Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Food/feed and environmental risk assessment of insect-resistant and herbicide-tolerant genetically modified maize 1507 x 59122 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (EFSA/GMO/NL/2005/15)

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

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

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Summary

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Environment Agency has requested the Norwegian Food Safety Authority 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 sectorial responsibility. 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 scientific risk assessments of 39 GMOs and products containing or consisting of GMOs that are authorized in the European Union. 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 requests VKM to consider whether updates or other changes to earlier submitted assessments are necessary.

The insect-resistant and herbicide-tolerant genetically modified maize 1507 x 59122 from Dow AgroSciences and Pioneer Hi-Bred International, Inc. is approved under Regulation (EC) No 1829/2003 for food and feed uses, import and processing since 28 July 2010 (Commission Decision 2010/432/EC).

Genetically modified maize 1507 x 59122 has previously been risk assessed by the VKM Panel on Genetically Modified Organisms (GMO), commissioned by the Norwegian Food Safety Authority and the Norwegian Environment Agency related to the EFSAs public hearing of the applications EFSA/GMO/NL/2005/15 and EFSA/GMO/NL/2005/28 in 2007 (VKM 2007a, 2008a). In addition, 1507 x 59122 has been evaluated by the VKM GMO Panel as single events and as a component of several stacked GM maize events (VKM 2004, VKM 2005a,b, VKM 2007b,c, VKM 2008b,c, VKM 2009a,b, VKM 2012).

The risk assessment of the maize 1507 x 59122 is based on information provided by the applicant in the applications EFSA/GMO/NL/2005/15 and EFSA/GMO/NL/2005/28, 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 1507 x 59122 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) and the selection of comparators for the risk assessment of GM plants (EFSA 2011b).

The scientific risk assessment of maize 1507 x 59122 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 1507 x 59122 was produced by conventional breeding between inbred lines of maize containing the 1507 and 59122 events. The hybrid was developed to provide protection against certain lepidopteran and coleopteran target pests, and to confer tolerance to glufosinate-ammonium herbicides.

Molecular characterisation

As conventional breeding methods were used in the production of maize 1507 x 59122, no additional genetic modification was involved. Southern and PCR analyses demonstrated that the recombinant insert in the single 1507 and 59122 events were retained in maize stack 1507 x 59122. Genetic stability of the inserts has been demonstrated in the parental lines 1507 and 59122. Phenotypic analyses demonstrated stability of the insect resistance and herbicide tolerance traits in the hybrid. The expression levels of Cry1F, Cry34Ab1/Cry35Ab1 and PAT proteins in seeds and forage were considered comparable with those in the single events.

Comparative assessment

Comparative analyses of data from field trials located at representative sites and environments in the USA and Europe indicate that maize stack 1507 x 59122 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, with the exception of the lepidopteran and coleopteran-protection traits and herbicide tolerance, conferred by the expression of the Cry1F, Cry34Ab1/Cry35Ab1 and PAT proteins. Based on the assessment of available data, the VKM GMO Panel is of the opinion that conventional crossing of maize 1507 and 59122 to produce the hybrid 1507 x 59122 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 studies in rats and broilers indicate that maize 1507 x 59122 is nutritionally comparable to conventional maize. Bioinformatics analyses have not disclosed expression of any known ORFs in the parental maize events, and none of the newly expressed proteins show resemblance to any known toxins or IgE allergens. None of the proteins have 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 maize 1507 x 59122.

Based on the current knowledge, the VKM GMO Panel concludes that 1507 x 59122 maize is nutritionally equivalent to conventional maize varieties, and that it is unlikely that newly expressed proteins introduce a toxic or allergenic potential in food and feed derived from maize 1507 x 59122 compared to conventional maize.

Environmental risk assessment

The scope of the application EFSA/GMO/NL/2005/15 includes import and processing of maize 1507 x 59122 for food and feed uses. Considering the intended uses of maize 1507 x 59122, excluding cultivation, the environmental risk assessment is concerned with accidental release into the

environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize 1507 x 59122.

Maize 1507 x 59122 has no altered survival, multiplication or dissemination characteristics, and there are no indications of an increased likelihood of spread and establishment of feral maize plants in the case of accidental release into the environment of seeds from maize 1507 x 59122. 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 1507 x 59122 or its processed products compared to conventional maize. Based on current knowledge, it is also unlikely that the Cry1F, Cry34Ab1 and Cry35Ab1 proteins will increase the allergenic potential of food and feed derived from maize 1507 x 59122 compared to conventional maize varieties. The VKM GMO Panel likewise concludes that maize 1507 x 59122, based on current knowledge, is comparable to conventional maize varieties concerning environmental risk in Norway with the intended usage.

Keywords

Maize, *Zea mays* L., genetically modified maize 1507 x 59122, EFSA/GMO/NL/2005/15, insect-resistance, herbicide-tolerance, Cry proteins, *cry34Ab1, cry35Ab1, cry1F*, PAT, glufosinate-ammonium, 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 har Miljødirektoratet (tidligere Direktoratet for Naturforvalting) bedt Mattilsynet om vurderinger 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. På den bakgrunnen har Mattilsynet, i brev av 13. februar 2013 (ref. 2012/150202), bedt Vitenskapskomiteen for mattrygghet (VKM) om å utarbeide endelige vitenskapelige risikovurderinger av 39 GMOer og avledete produkter som inneholder eller består av genmodifiserte organismer, innen Mattilsynets sektoransvar. VKM er bedt om endelige risikovurderinger for de EU-godkjente søknader hvor VKM ikke har avgitt endelig risikovurdering. I tillegg er VKM bedt om å vurdere hvorvidt det er nødvendig med oppdatering eller annen endring av de endelige risikovurderingene som VKM tidligere har levert.

Den insektsresistente og herbicidtolerante maishybriden 1507 x 59122 (unik kode DAS-Ø15Ø7-1 x DAS-59122-7) fra Dow AgroScience og Pioneer Hi-Bred International ble godkjent til import, videreforedling og til bruk som mat og fôr under EU-forordning 1829/2003 i 2010 (søknad EFSA/GMO/NL/2005/15, Kommisjonsbeslutning 2010/432/EC).

Maishybriden har tidligere vært vurdert av VKMs faggruppe for genmodifiserte organismer med hensyn på mulig helserisiko i forbindelse med EFSAs offentlige høring av søknaden i 2007 (VKM 2007a). En søknad om godkjenning av maishybrid 1507 x 59122 til dyrking (EFSA/GMO/NL/2005/28), som var på offentlig høring høsten 2007, er også vurdert av faggruppen med hensyn på mulig miljørisiko (VKM 2008a). Foreldrelinjene 1507 og 59122 er også tidligere risikovurdert av VKM, både som enkelt-eventer og i en rekke andre hybrider (VKM 2004, 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. Videre er agronomiske egenskaper, potensiale for utilsiktede effekter på fitness, genoverføring og effekter på ikke-målorganismer vurdert.

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

F_1-hybriden 1507 x 59122 er resultat av konvensjonelle kryssinger mellom de genmodifiserte maislinjene 1507 og 59122. Kryssingene er utført for å utvikle en maishybrid med resistens mot visse skadegjørere i sommerfuglordenen Lepidoptera og billeslekten *Diabrotica*, samt toleranse mot herbicider med virkestoff glufosinat-ammonium.

Foreldrelinjen 1507 har fått innsatt et *cry1F*-gen fra bakterien *Bacillus thuringiensis* var. *aizawai* og et *pat*-gen, som er isolert fra *Streptomyces viridochromogenes*. *Cry1F*-genet koder for et δ-endotoksin og

gir resistens mot enkelte arter i sommerfuglordenen Lepidoptera, eksempelvis maispyralide (*Ostrinia nubilatis*) og nattflyarten *Sesamia nonagrioides*. *Pat*-genet koder for enzymet fosfinotricin acetyltransferase (PAT), som acetylerer og inaktiverer glufosinat-ammonium, virkestoffet i fosfinotricin-herbicider av typen Finale. Fosfinotricin er et ikke-selektivt kontaktherbicid som hemmer glutaminsyntetase. Enzymet deltar i assimilasjonen av nitrogen og katalyserer omdanning av glutamat og ammonium til aminosyren glutamin. Hemming av glutaminsyntetase fører til akkumulasjon av ammoniakk, og til celledød i planten. De transgene maisplantene vil derfor tolerere høyere doser av sprøytemiddelet glufosinat sammenlignet med konkurrerende ugras.

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.

Molekylær karakterisering
Maishybriden 1507 x 59122 er dannet ved konvensjonell kryssing mellom maislinjene 59122 og 1507. 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 Cry1F-, Cry34Ab1-, Cry35Ab1- og PAT- proteiner i vegetativt vev og frø er sammenlignbare med uttrykk av tilsvarende proteinprodukter i foreldrelinjene.

Komparative analyser
Feltforsøk over en vekstsesong i henholdsvis Nord-Amerika og Europa viser små eller ingen signifikante forskjeller mellom den transgene maishybriden 1507 x 59122 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 1507 x 59122 og umodifisert kontroll, og at de innsatte genene i 1507 x 59122 ikke har medført utilsiktede endringer i egenskaper knyttet til vekst og utvikling hos maisplantene.

Helserisiko
Fôringsstudier utført på rotter og broiler med mais 1507x 59122, har ikke indikert helseskadelige effekter av maislinjen sammenlignet med umodifisert mais. 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).

Eksponeringsstudier på gnagere indikerer ingen toksisitet relatert til proteinene Cry1F, Cry34Ab1, Cry35Ab1, PAT og CP4 EPSPS. Denne typen studier gir derimot ingen tilleggsinformasjon om mulige helseskadelige egenskaper ved mais 1507 x NK603.

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at mais 1507 x 59122 er næringsmessig vesentlig lik konvensjonell mais, og at det er lite trolig at de nye proteinene vil introdusere et toksisk eller allergent potensiale i mat og fôr basert på mais 1507 x 59122 sammenliknet med konvensjonelle maissorter.

Miljørisiko
Søknaden gjelder godkjenning av maishybrid 1507 x 59122 for import, prosessering og til bruk i næringsmidler og fôrvarer, og omfatter ikke dyrking. Med bakgrunn i tiltenkt bruksområde er miljørisikovurderingen avgrenset til mulige effekter av utilsiktet frøspredning i forbindelse med

transport og prosessering, samt indirekte eksponering gjennom gjødsel fra husdyr fôret med genmodifisert mais.

Det er ingen indikasjoner på økt sannsynlighet for spredning, etablering og invasjon av maislinjen i naturlige habitater eller andre arealer utenfor jordbruksområder som resultat av frøspill i forbindelse med transport og prosessering. Risiko for utkryssing med dyrkede sorter vurderes av GMO panelet til å være ubetydelig. Ved foreskreven bruk av maislinjen 1507 x 59122 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 1507 x 59122 eller prosesserte produkter sammenliknet med konvensjonell mais. Basert på dagens kunnskap er det også lite trolig at Cry1F, Cry34Ab1 eller Cry35Ab1 proteinene vil øke det allergene potensialet til mat og fôr produsert fra mais 1507 x 59122 sammenliknet med konvensjonelle maissorter. Faggruppen finner at maishybrid 1507 x 59122, 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
Cry1F	Cry1 class crystal protein from *Bacillus thuringiensis* var. *aizawai*
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
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
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 30 May 2005, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands an application (Reference EFSA/GMO/NL/2005/15) for authorisation of the insect-resistant and herbicide tolerant genetically modified (GM) maize 1507 x 59122 (Unique Identifier DAS-Ø15Ø7-1 x DAS-59122-7), submitted by Dow AgroScience and Pioneer Hi-Bred International, Inc. within the framework of Regulation (EC) No 1829/2003.

The scope of the application covers:

- Import and processing of maize 1507 x 59122
- GM plants for food and feed use
- Food and feed, containing or consisting of maize 1507 x 59122
- Food and feed produced from maize 1507 x 59122
- Food containing ingredients produced from maize 1507 x 59122

After receiving the application EFSA/GMO/NL/2005/15 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 6 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 21 April 2009 (EFSA 2009b), and maize 1507 x 59122 was approved for food and feed uses, import and processing in 28 July 2010 (Commission Decision 2010/432/EC).

An application for authorisation of maize 1507 x 59122 for cultivation in the EU was submitted by Dow AgroScience in December 2005 (EFSA/GMO/NL/2005/28). VKM participated in the 90 days public consultation of the application in autumn 2007, and submitted a preliminary opinion in May 2008 (VKM 2008a). The clock for the application was however stopped by EFSA in September 2007, pending the finalization of the risk assessment of the parental line 59122 (application EFSA/GMO/NL/2005/23). The EFSA GMO Panel adopted its scientific opinion on maize 59122 in March 2013 (EFSA 2013), and the clock for application EFSA/GMO/NL/2005/28 was restarted.

Scientific opinions on the parental lines of the stack 1507 x 59122 have previously been submitted by the VKM GMO Panel (VKM 2004, 2005a, 2008b). In addition, maize 1507 and 59122 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 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.

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Environment Agency has requested the Norwegian Food Safety Authority 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 1507 x 59122 has been obtained from traditional breeding methods between progeny (inbred lines) of the genetically modified maize lines 1507 and 59122.

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

The parental line 59122 expresses the *cry34Ab1* and *cry34Ab1*genes from *B. 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.*

None of the target pests for maize 1507 and maize 59122 are present in the Norwegian agriculture. The PAT protein expressed in maize 1507 and 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 1507 x 59122 has been evaluated with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed.

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

The environmental risk assessment of the genetically modified maize 1507 x 59122 is based on information provided by the applicant in the applications EFSA/GMO/NL/2005/15 and EFSA/GMO/NL/2005/28, 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 1507 x 59122

According to the applicant, conventional breeding methods were used to develop the insect-resistant and herbicide-tolerant maize 1507 x 59122, and no genetic modification was involved. The two inserts present in maize 1507 x 59122 were derived from two independent events: 1507 and 59122, and combines resistance to certain lepidopteran and coleopteran pests, and tolerance to glufosinate-ammonium based herbicides.

