Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Food/feed and environmental risk assessment of insect-resistant and herbicide-tolerant genetically modified maize 59122 x NK603 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (EFSA/GMO/UK/2005/20)

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

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Summary

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

The insect-resistant and herbicide-tolerant genetically modified maize 59122 x NK603 from Pioneer Hi-Bred International, Inc. (Unique Identifier DAS-59122-7 x MONØØ6Ø3-6) is approved under Regulation (EC) No 1829/2003 for food and feed uses, import and processing since 30 October 2009 (Commission Decision 2009/815/EC).

Genetically modified maize 59122 x NK603 has previously been risk assessed by the VKM Panel on Genetically Modified Organisms (GMO), commissioned by the Norwegian Food Safety Authority related to the EFSAs public hearing of the application EFSA/GMO/UK/2005/20 in 2007 (VKM 2007a). In addition 59122 x NK603 has been evaluated by the VKM GMO Panel as single events and as a component of several stacked GM maize events (VKM 2005a,b,d, VKM 2007b,c, VKM 2008b,c, VKM 2009a,b, VKM 2012).

The food/feed and environmental risk assessment of the maize 59122 x NK603 is based on information provided by the applicant in the application EFSA/GMO/UK/2005/20, and scientific comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment also considered other peer-reviewed scientific literature as relevant.

The VKM GMO Panel has evaluated 59122 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 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 59122 x NK603 include molecular characterisation of the inserted DNA and expression of novel proteins, comparative assessment of agronomic and phenotypic characteristics, nutritional assessments, toxicology and allergenicity, unintended effects on plant fitness, potential for gene transfer, interactions between the GM plant and target and non-target organisms, effects on biogeochemical processes.

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

The genetically modified maize stack 59122 x NK603 was produced by conventional breeding between inbred lines of maize containing the 59122 and NK603 events. The hybrid was developed to provide protection against certain coleopteran target pests, and to confer tolerance to glufosinate-ammonium and glyphosate herbicides.

Molecular characterisation

Southern and PCR analyses has been performed and indicate that the recombinant inserts in the single maize events 59122 and NK603 are retained in maize stack 59122xNK603. Genetic stability of the inserts has previously been demonstrated in the parental lines 59122 and NK603. The level of Cry34Ab1/Cry35Ab1, PAT and CP4 EPSPS proteins in seed and forage from the stacked event were measured using ELISA and are comparable to the levels in the single events. Phenotypic analyses also indicate stability of the insect resistance and herbicide tolerance traits of the stacked event.

Comparative assessment

Comparative analyses of data from field trials located at representative sites and environments in North America indicate that maize stack 59122 x NK603 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, with the exception of the introduced insect resistance and herbicide tolerance, conferred by the expression of the Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins. Based on the assessment of available data, the VKM GMO Panel is of the opinion that conventional crossing of maize 59122 and NK603 to produce the hybrid 59122 x NK603 does not result in interactions that cause compositional, agronomic and phenotypic changes that would raise safety concerns.

Food and feed risk assessment

Whole food feeding study has not been performed using 59122 x NK603 maize. The applicant has, however, provided a nutritional study on broilers using the triple stacked event 59122 x 1507 x NK603 maize as test material. 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 toxicity tests in rodents have not indicated toxic effects of the newly expressed proteins. However, these tests do not provide any additional information about possible adverse effects of the stacked event maize 59122 x NK603.

Based on the current knowledge, the VKM GMO Panel concludes that 59122 x NK603 maize is nutritionally equivalent to its conventional maize, and that it is unlikely that newly expressed proteins will introduce a toxic or allergenic potential of food/feed derived from maize 59122 x NK603 compared to conventional maize.

Environmental risk assessment

The scope of the application EFSA/GMO/UK/2005/20 includes import and processing of maize stack 59122 x NK603 for food and feed uses. Considering the intended uses of maize 59122 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 59122 x NK603.

Maize 59122 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 59122 x NK603. Maize is the only representative of the genus Zea in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation. The VKM GMO Panel considers the risk of gene flow from occasional feral GM maize plants to conventional maize varieties to be negligible in Norway. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered by the GMO Panel to be an issue.

Overall conclusion

The VKM GMO Panel has not identified toxic or altered nutritional properties of maize 59122 x NK603 or its processed products compared to conventional maize. Based on current knowledge, it is also unlikely that the Cry34Ab1 and Cry35Ab1 protein will increase the allergenic potential of food and feed derived from maize 59122 x NK603 compared to conventional maize varieties. The VKM GMO Panel likewise concludes that maize 59122 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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Keywords

Maize, *Zea mays* L., genetically modified maize 59122 x NK603, EFSA/GMO/NL/2005/20, insect-resistance, herbicide-tolerance, Cry proteins, *cry34Ab1, cry35Ab1,* 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 insektsresistente og herbicidtolerante maishybriden 59122 x NK603 (unik kode DAS-59122-7 x MONØØ6Ø3-6) fra Pioneer Hi-Bred International ble godkjent til import, videreforedling og til bruk som mat og fôr under EU-forordning 1829/2003 i 2009 (søknad EFSA/GMO/UK/2005/20, Kommisjonsbeslutning 2009/815/EU).

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 2007 (VKM 2007a). Foreldrelinjene 59122 og NK603 er også tidligere risikovurdert av VKM, både som enkelt-eventer og i en rekke andre hybrider (VKM 2005a,b, VKM 2007b,c, VKM 2008b,c, VKM 2009a,b, VKM 2012).

Risikovurderingen av den genmodifiserte maislinjen er basert på uavhengige vitenskapelige publikasjoner og dokumentasjon som er gjort tilgjengelig på EFSAs nettside EFSA GMO Extranet. Vurderingen er gjort i henhold til tiltenkt bruk i EU/EØS-området, og i overensstemmelse med miljøkravene i genteknologiloven med forskrifter, først og fremst forskrift om konsekvensutredning etter genteknologiloven. Videre er kravene i EU-forordning 1829/2003/EF, utsettingsdirektiv 2001/18/EF (vedlegg 2,3 og 3B) og veiledende notat til Annex II (2002/623/EF), samt prinsippene i EFSAs retningslinjer for risikovurdering av genmodifiserte planter og avledete næringsmidler (EFSA 2006, 2010, 2011a,b,c) lagt til grunn for vurderingen.

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

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

F_1-hybriden 59122 x NK603 er resultat av konvensjonelle kryssinger mellom de genmodifiserte maislinjene 59122 og NK603. Kryssingene er utført for å utvikle en maishybrid med resistens mot visse skadegjørere i billeslekten *Diabrotica*, samt toleranse mot herbicider med virkestoff glufosinat-ammonium og glyfosat.

Foreldrelinjen 59122 uttrykker en ny type *Bt*-toksin, som er resultat av introduksjon av to *cry*-gener (*cry34Ab1*og *cry35Ab1*) fra *B. thuringiensis* stamme PS149B1. Proteinene virker sammen som et binært toksin og gir plantene resistens mot angrep fra skadegjørere i slekten *Diabrotica*. I tillegg har maislinjen fått satt inn et *pat*-gen.

Foreldrelinje NK603 uttrykker CP4-EPSPS-proteiner, som et resultat av introduksjon av cp4-epspsgenet 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 59122 x NK603 er dannet ved konvensjonell kryssing mellom maislinjene 59122 og NK603. Spaltingsdata og PCR-analyser indikerer at de innsatte strukturer nedarves stabilt, og at antall, struktur og organisering av disse genkonstruksjonene er ekvivalent med de som finnes i foreldrelinjene. Nivåene av Cry34Ab1-, Cry35Ab1-, PAT- og CP4 EPSPS-proteiner i vegetativt vev og frø er sammenlignbare med uttrykk av tilsvarende proteinprodukter i foreldrelinjene.

Komparative analyser

Feltforsøk over en vekstsesong i Nord-Amerika viser små eller ingen signifikante forskjeller mellom den transgene maishybriden 59122 x NK603 og korresponderende, nær-isogene kontrollhybrider med hensyn på ernæringsmessig, morfologiske og agronomiske karakterer. Det er funnet statistiske forskjeller i enkeltparametere, men verdiene for de enkelte analyserte komponentene ligger innenfor typiske verdier for andre maissorter som er rapportert i litteraturen. Resultatene indikerer agronomisk og fenotypisk ekvivalens mellom 59122 x NK603 og umodifisert kontroll, og at de innsatte genene i 59122 x NK603 ikke har medført utilsiktede endringer i egenskaper knyttet til vekst og utvikling hos maisplantene.

Helserisiko

Fôringsstudie med hel mat er ikke utført med mais 59122 x NK603. Ingen negative helseeffekter relatert til mais 59122 x 1507 x NK603 ble rapportert fra fôringsstudie med hel mat utført på broilere.
Bioinformatikk-analyser viser ingen likheter mellom de introduserte proteinene 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).

Akutte oral-eksponeringsstudier indikerer ingen toksisitet relatert til proteinene Cry34Ab1, Cry35Ab1, PAT og CP4 EPSPS. Denne typen studier gir derimot ingen tilleggsinformasjon om mulige helseskadelige egenskaper ved mais 59122 x NK603.

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at mais 59122 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 59122 x NK603 sammenliknet med konvensjonelle maissorter.

Miljørisiko

Søknaden gjelder godkjenning av maishybrid 59122 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 59122 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 ernæringsmessige egenskaper til mais 59122 x NK603 eller prosesserte produkter sammenliknet med konvensjonell mais. Basert på dagens kunnskap er det også lite trolig at Cry34Ab1 eller Cry35Ab1 proteinene vil øke det allergene potensialet til mat og fôr produsert fra mais 59122 x NK603 sammenliknet med konvensjonelle maissorter. Faggruppen finner at maishybrid 59122 x NK603, ut fra dagens kunnskap og omsøkt bruk, er sammenlignbar med konvensjonell mais når det gjelder mulig miljørisiko i Norge.

Abbreviations and explanations

ALS	Acetolactate synthase, an enzyme that catalyses the first step in the synthesis of the branched-chain amino acids, valine, leucine, and isoleucine
AMPA	Aminomethylphosphonic acid, one of the primary degradation products of glyphosate
ARMG	Antibiotic resistance marker gene
BC	Backcross. Backcross breeding in maize is extensively used to move a single trait of interest (e.g. disease resistance gene) from a donor line into the genome of a preferred or “elite” line without losing any part of the preferred lines existing genome. The plant with the gene of interest is the donor parent, while the elite line is the recurrent parent. BC_1, BC_2 etc. designates the backcross generation number.
BLAST	Basic Local Alignment Search Tool. Software that is used to compare nucleotide (BLASTn) or protein (BLASTp) sequences to sequence databases and calculate the statistical significance of matches, or to find potential translations of an unknown nucleotide sequence (BLASTx). BLAST can be used to understand functional and evolutionary relationships between sequences and help identify members of gene families.
bp	Basepair
Bt	*Bacillus thuringiensis*
CaMV	Cauliflower mosaic virus
Codex	Set by The Codex Alimentarius Commission (CAC), an intergovernmental body to implement the Joint FAO/WHO Food Standards Programme. Its principle objective is to protect the health of consumers and to facilitate the trade of food by setting international standards on foods (i.e. Codex Standards)
Cry	Any of several proteins that comprise the crystal found in spores of *Bacillus thuringiensis*. Activated by enzymes in the insects midgut, these proteins attack the cells lining the gut, and subsequently kill the insect
Cry34/35Ab1	Binary crystal protein containing of Cry34Ab1 and Cry35Ab1.
Cry34Ab1	Cry34 class crystal protein from *Bacillus thuringiensis* stamme 149B1.
Cry35Ab1	Cry35 class crystal protein from *Bacillus thuringiensis* stamme 149B1.
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.
TI	Trait integration
TMDI	Theoretical Maximum Daily Intake
U.S. EPA	United States Environmental Protection Agency.
Maize growth stages:	*Vegetative* VE: emergence from soil surface V1: collar of the first leaf is visible V2: collar of the second leaf is visible Vn: collar of the leaf number 'n' is visible VT: last branch of the tassel is completely visible *Reproductive* R0: Anthesis or male flowering. Pollen shed begins R1: Silks are visible R2: Blister stage, 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 19 September 2005, the European Food Safety Authority (EFSA) received from the Competent Authority of United Kingdom an application (Reference EFSA/GMO/UK/2005/20) for authorisation of the insect-resistant and herbicide tolerant genetically modified (GM) maize 59122 x NK603 (Unique Identifier DAS-59122-7 x MONØØ6Ø3-6), submitted by Pioneer Hi-Bred International, Inc. within the framework of Regulation (EC) No 1829/2003.

The scope of the application covers:

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

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

After receiving the application EFSA/GMO/NL/2005/20 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 20 June 2007, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the EC and consulted nominated risk assessment bodies of the MS, including the Competent Authorities within the meaning of Directive 2001/18/EC (EC 2001), following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1929/2003, to request their scientific opinion. Within three months following the date of validity, all MS could submit via the EFSA GMO Extranet to EFSA comments or questions on the valid application under assessment. The VKM GMO Panel assessed the application in connection with the EFSA official hearing, and submitted a preliminary opinion in September 2007 (VKM 2007a). EFSA published its scientific opinion 19 November 2008 (EFSA 2008), and maize stack 59122 x NK603 was approved for food and feed uses, import and processing in 30 October 2009 (Commission Decision 2009/815/EC).

An application for authorisation of maize 59122 x NK603 for cultivation in the EU was submitted by Pioneer Hi-Bred International, Inc. in January 2006 (EFSA/GMO/UK/2006/29). The application was, however, withdrawn by the notifier in December 2006.

Scientific opinions on the parental lines of the stack 59122 x NK603 have previously been submitted by the VKM GMO Panel (VKM 2005a,b,d, 2008b). In addition, maize 59122 and NK603 have been evaluated by the VKM GMO Panel as a component of several stacked GM maize events under Directive 2001/18/EC and Regulation (EC) 1829/2003 (VKM 2005b, VKM 2007b,c, VKM 2008c, VKM 2009a,b, VKM 2012).

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

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

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

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

Assessment

1 Introduction

Maize 59122xNK603 has been obtained from traditional breeding methods between progeny (inbred lines) of the genetically modified maize lines 59122 and NK603.

The parental line 59122 expresses the *cry34Ab1* and *cry34Ab1*genes from *Bacillus thuringiensis*, conferring resistance to certain coleopteran target pests belonging to the genus *Diabrotica,* such as the larvae of western corn rootworm (*D. virgifera virgifera*), northern corn rootworm (*D. barberi*) and the southern corn rootworm (*D. undecimpunctata howardi*). Maize 59122 also expresses the PAT protein from *S. viridochromogenes.*

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 59122 are present in the Norwegian agriculture. The PAT protein expressed in maize 59122 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 59122xNK603 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 59122xNK603 is based on information provided by the applicant in the applications EFSA/GMO/UK/2005/20, and scientific opinions and comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment is also based on a review and assessment of relevant peer-reviewed scientific literature.

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

2 Molecular characterisation

2.1 Evaluation of relevant scientific data

2.1.1 Method of production of maize 59122xNK603

Conventional breeding methods were used to develop maize 59122xNK603. The three inserts present in maize 59122xNK603 were derived from two independent events: 59122 and NK603, and combines resistance to corn rootworm larvae (Coleoptera: Chrysomelidae; *Diabrotica* spp.) and tolerance to glufosinate-ammonium and glyphosate based herbicides.

2.1.2 Summary of evaluation of the single events

2.1.2.1 Maize 59122

The gene modified maize strain 59122 expresses herbicide and insect tolerance through *Agrobacterium tumefaciens* mediated transformation of maize cells, with the insertion of a linear DNA fragment of 7390 bp from the binary vector PHP17662 into the maize genome. The DNA fragment does not contain an antibiotic resistance gene. Transformation of 59122 maize resulted in the stable insertion of the T-DNA region into the maize genome. The T-DNA region in PHP17662 contained the *cry*34Ab1, *cry*35Ab1 and *pat* coding sequences and the necessary regulatory components to regulate gene expression.

The maize-optimised *cry34Ab1* gene was derived from *Bacillus thuringiensis* strain PS149B1. *Cry34Ab1* encodes a protein comprising 123 amino acids. The amino acid sequence of the Cry34Ab1 protein (14 kDa) encoded by the maize-optimised *cry*34Ab1 gene is identical to the Cry34Ab1 protein (14 kDa) expressed in the bacteria. Expression of the maize-optimised *cry34Ab1* gene is regulated by the ubiquitin promoter from *Zea mays* (*ubi*1ZM). Termination of transcription for the maize-optimised *cry*34Ab1 gene is controlled by the terminator sequence from the *Solanum tuberosum* proteinase inhibitor II gene (*pinII*).

The maize-optimised *cry35Ab1* gene was derived from *Bacillus thuringiensis* strain PS149B1. *Cry35Ab1* encodes a protein comprising 383 amino acids. The amino acid sequence of the Cry35Ab1 protein (44 kDa) encoded by the maize-optimised *cry35Ab1* gene is identical to the Cry35Ab1 protein expressed by the bacteria. Expression of the maize-optimised *cry35Ab1* gene is regulated by the promoter from the *Triticum aestivum* peroxidase gene and its native leader. Termination of transcription is controlled by the terminator sequence from *Solanum tuberosum* proteinase inhibitor II gene (*pinII*).

The Cry34Ab1 and Cry35Ab1 proteins act together in conferring resistance against certain coleopteran insect pests, such as *Diabrotica* spp. which are important maize pests.

Maize 59122 also express the phosphinothricin-N-acetyltransferase (PAT) protein from *Streptomyces viridochromogenes* (previously described).

The level of the proteins Cry34Ab1, Cry35Ab1 and PAT were analysed by ELISA. Samples were collected from 11 different experimental fields in Chile, US and Canada in 2002/2003, and 3 and 6 in Europe in 2003 and 2004, respectively. Samples were collected at four different developmental stages. Cry34Ab1 and Cry35Ab1 was detected in leaves, pollen, seeds roots, stalk, and whole plants, whereas PAT was detected in leaves, roots, stalk and whole plant. The levels of PAT in seeds and pollen were below the detection limit. The expression of Cry34Ab1 and Cry35Ab1 varied between the different tissues of the plants and between experimental fields. The concentration of Cry35Ab1 in pollen was either low or below detection levels, whereas the concentration of Cry34Ab1 varied between 50 and 74 µg/g dw. In samples collected in Europe the concentrations of Cry34Ab1 and Cry35Ab1 in seeds were measured to be 61.8 ± 16.5 and 2.34 ± 0.475 µg/g dw, respectively, whereas samples from Chile and US/Canada showed 36.4 ± 8.9 og 2.0 ± 0.7 µg/g dw, respectively. The variation in protein concentration amongst samples collected from random blocks with and without herbicide treatment was shown to be higher than the variation between the experimental fields. The expression of PAT was generally low in all samples it was detected. Results from whole plant extracts in Europe showed concentrations of 0.0807 ± 0.0800 µg/g dw.

Western blot analysis and detection with polyclonal antibodies showed that the Cry34Ab1, Cry35Ab1 and PAT proteins all had the expected molecular weights. Cry35Ab1 produced a double protein band, which was explained by proteolytic cleavage of a C-terminal fragment by plant proteases. No indications of fusion proteins were found. Studies performed to detect coding sequences in the maize strain 59122, did not disclose any ORFs that could lead to the expression of peptides larger than a 100 amino acids.

Southern blot and sequence analysis showed that nearly a full length copy of the PHP17662 recombinant DNA fragment (7343 bp out of the 7390 bp fragment) is inserted in the maize genome. The 59122 maize does not contain fragments from the vector backbone portion of binary vector PHP17662, in particular the tetracycline and spectinomycin resistance genes, the *vir*G gene and other backbone sequences not intended for transformation. In addition, PCR amplification and sequence analysis showed that the 5' and 3' regions flanking the 59122 maize insert are of maize genomic origin. A 22 bp are missing from the 5' end and 25 bp from the 3' end of the fragment. The fragment contains all genes (*pat, cry34Ab1* and *cry35Ab1*) and respective regulatory sequences of the insert. Two base modifications have also been identified in the non-coding region of the fragment, but none of these affect the ORFs of the fragment. A 2593 bp of the 5'-, and 1986 bp of the 3' - flanking sequences have also been sequenced, where small regions display homology to e.g. chromosomal sequences and various expressed sequence tags, ESTs. The longest region of these is 179 bp. None of the flanking sequences contain coding regions to known proteins. The contents of genes and regulatory elements in the recombinant DNA fragment are outlined in Figure 1.

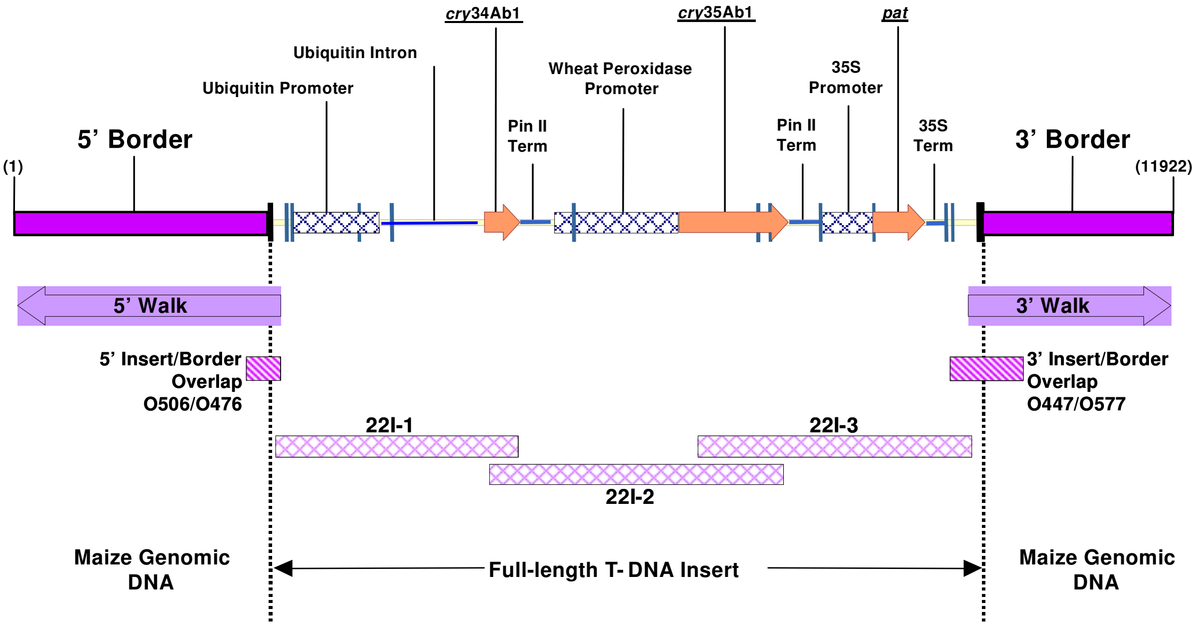


Figure 1. Restriction map of the various gene elements of the recombinant DNA fragment inserted in the genome of the maize strain 59122.

2.1.2.2 Maize NK603

The maize line AW x CW, a proprietary maize cell culture, was transformed by acceleration to develop the NK603 maize event. Conventional breeding methods were used to backcross plants generated from the initial transformation into a recurrent, desired inbred maize line with a genetic background of interest to the breeder.

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

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

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

In 1999, forage and grain tissues were produced in European field trials at four sites. Four replications were used at each of the four sites. CP4 EPSPS protein levels were measured in maize forage and grain. All protein values are expressed as micrograms (µg) of the specific protein per gram (g) of tissue on a fresh weight (fw) basis. Control maize samples were below the Limit of Detection (LOD) for CP4 EPSPS protein. In maize 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 recognizes both these proteins expressed in NK603.

