Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

VKM Report 2014: 09

Final health and environmental risk assessment of genetically modified maize 59122

Food/feed and environmental risk assessment of insect-resistant and herbicide-tolerant genetically modified maize 59122 from Pioneer Hi-Bred/Mycogen Seeds for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (EFSA/GMO/NL/2005/12)

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

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

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Summary

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

The herbicide-tolerant and insect-resistant genetically modified maize 59122 from Pioneer Hi-Bred/Mycogen Seeds (Unique Identifier DAS-59122-7) is approved under EU Regulation 1829/2003/EC for food and feed uses, import and processing since 24 October 2007 (Application EFSA/GMO/NL/2005/12, Commission Decision 2007/702/EC). An application for granting consent to all uses of 59122 maize, including cultivation, was submitted by Pioneer in accordance with articles 5 and 17 of the Regulation (EC) No. 1829/2003 21 October, 2005 (EFSA/GMO/NL/2005/23).

VKM participated in the 90 days public consultation of the application for placing on the market of maize 59122 for food and feed uses, import and processing (EFSA/GMO/NL/2005/12) in 2005, and submitted a preliminary opinion in December 2005 (VKM 2005a). Maize 59122 has also been assessed as food and feed by the VKM GMO Panel, commissioned by the Norwegian Environment Agency and the Norwegian Food Safety Authority in connection with the national finalisation of the application in 2008 (VKM 2008a). Maize 59122 has also been evaluated by the VKM GMO Panel as a component of several stacked GM maize events under Regulation (EC) 1829/2003 (VKM 2007a,b,c, VKM 2008b, VKM 2009, VKM 2012a,b, VKM 2013a,b,c,d). Due to the publication of new scientific literature and updated guidelines for risk assessment of genetically modified plants, the VKM GMO Panel has decided to deliver an updated food/feed and environmental risk assessment of event 59122.

The updated food/feed and environmental risk assessment of the maize 59122 is based on information provided by the applicant in the applications EFSA/GMO/NL/2005/12 and EFSA/GMO/NL/2005/23 and scientific comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment also considered other peer-reviewed scientific literature as relevant.

The VKM GMO Panel has evaluated 59122 with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed. The Norwegian Scientific Committee for Food Safety has also decided to take account of the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2011a), the environmental risk assessment of GM plants (EFSA 2010a), selection of comparators for the risk assessment of GM plants (EFSA 2011b) and for the post-market environmental monitoring of GM plants (EFSA 2011c).

The scientific risk assessment of maize 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, target and non-target organisms, and effects on biogeochemical processes.

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

Genetically modified maize 59122 expresses the *cry34Ab1* and *cry35Ab1* genes from *Bacillus thuringiensis*, conferring resistance to certain coleopteran target pests belonging to the genus *Diabrotica*, such as the larvae of western corn rootworm (*D. virgifera virgifera*), northern corn rootworm (*D. barberi*) and the southern corn rootworm (*D. undecimpunctata howardi*). None of the target pests for maize 59122 are present in the Norwegian agriculture. Maize 59122 also expresses the phosphinothricin-N-acetyltransferase (*pat*) gene, from the soil bacterium *Streptomyces viridochromogenes*. The encoded PAT protein confers tolerance to the herbicidal active substance glufosinate-ammonium. The PAT protein produced by maize 59122 has been used as a selectable marker to facilitate the selection process of transformed plant cells and is not intended for weed management purposes.

Molecular characterisation
Appropriate analyses of the transgenic DNA insert, its integration site, number of inserts and flanking sequences in the maize genome, have been performed. The results show that only one copy of the insert is present in maize 59122. Homology searches with databases of known toxins and allergens have not indicated any potential production of harmful proteins or polypeptides caused by the genetic modification in maize 59122. Southern blot analyses and segregation studies show that the introduced genes *cry34Ab1, cry35Ab1* and *pat* are stably inherited and expressed over several generations along with the phenotypic characteristics of maize 59122. The VKM GMO Panel considers the molecular characterisation of maize 59122 satisfactory.

Comparative assessment
Comparative analyses of maize 59122 to its non-GM conventional counterpart have been performed during multiple field trials in representative areas for maize cultivation in Chile (2002/2003), North America (2003, 2004) and Europe (2003, 2004). With the exception of small intermittent variations, no biologically significant differences were found between maize 59122 and the conventional non-GM control. Based on the assessment of available data, the VKM GMO Panel concludes that maize 59122 is compositionally, agronomical and phenotypically equivalent to its conventional counterpart, except for the introduced characteristics.

Food and feed risk assessment
A 90-day subchronic feeding study in rats, as well as whole food feeding studies on broilers, laying hens, lactating dairy cows, feedlot steers, and growing-finishing pigs, have not indicated any adverse effects of maize 59122, and shows that maize 59122 is nutritionally equivalent to conventional maize. The PAT, Cry34Ab1 and Cry35Ab1 proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE-mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize 59122 is nutritionally equivalent to conventional maize varieties. It is unlikely that the PAT, Cry34Ab1 and Cry35Ab1 proteins will introduce a toxic or allergenic potential in food or feed based on maize 59122 compared to conventional maize.

Environmental risk assessment
Considering the intended uses of maize 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 59122.

Maize 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 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.

Overall conclusion

Based on current knowledge, the VKM GMO Panel concludes that maize 59122 is nutritionally equivalent to conventional maize varieties. It is unlikely that the PAT, Cry34Ab1 and Cry35Ab1 proteins will introduce a toxic or allergenic potential in food or feed based on maize 59122 compared to conventional maize.

The VKM GMO Panel likewise concludes that maize 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 59122, EFSA/GMO/NL/2005/12, insect resistance, Cry34Ab1, Cry35AB1, herbicide tolerance, *pat* gene, *PAT* protein, glufosinate ammonium, food and feed safety assessment, environmental risk assessment, Regulation (EC) No 1829/2003

Norsk sammendrag

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

Den insektsresistente og herbicidtolerante maislinjen 59122 (unik kode DAS-59122-7) fra Pioneer Hi-Bred/Mycogen Seeds ble godkjent til import, videreforedling og til bruk som mat og fôr under EU-forordning 1829/2003 24. oktober 2007 (søknad EFSA/GMO/NL/2005/12, Kommisjonsbeslutning 2007/702/EU). En søknad om godkjenning av mais 59122 under forordning 1829/2003 for alle bruksområder, inkludert dyrking ble fremmet av Pioneer 21. oktober 2005 (EFSA/GMO/NL/2005/23).

Maislinjen ble første gang vurdert av VKMs faggruppe for GMO i 2005 (VKM 2005a). Den foreløpige risikovurderingen ble utført på oppdrag fra Mattilsynet i forbindelse med EFSAs høring av søknad EFSA/GMO/NL/2005/12, og inkluderte vurderinger av potensielle helseeffekter ved bruk av 59122 som næringsmiddel og fôrvare. I forbindelse med vurdering av markedsadgang i Norge, utarbeidet VKM en endelig helse- og miljørisikovurdering av mais 59122 i 2008 på oppdrag fra Mattilsynet og Miljødirektoratet (VKM 2008a). VKMs faggruppe for GMO har også risikovurdert en rekke maishybrider der 59122 inngår som en av foreldrelinjene (VKM 2007a,b,c, VKM 2008b, VKM 2009, VKM 2012a,b, VKM 2013a,b,c,d). Etablering av nye, reviderte retningslinjer for helse- og miljørisikovurderinger av genmodifiserte planter og publisering av ny vitenskapelig litteratur har medført at VKM har valgt å utarbeide en ny, oppdatert helse- og miljørisikovurdering av mais 59122.

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, 2011 a,b,c) lagt til grunn for vurderingen.

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

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

Den genmodifiserte maislinjen 59122 uttrykker en ny type *Bt*-toksin, som er resultat av introduksjon av to *cry*-gener (*cry34Ab1*og *cry35Ab1*) fra jordbakterien *Bacillus thuringiensis*, stamme PS149B1. Proteinene virker sammen som et binært toksin og gir plantene resistens mot angrep fra arter i billeslekten *Diabrotica* som *D. virgifera virgifera* (’Western Corn Rootworm), *D. barberi* (’Northern Corn Rootworm’) og *D. undecimpunctata howardi* (’Southern Corn Rootworm’). Ingen av disse

artene er påvist i Norge. Genkonstruksjonen består også av et *pat*-gen fra *Streptomyces viridochromogenes*. Genet koder for enzymet fosfinotricin acetyltransferase (PAT), som acetylerer og inaktiverer glufosinat-ammonium, virkestoffet i fosfinotricin-herbicider. I henhold til søker er *pat*-genet kun introdusert som markør ved seleksjon av transformerte planter.

Molekylær karakterisering
Adekvate analyser av det transgene DNA-innskuddet, dets integreringssete, antall integreringer og flankerende DNA-sekvenser i mais-genomet, har blitt utført. Resultatene viser at kun ett transgent innskudd er til stede i mais 59122. Homologisøk i databaser over kjente toksiner og allergener indikerer at genmodifiseringen ikke har ført til potensiell produksjon av skadelige proteiner eller polypeptider i mais 59122. Southern blot og segresjons analyser viser at de introduserte genene *cry34Ab1*, *cry35Ab1* og *pat* er stabilt uttrykt og nedarvet over flere generasjoner, og i samsvar med de fenotypiske egenskapene til mais 59122. VKMs faggruppe for genmodifiserte organismer vurderer den molekylære karakteriseringen av mais 59122 som tilfredsstillende.

Komparative analyser
Komparative analyser av mais 59122 og tilhørende umodifisert kontroll («konvensjonell motpart») er basert på feltforsøk i representative områder for maisdyrking i Chile (2002/2003), Nord-Amerika (2003 og 2004) og Europa (2003 og 2004). Med unntak av enkelte små variasjoner viste studiene ingen biologisk relevante forskjeller mellom mais 59122 og dens konvensjonelle motpart. Basert på vurdering av tilgjengelig data, konkluderer VKMs faggruppe for GMO at mais 59122 er ernæringsmessig, morfologisk og agronomisk vesentlig lik dens konvensjonelle motpart, med unntak av de introduserte egenskapene.

Helserisiko
Fôringsstudier utført på rotter, broiler, høns, melkekyr, kjøttfe og gris har ikke indikert helseskadelige effekter av mais 59122. Disse studiene indikerer også at mais 59122 er ernæringsmessig vesentlig lik konvensjonell mais. Proteinene Cry34Ab1, Cry35Ab1 og PAT viser ingen likhetstrekk til andre kjente toksiner eller allergener, og er heller ikke rapporterte å ha forårsaket IgE-medierte allergiske reaksjoner. Enkelte studier har derimot indikert at noen typer Cry-proteiner kan forsterke andre allergiske reaksjoner, dvs. fungere som adjuvans. Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at mais 59122 er ernæringsmessig vesentlig lik konvensjonell mais, og at det er lite trolig at proteinene Cry34Ab1, Cry35Ab1 og PAT vil introdusere et toksisk eller allergent potensiale i mat eller fôr basert på mais 59122 sammenliknet med konvensjonelle maissorter.

Miljørisiko
Med bakgrunn i tiltenkt bruksområde er miljørisikovurderingen avgrenset til mulige effekter av utilsiktet frøspredning i forbindelse med transport og prosessering, samt indirekte eksponering gjennom gjødsel fra husdyr fôret med genmodifisert mais.

Det er ingen indikasjoner på økt sannsynlighet for spredning, etablering og invasjon av maislinjen i naturlige habitater eller andre arealer utenfor jordbruksområder som resultat av frøspill i forbindelse med transport og prosessering. Risiko for utkryssing med dyrkede sorter vurderes av GMO-panelet til å være ubetydelig. Ved foreskreven bruk av maislinjen 59122 antas det ikke å være risiko for utilsiktede effekter på målorganismer, ikke-målorganismer eller på abiotisk miljø i Norge.

Samlet vurdering
Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO med at maislinje 59122 er ernæringsmessig ekvivalent med konvensjonell mais. Det er lite trolig at proteinene Cry34Ab1, Cry35Ab1 og PAT vil introdusere et toksisk eller allergent potensiale i mat eller fôr basert på mais 59122 sammenliknet med konvensjonelle maissorter.

Likeledes finner faggruppen, basert på dagens kunnskap, det lite trolig at forskreven bruk av maislinje 59122 vil medføre noen økt miljørisiko i Norge sammenliknet med konvensjonell mais.