2.1.2 Summary of evaluation of the single events

2.1.2.1 Maize 1507

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

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

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

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

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

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

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

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

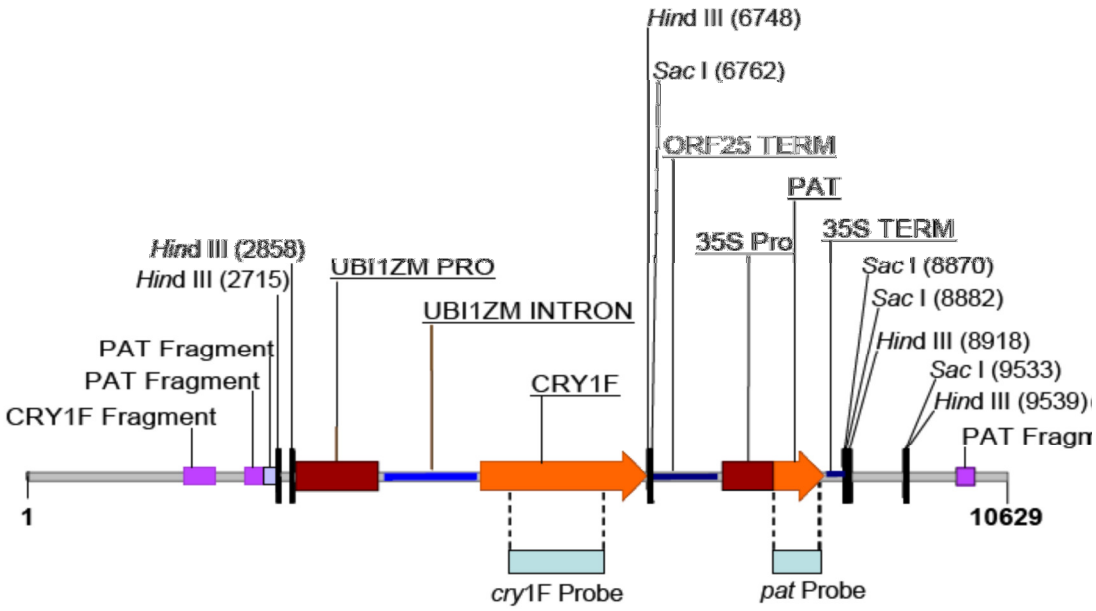

Figure 1. Restriction map of the various gene elements of the recombinant DNA fragment inserted in the genome of the maize strain 1507.

2.1.2.2 Maize 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 components to regulate gene expression.

The maize *cry34Ab1* gene is 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 *cry*34Ab1 gene is identical to the Cry34Ab1 protein (14 kDa) expressed in the bacteria. Expression of the maize *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 *cry35Ab1* gene is 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 *cry35Ab1* gene is identical to the Cry35Ab1 protein expressed by 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 expresses the phosphinothricin-N-acetyltransferase (PAT) protein from *Streptomyces viridochromogenes* (previously described).

The levels 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 only 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 USA/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 100 amino acids.

Southern blot and sequence analysis show 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 or other backbone sequences not intended for transformation. In addition, PCR amplification and sequence analysis have confirmed 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 2.

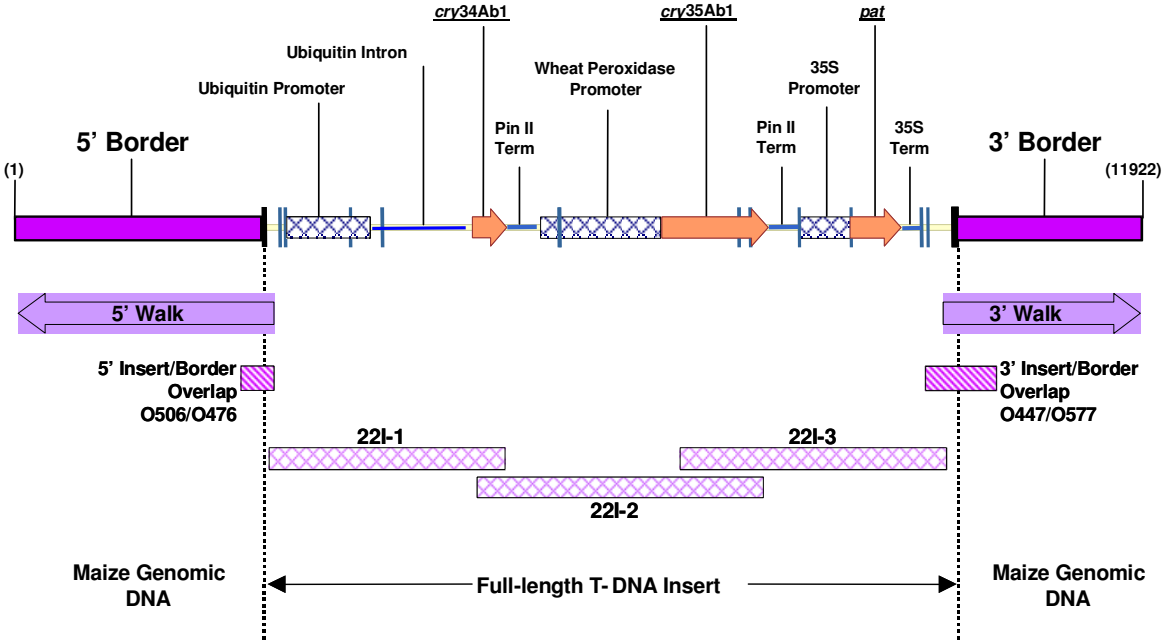

Figure 2. Restriction map of the various gene elements of the recombinant DNA fragment inserted in the genome of the maize strain 59122.

2.1.3 Transgene constructs in maize 1507 x 59122

According to the applicant, the 1507 x 59122 maize has been obtained by conventional crossbreeding of two genetically modified parental maize lines. No new genetic modification was used for the development of the 1507 x 59122 maize.

Using the *cry1F*, *cry34Ab1*, *cry35Ab1* and *pat* probes, southern blot hybridization showed intactness of the inserts, including their flanking sequences, present in 1507x59122 maize compared to the inserts in the 1507 and the 59122 maize. These Southern analyses with the inserted gene probes showed that the insertions in the 1507 maize and the 59122 maize were equivalent to that of 1507 x 59122 maize indicating that it was a successful cross of the two lines: the 1507 maize and the 59122 maize.

Hind III digestion was selected for comparing the 1507 x 59122 maize to 1507 maize. *Hin*d III sites are indicated on the 1507 maize insertion map in Figure 1. Two bands were expected to hybridize to the *cry*1F probe based on the insertion map, a single band of 3890 bp and one greater than 2715 bp (Figure 1). Consistent with the insertion map, two fragments, one of 3890 bp and one of 4200 bp, were observed in all of the samples of the 1507 maize and the 1507x59122 maize. Indicating that the inserts in 1507 maize and 1507 x 59122 maize are equivalent to each other. Using the *pat* probe and *Hind* III digestion, three bands were expected to hybridize to the 1507 maize insert, a single band of 2170 bp, one of approximately greater than 2715 bp, and a third band of approximately greater than 1090 based on the 1507 maize insertion map (Figure 1). In addition, the T-DNA of PHP17662 was also expected to hybridize to the *pat* probe, resulting in an internal fragment of 6963 bp (Figure 1). Three bands were observed in 1507 maize, one of 2170 bp, one of approximately 2300 bp and a faintly hybridizing band of approximately 4100 bp. A single band of 6963 bp was observed in the 59122 maize.

Sac I digestion was selected for comparing 1507x59122 maize to 59122 maize. *Sac* I sites are indicated on the T-DNA insertion in 59122 maize in Figure2. Hybridization of the *cry*34Ab1 probe with individual plants containing the DAS-59122-7 insertion was expected to result in a border fragment of approximately 3400 bp based on the T-DNA insertion map (Figure 2). This fragment was observed in both the 59122 maize and the 1507x59122 maize. The 59122 maize and the 1507x59122 maize exhibited the same hybridization pattern with the *cry*34Ab1 probe, indicating that the insert present in the 59122 maize was equivalent to that found in the 1507x59122 maize. Using the *cry*35Ab1 probe, three internal bands, one of 1855 bp, one of 1941 bp and one of 123 bp, were expected to hybridize in the *Sac* I digestion based on the T-DNA map derived from binary vector PHP17662 and consistent with the T-DNA insertion in 59122 maize. The 1855 bp and 1941 bp fragments were observed in both the 59122 maize and the 1507x59122 maize, indicating that the 1507x59122 maize contained the same insertion as the 59122 maize. The predicted 123 bp fragment was not detected, as fragments below approximately 1000 bp ran off the gel during electrophoresis and were not transferred to the nylon membrane.

The *pat* probe was expected to hybridize to both the 1507 maize and the 59122 maize. For the 59122 maize, a band of 1855 bp was expected to hybridize with the *Sac* I digestion. For 1507 maize, three bands were expected to hybridize, a band of 2108 bp, a band greater than 1096 bp, and a band greater than 6762 bp (Table 4). A 1855 bp band was observed in 59122 maize and three bands were observed in 1507 maize, a band of 2108 bp, a band of approximately 5700 bp, and a band approximately 8576 bp. All four fragments were observed in the 1507x59122 maize, indicating that the 1507x59122 maize contained the same insertion as those found in the 1507 maize and the 59122 maize.

None of the gene probes, *cry*1F, *pat*, *cry*34Ab1, or *cry*35Ab1 hybridized to control samples analyzed in Southern analysis. C*ry*1F did not hybridize to 59122 maize or PHP17662 plasmid control nor did *cry*34Ab1 and *cry*35Ab1 hybridize to 1507 maize or PHP8999 plasmid control.

2.1.4 Information on the expression of insert

Two field studies have been carried out in order to estimate the level of expression of the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in forage and grain obtained from 1507x59122 maize (Table 1 and 2). One field study was carried out, in Europe in 2004, in order to estimate the level of Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in forage and grain obtained from the 1507x59122 maize. The field study was conducted at five field sites located in major maize growing regions of: Spain (three locations), Hungary (one location) and Bulgaria (one location). These locations are representative of regions where maize is commercially grown in Europe. Another field study was conducted at five field sites located in the major maize growing regions of U.S. and Canada in 2003. These locations are representative of regions where maize is commercially grown in North America and are comparable to regions where the maize varieties would be suitable as commercial products in the EU. Another field study was conducted at five field sites located in the major maize growing regions of U.S. and Canada in 2003. These locations are representative of regions where maize is commercially grown in North America and are comparable to regions where the maize varieties would be suitable as commercial products in the EU.

Levels of Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in grain from 1507x59122 maize was characterized using a specific Enzyme Linked Immunosorbent Assay (ELISA) developed specifically for each protein. In the European study, Cry1F, Cry34Ab1 and Cry35Ab1 proteins was detected in leaf, pollen, silk, stalk, whole plant, grain, and senescent whole plant tissue samples from the 1507x59122 maize throughout the growing season. With the exception of R1 pollen, measurable concentration of the PAT protein was detected in all tissues assayed for the 1507x59122 maize. The forage and grain samples were taken from plots that were sprayed with glufosinate-ammonium herbicide or unsprayed. Levels of Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins, in forage and grain, were comparable regardless of the application of glufosinate-ammonium herbicide. The results are summarized in Table 1. In the U.S. and Canadian study grain samples were taken from plots that were sprayed with glufosinate-ammonium herbicide or unsprayed. The results obtained from the expression analysis have been summarized in Table 2. Levels of Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins were comparable to each other, regardless of the application of glufosinate-ammonium herbicide.

Cry1F
In the European study, the level of Cry1F protein ranged, in forage, from **8.34 to 12.5** µg/g dry weight and, in grain, from **1.02 to 3.48** µg/g dry weight. In the U.S. and Canadian study, the level in grain ranged from **1.70 to 2.04** µg/g dry weight. These results are comparable to expression level of Cry1F protein in grain from 1507 maize, which ranged from **1.2 to 3.1** µg/g dry weight.

Cry34Ab1
In the European study, the Cry34Ab1 was expressed, in forage, at levels ranging from **75.1 to 127** µg/g dry weight and in grain from **20.4 to 120** µg/g dry weight. In the U.S. and Canadian study, the level in grain ranged from **42.9 to 45.7** µg/g dry weight. These results are comparable to the levels of the Cry34Ab1 protein in 59122 maize, which ranged, in forage, from **90.1 to 100** µg/g dry weight (mean range across EU sites in 2003-2004) and in grain from **39.0 to 40.4** µg/g dry weight.

Cry35Ab1
In the European study, the Cry35Ab1 protein was detected, in forage at levels from **30.5 to 58.0** µg/g dry weight and in grain, from **0.29 to 1.50** µg/g dry weight. In the U.S. and Canadian study, the levels in grain ranged from **1.41 to 1.61** µg/g dry weight. These results are comparable to the levels of the Cry34Ab1 protein in 59122 maize, which are in the same order of magnitude as expression levels in 59122 maize, which ranged in forage from **41.3 to 52.5**µg/g dry weight (mean range across EU sites in 2003-2004) and in grain from **1.05 to 1.11** µg/g dry weight.

PAT

In the European study, levels of combined expression, from 1507 maize and 59122 maize, of the PAT protein in 1507x59122 maize, ranged, in forage, from **1.87 to 6.15** µg/g dry weight and in grain from **0.00 to 0.210** µg/g dry weight. In the U.S. and Canadian study, levels of combined mean expression of the PAT protein ranged from **N.D. to 0.44** µg/g dry weight. These results are comparable with the levels of the PAT protein in 1507 maize and 59122 maize, which were generally below their limit of detection.

Table 1. Levels of the Cry1F, Cty34Ab1, Cry35Ab1 and PAT proteins in grain and forage from 1507 x 59122 maize plants sprayed with glufosinate and unsprayed. Data from field trials in Europe in 2004 (Buffington 2005, Unpublished technical report).