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

Southern blot analysis was used to study the insert number, the copy number, the integrity of the inserted promoters, coding regions, and polyadenylation sequences, and the presence or absence of the plasmid backbone sequence. Polymerase chain reaction (PCR) was performed to verify the sequences at the 5' and 3' ends of the insert. Further, PCR analysis and subsequent DNA sequencing of four overlapping products spanning the length of the insert in NK603 were undertaken to characterize of the inserted DNA in NK603 (Kesterson et al., 2002a). Genomic DNA from the NK603 maize and control (B73) were digested with the restriction enzyme *StuI*. The result suggested that NK603 contains one 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 was used for characterization of the insert and the sequences flanking the insert. The results indicate that these sequences are native to the maize genome. These data indicate that only the expected full-length CTP2-CP4 EPSPS and CTP2-CP4 EPSPS L214P proteins are encoded by the insert in NK603. The contents of genes and regulatory elements in the recombinant DNA fragment are outlined in Figure 2.

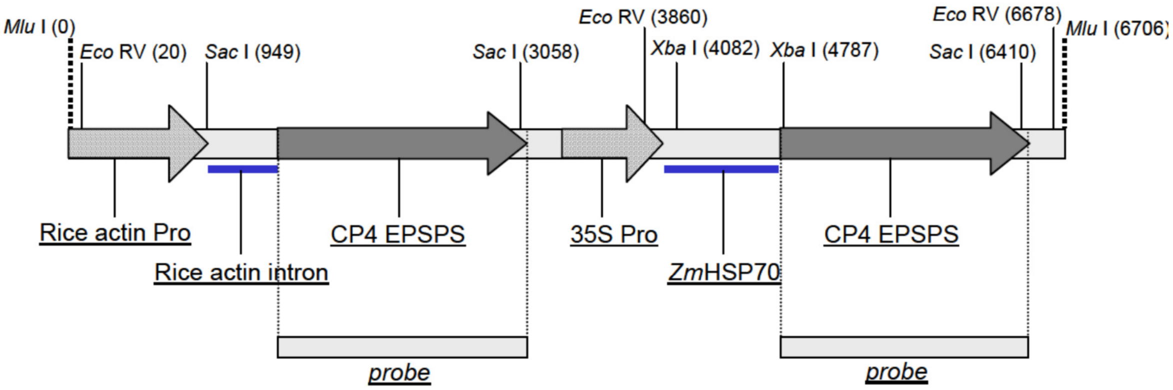


Figure 2. Restriction map of the various gene elements of the recombinant DNA fragment inserted in the genome of the maize strain NK603.

2.1.3 Transgene constructs in 59122 x NK603 maize

The 59122xNK603 maize was obtained by conventional crossing between two genetically modified oilseed rape events: 59122 and NK603 maize. No new genetic modification was used for the development of the 59122xNK603 maize.

A detailed molecular analysis was conducted to investigate the copy number, structure and organization of the inserts found in 59122xNK603 maize. Genomic DNA was extracted from leaves harvested from 59122, NK603 and 59122xNK603 maize plants. The DNA samples were analyzed by the Southern blot method using different restriction enzymes and genetic probes specific for the 59122 or NK603 maize inserts, respectively. Along with the 59122, NK603 and 59122xNK603 maize genomic DNA, positive control DNA was analyzed as well as negative control DNA containing genomic DNA from non-GM control maize with comparable genetic background to 59122xNK603 maize.

Samples of genomic DNA from four individual 59122xNK603 maize plants and from four individual 59122 maize plants were digested with the restriction enzyme *Sac* I and subjected to Southern blot analysis with the *cry*34Ab1, *cry*35Ab1 and *pat* gene probes. Hybridization of the *cry*34Ab1 gene probe with the *Sac* I digested genomic DNA from 59122 maize was expected to result in a single right border fragment of more than 3217 bp. A single hybridization fragment of approximately 3400 bp was observed for both the 59122 and 59122 x NK603 maize genomic DNA.

Sac I digestion of the 59122 maize genomic DNA and hybridization with the *cry*35Ab1 probe was expected to result in three internal hybridization fragments of 1941 bp, 1855 bp and 123 bp respectively. However, the predicted 123 bp fragment was not detected in the 59122 maize. The applicant concludes that fragments below approximately 1000 bp are run off the gel during electrophoresis and, therefore, are not transferred to the nylon membrane. The 1941 and 1855 bp hybridization fragments were observed for both the 59122 and 59122xNK603 maize genomic DNA using the *cry*35Ab1 gene probe in combination with *Sac* I digestion. The 1855 bp hybridization fragment for the *pat* gene probe was observed for both the 59122 and 59122 x NK603 maize genomic DNA.

In order to study the molecular equivalence and copy number of the insert present in NK603 maize to that present in 59122xNK603 maize, samples of genomic DNA from four individual 59122xNK603 maize plants and from four individual NK603 maize plants were digested with the restriction enzyme *Eco*R V and subjected to Southern blot analysis with the *cp*4 *epsps* probe. Hybridization of the *cp*4 *epsps* probe with the *Eco*R V digested NK603 maize genomic DNA was expected to result in two hybridization fragments of 3840 and 2818 bp respectively. Hybridization fragments of 3840 bp and 2818 bp were observed for both the NK603 and 59122xNK603 maize genomic DNA.

The results obtained from Southern Blot analyses indicate molecular equivalence, and identical copy number of the inserts present in 59122xNK603 maize to those present in 59122 and NK603 maize, respectively.

2.1.3.1 Information on the expression of insert

Two field studied was carried out in order to determine the level of the Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins in grain obtained from 59122xNK603 maize. One field study was conducted during the 2003 growing season at six field sites, of which five were located in the USA and one was located in Canada (EFSA-GMO-UK-2005-20). Levels of the Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins in grain from 59122xNK603 maize were determined using a specific Enzyme Linked Immunosorbent Assay (ELISA) developed for each protein. Another field study was conducted during the 2004 growing season at five locations in Europe: three locations in Spain, one location in Bulgaria and one location in Hungary (EFSA-GMO-UK-2006-29). Levels of the Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins in forage and grain from 59122xNK603 maize were determined using a specific Enzyme Linked Immunosorbent Assay (ELISA) developed for each protein.

In the 2003 study, the 59122xNK603 maize grain samples were taken from plots that were sprayed with glyphosate herbicide only; from plots that were sprayed with glufosinate-ammonium herbicide only; and from plots sprayed with glyphosate herbicide followed by glufosinate-ammonium herbicide. The results obtained from the analysis have been summarised in Table 1. Levels of the Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins in grain from 59122xNK603 maize were comparable regardless of the herbicide treatment (Table 1). In the 2004 study, the 59122xNK603 maize forage and grain samples were taken from plots that were sprayed with two sequential applications of glyphosate herbicide; from plots that were sprayed with two sequential applications of glufosinate-ammonium herbicide; and from plots sprayed with glyphosate herbicide followed by glufosinate-ammonium herbicides. The results obtained from the analysis have been summarized in Table 2. Expression levels of the Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins in forage and grain from 59122xNK603 maize were comparable regardless of the herbicide treatment (Table 2).

Cry34Ab1:
In the 2003 study, the level of the Cry34Ab1 protein in grain from 59122xNK603 maize ranged from **21.8 to 53.6** µg/g grain dry weight, while in the 2004 study the level ranged from **19.0 to 104** µg/g grain dry weight. In 59122 maize grain, field trials in USA, Canada and Chile, the Cry34Ab1 level ranged from **19.5 to 84.8** µg/g grain dry weight.

In the 2004 study, the level of the Cry34Ab1 protein in forage from 59122xNK603 maize ranged from **85.8 to 138** µg/g forage dry weight. In forage from the 59122 maize the Cry34Ab1 level ranged from **47.1 to 113** µg/g forage dry weight.

Cry35Ab1:
In the 2003 study, the level of the Cry35Ab1 protein in grain from 59122xNK603 maize ranged from **0.530 to 3.31** µg/g grain dry weight, while in the 2004 study the level ranged from **0.450 to 3.72** µg/g grain dry weight. In 59122 maize grain, field trials in USA, Canada and Chile, the Cry35Ab1 expression level ranged from **0.48 to 4.8** µg/g grain dry weight.

In the 2004 study , the level of the Cry35Ab1 protein in forage from 59122 x NK603 maize ranged from **22.8 to 49.2** µg/g forage dry weight. In forage from the 59122 maize the Cry35Ab1 level ranged from **15.8 to 76.6** µg/g forage dry weight.

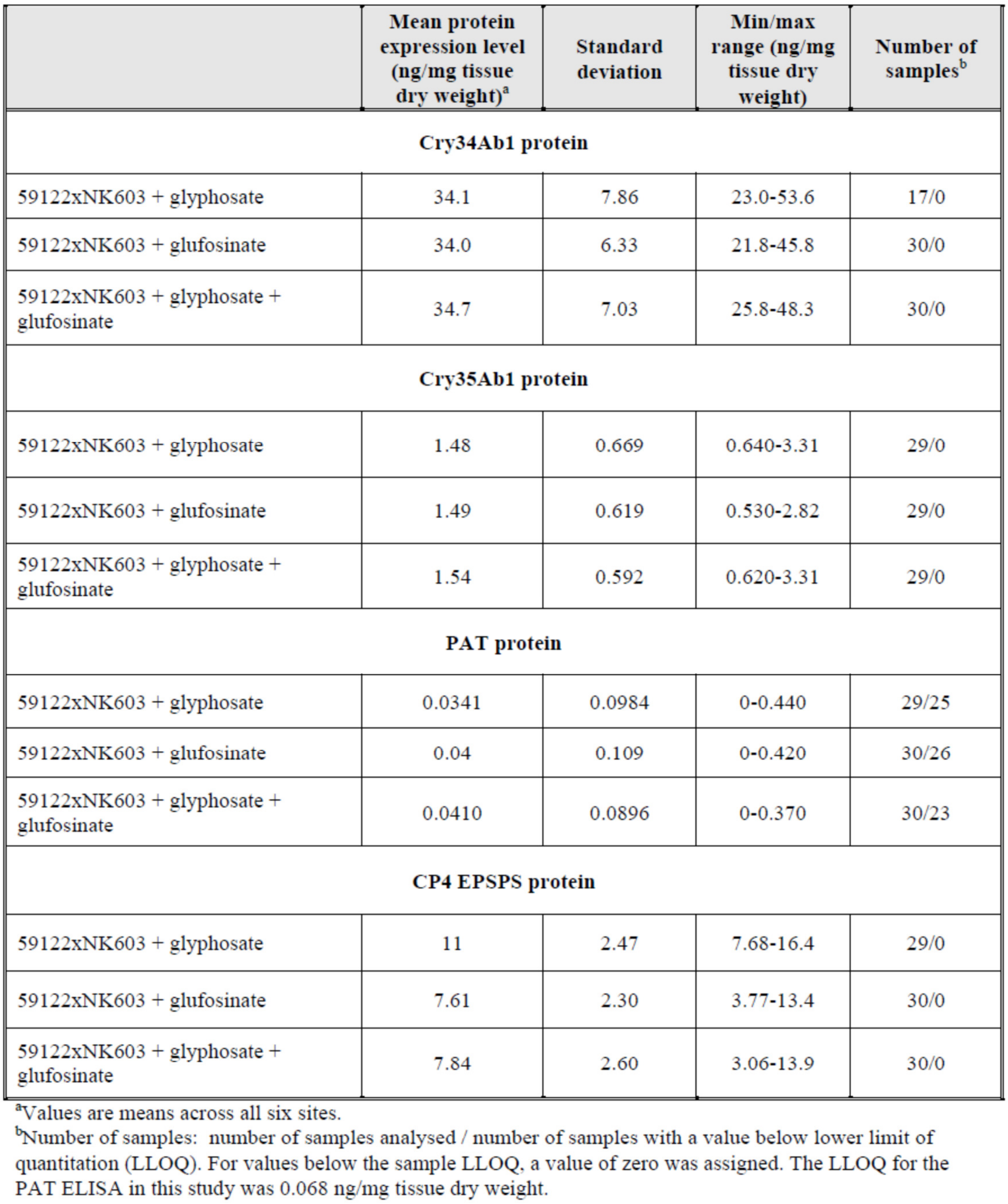
PAT:
In the 2003 study, the level of the PAT protein in grain from 59122xNK603 maize ranged from below the lower limit of quantitation of the assay, which was **0.068** µg/g grain dry weight, to **0.440** µg/g grain dry weight, while in the 2004 study the level was below the lower limit of quantitation of the assay, which was **0.068** µg/g grain dry weight. In 59122 maize grain, field trials in USA, Canada and Chile, the PAT expression level ranged from below the lower limit of quantification of the PAT ELISA assay used to **0.94** µg/g grain dry weight. .

In the 2004 study, the level of the PAT protein in forage from 59122 x NK603 maize ranged from **0.77 to 4.14** µg/g forage dry weight. In forage from the 59122 maize the PAT expression level ranged from below the lower limit of quantitation, which was 0.068 µg/g grain dry weight, to **0.240** µg/g grain dry weight.

CP4 EPSPS:
In the 2003 study, the level of the CP4 EPSPS protein in grain from 59122xNK603 maize ranged from **3.06 to 16.4** µg/g grain dry weight, while in the 2004 study, the level ranged from **0.940 to 8.43** µg/g grain dry weight.

In terms of µg/g fresh weight, the level of the CP4 EPSPS protein in grain from 59122xNK603 maize seems to be comparable to the CP4 EPSPS protein in grain from NK603maize. The level of the CP4 EPSPS protein in 59122 x NK603 maize grain ranged from **2.601 to 13.94** µg/g fresh weight, while in NK603 maize the level ranged from **6.9 to 15.6** µg/g fresh weight.

Table 1. Protein expression level of the Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins in grain from 59122 x NK603 maize plants sprayed with glyphosate; sprayed with glufosinate-ammonium; or sprayed with glyphosate followed by glufosinate-ammonium (USA and Canada, 2003).



	Mean protein expression level (ng/mg tissue dry weight)[a]	Standard deviation	Min/max range (ng/mg tissue dry weight)	Number of samples[b]
Cry34Ab1 protein				
59122xNK603 + glyphosate	34.1	7.86	23.0-53.6	17/0
59122xNK603 + glufosinate	34.0	6.33	21.8-45.8	30/0
59122xNK603 + glyphosate + glufosinate	34.7	7.03	25.8-48.3	30/0
Cry35Ab1 protein				
59122xNK603 + glyphosate	1.48	0.669	0.640-3.31	29/0
59122xNK603 + glufosinate	1.49	0.619	0.530-2.82	29/0
59122xNK603 + glyphosate + glufosinate	1.54	0.592	0.620-3.31	29/0
PAT protein				
59122xNK603 + glyphosate	0.0341	0.0984	0-0.440	29/25
59122xNK603 + glufosinate	0.04	0.109	0-0.420	30/26
59122xNK603 + glyphosate + glufosinate	0.0410	0.0896	0-0.370	30/23
CP4 EPSPS protein				
59122xNK603 + glyphosate	11	2.47	7.68-16.4	29/0
59122xNK603 + glufosinate	7.61	2.30	3.77-13.4	30/0
59122xNK603 + glyphosate + glufosinate	7.84	2.60	3.06-13.9	30/0

[a] Values are means across all six sites.

[b] Number of samples: number of samples analysed / number of samples with a value below lower limit of quantitation (LLOQ). For values below the sample LLOQ, a value of zero was assigned. The LLOQ for the PAT ELISA in this study was 0.068 ng/mg tissue dry weight.

Table 2. **Protein expression level of the Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins in forage and grain from 59122 x NK603 maize plants sprayed with two sequential applications of glyphosate herbicide; sprayed with two sequential applications of glufosinateammonium herbicide; or sprayed with glyphosate followed by glufosinate-ammonium herbicides (Europe locations, 2004) (Buffington, 2005).**

	Mean protein expression level (ng/mg tissue dry weight)[a]	Standard deviation	Min/max range (ng/mg tissue dry weight)	Number of samples
Cry34Ab1 protein				
59122xNK603 + glyphosate				
Forage	122	14.2	111 - 138	5
Grain	45.0	10.4	27.1 - 62.4	15
59122xNK603 + glufosinate-ammonium				
Forage	123	6.80	114 - 131	5
Grain	46.9	19.2	19.0 - 104	15
59122xNK603 + glyphosate + glufosinate				
Forage	106	18.4	85.8 – 128	5
Grain	52.1	14.9	30.4 – 78.1	15
Cry35Ab1 protein				
59122xNK603 + glyphosate				
Forage	33.6	5.55	27.2 – 37.4	5
Grain	1.04	0.355	0.630 – 1.71	15
59122xNK603 + glufosinate-ammonium				
Forage	36.7	9.62	22.8 – 49.2	5
Grain	1.22	0.811	0.450 – 3.72	15
59122xNK603 + glyphosate + glufosinate				
Forage	31.4	8.11	23.0 – 44.2	5
Grain	1.28	0.659	0.520 – 2.52	15
PAT protein				
59122xNK603 + glyphosate				
Forage	2.12	0.749	1.29 – 2.75	5
Grain	ND[b]	ND[b]	ND[b]	15
59122xNK603 + glufosinate-ammonium				
Forage	2.87	1.19	0.920 – 4.14	5
Grain	ND[b]	ND[b]	ND[b]	15
59122xNK603 + glyphosate + glufosinate				
Forage	2.06	1.16	0.770 – 3.65	5
Grain	ND[b]	ND[b]	ND[b]	15
CP4 EPSPS protein				
59122xNK603 + glyphosate				
Forage	68.4	13.0	53.6 – 77.6	5
Grain	5.31	1.61	3.11 – 8.02	15
59122xNK603 + glufosinate-ammonium				
Forage	68.3	18.1	38.4 – 80.4	5
Grain	4.74	1.59	2.10 – 8.14	15
59122xNK603 + glyphosate + glufosinate				
Forage	59.2	14.2	43.0 – 81.7	5
Grain	5.05	2.23	0.940 – 8.43	15

[a] Values are means across all five sites.
[b] ND: not detected. The LLOQ for the PAT ELISA in this study was 0.068 ng/mg tissue dry weight.

2.1.3.2 Parts of the plant where the insert is expressed

According to the applicant, the Cry34Ab1, Cry35Ab1 and CP4 EPSPS proteins are expressed in the leaf, root, pollen, stalk and grain of the 59122xNK603 maize. The PAT protein is expressed in the leaf, root and stalk of the 59122xNK603 maize. The level of the PAT protein in 59122xNK603 maize grain and pollen was below the lower limit of quantitation of the assay used. In addition, according to the applicant, the levels of the Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins in the different tissues of 59122xNK603 maize were comparable regardless of herbicide treatment.

2.1.3.3 Potential fusion proteins

Southern Blot analyses conducted on 59122xNK603 maize indicate molecular equivalence and identical copy number of the inserts present in 59122xNK603 maize to those present in 59122 and NK603 maize, respectively.

2.1.3.4 Inheritance and genetic stability of inserted DNA

According to the data from the applicant, the parental maize lines 59122 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. Interactions of the transgene inserts are therefore expected to be minimal during conventional breeding of the genetically modified maize lines 59122 and NK603.

Southern blot analyses, carried out on 83 individual plants from a single 59122xNK603 maize generation, indicate that the integrity of the inserts in the single events in 59122 and NK603 maize are preserved in the hybrid 59122 x NK603. Furthermore, protein expression levels, phenotypic characteristics and agronomic performance, indicate that the integrity of the inserts inherited from the single events is preserved in maize stack 59122 x NK603.

2.2 Conclusion

Southern and PCR analyses indicate that the recombinant inserts in the single maize events 59122 and NK603 are retained in maize stack 59122xNK603. Genetic stability of the inserts has previously been demonstrated in the parental lines 59122 and NK603. The level of Cry34Ab1/Cry35Ab1, PAT and CP4 EPSPS proteins in seed and forage from the stacked event are comparable to the levels in the single events. Phenotypic analyses also indicate stability of the insect resistance and herbicide tolerance traits of the stacked event.

3 Comparative assessment

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

3.1.1 Experimental design & statistical analysis

In the application EFSA/GMO/UK/2005/20 for food and feed uses, import and processing of maize 59122xNK603 within the European Union, the applicant present compositional data from seed and forage material collected in field trials in the North America during the 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 five separate sites in commercial maize-growing regions of the USA (Iowa, Indiana and Nebraska) and one field site in Ontario, Canada. These trials compared the composition of maize 59122xNK603 with a conventional counterpart having a genetic background representative of the test entry 59122 x NK603. The test of equivalence is used to verify whether the agronomic, phenotypic and compositional characteristics of the GM plant fall within the normal range of natural variation. Such a range of natural variation is estimated from a set of non-GM reference varieties with a history of safe use (EFSA 2010b) and therefore allows comparisons of the GM plant with a similar food or feed produced without the help of genetic modification and for which there is a well-established history of safe use. In this application, comparisons with baseline data on commercial maize, compiled from publicly available literature, have been used in the comparisons with maize 59122xNK603 for considerations of natural variations.

At each trial site, maize 59122xNK603 and the conventional counterpart were planted following a randomized 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. Fertilizer, 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. 59122xNK603 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. Maize 59122 x NK603 grown for agronomic analysis received one application of glyphosate followed by one application of glufosinate-ammonium. Plots untreated with the target herbicides were not included in the field study.

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 analyzed. 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.

3.2 Compositional Analysis

The nutritional analysis was undertaken on a broad range of compounds in grain from 59122xNK603 maize in accordance with OECD guidelines for assessment of GM maize (OECD, 2002). The objective was to determine that 59122xNK603 maize treated with glyphosate, glufosinate-ammonium, or glyphosate followed by glufosinate-ammonium herbicides, was equivalent to non-GM control maize with comparable genetic background. Grain samples from 59122xNK603 maize (all herbicide treatments) and non-GM control maize with comparable genetic background were collected and analyzed for nutrient composition, including: crude protein, crude fat, crude fiber, acid detergent fiber (ADF), neutral detergent fiber (NDF), ash, carbohydrates, 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).

Compositional analysis of maize forage included the determination of proximates (crude protein, crude fat, ash), crude fiber, acid detergent fiber (ADF), natural detergent fiber (NDF), carbohydrates and minerals (calcium and phosphorus).

According to the applicant, and in accordance with OECD guidelines (OECD, 2002), substantial equivalence was evaluated by comparing the mean nutrient composition values of each 59122xNK603 maize entry to non-GM maize with comparable genetic background, and mean nutrient composition values of the 59122xNK603 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.

Forage:
Proximates and fiber:
No statistically significant differences were observed for mean crude fat, crude fiber, ADF, NDF, or ash in the across location summary analysis. Mean crude protein and carbohydrates values across locations in the 59122xNK603 + glyphosate hybrid were significant different ($P<0.05$) (Table 1 – appendix). Significant differences for crude protein were only observed at two of six locations. Significant differences for carbohydrates were only observed at one of the six locations. The across locations mean values for all forage proximate, fiber and carbohydrate analytes for test and control hybrids were within reported literature ranges (Table 3 – appendix).

Minerals:
Mean calcium and phosphorus values across locations in the 59122xNK603 + glyphosate hybrid were significant different ($P<0.05$) (Table 2 – appendix). No statistically significant differences for calcium were observed at any of the individual locations. Statistically significant differences for phosphorus were only observed at two of the six locations. The mean values for calcium and phosphorus for the test and control hybrids across locations were within reported literature ranges (Table 3 – appendix).

Grain:

Proximates and fibers:

No statistically significant (P<0.05) differences were observed across locations between 59122xNK603 maize treated with ***glyphosate*** and non-GM control maize with regard to crude fiber or NDF values. Statistically significant differences were observed for crude protein, crude fat, ADF, ash and carbohydrates in the analysis across locations (Table 4 – appendix). However, when analyzed on a per location basis, these differences were not consistently observed. No statistically significant differences were observed between 59122 x NK603 and the non-GM control maize for crude fat and ADF at any of the six individual locations. With regard to crude protein and ash, no statistically significant differences were observed at three out of the six individual locations. With regard to carbohydrates, no statistically significant differences were observed at two out of the six individual locations.