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
ARMG	Antibiotic resistance marker gene
BC	Backcross. Backcross breeding in maize is extensively used to move a single trait of interest (e.g. disease resistance gene) from a donor line into the genome of a preferred or "elite" line without losing any part of the preferred lines existing genome. The plant with the gene of interest is the donor parent, while the elite line is the recurrent parent. BC_1, BC_2 etc. designates the backcross generation number.
BLAST	Basic Local Alignment Search Tool. Software that is used to compare nucleotide (BLASTn) or protein (BLASTp) sequences to sequence databases and calculate the statistical significance of matches, or to find potential translations of an unknown nucleotide sequence (BLASTx). BLAST can be used to understand functional and evolutionary relationships between sequences and help identify members of gene families.
bp	Basepair
Bt	*Bacillus thuringiensis*
CaMV	Cauliflower mosaic virus
Codex	Set by The Codex Alimentarius Commission (CAC), an intergovernmental body to implement the Joint FAO/WHO Food Standards Programme. Its principle objective is to protect the health of consumers and to facilitate the trade of food by setting international standards on foods (i.e. Codex Standards).
Cry	Any of several proteins that comprise the crystal found in spores of *Bacillus thuringiensis*. Activated by enzymes in the insects midgut, these proteins attack the cells lining the gut, and subsequently kill the insect.
Cry34/35Ab1	Binary crystal protein containing of Cry34Ab1 and Cry35Ab1. Provide protection against certain coleopteran target pests.
Cry34Ab1	Cry34 class crystal protein from *Bacillus thuringiensis* strain 149B1
Cry35Ab1	Cry35 class crystal protein from *Bacillus thuringiensis* strain 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

EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	Environmental risk assessment
E-score	Expectation score
EU	European Union
fa	Fatty acid
FAO	Food and Agriculture Organisation
FIFRA	US EPA Federal Insecticide, Fungicide and Rodenticide Act
Fitness	Describes an individual's ability to reproduce successfully relative to that of other members of its population.
fw	Fresh weight
fwt	Fresh weight tissue
GAT	Glyphosate N-acetyltransferase
GLP	Good Laboratory Practice
Glufosinate-ammonium	Broad-spectrum systemic herbicide
GM	Genetically Modified
GMO	Genetically Modified Organism
GMP	Genetically Modified Plant
H	Hybrid
ha	Hectare
ILSI	International Life Sciences Institute
IPM	Integrated Pest Management
IRM	Insect Resistance Management
Locus	The position/area that a given gene occupies on a chromosome
LOD	Limit of detection
LOQ	Limit of quantification
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization-Time Of Flight. A mass spectrometry method used for detection and characterisation of biomolecules, such as proteins, peptides, oligosaccharides and oligonucleotides, with molecular masses between 400 and 350,000 Da.
MCB	Mediterranean corn borer, *Sesamia nonagrioides*
mRNA	Messenger RNA
MT	Norwegian Food Safety Authority (Mattilsynet)
NDF	Neutral detergent fibre, measure of fibre used for animal feed analysis. NDF measures most of the structural components in plant cells (i.e. lignin, hemicellulose and cellulose), but not pectin.
Northern blot	Northern blot is a technique used to study gene expression by detection of RNA or mRNA separated in a gel according to size.

NTO	Non-target organism
Nicosulfuron	Herbicide for maize that inhibits the activity of acetolactate synthase
Near-isogenic lines	Term used in genetics/plant breeding, and defined genetic lines that are identical except for differences at a few specific locations or genetic loci.
OECD	Organisation for Economic Co-operation and Development
ORF	Open Reading Frame, in molecular genetics defined as a reading frame that can code for amino acids between two stop codons (without stop codons).
OSL	Over season leaf
OSR	Over season root
OSWP	Over season whole plant
pat	*Phosphinothricin-Acetyl-Transferase* gene
PAT	Phosphinothricin-Acetyl-Transferase protein
PCR	Polymerase chain reaction, a technique to amplify DNA by copying it
R0	First transformed generation, parent
RNA	Ribonucleic acid
RP	Recurrent parent
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis. Technique to separate proteins according to their approximate size
SAS	Statistical Analysis System
SD	Standard deviation
Southern blot	Method used for transfer of electrophoresis-separated DNA fragments to a filter membrane and possible subsequent fragment detection by probe hybridisation
T-DNA	Transfer DNA, the transferred DNA of the tumour-inducing (Ti) plasmid of some species of bacteria such as *Agrobacterium tumefaciens* and *A. rhizogenes*, into plant's nuclear genome. The T-DNA is bordered by 25-base-pair repeats on each end. Transfer is initiated at the left border and terminated at the right border and requires the *vir* genes of the Ti plasmid.
TI	Trait integrated
TMDI	Theoretical Maximum Daily Intake
U.S. EPA	United States Environmental Protection Agency.
Maize growth stages	*Vegetative* VE: emergence from soil surface V1: collar of the first leaf is visible V2: collar of the second leaf is visible Vn: collar of the leaf number 'n' is visible VT: last branch of the tassel is completely visible *Reproductive* R0: Anthesis or male flowering. Pollen shed begins

	R1: Silks are visible
	R2: Blister stage. The kernels are filled with a clear nourishing endosperm fluid and the embryo can be seen
	R3: Milk stage. The kernels endosperm is milky white.
	R4: Dough stage. The kernels endosperm has developed to a white paste
	R5: Dent stage. If the genotype is a dent type, the grains are dented
	R6: Physiological maturity
Western blot	Technique used to transfer proteins separated by gel electrophoresis by 3-D structure or denatured proteins by the length of the polypeptide to a membrane, where they might be identified by antibody labelling.
WHO	World Health Organisation
ZM	*Zea maize* L.

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Background

On 29 January 2005, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands an application (Reference EFSA/GMO/NL/2005/12) for authorisation of the genetically modified insect-resistant and herbicide-tolerant maize 59122 (Unique Identifier DAS-59122-7), submitted by Pioneer Hi-Bred International and Dow AgroSciences LLC within the framework of Regulation (EC) No 1829/2003.

The scope of the application covers:

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

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

After receiving the application EFSA/GMO/NL/2005/12 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 16 September 2005, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the EC and consulted nominated risk assessment bodies of the MS, including the Competent Authorities within the meaning of Directive 2001/18/EC (EC 2001), following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1929/2003, to request their scientific opinion. Within three months following the date of validity, all MS could submit via the EFSA GMO Extranet to EFSA comments or questions on the valid application under assessment. The VKM GMO Panel assessed the application in connection with the EFSA official hearing, and submitted a preliminary opinion in December 2005 (VKM 2005a). EFSA published its scientific opinion 23 March 2007 (EFSA 2007), and maize 59122 was approved for food and feed uses, import and processing October 24, 2007 (Commission Decision 2007/702/EC).

An application for authorisation of maize 59122 for food and feed uses, import, processing and cultivation in the EU was submitted by Pioneer Hi-Bred/Dow AgroSciences 21 October 2005 (EFSA/GMO/NL/2005/23). After receiving additional information requested under completeness check, EFSA declared the application as valid on 9 March 2007. On 26 March 2013 the EFSA GMO Panel adopted its scientific opinion on maize 59122 (EFSA 2013a). Shortly after publishing its opinion and as part of its continuous process of screening all relevant scientific literature, EFSA identified a gap in the data provided by the applicant to support the findings on non-target organisms (honeybees and ladybirds). Consequently, the GMO Panel has revised its previous opinion to indicate that it cannot conclude on both these issues and recommends the applicant provide the necessary data so that a full environmental risk evaluation can be completed. The statement supplementing the environmental risk assessment conclusions and risk management recommendations on maize 59122 in the light of new scientific information on non-target organisms and regionally sensitive areas was published by EFSA 23 October 2013 (EFSA 2013b).

Maize 59122 has previously been assessed as food and feed by the VKM GMO Panel commissioned by the Norwegian Environment Agency and the Norwegian Food Safety Authority in connection with the national finalisation of the procedure of the application EFSA/GMO/NL/2005/12 in 2008 (VKM 2008a). Due to the publication of new scientific literature and updated guidelines for risk assessment of genetically modified plants, the VKM GMO Panel has decided to deliver an updated food/feed and environmental risk assessment of maize 59122.

Maize 59122 has also been evaluated by the VKM GMO Panel as a component of several stacked GM maize events under Regulation (EC) 1829/2003 (VKM 2007a,b, VKM 2008b, VKM 2009, VKM 2012a,b, VKM 2013a,b,c,d).

Exemption of the authorisation requirements of 19 existing products in Norway
Through the Agreement of the European Economic Area (EEA), Norway is obliged to implement the EU regulations on GM food and feed (regulations 1829/2003, 1830/2003 et al). Until implementation of these regulations, Norway has a national legislation concerning processed GM food and feed products that are harmonised with the EU legislation. These national regulations entered into force 15 September 2005. For genetically modified feed and some categories of genetically modified food, no requirements of authorisation were required before this date. Such products that were lawfully placed on the Norwegian marked before the GM regulations entered into force, the so-called existing products, could be sold in a transitional period of three years when specific notifications were sent to the Norwegian Food Safety Authority. Within three years after 15. September 2005, applications for authorisation should be sent to the Authority before further marketing. Four fish feed producing companies have once a year since 2008, applied for an exemption of the authorisation requirements of 19 existing products, including maize 59122. These 19 GM events are all authorised in the EU, and the Norwegian Food Safety Authority has granted exemption for a period of one year each time.
http://www.mattilsynet.no/planter_og_dyrking/genmodifisering/fire_virksomheter_har_faatt_dispensasjon_fra_kravet_om_godkjenning_av_genmodifisert_fiskefor.10951

Terms of reference

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

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

The Norwegian Environment Agency

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

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

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

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

The Norwegian Food Safety Authority

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

The Norwegian Food Safety Authority has therefore, by letter dated 13 February 2013 (ref. 2012/150202), requested the Norwegian Scientific Committee for Food Safety (VKM) to carry out final scientific risk assessments of 39 GMOs and products containing or consisting of GMOs that are authorized in the European Union.

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

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

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

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

Assessment

1 Introduction

Genetically modified maize 59122 was developed to provide protection against 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*). The insect resistance is achieved through expression of the *cry34Ab1* and *cry*35Ab1 genes from *Bacillus thuringiensis* (*Bt*) strain PS149B1, a common soil bacterium. The mode of action of *Bt*-proteins is to bind selectively to specific receptors on the epithelial surface of the midgut of larvae of susceptible insect species, leading to death of larvae through pore formation and cell burst and subsequently septicaemia (reviewed by OECD 2007). None of the target pests of maize 59122 are present in the Norwegian agriculture.

Maize 59122 also produces the PAT (phosphinothricin-N-acetyltransferase) protein from *Streptomyces viridochromogenes*, another common soil bacterium, conferring tolerance to the broad spectrum herbicide DL-phosphinothricin, also known as glufosinate-ammonium. PAT acetylates the active compound L-phosphinothricin which forms the inactive product N-acetyl-L-phosphinothricin. The PAT protein produced in maize 59122 was used as a selectable marker to facilitate the selection process of transformed plant cells and is not intended for weed management purposes.

Maize 59122 (Unique Identifier DAS-59122-7) 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 taken into account the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2011a), the environmental risk assessment of GM plants (EFSA 2010a), the selection of comparators for the risk assessment of GM plants (EFSA 2011b), and for the post-market environmental monitoring of GM plants (EFSA 2011c).

The food/feed and environmental risk assessment of the genetically modified maize 59122 is based on information provided by the applicant in the applications EFSA/GMO/2005/19, EFSA/GMO/NL/2005/230, relevant peer-reviewed scientific literature, and scientific opinions and comments from EFSA and other member states made available on the EFSA website GMO Extranet. A risk analysis report from the "Food Standards Australia New Zealand" in 2005 (FSANZ 2005), has also been taken into account.

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

2 Molecular characterisation

2.1 Information related to the genetic modification

Maize 59122 has been genetically modified to constitutively express the three genes: *cry34Ab1*, *cry35Ab1*, and *pat* in order to produce the Cry34Ab1, Cry35Ab1 and PAT proteins. The Cry34Ab1 and Cry35Ab1 proteins act together to protect the plants from certain coleopteran insect pests, such as corn rootworm larvae (*Diabrotica* spp.). The PAT protein, used as a selectable marker, confers tolerance to glufosinate-ammonium herbicides. No other traits have been introduced or modified in maize event 59122.