Hybrid	Tissue	Mean (µg/g d.w.)	Standard Deviation	Range (µg/g d.w.)
Cry1F Protein				
1507 x 59122 (untreated)	Grain	2.23	0.629	1.02-3.48
1507 x 59122 (untreated)	Forage	10.8	1.27	9.51-12.5
1507 x 59122 +GA[1]	Grain	2.01	0.489	1.42-3.06
1507 x 59122 +GA	Forage	9.61	1.43	8.34-11.8
Cry34Ab1 Protein				
1507 x 59122 (untreated)	Grain	43.5	22.9	22.4-110
1507 x 59122 (untreated)	Forage	105	13.8	90.1-127
1507 x 59122 +GA	Grain	51.6	28.0	20.4-120
1507 x 59122 +GA	Forage	100	16.3	75.1-118
Cry35Ab1 Protein				
1507 x 59122 (untreated)	Grain	0.591	0.318	0.34-1.30
1507 x 59122 (untreated)	Forage	38.1	8.11	30.5-51.7
1507 x 59122 +GA	Grain	0.680	0.417	0.29-1.50
1507 x 59122 +GA	Forage	43.4	9.54	32.4-58.0
PAT Protein				
1507 x 59122 (untreated)	Grain	0.0240	0.0515	0.000-0.150
1507 x 59122 (untreated)	Forage	3.79	1.43	1.87-5.26
1507 x 59122 +GA	Grain	0.0473	0.0856	0.000-0.210
1507 x 59122 +GA	Forage	4.34	1.70	1.88-6.15

[1] Plots treated with glufosinate-ammonium (GA)

Table 2. Expression of Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in grain from 1507x59122 maize plants sprayed with glufosinate and unsprayed. Data from field trials in USA and Canada in 2003 (Buffington 2004, Unpublished technical report**).**

Hybrid	**Mean (µg/g d.w.)**	**Standard Deviation**	**Min/max range (µg/g d.w.)**
Cry1F Protein			
1507 x 59122 (untreated)	1.70	0.58	0.56/2.86
1507 x 59122 +GA[1]	2.04	0.74	0.96/3.81
Cry34Ab1 Protein			
1507 x 59122 (untreated)	42.9	11.7	23.5/69.1
1507 x 59122 +GA	45.7	9.5	33.6/63.3
Cry35Ab1 Protein			
1507 x 59122 (untreated)	1.41	0.50	0.82/2.78
1507 x 59122 +GA	1.61	0.70	0.64/3.35
PAT Protein			
1507 x 59122 (untreated)	0.10	0.14	N.D./0.44
1507 x 59122 +GA	0.11	0.40	N.D./0.37

[1] Plots treated with glufosinate-ammonium (GA)

ORF sequence comparisons

Out of a potential maximum number of twelve ORFs, only one ORF (referred to as RB-2 ORF) was identified that spans the right T-DNA border of the 59122 maize. The hypothetically translated amino acid sequence of the RB-2 ORF consists of 45 amino acids.

Bioinformatics analysis including a sequence comparison against databases of known toxic and allergenic proteins has been carried out with the deduced amino acid sequence of the RB-2 ORF. Absence of any significant homology to known protein toxins was determined through a global sequence homology search for the RB-2 ORF amino acid sequence against the GenPept "nr" and Uniprot datasets using the BLASTP 2.2.11 algorithm. A cutoff expectation value (E-value) of 1.0 was used to detect biological meaningful homology between the deduced amino acid sequence of the RB-2 ORF and proteins in the database. In the case of the amino acid sequence of the RB-2 ORF no stretches of six, seven, eight or more contiguous amino acids were found to be identical to strings found in any of the known protein allergens.

Overall, the results of the bioinformatics analyses indicate that there are neither potential fusion proteins with significant sequence homology to known protein toxins nor potential fusion proteins with significant sequence similarity to known protein allergens in the 59122 maize.

2.1.5 Inheritance and stability of inserted DNA

Both, the 1507 maize and the 59122 maize, incorporated a single DNA insert containing a single copy of the inserted DNA fragment, at different loci, in the maize genome. Southern blot analyses have shown that the integrity of the inserts in the single events in 1507 and 59122 maize are preserved in the hybrid 1507 x 59122.

Segregation analysis has shown that both: 1507 maize and 59122 maize inserts are inherited in a Mendelian fashion, i.e. the inserts are stably inherited as single, independent and dominant genes.

The maize strain Hi-II with the 1507 event was crossbred with one of Pioneers elite strains and back crossed over six generations. Genetic stability of the inserted gene construct was shown by segregation- and southern blot – analysis. In addition, field studies have shown over several growth seasons in Europe and the US that the inserted genes are stably incorporated in the maize genome.

Genetic stability of the inserted gene construct was evaluated through Southern blot and segregation analysis of four different generations (T1S1, T1S2, BC1 and BC2S1). The breeding strain Hi-II with the 59122 event (T0) was crossbred with the inbred elite strain PH098B to make the F_1 generation. The F_1 plants were self-pollinated to generate the T1S and T1S2 generations. To produce the BC1-hybride the F1-plants were crossed and backcrossed with the inbred strain 05F, and then crossed with yet another inbred strain, 581. To produce the BC2S1 generation, F1 plants were crossed and backcrossed twice with the inbred strain 581, and finally self-pollinated. Analysis of the progeny from the BC2S1 generation displayed the expected Mendelian inheritance of herbicide tolerance and expression of Cry34Ab1. Analyses of Cry34Ab1/35Ab1 and PAT expression data from field studies spanning two growth seasons in Europe, North- and South- America indicate phenotypic stability.

2.2 Conclusion

Southern blot and PCR analyses have shown that the recombinant inserts in the parental maize events 1507 and 59122 are retained in the stacked maize 1507 x 59122. Genetic stability of the inserts has previously been demonstrated in the parental events. The levels of Cry1F, Cry34Ab1, Cry35Ab1and PAT 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 in the stacked event.

The VKM Panel on GMO considers the molecular characterisation of maize 1507 x 59122 and its parental events 1507 and 59122 as adequate.

3 Comparative assessment

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

3.1.1 Experimental design & statistical analysis

Application EFSA/GMO/NL/2005/15
In the application EFSA/GMO/NL/2005/15 for food and feed uses, import and processing of maize 1507 x 59122 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, agronomic data derived from material obtained from field trials with the single events and the respective comparators were provided by the applicant.

The field trials in North America were performed at five separate sites in commercial maize-growing regions of the USA (Iowa, Indiana and Nebraska) and two field sites in Ontario, Canada. These trials compared the composition of maize 1507 x 59122 with a conventional counterpart having a genetic background representative of the test entry 1507 x 59122 (near-isoline hybrid, Pioneer brand commercial hybrid 36B08). Upon request of the EFSA GMO Panel, the applicant provided additional information on the breeding scheme used to produce the conventional control maize. According to EFSA, the pedigree information on the control, non-GM maize showed that the control had a genetic background comparable with that of maize 1507 x 59122 and thus represented an appropriate comparator for the F_1 hybrid 1507 x 59122 in the field trials (EFSA 2009b).

No conventional commercial reference varieties were included in the field trials and the comparative assessments. 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 1507 x 59122 for consideration of natural variations.

At each trial site, maize 1507 x 59122 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. Plots of the test entry 1507 x 59122 maize either received two sequential applications of herbicide containing glufosinate-ammonium or were unsprayed. The first application was applied at a rate that ranged from 0.36 to 0.38 lb ai/A (pounds of active ingredients per acre) at the V4 growth stage. The second application, at V7 growth stage, was applied at a rate ranging from 0.44 to 0.45 lb ai/A. The agronomic and phenotypic analyses were carried out from the same fields as the compositional analyses, but only from plots with the non GM-control and plots with the test entry 1507 x 59122 treated with glufosinate.

Analysis of variance (ANOVA) was conducted according to a randomized complete block design, and agronomic characteristics data were statistically analysed to test for differences between the test entry and the conventional control. Data analysis was completed on the following agronomic characteristics: stalk lodging, root lodging, stay green, disease incidence and insect damage. However, since no differences were identified, the applicant has not reported any statistical analysis on these characteristics. Statistical analysis was performed on data on maize material from both individual and combined field trial sites.

Compositional analyses, including a comparative assessment of the 1507x59122 maize with non-GM maize of comparable genetic background has been carried out. Grain samples from 1507x59122 maize (all herbicide treatments) and non-GM control maize were collected and analyzed for nutrient composition, including: proximates, fiber, fatty acids, amino acids, minerals, vitamins, secondary metabolites, and anti-nutrients. Statistical analysis of agronomic characteristics and nutrient composition data was conducted using SAS/STAT software, Version 8.2 to generate analysis of variance (ANOVA), means, and standard deviations.

Two separate statistical analyses were carried out on the composition data. For the first analysis, the data from all replicates and all locations were combined and analyzed. Least-square means and standard deviation were calculated for the data across all 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 of the separate locations. 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. In addition, comparisons with baseline data on commercial maize, compiled from publicly available literature, were used in the comparisons with the 1507x59122 maize.

Application EFSA/GMO/NL/2005/28

The application EFSA/GMO/NL/2005/28, covering authorisation of maize 1507 x 59122 for all food and feed uses, including cultivation, include results from field trials with maize 1507 x 59122 in Europe in 2004. The study was conducted at five separate field locations, with three locations in Spain, one in Hungary and one in Bulgaria. At each trial site, maize 1507 x 59122 and the conventional counterpart were planted following a randomized complete block design containing four blocks: three blocks were used for the compositional analysis and the additional block was used for protein expression analysis. Each block contained maize stack 1507 x 59122 and a non-GM control for comparison. Plots of the 1507 x 59122 maize either was left untreated or was treated with two applications of an herbicide containing the active ingredient glufosinate-ammonium. Agronomic characteristics of the untreated test line and the non-transgenic near isoline control were recorded over the course of the growing season.

Statistical analysis of agronomic characteristics and nutrient composition data was conducted using SAS/STAT software, Version 8.2 to generate analysis of variance (ANOVA), means, and standard deviations.

Two separate statistical analyses were carried out on the composition data. For the first analysis, the data from all replicates and all locations were combined and analyzed. Least-square means and standard deviation were calculated for the data across all 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 of the separate locations. 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. This indicates that there was a 5% chance that the non-significant differences in response variables were expected to be declared significant (false discovery) due to

sampling variability. In addition, the baseline data on commercial maize, compiled from publicly available literature, was used in the comparisons with maize 1507 x 59122.

3.2 Compositional analysis

The North American field trials (Application EFSA/GMO/NL/2005/15)
Considering that the application does not include authorisation for the cultivation of 1507x59122 maize seed products, only the results of the nutritional analysis on grain is presented in the application. Nutritional analysis for forage from the 1507x59122 maize (treated and untreated) and control maize is presented in Table 1 and 2- appendix (Buffington 2004, Unpublished technical report).

Grain and forage samples from the 1507 x 59122 maize and control maize were collected and analysed for nutrient composition. Forage was analysed for crude protein, crude fat, ash, crude fiber, ADF, NDF, carbohydrates. Analysis of grain included: proximates (crude protein, crude fat, ash) crude fiber, ADF, NDF, carbohydrates, fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acids), amino acids (methionine, cysteine, 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, raffinose, furfural, p-coumaric acid, and ferulic acid), and anti-nutrients (phytic acid and trypsin inhibitor). Statistical analyses were conducted with data combined across all five locations as well as on a per location basis using data from the 3 replicates at each of the individual locations.

The European field trials (Application EFSA/GMO/NL/2005/28)
According to the applicant (Buffington 2005), the compositional analysis was undertaken on a broad range of compounds in 1507x59122 maize forage and grain, in accordance with OECD guidelines for assessment of GM maize (OECD 2002). However, data from nutritional analysis of the untreated 1507x59122 maize (both forage and grain) are missing in the application.

Forage samples from 1507x59122 maize (all herbicide treatments) and non-GM control maize were collected and analyzed for nutrient composition, including: crude protein, crude fat, crude fiber, ADF, NDF, ash, carbohydrates, calcium, phosphorus. Conversely, grain samples from 1507x59122 maize (unsprayed or sprayed with glufosinate-ammonium herbicide) and control maize were collected and analyzed for nutrient composition, including: proximates (crude protein, crude fat, ash) crude fiber, ADF, NDF, carbohydrates, fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acids), amino acids (methionine, cysteine, 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, raffinose, furfural, p-coumaric acid, and ferulic acid), and anti-nutrients (phytic acid and trypsin inhibitor). Statistical analyses were conducted with data combined across all five locations as well as on a per location basis using data from the 3 replicates at each of the individual locations.

Proximates and fiber analysis (forage)
In the field trials in North America, no statistically significant differences were observed for crude protein, crude fat, ADF, NDF or carbohydrates in the across location summary analysis for forage from 1507x59122 maize sprayed with **glufosinate-ammonium herbicide** (Table 1 - appendix). The across location mean values for grain proximate, fiber, and carbohydrate analytes in 1507 x 59122 maize and control entries were within reported literature ranges (Table 3 - appendix).

In the European study, no statistically significant differences were observed for crude protein, crude fat, crude fiber, ADF, ash, and carbohydrates mean values in the across location analysis for **unsprayed** forage from 1507x59122 maize. NDF mean value across locations for the 1507x59122 (untreated) and control hybrids were significantly different (non-adjusted P-value<0.05). Statistically significant differences for NDF were only observed at one of the five individual locations. After the observed probability was adjusted using the false discovery rate, NDF value were not considered statistically significantly different (adjusted P-value>0.05) (Table 4 - appendix). The range of individual values for proximates and fiber for the 1507x59122 (untreated) and control hybrids were within the tolerance intervals and combined historical ranges (Buffington, 2005, Unpublished technical report).

No statistically significant differences were observed for crude protein, crude fat, crude fiber, ADF, NDF and carbohydrates mean values in the across location analysis for forage from 1507x59122 maize sprayed with **glufosinate-ammonium herbicide**. Ash mean value across locations for the 1507x59122 maize sprayed with glufosinate-ammonium herbicide and control maize hybrids were statistically significantly different (non-adjusted P-value<0.05) (Table 4 - appendix). No statistically significant differences for ash were observed at any of the five locations. After the observed probability was adjusted using the false discovery rate, ash values were not considered statistically significantly different (adjusted P-value>0.05). The range of individual values for crude protein and ash for the 1507x59122 maize sprayed with glufosinate-ammonium herbicide and control maize hybrids were within the tolerance intervals and/or combined historical ranges (Buffington 2005, Unpublished technical report).