No statistically significant differences were observed across locations between 59122 x NK603 maize treated with ***glufosinate*** and non-GM control maize with regard to ADF, crude fiber or NDF. Statistically significant differences were observed for crude protein, crude fat, ash and carbohydrates in the analysis across locations (Table 5 – appendix)). However, when analyzed on a per location basis, these differences were not consistently observed. No statistically significant differences were observed at five out of the six individual locations for crude fat. No statistically significant differences were observed at three out of the six individual locations for ash. No statistically significant differences were observed at one of the six individual locations for crude protein and carbohydrates

No statistically significant differences were observed across locations between 59122 x NK603 maize treated with ***glyphosate followed by glufosinate*** and non-GM control maize with regard to crude fat or crude fiber. Statistically significant differences between 59122 x NK603 maize and non-GM control maize were observed for crude protein, ADF, NDF, ash and carbohydrates (Table 6 – appendix). However, when analysed on a per location basis, these differences were not consistently observed. No statistically significant differences were observed at five out of the six individual locations for ADF and NDF. No statistically significant differences were observed at two out of the six individual locations for crude protein and ash. No statistically significant differences were observed at one out of the six locations for carbohydrates. In addition, all mean values for proximates, fiber and carbohydrates in grain from 59122 x NK603 maize treated with glyphosate followed by glufosinate and in grain from non-GM control maize were within reported literature ranges (Table 7 – appendix)).

Fatty acids:

No statistically significant differences were observed across locations between 59122 x NK603 maize treated with ***glyphosate*** and non-GM control maize with regard to linolenic acid. Statistically significant differences were observed for palmitic acid, stearic acid, oleic acid and linolenic acid in the analysis across locations (Table 8 - appendix). However, when analyzed on a per location basis, these differences were not consistently observed. No statistically significant differences were observed for palmitic acid and stearic acid at four out of the six individual locations. With regard to oleic and linoleic acid, the mean values obtained from the analysis on a per location basis were not statistically different from the control at two out of the six individual locations.

No statistically significant differences were observed across locations between 59122 x NK603 maize treated with ***glufosinate*** and non-GM control maize with regard to linolenic acid. Statistically significant differences were observed for palmitic acid, stearic acid, oleic acid and linoleic acid in the analysis across locations (Table 9 - appendix). However, when analyzed on a per location basis, these differences were not consistently observed. No statistically significant differences were observed for palmitic acid at any of the six individual locations. No statistically significant differences were observed for stearic acid at four out of the six individual locations. No statistically significant differences were observed for oleic and linoleic acid at two out of the six individual locations.

No statistically significant differences were observed across locations between 59122 x NK603 maize treated with ***glyphosate followed by glufosinate*** and non-GM control maize with regard to linolenic acid. Statistically significant differences between 59122 x NK603 maize and non-GM control maize were observed for palmitic acid, stearic acid, oleic acid and linoleic acid in the analysis across locations (Table 10 - appendix). However, when analysed on a per location basis no statistically significant differences between 59122 x NK603 maize and non-GM control maize were observed at five out of six individual locations for palmitic acid. No statistically significant differences were observed at four and three out of the six individual locations for stearic and oleic acid respectively. Statistically significant differences for linoleic acid were observed at six locations. However, statistically significant differences for linoleic acid were not consistently observed across locations for grain from 59122 x NK603 maize treated with glyphosate, nor for grain from 59122 x NK603 maize treated with glufosinate, indicating the absence of an obvious trend. Furthermore, all mean values for fatty acids in grain from 59122 x NK603 maize treated with glyphosate followed by glufosinate and in grain from non-GM control maize were within reported literature ranges (Table 11 – appendix).

Amino acids:
Statistically significant differences were observed across locations between 59122 x NK603 maize treated with ***glyphosate*** and non-GM control maize with regard to methionine, cystine, lysine, tryptophan, threonine, isoleucine, histidine, valine, leucine, arginine, phenylalanine, glycine, alanine, aspartic acid, glutamic acid, proline, serine or tyrosine (Table 12 - appendix). However, when analyzed on a per location basis, these differences were not consistently observed. No statistically significant differences were observed at any of the six individual locations for methionine and cystine. No statistically significant differences were observed on a per location basis for five out of the six individual locations for lysine, glycine and tyrosine. No statistically significant differences were observed on a per location basis for four out of the six individual locations for tryptophan, threonine, aspartic acid and serine. No statistically significant differences were observed on a per location basis for three out of the six individual locations for isoleucine, histidine, valine, leucine, arginine, phenylalanine, glutamic acid and proline. No statistically significant differences were observed on a per location basis for two out of the six individual locations for alanine. In addition, all mean values for amino acids in grain from 59122 x NK603 maize treated with glyphosate and in grain from non-GM control maize were within reported literature ranges.

Statistically significant differences were observed across locations between 59122 x NK603 maize treated with ***glufosinate*** and non-GM control maize with regard to methionine, cystine, lysine, tryptophan, threonine, isoleucine, histidine, valine, leucine, arginine, phenylalanine, glycine, alanine, aspartic acid, glutamic acid, proline, serine and tyrosine (Table 13 - appendix). However, when analyzed on a per location basis, these differences were not consistently observed. No statistically significant differences were observed at five out of the six individual locations for lysine and glycine. No statistically significant differences were observed on a per location basis for four out of the six individual locations for methionine, cystine, tryptophan, threonine, histidine, valine, aspartic acid, glutamic acid, proline, serine and tyrosine. No statistically significant differences were observed on a per location basis for three out of the six individual locations for isoleucine, leucine and alanine. No statistically significant differences were observed on a per location basis for two out of the six individual locations for arginine and phenylalanine.

Statistically significant differences were observed across locations between 59122 x NK603 maize treated with ***glyphosate followed by glufosinate*** and non-GM control maize with regard to methionine, cystine, lysine, tryptophan, threonine, isoleucine, histidine, valine, leucine, arginine, phenylalanine, glycine, alanine, aspartic acid, glutamic acid, proline, serine or tyrosine (Table 14 - appendix). However, when analysed on a per location basis, these differences were not consistently observed. No statistically significant differences were observed at any of the six individual locations for lysine. No statistically significant differences were observed on a per location basis at five out of the six individual locations for cystine. No statistically significant differences were observed on a per location basis at four out of the six individual locations for methionine, tryptophan, threonine, histidine, aspartic acid, proline, serine and tyrosine. No statistically significant differences were observed on a per location basis for three out of the six individual locations for isoleucine, phenylalanine, glycine, alanine and glutamic acid. No statistically significant differences were observed on a per location basis for two out of the six individual locations for valine and leucine. No statistically significant differences were observed on a per location basis at one out of the six individual locations for arginine. In addition, all mean values for amino acids in grain from 59122 x NK603 maize treated with glyphosate followed by glufosinate and in grain from non-GM control maize were within reported literature ranges (Table 15 - appendix).

<u>*Minerals:*</u>
Statistically significant differences between 59122 x NK603 maize treated with ***glyphosate*** and non-GM control maize were observed for magnesium, manganese, phosphorus and potassium in the analysis across locations. No statistically significant differences were observed across locations for calcium, copper, iron, sodium or zinc (Table 16 - appendix). However, when analyzed on a per location basis, these differences were not consistently observed. No statistically significant differences were observed between 59122 x NK603 maize and non-GM control maize for magnesium at five of the six individual locations. No statistically significant differences were observed between 59122 x NK603 maize and non-GM control maize for manganese and potassium at four of the six individual locations. With regard to phosphorus, the mean values obtained from the analysis on a per location basis were not significantly different from non-GM control maize at three of the six locations.

No statistically significant differences were observed across locations between 59122 x NK603 maize treated with ***glufosinate*** and non-GM control maize with regard to calcium, copper, iron, sodium or zinc. Statistically significant differences were observed for magnesium, manganese, phosphorus and potassium in the analysis across locations (Table 17 - appendix). However, when analyzed on a per location basis, these differences were not consistently observed. No statistically significant differences were observed at three of the six individual locations for magnesium, manganese and potassium. No statistically significant differences were observed at two of the six individual locations for phosphorus.

No statistically significant differences were observed across locations between 59122 x NK603 maize treated with ***glyphosate followed by glufosinate*** and non-GM control maize with regard to calcium, copper, sodium or zinc. Statistically significant differences were observed for iron, magnesium, manganese, phosphorus and potassium in the analysis across locations (Table 18 - appendix). However, when analyzed on a per location basis, these differences were not consistently observed. No statistically significant differences were observed at any of the six individual locations for iron. No statistically significant differences were observed at four out of the six individual locations for magnesium, manganese and potassium. No statistically significant differences were observed at one out of the six individual locations for phosphorus. In addition, all mean values for minerals in grain from 59122 x NK603 maize treated with glyphosate followed by glufosinate and in grain from non-GM control maize were within the reported literature ranges (Table 19 - appendix).

Vitamins:
No statistically significant differences were observed across locations between 59122 x NK603 maize treated with ***glyphosate*** and non-GM control maize with regard to vitamin B1, folic acid or vitamin E. Levels of vitamin B2 in both the 59122 x NK603 maize grain and non- GM control maize grain were below the lower limit of quantitation (LLOQ) of the assay used in this analysis (Table 20 - appendix). Statistically significant differences were observed for beta-carotene. However for beta-carotene, the values obtained from the analysis on a per location basis were not statistically different between 59122 x NK603 maize and the non-GM control maize at two out of the six individual locations.

No statistically significant differences were observed across locations between 59122 x NK603 maize treated with ***glufosinate*** and non-GM control maize with regard to vitamin B1, folic acid and vitamin E. Levels of vitamin B2 for both the 59122 x NK603 maize grain and non- GM control maize grain were below the LLOQ for the assay used in this analysis. Statistically significant differences were observed between 59122 x NK603 maize and non-GM control maize for beta-carotene in the analysis across locations (Table 21 - appendix). However, when analyzed on a per location basis, these differences were not consistently observed. No statistically significant differences were observed at two out of the six individual locations for beta-carotene values.

No statistically significant differences were observed across locations between 59122 x NK603 maize treated with ***glyphosate followed by glufosinate*** and non-GM control maize with regard to folic acid and vitamin E. Levels of vitamin B2 for both the 59122 x NK603 maize grain and non-GM control maize grain were below the LLOQ for the assay used in this analysis. Statistically significant differences were observed between 59122 x NK603 maize and non-GM control maize for beta-carotene and vitamin B1 in the analysis across locations (Table 22 - appendix). However, when analyzed on a per location basis, these differences were not consistently observed. No statistically significant differences were observed at five out of the six individual locations for vitamin B1 and no statistically significant differences were observed at four out of the six individual locations for beta-carotene. In addition, all mean values for vitamins in 59122 x NK603 maize and non-GM control maize were within reported literature ranges with the exception of vitamin B1 values that for both the 59122 x NK603 maize grain and the non-GM control maize grain were above the reported literature range (Table 23 - appendix).

Secondary metabolites and anti-nutrients:
No statistically significant differences were observed across locations between 59122 x NK603 maize treated with ***glyphosate*** and non-GM control maize with regard to ferulic acid and trypsin inhibitor. Levels of furfural in both 59122 x NK603 maize treated with *glyphosate* and non-GM control maize were below the LLOQ of the assay used in this analysis (Table 24 and 28 - appendix). Statistically significant differences were observed for inositol, p-coumaric acid, raffinose and phytic acid (Table 24 and 28 - appendix). However, when analyzed on a per location basis, no statistically significant differences were observed at any of the six individual locations for p-coumaric acid. No statistically significant differences were observed at four out of the six individual locations for inositol and raffinose. No statistically significant differences were observed at three out of the six individual locations for phytic acid.

No statistically significant differences were observed across locations between 59122 x NK603 maize treated with ***glufosinate*** and non-GM control maize with regard to inositol, p-coumaric acid or ferulic acid. Levels of furfural in both 59122 x NK603 maize grain treated with *glufosinate* and non- GM control maize grain were below the LLOQ of the assay used in this analysis (Table 25 - appendix). Statistically significant differences between 59122 x NK603 maize and non-GM control maize were observed for raffinose, phytic acid and trypsin inhibitor in the analysis across locations (Table 29 - appendix). However, when analyzed on a per location basis, these differences were not consistently observed. No statistically significant differences were observed at five out of the six individual locations for raffinose and trypsin inhibitor and no statistically significant differences were observed at three out of the six individual locations for phytic acid.

No statistically significant differences were observed across locations between 59122 x NK603 maize treated with ***glyphosate followed by glufosinate*** and non-GM control maize with regard to inositol, p-coumaric acid, ferulic acid or trypsin inhibitor. Levels of furfural in both 59122 x NK603 maize grain and non-GM control maize grain were below the LLOQ of the assay used in this analysis (Table 26 and 30 - appendix). Statistically significant differences between 59122 x NK603 maize treated with *glyphosate followed by glufosinate* and non- GM control maize were observed for raffinose and phytic acid in the analysis across locations. However, when analyzed on a per location basis, these differences were not consistently observed. No statistically significant differences were observed at any of the six individual locations for raffinose. No statistically significant differences were observed at five out of the six individual locations for phytic acid. In addition, all mean values for secondary metabolites and anti-nutrients in grain from 59122 x NK603 maize treated with glyphosate followed by glufosinate and in grain from non-GM control maize were within reported literature ranges (Table 27 and 31 -appendix).

3.3 Agronomic and phenotypic characters

During field trials over at six different locations in North America in the growth season 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. Both in the field trials in USA and Canada, the early population/germination, seeding vigour, time to silking, time to 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, were measured. Yield/grain yield was not measured in these trials.

Analyses of variance across trial locations showed statistically significant differences between maize 59122 x NK603 (treated with glyphosate and glufosinate ammonium) and the corresponding conventional counterpart for mean time to silking, time to pollen shed and plant height ($p<0.05$) (Table 32 - appendix). On average, maize 59122 x NK603 plants were taller than the conventional counterpart (95 vs. 91 cm) and had higher number of accumulated heat units before 50 % of the plants were silking and shedding pollen (1242 vs. 1226 GDU, and 1277 vs. 1260 GDU) compared with the conventional counterpart. However, significant differences for these parameters were only observed at one of the test locations. No statistically significant differences between the transgenic maize 59122 x NK603 and the comparator were detected for any of the other assessed phenotypic characteristics in the across location analysis ($p>0.05$).

3.4 Conclusion

Comparative analyses of data from field trials located at representative sites and environments in the USA and Europe indicate that maize stack 59122 x NK603 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, with the exception of the herbicide tolerance, conferred by the expression of Cry34Ab1/Cry35Ab1, PAT and CP4 EPSPS proteins. Based on the assessment of available data, the VKM GMO Panel is of the opinion that conventional crossing of maize 59122 and NK603 to produce the hybrid 59122 x NK603 does not result in interactions that cause compositional, agronomic and phenotypic changes that would raise safety concerns.

4 Food /feed risk assessment

4.1 Product description and intended uses

According to the applicant, all 59122xNK603 maize products will be produced and processed for use in food, animal feed and industrial products in the same way as other commercial maize. The 59122xNK603 maize and all food, feed and processed products derived from 59122xNK603 maize are expected to replace a portion of similar products from commercial maize, with total consumption of maize products remaining unchanged. Therefore, the applicant concludes that, 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 of both DNA and proteins are denatured, which also applies to Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins (Hammond et al. 2011).

4.3 Toxicological assessment

4.3.1 Toxicological assessment of the newly expressed protein

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. The study was performed in accordance with the principles of Good Laboratory of O.E.C.D. (Organization 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 PAT (phosphoacetyl transferase) protein (> 95% purity), a protein encoded by the *pat* 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 with 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 (Hèrouet et al. 2005).

PAT Microbial Protein (FL), which was 84% pure microbial protein, was evaluated for acute oral toxicity. 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 observation. All mice except one female gained weight over the duration of the study. There were no gross pathological lesions for any animal on study. Under the condition of this study, the acute oral LD_{50} of PAT Microbial protein (FL) in male and female CD-1 mice was greater than 6000 mg/kg (Brooks and DeWildt, 2000).

*Acute oral exposure of **Cry34Ab1** and **Cry34Ab2** proteins in rodents*

The potential toxicity of the Cry34Ab1 and Cry35Ab1 proteins to humans and animals was examined in acute oral toxicology studies. The equivalent microbially-derived Cry34Ab1 and Cry35Ab1 proteins were evaluated either separately or as a Cry34Ab1/Cry35Ab1 protein mixture for acute toxicity potential in mice (Brooks and DeWildt 2000a; Brooks and DeWildt 2000b; Brooks and DeWildt 2000c).

The Cry34Ab1 protein was evaluated for acute oral toxicity and the highest dose tested was 5000 mg of test material per kg body weight. When adjusted for purity of the test material (54% pure; Brooks and DeWildt 2000a), the dose was 2700 mg Cry34Ab1 protein per kg body weight. During the two-week observation period, mortality and/or clinical or behavioural signs of pathology as well as body weights were recorded. Gross necropsies were conducted at the end of the study. No mortality occurred during the course of the study. Additionally, no adverse clinical signs were observed during the study and no adverse findings were noted at necropsy. The relatively high dose tested in this study did not give rise to any toxicity and therefore the acute LD_{50} for Cry34Ab1 protein could not be determined and is estimated to be higher than 2700 mg Cry34Ab1 per kg body weight.

The Cry35Ab1 protein was evaluated for acute oral toxicity and the highest dose tested was 5000 mg of test material per kg body weight. When adjusted for purity of the test material (37% pure; Brooks and DeWildt 2000b), the dose was 1850 mg Cry35Ab1 protein per kg body weight. During the two-week observation period, mortality and/or clinical or behavioural signs of pathology as well as body weights were recorded. Gross necropsies were conducted at the end of the study. No mortality occurred during the course of the study. Additionally, no adverse clinical signs were observed during the study and no adverse findings were noted at necropsy. The relatively high dose tested in this study did not give rise to any toxicity and therefore the acute LD_{50} for Cry35Ab1 protein could not be determined and is estimated to be higher than 1850 mg Cry35Ab1 per kg body weight.

Finally, a mixture of Cry34Ab1 and Cry35Ab1 proteins was evaluated for acute oral toxicity in mice and the highest dose tested was 5000 mg of test material per kg body weight. When adjusted for purity of the test material (54% pure for Cry34Ab1 protein and 37% pure for the Cry35Ab1 protein (Brooks and DeWildt, 2000c), the mixture contained 482 mg Cry34Ab1 protein per kg body weight and 1520 mg Cry35Ab1 protein per kg body weight. During the two-week observation period, mortality and/or clinical or behavioural signs of pathology as well as body weights were recorded. Gross necropsies were conducted at the end of the study. No mortality occurred during the course of the study. Additionally, no adverse clinical signs were observed during the study that was treatment related and no adverse findings were noted at necropsy. Therefore, the acute oral LD_{50} for a mixture of Cry34Ab1 and Cry35Ab1 proteins could not be determined and is estimated to be higher than 2000 mg/kg body weight of an equimolar mixture of the pure Cry34Ab1 and Cry35Ab1 proteins.

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

Monsanto has conducted an acute toxicity study (MSL-13077, 1993) conducted 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 is >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 randomized 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 the mice at dosages of 49, 154 and 572 mg/kg body weight respectively. These doses correspond to 40, 100 and 400 mg/kg of CP4 EPSPS protein based on the level of purity of the protein and ELISA analyses of the dosing solutions. A control group received bovine serum albumin (BSA) at a dosage of 363 mg/kg in the same solution and delivery volume as the test substance. The second control group was administered the carrier solution only, 50 mM sodium bicarbonate.

At defined stages throughout the duration of the study, clinical observations were performed for mortality and signs of toxicity, and body weights and food consumption measured. Signs of toxicity include such occurrences as changes in the skin and fur, eyes and mucous membranes, respiratory, autonomic and central nervous systems as well as behavioral 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 protein, when compared with the carrier control group. The data were evaluated according to a decision-tree analysis procedure which, depending on the results of early statistical tests, determined further statistical analysis applied to detect group differences and analyse for trends. All animals survived to the scheduled termination of the study, and there were no clinical signs observed that could be related to the test material.

EHL decision-tree analysis (two-tailed): Terminal body weights were evaluated by decision-tree statistical analyses which, depending on the results of tests for normality (2) and homogeneity of variances [Bartlett’s,Test (3)], utilized 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 analyze for trend.

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 Good Laboratory of O.E.C.D. (Organization 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 in certain circumstances; justification for use of a 14-day exposure period should be provided. The duration of this repeated dose oral toxicity was 14-day exposure period. No justification for using 14-days has been given in the dossier of the applicant.

Animals of group 1 received a standard diet and rats of groups 2, 3 and 4 were fed a low protein diet, which was adjusted to similar protein content as that of group 1 by using soybean derived protein. Protein was administered by feed admixture in powdered diet to Wistar rats of 0 (group 1), 0.5 % PAT-protein + 4.5 % soyprotein (group 2), 5 % PAT-protein (group 3) and 5% soyprotein (group 4) for a period of 14 days. The study comprised four groups each with five male and five female rats. The mean intake of PAT-protein over the treatment period was: 0.712 mg/kg body weight/day for males in group 2; 703 mg/kg body weight/day for females in group 2; 7965 mg/kg body weight/day for males in group 3 and 7619 mg/kg body weight/day for females in group 3.

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 hematology 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 male rats of groups 2, 3 and 4 and slightly higher triglyceride level 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 slightly similar to that of groups 2 and 3. The above changes are according to the applicant considered to reflect differences in the dietary composition and to be unrelated to PAT Protein itself. Further, the increased total cholesterol and phospholipid levels are found to be in a similar range when comparing group 3 (low protein diet + 5 % PAT-protein) with group 4 (low protein diet + 5 % soya protein). The results may suggest a similar nutritional value of both proteins.

*Repeated dose 28-day oral toxicity study of **Cry34Ab1** and **Cry35Ab1** protein in rodents*

Five male and five female CD-1 mice per group were given test diets formulated to supply 0/0, 1.97/0.078, 19.7/0.78, or 197/7.8 milligrams Cry34/35Ab1 proteins respectively, per kilogram body weight per day (mg/kg/day, mkd). These values corresponded to nominal time-weighted average concentrations of 0/0, 1.84/0.073, 18.4/0.73, and 195/7.7 mg/kg/day for males and 0/0, 2.13/0.085, 19.8/0.79, and 202/8 mg/kg/day for females, of Cry34/35Ab1 proteins, respectively. Actual concentrations of Cry34/35Ab1 proteins were higher in all dose groups based on analytical results, with the exception of the lower concentration of Cry35Ab1 in the low-dose group. Additional groups of five male and five female mice were fed diets containing bovine serum albumin (BSA) at the rate of 204.8 mg/kg body weight /day to serve as a protein control group. The nominal time-weighted average concentrations of BSA were 189.3 and 202.1 mg/kg/day for males and females, respectively. The Cry34/35Ab1 protein treatment groups were statistically compared to BSA-control group. Parameters evaluated were daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, body weights, feed consumption, hematology, clinical chemistry, selected organ weights, and gross and histopathologic examinations. There were no treatment-related effects on any parameter (Juberg et al 2009).

