conditions. It is considered very unlikely that maize 1507 x 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 1507 x NK603 relative to its conventional counterpart. A series of field trials with maize 1507 x NK603 were carried out across six locations in Chile in 2002/2003 (application EFSA/GMO/UK/2004/05). In addition, agronomic observations performed in field trials in the EU in 2003 (Spain and Bulgaria) have been provided by the applicant in application EFSA/GMO/UK/2005/17. Information on phenotypic (e.g. crop physiology, morphology, development) and agronomic characteristics was provided to assess the agronomic performance of maize 1507 x NK603 in comparison with its conventional counterpart (see section 3.1). Data from the field trials in Chile showed no statistically significant differences between maize 1507 x NK603 (treated with glyphosate and glufosinate ammonium) and the corresponding conventional counterpart for any of the agronomic characteristics measured ($p>0.05$). Similarly, no unexpected changes in pollen production, seed production, seed viability and germination were observed for maize stack 1507 x NK603 when compared with the conventional counterpart.

Data from field trials in Europe shows some statistical significant differences between maize 1507 x NK603 and non-GM control maize at individual field sites, e.g. for degree units to 50 % pollen shed and 50 % silking, ear height and final population count ($p<0.05$). These differences were however small in magnitude and were not consistently observed over locations. In the European field trials mean time to silking and plant height values across locations for the maize 1507 x NK603 and control maize were statistically different ($p<0.05$). The VKM GMO Panel is of the opinion that they do not raise any environmental safety concern.

In addition to the data presented by the applicant, the VKM GMO Panel is not aware of any scientific reports indicative of increased establishment or spread of maize 1507 x NK603, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of maize 1507 x NK603 are unchanged, insect resistance and glufosinate tolerance are not likely to provide a selective advantage outside of cultivation in Europe. The VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects based on establishment and survival of maize 1507 x 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 1507 x NK603. This means that micro-organisms in the digestive tract in humans and animals (both domesticated animals and other animals feeding on fresh or decaying plant material from the transgenic maize line) may be exposed to transgenic DNA.

Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation with which maize can hybridise and form backcross progeny

(Eastham & Sweet 2002; OECD 2003). Vertical gene transfer in maize therefore depends on cross-pollination with other conventional or organic maize varieties. All maize varieties which are cultivated in Europe can interbreed. In addition, unintended admixture/adventitious presences of genetically modified material/transgenes in seeds represent a possible way for gene flow between different production systems.

5.2.1 Plant to micro-organisms gene transfer

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

Based on established scientific knowledge of the barriers for gene transfer between unrelated species and the experimental research on horizontal transfer of genetic material from plants to microorganisms, there is today little evidence pointing to a likelihood of random transfer of the transgenes present in maize 1507 x 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 1507 x NK603 will transfer and establish in the genome of microorganisms in the environment or in the intestinal tract of humans or animals. In the rare, but theoretically possible case of transfer of the *cry*, *pat* and CP4 EPSPS genes from 1507 x NK603 to soil bacteria, no novel property would be introduced into or expressed in the soil microbial communities; as these genes are already present in other bacteria in soil. Therefore, no positive selective advantage that would not have been conferred by natural gene transfer between bacteria is expected.

5.2.2 Plant to plant gene flow

Considering the intended uses of maize 1507 x 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 1507 x NK603 has no altered survival, multiplication or dissemination characteristics, the VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this GM maize in Norway will not differ from that of conventional maize varieties. The likelihood of cross-pollination between cultivated maize and the occasional feral maize plants resulting from grain spillage is considered extremely low.

5.3 Interactions between the GM plant and target organisms

Maize 1507 expresses the *cry1F* gene and was developed to provide protection against a variety of target pests of the order Lepidoptera. Two Lepidoptera pests are primarily targeted by maize 1507; *Ostrinia nubilalis* (European corn borer, ECB) and *Sesamia nonagrioides* (Mediterranean corn borer, MCB). The European corn borer is widely distributed in Europe covering the Iberian Peninsula, Czech Republic and Slovakia, southwest of France, northern Italy and the southern regions of Germany and Poland. The Mediterranean corn borer is present in the Mediterranean region (Andreadis 2011). There are ten reports of *O. nubilalis* in Norway, restricted to the counties of Vestfold, Telemark, Aust-Agder and Vest Agder. *Sesamia* spp. has not been reported in Norway. There are no reports of *O. nubilalis* attaining pest status in Norway, and the Plant Clinic (Planteklinikken) at Bioforsk has never received samples of this pest or plant material damaged by this pest (K. Ørstad pers. com.). Consequently, there are no insecticides authorised or previous applications for registrations of insecticides against this herbivore in Norway.

Considering the intended uses of maize 1507 x NK603, excluding cultivation, the environmental exposure is limited to exposure through manure and faeces from the gastrointestinal tract mainly of animals fed on the GM maize as well as to the accidental release into the environment of GM seeds during transportation and processing and subsequently to potential occurrence of sporadic feral plants. Thus the level of exposure of target organisms to Cry1F proteins is likely to be extremely low and of no ecological relevance.

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

Considering the intended uses of maize stack 1507 x NK603, excluding cultivation, the environmental risk assessment is concerned with accidental release of GM maize viable grains into the environment during transportation and processing, and exposure through manure and faeces from the gastrointestinal tracts of animals fed the GM maize.

Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only very low amounts would remain intact to pass out in faeces (e.g. Lutz et al. 2005, Guertler et al. 2008; Paul et al. 2010). There would subsequently, be further degradation of the Cry proteins in the manure and faeces due to microbial processes. In addition, there will be further degradation of Cry proteins in soil, reducing the possibility for the exposure of potentially sensitive non-target organisms. Although Cry proteins bind rapidly on clays and humic substances in the soil and thereby reducing their availability to microorganisms for degradation, there is little evidence for the accumulation of Cry proteins from GM plants in soil (Icoz & Stotzky 2009).

Data supplied by the applicant indicate that a limited amount of the Cry1F proteins enters the environment due to expression in the grains (mean value of 2.04 µg/g d.w., respectively). In addition, the data show that at least 99% of microbially produced Cry1F protein was rapidly degraded in simulated gastric fluid.

In conclusion, the VKM GMO Panel considers that the exposure of potentially non-target organisms to the Cry1F protein is likely to be very low and of no biological relevance.

5.5 Potential interactions with the abiotic environment and biochemical cycles

Considering the intended uses of maize 1507 x NK603, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with the abiotic environment and biogeochemical cycles were not considered an issue by the VKM GMO Panel.

5.6 Post-market environmental monitoring

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

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

The objective of general surveillance is to identify unanticipated adverse effects of the GM plant or its use on human health and the environment that were not predicted or specifically identified during the ERA. In contrast to case-specific monitoring, the general status of the environment that is associated

with the use of the GM plant is monitored without any preconceived hypothesis, in order to detect any possible effects that were not anticipated in the ERA, or that are long-term or cumulative.