Mineral analysis (forage)
In the field trials in North America, no statistically significant differences were observed for calcium and phosphorus in the across location summary analysis for forage from 1507x59122 maize sprayed with **glufosinate-ammonium herbicide** (Table 2 - appendix). The across location mean values for grain calcium and phosphorus in 1507 x 59122 maize and control entries were within reported literature ranges (Table 3 - appendix) (Buffington 2004, Unpublished technical report).

In the European study, no statistically significant differences were observed for calcium mean values in the across location analysis. Phosphorus mean value across locations for the **unsprayed** 1507x59122 and control hybrids were significantly different (non-adjusted p-value<0.05) (Table 4 - appendix). No statistically significant differences for phosphorus were observed at any of the five locations. After the observed probability was adjusted using the false discovery rate, phosphorus values were not considered statistically significantly different (adjusted p-value>0.05). The range of individual values for minerals for the 1507x59122 (untreated) and control hybrids were within the tolerance intervals and combined historical ranges (Buffington, 2005, Unpublished technical report).

Proximates and fiber analysis (grain)
In the field trials in North America, no statistically significant differences were observed for crude protein, crude fat, ADF, NDF or carbohydrates in the across location summary analysis for **unsprayed** grain from 1507 x 59122 maize. Mean ash value across locations in 1507x59122 maize was significantly different (p <0.05). However, no statistically significant differences for ash mean values were observed at any of the five individual locations. Significant differences for ash were observed at only one of the five individual locations. The across location mean values for grain proximate, fiber, and carbohydrate analytes in 1507x59122 maize and control entries were within reported literature ranges (Buffington 2004, Unpublished technical report).

No statistically significant differences were observed for crude protein, crude fat, ADF, NDF or carbohydrates in the across location summary analysis for grain from 1507x59122 maize sprayed with **glufosinate-ammonium herbicide** (Table 5 - appendix). Mean crude fiber and ash values across locations for the test entry were statistically different (P<0.05). However, no statistically significant differences for crude fiber mean values were observed at any of the five individual locations. Significant differences for ash were observed at only one of the five individual locations. The across

location mean values for proximates, fiber and carbohydrates for the test and control entries were within reported literature ranges (Table 6 - appendix) (Buffington 2004, Unpublished technical report).

In the field trials in Europe, no statistically significant differences were observed for crude fat, ADF, crude fiber, NDF, and ash mean values in the across location analysis for **unsprayed** grain from 1507x59122 maize. Crude protein and carbohydrates mean values across locations for 1507x59122 maize and control maize were statistically significantly different (non-adjusted P-value<0.05) (Table 7 - appendix). Statistically significant differences for crude protein and carbohydrates were only observed at two and three of the five locations, respectively. After the observed probability was adjusted using the false discovery rate, crude protein and carbohydrates values were not considered statistically significantly different (adjusted P-value>0.05). The range of individual values for crude protein and carbohydrates for the 1507x59122 maize sprayed with glufosinate-ammonium herbicide and control maize hybrids were within the tolerance intervals and combined historical ranges (Buffington 2005, Unpublished technical report).

No statistically significant differences were observed for crude fat, ADF, crude fiber, NDF, and ash mean values in the across location analysis for grain from 1507x59122 maize sprayed with **glufosinate-ammonium herbicide**. Crude protein and carbohydrates mean values across locations for 1507x59122 maize and control maize were statistically significantly different (non-adjusted P-value<0.05) (Table 7 - appendix). Statistically significant differences for crude protein and carbohydrates were only observed at two and three of the five locations, respectively. After the observed probability was adjusted using the false discovery rate, crude protein and carbohydrates values were not considered statistically significantly different (adjusted P-value >0.05). The range of individual values for crude protein and carbohydrates for 1507x59122 maize and control maize were within the tolerance intervals and combined historical ranges (Buffington 2005, Unpublished technical report).

Fatty acids analysis (grain)
In the field trials in North America, no statistically significant differences were observed for stearic acid, oleic acid, linoleic acid, or linolenic acid mean values in the across location summary analysis for **unsprayed** grain from 1507x59122 maize. The mean palmitic acid value across locations for the test entry was statistically different (P<0.05). Significant differences for palmitic acid were only observed at two of the five individual locations, but since differences were not consistently observed and as a consequence there is no obvious trend, they were not considered to be meaningful.

No statistically significant differences were observed for stearic acid, linoleic acid, or linolenic acid mean values in the across location summary analysis for grain from 1507x59122 maize sprayed with **glufosinate-ammonium herbicide** (Table 8 - appendix). Mean palmitic acid and oleic acid values across locations for the test entry and control were statistically different (P<0.05). However, no statistically significant differences for palmitic acid and oleic acid mean values were observed at individual locations. The across location mean values for fatty acids in grain for the test and control entry were within reported literature ranges (Table 9 - appendix).

In the field trials in Europe, no statistically significant differences were observed for stearic acid and oleic acid mean values in the across location analysis for **unsprayed** grain from 1507x59122 maize. Palmitic acid, linoleic acid, and linolenic acid mean values across locations for 1507x59122 maize and control maize were statistically significantly different (non-adjusted P-value<0.05) (Table 7 - appendix). Statistically significant differences for palmitic acid, linoleic acid, and linolenic acid were only observed at four, two, and three of the five locations, respectively. After the observed probability was adjusted using the false discovery rate, palmitic acid and linoleic acid mean values were considered significantly different (adjusted P-value<0.05), while linolenic acid mean values were not considered statistically significantly different (adjusted P-value>0.05).

No statistically significant differences were observed for oleic acid mean values in the across location analysis for grain from 1507x59122 maize sprayed with **glufosinate-ammonium herbicide**. Palmitic acid, stearic acid, linoleic acid, and linolenic acid mean values across locations for 1507x59122 maize and control maize were statistically significantly different (non-adjusted P-value<0.05) (Table 7 - appendix). Statistically significant differences for palmitic acid, stearic acid, linoleic acid, and linolenic acid were observed at four, two, two, and four of the five locations, respectively. After the observed probability was adjusted using the false discovery rate, palmitic acid, stearic acid, linoleic acid, and linolenic acid mean values were not considered statistically significantly different (adjusted P-value>0.05). The range of individual values for palmitic acid, stearic acid, linoleic acid, and linolenic acid for 1507x59122 maize and control maize were within the tolerance intervals and combined historical ranges (Buffington, 2005, Unpublished technical report).

Amino acids analysis (grain)

In the field trials in North America, no statistically significant differences were observed for methionine, cystine, lysine, threoine, isoleucine, histidine, valine, leucine, arginine, glycine, alanine, aspartic acid, glutamic acid, proline, serine, or tyrosine in the across location summary analysis for **unsprayed** grain from 1507x59122 maize. Mean tryptophan and phenylalanine values across locations for the test entry and were statistically different (P<0.05). Significant differences for tryptophan were observed at one of the five individual locations. Significant differences for phenylalanine were observed at two individual locations. The across location mean values for amino acids in the test and control entries were within reported literature ranges.

No statistically significant differences were observed for methionine, cystine, lysine, tryptophan, threoine, isoleucine, histidine, valine, leucine, arginine, phenylalanine, glycine, alanine, aspartic acid, glutamic acid, proline, serine, or tyrosine in the across location summary analysis for grain from 1507x59122 maize sprayed with **glufosinate-ammonium herbicide** (Table 10 - appendix). The across location mean values for amino acids in grain for the test and control entries were within reported literature ranges (Table 11 - appendix).

In the field trials in Europe, no statistically significant differences were observed for methionine, cystine, lysine, tryptophan, and arginine mean values in the across location analysis for **unsprayed** grain from 1507x59122 maize. Threonine, isoleucine, histidine, valine, leucine, phenylalanine, glycine, alanine, aspartic acid, glutamic acid, proline, serine, and tyrosine mean values across locations for 1507x59122 maize and control maize were statistically significantly different (non-adjusted P-value<0.05) (Table 7 - appendix). Statistically significant differences for threonine and serine were only observed at one of the five locations. Statistically significant differences for histidine, glycine, and aspartic acid were observed at two of the five locations. Significant differences for isoleucine, valine, leucine, phenylalanine, alanine, glutamic acid, proline, and tyrosine were observed at three of the five locations. After the observed probability was adjusted using the false discovery rate, threonine, histidine, and glycine mean values were not considered statistically significantly different (adjusted P-value>0.05), while isoleucine, valine, leucine, phenylalanine, alanine, aspartic acid, glutamic acid, proline, serine, and tyrosine mean values were considered significantly different (adjusted P-value<0.05). With the exception of threonine and glycine, the range of individual values for isoleucine, histidine, valine, leucine, phenylalanine, alanine, aspartic acid, glutamic acid, proline, serine, and tyrosine for 1507x59122 maize and control maize were within the tolerance intervals and combined ranges (Buffington, 2005, Unpublished technical report).

No statistically significant differences were observed for methionine, cystine, lysine, tryptophan, threonine, histidine, arginine, glycine, glutamic acid, proline, and serine mean values in the across location analysis for grain from 1507x59122 maize sprayed with **glufosinate-ammonium herbicide.** Isoleucine, valine, leucine, phenylalanine, alanine, aspartic acid, and tyrosine mean values across locations for 1507x59122 maize and control maize were statistically significantly different (non-

adjusted P-value <0.05) (Table 7 - appendix). No statistically significant differences for aspartic acid were observed at any of the five locations. Statistically significant differences for isoleucine, valine, leucine, phenylalanine, alanine, and tyrosine were observed at three, three, two, two, three, and three, respectively, of the five locations. After the observed probability was adjusted using the false discovery rate, isoleucine, valine, leucine, phenylalanine, alanine, aspartic acid, and tyrosine mean values were not considered statistically significantly different (adjusted P-value>0.05). The range of individual values for isoleucine, valine, leucine, phenylalanine, alanine, aspartic acid, and tyrosine mean for 1507x59122 maize and control maize were within the tolerance intervals and combined ranges (Buffington 2005, Unpublished technical report).

Minerals analysis (grain)
In the field trials in North America, no statistically significant differences were observed for calcium, copper, magnesium, manganese, sodium, or zinc in the across location summary analysis for **unsprayed** grain from 1507x59122 maize. Mean iron, phosphorus, and potassium values across locations for the test entry were statistically different (P<0.05). Significant differences for iron and phosphorus were observed at one of the five individual locations. Significant differences for potassium were observed at three individual locations. The across location mean values for minerals in test and control entries were within reported literature ranges.

No statistically significant differences were observed for calcium, copper, magnesium, manganese, sodium, or zinc in the across location summary analysis for grain from 1507x59122 maize sprayed with **glufosinate-ammonium herbicide** (Table 12 - appendix). Mean iron, phosphorus, and potassium values across locations for the test entry were statistically different (P<0.05). Significant differences for iron and potassium were observed at one individual of the five locations. Significant differences for phosphorus were observed at three of the five individual locations. The across location mean values for minerals in test and control entries were within reported literature ranges (Table 13 - appendix).

In the field trials in Europe, no statistically significant differences were observed for copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc mean values in the across location analysis for **unsprayed** grain from 1507x59122 maize. Mean calcium values across locations for 1507x59122 maize and control maize were statistically significantly different (non-adjusted P-value<0.05) (Table 7 - appendix). Statistically significant differences for calcium were observed at four of the five locations. After the observed probability was adjusted using the false discovery rate, calcium mean values were considered statistically significantly different (adjusted P-value<0.05).

No statistically significant differences were observed for copper, iron, magnesium, phosphorus, sodium, and zinc mean values in the across location analysis for grain from 1507x59122 maize sprayed with **glufosinate-ammonium herbicide**. Calcium, manganese, and potassium mean values across locations for 1507x59122 maize and control maize were statistically significantly different (non-adjusted P-value<0.05) (Table 7 - appendix). Statistically significant differences for calcium, manganese, and potassium were observed at four, one, and two, respectively, of the five locations. After the observed probability was adjusted using the false discovery rate, calcium mean values were considered statistically significantly different (adjusted P-value<0.05), while manganese and potassium were not considered statistically significantly different (adjusted P-value>0.05). The range of individual values for calcium, manganese, and potassium for 1507x59122 maize and control maize were within the tolerance intervals and/or combined ranges (Buffington 2005, Unpublished technical report).

Vitamins analysis (grain)

In the field trials in North America, levels of vitamin B2 were below the limit of quantitation for the assay used in this analysis for **unsprayed** grain from the 1507x59122 maize. No statistically significant differences were observed for vitamin B1 and vitamin E in the across location summary analysis. Mean beta-carotene and folic acid values across locations in the test entry were significantly different (P<0.05). However, no statistically significant differences for beta-carotene mean values were observed at individual locations. Significant differences for folic acid were only observed at one of the five individual locations.

No statistically significant differences were observed for beta-carotene, vitamin B1, folic acid or vitamin E in the across location summary analysis for grain from 1507x59122 maize sprayed with **glufosinate-ammonium herbicide** (Table 14 – appendix). Levels of vitamin B2 were below the limit of quantitation for the assay used in this analysis The across location mean values for vitamins in grain for the test and control entries were within reported literature ranges, where applicable (Table 15 - appendix).

In the field trials in Europe, no statistically significant differences were observed for beta-carotene, vitamin B1, folic acid, and vitamin E mean values in the across location analysis between **unsprayed** 1507x59122 maize and control maize grain (Table 7 - appendix). Levels of vitamin B2 were below the limit of quantitation for the assay used in this analysis (Buffington 2005, Unpublished technical report).

Further, no statistically significant differences were observed between 1507x59122 maize sprayed with **glufosinate-ammonium herbicide** and control maize and for beta-carotene, vitamin B1, folic acid, and vitamin E mean values in the across location analysis (Table 7 - appendix). Levels of vitamin B2 were below the limit of quantitation for the assay used in this analysis (Buffington 2005, Unpublished technical report).