The study evaluated the potential toxicity of the combination of microbially derived Cry34Ab1 and Cry35Ab1 insecticidal crystal proteins, referred to as Cry34/35Ab1, in mice following dietary administration for 28 days. Five male and five female CD-1 mice per group were given test diets formulated to supply 0/0, 1.97/0.078, 19.7/0.78, or 197/7.8 milligrams Cry34/35Ab1 proteins respectively, per kilogram body weight per day (mg/kg/day, mkd). These values corresponded to nominal time-weighted average concentrations of 0/0, 1.84/0.073, 18.4/0.73, and 195/7.7 mg/kg/day for males and 0/0, 2.13/0.085, 19.8/0.79, and 202/8 mg/kg/day for females, of Cry34/35Ab1 proteins, respectively. Actual concentrations of Cry34/35Ab1 proteins were higher in all dose groups based on analytical results, with the exception of the lower concentration of Cry35Ab1 in the low-dose group. Additional groups of five male and five female mice were fed diets containing of 204.8 mg/kg body weight /day bovine serum albumin (BSA) serving as a protein control group. The nominal time-weighted average concentrations of BSA were 189.3 and 202.1 mg/kg/day for males and females, respectively. The Cry34/35Ab1 protein treatment groups were statistically compared to BSA-control group. Parameters evaluated were daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, body weights, feed consumption, hematology, clinical chemistry, selected organ weights, and gross and histopathological examinations. There were no treatment-related effects on any parameter (Thomas et al. 2006, Dow AgroSciences unpublished internal report.).

4.3.2 Toxicological assessment of the whole GM food/feed

The applicant has provided a nutritional study on broilers using the triple stacked event 59122x1507xNK603 maize as test material.

A poultry feeding study was conducted with diets containing grain from 59122x1507xNK603 maize. According to the applicant, the poultry feeding study with the 59122x1507xNK603 maize is relevant for the safety assessment of the 59122xNK603 maize. The 59122x1507xNK603 maize has been obtained from traditional breeding methods between progeny of three genetically modified maizes. Since the 59122x1507xNK603 maize contains the 59122 maize insert and the NK603 maize insert, the applicant concludes that absence of adverse effects of the 59122x1507xNK603 maize grain in a 42-day poultry study provides the necessary scientific information to also infer the absence of adverse effects on broiler chickens from feeding with 59122xNK603 maize.

The poultry feeding study with diets containing grain from 59122x1507xNK603 maize was conducted over a 42-day period. The 59122x1507xNK603 maize grains used in this study were produced from plants that received either two sequential treatments with glufosinate herbicide, two sequential treatments with glyphosate herbicide or treatments of glyphosate followed by glufosinate herbicide. For comparison, diets containing grain from non-GM maize with comparable genetic background and from three types of commercial maize (33P66, 33J56 and 33R77) were also fed to the chickens. The chickens fed the 59122 x1507xNK603 maize grain, non-GM control maize grain or commercial maize grain were observed for overall health, behavioral 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, weight gain, feed efficiency, organ yields, carcass and parts (breast, thigh, wing and leg) yields and abdominal fat were analyzed for statistical differences between the chickens fed the

59122x1507xNK603 maize diet and chickens fed the non-GM control maize diet at $p \leq 0.05$. In addition, tolerance intervals were calculated for performance and carcass traits that are expected to contain, with 95% confidence, 99% of the values obtained for the chickens fed the three commercial non-GM maize diets. Data from the chickens fed the 59122x1507xNK603 maize diet and chickens fed the diet with non-GM control maize with comparable genetic background were then evaluated to determine if observed values were contained within each tolerance interval.

No statistically significant differences were observed on mortality, body weight, body weight gain or feed efficiency between chickens fed a diet containing grain from 59122x1507xNK603 maize or chickens fed a non-GM control maize diet. No statistically significant differences were observed for overall organ yields between chickens fed a diet containing grain from 59122x1507xNK603 maize or chickens fed a non-GM control maize diet. Kidney yields, within females, were slightly lower for the 59122x1507xNK603 maize fed chickens compared to chickens fed the non-GM control maize diet. However, the observed values for female kidney yield for both the 59122 x 1507 x NK603 maize fed and the non-GM control fed chickens are still well within the tolerance range calculated from the kidney yields from chickens fed the three commercial non-GM maize diets.

No statistically significant differences were observed for carcass yield, breast, thigh, wing and leg yield or overall abdominal fat between chickens fed a diet containing grain from 59122x1507xNK603 maize or chickens fed a non-GM control maize diet. Abdominal fat, within males, was slightly lower for the 59122 x 1507 x NK603 maize fed chickens compared to chickens fed the non-GM control maize diet. However, the observed values for male abdominal fat for both the 59122 x 1507 x NK603 maize fed and the non-GM control fed chickens are still well within the tolerance range calculated from the abdominal fat from chickens fed the three commercial non-GM maize diets.

Based on the results from this study, the applicant concludes that 59122xNK603 maize is nutritionally equivalent to non-GM maize with comparable genetic background and to commercial maize. In addition, the results obtained further indicate the safety of the Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins expressed in 59122xNK603 maize.

Further, no subchronic adverse effects were observed in a 90-day study where rats were fed with diets prepared with the 59122 maize (Malley et al 2007). A published 90-day study in rats conducted with diets prepared with NK603 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 (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 2012), 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

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 food allergies are mediated by IgE and are characteristic of type-I reactions. According to Regulation (EC) No. 1829/2003 the applicant shall assess post-translational modifications of expressed proteins, and assess gluten-sensitive enteropathy or other enteropathies which are not IgE-mediated.

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 protein

The applicant has performed a weight-of-evidence approach (Metcalfe et al.,1996; FAO/WHO 2001; Codex 2003) for an overall assessment of the IgE allergenic potential of the Cry34Ab1, Cry35Ab1, 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 NK603 (EFSA-GMO-RX-NK603) and 59122 (EFSA-GMO-NL-2005-12, EFSA-GMO-NL-2005-23), and were based on the following aspects:

i) The sources of the transgenes genes: *B. thuringiensis* (*cry-genes*), *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 and animals
iii) The Cry34Ab1 and Cry35Ab1 proteins do not show significant amino acid sequence similarity to known protein toxins, and don't share immunologically relevant sequence similarity with known allergens
iv) The Cry34Ab1 and Cry35Ab1 proteins are rapidly degraded, as shown by SDS-PAGE, under simulated gastric fluid digestive conditions
v) The Cry34Ab1 and Cry35Ab1 proteins have been considered as heat labile, since biological activity of Cry1F was lost after exposure at 75oC for 30 minutes, while the Cry34Ab1 and Cry35Ab1 proteins lost theirs after exposure at 60 oC for 30 minutes

vi) The proteins Cry34Ab1, Cry35Ab1 are not glycosylated
vii) The PAT protein has been the subject of previous safety assessments for genetically modified plants and found to have no potential for allergenicity
viii) The PAT protein lacks homology to known toxins or 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 maize 59122 x NK603 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 59122 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 59122 x NK603 or the parental events 59122 and NK603 with the exception of the introduced traits, no increased allergenicity is anticipated for maize 59122 x NK603. Moreover, maize is not considered a common allergenic food.

4.4.3 Adjuvanticity

According to the EFSA guidance document for risk assessment of food and feed from GM plants (EFSA 2011b), adjuvants are substances that, when co-administered with an antigen increase the immune response to the antigen and therefore might increase the allergic response. In cases when known functional aspects of the newly expressed protein or structural similarity to known strong adjuvants may indicate possible adjuvant activity, the possible role of these proteins as adjuvants should be considered. As for allergens, interactions with other constituents of the food matrix and/or processing may alter the structure and bioavailability of an adjuvant and thus modify its biological activity.

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 2012b)

4.5 Nutritional assessment of GM food/feed

Compositional analyses of maize 59122 x NK603 indicate nutritional equivalence to the non-GM control maize with comparable genetic background and to the published range of values in the literature. The applicant has provided a nutritional study on broilers using the triple stacked event 59122x1507xNK603 maize as test material.

4.5.1 Intake information/exposure assessment

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

The expression levels of the Cry34Ab1, Cry35Ab1 and CP4 EPSPS proteins in grain from 59122 x NK603 maize ranged from 21.8 to 53.6 µg/g grain dry weight, from 0.53 to 3.31 µg/g grain dry weight, and from 3.06 to 16.4 µg/g grain dry weight, respectively. Expression of the PAT protein in 59122 x NK603 grain ranged from below the lower limit of quantitation of the assay to 0.44 µg/g grain dry weight.

Since all foods from maize are derived from grains, the estimated maximum daily intake for a Norwegian adult of Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins is calculated to be 236 µg, 15 µg, 2 µg and 28 µg, respectively, based on grain dry weight. 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). Some farm animals such as pigs and poultry which are fed diets formulated with up to 80% maize, are exposed to Cry1F, Cry34Ab1 and Cry35Ab1 levels that are close to 100 times above the TTC level of 1,8 mg/animal/day.

This dietary exposure assessment is very conservative. It assumes that all maize consumed consists of 59122 x NK603 maize and that protein levels are not reduced by processing. The comparable composition and nutritional value of the 59122 x NK603 maize, together with the results of the assessment of dietary intake and nutritional impact, indicate that food products derived from 59122 x NK603 maize are nutritionally equivalent to food products derived from commercial maize. Hence, anticipated dietary intake, of maize derived foods and feeds is not expected to change.

4.5.2 Nutritional assessment of feed derived from the GM plant

Based on the compositional analyses comprising proximates, minerals, fatty acids, amino acids, vitamins, secondary metabolites and anti-nutrients of forage and grain samples from 59122 x NK603 maize; nutritional equivalence shown in a poultry feeding study; and, safety evaluation of the Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins expressed in 59122 x NK603 maize, the 59122xNK603 maize and derived feed products seem to be substantially equivalent to, nutritionally equivalent, and as safe as commercial maize and derived feed products.

4.5.3 Post-Market Monitoring of GM food/feed

Based on the safety assessment discussed throughout section 4.3, no risks to human and animal health have been identified from the human food and animal feed use of 59122xNK603 maize as compared to human food and animal feed use of commercial maize. In addition, the nutritional characteristics and use of food, feed and processed products derived from 59122xNK603 maize seem to be similar to food, feed and processed products derived from commercial maize.

4.6 Conclusion

Whole food feeding study has not been performed using 59122 x NK603 maize. The applicant has, however, provided a nutritional study on broilers using the triple stacked event 59122 x 1507 x NK603 maize as test material. 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 toxicity tests in rodents have not indicated toxic effects of the newly expressed proteins. However, these tests do not provide any additional information about possible adverse effects of the stacked event maize 59122 x NK603.

Based on the current knowledge, the VKM GMO Panel concludes that 59122 x NK603 maize is nutritionally equivalent to its conventional maize, and that it is unlikely that newly expressed proteins will introduce a toxic or allergenic potential of food/feed derived from maize 59122 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 59122 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 59122 x NK603 under infestation conditions. It is considered very unlikely that maize 59122 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 59122 x NK603 relative to its conventional counterpart. A series of field trials with maize 59122 x NK603 were carried out across 6 locations in the USA and Canada in 2003 (application EFSA/GMO/UK/2005/20). In addition, agronomic observations performed in field trials in the EU in 2004 (Spain, Hungary and Bulgaria) have been provided by the applicant in application EFSA/GMO/UK/2005/30 (maize stack 59122 x 1507 x NK603). Information on phenotypic (e.g. crop physiology, morphology, development) and agronomic (e.g. grain yield) characteristics was provided to assess the agronomic performance of maize 59122 x NK603 in comparison with its conventional counterpart (see section 3.1). Data from the field trials in the USA and Canada shows some statistical significant differences for plant height, time to silking and pollen shed). 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 59122 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 59122 x NK603, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of maize 59122 x NK603 are unchanged, insect resistance, glufosinate and glyphosate tolerance are not likely to provide a selective advantage outside of cultivation in Europe. The VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects based on establishment and survival of maize 59122 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 59122 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 59122 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 59122 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 59122 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 59122 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 59122 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 59122 was transformed to co-express the *cry34Ab1* and *cry35Ab1* genes from *Bacillus thuringiensis.* The binary insecticidal toxin is made of two components, the Cry34Ab1 and Cry35Ab proteins, acting together and conferring resistance to coleopteran insect pests belonging to the genus *Diabrotica,* such as larvae of western corn rootworm (WCR; *D. virgifera virgifera*) and the northern corn rootworm (NCR; *D. barberi*). WCR has been introduced to Europe from North America, where it is native and widespread (Miller et al. 2005, ref. EFSA 2013). *D. virgifera virgifera* was first detected in Serbia in 1992, but has since spread across the continent, resulting in well-established populations in approximately 19 European countries (EC 2012). There have been no reports of *D. virgifera virgifera* in Norway (http://www.faunaeur.org/distribution.php)

Considering the intended uses of maize 59122 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 Cry34Ab and Cry35Ab1 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 59122 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 Cry34Ab1 and Cry35Ab1 proteins enters the environment due to expression in the grains (mean value of 45.7 and 1.61µg/g d.w., respectively). In addition, the data show that at least 99% of microbially produced Cry34Ab1 and Cry35Ab1 proteins were rapidly degraded in simulated gastric fluid.

In conclusion, the VKM GMO Panel considers that the exposure of potentially non-target organisms to the binary Cry34Ab1 and Cry35Ab1 proteins 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 59122 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 59122 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

The scope of the application EFSA/GMO/NL/2005/20 includes import and processing of maize 59122 x NK603 for food and feed uses. Considering the intended uses of maize 59122 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 59122 x NK603.

Maize 59122 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 59122 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 **r**esistance to one or two broad spectrum herbicides could entail higher levels of herbicide residue cocktails compared to plants produced by conventional farming practice.

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 stacked plants as a result of the application of plant-protection products fall outside the remit of the Norwegian VKM Panels.

7 Conclusions

Molecular characterisation

Southern and PCR analyses has been performed and indicate that the recombinant inserts in the single maize events 59122 and NK603 are retained in maize stack 59122xNK603. Genetic stability of the inserts has previously been demonstrated in the parental lines 59122 and NK603. The level of Cry34Ab1/Cry35Ab1, PAT and CP4 EPSPS proteins in seed and forage from the stacked event were measured using ELISA and are comparable to the levels in the single events. Phenotypic analyses also indicate stability of the insect resistance and herbicide tolerance traits of the stacked event.

Comparative assessment

Comparative analyses of data from field trials located at representative sites and environments in North America indicate that maize stack 59122 x NK603 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, with the exception of the introduced insect resistance and herbicide tolerance, conferred by the expression of the Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins. Based on the assessment of available data, the VKM GMO Panel is of the opinion that conventional crossing of maize 59122 and NK603 to produce the hybrid 59122 x NK603 does not result in interactions that cause compositional, agronomic and phenotypic changes that would raise safety concerns.

Food and feed risk assessment

Whole food feeding study has not been performed using 59122 x NK603 maize. The applicant has, however, provided a nutritional study on broilers using the triple stacked event 59122 x 1507 x NK603 maize as test material. 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 toxicity tests in rodents have not indicated toxic effects of the newly expressed proteins. However, these tests do not provide any additional information about possible adverse effects of the stacked event maize 59122 x NK603.

Based on the current knowledge, the VKM GMO Panel concludes that 59122 x NK603 maize is nutritionally equivalent to its conventional maize, and that it is unlikely that newly expressed proteins will introduce a toxic or allergenic potential of food/feed derived from maize 59122 x NK603 compared to conventional maize.

Environmental risk assessment

The scope of the application EFSA/GMO/UK/2005/21 includes import and processing of maize 59122 x NK603 for food and feed uses. Considering the intended uses of maize 59122 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 59122 x NK603.

Maize 59122 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 59122 x NK603. Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation. The VKM GMO Panel considers the risk of gene flow from occasional feral GM maize plants to conventional maize varieties to be negligible in Norway. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered by the GMO Panel to be an issue.

Overall conclusion

The VKM GMO Panel has not identified toxic or altered nutritional properties of maize 59122 x NK603 or its processed products compared to conventional maize. Based on current knowledge, it is also unlikely that the Cry34Ab1 and Cry35Ab1 protein will increase the allergenic potential of food and feed derived from maize 59122 x NK603 compared to conventional maize varieties. The VKM GMO Panel likewise concludes that maize 59122 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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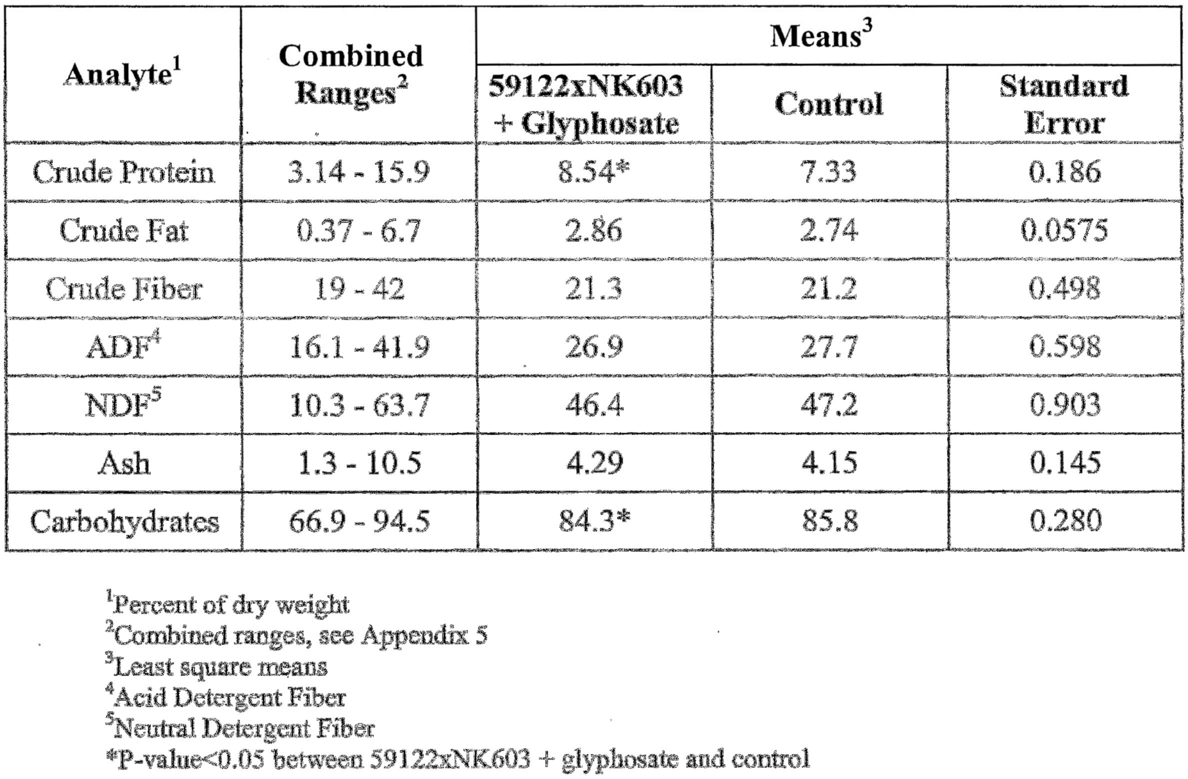
Appendix

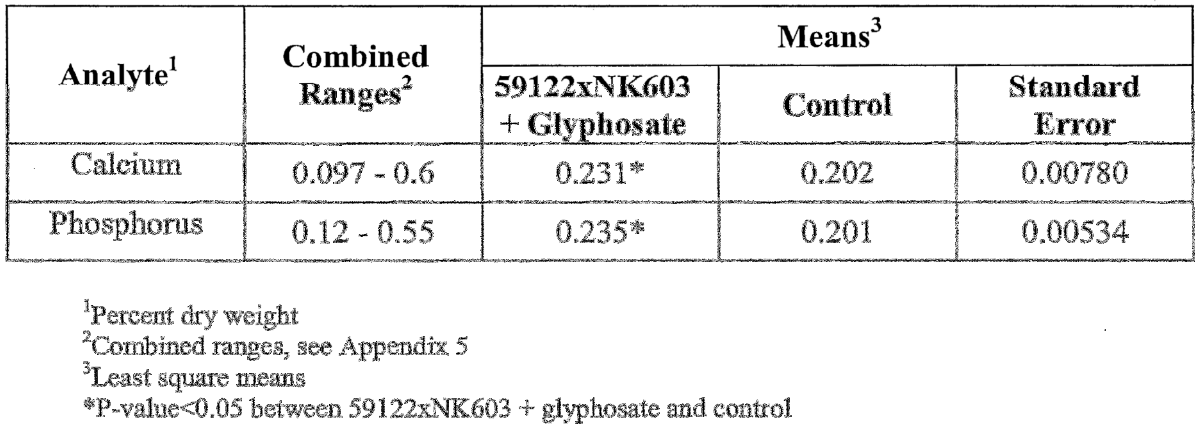
Table 1. Summary Analysis of Proximates and Fiber in Forage for the 59122 x NK603 + Glyphosate and Control Hybrids (North America 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glyphosate	Control	Standard Error
Crude Protein	3.14 - 15.9	8.54*	7.33	0.186
Crude Fat	0.37 - 6.7	2.86	2.74	0.0575
Crude Fiber	19 - 42	21.3	21.2	0.498
ADF[4]	16.1 - 41.9	26.9	27.7	0.598
NDF[5]	10.3 - 63.7	46.4	47.2	0.903
Ash	1.3 - 10.5	4.29	4.15	0.145
Carbohydrates	66.9 - 94.5	84.3*	85.8	0.280

[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 59122xNK603 + glyphosate and control

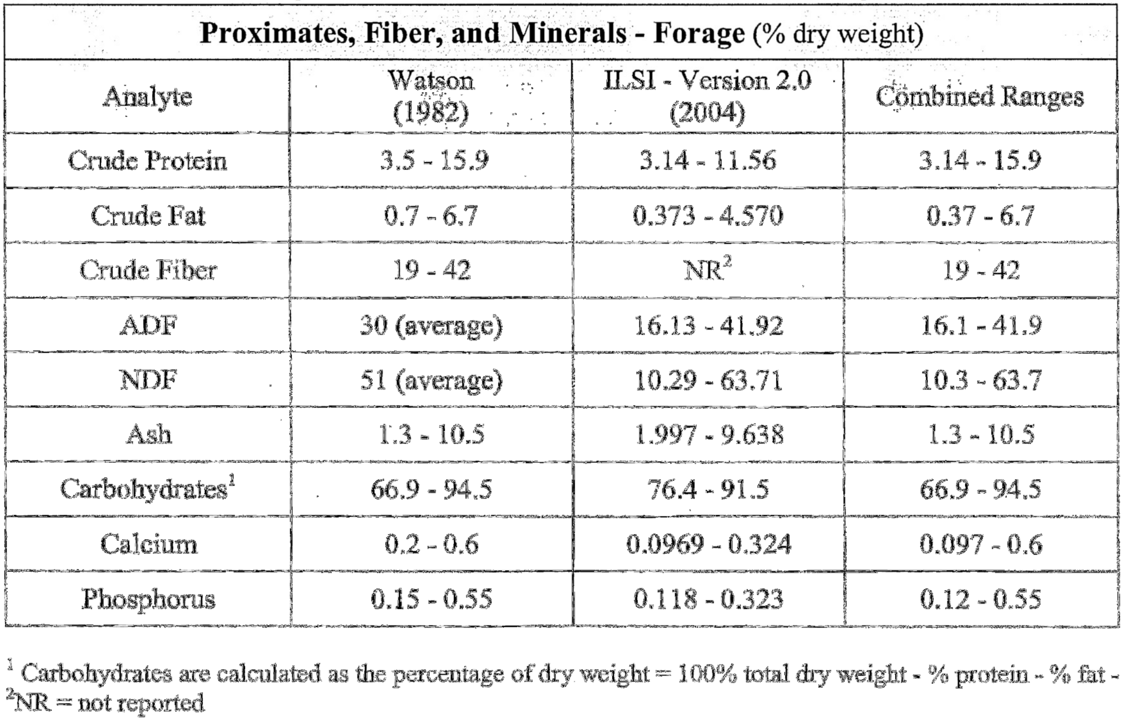
Table 2. Summary Analysis of Minerals in Forage for the 59122 x NK603 + Glyphosate and Control Hybrids (North America 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glyphosate	Control	Standard Error
Calcium	0.097 - 0.6	0.231*	0.202	0.00780
Phosphorus	0.12 - 0.55	0.235*	0.201	0.00534

[1] Percent dry weight
[2] Combined ranges, see Appendix 5
[3] Least square means
*P-value<0.05 between 59122xNK603 + glyphosate and control

Table 3. Literature Ranges of Proximates, Fiber and Mineral in Forage

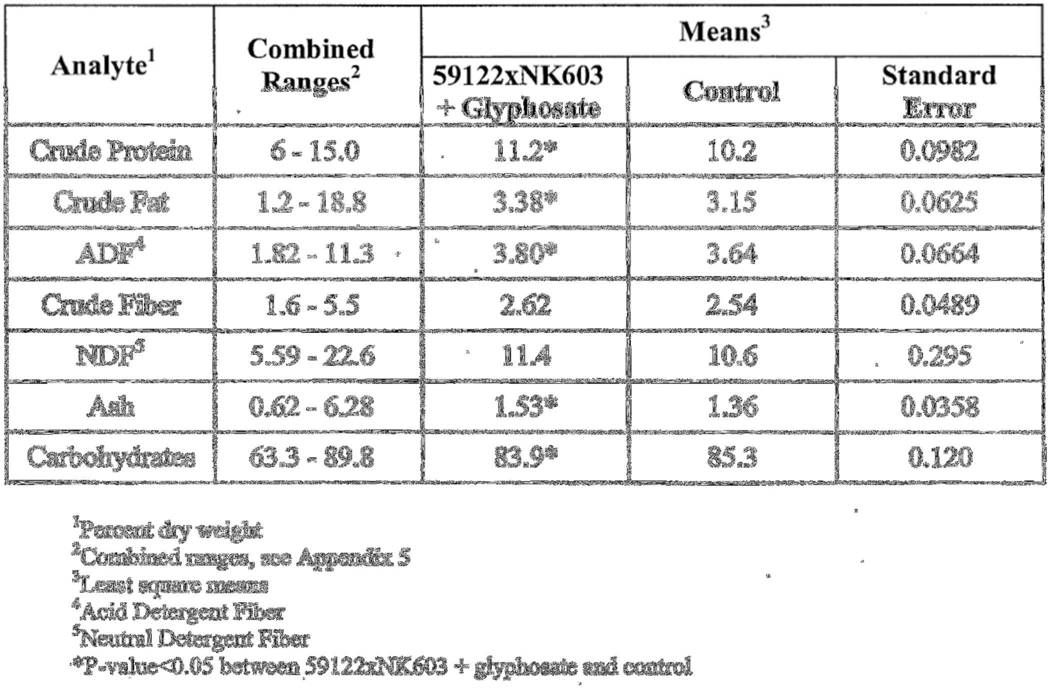


Proximates, Fiber, and Minerals - Forage (% dry weight)			
Analyte	Watson (1982)	ILSI - Version 2.0 (2004)	Combined Ranges
Crude Protein	3.5 - 15.9	3.14 - 11.56	3.14 - 15.9
Crude Fat	0.7 - 6.7	0.373 - 4.570	0.37 - 6.7
Crude Fiber	19 - 42	NR[2]	19 - 42
ADF	30 (average)	16.13 - 41.92	16.1 - 41.9
NDF	51 (average)	10.29 - 63.71	10.3 - 63.7
Ash	1.3 - 10.5	1.997 - 9.638	1.3 - 10.5
Carbohydrates[1]	66.9 - 94.5	76.4 - 91.5	66.9 - 94.5
Calcium	0.2 - 0.6	0.0969 - 0.324	0.097 - 0.6
Phosphorus	0.15 - 0.55	0.118 - 0.323	0.12 - 0.55

[1] Carbohydrates are calculated as the percentage of dry weight = 100% total dry weight - % protein - % fat - % ash.