No specific environmental impact of genetically modified maize 1507 x 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

Considering the intended uses of maize 1507 x NK603, excluding cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize 1507 x NK603.

Maize 1507 x 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 1507 x NK603. Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation. The risk of gene flow from occasional feral GM maize plants to conventional maize varieties is negligible. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered to be an issue.

6 Data gaps

Adjuvanticity

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

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

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

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

Herbicide residue levels

Herbicide residue levels on plants with engineered 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 Conclusion

Molecular characterisation
Southern blot and PCR analyses have indicated that the recombinant inserts in the parental maize events1507 and NK603 are retained in the stacked maize 1507 x NK603. Genetic stability of the inserts has previously been demonstrated in the parental events. Protein measurements show comparable levels of Cry1F, PAT and CP4 EPSPS proteins in the stacked maize 1507 x NK603 and the parental lines. Phenotypic analyses also indicated stability of the insect resistance and herbicide tolerance traits.

The VKM Panel on GMO considers the molecular characterisation of maize 1507 x NK603 and its parental events 1507 and NK603 as adequate.

Comparative assessment
Comparative analyses of the compositional, agronomic and phenotypic characteristics of maize stack 1507 x NK603 and near-isogenic comparators were performed during multiple field trials in Chile and Europe in 2002/2003. With the exception of small intermittent variations, the results show no indications of unwanted unintentional effects, and that maize stack 1507 x NK603 is compositionally, agronomically and phenotypically equivalent to its comparators, with the exception of the introduced insect resistance and herbicide tolerance traits.

Food and feed risk assessment
Whole food feeding studies on rats have not indicated any adverse effects of the parental maize lines 1507 and NK603. No rodent whole food feeding study has been performed on the stacked maize 1507 x NK603; the applicant has however provided a nutritional feeding study performed on broilers. No adverse effects were observed in the study. Bioinformatics analyses have not revealed expression of any known ORFs in the parental maize lines, and none of the newly expressed proteins show resemblance to any known toxins or IgE allergens. Nor have the newly expressed proteins been reported to cause IgE mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Acute and repeated dose toxicity tests in rodents have not indicated toxic effects of the Cry1F, PAT or CP4 EPSPS proteins. However, these tests do not provide any additional information about possible adverse effects of maize 1507 x NK603.

Based on current knowledge, the VKM GMO Panel concludes that the stacked maize 1507 x NK603 is nutritionally equivalent to conventional maize varieties, and that it is unlikely that the newly expressed proteins introduce a toxic or allergenic potential in food and feed derived from maize 1507 x NK603 compared to conventional maize.

Environmental risk
Considering the intended uses of maize 1507 x NK603, excluding cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize 1507 x NK603.

Maize 1507 x 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 1507 x NK603. Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation. The risk of gene flow from occasional feral GM maize plants to conventional

maize varieties is negligible. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered to be an issue.

Overall conclusion

The VKM GMO Panel has not identified toxic or altered nutritional properties in maize 1507 x NK603 or its processed products compared to conventional maize. Based on current knowledge, it is also unlikely that the Cry1F protein will increase the allergenic potential of food and feed derived from maize 1507 x NK603 compared to conventional maize varieties. The VKM GMO Panel likewise concludes that maize 1507 x 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

Analyses of forage, 1507 x NK603 + Glyphosate and control. (Chile)

Table 1. Summary of proximates and fibres - Forage

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate	Control	Standard Error
Crude Protein	3.14 – 15.9	6.84	6.95	0.139
Crude Fat	0.37 – 6.7	2.70	2.62	0.0671
Crude Fiber	19 – 42	22.8	23.1	0.422
ADF	16.1 – 41	28.9	28.5	0.541
NDF	20.3 – 63.7	46.7	47.0	0.681
Ash	1.3 – 10.5	5.15	5.09	0.0938
Carbohydrates[4]	66.9 – 94.5	85.3	85.3	0.224

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
[4]Carbohydrates are calculated using the following formula = 100% - % protein - % ash-%fat
*P-value<0.05 between test and control

Table 2. Summary of minerals - Forage

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x NK603 + Glyphosate	Control	Standard Error
Calcium	0.10 – 0.6	0.245	0.247	0.00451
Phosphorus	0.14 – 0.55	0.199	0.208	0.00325

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
*P-value<0.05 between test and control

Analyses of grain, 1507 x NK603 + Glyphosate and control. (Chile)

Table 3. Summary of proximates and fibres - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x NK603 + Glyphosate	Control	Standard Error
Crude Protein	6 – 15.0	9.35	9.49	0.109
Crude Fat	1.2 – 18.8	4.25	4.04	0.108
Crude Fiber	1.6 – 5.5	2.3	2.2	0.12
ADF	1.82 – 11.3	3.9	3.8	0.14
NDF	5.59 – 22.6	11.3	10.3	0.392
Ash	0.62 – 6.28	1.78	1.35	0.270
Carbohydrates[4]	63.3 – 89.8	84.6	85.1	0.291

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
[4]Carbohydrates are calculated using the following formula = 100% - % protein - % ash-%fat
*P-value<0.05 between test and control

Table 4. Summary of fatty acids - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x NK603 + Glyphosate	Control	Standard Error
Palmitic acid	7 – 19	12.3*	12.2	0.0339
Stearic acid	0 – 4.17	1.69	1.65	0.0149
Oleic acid	18.6 – 46	29.1	29.7	0.216
Linoleic acid	34.0 – 70	55.3	55.0	0.198
Linolenic acid	0 – 2.0	1.23*	1.19	0.00851

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
*P-value<0.05 between test and control

Table 5. Summary of amino acids - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate	Control	Standard Error
Methionine	0.1 – 0.46	0.21	0.22	0.0058
Cysteine	0.08 – 0.32	0.23	0.24	0.0067
Lysine	0.05 – 0.55	0.29	0.29	0.010
Tryptophan	0.04 – 0.13	0.06	0.07	0.002
Threonine	0.21 – 0.58	0.52	0.50	0.012
Isoleucine	0.19 – 0.71	0.33	0.32	0.0044
Histidine	0.15 – 0.40	0.27	0.27	0.0045
Valine	0.21 – 0.85	0.45	0.44	0.0059
Leucine	0.43 – 2.41	1.19	1.19	0.0193
Arginine	0.22 – 0.64	0.31	0.30	0.0045
Phenylalanine	0.04 – 0.83	0.48	0.47	0.0065
Glycine	0.24 – 0.50	0.39	0.39	0.0077
Alanine	0.37 – 1.20	0.58	0.58	0.0095
Aspartic Acid	0.37 – 0.95	0.63	0.62	0.0085
Glutamic Acid	0.89 – 3.04	1.83	1.82	0.0281
Proline	0.43 – 1.46	0.94	0.94	0.013
Serine	0.24 – 0.91	0.50	0.50	0.013
Tyrosine	0.11 – 0.79	0.24	0.23	0.0096