Secondary metabolites analysis (grain)
In the field trials in North America, no statistically significant differences were observed for inositol, p-coumaric acid, or ferulic acid in the across location summary analysis for **unsprayed** grain from 1507x59122 maize. Levels of furfural were below the limit of quantitation for the assay used in this analysis. The across location mean values for secondary metabolites in grain for the test and control entries were within reported literature ranges.

No statistically significant differences were observed for inositol in the across location summary analysis of grain from 1507x59122 maize sprayed with **glufosinate-ammonium herbicide** (Table 16 - appendix). Levels of furfural were below the limit of quantitation for the assay used in this analysis. Mean p-coumaric acid and ferulic acid values across locations in the test entry were significantly different (P<0.05). However, no statistically significant differences for ferulic acid mean values were observed at individual locations. Significant differences for p-coumaric acid were observed at one of the five locations. The across location mean values for secondary metabolites in grain for the test and control entries were within reported literature ranges (Table 17 - appendix).

In the field trials in Europe, no statistically significant differences were observed for raffinose, inositol, p-coumaric acid, and ferulic acid in the across location summary analysis between **unsprayed** 1507x59122 maize and control maize grain (Table 7 - appendix). Levels of furfural were below the limit of quantitation for the assay used in this analysis (Buffington, 2005, Unpublished technical report).

No statistically significant differences were observed between 1507x59122 maize sprayed with **glufosinate-ammonium herbicide** and control maize for raffinose, inositol, p-coumaric acid, and

ferulic acid in the across location summary analysis (Table 7 - appendix). Levels of furfural were below the limit of quantitation for the assay used in this analysis (Buffington 2005).

Anti-nutrients analysis (grain)
In the field trials in North America, no statistically significant differences were observed for phytic acid in the across location summary analysis for **unsprayed** grain from 1507x59122 maize. Mean raffinose and trypsin inhibitor values across locations in the test entry were significantly different ($P<0.05$). Significant differences for raffinose and trypsin inhibitor were observed at one (separate) of the five individual locations.

No statistically significant differences were observed for phytic acid in the across location summary analysis of grain from 1507x59122 maize sprayed with **glufosinate-ammonium herbicide** (Table 18 appendix). Mean raffinose and trypsin inhibitor values across locations in the test entry were significantly different ($P<0.05$). Significant differences for raffinose and trypsin inhibitor were observed at one individual location. Mean values for secondary metabolites in grain reported literature are presented in Table 17 – appendix.

In the field trials in Europe, no statistically significant differences were observed between **unsprayed** 1507x59122 maize and control maize for phytic acid, and trypsin inhibitor in the across location summary analysis (Table 7 - appendix) (Buffington, 2005, Unpublished technical report).

No statistically significant differences were observed between 1507x59122 maize sprayed with **glufosinate-ammonium herbicide** and control maize, for phytic acid, and trypsin inhibitor in the across location summary analysis (Table 7 - appendix) (Buffington, 2005, Unpublished technical report).

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, number of accumulated heat units when approximately 50 % of the plants are silking or shedding pollen, plant height, ear height, number of stalk and root lodged plants, final stand count, stay green, pollen shape, disease incidence and insect damage, were measured.

Analyses of variance across trial locations showed statistically significant differences between maize 1507 x 59122 (treated with glufosinate ammonium) and the corresponding conventional counterpart for early population, plant height and final population (number of viable plants remaining at maturity ($p<0.05$) (Table 20 - appendix). None of these differences were consistently observed over locations.

In 2004, corresponding agronomic and phenotypic characters were measured for maize stack 1507 x 59122 and the non-GM control maize in field trials at five locations in Europe. Analyses of variance across trial locations showed statistically significant differences between the transgenic maize 59122 x 1507 x NK603 (untreated) and the comparator for plant height and number of accumulated heat units to 50 % silking ($p<0.05$) (Table 21 - appendix). On average 1507 x 59122 maize plants had a higher number of accumulated heat units before 50 % of the plants were silking and was significant lower compared with the conventional counterpart. Significant differences for time to silking and plant height were observed at one and three of the five locations, respectively. In the European field trials, no statistically significant differences between the transgenic maize 1507 x 59122 and the comparator were observed for the characteristics mean early population, final population, time to pollen shed, ear

height, stalk lodging, root lodging, seedling vigour, stay green, disease incidence, insect damage and pollen viability values in the across location analysis ($p>0.05$). The VKM GMO Panel is of the opinion that the observed differences are not biologically relevant.

The information regarding the comparative analysis of agronomic and phenotypic data in the applications EFSA/GMO/NL/2005/15 and EFSA/GMO/NL/2005/28 has earlier been assessed by the VKM GMO Panel in the frame of EFSAs official hearing of the applications in 2007 (VKM 2007a, 2008b).

3.3 Conclusion

Comparative analyses of data from field trials located at representative sites and environments in the USA and Europe indicate that maize stack 1507 x 59122 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart with the exception of the herbicide tolerance, conferred by the expression of the Cry1F, Cry34Ab1/Cry35Ab1 and PAT proteins. Based on the assessment of available data, the VKM GMO Panel is of the opinion that conventional crossing of maize 1507 and 59122 to produce the hybrid 1507 x 59122 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

The genetic modification in 1507 x 59122 maize will not impact the existing production processes used for maize. All 1507 x 59122 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 1507 x 59122 maize and all food, feed and processed products derived from 1507 x 59122 maize are expected to replace a portion of similar products from commercial maize, with total consumption of maize products remaining unchanged. The total anticipated intake/extent of use of maize and all food, 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 proteins are denatured, which also applies to CRY1F, CRY34Ab1, CRY35Ab1 and PAT proteins (Hammond & Jez 2011).

4.3 Toxicological assessment

4.3.1 Toxicological assessment of the newly expressed protein

Given that the 1507x59122 maize was obtained by traditional breeding methods between progeny of genetically modified 1507 maize and 59122 maize their corresponding inserts will be inherited by 1507x59122 maize. Consequently, maize stack 1507x59122 expresses the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins. The safety of the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins for animal and human health has already been demonstrated as part of the safety evaluation of parental lines 1507 and 59122.

The *cry34Ab1, cry35Ab1* and *cry1F* genes were originally obtained from *Bacillus thuringiensis* strain. There is no evidence of Cry34Ab1, Cry35Ab1 or Cry1F having harmful effects on the health of humans or animals (EPA, 1995a; McClintock *et. al*., 1995; EPA, 1996). The Cry1F, Cry34Ab1 and Cry35Ab1 proteins have a very specific mode of action and selective toxicity against certain lepidopteran and coleopteran insect pests (target organisms).

The *pat* gene was originally obtained from *Streptomyces viridochromogenes* strain Tü494 which has no known toxic or pathogenic potential. The PAT protein is enzymatically active but it has high substrate specificity to the active ingredient L-phosphinothricin (L-PPT) of glufosinate-ammonium. The PAT protein has already been found safe to human health during the assessment of glufosinate-ammonium tolerant maize (OECD, 1999).

4.3.1.1 Acute 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).

In the second study, 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 2000a).

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

The potential toxicity of the Cry1F protein to *humans and animals* was specifically examined in an acute oral toxicology study where Cry1F protein was evaluated for acute toxicity in mice (Kuhn, 1998ok). The test substance, Cry1F *bacillus thuringiensis subsp. aizawai* Delta-toksin, was evaluated for its acute oral toxicity potential in albino mice when administrated as a gavage dose at a level of 5050 mg/kg to males and females. The test substance was administrated as a 15% w/v concentration in 2% w/v aqueous carboxymethyl cellulose. No mortality occurred during the study. There were no clinical signs of toxicity exhibited at any time throughout the study. There was no meaningful effect on body weight gain. The gross necropsy conducted at termination of the study revealed no observable abnormalities. The acute oral LD_{50}, as indicated by the data, was determined to be greater than 5050 mg/kg.

*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 2000b; Brooks and DeWildt 2000c; Brooks and DeWildt 2000d (Unpublished technical reports, see application 2005-12-NL-59122, Dow AgroSciences,,).

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 2000b), 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 2000c), 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, 2000d), 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.

The lack of toxicity of the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins to human and animal health was anticipated since the Cry and PAT proteins have a long history of safe use and they all lack a toxicity mechanism in mammals.

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, Unpublished technical report. AgrEvo Company). 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 under certain circumstances; justification for use of a 14-day

exposure period should be provided. The duration of this repeated dose oral toxicity study was 14-days. No justification for using 14-days has been found in the dossier from the applicant.

The study comprised four groups of five male and five female Wistar rats in each group. The rats in group 1 received a standard diet without PAT protein, whereas rats in group 2, 3 and 4 received diets with the inclusion of PAT and/or soybean protein: group 1 (standard diet), group 2 (0.5 % PAT + 4.5 % soybean), group 3 (5 % PAT), group 4 (5 % soybean), for a period of 14 days.

The mean intake of PAT-protein in group 2 over the treatment period was 712 mg/kg body weight/day for males and 703 mg/kg body weight/day for females. In group 3 the mean intake of PAT-protein was 7965 mg/kg body weight/day for males and 7619 mg/kg body weight/day for females.

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

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

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

42-day feeding study on broiler chickens
Additionally, the wholesomeness and safety of the 1507x59122 maize has been shown in a 42-day feeding study using broiler chickens. A poultry feeding study was conducted to confirm the nutritional equivalence of the 1507x59122 maize with its non-GM commercial maize equivalent (Delaney and Smith 2004, unpublished report of Pioneer Hi-Bred). Chickens were fed over a 42-day period with one diet containing 1507x59122 maize grains (treated with glufosinate-ammonium herbicide). For comparison, diets produced from grain of non-GM maize with the comparable genetic background and three non-GM commercial maize diets were also fed. Poultry studies are considered to be very useful because they utilize a fast growing organism that can eat a high percentage of maize in the diet, thus, it is very sensitive to potentially toxic effects of dietary components (OECD 2003 a).

The chickens 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.

The results of the study indicate shown that, as 1507 maize and 59122 maize, 1507x59122 maize is nutritionally equivalent to non-GM maize (EFSA 2004a; EFSA 2005a; EFSA 2005b, EFSA 2007, and EFSA-GMO-NL-2005-23 (EFSA). These findings also provide further confirmation of the safety of the proteins Cry1F, Cry34Ab1, Cry35Ab1 and PAT expressed in the 1507x59122 maize. The applicant concludes that the 1507x59122 maize is nutritionally equivalent to, and as safe as, non-GM commercial maize.

90-days feeding study on rats (sub-chronic toxicity testing)
The purpose of the study was to evaluate in rats the potential health effects of consuming a rodent diet formulated with the combined trait maize product 1507x59122. A group of young adult male and female Crl:CD(SD) rats (12/sex) was fed a test diet formulated with 1507x59122 maize. For comparison, four additional groups of rats (12/sex/group) were fed diets formulated with either a near-isogenic, non-transgenic maize (control grain: 091) or one of three non-transgenic commercial maize (reference grains: 3573, 35P12, and 36G12). All maize grain was incorporated into PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 at a concentration of approximately 34% w/w. All diets were fed for approximately 13 weeks (92-93 days for males; 93-94 days for females). Body weights and food consumption were evaluated daily for the first week, then weekly. Detailed clinical observations were made weekly. Neurobehavioral and ophthalmological assessments were performed prior to the start of dietary exposure and near the end of the exposure period. Clinical pathology endpoints were also evaluated near the end of the exposure period. After at least 13 weeks of dietary exposure, rats were euthanized and given a gross and microscopic pathological examination. All maize grain (from control, reference, and test sources) contained similar concentrations of proximate analytes, fiber, fatty acids, amino acids, vitamins, secondary plant metabolites, anti-nutrients, and minerals. All diets contained comparable levels of proximate analytes, fiber, energy, amino acids, vitamins, and minerals with one exception: copper concentration was over 3-fold higher in the control diet compared with the test diet. Grain and dietary contaminants (e.g., heavy metals, mycotoxins, pesticides) were either not detected or were present at concentrations below levels that would be expected to impact animal health. Molecular characterization of the grains used in formulating the diets demonstrated that genes encoding events 1507 and 59122 were present in the 1507 x 59122 maize grain, and were absent from the control and reference maize grain. The transgenic proteins Cry1F, Cry34Ab1, and Cry35Ab1 were quantified by antibody-specific enzyme-linked immunosorbent assay (ELISA) in the experimental maize grain, and were present and homogeneously distributed in the diet formulated with this grain; the PAT protein concentration was below the lower limit of quantitation (LLOQ) in the experimental grain and diet. The Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins were not detected in the control or reference grain or diets. Analysis of the

experimental test diet near the beginning and end of the study demonstrated that the detected transgenic proteins were stable over the course of the study. Biological activity of Cry1F protein was demonstrated in the experimental diet but not in the control or reference diets based on a European corn borer (ECB) specific bioassay, and were maintained for the duration of the study. All rats survived to scheduled euthanasia. No toxicologically significant, diet-related differences were observed between groups fed the 1507x59122 maize diet and groups fed a diet containing non-transgenic, near isogenic maize with respect to body weight, body weight gain, food consumption, food efficiency, clinical signs of toxicity, or ophthalmological observations. No effects attributed to test diet exposure were observed on any neurobehavioral assessments (forelimb and hindlimb grip strength, sensory function observations, motor activity), clinicalpathology (hematology, coagulation, clinical chemistry, and urinalysis parameters), organ weights, or gross or microscopic pathology.

Under the conditions of the study, no toxicologically significant differences were observed in rats consuming a diet containing the 1507x59122 maize compared with rats fed a diet containing non-transgenic, near isogenic maize).

In addition, no sub-chronic adverse effects were observed in a 90-day feeding study in rats conducted with diets prepared with 1507 maize (MacKenzie 2003). Further, no sub-chronic adverse effects were observed in a 90-days study where rats were fed diets prepared with the 59122 maize (Malley 2004, Unpublished technical report).

The applicant concludes that evaluation of the nutrient composition of the 1507x59122 maize has proved its equivalency to non-GM control maize with comparable genetic background.