2.1.1 Description of the methods used for the genetic modification

The binary vector PHP17662 was used in the *Agrobacterium* mediated transformation of 59122 maize (Figure 1). Initially, the *cry34Ab1*, *cry35Ab1*, and *pat* genes, all modified for optimal expression in maize, were cloned from either *Bacillus thuringiensis* strain PS149B1 (*cry*34/35*)* or *Streptomyces viridochromogenes* (*pat*). The three transgenes and necessary regulatory elements were then assembled and introduced to an intermediate vector to produce the vector PHP17661. The PHP17661 vector contained the right border T-DNA sequence linked with the *ubi*1ZM promoter, the maize-optimised *cry34Ab1* gene, the pinII terminator, the wheat peroxidase promoter, the maize-optimised *cry35Ab1* gene, a second pinII terminator, the CaMV 35S promoter, the plant-optimised *pat* gene and the CaMV 35S terminator fused with the left border T-DNA sequence; plus a vector backbone portion including a spectinomycin resistance gene, the ColE1 *ori* and the *cos* site of phage lambda. Vector PHP17661 was then integrated through homologous recombination with a new vector, vector PHP10523, to form the vector PHP17662. To achieve this, the *Escherichia coli* strain HB101, containing vector PHP17661, was mated with *Agrobacterium* strain LBA4404 containing vector PHP10523.

According to the applicant, transformation of 59122 maize resulted in the stable insertion of the T-DNA region (Figure 2) from binary vector PHP17662 (Figure 1) in the maize genome. The T-DNA region contains the *cry34Ab1*, *cry35Ab1* and *pat* coding sequences and the necessary regulatory components to regulate gene expression. The plant regenerated from these maize cells produces the Cry34Ab1, Cry35Ab1 and PAT proteins and is referred to as maize 59122.

2.1.2 Nature and source of vector used

An overview of the genetic elements that constitute binary vector PHP17662, including their size, origin and role, is provided in Table 1. The T-DNA region of PHP17662 is 7390 bp long. A schematic representation of the PHP17662 T-DNA is shown in Figure 2. A complete description of the size, position, origin and intended function of the DNA sequences contained in the T-DNA region is presented in Table 2.

2.1.2.1 Size, source of donor organism(s) and intended function of each constituent fragment of the region intended for insertion

The maize-optimised *cry34Ab1* gene encodes a Cry34Ab1 protein (14 kDa; 123 amino acids) with an amino acid sequence identical to the native Cry34Ab1 derived from *Bacillus thuringiensis* strain PS149B1. Expression of maize-optimised *cry34Ab1* is regulated by the 1993 bp long ubiquitin promoter from *Zea mays* (*ubi*1ZM), including the 5'UTR and intron. Termination of transcription is controlled by the 315 bp long terminator sequence from the *Solanum tuberosum* proteinase inhibitor II gene.

Likewise, the maize-optimised *cry35Ab1* gene encodes a Cry35Ab1 protein (44 kDa; 383 amino acids) with an amino acid sequence identical to the native Cry35Ab1 protein (44 kDa) derived from *Bacillus thuringiensis* strain PS149B1. Expression of maize-optimised *cry35Ab1* is regulated by the 1298 bp long promoter from the *Triticum aestivum* peroxidase gene and its native leader. Termination of transcription is controlled by the 315 bp long terminator sequence from *Solanum tuberosum* proteinase inhibitor II gene.

The sequence of the *pat* gene in the PHP17662 T-DNA is 552 bp long and has been optimised for expression in plants. It is based on the native *pat* gene from *Streptomyces viridochromogenes*. The CaMV 35S promoter and terminator sequences from cauliflower mosaic virus regulate expression of *pat* and consist of 530 bp and 194 bp, respectively. Cauliflower mosaic virus is a DNA caulimovirus with a host range restricted primarily to cruciferous plants. It has a double stranded DNA genome within which two distinct promoters, producing 19S and 35S transcripts. The 35S promoter and its variants with enhanced transcriptional activity are constitutively active in several plant species, including maize.

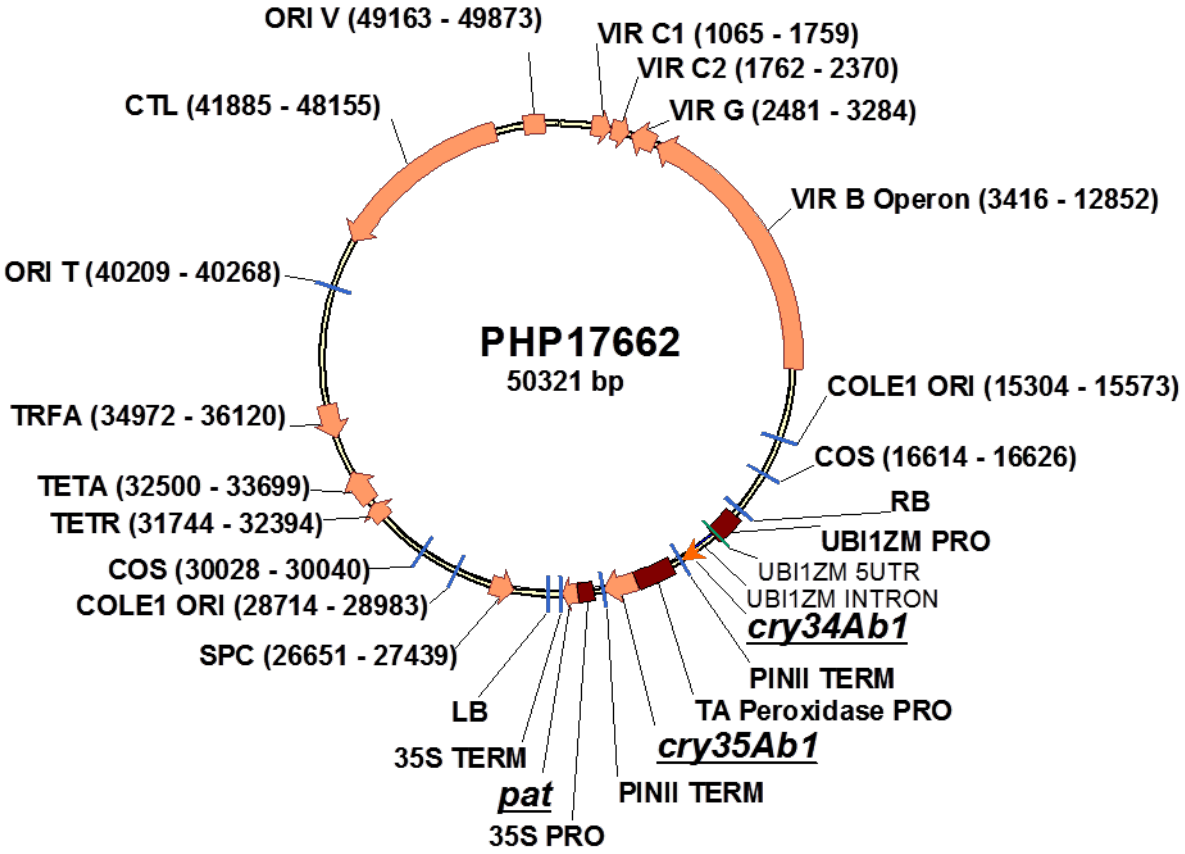

Figure 1. Schematic representation of binary vector PHP17662 used for transformation of 59122 maize.

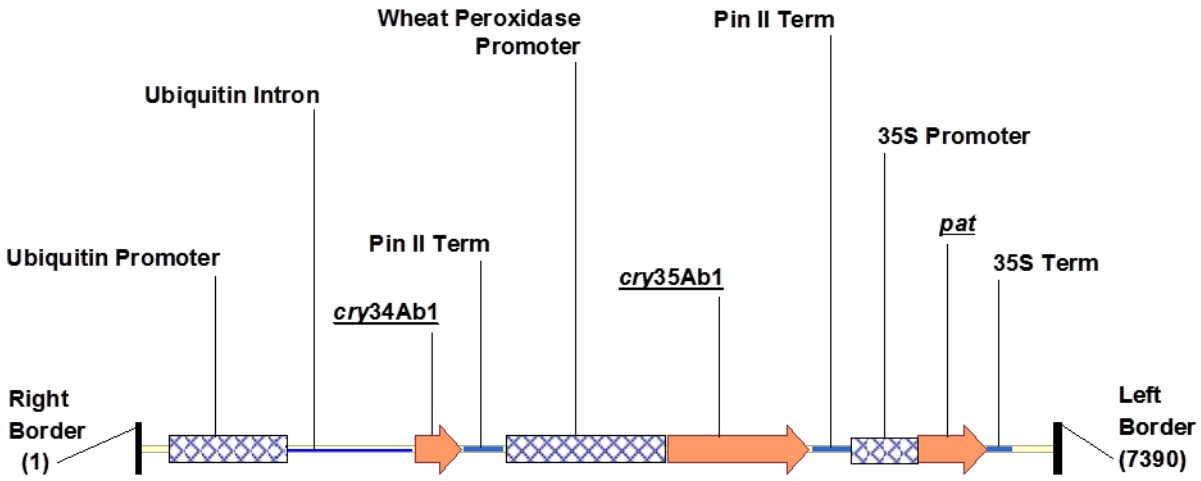

Figure 2. Schematic representation of the 7390 bp T-DNA region from binary vector PHP17662.

Table 1. Genetic elements present in binary vector PHP17662 used for transformation of maize 59122.

Location on binary vector PHP17662 (bp to bp)	Genetic element	Size (bp)	Source and function
1-1064	Backbone sequence	1064	Backbone sequence from vector pSB1 (Komari *et al*., 1996). No function ascribed.
1065 – 1759	VIR C1	695	*vir*C1 gene region from *vir*C operon from *Agrobacterium tumefaciens* (Komari *et al*., 1986). Virulence gene required for efficient and accurate T-DNA transfer.
1760-1761	Backbone sequence	2	Backbone sequence from vector pSB1 (Komari *et al*., 1996). No function ascribed.
1762 – 2370	VIR C2	609	*vir*C2 gene region from *vir*C operon from *Agrobacterium tumefaciens* (Komari *et al*., 1986). Virulence gene required for efficient and accurate T-DNA transfer.
2371-2480	Backbone sequence	110	Backbone sequence from vector pSB1 (Komari *et al*., 1996). No function ascribed.
2481 – 3284	VIR G	804	*vir*G gene region from *vir*G operon from *Agrobacterium tumefaciens* (Komari *et al*., 1986). Virulence gene required for efficient and accurate T-DNA transfer.
3285-3415	Backbone sequence	131	Backbone sequence from vector pSB1 (Komari *et al*., 1996). No function ascribed.
3416 – 12852	VIR B	9437	*vir*B operon region from *Agrobacterium tumefaciens* plasmid (Komari *et al*., 1986). Virulence region required for efficient and accurate T-DNA transfer.
12853-15303	Backbone sequence	2451	Backbone sequence from vector pSB1 (Komari *et al*., 1996). No function ascribed.
15304 - 15573	COL E1	270	ColE1 origin of replication region from *E. coli*.

(*Table continues on following page*)

Table 1 (continued from previous page): Genetic elements present in binary vector PHP17662 used for transformation of maize 59122.

15574-16613	Backbone sequence	1040	Backbone sequence from vector pSB1 (Komari *et al*., 1996). No function ascribed.
16614 –16626	COS	13	*cos* site (cohesive ends) from bacteriophage lambda.
16627-18085	Backbone sequence	1459	Backbone sequence from vector pSB1 (Komari *et al*., 1996). No function ascribed.
18086 – 25475	T-DNA region	7390	See Table 2 for a complete description of genetic elements in the T-DNA region of binary vector PHP17662.
25476-26650	Backbone sequence	1175	Backbone sequence from vector pSB1 (Komari *et al*., 1996). No function ascribed.
26651 - 27439	SPC	789	Spectinomycin resistance gene (*spc*) from transposon Tn7 (Fling *et al*., 1985).
27440-28713	Backbone sequence	1274	Backbone sequence from vector pSB1 (Komari *et al*., 1996). No function ascribed.
28714 - 28983	COL E1	270	ColE1 origin of replication region from *E. coli*.
28984-30027	Backbone sequence	1044	Backbone sequence from vector pSB1 (Komari *et al*., 1996). No function ascribed.
30028 – 30040	COS	13	*cos* site (cohesive ends) from bacteriophage lambda.
30041-31743	Backbone sequence	1703	Backbone sequence from vector pSB1 (Komari *et al*., 1996). No function ascribed.
31744 – 32394	TET R	651	Tetracycline resistance regulation gene (*tet*R; Waters *et al*., 1983; Speer *et al*., 1992).
32395-32499	Backbone sequence	105	Backbone sequence from vector pSB1 (Komari *et al*., 1996). No function ascribed.
32500 – 33699	TET A	1200	Tetracycline resistance gene (*tet*A; Waters *et al*., 1983), which encodes a tetracycline efflux protein (Speer *et al*., 1992).