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
*P-value<0.05 between test and control

Table 6. Summary of minerals - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate	Control	Standard Error
Calcium	0.00216 – 0.1	0.00356	0.00367	0.0000891
Phosphorus	0.21 – 0.75	0.327	0.322	0.00458
Copper	0.000085 – 0.001	0.000153	0.000146	0.00000421
Iron	0.0001 – 0.01	0.00214	0.00209	0.0000652
Magnesium	0.08 – 1.0	0.111	0.116	0.00207
Manganese	0.00007 – 0.0054	0.000481*	0.000543	0.0000098
Potassium	0.28 – 0.72	0.395*	0.374	0.00332
Sodium	0.0 – 0.15	0.000917	0.000976	0.000137
Zinc	0.00065 – 0.0037	0.00178*	0.00202	0.0000315

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
*P-value<0.05 between test and control

Table 7. Summary of vitamins - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x NK603 + Glyphosate	Control	Standard Error
Beta Carotene	2.5 (Ave.)	5.16	5.14	0.191
Vitamin B1	1.0– 8.6	8.40*	9.13	0.120
Vitamin B2	0.25 – 16.5	ND	ND	ND
Folic Acid	0.1– 683	0.756	0.730	0.0172
Vitamin E[4]	1.5– 68.7	18.3*	16.9	0.314

[1] dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
[4]Measured as α–tocopherol
ND: Analyte not detected
*P-value<0.05 between test and control

Table 8. Summary of secondary metabolites and anti-nutrients - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x NK603 + Glyphosate	Control	Standard Error
Secondary Metabolites				
Inositol	NR	0.027	0.026	0.0012
Raffinose	0.04 – 0.31	0.15	0.16	0.010
Furfural	NR	ND	ND	ND
Coumaric Acid	0.003 – 0.058	0.017	0.016	0.0012
Ferulic Acid	0.02 – 0.37	0.171	0.155	0.0104
Anti-nutrients				
Phytic acid	0.45 – 1.29	0.926	0.954	0.0549
Trypsin Inhibitor (TIU/g)	NR	2.59	2.54	0.0535

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
NR: Literature range not reported
ND Analyte not detected
*P-value<0.05 between test and control

Analyses of forage, 1507 x NK603 + Glufosinate and control. (Chile)

Table 9. Summary of proximates and fibres - Forage

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glufosinate	Control	Standard Error
Crude Protein	3.14 – 15.9	6.80	6.95	0.113
Crude Fat	0.37 – 6.7	2.70	2.62	0.0641
Crude Fiber	19 – 42	22.7	23.1	0.398
ADF	16.1 – 41	28.9	28.5	0.490
NDF	20.3 – 63.7	47.2	47.0	0.609
Ash	1.3 – 10.5	5.16	5.09	0.0950
Carbohydrates[4]	66.9 – 94.5	85.3	85.3	0.175

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
[4]Carbohydrates are calculated using the following formula = 100% - % protein - % ash-%fat
*P-value<0.05 between test and control

Table 10. Summary of minerals - Forage

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x NK603 + Glufosinate	Control	Standard Error
Calcium	0.10 – 0.6	0.250	0.247	0.00555
Phosphorus	0.14 – 0.55	0.205	0.208	0.00374

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
*P-value<0.05 between test and control

Analyses of grain, 1507 x NK603 + Glufosinate and control. (Chile)

Table 11. Summary of proximates and fibres - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x NK603 + Glufosinate	Control	Standard Error
Crude Protein	6 – 15.0	9.27	9.49	0.112
Crude Fat	1.2 – 18.8	4.28*	4.04	0.0734
Crude Fiber	1.6 – 5.5	2.4	2.2	0.085
ADF	1.82 – 11.3	3.7	3.8	0.12
NDF	5.59 – 22.6	11.0	10.3	0.322
Ash	0.62 – 6.28	1.39	1.35	0.0216
Carbohydrates[4]	63.3 – 89.8	85.1	85.1	0.145

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
[4]Carbohydrates are calculated using the following formula = 100% - % protein - % ash-%fat
*P-value<0.05 between test and control

Table 12. Summary of fatty acids - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x NK603 + Glufosinate	Control	Standard Error
Palmitic acid	7 – 19	12.4*	12.2	0.0470
Stearic acid	0 – 4.17	1.71*	1.65	0.0156
Oleic acid	18.6 – 46	29.1*	29.7	0.192
Linoleic acid	34.0 – 70	55.2	55.0	0.178
Linolenic acid	0 – 2.0	1.22*	1.19	0.0102

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
*P-value<0.05 between test and control

Table 13. Summary of amino acids - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x NK603 + Glufosinate	Control	Standard Error
Methionine	0.1 – 0.46	0.20*	0.22	0.0043
Cysteine	0.08 – 0.32	0.22*	0.24	0.0063
Lysine	0.05 – 0.55	0.29	0.29	0.0097
Tryptophan	0.04 – 0.13	0.06	0.07	0.002
Threonine	0.21 – 0.58	0.50	0.50	0.0093
Isoleucine	0.19 – 0.71	0.33	0.32	0.0050
Histidine	0.15 – 0.40	0.27	0.27	0.0043
Valine	0.21 – 0.85	0.44	0.44	0.0065
Leucine	0.43 – 2.41	1.19	1.19	0.0206
Arginine	0.22 – 0.64	0.30	0.30	0.0046
Phenylalanine	0.04 – 0.83	0.47	0.47	0.0074
Glycine	0.24 – 0.50	0.38	0.39	0.0069
Alanine	0.37 – 1.20	0.58	0.58	0.0099
Aspartic Acid	0.37 – 0.95	0.63	0.62	0.0086
Glutamic Acid	0.89 – 3.04	1.83	1.82	0.0300
Proline	0.43 – 1.46	0.93	0.94	0.014
Serine	0.24 – 0.91	0.50	0.50	0.013
Tyrosine	0.11 – 0.79	0.24	0.23	0.0074

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
*P-value<0.05 between test and control

Table 14. Summary of minerals - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x NK603 + Glufosinate	Control	Standard Error
Calcium	0.00216 – 0.1	0.00373	0.00367	0.0000877
Phosphorus	0.21 – 0.75	0.320	0.322	0.00473
Copper	0.000085 – 0.001	0.000149	0.000146	0.00000357
Iron	0.0001 – 0.01	0.00201	0.00209	0.0000488
Magnesium	0.08 – 1.0	0.110*	0.116	0.00215
Manganese	0.00007 – 0.0054	0.000467*	0.000543	0.0000108
Potassium	0.28 – 0.72	0.389*	0.374	0.00336
Sodium	0.0 – 0.15	0.000920	0.000976	0.000173
Zinc	0.00065 – 0.0037	0.00173*	0.00202	0.0000317