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, Cry1F and PAT proteins, which includes:

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

These assessments have previously been described by the applicant for the single maize events 1507 (EFSA-GMO-NL-2004-02, EFSA-GMO-RX-1507)) and 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*) and *S. viridochromogenes* (*pat*) have no history of causing allergy
ii) History of safe use of Cry proteins as microbial pesticides (EPA, 1998), no indications of Cry proteins originating from *Bacillus thuringiensis* having harmful effects on the health of humans and animals
iii) The Cry1F, 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 Cry1F, Cry34Ab1 and Cry35Ab1 proteins are rapidly degraded, as shown by SDS-PAGE, under simulated gastric fluid digestive conditions
v) The Cry1F, 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 Cry1F, 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

The information listed above indicates that the newly expressed proteins in maize 1507 x 59122 lack IgE allergenic potential with regard to human and animal health. However, it does not cover allergic reactions that are not IgE mediated, e.g. some gluten-sensitive enteropathies or other enteropathies that are not IgE-mediated.

4.4.2 Assessment of the allergenicity of the whole GM plant

Allergenicity of the maize 1507 x 59122 could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, e.g. through qualitative or quantitative modifications of the expression of endogenous proteins. However, given that no biologically relevant agronomic or compositional changes have been identified in maize 1507 x 59122 or the parental events 1507 and 59122 with the exception of the introduced traits, no increased allergenicity is anticipated for maize 1507 x 59122. Moreover, maize is not considered a common allergenic food.

4.4.3 Assessment of the allergenicity of proteins from the GM plant

Allergenicity of the maize 1507 x 59122 could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, e.g. through qualitative or quantitative modifications of the expression of endogenous proteins. However, given that no biologically relevant agronomic or compositional changes have been identified in maize 1507 x 59122 or the parental events 59122 and 1507 with the exception of the introduced traits, no increased allergenicity is anticipated for maize 1507 x 59122. Moreover, maize is not considered a common allergenic food.

4.4.4 Adjuvanticity

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

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 1507x59122 indicate nutritional equivalence to the non-GM control maize with comparable genetic background and to the published range of values in the literature. The nutritional equivalence between 1507x59122 maize and non-GM control maize has been further shown by the results of a poultry feeding study where broiler chickens were fed over a 42-day period with diets containing grain from the 1507x59122 maize, grain from non-GM control maize with comparable genetic background, or yellow dent grain from commercial maize (Delaney and Smith 2004). In addition, no sub-chronic adverse effects were observed in the 90-days feeding study in rats conducted with diets prepared with the 1507x59122 maize (MacKenzie 2006).

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).

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The maximum expression levels of the proteins Cry1F, Cry34Ab1 and Cry35Ab1 are 3.48, 120 and 1,5 µg/g measured in grain from 1507x59122 maize. PAT-protein is below detection level. Since all foods from maize are derived from grains, the estimated maximum daily intake for a Norwegian adult of the Cry-proteins (i.e. Cry1F, Cry34Ab1, Cry35Ab) and PAT proteins would correspond to 550 and 6.2 µg/person/day, 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 (Cramer 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 1507 x 59122 maize and that protein levels are not reduced by processing. The comparable composition and nutritional value of the 1507 x 59122 maize, together with the results of the assessment of dietary intake and nutritional impact, indicate that food products derived from 1507x59122 maize are nutritionally equivalent to food products derived from commercial maize. Hence, anticipated dietary intake is not expected to change.

4.5.2 Nutritional assessment of feed derived from the GM plant

According to the applicant, the 1507 x 59122 maize and derived feed products are substantially equivalent to, nutritionally equivalent to and as safe as commercial maize and derived feed products. This is based on the compositional analyses comprising proximates, minerals, fatty acids, amino acids, vitamins, secondary metabolites and anti-nutrients of forage and grain samples from 1507 x 59122; nutritional equivalence shown in a poultry feeding study; and, safety evaluation of the Cry34Ab1, Cry35Ab1, Cry1F and PATmproteins expressed in 1507 x 59122 maize.

4.6 Conclusion

Whole food feeding studies in rats and broilers indicate that maize 1507 x 59122 is nutritionally comparable to conventional maize. Bioinformatics analyses have not disclosed expression of any known ORFs in the parental maize events, and none of the newly expressed proteins show resemblance to any known toxins or IgE allergens. None of the proteins have 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 maize 1507 x 59122.

Based on the current knowledge, the VKM GMO Panel concludes that 1507 x 59122 maize is nutritionally equivalent to conventional maize varieties, and that it is unlikely that newly expressed proteins introduce a toxic or allergenic potential in food and feed derived from maize 1507 x 59122 compared to conventional maize.

5 Environmental risk assessment

5.1 Unintended effects on plant fitness due to the genetic modification

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

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

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

It is considered very unlikely that the establishment, spread and survival of maize 1507 x 59122 would be increased due to the insect resistance and herbicide tolerance traits. The herbicide tolerant trait can only be regarded as providing a selective advantage for the GM maize plant where and when glufosinate ammonium-based herbicides are applied. Glufosinate ammonium-containing herbicides have been withdrawn from the Norwegian market since 2008, and the substance will be phased out in the EU in 2017 for reasons of reproductive toxicity. Similarly insect resistance against certain lepidopteran and coleopteran pests provides a potential advantage in cultivation of 1507 x 59122 under infestation conditions. It is considered very unlikely that maize 1507 x 59122 plants or their progeny will differ from conventional maize cultivars in their ability to survive as volunteers until subsequent seasons, or to establish feral populations under European environmental conditions.

Field trials carried out by the applicant do not indicate altered fitness of maize 1507 x 59122 relative to its conventional counterpart. A series of field trials with maize 1507 x 59122 were carried out across 5 locations in the USA and Canada in 2003 (application EFSA/GMO/NL/2005/15). 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/NL/2005/28. 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 1507 x 59122 in comparison with its conventional counterpart (see section 3.1). Data from the field trials in North America shows some statistical significant differences at individual field sites, e.g. for plant height and early and final population count. These differences were however small in magnitude and were not consistently observed over locations. In the European field trials mean time to silking and plant height values across locations for the maize 1507 x 59122 and control maize were statistically different ($p<0.05$). The VKM GMO Panel is of the opinion that they do not raise any environmental safety concern.

In addition to the data presented by the applicant, the VKM GMO Panel is not aware of any scientific reports indicative of increased establishment or spread of maize 1507 x 59122, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of maize 1507 x 59122 are unchanged, insect resistance and glufosinate tolerance are not likely to provide a selective advantage outside of cultivation in Europe. The VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects based on establishment and survival of maize 1507 x 59122 will not differ from that of conventional maize varieties.

5.2 Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or vertical gene flow via pollen or seed dispersal. Exposure of microorganisms to transgenic DNA occurs during decomposition of plant material remaining in the field after harvest or comes from pollen deposited on cultivated areas or the field margins. Transgenic DNA is also a component of a variety of food and feed products derived from maize 1507 x 59122. 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 2004b, 2009a; Bensasson et al. 2004; VKM 2005c).

Based on established scientific knowledge of the barriers for gene transfer between unrelated species and the experimental research on horizontal transfer of genetic material from plants to microorganisms, there is today little evidence pointing to a likelihood of random transfer of the transgenes present in maize 1507 x 59122 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 feces from the control group. Rizzi et al. (2012) provides an extensive review of the fate of feed-derived DNA in the gastrointestinal system of mammals.

In conclusion, the VKM GMO Panel consider it is unlikely that the introduced gene from maize 1507 x 59122 will transfer and establish in the genome of microorganisms in the environment or in the intestinal tract of humans or animals. In the rare, but theoretically possible case of transfer of the *cry* and *pat* genes from 1507 x 59122 to soil bacteria, no novel property would be introduced into or expressed in the soil microbial communities; as these genes are already present in other bacteria in soil. Therefore, no positive selective advantage that would not have been conferred by natural gene transfer between bacteria is expected.

5.2.2 Plant to plant gene flow

Considering the intended uses of maize 1507 x 59122 (excluding cultivation) and the physical characteristics of maize seeds, possible pathways of gene dispersal are grain spillage and dispersal of pollen from potential transgenic maize plants originating from accidental grain spillage during transport and/or processing.

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

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

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

As maize 1507 x 59122 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

Genetically modified maize 1507 was transformed to provide protection against lepidopteran and coleopteran pests.

Maize Cry1F was developed to provide protection against a variety of target pests of the order Lepidoptera. Two Lepidoptera pests are primarily targeted by 15070; *Ostrinia nubilalis* (European corn borer, ECB) and *Sesamia nonagrioides* (Mediterranean corn borer, MCB). The European corn borer is widely distributed in Europe covering the Iberian Peninsula, Czech Republic and Slovakia, southwest of France, northern Italy and the southern regions of Germany and Poland. The Mediterranean corn borer is present in the Mediterranean region (Andreadis 2011). There are ten reports of *O. nubilalis* in Norway, restricted to the counties of Vestfold, Telemark, Aust-Agder and Vest Agder. *Sesamia* spp. has not been reported in Norway. There are no reports of *O. nubilalis* attaining pest status in Norway, and the Plant Clinic (Planteklinikken) at Bioforsk has never received samples of this pest or plant material damaged by this pest (K. Ørstad pers. com.). Consequently, there are no insecticides authorised or previous applications for registrations of insecticides against this herbivore in Norway.

Maize 59122 expresses the *cry34Ab1* and *cry35Ab1* genes from *Bacillus thuringiensis,* 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 1507 x 59122, excluding cultivation, the environmental exposure is limited to exposure through manure and feces from the gastrointestinal tract mainly of animals fed on the GM maize as well as to the accidental release into the environment of GM seeds during transportation and processing and subsequently to potential occurrence of sporadic feral plants. Thus the level of exposure of target organisms to Cry1F, 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 1507 x 59122, excluding cultivation, the environmental risk assessment is concerned with accidental release of GM maize viable grains into the environment during transportation and processing, and exposure through manure and faeces from the gastrointestinal tracts of animals fed the GM maize.

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

Data supplied by the applicant indicate that a limited amount of the Cry1F, Cry34Ab1 and Cry35Ab1 proteins enters the environment due to expression in the grains (mean value of 2.04, 45.7 and 1.61 µg/g d.w., respectively). In addition, the data show that at least 99% of microbially produced Cry1F and Cry34Ab1/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 Cry1F and 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 1507 x 59122, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with the abiotic environment and biogeochemical cycles were not considered an issue by the VKM GMO Panel.

5.6 Conclusion

The scope of the application EFSA/GMO/NL/2005/15 includes import and processing of maize 1507 x 59122 for food and feed uses. Considering the intended uses of maize 1507 x 59122, excluding cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize 1507 x 59122.

Maize 1507 x 59122 has no altered survival, multiplication or dissemination characteristics, and there are no indications of an increased likelihood of spread and establishment of feral maize plants in the case of accidental release into the environment of seeds from maize 1507 x 59122. 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.

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.

Conclusion

Molecular characterisation

As conventional breeding methods were used in the production of maize 1507 x 59122, no additional genetic modification was involved. Southern and PCR analyses demonstrated that the recombinant insert in the single 1507 and 59122 events were retained in maize stack 1507 x 59122. Genetic stability of the inserts has been demonstrated in the parental lines 1507 and 59122. Phenotypic analyses demonstrated stability of the insect resistance and herbicide tolerance traits in the hybrid. The expression levels of Cry1F, Cry34Ab1/Cry35Ab1 and PAT proteins in seeds and forage were considered comparable with those in the single events.

Comparative assessment

Comparative analyses of data from field trials located at representative sites and environments in the USA and Europe indicate that maize stack 1507 x 59122 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart and to other conventional maize varieties, with the exception of the herbicide tolerance, conferred by the expression of the Cry1F, Cry34Ab1/Cry35Ab1 and PAT proteins. Based on the assessment of available data, the VKM GMO Panel is of the opinion that conventional crossing of maize 1507 and 59122 to produce the hybrid 1507 x 59122 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 studies in rats and broilers indicate that maize 1507 x 59122 is nutritionally comparable to conventional maize. Bioinformatics analyses have not disclosed expression of any known ORFs in the parental maize events, and none of the newly expressed proteins show resemblance to any known toxins or IgE allergens. None of the proteins have 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 maize 1507 x 59122.

Based on the current knowledge, the VKM GMO Panel concludes that 1507 x 59122 maize is nutritionally equivalent to conventional maize varieties, and that it is unlikely that newly expressed proteins introduce a toxic or allergenic potential in food and feed derived from maize 1507 x 59122 compared to conventional maize.

Environmental risk assessment

The scope of the application EFSA/GMO/NL/2005/15 includes import and processing of maize 1507 x 59122 for food and feed uses. Considering the intended uses of maize 1507 x 59122, excluding cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize 1507 x 59122.

Maize 1507 x 59122 has no altered survival, multiplication or dissemination characteristics, and there are no indications of an increased likelihood of spread and establishment of feral maize plants in the case of accidental release into the environment of seeds from maize 1507 x 59122. 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 1507 x 59122 or its processed products compared to conventional maize. Based on current knowledge, it is also unlikely that the Cry1F, Cry34Ab1 and Cry35Ab1 protein will increase the allergenic potential of food and feed derived from maize 1507 x 59122 compared to conventional maize varieties. The VKM GMO Panel likewise concludes that maize 1507 x 59122, 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 fibers in forage (Buffington 2004).

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x59122 - Glufosinate	Control	Standard Error
Crude Protein	3.14 - 15.9	7.21	7.27	0.213
Crude Fat	0.37 - 6.7	2.71	2.71	0.0725
Crude Fiber	19 - 42	21.6	21.1	0.417
ADF[4]	16.1 - 41.9	28.2	28.4	0.677
NDF[5]	10.3 - 63.7	47.9	47.5	1.06
Ash	1.3 - 10.5	3.89	3.91	0.127
Carbohydrates	66.9 - 94.5	86.2	86.1	0.289

[1]Percent of dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
[4]Acid Detergent Fiber
[5]Neutral Detergent Fiber

Table 2. Summary analysis of Minerals in forage (Buffington 2004).