(*Table continues on following page*)

Table 1 (continued from previous page): Genetic elements present in binary vector PHP17662 used for transformation of maize 59122

33700-34971	Backbone sequence	1272	Backbone sequence from vector pSB1 (Komari *et al*., 1996). No function ascribed.
34972 – 36120	TRF A	1149	Trans-acting replication gene (*trf*A), required for initiation of plasmid replication (Thomas *et al*., 1980).
36121-40208	Backbone sequence	4090	Backbone sequence from vector pSB1 (Komari *et al*., 1996). No function ascribed.
40209 – 40268	ORI T	60	*ori*T, origin of transfer region.
40269-41884	Backbone sequence	1616	Backbone sequence from vector pSB1 (Komari *et al*., 1996). No function ascribed.
41885 – 48155	CTL	6271	Central control operon region, required for active partitioning of the plasmid (Thomas, 2000).
48156-49162	Backbone sequence	1007	Backbone sequence from vector pSB1 (Komari *et al*., 1996). No function ascribed.
49163 – 49873	ORI V	711	*ori*V, origin of replication region.
49874-50321	Backbone sequence	448	Backbone sequence from vector pSB1 (Komari *et al*., 1996). No function ascribed.

Note: a schematic representation of binary vector PHP17662 is provided in Figure 1.

Table 2. Complete description of genetic elements in the 7390 bp T-DNA region from binary vector PHP17662.

Location on binary vector PHP17662 (bp to bp)	Location on PHP17662 T-DNA (bp to bp)	Genetic element	Size (bp)	Function
18086 - 18262	1 – 177	RB	177	Right T-DNA border region from Ti plasmid of *Agrobacterium tumefaciens*. T-DNA right border 25 bp repeat region located from bp 1 to bp 25.
18263 - 18333	178 – 248	Polylinker region	71	Region required for the cloning of the genetic elements.
18334 - 20326	249 – 2241	*ubi*1ZM promoter	1993	Ubiquitin promoter from *Zea mays* including 5'UTR (bp 1149 to bp 1231) and intron (bp 1232 to bp 2241) (Christensen *et al*., 1992).
20327 - 20354	2242 – 2269	Polylinker region	28	Region required for the cloning of the genetic elements.
20355 - 20726	2270 – 2641	*cry*34Ab1	372	Maize-optimised *cry*34Ab1 gene encoding the 14 kDa delta-endotoxin parasporal crystal protein from *Bacillus thuringiensis* strain PS149B1 (Ellis *et al*., 2002). Coding region from start codon through stop codon.
20727 - 20749	2642 – 2664	Polylinker region	23	Region required for cloning of the genetic elements.
20750 - 21064	2665 – 2979	PINII terminator	315	Terminator sequence from *Solanum tuberosum* proteinase inhibitor II gene (An *et al*., 1989).
21065 - 21090	2980 – 3005	Polylinker region	26	Region required for cloning of the genetic elements.
21091 - 22388	3006 – 4303	TA peroxidase promoter	1298	*Triticum aestivum* peroxidase promoter (wheat peroxidase); (Hertig *et al*., 1991).
22389 - 22404	4304 – 4319	Polylinker region	16	Region required for cloning of the genetic elements.
22405 - 23556	4320 – 5471	*cry*35Ab1	1152	Maize-optimised *cry*35Ab1 gene encoding the 44 kDa delta-endotoxin parasporal crystal protein from *Bacillus thuringiensis* strain PS149B1 (Ellis *et al*., 2002). Coding region from start codon through stop codon.
23557 - 23579	5472 – 5494	Polylinker region	23	Region required for cloning of the genetic elements.

(Table continues on following page)

Table 2 (continued from previous page): Complete description of genetic elements in the 7390 bp T-DNA region from binary vector PHP17662.

23580 - 23894	5495 – 5809	PINII terminator	315	Terminator sequence from *Solanum tuberosum* proteinase inhibitor II gene (An *et al*., 1989).
23895 - 23896	5810 – 5811	Polylinker region	2	Region required for cloning of the genetic elements.
23897 – 24426	5812 – 6341	CaMV 35S promoter	530	35S promoter from Cauliflower Mosaic Virus, Strasbourg strain (Hohn *et al*., 1982; Pietrzak, *et al*., 1986).
24427 - 24445	6342 – 6360	Polylinker region	19	Region required for cloning of the genetic elements.
24446 - 24997	6361 – 6912	*pat*	552	Plant-optimised phosphinothricin acetyltransferase coding sequence from *Streptomyces viridochromogenes*. Coding region from start codon through stop codon (Wohlleben *et al*., 1988).
24998 – 25016	6913 – 6931	Polylinker region	19	Region required for cloning of the genetic elements.
25017 - 25210	6932 – 7125	CaMV 35S terminator	194	35S terminator from Cauliflower Mosaic Virus (Hohn *et al*., 1982).
25211 - 25393	7126 – 7308	Polylinker region	183	Region required for cloning of the genetic elements.
25394 - 25475	7309 – 7390	LB	82	Left T-DNA border region from Ti plasmid of *Agrobacterium tumefaciens*. T-DNA Left Border 25 bp repeat region located from bp 7366 to bp 7390.

Note: a schematic representation of the T-DNA region of binary vector PHP17662 is provided in Figure 2.

2.2 Information relating to the GM plant

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

Maize 59122 produces the insecticidal proteins Cry34Ab1 and Cry35Ab1, and the PAT protein which confers tolerance to glufosinate-ammonium herbicides. The Cry34Ab1 and Cry35Ab1 proteins act together to protect the plants from certain coleopteran insect pests, such as corn rootworm larvae (*Diabrotica* spp.). The PAT protein was used as a selectable marker. No other traits have been introduced or modified in maize event 59122.

2.2.2 Information on the sequences actually inserted or deleted

Southern blot and sequence analyses indicate that a single full-length copy of the T-DNA region from binary vector PHP17662 was inserted in the genome of maize 59122, with the exception of the last 22 bp at the 5' (right) end, and the last 25 bp at the 3' (left) end of the T-DNA borders, which were lost during the integration process. The analyses also show the absence of the tetracycline and spectinomycin resistance genes, the *vir*G gene, and regions immediately outside of the left and right T-DNA borders. These results show that maize event 59122 does not contain fragments from the vector backbone portion of binary vector PHP17662.

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

The T-DNA insert as well as the 5' and 3' flanking genomic regions of the 59122 maize have been sequenced and characterised. PCR analyses of the DNA flanking the insert indicate that both regions correspond to maize genomic DNA.

Southern blot analysis was performed on leaf samples from four different generations of 59122 maize, these were designated: T1S1, T1S2, BC1 and BC2S1.

Genomic DNA from 59122 maize was digested with the restriction enzymes *Hind* III, *Sac* I or *Nco* I and hybridised with probes for: *cry34Ab1*, *cry35Ab1*, *pat*, ubiquitin promoter, ubiquitin intron, pinII terminator, wheat peroxidase promoter, or the 35S promoter (Figure 3). Three of the probes (ubiquitin intron, ubiquitin promoter and the wheat peroxidase promoter) were not only homologous to regions of the PHP17662 T-DNA, but were also homologous to endogenous maize genomic sequences. For these probes, the hybridisation patterns in non-GM maize lines were used to identify endogenous hybridisation bands in the 59122 maize DNA.

Positive control was non-GM maize DNA (Hi-II maize) spiked with PHP17662 plasmid DNA, digested with *Hind* III, *Sac* I or *Nco* I. Samples of genomic DNA from different non-GM maize lines were used as negative controls.

***Hind* III**

Digestion with *Hind* III was expected to release an internal hybridising fragment (6963 bp) from the T-DNA insert in maize 59122, which should contain the complete plant transcription unit (promoter/gene/terminator) for all three genes in the T-DNA (*cry*34/35Ab1 and *pat*). If intact, this fragment should therefore hybridise with all probes covering the length of the T-DNA. Southern blot analysis show that the digestion with *Hind* III of genomic DNA from 59122 maize and positive control, produced the same predicted internal hybridising fragment (6963 bp), indicative of an intact T-DNA insert in 59122 maize.

***Sac* I**

Sac I digestion was expected to result in three internal hybridising fragments of 1941 bp, 1855 bp and 123 bp, respectively. The 1941 bp fragment was expected to hybridise with the probes for the wheat peroxidase promoter and *cry35Ab1*. The 1855 bp fragment was expected to hybridise with the probes for *cry35Ab1*, *pinII* terminator, 35S promoter, and *pat*. The smallest fragment (123 bp) was predicted to hybridise with the *cry35Ab1* probe.

The Southern blot analyses show that the digestion with *Sac* I formed both of the biggest fragments, and that they hybridised with the selected probes. The predicted 123 bp fragment was however not detected with the *cry35Ab1* probe, as fragments below approximately 1000 bp were run off the gel during electrophoresis and therefore not transferred to the nylon membrane prior to the hybridisation.

***Nco* I**

Nco I digestion was expected to result in two internal hybridising fragments of 1915 bp and 2607 bp, respectively. The 1915 bp fragment was expected to hybridise with the probes for the ubiquitin intron, *cry34Ab1*, pinII terminator, and the wheat peroxidase promoter. The 2607 bp fragment was expected to hybridise with the probes for the wheat peroxidase promoter, *cry35Ab1*, and the pinII terminator. The Southern blot analyses show that both hybridisation fragments were formed with the selected probes as predicted.

Copy number

In order to determine the copy number of T-DNA in 59122 maize, genomic DNA from maize 59122 and positive control (Hi-II maize genomic DNA spiked with plasmid PHP17662) was digested with the *Xho* I, *Sac* I, *Bsa* I or *Nco* I restriction enzymes (Figure 3). Samples of genomic DNA from different non-GM maize lines were used as negative control.

***Xho* I**

The *Xho* I restriction enzyme has one cleavage site located within the T-DNA region from binary vector PHP17662, and two cleavage sites located in the maize genome. The genomic cleavage sites flank the left and right borders of the T-DNA insert in maize 59122. Genomic DNA from maize 59122 digested with *Xho* I was therefore expected to form a single hybridisation fragment (if only one copy of the T-DNA insert was present) with the probes for *cry34Ab1*, *cry35Ab1*, *pat*, ubiquitin intron, pinII terminator, and the 35S promoter, and two hybridisation fragments with the ubiquitin promoter probe (Because of the two genomic cleavage sites in maize 59122).

The Southern blot analyses show that hybridisation with the *cry34Ab1*, *cry35Ab1*, *pat*, ubiquitin intron, pinII terminator, and 35S promoter probes, resulted in a single ~ 8000 bp fragment, whereas the ubiquitin promoter probe formed the ~ 8000 bp fragment and a second ~ 1500 bp fragment, as expected.

***Bsa* I**

The *Bsa* I restriction enzyme has one cleavage site located within the T-DNA region from binary vector PHP17662, and one cleavage site in the maize genome flanking the left border of the T-DNA insert in maize 59122.

Genomic DNA from maize 59122 digested with *Bsa* I was therefore expected to form a single hybridisation fragment with the probes for *cry34Ab1*, *cry35Ab1*, ubiquitin promoter, ubiquitin intron, wheat peroxidase promoter, pinII terminator and the 35S promoter. In addition, a single hybridisation fragment was expected with the *pat* probe.

The Southern blot analyses show that a single ~ 6400 bp hybridisation fragment containing the T-DNA right border region was formed with the probes for *cry34Ab1*, *cry35Ab1*, ubiquitin promoter, ubiquitin intron, wheat peroxidase promoter, pinII terminator and the 35S promoter. The *pat* probe formed a single hybridisation fragment of ~2800 bp, containing the T-DNA left border region.

***Nco* I and *Sac* I**

The two enzymes *Nco* I and *Sac* I were used to confirm the presence of only single copies of the left and right border regions of the T-DNA insert in maize 59122. For the left border region, Genomic DNA from maize 59122 was digested with *Nco* I and hybridised with the *pat* and 35S promoter probes, which resulted in a single ~ 3400 bp hybridisation fragment containing the T-DNA left border region. For the right border region, Genomic DNA from maize 59122 was digested with *Sac* I and hybridised with the probes for *cry34Ab1*, ubiquitin promoter, ubiquitin intron, wheat peroxidase promoter and the pinII terminator, which resulted in a single ~ 3400 bp hybridisation fragment containing the T-DNA right border region.