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
*P-value<0.05 between test and control

Table 15. Summary of vitamins - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x NK603 + Glufosinate	Control	Standard Error
Beta Carotene	2.5 (Ave.)	5.30	5.14	0.207
Vitamin B1	1.0– 8.6	8.27*	9.13	0.116
Vitamin B2	0.25 – 16.5	ND	ND	ND
Folic Acid	0.1– 683	0.772	0.730	0.0163
Vitamin E[4]	1.5– 68.7	18.8*	16.9	0.207

[1] dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
[4]Measured as α-tocopherol
ND: Analyte not detected
*P-value<0.05 between test and control

Table 16. Summary of secondary metabolites and anti-nutrients - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glufosinate	Control	Standard Error
Secondary Metabolites				
Inositol	NR	0.027	0.026	0.00079
Raffinose	0.04 – 0.31	0.15	0.16	0.010
Furfural	NR	ND	ND	ND
Coumaric Acid	0.003 – 0.058	0.018*	0.016	0.00084
Ferulic Acid	0.02 – 0.37	0.181*	0.155	0.00789
Anti-nutrients				
Phytic acid	0.45 – 1.29	0.916	0.954	0.0562
Trypsin Inhibitor (TIU/g)	NR	2.63	2.54	0.0719

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
NR: Literature range not reported
ND Analyte not detected
*P-value<0.05 between test and control

Analyses of forage, 1507 x NK603 + Glyphosate f.b. Glufosinate, and control. (Chile)

Table 17. Summary of proximates and fibres - Forage

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603+Glyphosate f.b. Glufosinate	Control	Standard Error
Crude Protein	3.14 – 15.9	7.00	6.95	0.113
Crude Fat	0.37 – 6.7	2.76*	2.62	0.0589
Crude Fiber	19 – 42	21.7	23.1	0.586
ADF	16.1 – 41	28.3	28.5	0.424
NDF	20.3 – 63.7	44.9	47.0	1.10
Ash	1.3 – 10.5	5.18	5.09	0.0840
Carbohydrates[4]	66.9 – 94.5	85.1	85.3	0.162

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
[4]Carbohydrates are calculated using the following formula = 100% - % protein - % ash-%fat
*P-value<0.05 between test and control

Table 18. Summary of minerals - Forage

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603+ Glyphosate f.b. Glufosinate	Control	Standard Error
Calcium	0.10 – 0.6	0.238	0.247	0.00635
Phosphorus	0.14 – 0.55	0.201	0.208	0.00445

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
*P-value<0.05 between test and control

Analyses of grain, 1507 x NK603 + Glyphosate f.b. Glufosinate, and control. (Chile)

Table 19. Summary of proximates and fibres - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603+ Glyphosate f.b. Glufosinate	Control	Standard Error
Crude Protein	6 – 15.0	9.43	9.49	0.100
Crude Fat	1.2 – 18.8	4.23	4.04	0.0738
Crude Fiber	1.6 – 5.5	2.2	2.2	0.098
ADF	1.82 – 11.3	3.7	3.8	0.13
NDF	5.59 – 22.6	10.7	10.3	0.364
Ash	0.62 – 6.28	1.37	1.35	0.0209
Carbohydrates[4]	63.3 – 89.8	85.0	85.1	0.132

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
[4]Carbohydrates are calculated using the following formula = 100% - % protein - % ash-%fat
*P-value<0.05 between test and control

Table 20. Summary of fatty acids - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603+ Glyphosate f.b. Glufosinate	Control	Standard Error
Palmitic acid	7 – 19	12.6*	12.2	0.141
Stearic acid	0 – 4.17	1.77*	1.65	0.0296
Oleic acid	18.6 – 46	29.4	29.7	0.245
Linoleic acid	34.0 – 70	54.7	55.0	0.329
Linolenic acid	0 – 2.0	1.18	1.19	0.0205

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
*P-value<0.05 between test and control

Table 21. Summary of amino acids - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603+ Glyphosate f.b. Glufosinate	Control	Standard Error
Methionine	0.1 – 0.46	0.21*	0.22	0.0041
Cysteine	0.08 – 0.32	0.22*	0.24	0.0055
Lysine	0.05 – 0.55	0.31	0.29	0.0091
Tryptophan	0.04 – 0.13	0.06	0.07	0.002
Threonine	0.21 – 0.58	0.50	0.50	0.0098
Isoleucine	0.19 – 0.71	0.33	0.32	0.0053
Histidine	0.15 – 0.40	0.27	0.27	0.0044
Valine	0.21 – 0.85	0.45	0.44	0.0068
Leucine	0.43 – 2.41	1.21	1.19	0.0235
Arginine	0.22 – 0.64	0.30	0.30	0.0043
Phenylalanine	0.04 – 0.83	0.48	0.47	0.0079
Glycine	0.24 – 0.50	0.39	0.39	0.0077
Alanine	0.37 – 1.20	0.59	0.58	0.011
Aspartic Acid	0.37 – 0.95	0.65*	0.62	0.0098
Glutamic Acid	0.89 – 3.04	1.86	1.82	0.0338
Proline	0.43 – 1.46	0.95	0.94	0.015
Serine	0.24 – 0.91	0.51	0.50	0.014
Tyrosine	0.11 – 0.79	0.23	0.23	0.0065

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
*P-value<0.05 between test and control

Table 22. Summary of minerals - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603+ Glyphosate f.b. Glufosinate	Control	Standard Error
Calcium	0.00216 – 0.1	0.00363	0.00367	0.0000696
Phosphorus	0.21 – 0.75	0.331	0.322	0.00420
Copper	0.000085 – 0.001	0.000145	0.000146	0.00000347
Iron	0.0001 – 0.01	0.00216	0.00209	0.0000951
Magnesium	0.08 – 1.0	0.115	0.116	0.00185
Manganese	0.00007 – 0.0054	0.000481*	0.000543	0.0000102
Potassium	0.28 – 0.72	0.397*	0.374	0.00326
Sodium	0.0 – 0.15	0.000899	0.000976	0.000159
Zinc	0.00065 – 0.0037	0.00174*	0.00202	0.000035

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
*P-value<0.05 between test and control

Table 23. Summary of vitamins - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603+ Glyphosate f.b. Glufosinate	Control	Standard Error
Beta Carotene	2.5 (Ave.)	5.05	5.14	0.172
Vitamin B1	1.0– 8.6	8.23*	9.13	0.130
Vitamin B2	0.25 – 16.5	ND	ND	ND
Folic Acid	0.1– 683	0.764*	0.730	0.0131
Vitamin E[4]	1.5– 68.7	19.1*	16.9	0.200

[1] dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
[4]Measured as α-tocopherol
ND: Analyte not detected
*P-value<0.05 between test and control