[2] NR = not reported

Table 4. Summary Analysis of Proximates and Fiber in Grain for the 59122 x NK603 + Glyphosate and Control Hybrids (North America 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glyphosate	Control	Standard Error
Crude Protein	6 - 15.0	11.2*	10.2	0.0982
Crude Fat	1.2 - 18.8	3.38*	3.15	0.0625
ADF[4]	1.82 - 11.3	3.80*	3.64	0.0664
Crude Fiber	1.6 - 5.5	2.62	2.54	0.0489
NDF[5]	5.59 - 22.6	11.4	10.6	0.295
Ash	0.62 - 6.28	1.53*	1.36	0.0358
Carbohydrates	63.3 - 89.8	83.9*	85.3	0.120

[1] Percent dry weight

[2] Combined ranges, see Appendix 5

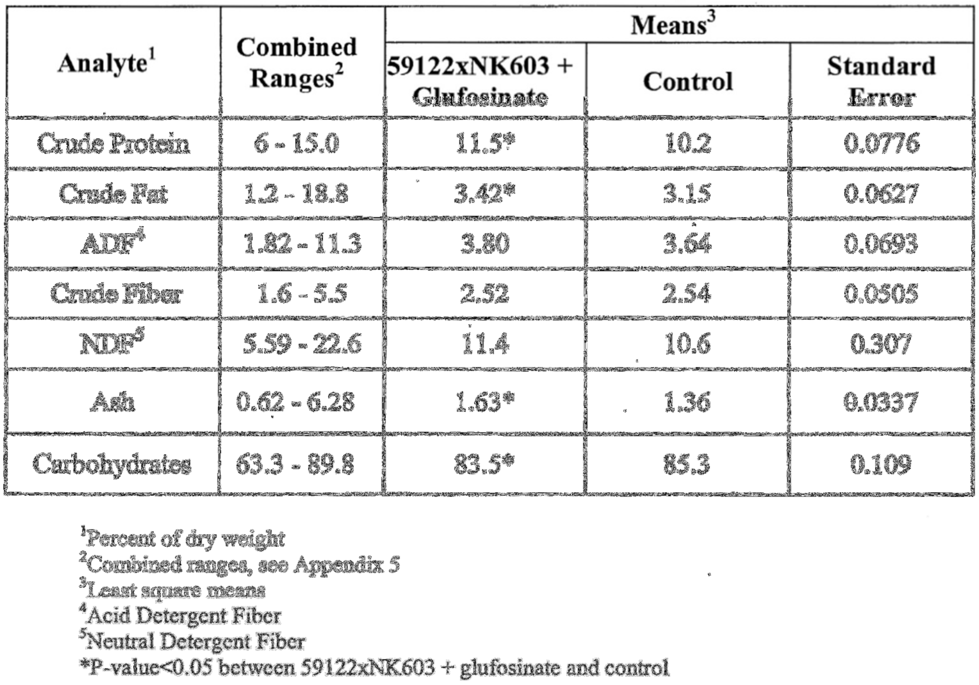
[3] Least square means

[4] Acid Detergent Fiber

[5] Neutral Detergent Fiber

*P-value<0.05 between 59122xNK603 + glyphosate and control

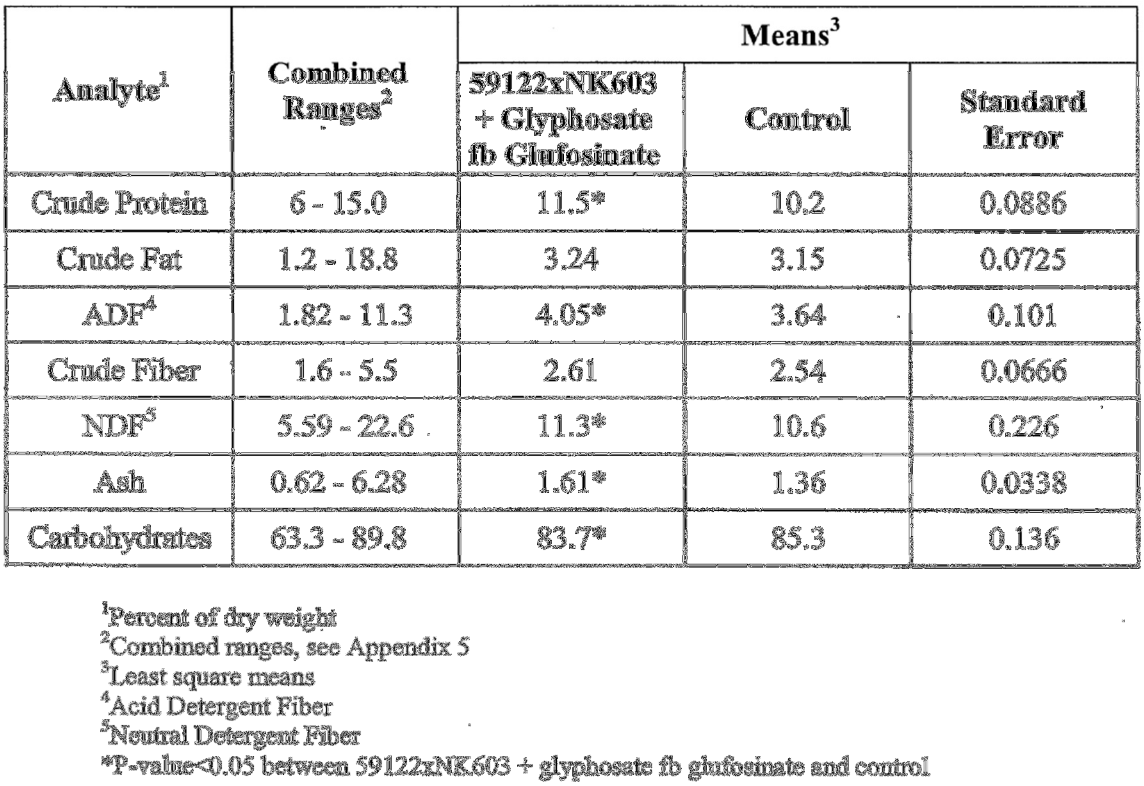
Table 5. Summary Analysis of Proximates and Fiber in Grain for the 59122 x NK603 + Glufosinate and Control Hybrids (North America, 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glufosinate	Control	Standard Error
Crude Protein	6 - 15.0	11.5*	10.2	0.0776
Crude Fat	1.2 - 18.8	3.42*	3.15	0.0627
ADF[4]	1.82 - 11.3	3.80	3.64	0.0693
Crude Fiber	1.6 - 5.5	2.52	2.54	0.0505
NDF[5]	5.59 - 22.6	11.4	10.6	0.307
Ash	0.62 - 6.28	1.63*	1.36	0.0337
Carbohydrates	63.3 - 89.8	83.5*	85.3	0.109

[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 59122xNK603 + glufosinate and control

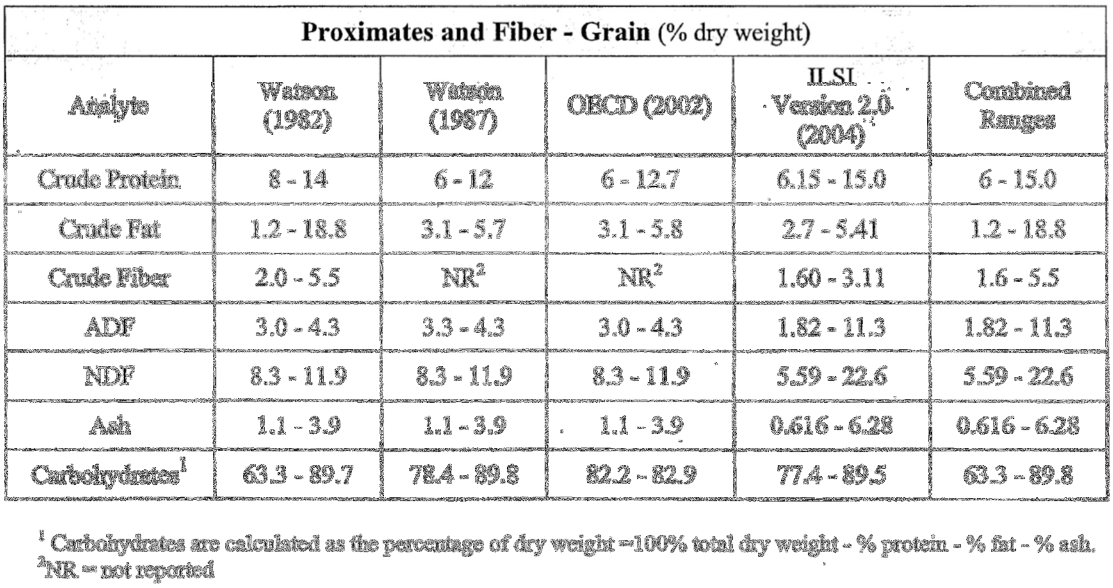
Table 6. Summary of Analysis of Proximates and Fiber in Grain for the 59122 x NK603 + Glyphosate fb Glufosinate and Control Hybrids (North America, 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glyphosate fb Glufosinate	Control	Standard Error
Crude Protein	6 - 15.0	11.5*	10.2	0.0886
Crude Fat	1.2 - 18.8	3.24	3.15	0.0725
ADF[4]	1.82 - 11.3	4.05*	3.64	0.101
Crude Fiber	1.6 - 5.5	2.61	2.54	0.0666
NDF[5]	5.59 - 22.6	11.3*	10.6	0.226
Ash	0.62 - 6.28	1.61*	1.36	0.0338
Carbohydrates	63.3 - 89.8	83.7*	85.3	0.136

[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 59122xNK603 + glyphosate fb glufosinate and control

Table 7. Literature Ranges of Proximates and Fibers in Grain

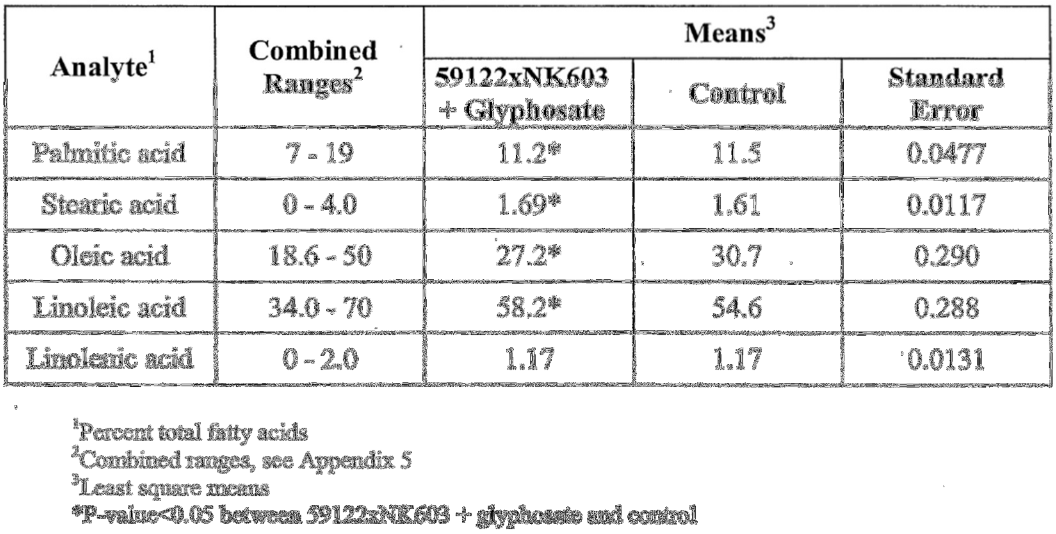


Proximates and Fiber - Grain (% dry weight)					
Analyte	Watson (1982)	Watson (1987)	OECD (2002)	ILSI Version 2.0 (2004)	Combined Ranges
Crude Protein	8 - 14	6 - 12	6 - 12.7	6.15 - 15.0	6 - 15.0
Crude Fat	1.2 - 18.8	3.1 - 5.7	3.1 - 5.8	2.7 - 5.41	1.2 - 18.8
Crude Fiber	2.0 - 5.5	NR[2]	NR[2]	1.60 - 3.11	1.6 - 5.5
ADF	3.0 - 4.3	3.3 - 4.3	3.0 - 4.3	1.82 - 11.3	1.82 - 11.3
NDF	8.3 - 11.9	8.3 - 11.9	8.3 - 11.9	5.59 - 22.6	5.59 - 22.6
Ash	1.1 - 3.9	1.1 - 3.9	1.1 - 3.9	0.616 - 6.28	0.616 - 6.28
Carbohydrates[1]	63.3 - 89.7	78.4 - 89.8	82.2 - 82.9	77.4 - 89.5	63.3 - 89.8

[1] Carbohydrates are calculated as the percentage of dry weight =100% total dry weight - % protein - % fat - % ash.

[2] NR = not reported

Table 8. Summary Analysis of Fatty Acids in Grain for the 59122 x NK603 + Glyphosate and Control Hybrids (North America 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glyphosate	Control	Standard Error
Palmitic acid	7 - 19	11.2*	11.5	0.0477
Stearic acid	0 - 4.0	1.69*	1.61	0.0117
Oleic acid	18.6 - 50	27.2*	30.7	0.290
Linoleic acid	34.0 - 70	58.2*	54.6	0.288
Linolenic acid	0 - 2.0	1.17	1.17	0.0131

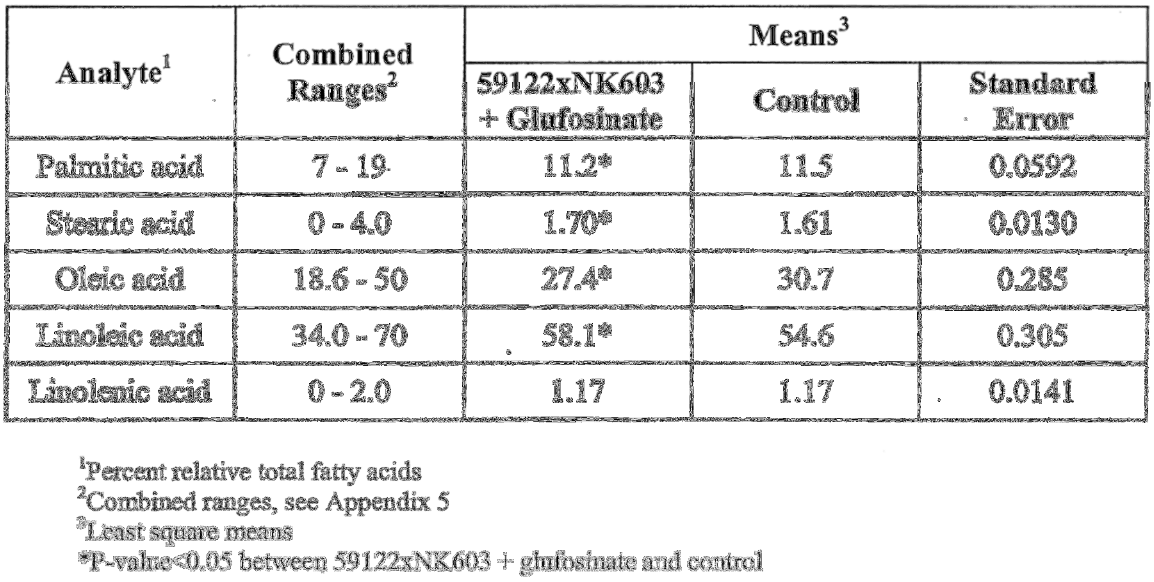
[1] Percent total fatty acids

[2] Combined ranges, see Appendix 5

[3] Least square means

*P-value<0.05 between 59122xNK603 + glyphosate and control

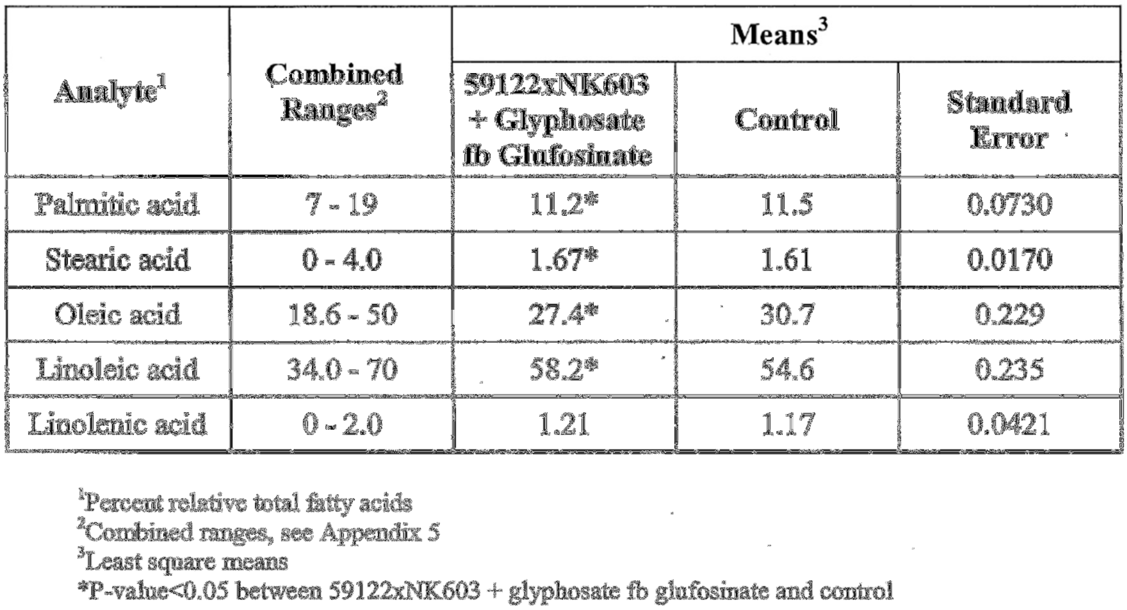
Table 9. Summary Analysis of Fatty Acids in Grain for the 59122 x NK603 + Glufosinate and Control Hybrids (North America 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glufosinate	Control	Standard Error
Palmitic acid	7 - 19	11.2*	11.5	0.0592
Stearic acid	0 - 4.0	1.70*	1.61	0.0130
Oleic acid	18.6 - 50	27.4*	30.7	0.285
Linoleic acid	34.0 - 70	58.1*	54.6	0.305
Linolenic acid	0 - 2.0	1.17	1.17	0.0141

[1] Percent relative total fatty acids
[2] Combined ranges, see Appendix 5
[3] Least square means
*P-value<0.05 between 59122xNK603 + glufosinate and control

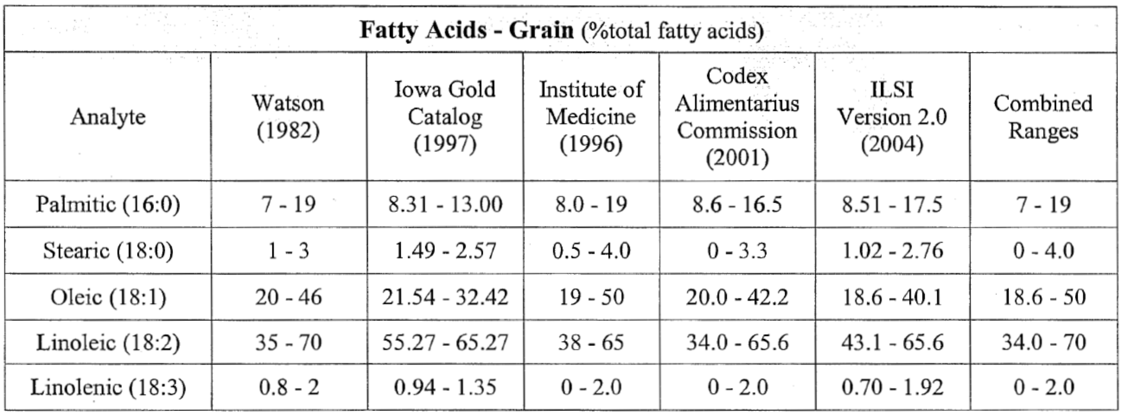
Table 10. Summary of Analysis of Fatty Acids in Grain for the 59122 x NK603 + Glyphosate fb Glufosinate and Control Hybrids (North America 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glyphosate fb Glufosinate	Control	Standard Error
Palmitic acid	7 - 19	11.2*	11.5	0.0730
Stearic acid	0 - 4.0	1.67*	1.61	0.0170
Oleic acid	18.6 - 50	27.4*	30.7	0.229
Linoleic acid	34.0 - 70	58.2*	54.6	0.235
Linolenic acid	0 - 2.0	1.21	1.17	0.0421