Southern blot analysis with the negative control samples did not hybridise to the probes, while the plasmid positive controls the same expected hybridisation fragments were observed.

***spc*, *tet*, *virG*, LB backbone and RB backbone probes**

The 59122 maize genomic DNA was hybridised to a number of DNA probes to investigate if any plasmid backbone from of PHP17662 (*i.e.* outside the PHP17662 T-DNA region) had been transferred to 59122 maize during the transformation. Southern blot analysis showed no hybridisation signals in the DNA samples from 59122 maize when hybridised to the probes for *spc* (spectinomycin), *tet* (tetracycline), *virG* (plasmid virulence gene), LB backbone or RB backbone. The expected hybridisation bands were observed in the positive control samples.

The results of the Southern blot analyses shows absence of antibiotic resistance genes and vector backbone in 59122 maize, and the presence of an intact and full-length T-DNA insert.

PCR

PCR analysis was used to determine the sequence of the inserted T-DNA in 59122 maize. In addition, cloning and sequencing of the flanking border regions was performed to characterise the T-DNA insertion site in the genome of 59122 maize. In total, 11922 bp of 59122 maize genomic sequence was determined, which included 2593 bp of 5' flanking border sequence, 1986 bp of 3' flanking border sequence, and 7343 bp of T-DNA insert.

Two nucleotide differences were observed in the non-translated wheat peroxidase promoter region of the T-DNA insert in maize 59122. The adenine nucleotide at position 3955 of the T-DNA sequence (corresponds to position 22040 on the PHP17662 sequence) was found to be a cytosine nucleotide in the 59122 maize insert, and the adenine nucleotide at position 3991 of the PHP17662 T-DNA sequence (corresponds to position 22076 on the PHP17662 sequence) was found to be a guanine nucleotide in the 59122 maize insert. The two nucleotide changes occurred in a non-translated part of the T-DNA. According to the applicant, neither of the observed base changes affected the open reading frame composition of the T-DNA insert.

To verify the amplified flanking border sequences, event-specific PCR amplifications were performed on genomic DNA from 59122 maize. Primers were designed based on the sequences obtained from the genome walking experiments. According to the applicant, these primer sets gave negative results with the control samples. Subsequently, it was investigated whether the characterised sequences at the 5' and 3' end of the T-DNA insert were of maize genomic origin. PCR primer sets were designed with homology to the 5' and 3' genomic border regions flanking the 59122 maize insert. According to the applicant, the PCR products (amplicons) from the 5' genomic border region, were of the expected size (136 bp and 263 bp, respectively) in all maize samples tested. Similarly, the 3' genomic border region was of the expected size (227 bp and 492 bp, respectively) in all samples tested. Since these amplicons were amplified in 59122 maize, as well as in the non-GM maize samples, the results indicate that the 5' and 3' genomic border regions flanking the 59122 maize T-DNA insert are of maize genomic origin. This was verified by performing a local alignment of the complete sequence of binary vector PHP17662 with the amplified sequence of the 59122 maize genomic borders. The alignment showed no significant homologies, showing that the border regions flanking the T-DNA insert in 59122 maize did not contain fragments of the transforming plasmid PHP17662.

BLAST

BLAST sequence analysis of the amplified genomic border regions of 59122 maize was performed. Analysis of the 5' border region found two areas with significant homology to maize genomic and EST (Expressed Sequence Tag) sequences. The first area encompasses 179 bp and displays similarity to several molecular markers, chromosomal sequences and consensus sequences obtained by alignment of various ESTs. The second area encompasses 74 bp that shows similarity to a number of different maize ESTs and genomic sequences. The 3' border region also had two non-contiguous regions of similarity to maize genomic DNA sequences. A first region of 162 bp showed similarity to the 3' untranslated end of two genomic *Zea mays* alcohol dehydrogenase (*adh1*) genes as well as to several EST sequences. A second region of 57 bp showed similarity to non-coding regions from multiple maize genomic sequences.

Individual GenBank accessions displaying similarity to the genomic border regions of 59122 maize were examined to determine if 59122 maize insertion occurred in a characterised protein sequence. According to the applicant, none of the regions of similarity occurred within any known protein coding sequence.

ORF

Analysis of the 5' and 3' junction regions of the 59122 maize insert sequence for the presence of potential open reading frames (ORFs) was carried out. Based on the sequence data as obtained by PCR analysis of the 59122 maize insert, the presence of novel ORFs in the 5' and 3' junction regions between the maize genomic border sequence and the T-DNA insert in maize 59122 was investigated. According to the applicant, no ORFs of significant size (> 100 amino acids) were identified in the 5' or 3' border junction regions, indicating that no ORFs were generated as a result of the T-DNA insertion. Additionally, the homology searches did not indicate the presence of endogenous maize ORFs in the border regions that might have been interrupted by the T-DNA insertion in maize 59122.

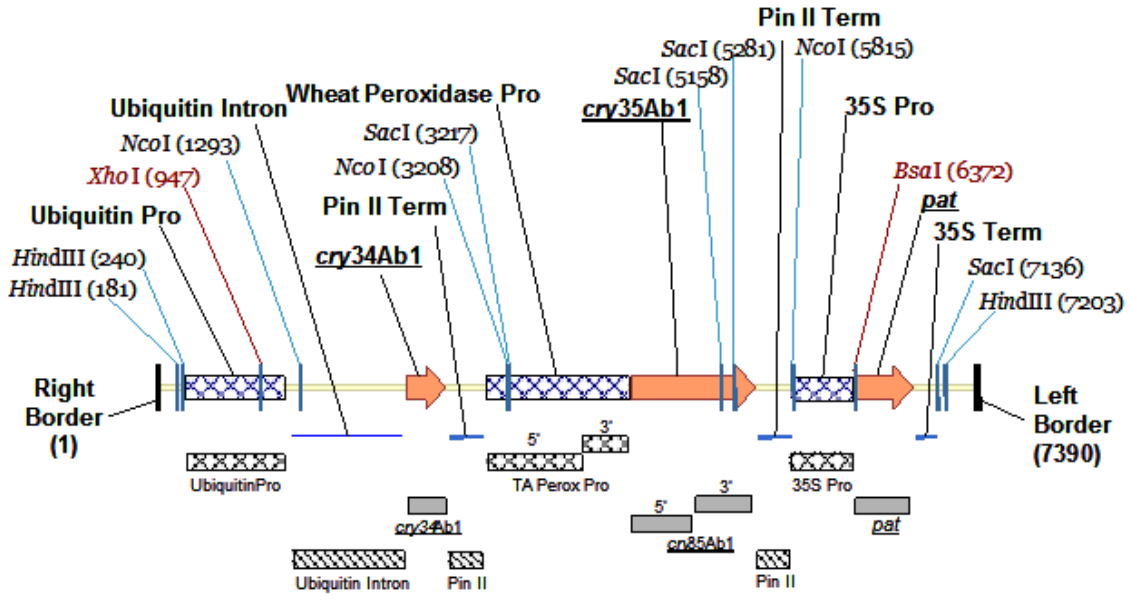

Figure 3. Restriction map of the T-DNA region from binary vector PHP17662, indicating *Sac* I, *Hind* III, *Xho* I, *Nco* I and *Bsa* I restriction sites and gene probes used for Southern analysis.

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

The structural organisation of the insert in 59122 was analysed by Southern blot, and validation of the insert was performed by DNA sequencing. The results of the molecular characterisation showed that 59122 contains a single DNA insert, containing one intact copy of the restriction fragment from binary vector PHP17662. No other detectable fragments from plasmid PHP17662 were found in maize 59122.

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

Not applicable.

2.2.2.4 Chromosomal location(s) of insert(s) (nucleus, chloroplasts, mitochondria or maintained in a non-integrated form) and methods for its determination

Segregation data of 59122 maize progeny established the location and stability of the inserted T-DNA, and statistical analysis showed that the insert segregates according to standard Mendelian patterns, as expected for a single insertion site in the nuclear genome of maize 59122.

2.3 Information on the expression of the insert

Protein levels of Cry34Ab1, Cry35Ab1 and PAT have been measured in a range of tissues from samples of 59122 maize during field trials in Chile, USA and Canada. The levels were measured with specific Enzyme Linked Immunosorbent Assay (ELISA) systems developed for each protein. Complete results are summarised in Tables 3, 4 and 5.

All field trials were performed in representative maize growing regions of each country. Six field locations were used in Chile (2002, 2003), three locations in USA (2003) and two locations in Canada (2003). Samples of leaf and root tissue were collected at the V9 (nine leaf stage), the R1 (silking stage, 50% pollen shed), the R4 (24-28 days after silking) and the R6 (physiological maturity) developmental stages in all trials. Additional, root samples were collected during the V6 (all plant parts are present) developmental stage in USA and Canada. Samples of pollen and stalk were collected at approximately the R1 stage of development. Whole plants were collected at the V9, R1 and the R6 stage. Mature grain (R6 stage) and forage (R4 stage) were also analysed.

Chile
At each location in Chile, the 59122 maize was compared to non-GM control maize with a comparable genetic background. Grain samples were collected once the plants had reached physiological maturity (R6 stage). Per location, five samples were collected from the 59122 maize and one sample was collected from the non-GM control maize. Each grain sample consisted of a single ear collected from a self-pollinated plant. The mean levels of Cry34Ab1 and Cry35Ab1 proteins in 59122 maize grain from the Chilean field trials were 49.7 and 0.99 ng/mg dry weight (dw) (= 49.7 and 0.99 µg/g dw), respectively. The level of PAT protein was below the lower limit of quantitation of the assay (LOQ ≥ 0.06 ng/mg dw) in all samples analysed. Results are summarised in Table 3.

USA and Canada
In USA, the field trials were conducted at Richland (Iowa); Noblesville (Indiana), York (Nebraska), and a fourth location which was omitted from the study due to severe flooding of the fields. In Canada, the field trials were conducted at Branchton (Ontario) and Thorndale (Ontario). At each location, the trial consisted of 59122 maize sprayed with glufosinate-ammonium (sprayed 59122 maize), 59122 maize not sprayed with glufosinate-ammonium (non-sprayed 59122 maize) and non-GM control maize with a comparable genetic background.

Grain samples were collected once the plants had reached physiological maturity (R6 stage). Per location, five samples were collected from the sprayed and non-sprayed 59122 maize, and one sample was collected from the non-GM control maize. Each grain sample consisted of a single ear collected from a self-pollinated plant.

The mean levels of Cry34Ab1 and Cry35Ab1 proteins in grain from sprayed 59122 maize from the US and Canadian field trials were 39.6 and 1.98 ng/mg dw (= 39.6 and 1.98 µg/g dw), respectively. The mean level of PAT protein in sprayed 59122 maize grain was 0.1 ng /mg dw (= 0.1 µg/g dw). The mean levels of Cry34Ab1 and Cry35Ab1 proteins in grain from non-sprayed 59122 maize grain were 36.4 and 2.0 ng/mg dw (= 36.4 and 2.0 µg/g dw), respectively. The mean level of PAT protein in non-sprayed 59122 maize grain was 0.1 ng /mg dw (= 0.1 µg/g dw).

Levels of Cry34Ab1, Cry35Ab1 and PAT proteins in sprayed and non-sprayed 59122 maize grain from the USA and Canada field trials (2003) are summarised in Tables 4 and 5.

Table 3. Summary of Cry34Ab1, Cry35Ab1 and PAT protein levels (ng/mg tissue dw) in tissues collected from 59122 maize from field trials in Chile in 2002-2003 (Essner & Coats 2003).

Tissue (growth stage)[a]	Mean[b] Cry34Ab1 expression (ng/mg tissue dry weight)	Standard deviation	Mean[b] Cry35Ab1 expression (ng/mg tissue dry weight)	Standard deviation	Mean[b] PAT expression (ng/mg tissue dry weight)	Standard deviation
Leaf (V9 stage)	49.5	7.79	40.7	7.29	11.1	3.68
Leaf (R1 stage)	80.6	12.4	52.2	12.9	11.2	3.49
Leaf (R4 stage)	220	37.5	85.3	18.9	8.13	3.02
Leaf (R6 stage)	163	83.6	54.4	22.2	0.38	0.46
Root (V9 stage)	38.8	8.28	8.06	2.98	0.47	0.15
Root (R1 stage)	36.8	8.54	5.08	1.57	0.27	0.12
Root (R4 stage)	49.1	9.23	3.50	0.85	0.09	0.12
Root (R6 stage)	49.7	19.6	3.10	2.43	0.08	0.11
Whole plant (V9 stage)	31.5	15.5	7.36	2.19	0.18	0.13
Whole plant (R1 stage)	45.4	13.5	12.3	3.54	0.13	0.23
Senescent whole plant	76.5	10.3	13.9	1.91	0	0
Pollen (R1 stage)	74.4	6.57	0.02	0.04	0	0
Stalk (R1 stage)	32.9	4.14	10	2.26	0.13	0.03
Forage (R4 stage)	53.1	19.1	12.4	2.77	0	0
Grain (Physiol. maturity)	49.7	16.2	0.99	0.33	0	0

[a] Iowa State University (1993).