Table 24. Summary of secondary metabolites and anti-nutrients - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 +Glyphosate f.b. Glufosinate	Control	Standard Error
Secondary Metabolites				
Inositol	NR	0.027	0.026	0.00086
Raffinose	0.04 – 0.31	0.16	0.16	0.013
Furfural	NR	ND	ND	ND
Coumaric Acid	0.003 – 0.058	0.017	0.016	0.0011
Ferulic Acid	0.02 – 0.37	0.164	0.155	0.0107
Anti-nutrients				
Phytic acid	0.45 – 1.29	0.921	0.954	0.0486
Trypsin Inhibitor (TIU/g)	NR	2.55	2.54	0.0732

[1]Percent of dry weight
[2]Combined Ranges, See Appendix 3
[3]Least square means
NR: Literature range not reported
ND Analyte not detected
*P-value<0.05 between test and control

Analyses of forage, 1507 x NK603 + Glyphosate, and control. (Europe)

Table 25. Summary of proximates and fibre - Forage

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate	Control	Standard Error
Crude Protein	3.14 - 15.9	8.25	7.94	0.175
Crude Fat	0.37 - 6.7	2.64	2.42	0.0999
Crude Fiber	19 - 42	22.4	22.5	0.451
ADF[4]	16.1 - 41.9	29.3	29.7	0.618
NDF[5]	10.3 - 63.7	51.6	52.7	0.882
Ash	1.3 - 10.5	5.28	4.99	0.189
Carbohydrates	66.9 - 94.5	83.8	84.7	0.352

[1]Percent of dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
[4]Acid Detergent Fiber
[5]Neutral Detergent Fiber
*P-value<0.05 between 1507xNK603 + glyphosate and control

Table 26. Summary of minerals - forage

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate	Control	Standard Error
Calcium	0.097 - 0.6	0.238	0.258	0.0120
Phosphorus	0.12 - 0.55	0.221	0.232	0.00753

[1]Percent dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
*P-value<0.05 between 1507xNK603+ glyphosate and control

Analyses of grain, 1507 x NK603 + Glyphosate, and control. (Europe)

Table 27. Summary of proximates and fibres - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate	Control	Standard Error
Crude Protein	6 - 15.0	10.8*	9.89	0.183
Crude Fat	1.2 - 18.8	4.31	4.21	0.0528
ADF[4]	1.82 - 11.3	3.10*	2.83	0.0744
Crude Fiber	1.6 - 5.5	2.1	2.0	0.035
NDF[5]	5.59 - 22.6	9.60*	8.90	0.185
Ash	0.62 - 6.28	1.57	1.50	0.0520
Carbohydrates	63.3 - 89.8	85.7*	86.8	0.153

[1]Percent dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
[4]Acid Detergent Fiber
[5]Neutral Detergent Fiber
*P-value<0.05 between 1507xNK603 + glyphosate and control

Table 28. Summary of fatty acids - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate	Control	Standard Error
Palmitic acid	7 - 19	10.9*	11.3	0.0238
Stearic acid	0 - 4.0	1.52*	1.47	0.00801
Oleic acid	18.6 - 50	27.0	26.7	0.212
Linoleic acid	34.0 - 70	59.1	59.0	0.217
Linolenic acid	0 - 2.0	1.20	1.17	0.00899

[1]Percent total fatty acids
[2]Combined ranges, see Appendix 5
[3]Least square means
*P-value<0.05 between 1507xNK603 + glyphosate and control

Table 29. Summary of amino acids - grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate	Control	Standard Error
Methionine	0.10 - 0.46	0.25*	0.23	0.0043
Cystine	0.08 - 0.32	0.24*	0.21	0.0043
Lysine	0.05 - 0.56	0.33	0.31	0.0093
Tryptophan	0.04 - 0.13	0.08*	0.07	0.001
Threonine	0.22 - 0.65	0.52*	0.46	0.014
Isoleucine	0.20- 0.71	0.36	0.35	0.0073
Histidine	0.15 - 0.42	0.28	0.26	0.0072
Valine	0.21 - 0.85	0.45	0.43	0.0082
Leucine	0.64 - 2.41	1.31	1.24	0.0311
Arginine	0.22 - 0.64	0.33*	0.30	0.011
Phenylalanine	0.26 - 0.83	0.54*	0.50	0.011
Glycine	0.26 - 0.50	0.43	0.41	0.0079
Alanine	0.44 - 1.20	0.86	0.82	0.018
Aspartic Acid	0.40 - 0.95	0.75*	0.69	0.012
Glutamic Acid	1.04 - 3.04	2.21*	2.06	0.0461
Proline	0.53 - 1.46	0.98	0.92	0.023
Serine	0.24 - 0.91	0.55	0.53	0.011
Tyrosine	0.11 - 0.79	0.28	0.25	0.012

[1]Percent dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
*P-value<0.05 between 1507xNK603 + glyphosate and control

Table 30. Summary of minerals - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate	Control	Standard Error
Calcium	0.00216 - 0.1	0.00387*	0.00477	0.000110
Copper	0.000073 - 0.001	0.000124	0.000129	0.00000618
Iron	0.0001 - 0.01	0.00195	0.00187	0.0000302
Magnesium	0.08 - 1.0	0.121*	0.117	0.00156
Manganese	0.00007 - 0.0054	0.000458*	0.000509	0.00000872
Phosphorus	0.21 - 0.75	0.328*	0.299	0.00325
Potassium	0.27 - 0.72	0.389*	0.352	0.00377
Sodium	0.0 - 0.15	0.000344	0.000421	0.0000871
Zinc	0.00065 - 0.0037	0.00204*	0.00218	0.0000295

[1]Percent dry weight
[2]Combined ranges, see Appendix 3
[3]Least square means
*P-value<0.05 between 1507xNK603 + glyphosate and control

Table 31. Summary of vitamins - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate	Control	Standard Error
Beta-carotene	0.53 - 16.4	6.27*	8.10	0.359
Vitamin B1	1.3 - 8.6	8.89*	7.69	0.124
Vitamin B2	0.25 - 5.6	ND[5]	ND[5]	ND[5]
Folic Acid	0.15 - 683	1.02*	0.750	0.0156
Vitamin E[4]	1.5 - 68.7	11.3*	8.24	0.199

[1]mg/kg dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
[4]Measured as α-tocopherol
[5]ND: Not Detected
*P-value<0.05 between 1507xNK603 + glyphosate and control

Table 32. Summary of secondary metabolites and anti-nutrients - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate	Control	Standard Error
Inositol	0.0138 - 0.257	0.015*	0.024	0.00068
Furfural	0.0003 - 0.0005	ND[5]	ND[5]	ND[5]
P-Coumaric Acid	0.003 - 0.058	0.021	0.021	0.00068
Ferulic Acid	0.02 - 0.373	0.166	0.170	0.00330