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x59122 - Glufosinate	Control	Standard Error
Calcium	0.097 - 0.6	0.196	0.197	0.0111
Phosphorus	0.12 - 0.55	0.207	0.201	0.00530

[1]Percent dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means

Table 3. Litterature ranges for proximats and fibers 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 nutrient composition results in forage across locations in Europe (Buffington 2005).**

Analyte	Least Square Means (Range[1])			Standard Error	Tolerance Interval[2]	FDR[3] Adjusted P-value (Non-adjusted P-value)		Combined Ranges[4]
	Control	1507x59122 (Untreated)	1507x59122 + Glufosinate			1507x59122 (Untreated)	1507x59122 + Glufosinate	
Proximates, Fiber, and Minerals Composition (% Dry Weight)								
Crude Protein	7.39 (5.14 – 9.40)	7.43 (5.55 – 9.78)	8.03 (4.66 – 10.3)	0.585	1.40 – 14.8	0.583 (0.0363)	0.566 (0.358)	3.14 – 15.9
Crude Fat	2.35 (1.41 – 3.71)	2.19 (0.961 – 2.83)	2.28 (1.11 – 2.83)	0.214	0.998 – 3.58	0.555 (0.361)	0.693 (0.616)	0.37 – 6.7
Crude Fiber	22.9 (15.7 – 26.3)	23.6 (19.5 – 29.0)	23.3 (19.4 – 31.3)	1.35	10.3 – 35.7	0.555 (0.368)	0.693 (0.637)	19 – 42
ADF	27.6 (21.0 – 33.3)	29.1 (24.2 – 35.7)	29.6 (25.6 – 37.8)	1.40	12.9 – 42.7	0.443 (0.240)	0.282 (0.139)	16.1 – 41.9
NDF	49.6 (36.1 – 55.6)	53.4[5] (42.1 – 59.7)	51.3 (34.2 – 63.9)	2.89	21.3 – 81.9	0.156 (0.0473)	0.174 (0.0539)	20.3 – 63.7
Ash	3.99 (2.46 – 5.09)	4.43 (3.31 – 6.63)	4.59[5] (2.90 – 5.96)	0.341	[illegible] – 9.97	0.173 (0.0545)	0.115 (0.0316)	1.3 – 10.5
Carbohydrates	85.0 (82.5 – 90.6)	85.2 (79.8 – 88.1)	85.2 (81.2 – 86.6)	0.722	74.3 – 95.0	0.749 (0.677)	0.395 (0.285)	66.9 – 94.5
Calcium	0.217 (0.0946 – 0.403)	0.229 (0.0935 – 0.350)	0.230 (0.165 – 0.444)	0.0400	0.000 – 0.529	0.463 (0.281)	0.395 (0.214)	0.077 – 0.6
Phosphorus	0.206 (0.135 – 0.256)	0.224[5] (0.153 – 0.319)	0.253[5] (0.177 – 0.290)	0.0139	0.000 – 0.342	0.145 (0.0413)	0.103 (0.00498)	0.12 – 0.35

[1]Range denotes the lowest and highest individual value across locations.
[2]Negative tolerance limits have been set to zero.
[3]False Discovery Rate
[4]Combined ranges are taken from published literature for maize (*2, 5, 6, 8, 10, and 11*).
[5]Non-adjusted P-value <0.05

Table 5. Summary analysis of proximates and fibers in grain (Buffington 2004).

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x59122 - Glufosinate	Control	Standard Error
Crude Protein	6 - 15.0	10.6	10.5	0.108
Crude Fat	1.2 - 18.8	3.74	3.61	0.0625
ADF[4]	1.82 - 11.3	3.73	3.62	0.111
Crude Fiber	1.6 - 5.5	2.27	2.28	0.0505
NDF[5]	5.59 - 22.6	10.4	10.6	0.374
Ash	0.62 - 6.28	1.54*	1.48	0.0182
Carbohydrates	63.3 - 89.8	84.1	84.4	0.123

[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 1507x59122 - glufosinate and control

Table 6. Litterature ranges for proximats and fibers in grain (Buffington 2004).

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 7. Summary analysis of nutrient composition results in grain across locations in Europe (Buffington 2005).

Analyte	Least Square Means (Range[1])			Standard Error	Tolerance Interval[2]	FDR[3] Adjusted P-value (Non-adjusted P-value)		Combined Ranges[4]
	Control	1507×59122 (Untreated)	1507×59122 + Glufosinate			1507×59122 (Untreated)	1507×59122 + Glufosinate	
Proximates and Fiber Composition (% Dry Weight)								
Crude Protein	9.89 (8.89 – 11.3)	11.0[b] (8.74 – 12.4)	10.7[b] (8.93 – 13.0)	0.419	4.11 – 15.6	0.0186 (0.00388)	0.115 (0.0272)	6 – 15.0
Crude Fat	4.24 (3.58 – 5.01)	4.39 (3.99 – 5.08)	4.46 (3.88 – 5.39)	0.194	1.84 – 6.48	0.288 (0.203)	0.285 (0.139)	1.2 – 18.8
ADF	2.16 (1.64 – 2.74)	2.27 (1.84 – 2.94)	2.23 (1.57 – 2.74)	0.135	0.958 – 4.49	0.291 (0.110)	0.427 (0.226)	1.82 – 11.3
Crude Fiber	2.78 (2.13 – 3.48)	2.46 (1.70 – 3.19)	2.65 (1.80 – 3.70)	0.141	1.18 – 3.64	0.307 (0.129)	0.630 (0.530)	1.6 – 5.5
NDF	9.35 (6.45 – 11.4)	9.55 (6.48 – 11.7)	9.61 (6.61 – 12.8)	0.659	2.34 – 20.6	0.674 (0.554)	0.548 (0.444)	5.59 – 22.6
Ash	1.44 (1.28 – 1.58)	1.52 (1.37 – 1.68)	1.54 (1.35 – 1.67)	0.0447	0.338 – 2.54	0.390 (0.150)	0.289 (0.118)	0.616 – 6.28
Carbohydrates	84.4 (82.2 – 85.8)	83.1[b] (81.3 – 85.7)	83.3[b] (80.8 – 85.5)	0.500	78.2 – 91.6	0.0376 (0.00670)	0.115 (0.0176)	63.3 – 89.8
Fatty Acids Composition (% Total Fatty Acids)								
Palmitic acid	11.6 (10.8 – 12.6)	10.7[b] (9.95 – 12.4)	10.7[b] (10.1 – 12.0)	0.350	4.85 – 19.3	0.0224 (0.00310)	0.103 (0.00968)	7 – 19
Stearic acid	1.53 (1.40 – 1.73)	1.50 (1.37 – 1.66)	1.46[b] (1.34 – 1.61)	0.0704	0.425 – 2.04	0.175 (0.0569)	0.115 (0.0238)	0 – 4.0
Oleic acid	23.3 (20.8 – 25.8)	23.1 (20.5 – 26.0)	23.1 (20.7 – 26.0)	0.777	0.000 – 73.4	0.367 (0.165)	0.415 (0.263)	19.6 – 50
Linoleic acid	62.0 (58.8 – 64.1)	63.3[b] (60.0 – 65.9)	63.2[b] (60.3 – 65.7)	0.790	21.4 – 97.3	0.0376 (0.00590)	0.115 (0.0192)	34.0 – 70
Linolenic acid	1.10 (0.866 – 1.21)	0.947[b] (0.825 – 1.17)	0.951[b] (0.811 – 1.07)	0.0442	0.000 – 2.91	0.0456 (0.0126)	0.115 (0.0177)	0 – 2.0
Amino Acids Composition (% Dry Weight)								
Methionine	0.246 (0.169 – 0.309)	0.248 (0.212 – 0.294)	0.239 (0.209 – 0.275)	0.00991	0.0623 – 0.935	0.811 (0.752)	0.836 (0.417)	0.10 – 0.46
Cystine	0.186 (0.125 – 0.238)	0.188 (0.156 – 0.225)	0.189 (0.139 – 0.246)	0.00871	0.0531 – 0.360	0.868 (0.839)	0.788 (0.690)	0.08 – 0.32
Lysine	0.326 (0.246 – 0.428)	0.313 (0.249 – 0.427)	0.312 (0.258 – 0.388)	0.0142	0.214 – 0.537	0.879 (0.423)	0.814 (0.355)	0.05 – 0.56
Tryptophan	0.0612 (0.0541 – 0.0690)	0.0631 (0.0585 – 0.0673)	0.0624 (0.0506 – 0.0705)	0.00171	0.000 – 0.134	0.509 (0.353)	0.631 (0.558)	0.04 – 0.13
Threonine	0.354 ([illegible])	0.343[b] ([illegible])	0.339 ([illegible])	0.0226	0.136 – 0.660	0.0743 (0.0185)	0.174 (0.0581)	0.22 – 0.66
Isoleucine	0.355 ([illegible])	0.387[b] (0.323 – 0.439)	0.389[b] (0.331 – 0.451)	0.0142	0.123 – 0.582	0.0199 (0.00169)	0.165 (0.0142)	0.29 – 0.71
Histidine	0.274 (0.232 – 0.359)	0.313[b] (0.258 – 0.342)	0.298 (0.252 – 0.347)	0.00924	0.142 – 0.389	0.0456 (0.0153)	0.269 (0.110)	0.15 – 0.42
Valine	0.439 ([illegible])	0.404[b] ([illegible])	0.469[b] (0.405 – 0.539)	0.0152	0.179 – 0.636	0.0298 (0.00259)	0.115 (0.0232)	0.21 – 0.85

Table 7 (continued)

Analyte	Least Square Means (Range[1])			Standard Error	Tolerance Interval[2]	FDR[3] Adjusted P-value (Non-adjusted P-value)		Combined Ranges[4]
	Control	1507x59122 (Untreated)	1507x59122 + Glufosinate			1507x59122 (Untreated)	1507x59122 + Glufosinate	
Amino Acids Composition (% Dry Weight)								
Leucine	1.23 (1.04 – 1.56)	1.47[5] (1.13 – 1.76)	1.36[5] (1.08 – 1.67)	0.0635	0.333 – 2.10	0.0199 (0.00120)	0.115 (0.0204)	0.64 – 2.41
Arginine	0.351 (0.294 – 0.422)	0.367 (0.332 – 0.410)	0.367 (0.335 – 0.409)	0.00740	0.162 – 0.480	0.325 (0.188)	0.260 (0.0992)	0.22 – 0.64
Phenylalanine	0.559 (0.485 – 0.713)	0.658[5] (0.525 – 0.757)	0.615[5] (0.497 – 0.719)	0.0255	0.180 – 0.774	0.0199 (0.00120)	0.115 (0.0245)	0.26 – 0.53
Glycine	0.466 (0.401 – 0.597)	0.524[5] (0.417 – 0.607)	0.499 (0.436 – 0.576)	0.0155	0.205 – 0.528	0.0656 (0.0158)	0.260 (0.114)	0.26 – 0.50
Alanine	0.828 (0.706 – 1.08)	0.968[5] (0.776 – 1.10)	0.911[5] (0.727 – 1.07)	0.0364	0.298 – 1.27	0.0208 (0.00260)	0.115 (0.0278)	0.44 – 1.20
Aspartic Acid	0.709 (0.606 – 0.861)	0.789[5] (0.646 – 0.927)	0.751[5] (0.645 – 0.821)	0.0243	0.332 – 1.02	0.0199 (0.000500)	0.164 (0.0468)	0.40 – 0.95
Glutamic Acid	2.19 (1.92 – 2.65)	2.56[5] (1.97 – 3.06)	2.38 (1.94 – 2.90)	0.106	0.742 – 3.26	0.0208 (0.00220)	0.179 (0.0595)	1.04 – 3.04
Proline	1.11 (0.947 – 1.34)	1.26[5] (1.02 – 1.45)	1.17 (1.03 – 1.30)	0.0422	0.501 – 1.84	0.0208 (0.00250)	0.293 (0.154)	0.53 – 1.46
Serine	0.569 (0.497 – 0.711)	0.659[5] (0.524 – 0.749)	0.599 (0.498 – 0.711)	0.0198	0.209 – 0.780	0.0451 (0.00870)	0.293 (0.156)	0.24 – 0.91
Tyrosine	0.259 (0.138 – 0.379)	0.320[5] (0.247 – 0.404)	0.314[5] (0.240 – 0.371)	0.0191	0.138 – 0.435	0.0291 (0.00420)	0.115 (0.00740)	0.11 – 0.79
Minerals Composition (% Dry Weight)								
Calcium	0.00466 (0.00386 – 0.00596)	0.00487[5] (0.00381 – 0.00622)	0.00499[5] (0.00366 – 0.00648)	0.000297	0.000 – 0.00961	0.00830 (0.000100)	0.00430 (0.000100)	0.00216 – 0.1
Copper	0.000152 (0.0000972 – 0.000354)	0.000199 (0.000110 – 0.000924)	0.000245 (0.000102 – 0.00123)	0.0000815	0.000 – 0.00114	0.579 (0.426)	0.260 (0.116)	0.000073 – 0.001
Iron	0.00194 (0.00124 – 0.00364)	0.00175 (0.00131 – 0.00236)	0.00181 (0.00129 – 0.00309)	0.000215	0.000898 – 0.00274	0.558 (0.390)	0.630 (0.522)	0.0001 – 0.01
Magnesium	0.122 (0.101 – 0.196)	0.122 (0.0901 – 0.162)	0.123 (0.0980 – 0.210)	0.00657	0.0613 – 0.193	0.973 (0.969)	0.873 (0.873)	0.08 – 1.0
Manganese	0.000837 (0.000625 – 0.00105)	0.000778 (0.000493 – 0.00115)	0.000739[5] (0.000539 – 0.00102)	0.0000393	[illegible]137 – 0.00102	0.238 (0.0829)	0.115 (0.0137)	0.00007 – 0.0054
Phosphorus	0.288 (0.239 – 0.420)	0.297 (0.231 – 0.375)	0.302 (0.246 – 0.455)	0.0121	0.203 – 0.533	0.674 (0.540)	0.501 (0.330)	0.21 – 0.75
Potassium	0.329 (0.294 – 0.400)	0.344 (0.313 – 0.388)	0.355[5] (0.298 – 0.445)	0.00794	0.000 – 0.835	0.367 (0.164)	0.115 (0.0235)	0.27 – 0.72
Sodium	0.00187 (0.00116 – 0.00262)	0.00199 (0.00152 – 0.00238)	0.00206 (0.00131 – 0.00447)	[illegible]	0.000 – 0.000999	0.674 (0.522)	0.501 (0.360)	0.0 – 0.15
Zinc	0.00161 (0.00108 – 0.00181)	0.00165 (0.00107 – 0.00192)	0.00167 (0.00119 – 0.00195)	0.0000739	0.00113 – 0.00254	0.638 (0.485)	0.501 (0.345)	0.00065 – 0.0037