[b] Values are means across all six sites from mean values calculated from the analysis of (i) five individual samples per site for leaf at the V9 and R1 stage; root, pollen and stalk at the R1 stage, and grain at the R6 stage; and (ii) three individual samples per site for leaf at the R4 and R6 stage, root at the V9, R4 and R6 stage; and (iii) one pooled sample per site derived from three whole plant samples for the whole plant stage at the V9, R1, R4 and R6 stage and forage at the R4 stage.

Table 4. Summary of Cry34Ab1, Cry35Ab1 and PAT protein levels (ng/mg tissue dw) in tissues collected from 59122 maize sprayed with glufosinate-ammonium herbicide from field trials in USA and Canada in 2003 (Buffington 2004).

Tissue (growth stage)[a]	Mean[b] Cry34Ab1 expression (ng/mg tissue dry weight)	Standard deviation	Mean[b] Cry35Ab1 expression (ng/mg tissue dry weight)	Standard deviation	Mean[b] PAT expression (ng/mg tissue dry weight)	Standard deviation
Leaf (V9 stage)	56.1	14.2	58.3	13.2	10.6	7.8
Leaf (R1 stage)	73.8	13.2	66.8	15.4	3.6	3.2
Leaf (R4 stage)	292.8	54.8	110.2	18.0	13.2	7.0
Leaf (R6 stage)	93.2	117.5	67.5	51.8	0.40	0.71
Root (V6 stage)	37.8	11.4	15.8	9.1	0.74	0.40
Root (V9 stage)	33.3	11.2	14.4	8.9	0.73	0.51
Root (R1 stage)	39.2	10.7	9.9	3.6	0.34	0.26
Root (R4 stage)	45.3	11.9	5.8	2.2	0.30	0.14
Root (R6 stage)	45.8	26.6	9.1	3.5	0.21	0.15
Whole plant (V9 stage)	78.6	10.9	55.1	10	4.6	5.0
Whole plant (R1 stage)	75.9	9.5	45.9	7.4	5.1	2.0
Senescent whole plant	145	17.9	47.1	11.3	0.2	0.2
Pollen (R1 stage)	62.6	6.3	0.10	0.17	0	0
Stalk (R1 stage)	52.3	12.9	19.4	7.4	0.48	0.8
Forage (R4 stage)	97.1	17.7	33.8	5.9	2.5	1.3
Grain (Physiol. maturity)	39.6	8.8	1.98*	9.8	0.1	0.2

[a] Iowa State University (1993).

[b] Values are means across all five sites from mean values calculated from the analysis of (i) five individual samples per site for leaf at the V9 and R1 stage; root, pollen and stalk at the R1 stage, and grain at the R6 stage; and (ii) three individual samples per site for leaf at the R4 and R6 stage, root at the V6, V9, R4 and R6 stage; and (iii) one pooled sample per site derived from three whole plant samples for the whole plant stage at the V9, R1, R4 and R6 stage and forage at the R4 stage.

* This mean Cry35Ab1 protein expression value was calculated for 21 grain samples from sprayed 59122 maize collected across 5 field trial locations in the USA and Canada. Expression values for 4 grain samples from sprayed 59122 maize were considered as outliers and were not included in the calculation.

Table 5. Summary of Cry34Ab1, Cry35Ab1 and PAT protein levels (ng/mg tissue dw) in tissues collected from non-sprayed 59122 maize from field trials in USA and Canada in 2003 (Buffington 2004).

Tissue (growth stage)[a]	Mean[b] Cry34Ab1 expression (ng/mg tissue dry weight)	Standard deviation	Mean[b] Cry35Ab1 expression (ng/mg tissue dry weight)	Standard deviation	Mean[b] PAT expression (ng/mg tissue dry weight)	Standard deviation
Leaf (V9 stage)	54.5	9.6	52.3	10.4	7.2	6.8
Leaf (R1 stage)	69.0	9.3	65.9	9.8	2.3	2.3
Leaf (R4 stage)	266.4	39.3	97.1	14.1	11.4	5.2
Leaf (R6 stage)	93.0	117.2	69.9	53.9	0.25	0.52
Root (V6 stage)	35.6	7.8	15.5	6.2	0.53	0.22
Root (V9 stage)	35.4	10.5	12.3	5.5	0.42	0.23
Root (R1 stage)	36.5	12.1	10.0	4.9	0.29	0.23
Root (R4 stage)	43.7	22.1	5.3	3.3	0.18	0.12
Root (R6 stage)	46.3	23.4	7.2	2.9	0.24	0.22
Whole plant (V9 stage)	59.8	7.2	49.6	15.5	5.7	6.7
Whole plant (R1 stage)	77.5	6.4	47.4	2.8	5.4	1.9
Senescent whole plant	110.2	14.4	37.5	4.5	0.2	0.2
Pollen (R1 stage)	64.7	6.3	0.06	0.15	0	0
Stalk (R1 stage)	49.0	9.9	19.3	10.3	0.38	0.6
Forage (R4 stage)	87.7	9.5	28.1	4.2	2.4	1.2
Grain (Physiol. maturity)	36.4	8.9	2.0	0.7	0.1	0.1

[a] Iowa State University (1993).

[b] Values are means across all five sites from mean values calculated from the analysis of (i) five individual samples per site for leaf at the V9 and R1 stage; root, pollen and stalk at the R1 stage, and grain at the R6 stage; and (ii) three individual samples per site for leaf at the R4 and R6 stage, root at the V6, V9, R4 and R6 stage; and (iii) one pooled sample per site derived from three whole plant samples for the whole plant stage at the V9, R1, R4 and R6 stage and forage at the R4 stage.

2.3.1 Part of the plant where the insert is expressed

As described above, the levels of Cry34Ab1, Cry35Ab1 and PAT proteins in 59122 maize was analysed in leaf, root, pollen, stalk, forage, mature grain and whole plant tissues during field trials conducted in Chile in 2002-2003 and in USA and Canada in 2003.

2.3.2 Expression of potential fusion proteins

SDS-PAGE and Western blot analyses were used to determine if the Cry34Ab1, Cry35Ab1 and PAT proteins produced by 59122 maize were of the expected molecular weight and immunoreactivity.

According to the applicant, the Cry34Ab1 protein was detected as a single band of approximately 14 kDa, identical to the 14 kDa band detected for the microbially-derived Cry34Ab1 protein. No other bands indicative of a partial Cry34Ab1 protein or a fusion protein of greater molecular weight were observed for 59122 maize.

The Cry35Ab1 protein detected as two bands, a 44 kDa band and a band of approximately 40 kDa, commonly referred to as a "doublet". Characterisation of the microbially-derived protein fraction with Cry35Ab1 specific polyclonal rabbit antibodies also revealed the presence of a 44 kDa and an approximately 40 kDa protein band. No other bands indicative of a partial Cry35Ab1 protein or a fusion protein of greater molecular weight were observed in 59122 maize tissues. No immunoreactive proteins were detected in the negative control tissues.

The PAT protein is known to be a homodimer of approximately 43 kDa in its native form, and it is comprised of two components of 22-23 kDa. The PAT protein, produced by 59122 maize, was detected as a band of approximately 23 kDa. No other bands indicative of a partial PAT protein or a fusion protein of greater molecular weight were observed in 59122 maize tissues.

A bioinformatics analysis, including sequence comparisons against databases of known toxic and allergenic proteins, did not reveal any significant homologies between new potential ORFs in 59122 maize and known protein toxins or allergens..

The results indicate no presence of new potential fusion proteins in the 59122 maize that could be harmful to humans or animals.

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

2.4.1 Genetic stability of the insert in 59122

To examine the stability of the 59122 maize insert across generations, Southern blot analysis was conducted on four different 59122 maize generations: T1S1, T1S2, BC1 and BC2S1. These four generations were produced from an F1 generation and individually analysed in various Southern blot studies. Southern blot analysis of the individual T1S1, T1S2, BC1 and BC2S1 plants produced identical hybridising border fragments which are unique to the site of insertion, indicating stability of the 59122 maize insert across the four generations analysed.

To address stability within a single generation, 79 individual plants within a segregating BC2S1 generation, derived from the 59122 maize T0 transformant were analysed. All test plants that germinated (79 plants) were tested for the presence or the absence of the Cry34Ab1 and PAT proteins. Lateral flow devices (LFD) specific for the Cry34Ab1 protein were used to show presence or absence of Cry34Ab1, while herbicide leaf painting was conducted in order to show presence or absence of PAT production in the individual plants. In summary, these studies indicate that the 59122 maize insert is genetically stable, following a typical pattern of Mendelian inheritance.

2.4.2 Phenotypic stability of the GM plant

Results from the Cry34Ab1 and PAT detection assays for the 79 test plants derived from the segregating BC2S1 generation are presented in Table 6. The BC2S1 generation of 59122 maize was expected to segregate 3 positive to 1 negative for the phenotypes tested. Of the 79 plants, 55 tested positive for both assays and 24 tested negative for both assays (the latter plants are referred to as null segregants). A chi-square test for specified proportions was used to compare the observed segregation data of 55 positive: 24 negative to the hypothesised segregation ratio of 3:1. The analysis was carried out with the SAS FREQ procedure and did not indicate a significant deviation from the hypothesised ratio (p-value = 0.27) showing phenotypic stability of the introduced traits in 59122 maize. Based on this result, all 55 positive plants were selected for Southern blot analysis along with 23 of the 24 plants that tested negative in the Cry34Ab1 and PAT assays. All 55 individual plants analysed by Southern blot analysis with the *Sac* I restriction enzyme and the *cry34Ab1*, *cry35Ab1* or *pat* probes showed a consistent hybridisation pattern indicating that the 59122 insertion was equivalent in all individuals within the BC2S1 generation (Weber and Igo, 2003; Annex 8). None of the null segregants showed presence of the T-DNA insert.

Analyses of the Cry34Ab1, Cry35Ab1 and PAT protein levels across several locations in Chile, USA and Canada during different growing seasons (described in 2.3) also indicates phenotypic stability of the introduced traits in maize 59122.

Table 6. Segregation data of the 59122 maize insert within a single generation to confirm genetic stability of the 59122 maize. Segregation data obtained from the BC2S1 generation (Weber & Igo, 2003; Annex 8).

Generation	Observed ratio[a]	Expected ratio	Significant difference?[b]
BC2S1	55:24	3:1	No

[a] Data are expressed as number of observed maize plants tolerant to glufosinate-ammonium herbicide and positive for Cry34Ab1 expression:number of observed plants susceptible for both assays.
[b] Significant at alpha = 0.05

Another study analysed the Mendelian segregation of maize 59122 over eight generations (FSANZ 2005). The T0 generation of maize was out-crossed for one generation to the inbred line PH09B to produce T1 generation plants which were either self-pollinated to produce the T1S1 generation or out-crossed with inbred lines designated inbred B (DAS male) or inbred C (DAS female) to produce a number of backcrosses. Since the insert in maize line DAS-59122-7 was expected to segregate as a single dominant gene, each generation was sprayed with glufosinate-ammonium to eliminate susceptible plants to determine if the insert was segregating as expected. All plants found to be herbicide tolerant were also tested with Cry34Ab1 immunoassay lateral flow devices. All of the plants determined to be herbicide tolerant were also positive for CryAb341.

Significant deviations from the expected segregation ratio were observed in the BC1, BC4 and BC4S1 generations. According to the applicant the deviation in the BC1 generation was likely due to the small sample size used. A breeding error that would have allowed extra susceptible plants in the BC4 and BC2S1 generations is also a possibility. The deviation in the BC4S1 generation occurred only in one inbred background and was not seen in either inbred in the BC2S1 generation. Since the majority of the generations showed no significant deviations from the expected ratios, and the deviations that occurred were inconsistent across generations and inbreds, the significant differences observed were

likely to be due to experimental error(s). The results indicate that the insert in maize DAS-59122-7 was inherited as a Mendelian dominant gene.