[1]Percent dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
[4]NR: Not reported
[5]ND: Not Detected
*P-value<0.05 between 1507xNK603 + glyphosate and control

Table 33. Summary of anti-nutrients - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate	Control	Standard Error
Raffinose	0.04 - 0.31	0.08*	0.06	0.003
Phytic acid	0.29 - 1.29	0.948	0.859	0.0643
Trypsin Inhibitor (TIU/g)[4]	1.10 - 7.18	3.05	3.03	0.0801

[1]Percent dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
[4]Abbreviation: TIU, trypsin inhibitor units
*P-value<0.05 between 1507xNK603 + glyphosate and control

Analyses of forage, 1507 x NK603 + Glufosinate and control. (Europe)

Table 34. Summary of proximates and fibres - Forage

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glufosinate	Control	Standard Error
Crude Protein	3.14 - 15.9	7.80	7.94	0.160
Crude Fat	0.37 - 6.7	2.38	2.42	0.131
Crude Fiber	19 - 42	22.4	22.5	0.536
ADF[4]	16.1 - 41.9	29.1	29.7	0.599
NDF[5]	10.3 - 63.7	51.7	52.7	0.941
Ash	1.3 - 10.5	4.54	4.99	0.176
Carbohydrates[6]	66.9 - 94.5	85.3	84.7	0.261

[1]Percent of dry weight
[2]Combined rangea, see Appendix 5
[3]Least square means
[4]Acid Detergent Fiber
[5]Neutral Detergent Fiber
*P-value<0.05 between 1507xNK603 + glufosinate and control

Table 35. Summary of minerals - Forage

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glufosinate	Control	Standard Error
Calcium	0.097 - 0.6	0.227	0.258	0.0119
Phosphorus	0.12 - 0.55	0.226	0.232	0.00666

[1]Percent of dry weight
[2]Combined rangea, see Appendix 3
[3]Least square means
*P-value<0.05 between 1507xNK603 + glufosinate and control

Analyses of grain, 1507 x NK603 + Glufosinate and control. (Europe)

Table 36. Summary of proximates and fibres - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glufosinate	Control	Standard Error
Crude Protein	6 - 15.0	10.1	9.89	0.186
Crude Fat	1.2 - 18.8	4.34	4.21	0.0519
ADF[4]	1.82 - 11.3	3.23*	2.83	0.0665
Crude Fiber	1.6 - 5.5	2.2*	2.0	0.049
NDF[5]	5.59 - 22.6	9.87*	8.90	0.259
Ash	0.62 - 6.28	1.53	1.50	0.0480
Carbohydrates[6]	63.3 - 89.8	85.4*	86.8	0.272

[1]Percent of dry weight
[2]Combined ranges, see Appendix 3
[3]Least square means
[4]Acid Detergent Fiber
[5]Neutral Detergent Fiber
*P-value<0.05 between 1507xNK603 + glufosinate and control

Table 37. Summary of fatty acids - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glufosinate	Control	Standard Error
Palmitic acid	7 - 19	11.1*	11.3	0.0251
Stearic acid	0 - 4.0	1.55*	1.47	0.0101
Oleic acid	18.6 - 50	27.3*	26.7	0.184
Linoleic acid	34.0 - 70	58.6	59.0	0.191
Linolenic acid	0 - 2.0	1.16	1.17	0.00854

[1]Percent relative total fatty acids
[2]Combined ranges, see Appendix 5
[3]Least square means
*P-value<0.05 between 1507xNK603 + glufosinate and control

Table 38. Summary of amino acids - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glufosinate	Control	Standard Error
Methionine	0.10 - 0.46	0.23	0.23	0.0032
Cystine	0.08 - 0.32	0.23*	0.21	0.0044
Lysine	0.05 - 0.56	0.30	0.31	0.0052
Tryptophan	0.04 - 0.13	0.07	0.07	0.0008
Threonine	0.22 - 0.65	0.50*	0.46	0.014
Isoleucine	0.20- 0.71	0.35	0.35	0.0082
Histidine	0.15 - 0.42	0.27	0.26	0.0072
Valine	0.21 - 0.85	0.43	0.43	0.0083
Leucine	0.64 - 2.41	1.23	1.24	0.0321
Arginine	0.22 - 0.64	0.30	0.30	0.014
Phenylalanine	0.26 - 0.83	0.51	0.50	0.012
Glycine	0.26 - 0.50	0.42	0.41	0.0063
Alanine	0.44 - 1.20	0.81	0.82	0.019
Aspartic Acid	0.40 - 0.95	0.70	0.69	0.013
Glutamic Acid	1.04 - 3.04	2.08	2.06	0.0510
Proline	0.53 - 1.46	0.90	0.92	0.028
Serine	0.24 - 0.91	0.53	0.53	0.012
Tyrosine	0.11 - 0.79	0.27	0.25	0.010

[1]Percent of dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
*P-value<0.05 between 1507xNK603 + glufosinate and control

Table 39. Summary of minerals - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glufosinate	Control	Standard Error
Calcium	0.00216 - 0.1	0.00411*	0.00477	0.000122
Copper	0.000073 - 0.001	0.000130	0.000129	0.00000360
Iron	0.0001 - 0.01	0.00189	0.00187	0.0000282
Magnesium	0.08 - 1.0	0.117	0.117	0.00157
Manganese	0.00007 - 0.0054	0.000404*	0.000509	0.00000837
Phosphorus	0.21 - 0.75	0.318*	0.299	0.00399
Potassium	0.27 - 0.72	0.388*	0.352	0.00442
Sodium	0.0 - 0.15	0.000610	0.000421	0.000137
Zinc	0.00065 - 0.0037	0.00202*	0.00218	0.0000332

[1]Percent of dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
*P-value<0.05 between 1507xNK603 + glufosinate and control

Table 40. Summary of vitamins - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glufosinate	Control	Standard Error
Beta-carotene	0.53 - 16.4	6.45*	8.10	0.328
Vitamin B1	1.3 - 8.6	7.92	7.69	0.122
Vitamin B2	0.25 - 5.6	ND[5]	ND[5]	ND[5]
Folic Acid	0.15 - 683	0.928*	0.750	0.0207
Vitamin E[4]	1.5 - 68.7	9.09	8.24	0.435

[1]mg/kg dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
[4]Measured as α-tocopherol
[5]ND: not detected
*P-value<0.05 between 1507xNK603 + glufosinate and control

Table 41. Summary of secondary metabolites - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glufosinate	Control	Standard Error
Inositol	NR[4]	0.020*	0.024	0.00070
Furfural	0.0003 - 0.0005	ND[5]	ND[5]	ND[5]
P-Coumaric Acid	0.003 - 0.058	0.021	0.021	0.00087
Ferulic Acid	0.02 - 0.37	0.165	0.170	0.00380