[1] Percent relative total fatty acids
[2] Combined ranges, see Appendix 5
[3] Least square means
*P-value<0.05 between 59122xNK603 + glyphosate fb glufosinate and control

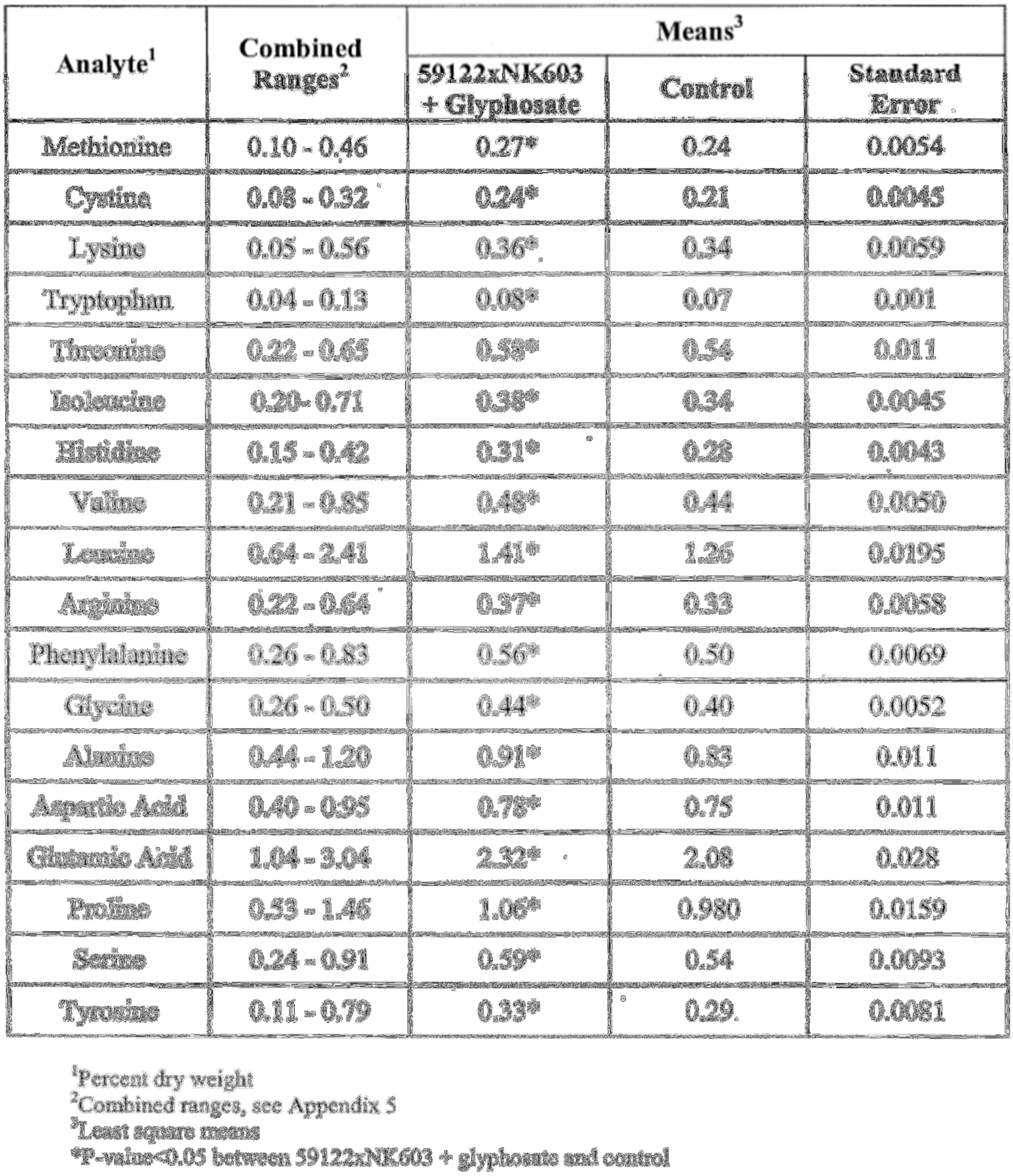
Table 11. Literature Ranges of Fatty Acids in Grain



Fatty Acids - Grain (%total fatty acids)

Analyte	Watson (1982)	Iowa Gold Catalog (1997)	Institute of Medicine (1996)	Codex Alimentarius Commission (2001)	ILSI Version 2.0 (2004)	Combined Ranges
Palmitic (16:0)	7 - 19	8.31 - 13.00	8.0 - 19	8.6 - 16.5	8.51 - 17.5	7 - 19
Stearic (18:0)	1 - 3	1.49 - 2.57	0.5 - 4.0	0 - 3.3	1.02 - 2.76	0 - 4.0
Oleic (18:1)	20 - 46	21.54 - 32.42	19 - 50	20.0 - 42.2	18.6 - 40.1	18.6 - 50
Linoleic (18:2)	35 - 70	55.27 - 65.27	38 - 65	34.0 - 65.6	43.1 - 65.6	34.0 - 70
Linolenic (18:3)	0.8 - 2	0.94 - 1.35	0 - 2.0	0 - 2.0	0.70 - 1.92	0 - 2.0

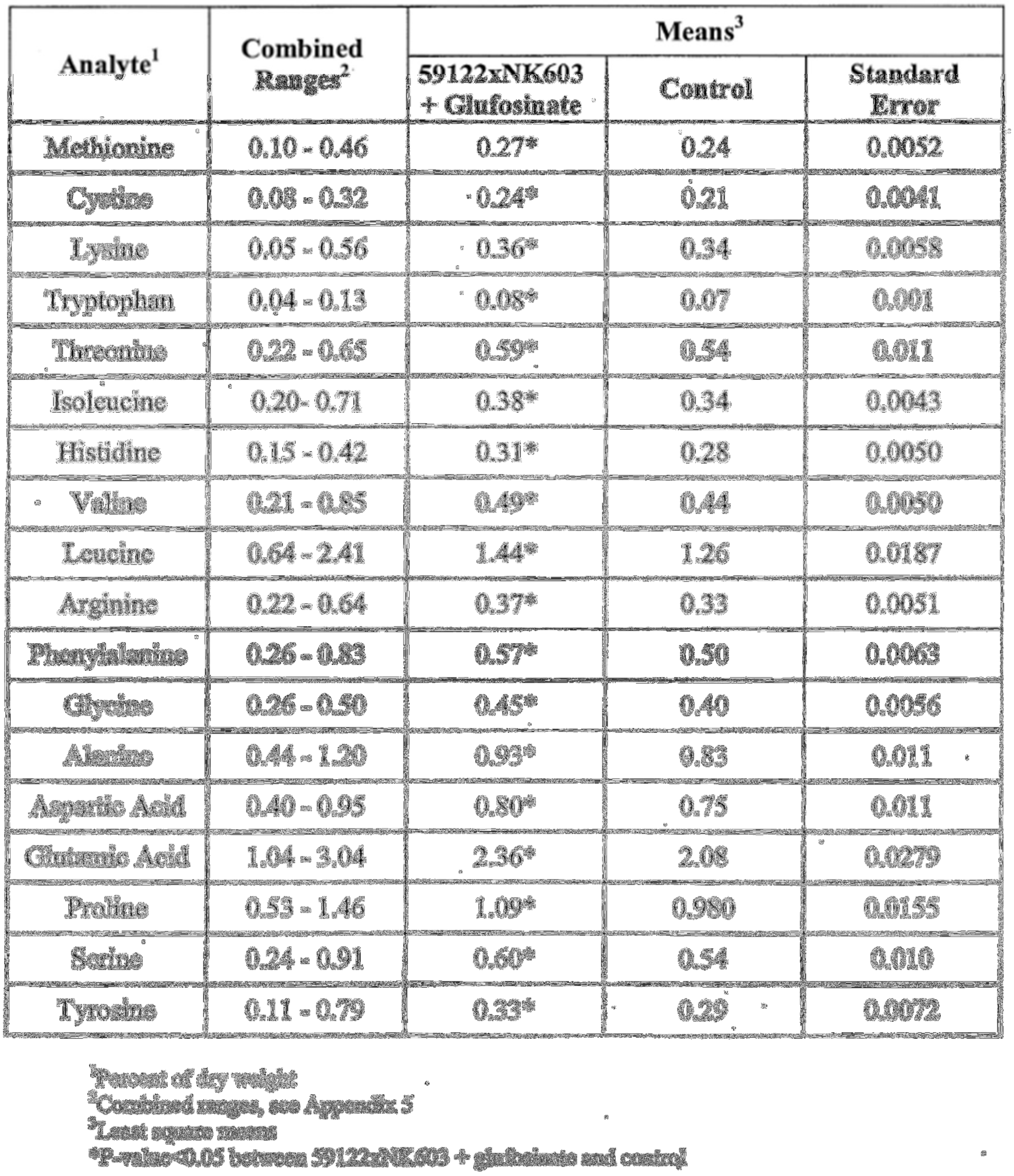
Table 12. Summary Analysis of Amino Acids in Grain for the 59122 x NK603 + Glyphosate and Control Hybrids (North America 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glyphosate	Control	Standard Error
Methionine	0.10 - 0.46	0.27*	0.24	0.0054
Cystine	0.08 - 0.32	0.24*	0.21	0.0045
Lysine	0.05 - 0.56	0.36*	0.34	0.0059
Tryptophan	0.04 - 0.13	0.08*	0.07	0.001
Threonine	0.22 - 0.65	0.58*	0.54	0.011
Isoleucine	0.20- 0.71	0.38*	0.34	0.0045
Histidine	0.15 - 0.42	0.31*	0.28	0.0043
Valine	0.21 - 0.85	0.48*	0.44	0.0050
Leucine	0.64 - 2.41	1.41*	1.26	0.0195
Arginine	0.22 - 0.64	0.37*	0.33	0.0058
Phenylalanine	0.26 - 0.83	0.56*	0.50	0.0069
Glycine	0.26 - 0.50	0.44*	0.40	0.0052
Alanine	0.44 - 1.20	0.91*	0.83	0.011
Aspartic Acid	0.40 - 0.95	0.78*	0.75	0.011
Glutamic Acid	1.04 - 3.04	2.32*	2.08	0.028
Proline	0.53 - 1.46	1.06*	0.980	0.0159
Serine	0.24 - 0.91	0.59*	0.54	0.0093
Tyrosine	0.11 - 0.79	0.33*	0.29	0.0081

[1] Percent dry weight
[2] Combined ranges, see Appendix 5
[3] Least square means
*P-value<0.05 between 59122xNK603 + glyphosate and control

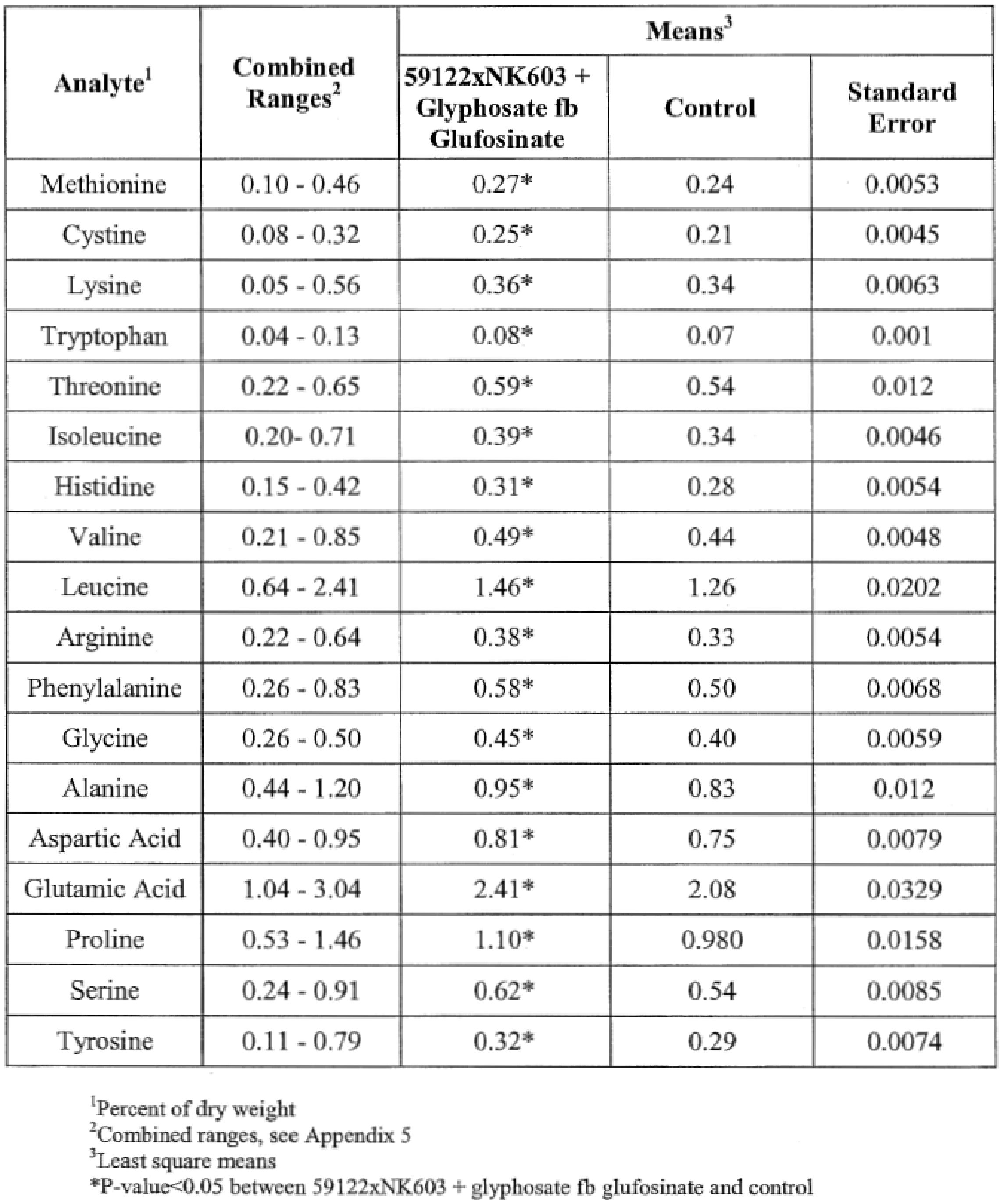
Table 13. Summary Analysis of Amino Acids in Grain for the 59122 x NK603 + Glufosinate and Control Hybrids (North America, 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glufosinate	Control	Standard Error
Methionine	0.10 - 0.46	0.27*	0.24	0.0052
Cystine	0.08 - 0.32	0.24*	0.21	0.0041
Lysine	0.05 - 0.56	0.36*	0.34	0.0058
Tryptophan	0.04 - 0.13	0.08*	0.07	0.001
Threonine	0.22 - 0.65	0.59*	0.54	0.011
Isoleucine	0.20- 0.71	0.38*	0.34	0.0043
Histidine	0.15 - 0.42	0.31*	0.28	0.0050
Valine	0.21 - 0.85	0.49*	0.44	0.0050
Leucine	0.64 - 2.41	1.44*	1.26	0.0187
Arginine	0.22 - 0.64	0.37*	0.33	0.0051
Phenylalanine	0.26 - 0.83	0.57*	0.50	0.0063
Glycine	0.26 - 0.50	0.45*	0.40	0.0056
Alanine	0.44 - 1.20	0.93*	0.83	0.011
Aspartic Acid	0.40 - 0.95	0.80*	0.75	0.011
Glutamic Acid	1.04 - 3.04	2.36*	2.08	0.0279
Proline	0.53 - 1.46	1.09*	0.980	0.0155
Serine	0.24 - 0.91	0.60*	0.54	0.010
Tyrosine	0.11 - 0.79	0.33*	0.29	0.0072

[1] Percent of dry weight
[2] Combined ranges, see Appendix 5
[3] Least square means
*P-value<0.05 between 59122xNK603 + glufosinate and control

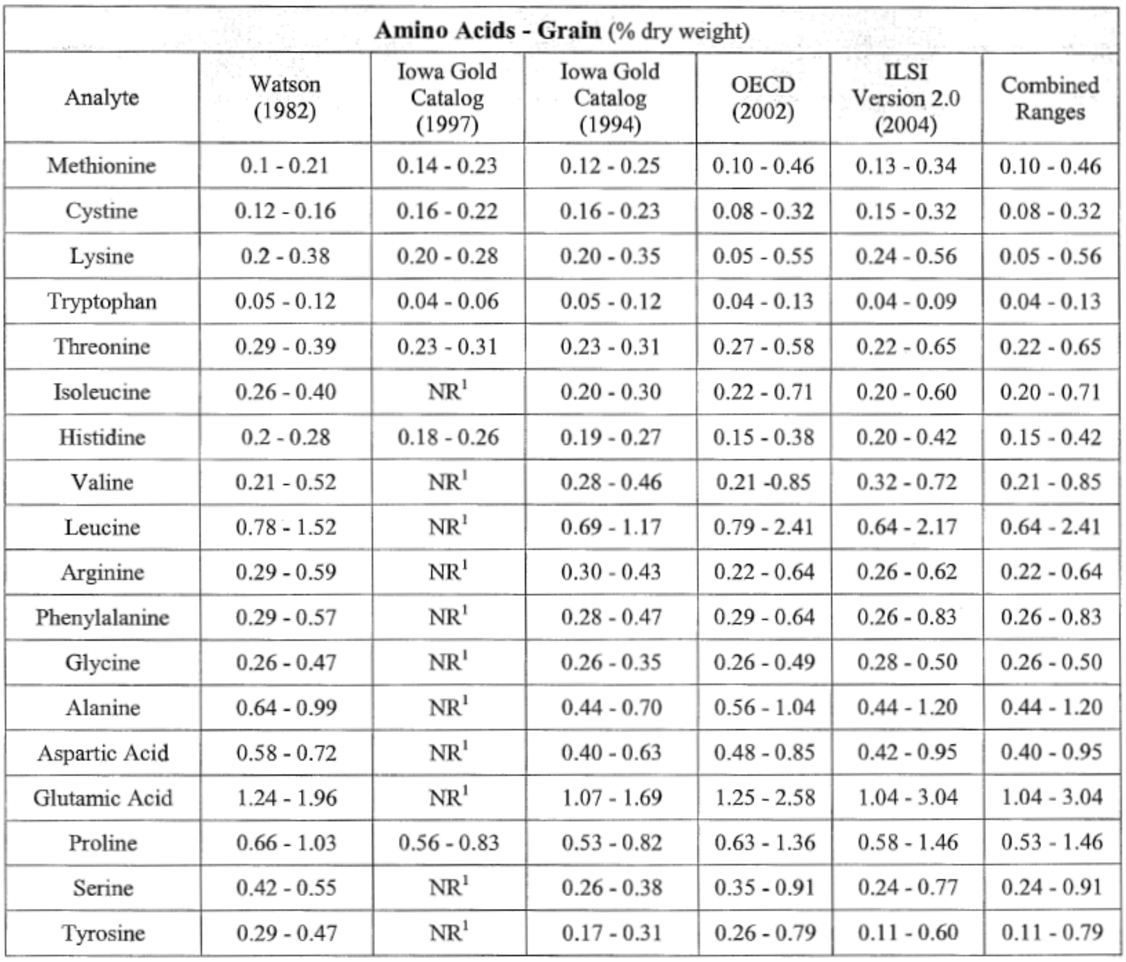
Table 14. Summary of Analysis of Amino Acids in Grain for the 59122 x NK603 + Glyphosate fb Glufosinate and Control Hybrids (North America 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glyphosate fb Glufosinate	Control	Standard Error
Methionine	0.10 - 0.46	0.27*	0.24	0.0053
Cystine	0.08 - 0.32	0.25*	0.21	0.0045
Lysine	0.05 - 0.56	0.36*	0.34	0.0063
Tryptophan	0.04 - 0.13	0.08*	0.07	0.001
Threonine	0.22 - 0.65	0.59*	0.54	0.012
Isoleucine	0.20- 0.71	0.39*	0.34	0.0046
Histidine	0.15 - 0.42	0.31*	0.28	0.0054
Valine	0.21 - 0.85	0.49*	0.44	0.0048
Leucine	0.64 - 2.41	1.46*	1.26	0.0202
Arginine	0.22 - 0.64	0.38*	0.33	0.0054
Phenylalanine	0.26 - 0.83	0.58*	0.50	0.0068
Glycine	0.26 - 0.50	0.45*	0.40	0.0059
Alanine	0.44 - 1.20	0.95*	0.83	0.012
Aspartic Acid	0.40 - 0.95	0.81*	0.75	0.0079
Glutamic Acid	1.04 - 3.04	2.41*	2.08	0.0329
Proline	0.53 - 1.46	1.10*	0.980	0.0158
Serine	0.24 - 0.91	0.62*	0.54	0.0085
Tyrosine	0.11 - 0.79	0.32*	0.29	0.0074

[1] Percent of dry weight
[2] Combined ranges, see Appendix 5
[3] Least square means
*P-value<0.05 between 59122xNK603 + glyphosate fb glufosinate and control

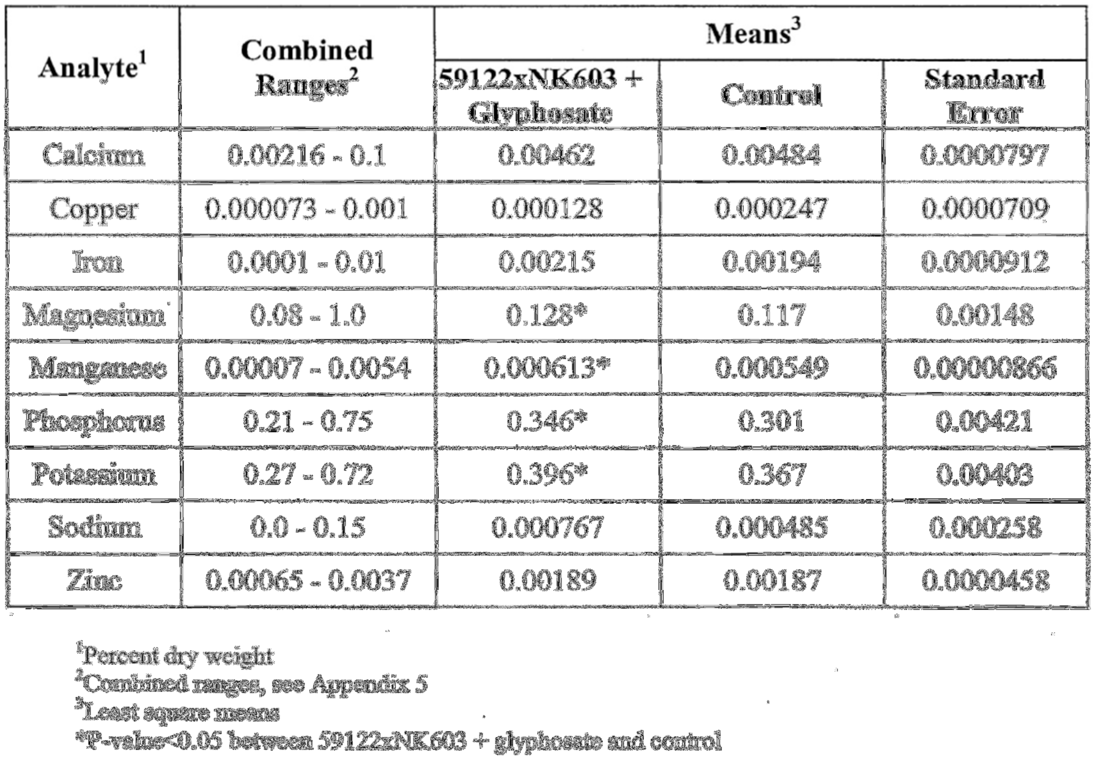
Table 15. Literature Ranges of Amino Acids in Grain