A Chi-square test across all generations with an expected ratio of 1:1 (2644:2750) resulted in no significant difference between expected and observed ratios, as did a test across all generations with an expected segregation ratio or 3:1 (1354:472).

2.5 Conclusion

Appropriate analyses of the transgenic DNA insert, its integration site, number of inserts and flanking sequences in the maize genome, have been performed. The results show that only one copy of the insert is present in maize 59122. Homology searches with databases of known toxins and allergens have not indicated any potential production of harmful proteins or polypeptides caused by the genetic modification in maize 59122. Southern blot analyses and segregation studies show that the introduced genes *cry34Ab1*, *cry35Ab1* and *pat* are stably inherited and expressed over several generations along with the phenotypic characteristics of maize 59122. The VKM GMO Panel considers the molecular characterisation of maize 59122 satisfactory.

3 Comparative assessment

3.1 Production of material for the comparative assessment

Several field studies have been performed with the genetically modified maize 59122. Studies at six locations were performed in Chile during the growing season 2002/2003, and at eight different North American locations in USA and Canada during 2003 and 2004 (Technical dossier, Annex 3, Annex 4 and Herman et al. 2007). Following a request for additional data from the EFSA GMO Panel, the applicant has also provided results from a study performed at three locations in Bulgaria in 2003, and from a total of six locations in Spain and Bulgaria in a study performed in 2004 (Technical dossier, Annex_03 and _04). The 59122 maize and non-GM control lines were grown in a randomised complete block design in all studies.

With the exception of the studies in Chile, all studies included analyses of 59122 maize both treated and untreated with glufosinate-ammonium to investigate potential effects of the herbicide on maize composition. Samples of forage, grain and other collected tissues from 59122 maize and respective non-GM control maize lines with comparable genetic backgrounds were analysed in all studies.

Following a request from the EFSA GMO Panel the applicant also provided additional information on the different breeding schemes used to produce the non-GM control lines in each study. The pedigree of these lines shows that they were representative of the different crosses and backcrosses used to produce the 59122 maize (Appendix, Figure 1).

Chile (2002/2003)
Nutrient content, agronomic characteristics and levels of the transgene proteins were assessed. The six locations used in the study comprised a randomised complete block design containing four blocks, and the plots were treated with conventional herbicides. Each block contained the 59122 maize and non-GM control.

Canada and USA (2003, 2004)
In 2003 field trials were performed at five field sites and in 2004 at four of these same sites, and in additional tree sites (in total eight different sites in 2003/2004). To test this, plots of 59122 maize were either left untreated or they received two sequential applications of glufosinate-ammonium herbicide. The 59122 maize and non-GM controls, were grown in a randomised complete block design.

Europe (2003, 2004)
Compositional data were obtained by analysis of forage and grain harvested from field trials conducted in commercial maize growing regions of Europe during two growing seasons. The field studies were performed at three separate locations in Bulgaria in 2003, and 2004 and at three corresponding locations in Spain 2004. Plots at each site were arranged in a randomised complete block design with four blocks. Each block contained the test hybrid 59122 and a near-isogenic non-transgenic control hybrid. For the field trials in the EU during the 2003 growing season, the 59122 maize was hybrid seed from a first backcross generation (BC1 hybrid) (Figure 1, Appendix). Likewise, the 59122 maize was hybrid seed from a fourth backcross generation (BC4 hybrid) in 2004. No conventional commercial reference varieties were included in the comparative assessments. According to the updated EFSA guidance on risk assessment of food and feed from genetically modified plants (EFSA 2011a), there should be at least three appropriate non-GM reference varieties of the crop that have a known history of safe use at each site. The test of equivalence is used to verify whether the agronomic, phenotypic and compositional characteristics of the GM plant fall within the normal range of natural variation. Such a range of natural variation is estimated from a set of non-GM reference varieties with a history of safe use (EFSA 2011b) and therefore allows comparisons of the GM plant with a similar food or feed produced without the help of genetic modification and for which there is a well-established history of safe use. These requirements were however not in place at the

time of submission of the application EFSA/GMO/NL/2005/12.

Plots of 59122 maize were either left untreated or treated two times sequentially with herbicides containing glufosinate-ammonium (V4 and V7 growth stage, see abbreviations and explanations). Grain and forage samples for compositional analyses were collected from three out of the four blocks per location. The remaining block was used for expression analysis of the Cry34Ab1, Cry35Ab1 and PAT proteins in 59122 maize tissues. Grain samples were collected once the plant had reached physiological maturity (R6 stage), while forage samples were collected at the dough growth stage (R4).

Two separate statistical analyses were carried out on the composition data. A combined analysis of variance (ANOVA) was conducted across the trial sites, using a linear mixed model (Proc Mixed, SAS, 1999). Least-square means and standard error of the means were calculated for the data across locations. 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. Statistically significant differences, both for the across location analysis and the individual location analysis, were identified at a 5% level of significance. In addition to the statistical analysis as described above, composition data obtained for 59122 maize forage and grain from six field locations in Europe (Spain/Bulgaria) during the 2004 growing season were also analysed for multiple comparisons.

As more tests are conducted simultaneously, the chance of obtaining at least one or more false discoveries becomes larger. In the study from 2004 a false discovery rate (FDR) evaluation, by Benjamini & Hochberg (1995) was therefore applied by the applicant. The FDR method has been shown to improve power and increase the opportunity of discriminating between true differences and false-positives. In the across location analysis of the 2004 Spain/Bulgaria study the range of individual values were compared to a 95% tolerance interval which was expected to contain 99% of the values for the analytes in question, as well as published literature values for maize.

3.2 Compositional Analysis

3.2.1 Chilean field trials (2002/2003)

Grain samples from maize 59122 and the near-siogenic control were collected and analysed for proximates, fibres, fatty acids, amino acids, minerals, vitamins, secondary metabolites, and anti-nutrients. Forage samples were analysed for proximates and minerals. Small but statistically significant differences were found between 59122 maize and the control for some forage proximates and minerals, and some grain proximates, fatty acids, amino acids, one mineral, and some vitamins. None of these differences were however found consistently over years and locations. All measured values also fell within the reported literature values for commercial maize varieties.

3.2.2 North American field trials (2003, 2004)

Forage samples were analysed for proximates and minerals. Grain samples were analysed for proximates, minerals, amino acids, fatty acids, vitamins, secondary metabolites and anti-nutrients. The results from both studies were published in 2007 (Herman et al. 2007), which indicated that the composition of 59122 maize is equivalent to that of non-GM maize.

3.2.3 European field trials

The compositional data from the European field trials include proximate and mineral analyses (fat, protein, total carbohydrate, acid detergent fibre (ADF), neutral detergent fibre (NDF), ash, phosphorus, and calcium) of forage, and the compositional analyses of grain: proximates (fat, protein, ash, moisture, carbohydrates, starch), fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acid), amino acids (eighteen amino acids including aromatic amino acids), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, selenium and zinc), vitamins (vitamin B1,

vitamin B2, folic acid, β-carotene, vitamin E), anti-nutrients (phytic acid, raffinose and trypsin inhibitor) and other secondary metabolites (inositol, furfural, p-coumaric acid, and ferulic acid).

In all studies the applicant also included typical ranges of values for the analytes reported in literature for commercial maize varieties (ILSI databases, OECD 2002, Watson 1982 and 1987). These are included in the tables of results and denoted combined ranges. All summary results tables from the two European field studies are included in the Appendix of this risk assessment.

3.2.3.1 Bulgaria (2003)

Forage

Proximate and fiber analysis
Mean crude protein, ADF, NDF, ash, and carbohydrate values across locations were statistically significant different between the 59122, treated with glufosinate, and the control hybrids (Table 1, Appendix). No differences were seen at individual locations for NDF, for ash, ADF, carbohydrates and crude protein from one three of the individual locations. The differences were small and all mean proximate values were within the reported literature values.

For the 59122 hybrid untreated with glufosinate and control hybrids mean concentration of ash and carbohydrate across locations were statistically different ($p<0.05$) (Table 2, Appendix). The difference were seen at one of three individual locations.

Mineral analysis
For phosphorus a significant difference ($p<0.05$) between the 59122, treated with glufosinate, and the control hybrids was observed in the across location analysis and also at two of the three individual locations (Table 3, Appendix). The levels of phosphorus was higher in hybrid 59122 + glufosinate (0.279 % of DM) than in the control hybrids (0.217 % of DM). Both minerals analysed in forage (calsium and phosphorus) were within the levels reported in the literature.

No statistically significant differences were observed for the mean calcium or phosphorus values in the 59122 untreated with glufosinate and control hybrids in the across location summary analysis. Moreover the measured values of minerals were within the reported literature ranges (Table 4, Appendix)

Grain

Proximates and fiber analysis
Mean crude protein, crude fat, crude fiber, ADF, NDF, ash, and carbohydrates values across locations for the 59122 hybrid treated with glufosinate and control hybrids were statistically different ($p<0.05$) (Table 5, Appendix). For crude protein, ADF and ash no such difference were seen at the individual locations. For the other proximates the statistically significant difference were seen at one of the three different locations. The across locations mean concentrations of proximates and fiber were all within the literature values.

Between the 59122 hybrid unsprayed with glufosinate and the control hybrids mean crude fiber and NDF values across locations were statistically significantly different ($p<0.05$) (Table 6, Appendix). The difference for crude fiber was not seen at any of the individual locations, while for NDF the difference were seen at two of the three individual locations. All mean values of grain proximates, from the across locations analysis were within the reported literature values.

Fatty acids analysis
The mean contents of oleic-, linoleic-, and linolenic acids were in the across locations analysis statistically significant different between the hybrid 59122 treated with glufosinate and control hybrids ($p<0.05$). For linolenic acid there was no difference seen at any of the individual sites, while for

linoleic acid the difference was observed at one of the three individual locations. For oleic acid the difference was observed at two of the locations. All values of fatty acids were within the literature values (Table 7, Appendix).

The mean palmitic acid values for the 59122 hybrid untreated with glufosinate were statistically significant from the control hybrids in the across location summary analysis ($p<0.05$). The difference was however, not seen at any of the three individual locations (Table 8, Appendix). Moreover, the values for all fatty acids across locations were within the reported literature values.

Amino acid analysis
Mean contents of lysine, thryptophane, threonine, leucine, arginine, phenylalanine, glycine, aspartic acid, glutamic acid, proline, and tyrosine values across locations for the hybrid 59122 treated with glufosinate were statistically significantly different from the control hybrids ($p<0.05$) (Table 9, Appendix). For most of these no difference were seen at the individual locations. In addition all mean levels of amino acid were within the reported literature levels.

No statistically significant differences were seen for the concentrations of any of the amino acids analysed in the 59122 untreated with glufosinate and the control hybrids in location summary analysis (Table 10, Appendix). In addition, the amino acids concentrations were all within the reported literature values.

Mineral analysis
A statistically significant difference was observed for the minerals calcium, copper, iron, manganese, phosphorus, potassium, and zinc values across locations for the 59122 treated with glufosinate and control hybrids ($p<0.05$) (Table 11, Appendix). For copper, manganese, and zinc no differences were seen at any of the individual locations. For calcium and phosphorus the differences were seen at only one of the three locations, while for potassium the differences were seen at two of the three locations. For iron the differences were seen at all three locations. All across location mean mineral values were within the reported literature values.

For the minerals manganese and potassium statistically significant mean values were observed, between the 59122 untreated with glufosinate and the control hybrids in the across locations summary analysis, but not in any of the individual locations (Table 12, Appendix). All mineral concentrations were within the reported literature values.

Vitamin analysis
The observed mean vitamin B1 value in the 59122 treated with glufosinate hybrid was higher than the reported literature values, and this was also the case at two of the three individual locations (Table 13, Appendix). The observed mean concentration of vitamin B1 in the control hybrids was also higher than the literature ranges. Since the across mean concentration of vitamin B1 in the 59122 treated with glufosinate and the control hybrids were similar and not statistically significant different, the observed deviation from literature values are not regarded to be caused by the genetic modification. Mean beta-carotene, vitamin B2, folic acid and vitamin E value were statistically significant different across locations. The mean concentrations of these vitamins were however, within published literature ranges.