[1] Percent of dry weight
[2] Combined ranges, see Appendix 5
[3] Least square means
[4] NR: Not Reported
[5] ND: Not Detected
*P-value<0.05 between 1507xNK603 + glufosinate and control

Table 42. Summary of anti-nutrients - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glufosinate	Control	Standard Error
Raffinose	0.04 - 0.31	0.07	0.06	0.003
Phytic acid	0.29 - 1.29	0.911	0.859	0.0772
Trypsin Inhibitor (TIU/g)[4]	1.10 - 7.18	3.06	3.03	0.0497

[1] Percent of dry weight
[2] Combined ranges, see Appendix 5
[3] Least square means
[4] Abbreviation: TIU, trypsin inhibitor units
*P-value<0.05 between 1507xNK603 + glufosinate and control

Analyses of forage, 1507 x NK603 + Glyphosate f.b. Glufosinate, and control (Europe)

Table 43. Summary of proximates and fibres - Forage

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate fb Glufosinate	Control	Standard Error
Crude Protein	3.14 - 15.9	7.79	7.94	0.196
Crude Fat	0.37 - 6.7	2.46	2.42	0.124
Crude Fiber	19 - 42	23.3	22.5	0.571
ADF[4]	16.1 - 41.9	30.3	29.7	0.614
NDF[5]	10.3 - 63.7	52.0	52.7	0.843
Ash	1.3 - 10.5	4.87	4.99	0.150
Carbohydrates	66.9 - 94.5	84.9	84.7	0.283

[1]Percent of dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
[4]Acid Detergent Fiber
[5]Neutral Detergent Fiber
*P-value<0.05 between 1507xNK603 + glyphosate fb glufosinate and control

Table 44. Summary of minerals - Forage

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate fb Glufosinate	Control	Standard Error
Calcium	0.097 - 0.6	0.228*	0.258	0.00960
Phosphorus	0.12 - 0.55	0.225	0.232	0.00640

[1]Percent of dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
*P-value<0.05 between 1507xNK603 + glyphosate fb glufosinate and control

Analyses of grain, 1507 x NK603 + Glyphosate f.b. Glufosinate, and control (Europe)

Table 45. Summary of proximates and fibres - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate fb Glufosinate	Control	Standard Error
Crude Protein	6 - 15.0	10.5*	9.89	0.163
Crude Fat	1.2 - 18.8	4.44*	4.21	0.0610
ADF[4]	1.82 - 11.3	3.32*	2.83	0.0807
Crude Fiber	1.6 - 5.5	2.1*	2.0	0.032
NDF[5]	5.59 - 22.6	9.66*	8.90	0.203
Ash	0.62 - 6.28	1.55	1.50	0.0498
Carbohydrates	63.3 - 89.8	85.5*	86.8	0.183

[1]Percent of dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
[4]Acid Detergent Fiber
[5]Neutral Detergent Fiber
*P-value<0.05 between 1507xNK603 + glyphosate fb glufosinate and control

Table 46. Summary of fatty acids - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate fb Glufosinate	Control	Standard Error
Palmitic acid	7 - 19	11.1*	11.3	0.0337
Stearic acid	0 - 4.0	1.52*	1.47	0.00914
Oleic acid	18.6 - 50	26.7	26.7	0.208
Linoleic acid	34.0 - 70	59.1	59.0	0.212
Linolenic acid	0 - 2.0	1.16	1.17	0.00894

[1]Percent relative total fatty acids
[2]Combined ranges, see Appendix 5
[3]Least square means
*P-value<0.05 between 1507xNK603 + glyphosate fb glufosinate and control

Table 47. Summary of amino acids - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate fb Glufosinate	Control	Standard Error
Methionine	0.10 - 0.46	0.25*	0.23	0.0036
Cystine	0.08 - 0.32	0.23*	0.21	0.0041
Lysine	0.05 - 0.56	0.32	0.31	0.0069
Tryptophan	0.04 - 0.13	0.08*	0.07	0.0008
Threonine	0.22 - 0.65	0.48*	0.46	0.011
Isoleucine	0.20- 0.71	0.36	0.35	0.0063
Histidine	0.15 - 0.42	0.28	0.26	0.0074
Valine	0.21 - 0.85	0.45*	0.43	0.0067
Leucine	0.64 - 2.41	1.29	1.24	0.0266
Arginine	0.22 - 0.64	0.33	0.30	0.011
Phenylalanine	0.26 - 0.83	0.53	0.50	0.010
Glycine	0.26 - 0.50	0.43*	0.41	0.0056
Alanine	0.44 - 1.20	0.85	0.82	0.016
Aspartic Acid	0.40 - 0.95	0.73*	0.69	0.013
Glutamic Acid	1.04 - 3.04	2.16	2.06	0.0411
Proline	0.53 - 1.46	0.98	0.92	0.022
Serine	0.24 - 0.91	0.55	0.53	0.011
Tyrosine	0.11 - 0.79	0.29*	0.25	0.0093

[1]Percent of dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
*P-value<0.05 between 1507xNK603 + glyphosate fb glufosinate and control

Table 48. Summary of minerals - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate fb Glufosinate	Control	Standard Error
Calcium	0.00216 - 0.1	0.00389*	0.00477	0.000117
Copper	0.000073 - 0.001	0.000138	0.000129	0.0000135
Iron	0.0001 - 0.01	0.00198*	0.00187	0.0000297
Magnesium	0.08 - 1.0	0.119	0.117	0.00147
Manganese	0.00007 - 0.0054	0.000442*	0.000509	0.00000845
Phosphorus	0.21 - 0.75	0.321*	0.299	0.00308
Potassium	0.27 - 0.72	0.389*	0.352	0.00372
Sodium	0.0 - 0.15	0.000418	0.000421	0.0000614
Zinc	0.00065 - 0.0037	0.00205*	0.00218	0.0000296

[1]Percent of dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
*P-value<0.05 between 1507xNK603 + glyphosate fb glufosinate and control

Table 49. Summary of vitamins - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate fb Glufosinate	Control	Standard Error
Beta-carotene	0.53 - 16.4	6.95	8.10	0.477
Vitamin B1	1.3 - 8.6	8.32*	7.67	0.221
Vitamin B2	0.25 - 5.6	ND[5]	ND[5]	ND[5]
Folic Acid	0.15 - 683	0.938*	0.750	0.0214
Vitamin E[4]	1.5 - 68.7	10.2*	8.24	0.164

[1]mg/kg dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
[4]Measured as α-tocopherol
[5]ND: not detected
*P-value<0.05 between 1507xNK603 + glyphosate fb glufosinate and control

Table 50. Summary of secondary metabolites -Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate fb Glufosinate	Control	Standard Error
Inositol	0.0138 - 0.257	0.022	0.024	0.00072
Furfural	0.0003 - 0.0005	ND[5]	ND[5]	ND[5]
P-Coumaric Acid	0.003 - 0.058	0.021	0.021	0.00079
Ferulic Acid	0.02 - 0.373	0.166	0.170	0.00399