Amino Acids - Grain (% dry weight)						
Analyte	Watson (1982)	Iowa Gold Catalog (1997)	Iowa Gold Catalog (1994)	OECD (2002)	ILSI Version 2.0 (2004)	Combined Ranges
Methionine	0.1 - 0.21	0.14 - 0.23	0.12 - 0.25	0.10 - 0.46	0.13 - 0.34	0.10 - 0.46
Cystine	0.12 - 0.16	0.16 - 0.22	0.16 - 0.23	0.08 - 0.32	0.15 - 0.32	0.08 - 0.32
Lysine	0.2 - 0.38	0.20 - 0.28	0.20 - 0.35	0.05 - 0.55	0.24 - 0.56	0.05 - 0.56
Tryptophan	0.05 - 0.12	0.04 - 0.06	0.05 - 0.12	0.04 - 0.13	0.04 - 0.09	0.04 - 0.13
Threonine	0.29 - 0.39	0.23 - 0.31	0.23 - 0.31	0.27 - 0.58	0.22 - 0.65	0.22 - 0.65
Isoleucine	0.26 - 0.40	NR[1]	0.20 - 0.30	0.22 - 0.71	0.20 - 0.60	0.20 - 0.71
Histidine	0.2 - 0.28	0.18 - 0.26	0.19 - 0.27	0.15 - 0.38	0.20 - 0.42	0.15 - 0.42
Valine	0.21 - 0.52	NR[1]	0.28 - 0.46	0.21 -0.85	0.32 - 0.72	0.21 - 0.85
Leucine	0.78 - 1.52	NR[1]	0.69 - 1.17	0.79 - 2.41	0.64 - 2.17	0.64 - 2.41
Arginine	0.29 - 0.59	NR[1]	0.30 - 0.43	0.22 - 0.64	0.26 - 0.62	0.22 - 0.64
Phenylalanine	0.29 - 0.57	NR[1]	0.28 - 0.47	0.29 - 0.64	0.26 - 0.83	0.26 - 0.83
Glycine	0.26 - 0.47	NR[1]	0.26 - 0.35	0.26 - 0.49	0.28 - 0.50	0.26 - 0.50
Alanine	0.64 - 0.99	NR[1]	0.44 - 0.70	0.56 - 1.04	0.44 - 1.20	0.44 - 1.20
Aspartic Acid	0.58 - 0.72	NR[1]	0.40 - 0.63	0.48 - 0.85	0.42 - 0.95	0.40 - 0.95
Glutamic Acid	1.24 - 1.96	NR[1]	1.07 - 1.69	1.25 - 2.58	1.04 - 3.04	1.04 - 3.04
Proline	0.66 - 1.03	0.56 - 0.83	0.53 - 0.82	0.63 - 1.36	0.58 - 1.46	0.53 - 1.46
Serine	0.42 - 0.55	NR[1]	0.26 - 0.38	0.35 - 0.91	0.24 - 0.77	0.24 - 0.91
Tyrosine	0.29 - 0.47	NR[1]	0.17 - 0.31	0.26 - 0.79	0.11 - 0.60	0.11 - 0.79

[1] NR = not reported

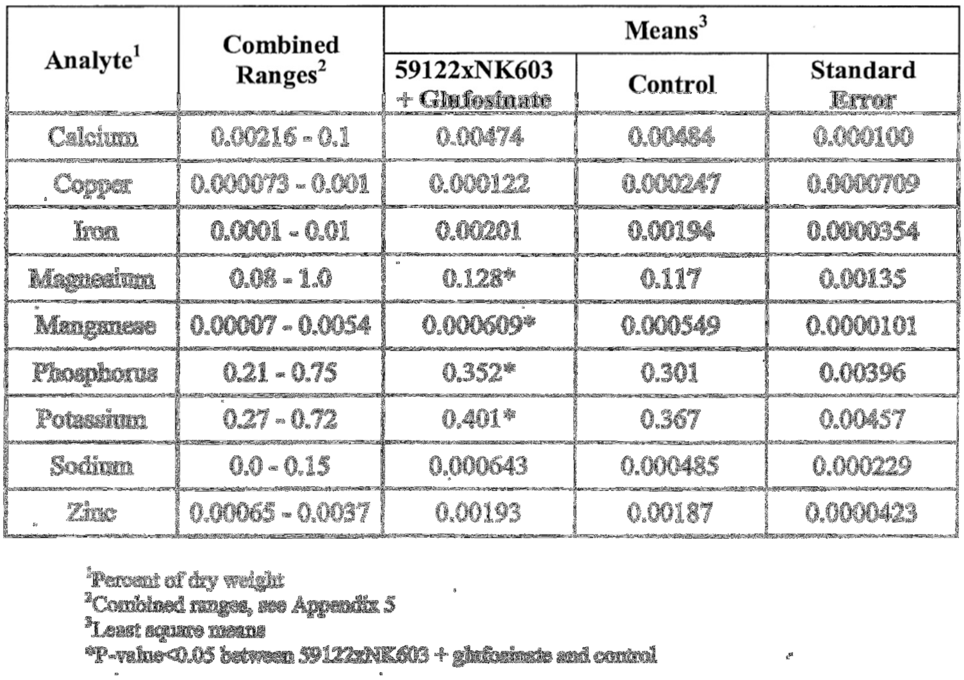
Table 16. Summary Analysis of Minerals in Grain for the 59122 x NK603 + Glyphosate and Control Hybrids (North America 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glyphosate	Control	Standard Error
Calcium	0.00216 - 0.1	0.00462	0.00484	0.0000797
Copper	0.000073 - 0.001	0.000128	0.000247	0.0000709
Iron	0.0001 - 0.01	0.00215	0.00194	0.0000912
Magnesium	0.08 - 1.0	0.128*	0.117	0.00148
Manganese	0.00007 - 0.0054	0.000613*	0.000549	0.00000866
Phosphorus	0.21 - 0.75	0.346*	0.301	0.00421
Potassium	0.27 - 0.72	0.396*	0.367	0.00403
Sodium	0.0 - 0.15	0.000767	0.000485	0.000258
Zinc	0.00065 - 0.0037	0.00189	0.00187	0.0000458

[1] Percent dry weight
[2] Combined ranges, see Appendix 5
[3] Least square means
*P-value<0.05 between 59122xNK603 + glyphosate and control

Table 17. Summary Analysis of Minerals in Grain for the 59122 x NK603 + Glufosinate and Control Hybrids (North America 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glufosinate	Control	Standard Error
Calcium	0.00216 - 0.1	0.00474	0.00484	0.000100
Copper	0.000073 - 0.001	0.000122	0.000247	0.0000709
Iron	0.0001 - 0.01	0.00201	0.00194	0.0000354
Magnesium	0.08 - 1.0	0.128*	0.117	0.00135
Manganese	0.00007 - 0.0054	0.000609*	0.000549	0.0000101
Phosphorus	0.21 - 0.75	0.352*	0.301	0.00396
Potassium	0.27 - 0.72	0.401*	0.367	0.00457
Sodium	0.0 - 0.15	0.000643	0.000485	0.000229
Zinc	0.00065 - 0.0037	0.00193	0.00187	0.0000423

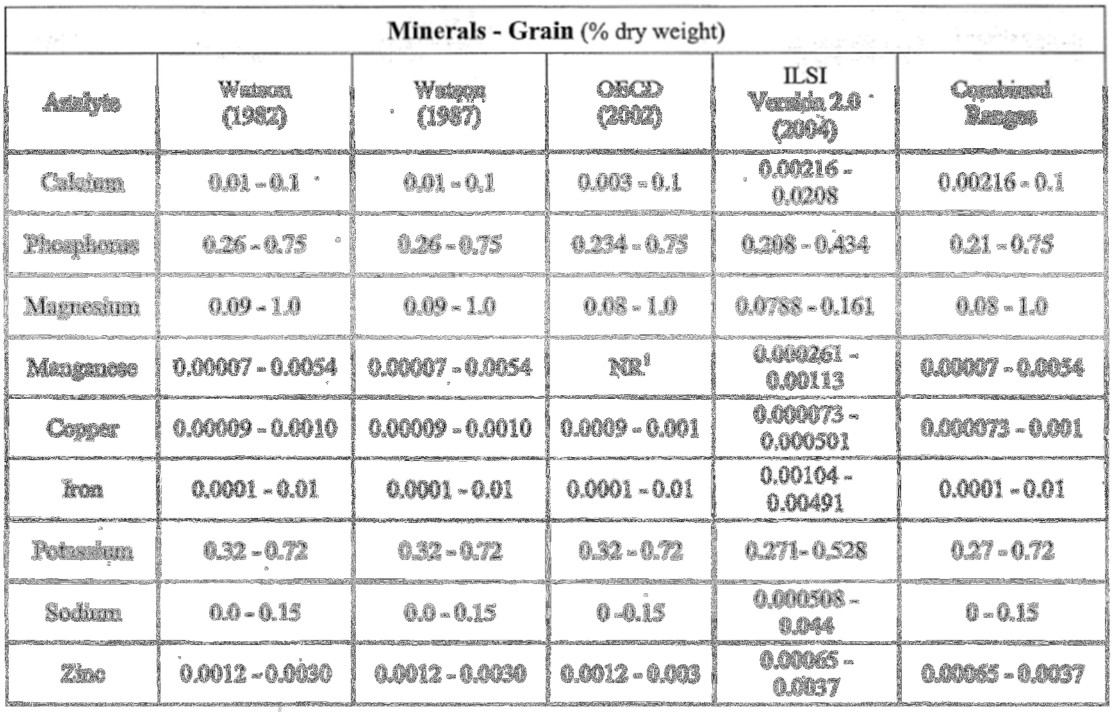
[1] Percent of dry weight
[2] Combined ranges, see Appendix 5
[3] Least square means
*P-value<0.05 between 59122xNK603 + glufosinate and control

Table 18. Summary of Analysis of Minerals in Grain for the 59122 x NK603 + Glyphosate fb Glufosinate and Control Hybrids (North America 2003)

Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glyphosate fb Glufosinate	Control	Standard Error
Calcium	0.00216 - 0.1	0.00490	0.00484	0.000111
Copper	0.000073 - 0.001	0.000125	0.000247	0.0000709
Iron	0.0001 - 0.01	0.00205*	0.00194	0.0000359
Magnesium	0.08 - 1.0	0.128*	0.117	0.00131
Manganese	0.00007 - 0.0054	0.000637*	0.000549	0.0000113
Phosphorus	0.21 - 0.75	0.351*	0.301	0.00377
Potassium	0.27 - 0.72	0.403*	0.367	0.00467
Sodium	0.0 - 0.15	0.000504	0.000485	0.000228
Zinc	0.00065 - 0.0037	0.00197	0.00187	0.0000491

[1] Percent of dry weight
[2] Combined ranges, see Appendix 5
[3] Least square means
*P-value<0.05 between 59122xNK603 + glyphosate fb glufosinate and control

Table 19. Literature Ranges of Minerals in Grain

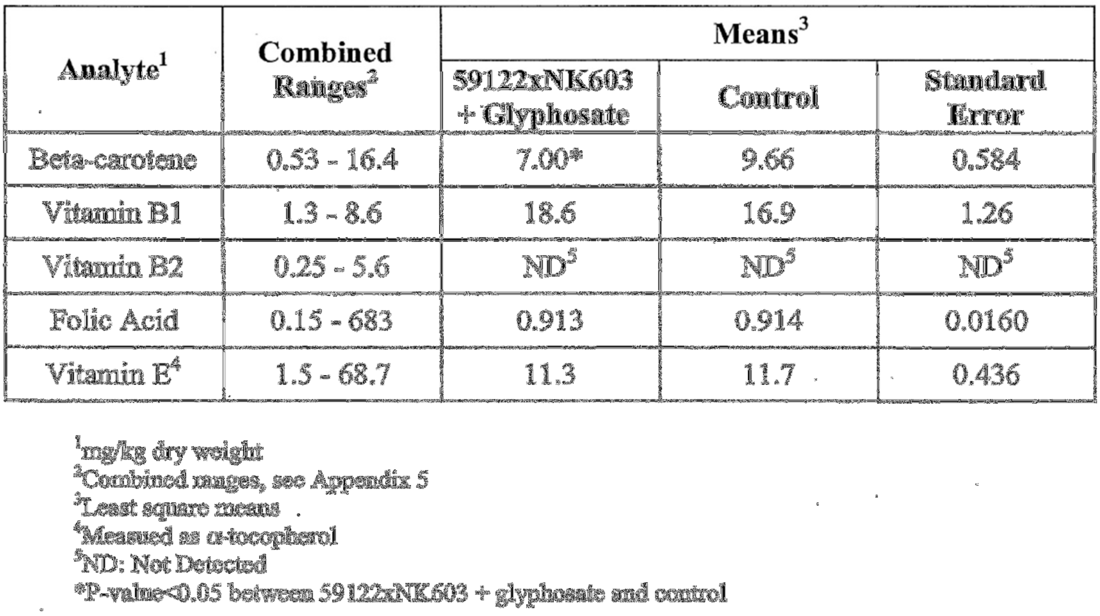


Minerals - Grain (% dry weight)

Analyte	Watson (1982)	Watson (1987)	OECD (2002)	ILSI Version 2.0 (2004)	Combined Ranges
Calcium	0.01 - 0.1	0.01 - 0.1	0.003 - 0.1	0.00216 - 0.0208	0.00216 - 0.1
Phosphorus	0.26 - 0.75	0.26 - 0.75	0.234 - 0.75	0.208 - 0.434	0.21 - 0.75
Magnesium	0.09 - 1.0	0.09 - 1.0	0.08 - 1.0	0.0788 - 0.161	0.08 - 1.0
Manganese	0.00007 - 0.0054	0.00007 - 0.0054	NR[1]	0.000261 - 0.00113	0.00007 - 0.0054
Copper	0.00009 - 0.0010	0.00009 - 0.0010	0.0009 - 0.001	0.000073 - 0.000501	0.000073 - 0.001
Iron	0.0001 - 0.01	0.0001 - 0.01	0.0001 - 0.01	0.00104 - 0.00491	0.0001 - 0.01
Potassium	0.32 - 0.72	0.32 - 0.72	0.32 - 0.72	0.271- 0.528	0.27 - 0.72
Sodium	0.0 - 0.15	0.0 - 0.15	0 -0.15	0.000508 - 0.044	0 - 0.15
Zinc	0.0012 - 0.0030	0.0012 - 0.0030	0.0012 - 0.003	0.00065 - 0.0037	0.00065 - 0.0037

[1] NR = not reported

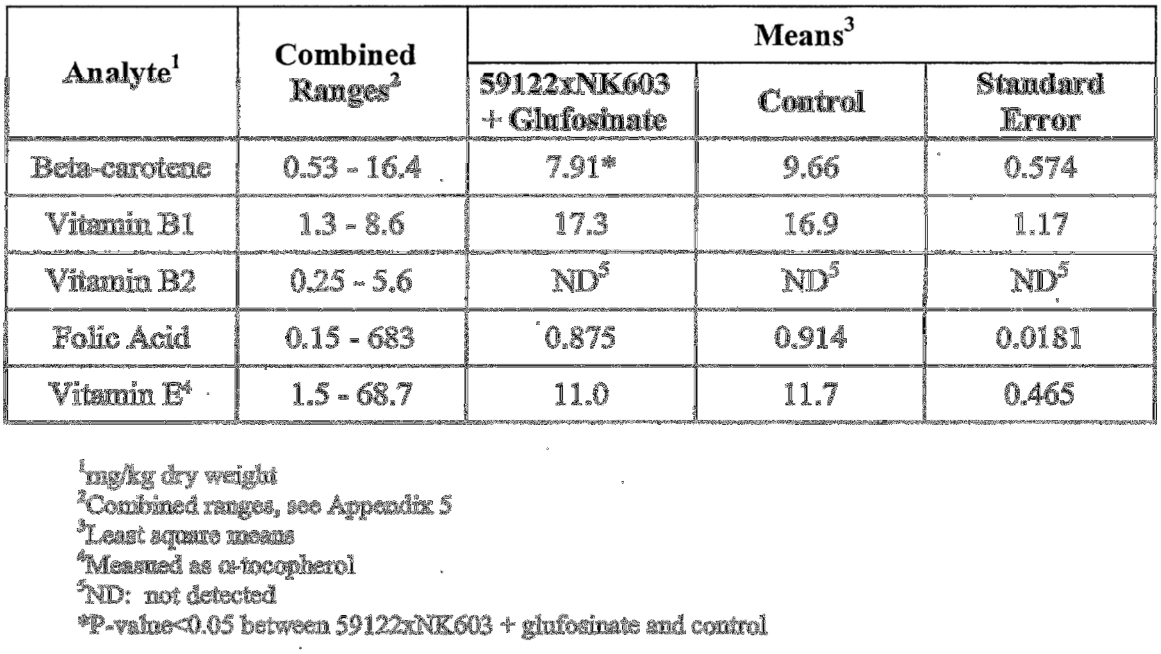
Table 20. Summary Analysis of Vitamins in Grain for the 59122 x NK603 + Glyphosate and Control Hybrids (North America 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glyphosate	Control	Standard Error
Beta-carotene	0.53 - 16.4	7.00*	9.66	0.584
Vitamin B1	1.3 - 8.6	18.6	16.9	1.26
Vitamin B2	0.25 - 5.6	ND[5]	ND[5]	ND[5]
Folic Acid	0.15 - 683	0.913	0.914	0.0160
Vitamin E[4]	1.5 - 68.7	11.3	11.7	0.436

[1] mg/kg dry weight
[2] Combined ranges, see Appendix 5
[3] Least square means
[4] Measued as α-tocopherol
[5] ND: Not Detected
*P-value<0.05 between 59122xNK603 + glyphosate and control

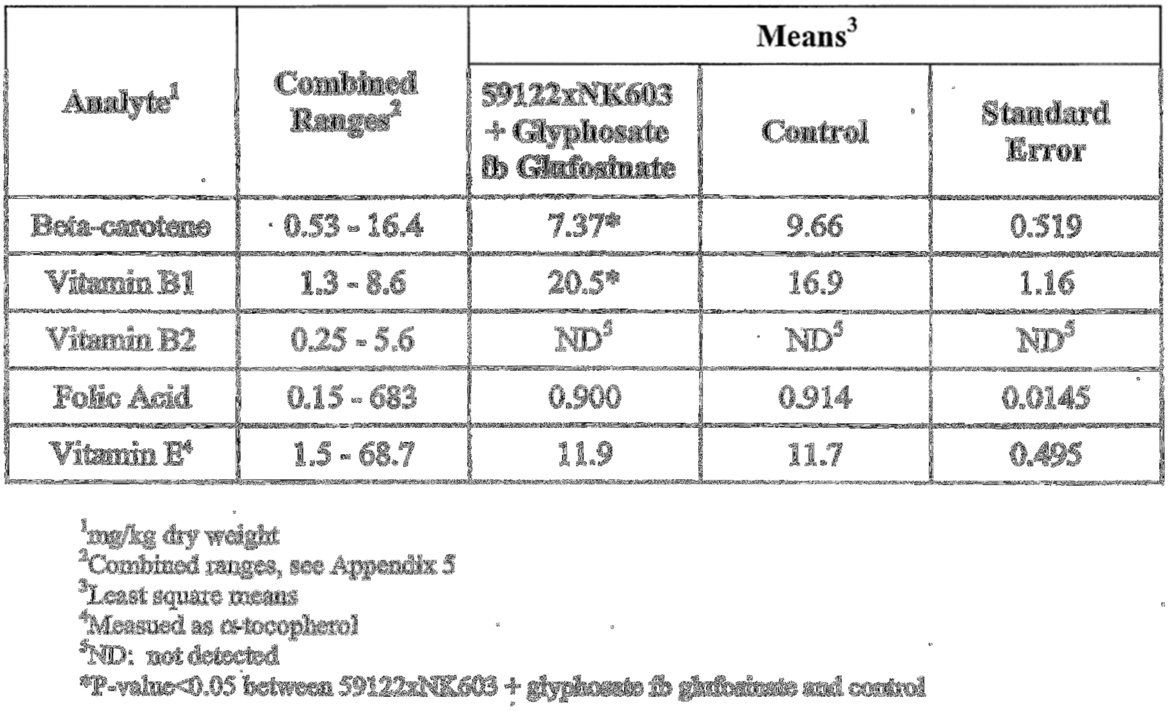
Table 21. Summary Analysis of Vitamins in Grain for the 59122 x NK603 + Glufosinate and Control Hybrids (North America, 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glufosinate	Control	Standard Error
Beta-carotene	0.53 - 16.4	7.91*	9.66	0.574
Vitamin B1	1.3 - 8.6	17.3	16.9	1.17
Vitamin B2	0.25 - 5.6	ND[5]	ND[5]	ND[5]
Folic Acid	0.15 - 683	0.875	0.914	0.0181
Vitamin E[4]	1.5 - 68.7	11.0	11.7	0.465

[1] mg/kg dry weight
[2] Combined ranges, see Appendix 5
[3] Least square means
[4] Measued as α-tocopherol
[5] ND: not detected
*P-value<0.05 between 59122xNK603 + glufosinate and control

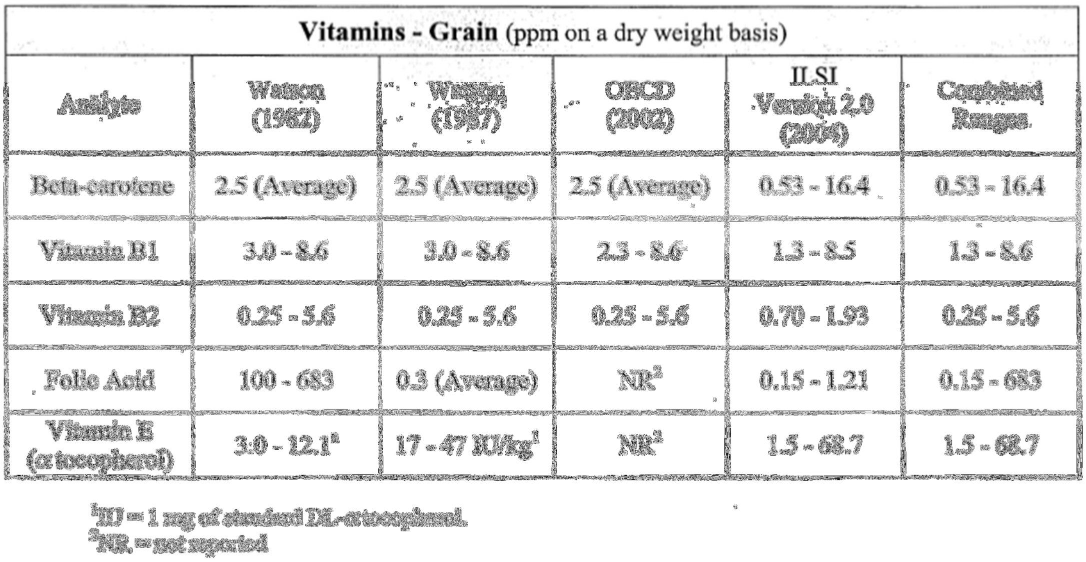
Table 22. Summary of Analysis of Vitamins in Grain for the 59122 x NK603 + Glyphosate fb Glufosinate and Control Hybrids (North America 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glyphosate fb Glufosinate	Control	Standard Error
Beta-carotene	0.53 - 16.4	7.37*	9.66	0.519
Vitamin B1	1.3 - 8.6	20.5*	16.9	1.16
Vitamin B2	0.25 - 5.6	ND[5]	ND[5]	ND[5]
Folic Acid	0.15 - 683	0.900	0.914	0.0145
Vitamin E[4]	1.5 - 68.7	11.9	11.7	0.495

[1] mg/kg dry weight
[2] Combined ranges, see Appendix 5
[3] Least square means
[4] Measued as α-tocopherol
[5] ND: not detected
*P-value<0.05 between 59122xNK603 + glyphosate fb glufosinate and control