Table 7 (continued)

Analyte	Least Square Means (Range[1])			Standard Error	Tolerance Interval[2]	FDR[3] Adjusted P-value (Non-adjusted P-value)		Combined Ranges[4]
	Control	1507x59122 (Untreated)	1507x59122 + Glufosinate			1507x59122 (Untreated)	1507x59122 + Glufosinate	
Vitamins Composition (mg/kg Dry Weight)								
Beta-carotene	9.45 (3.58 – 20.4)	9.04 (3.19 – 27.9)	9.63 (3.44 – 31.9)	4.37	0.000 – 30.9	0.500 (0.207)	0.700 (0.434)	0.53 – 16.4
Vitamin B1	14.9 (<3.00[5] – 25.2)	14.2 (<3.00[5] – 26.1)	12.5 (<3.00[5] – 27.0)	3.39	0.000 – 28.4	0.773 (0.700)	0.298 (0.158)	1.3 – 8.6
Vitamin B2	<1.00[5] (<1.00[5])	<1.00[5] (<1.00[5])	<1.00[5] (<1.00[5])	NA[6]	NA[6]	NA[6]	NA[6]	0.25 – 5.6
Folic Acid	0.554 (0.429 – 0.700)	0.564 (0.462 – 0.708)	0.575 (0.470 – 0.700)	0.0278	0.114 – 1.49	0.657 (0.506)	0.303 (0.168)	0.15 – 683
Vitamin E[7]	9.94 (3.81 – 18.8)	8.84 (0.902 – 13.2)	9.24 (1.12 – 17.7)	1.99	0.000 – 23.6	0.526 (0.389)	0.663 (0.579)	1.5 – 68.7
Secondary Metabolites Composition (% Dry Weight)								
Inositol	0.0183 (0.0114 – 0.0362)	0.0222 (0.0114 – 0.0416)	0.0243 (0.00807 – 0.0469)	0.00452	0.000 – 0.0437	0.430 (0.223)	0.207 (0.0756)	0.0138 – 0.257
Furfural	<0.000100[5] (<0.000100[5])	<0.000100[5] (<0.000100[5])	<0.000100[5] (<0.000100[5])	NA[6]	NA[6]	NA[6]	NA[6]	0.0003 – 0.0035
P-Coumaric Acid	0.0179 (0.0132 – 0.0245)	0.0170 (0.00817 – 0.0219)	0.0176 (0.0134 – 0.0283)	0.00427	0.000 – 0.0435	0.590 (0.390)	0.784 (0.702)	0.003 – 0.058
Ferulic Acid	0.158 (0.0680 – 0.193)	0.159 (0.0445 – 0.228)	0.148 (0.0303 – 0.217)	0.0464	0.0585 – 0.300	0.244 (0.0940)	0.514 (0.400)	0.02 – 0.373
Anti-Nutrients Composition (% Dry Weight or as Indicated)								
Raffinose	0.163 (<0.165[5] – 0.205)	0.181 (<0.165[5] – 0.301)	0.175 (<0.165[5] – 0.317)	0.0143	0.000 – 0.485	0.390 (0.197)	0.514 (0.300)	0.02 – 0.32
Phytic acid	0.678 (0.387 – 1.28)	0.717 (0.402 – 0.971)	0.686 (0.359 – 0.887)	0.0632	0.158 – 1.20	0.715 (0.400)	0.784 (0.729)	0.39 – 1.29
Trypsin Inhibitor[8]	3.86 (3.12 – 5.10)	3.11 (0.95 – 5.30)	3.19 (2.10 – 5.40)	0.479	1.25 – 5.65	0.737 (0.627)	0.485 (0.269)	1.10 – 7.18

[1]Range denotes the lowest and highest individual value across locations.
[2]Negative tolerance limits have been set to zero.
[3]False Discovery Rate
[4]Combined ranges are taken from published literature for maize (*2, 5, 6, 8, 10*, and *11*).
[5]<Lower Limit of Quantitation (LLOQ); Indicates that the values of the sample or samples were detected below the assay's LLOQ
[6]Statistical analysis was not available (NA), due to lack of measurable concentrations detected for this analyte.
[7]Measured as α-tocopherol
[8]Analyte reported in TIU/g (Abbreviation: TIU, trypsin inhibitor units)
[9]Non-adjusted P-value <0.05

Table 8. Summary analysis of fatty acids in grain (Buffington 2004).

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x59122 - Glufosinate	Control	Standard Error
Palmitic acid	7 - 19	12.3*	11.7	0.0833
Stearic acid	0 - 4.0	1.55	1.53	0.0140
Oleic acid	18.6 - 50	28.0	28.4	0.357
Linoleic acid	34.0 - 70	56.5	56.7	0.370
Linolenic acid	0 - 2.0	1.23	1.21	0.0173

[1]Percent total fatty acids
[2]Combined ranges, see Appendix 5
[3]Least square means
*P-value<0.05 between 1507x59122 - glufosinate and control

Table 9. Literature ranges for fatty acids in grain (Buffington 2004)

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 10. Summary analysis of amino acids in grain (Buffington 2004).

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x59122 - Glufosinate	Control	Standard Error
Methionine	0.10 - 0.46	0.23	0.24	0.0038
Cystine	0.08 - 0.32	0.22	0.23	0.0055
Lysine	0.05 - 0.56	0.34	0.33	0.0080
Tryptophan	0.04 - 0.13	0.07*	0.07	0.0007
Threonine	0.22 - 0.65	0.56	0.54	0.012
Isoleucine	0.20 - 0.71	0.38	0.38	0.0049
Histidine	0.15 - 0.42	0.32	0.31	0.0048
Valine	0.21 - 0.85	0.48	0.48	0.0039
Leucine	0.64 - 2.41	1.43	1.40	0.0224
Arginine	0.22 - 0.64	0.37	0.37	0.0061
Phenylalanine	0.26 - 0.83	0.57*	0.56	0.0080
Glycine	0.25 - 0.50	0.47	0.47	0.0068
Alanine	0.44 - 1.20	0.95	0.95	0.013
Aspartic Acid	0.40 - 0.95	0.83	0.84	0.012
Glutamic Acid	1.04 - 3.04	2.43	2.38	0.0383
Proline	0.53 - 1.46	1.06	1.06	0.0153
Serine	0.24 - 0.91	0.59	0.58	0.0095
Tyrosine	0.11 - 0.79	0.31	0.29	0.010

[1]Percent dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
*P-value<0.05 between 1507x59122 - glufosinate and control

Table 11. Literature ranges of amino acids in grain (Buffington 2004).

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 - 0.48	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 12. Summary analysis of minerals in grain (Buffington 2004).

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x59122 - Glufosinate	Control	Standard Error
Calcium	0.00216 - 0.1	0.00404	0.00431	0.000141
Copper	0.000073 - 0.001	0.000159	0.000148	0.00000508
Iron	0.0001 - 0.01	0.00205*	0.00190	0.0000485
Magnesium	0.08 - 1.0	0.116	0.112	0.00245
Manganese	0.00007 - 0.0054	0.000496	0.000517	0.0000103
Phosphorus	0.21 - 0.75	0.316*	0.291	0.00630
Potassium	0.27 - 0.72	0.423*	0.389	0.00654
Sodium	0.0 - 0.15	0.000482	0.000763	0.000112
Zinc	0.00065 - 0.0037	0.00165	0.00170	0.0000518

[1]Percent dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
*P-value<0.05 between 1507x59122 - glufosinate and control

Table 13. Literature ranges of minerals in grain (Buffington 2004).

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	0.000573 - 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 14. Summary analysis of vitamins in grain (Buffington 2004).

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x59122 - Glufosinate	Control	Standard Error
Beta-carotene	0.53 - 16.4	16.2*	13.9	0.859
Vitamin B1	1.3 - 8.6	4.00	4.23	0.0899
Vitamin B2	0.25 - 5.6	ND[5]	ND[5]	ND[5]
Folic Acid	0.15 - 683	0.938*	1.02	0.0242
Vitamin E[4]	1.5 - 68.7	11.6	11.4	0.504

[1]mg/kg dry weight
[2]Combined ranges, see Appendix 8
[3]Least square means
[4]Measured as α-tocopherol
[5]ND: Not Detected
*P-value<0.05 between 1507x59122 - glufosinate and control

Table 15. Literature ranges of vitamins in grain (Buffington 2004).

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 16. Summary analysis of secondary metabolites in grain (Buffington 2004).

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x59122 - Glufosinate	Control	Standard Error
Inositol	0.0138 - 0.257	0.026	0.024	0.00078
Furfural	0.0003 - 0.0005	ND[4]	ND[4]	ND[4]
P-Coumaric Acid	0.003 - 0.058	0.023	0.021	0.00066
Ferulic Acid	0.02 - 0.373	0.169	0.169	0.00453

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

Table 17. 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 18. Summary analysis of anti-nutrients in grain (Buffington 2004).

Analyte[1]	Combined Ranges[2]	Means[3]		
		1507x59122 - Glufosinate	Control	Standard Error
Raffinose	0.04 - 0.31	0.12[5]	0.094	0.0063
Phytic acid	0.29 - 1.29	0.815	0.787	0.0248
Trypsin Inhibitor (TIU/g)[4]	1.10 - 7.18	3.30[5]	2.91	0.0828

[1]Percent dry weight
[2]Combined ranges, see Appendix 5
[3]Least square means
[4]Abbreviations: TIU, trypsin inhibitor units
[5]T-value<0.05 between 1507x59122 - glufosinate and control

Table 19. 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]Abbreviations: TIU, trypsin inhibitor units
[2]NR = not reported

Table 20. Mean agronomic data from maize stack 1507 x 59122, sprayed with glufosinate-ammonium and non-GM control with comparable genetic background from field trials at five locations in the USA and Canada in 2003.

Entry	1507x59122 Maize sprayed with glufosinate-ammonium	Control hybrid
Germination/Early Population[1]	54*	54
Seedling Vigor[2]	7	7
GDU[7] 50% Silking[3]	1264	1240
GDU[7] 50% Pollen Shed[4]	1289	1276
Plant Height[5] (in)	98*	96
Ear Height[6] (in)	37	36
Stalk Lodging [7] (%)	0	1
Root Lodging [8] (%)	0	0
Final Population [9]	52*	52
Stay Green [10]	4	3
Disease Incidence [11]	8	8
Insect Damage[12]	8	7
Pollen Shape [13]	86	83

[1]Number of plants emerged per 60 seed planted.
[2]Visual estimate of average vigor evaluated on a 1 to 9 scale where 1 is short plants with small, thin leaves and 9 is tall plants with large, robust leaves.
[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.
[5]Measured from the soil surface to the tip of tassel), n=10.
[6]Measured from the soil surface to the base primary ear), n=10.
[7]Percent of plants broken below the primary ear.
[8]Percent of plants leaning ≥ 30° in the first ½ meter above the soil surface.
[9]Total number of viable plants (per plot) remaining at maturity
[10]Overall plant health at maturity evaluated on a 1 to 9 scale where 1 is completely dead and 9 is very green.
[11]Level of disease resistance at maturity evaluated on a 1 to 9 scale where 1 is poor resistance and 9 is high resistance or no visible disease.
[12]Level of destructive insect resistance at maturity evaluated on a 1 to 9 scale where 1 is poor resistance and 9 is high resistance or no damage.
[13]Pollen grains with collapsed walls after 120 minutes
[14] GDU: Growing Degree Units
*P-value<0.05 between 1507x59122 - glufosinate and the control

Table 21. Mean agronomic data from maize stack 1507 x 59122, untreated, and non-GM control with comparable genetic background, collected from field trials at five locations in the EU in 2004.

Entry	1507x59122 (Untreated)	Control
Germination/Early Population[1]	52/(45 – 57)	52/(40 – 59)
Seedling Vigor[2]	6	7
Time to Silking[3]	865[a]	838
Time to Pollen Shed[4]	841	823
Plant Height[5] (cm)	235	236
Ear Height[6] (cm)	95.1	95.8
Stalk Lodging[7] (%)	0.13	2.7
Root Lodging[8] (%)	0.13	2
Final Population[9]	51	50
Stay Green[10]	3	3
Disease Incidence[11]	6	6
Insect Damage[12]	7	6
Pollen Viability (Shape)[13]	87	83
Pollen Viability (Color)[14]	71	74

[1]Number of plants emerged per 60 seed planted.
[2]Visual estimate of average vigor evaluated on a 1 to 9 scale where 1 is short plants with small, thin leaves and 9 is tall plants with large, robust leaves.
[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.
[5]Measured from the soil surface to the tip of tassel), n=10.
[6]Measured from the soil surface to the base primary ear), n=10.
[7]Percent of plants broken below the primary ear.
[8]Percent of plants leaning ≥ 30° in the first ½ meter above the soil surface.
[9]Total number of viable plants (per plot) remaining at maturity
[10]Overall plant health at maturity evaluated on a 1 to 9 scale where 1 is completely dead and 9 is very green.
[11]Level of disease resistance at maturity evaluated on a 1 to 9 scale where 1 is poor resistance and 9 is high resistance or no visible disease.
[12]Level of destructive insect resistance at maturity evaluated on a 1 to 9 scale where 1 is poor resistance and 9 is high resistance or no damage.
[13]Pollen grains with collapsed walls after 120 minutes
[14]Pollen grains with pollen grains with intense yellow color after 120 minutes
[a] Non-adjusted P-value<0.05