[1]Percent of dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
[4]NR: Not Reported
[5]ND: Not Detected
*P-value<0.05 between 1507xNK603 + glyphosate fb glufosinate and control

Table 51. Summary of anti-nutrients - Grain

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507xNK603 + Glyphosate fb Glufosinate	Control	Standard Error
Raffinose	0.04 - 0.31	0.08*	0.06	0.004
Phytic acid	0.29 - 1.29	0.932	0.859	0.0547
Trypsin Inhibitor (TIU/g)[4]	1.10 - 7.18	3.13	3.03	0.0479

[1] Percent of dry weight
[2] Combined ranges, see Appendix 5
[3] Least square means
[4] Abbreviation: TIU, trypsin inhibitor units
*P-value<0.05 between 1507xNK603 + glyphosate fb glufosinate and control

Table 52. Mean agronomic data from maize stack 1507 x NK603, sprayed with glyphosate herbicide followed by glufosinate herbicide, and from non-GM control maize with comparable genetic background. Data from field trials at six locations in Chile (2002/2003 growing season).

Trait	1507xNK603 +glyphosate	1507xNK603 +glufosinate	1507xNK603 +glyphosate +glufosinate	Non-GM control
Germination/early population[1]	51	51	50	49
Growing degree units to reach 50% pollen shed[2]	1212	1203	1209	1225
Growing degree units to reach 50% silking[3]	1205	1208	1209	1213
Stalk lodging[4]	0	0	0	0
Root lodging[5]	0	0	0	0
Plant height[6] (cm)	311	305	305	308
Ear height[7] (cm)	132	122	123	140
Final population[8]	51	49	46	48
Stay green[9]	6	6	6	6
Disease incidence[10]	9	9	9	9
Insect damage[11]	9	9	9	9
Grain moisture[12] (%)	28.0	27.6	27.6	27.1
Pollen shape[13]	96	96	97	95
Pollen colour[14]	96	97	95	96

[1]Number of plants emerged per 60 seed planted
[2]Number of accumulated heat units when approximately 50% of the plants are shedding pollen
[3]Number of accumulated heat units when approximately 50% of the plants are silking
[4]Percent of plants broken below the primary ear
[5]Percent of plants leaning ≥ 30° in the first ½ meter above the soil surface
[6]Measured from the soil surface to the tip of tassel), n=10
[7]Measured from the soil surface to the base primary ear), n=10
[8]Total number of viable plants (per plot) remaining at maturity
[9]Overall plant health at maturity evaluated on a 1 to 9 scale where 1 is completely dead and 9 is very green
[10]Level of disease resistance at maturity evaluated on a 1 to 9 scale where 1 is poor resistance and 9 is high resistance or no visible disease. Note: low level of disease pressure in Chile
[11]Level of destructive insect resistance at maturity evaluated on a 1 to 9 scale where 1 is poor resistance and 9 is high resistance or no damage. Note: no target pest found in Chile
[12]% water content of grain at typical harvest maturity
[13]% pollen grains with collapsed walls after 120 minutes
[14] % pollen grains with intense yellow colour after 120 minutes

Table 53. Mean agronomic data from maize stack 1507 x NK603, sprayed with glyphosate herbicide followed by glufosinate herbicide, and from non-GM control maize with comparable genetic background. Data from field trials at five locations in Europe (2003 growing season).

Trait	1507xNK603 +glyphosate +glufosinate	Non-GM control
Germination/early population[1]	50	54
Seedling vigour[14]	7	8
Growing degree units to reach 50% pollen shed[2]	860*	833
Growing degree units to reach 50% silking[3]	877*	854
Stalk lodging[4]	1	8
Root lodging[5]	2	1
Plant height[6] (cm)	207	210
Ear height[7] (cm)	73*	81
Final population[8]	44*	46
Stay green[9]	5	2
Disease incidence[10]	7	8
Insect damage[11]	7	4
Pollen shape[12]	79	83
Pollen colour[13]	97	99

[1]Number of plants emerged per 60 seed planted
[2]Number of accumulated heat units when approximately 50% of the plants are shedding pollen
[3]Number of accumulated heat units when approximately 50% of the plants are silking
[4]Percent of plants broken below the primary ear
[5]Percent of plants leaning ≥ 30° in the first ½ meter above the soil surface
[6]Measured from the soil surface to the tip of tassel, n=10
[7]Measured from the soil surface to the base primary ear, n=10
[8]Total number of viable plants (per plot) remaining at maturity
[9]Overall plant health at maturity evaluated on a 1 to 9 scale where 1 is completely dead and 9 is very green
[10]Level of disease resistance at maturity evaluated on a 1 to 9 scale where 1 is poor resistance and 9 is high resistance or no visible disease
[11]Level of destructive insect resistance at maturity evaluated on a 1 to 9 scale where 1 is poor resistance and 9 is high resistance or no damage
[12] % pollen grains with collapsed walls after 120 minutes
[13] % pollen grains with intense yellow colour after 120 minutes
[14]Visual estimate of average vigour evaluated on a 1 to 9 scale where 1 is short plants with small, thin leaves and 9 is tall plants with large, robust leaves
*Statistically significant difference ($P < 0.05$)

Table 54. Data on time to pollen shed, time to silking, ear height and final population for maize stack 1507 x NK603, sprayed with glyphosate herbicide followed by glufosinate herbicide, and from non-GM control

maize with comparable genetic background. Data from field trials at five locations in Europe (2003 growing season).

	GDU[5] 50% pollen shed[1]	GDU[5] 50% silking[2]	Ear height[3] (cm)	Final population[4]
Tchavdazri, Bulgaria (EU1)				
1507xNK603 maize	857	829	64	45*
Non-GM maize	829	799	74	54
Letniza, Bulgaria (EU2)				
1507xNK603 maize	844*	812*	67*	52
Non-GM maize	781	769	83	56
Montañana Zaragoza, Spain (EU5)				
1507xNK603 maize	856	914	81	39*
Non-GM maize	840	900	87	41
Pastriz Zaragoza, Spain (EU6)				
1507xNK603 maize	930	976	61	39
Non-GM maize	914	961	60	40
Cogullada Zaragoza, Spain (EU7)				
1507xNK603 maize	813	856	94	43
Non-GM maize	799	840	98	42
Average				
1507xNK603 maize	860*	877*	73*	44*
Non-GM maize	833	854	81	46

[1]Number of accumulated heat units when approximately 50% of the plants are silking.
[2]Number of accumulated heat units when approximately 50% of the plants are shedding pollen.
[3]Measured from the soil surface to the base primary ear, n=10.
[4]Total number of viable plants (per plot) remaining at maturity
[5]GDU: Growing degree units
*Statistically significant difference ($P < 0.05$)