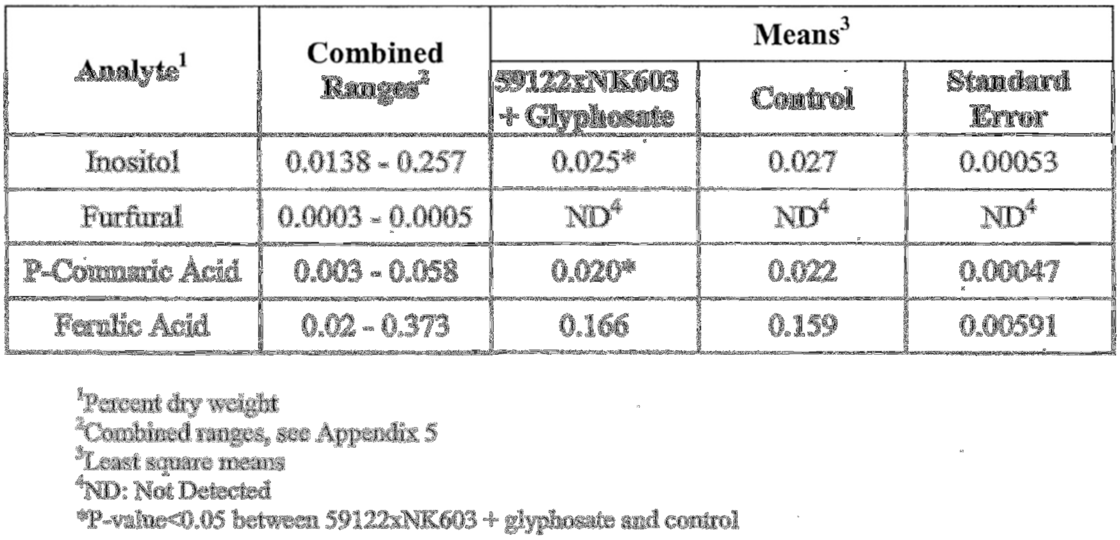
Table 23. Literature Ranges of Vitamins in Grain



Vitamins - Grain (ppm on a dry weight basis)					
Analyte	Watson (1982)	Watson (1987)	OECD (2002)	ILSI Version 2.0 (2004)	Combined Ranges
Beta-carotene	2.5 (Average)	2.5 (Average)	2.5 (Average)	0.53 - 16.4	0.53 - 16.4
Vitamin B1	3.0 - 8.6	3.0 - 8.6	2.3 - 8.6	1.3 - 8.5	1.3 - 8.6
Vitamin B2	0.25 - 5.6	0.25 - 5.6	0.25 - 5.6	0.70 - 1.93	0.25 - 5.6
Folic Acid	100 - 683	0.3 (Average)	NR[2]	0.15 - 1.21	0.15 - 683
Vitamin E (α tocopherol)	3.0 - 12.1[1]	17 - 47 IU/kg[1]	NR[2]	1.5 - 68.7	1.5 - 68.7

[1] IU = 1 mg of standard DL-α-tocopherol.
[2] NR = not reported

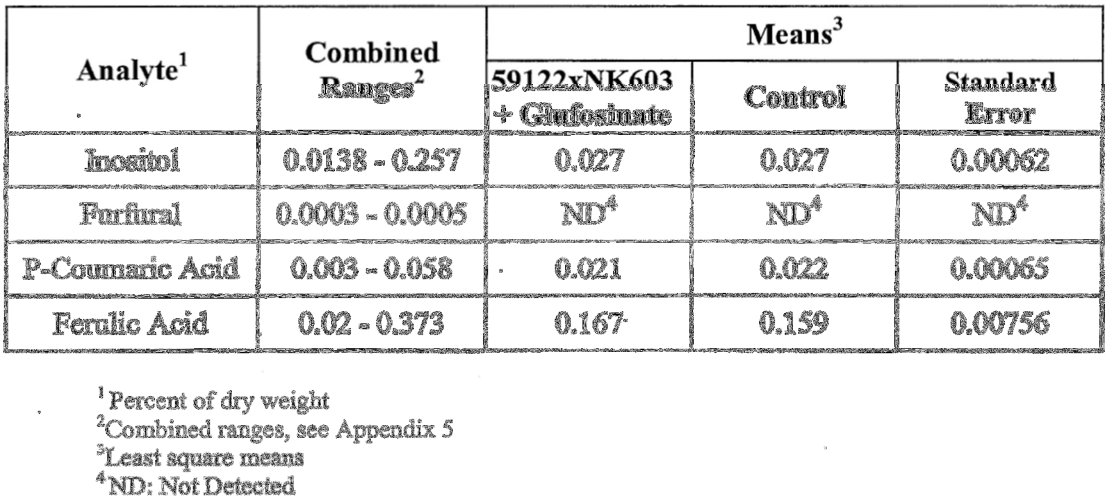
Table 24. Summary Analysis of Secondary Metabolites in Grain for the 59122 x NK603 + Glyphosate and Control Hybrids (North America 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glyphosate	Control	Standard Error
Inositol	0.0138 - 0.257	0.025*	0.027	0.00053
Furfural	0.0003 - 0.0005	ND[4]	ND[4]	ND[4]
P-Coumaric Acid	0.003 - 0.058	0.020*	0.022	0.00047
Ferulic Acid	0.02 - 0.373	0.166	0.159	0.00591

[1] Percent dry weight
[2] Combined ranges, see Appendix 5
[3] Least square means
[4] ND: Not Detected
*P-value<0.05 between 59122xNK603 + glyphosate and control

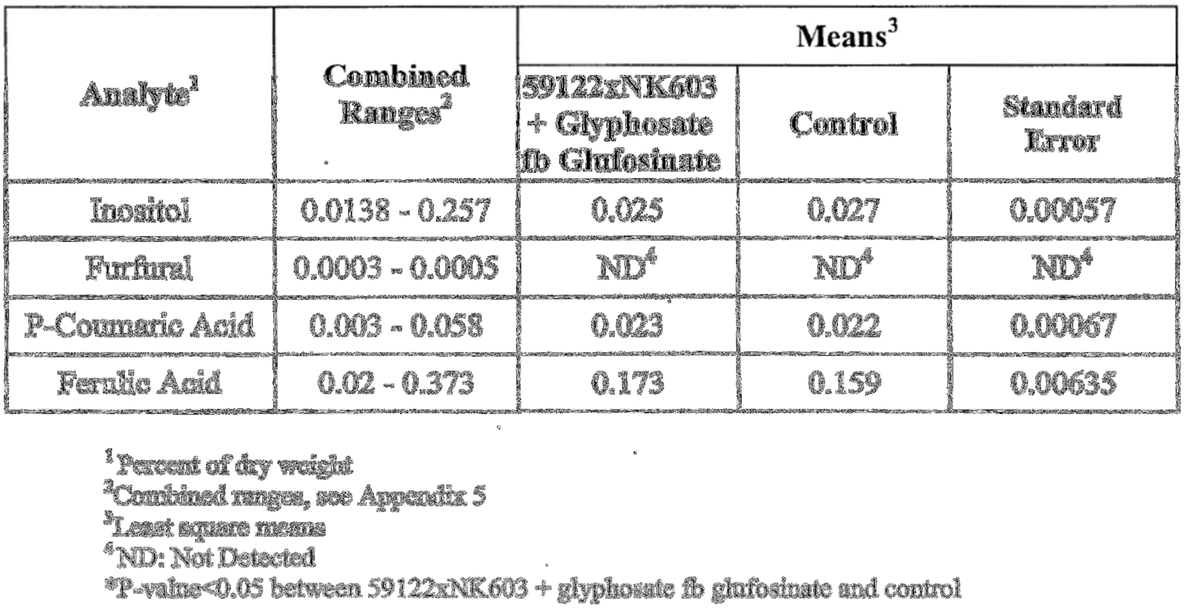
Table 25. Summary Analysis of Secondary Metabolites in Grain for the 59122 x NK603 + Glufosinate and Control Hybrids (North America, 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glufosinate	Control	Standard Error
Inositol	0.0138 - 0.257	0.027	0.027	0.00062
Furfural	0.0003 - 0.0005	ND[4]	ND[4]	ND[4]
P-Coumaric Acid	0.003 - 0.058	0.021	0.022	0.00065
Ferulic Acid	0.02 - 0.373	0.167	0.159	0.00756

[1] Percent of dry weight
[2] Combined ranges, see Appendix 5
[3] Least square means
[4] ND: Not Detected

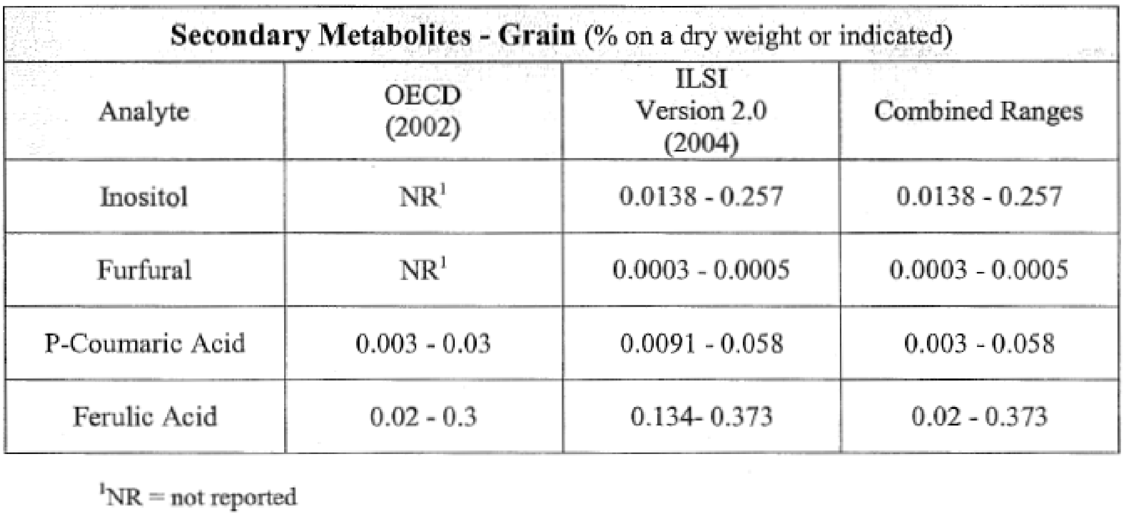
Table 26. Summary of Secondary Metabolites Analysis of in Grain for the 59122 x NK603 + Glyphosate fb Glufosinate and Control Hybrids (North America 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glyphosate fb Glufosinate	Control	Standard Error
Inositol	0.0138 - 0.257	0.025	0.027	0.00057
Furfural	0.0003 - 0.0005	ND[4]	ND[4]	ND[4]
P-Coumaric Acid	0.003 - 0.058	0.023	0.022	0.00067
Ferulic Acid	0.02 - 0.373	0.173	0.159	0.00635

[1] Percent of dry weight
[2] Combined ranges, see Appendix 5
[3] Least square means
[4] ND: Not Detected
*P-value<0.05 between 59122xNK603 + glyphosate fb glufosinate and control

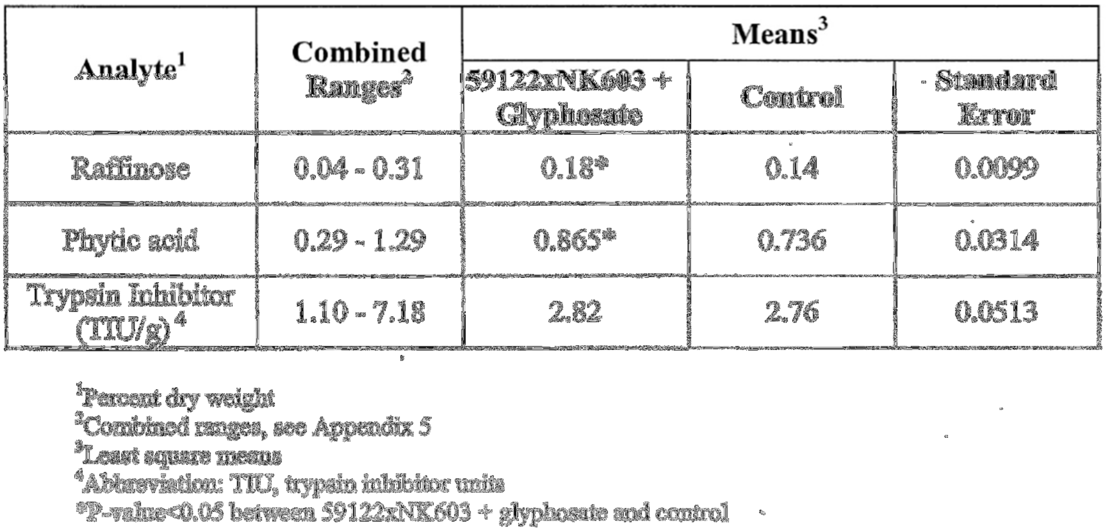
Table 27. Literature Ranges of Secondary Metabolites in Grain



Secondary Metabolites - Grain (% on a dry weight or indicated)			
Analyte	OECD (2002)	ILSI Version 2.0 (2004)	Combined Ranges
Inositol	NR[1]	0.0138 - 0.257	0.0138 - 0.257
Furfural	NR[1]	0.0003 - 0.0005	0.0003 - 0.0005
P-Coumaric Acid	0.003 - 0.03	0.0091 - 0.058	0.003 - 0.058
Ferulic Acid	0.02 - 0.3	0.134- 0.373	0.02 - 0.373

[1] NR = not reported

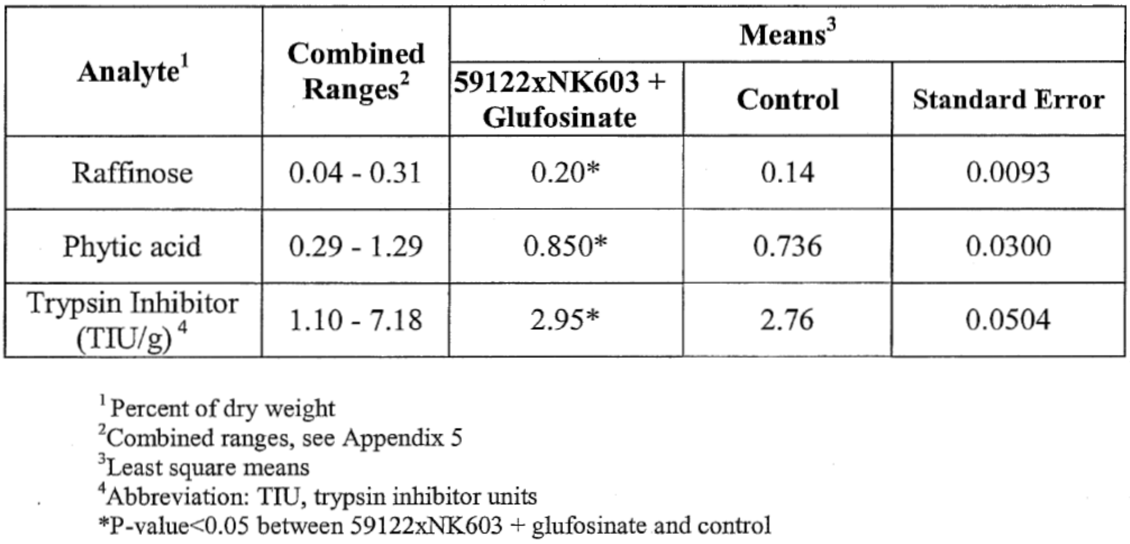
Table 28. Summary Analysis of Anti-Nutrients in Grain for the 59122 x NK603 + Glyphosate and Control Hybrids (North America 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glyphosate	Control	Standard Error
Raffinose	0.04 - 0.31	0.18*	0.14	0.0099
Phytic acid	0.29 - 1.29	0.865*	0.736	0.0314
Trypsin Inhibitor (TIU/g)[4]	1.10 - 7.18	2.82	2.76	0.0513

[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 59122xNK603 + glyphosate and control

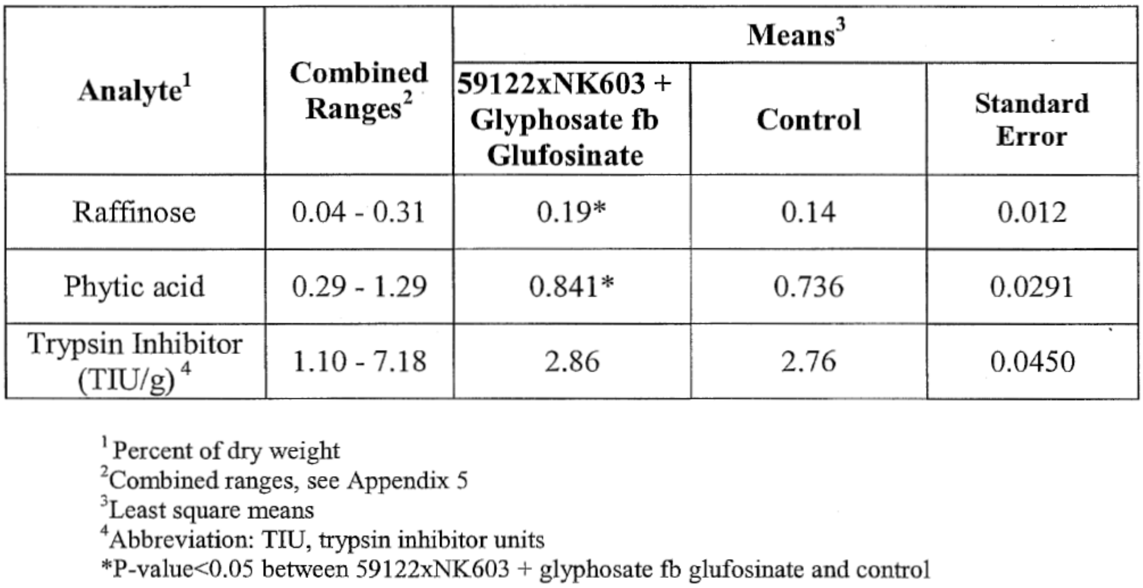
Table 29. Summary Analysis of Anti-Nutrients in Grain for the 59122 x NK603 + Glufosinate and Control Hybrids (North America 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glufosinate	Control	Standard Error
Raffinose	0.04 - 0.31	0.20*	0.14	0.0093
Phytic acid	0.29 - 1.29	0.850*	0.736	0.0300
Trypsin Inhibitor (TIU/g)[4]	1.10 - 7.18	2.95*	2.76	0.0504

[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 59122xNK603 + glufosinate and control

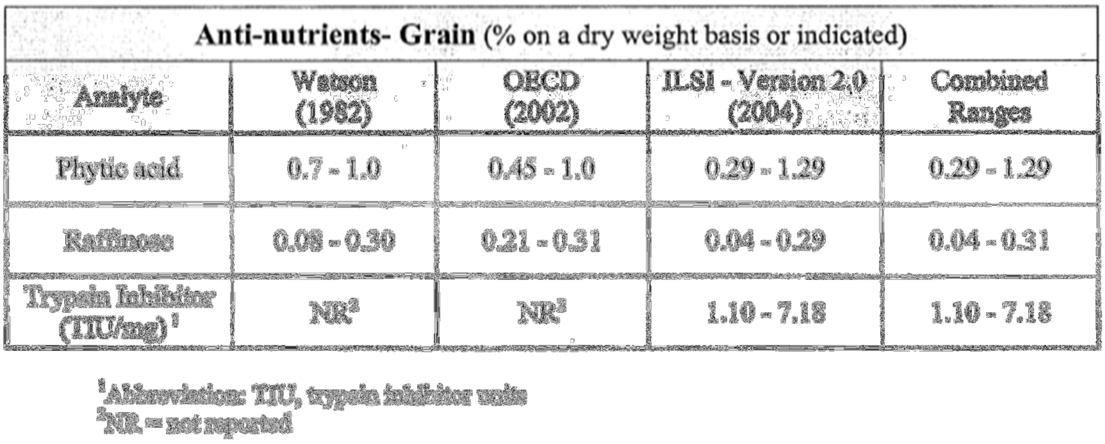
Table 30. Summary of Analysis of Anti-Nutrients in Grain for the 59122 x NK603 + Glyphosate fb Glufosinate and Control Hybrids (North America 2003)



Analyte[1]	Combined Ranges[2]	Means[3]		
		59122xNK603 + Glyphosate fb Glufosinate	Control	Standard Error
Raffinose	0.04 - 0.31	0.19*	0.14	0.012
Phytic acid	0.29 - 1.29	0.841*	0.736	0.0291
Trypsin Inhibitor (TIU/g)[4]	1.10 - 7.18	2.86	2.76	0.0450

[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 59122xNK603 + glyphosate fb glufosinate and control

Table 31. Literature Ranges of Anti-Nutrients in Grain



Anti-nutrients- Grain (% on a dry weight basis or indicated)				
Analyte	Watson (1982)	OECD (2002)	ILSI - Version 2.0 (2004)	Combined Ranges
Phytic acid	0.7 - 1.0	0.45 - 1.0	0.29 - 1.29	0.29 - 1.29
Raffinose	0.08 - 0.30	0.21 - 0.31	0.04 - 0.29	0.04 - 0.31
Trypsin Inhibitor (TIU/mg)[1]	NR[2]	NR[2]	1.10 - 7.18	1.10 - 7.18

[1] Abbreviation: TIU, trypsin inhibitor units
[2] NR = not reported

Table 32. Agronomic data (per location and across locations data) from maize stack 59122 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 USA and one location in Canada (2003 growing season).

	Germination/ Early population[1]	Seedling vigor[2]	GDU 50% silking[3]	GDU 50% pollen shed[4]	Plant height (cm)[5]	Ear height (cm)[6]	Stalk lodging (%)[7]	Root lodging (%)[8]	Stay Green[9]	Disease incidence[10]	Insect damage[11]	Pollen colour[12]	Pollen shape[13]
Location 1: Richland, Iowa													
59122xNK603	59	9	1166*	1190	101	39	2	0	3	8	7	95	100
Non-GM control	58	9	1123	1158	105	41	0	0	4	8	6	99	100
Location 2: Bagley, Iowa													
59122xNK603	53	6	1153	1251	85	34	0	0	7	5	7	43	62
Non-GM control	53	6	1153	1243	81	33	0	0	7	5	7	37	50
Location 3: Rochelle, Illinois													
59122xNK603	58	8	1247	1269	103	46	0	0	6	8	9	67	73
Non-GM control	58	8	1247	1269	103	46	0	0	6	8	9	83	85
Location 4: Noblesville, Indiana													
59122xNK603	51	8	1267	1282*	93	32	0	0	5	8	8	95	88
Non-GM control	51	8	1245	1245	92	32	0	4	4	8	7	92	90
Location 5: Geneva, Minnesota													
59122xNK603	45	7	1258	1276	94	43*	0	0	8	7	9	95	95
Non-GM control	46	8	1227	1250	93	40	0	0	8	8	9	95	95
Location 6: Branchton, Ontario													
59122xNK603	57	8	1362	1394	93*	32*	0	0	3	9	9	72	73
Non-GM control	57	8	1362	1394	91	30	0	0	3	9	9	72	78
Average													
59122xNK603	54	8	1242*	1277*	95*	38	0	0	5	8	8	78	82
Non-GM control	54	8	1226	1260	94	37	0	1	5	8	8	80	83

[1] Number of plants emerged per 60 seeds planted.

[2] A visual estimate was taken of seedling vigor of emerged plants. Seedling vigor was assessed using a 1 to 9 scale, where 1 correlates to short plants with small, thin leaves, and 9 correlates to tall plants with large, robust leaves.

[3] Number of accumulated heat units when approximately 50% of the plants are silking.

[4] Number of accumulated heat units when approximately 50% of the plants are shedding pollen.