Both for folic acid and vitamin E statistically significant difference between 59122 hybrid untreated with glufosinate and control hybrids were found in the across location summary analysis (Table 14, Appendix). Statistically significant differences were found for folic acid at two of the three individual locations. The mean vitamin B1 values for the 59122 untreated with glufosinate were higher than the reported literature values at two of three locations, while for the control hybrid the vitamin B1 levels was higher at one of the three locations. Since the across locations mean values for both the 59122 untreated with glufosinate and control hybrids not were statistically significant different the deviation in vitamin B1 compared to literature values suggests that the effect is not caused by the genetic modification.

Secondary metabolites analysis
A statistical significant difference were observed for the mean levels of inositol and p-coumaric acid across locations for the 59122 treated with glufosinate hybrid and the control hybrids ($p<0.05$) (Table 15, Appendix). These differences were not observed at all locations. The levels of furfural were below LOD. All levels of secondary metabolites were within the reported literature ranges.

No statistically significant difference between the 59122 untreated with glufosinate and control hybrids were found for the mean inositol, p-coumaric acid, or ferulic acid levels in the across location summary analysis, and the levels were within the reported literature values (Table 16, Appendix). Levels of furfural were below LOD.

Anti-nutrient analysis
For raffinose the mean values across locations were significantly different between the 59122 treated with glufosinate and control hybrids ($p<0.05$) (Appendix, Table 17). All levels of anti-nutrients were within the reported literature ranges.

The mean raffinose levels across locations was statistically significantly different between the hybrid 59122 untreated with glufosinate and control hybrids, but no difference was seen at individual locations (Appendix, Table 18). At one of the individual locations the raffinose levels were below the LOD for this analyte. All mean values for anti-nutrients across locations were within the reported literature values.

3.2.3.2. Results from field studies in Spain and Bulgaria (2004)

Forage

Proximates, fiber, and mineral analysis
Mean crude protein, crude fat, ash, carbohydrates, and phosphorus values were statistically significantly different ($p<0.05$) between the 59122 hybrid treated with glufosinate and control hybrids in the across location summary analysis (Table 19, Appendix). These differences were either not seen at individual locations (ash, carbohydrates) or were seen at two or four of the individual locations. All levels of proximates were within the ranges of both the tolerance intervals and combined historical ranges.

Mean crude protein, ash, carbohydrates, and phosphorus values across locations were statistically significantly different ($p<0.05$) between the 59122 hybrid untreated with glufosinate and control hybrids. Some of these were different only at one or two of the six individual locations. All levels of proximates were within both the tolerance intervals and combined historical ranges (Table 19, Appendix).

Grain

Proximates and fiber analysis
A statistically significant difference ($p<0.05$) was found between the 59122 treated with glufosinate and control hybrids in the levels of mean crude protein, crude fat, ash, and carbohydrates in the across location analysis and at some of the individual locations (Table 20, Appendix). All levels of proximates in glufosinate sprayed 59122 were within the ranges seen in both the tolerance intervals and combined historical ranges.

In the across location summary analysis significantly different ($p<0.05$) values for mean crude protein, crude fat, ash and carbohydrates were observed between the 59122 hybrid untreated with glufosinate and control hybrids (Table 20, Appendix). This difference for crude protein, crude fat, ash and carbohydrates was observed at three, three, one and five, respectively individual locations. All levels

of proximates in grain were within the ranges of both the tolerance intervals and combined historical ranges.

Fatty acid analysis
For the fatty acids stearic acid, linoleic acid, and linolenic acid statistically significant differences ($p<0.05$) were observed between the 59122 treated with glufosinate and control hybrids (Table 20, Appendix). For stearic acid none of these differences were seen at any of the six locations. For linoleic- and linolenic acid the differenes were seen at two and four, respectively, of the six locations. The ranges of values for all analysed fatty acids were within the tolerance intervals and combined historical ranges.

Mean palmitic acid, stearic acid, linoleic acid, and linolenic acid in 59122 untreated with glufosinate were statistically significant different ($p<0.05$) from the control at two, one, one, and four, respectively, locations. All levels of fatty acids in grain not treated with glufosinate were within the ranges of both the tolerance intervals and combined historical ranges (Table 20, Appendix).

Amino acid analysis
Mean content of threonine, histidine, aspartic acid, serine, and tyrosine values in the across locations summary analysis were statistically significant different ($p<0.05$) between the 59122 treated with glufosinate and control hybrids. In the single site analysis this difference was only observed for aspartic acid, the others were not statistically significant different at single sites. The range of all amino acids for 59122 treated with glufosinate and control hybrids, except threonine, was within the tolerance intervals and combined historical controls. Threonine levels were slightly higher than the upper range of historical control both for the control and 59122 hybrids. The difference was small (8.2%), and it was according to the applicant probably caused by the presence of an interfering peak in all sample chromatograms. This peak was a byproduct of the sample preparation method.

Mean thryptophan, threonine, isoleucine, histidine, valine, leucine, alanine, aspartic acid, glutamic acid, proline, serine, and tyrosine values were statistically significant ($p<0.05$) across locations between the 59122 untreated with glufosinate and control hybrids. These differences were seen at maximum three of the individual locations. The amino acid threonine exceeded the upper range of the tolerance interval and the historical controls. The range of all amino acids for 59122 and control hybrids, except threonine, were within the tolerance intervals and combined historical controls (Table 20, Appendix).

Mineral analysis
Mean calcium, copper, phosphorus, potassium, and zinc values across locations were statistically significant ($p<0.05$) between the 59122 treated with glufosinate and control hybrids in the across location summary analysis. At single locations no statistically significant differences were observed for potassium, while for calcium, copper, phosphorus, potassium, and zinc there was observed significant differences at six, three, two, three, and three, recpectively, of the six locations. All levels of minerals were within the tolerance intervals and combined historical controls.

The mean concentration of the minerals calcium, copper, phosphorus, potassium, and zinc were statistically significant different ($p<0.05$) between 59122 untreated with glufosinate and control hybrids in the across location analysis. The differences were observed at one location for copper, phosphorus, and zinc, respectively, and at two locations for calcium and potassium, respectively. All levels of minerals were within the tolerance intervals and combined historical controls (Table 20, Appendix).

Vitamin analysis
Levels of vitamin B2 were below LOD. All levels of vitamins in maize 59122 treated with glufosinate and control hybrids, except vitamin B2, were within the tolerance intervals and combined historical controls (Table 20, Appendix).

Mean folic acid in the across location analysis was statistically significant different ($p<0.05$) between the 59122 untreated with glufosinate and control hybrids, and this was also the case at three of the six individual locations. Levels of vitamin B2 was below LOD. All levels of vitamins, except vitamin B2, in the untreated 59122 were within the tolerance intervals and combined historical controls (Table 20, Appendix).

Secondary metabolites analysis
Mean levels of p-coumaric acid were statistically significant different ($p<0.05$) between maize 59122 treated with glufosinate and control hybrids in the across location summary analysis, and at one of the single sites. After adjustment with the false discovery rate, p-coumaric acid was no longer considered statistically significantly different. The amounts of furfural was below LOD. All levels of secondary metabolites were within the tolerance intervals and combined historical controls.

The amounts of furfural in 59122 untreated with glufosinate and control hybrids were also below LOD. All levels of secondary metabolites in 59122 untreated with glufosinate were within the tolerance intervals and combined historical controls (Table 20, Appendix).

Anti-nutrients analysis
Mean phytic acid levels across locations were statistically significantly different ($p<0.05$) between 59122 treated with glufosinate and the control hybrids, but this difference was not observed at any of the single sites. All levels of anti-nutrients were within the tolerance intervals and combined historical controls.

Mean phytic acid levels across locations were statistically significantly different ($p<0.05$) between the 59122 untreated with glufosinate and control hybrids (Table, Appendix). Statistically significant difference ($p<0.05$) were observed for phytic acid at two of six single locations. All levels of anti-nutrients were within the tolerance intervals and combined historical controls (Table 20, Appendix).

3.3 Agronomic and phenotypic characters

The information regarding the comparative analysis of phenotypic, agronomic and ecological data in application EFSA/GMO/NL/2005/12 was assessed by the VKM GMO Panel related to the national finalisation of the application in 2008 (VKM 2008a). No additional data are presented by the applicant in frame of the application EFSA/GMO/NL/23 covering authorisation of maize 59122 for all food and feed uses, including cultivation.

To summarise, the comparative assessments of phenotypic and agronomic characteristics of maize 59122 and conventional maize have been conducted in field trials over several growing seasons in Chile (six locations in 2002, 2003), USA (three locations in 2003) Canada (two locations in 2003), Bulgaria (three locations in 2003), and Bulgaria and Spain (three locations in 2004). During the field trials extensive agronomic data (e.g. grain yield, number of emerged plants, ear height, plant height, early population, final population), were collected for maize 59122 (treated and untreated with glufosinate-ammonium) and for the corresponding non-GM control.

Some statistically significant differences were detected in the European field trials during the 2004 growing season (e.g. mean early population, final population, plant height, and ear height). None of these differences were consistently observed over locations and years. The VKM GMO Panel concludes that the agronomic performance and phenotypic characteristics of maize 59122 are comparable to its non-transgenic counterpart except for the introduced traits.

In addition, data on yield performance for maize 59122 have been collected from four locations in USA during the 2003 growing season. At each location, three replications were grown and each replication included 59122 maize, non-GM control maize with comparable genetic background and non-GM control maize with comparable genetic background that was treated with a soil insecticide

against corn rootworm damage. Yield data that were collected, included: yield, harvest weight and test weight and are representative of the type of data used by commercial maize seed companies to develop elite maize varieties. Statistical analysis of yield data included analysis of variance (ANOVA) and statistically significant differences were identified at a 5% level of significance. No statistically significant differences were observed across locations between maize 59122 and the non-GM control maize (untreated or treated with a soil insecticide against corn rootworm damage), thereby confirming that 59122 maize is comparable with non-GM control maize regarding yield.

3.4 Conclusion

Comparative analyses of maize 59122 to its non-GM conventional counterpart have been performed during multiple field trials located at representative sites for maize cultivation in Chile (2002, 2003), Canada and USA (2003, 2004) and Europe (2003, 2004). Statistically significant differences between 59122 maize and non-GM controls were found in the studies from Europe in 2003 and 2004 for some analytes, either in forage or in grain. These differences were however not consistent over time and locations, and values of the analytes found in both 59122 maize and controls were all within the reported literature values. No biologically significant differences were found between maize 59122 and the conventional maize. Based on the assessment of available data from the field studies, the VKM GMO Panel concludes that maize 59122 is compositionally, agronomical and phenotypically equivalent to its conventional counterpart, except for the introduced characteristics, and that its composition fell within the normal ranges of variation observed among non-GM varieties.

4 Food and feed safety assessment

4.1 Product description and intended uses

The genetic modification in 59122 field maize will not impact the existing production processes used for maize. All 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 59122 field maize and all food, feed and processed products derived from 59122 field 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 of 59122 field maize includes many harsh processing steps, e.g. cooking, heating, high pressures, pH treatments, physical shearing, extrusion at high temperatures etc. under which the majority of DNA and proteins are denatured, which also applies to the Cry34Ab1, Cry35Ab1 and PAT proteins and to *cry34Ab1*, *cry35Ab1* and *pat* genes (Dien et al. 2002; Hammond & Jez 2011; Fernandes et al. 2013). Baking of the maize bread broa containing 11% of TC1507 maize flour and 20% MON810 maize flour, showed that the baking process sheared the DNA into small fragments, less than 1000 base pair (bp), the main part of sheared DNA was about 200 bp (Fernandes et al. 2013). In the unprocessed grain and all dry-milled fractions these proteins and DNA will probably be found in quantifiable amounts.

4.3 Toxicological assessment

4.3.1 Toxicology

The Cry34Ab1, Cry35Ab1 and PAT proteins produced by 59122 maize have been assessed in several acute – and repeated dose oral toxicity studies in rodents.

4.3.1.1 Toxicological assessment of the newly expressed protein

Phosphinothricin-N-acetyltransferase (PAT)
The PAT-protein originally obtained from *Streptomyces viridochromogenes* confers tolerance to the herbicidal active substance glufosinate-ammonium.

The PAT protein has no known toxic potential. The PAT protein is enzymatically active but has high substrate specificity to the active ingredient glufosinate. The PAT protein has already been found safe to human health during the assessment of glufosinate tolerant maize (EFSA 2013a,b; Herouet et al. 2005, US EPA 2005, 2010, VKM 2005, 2008, 2012, 2013).

Cry34Ab1 and Cry35Ab1 protein
Bacillus thuringiensis crystal proteins of the Cry34 and Cry35 classes are toxins showing activity on the western corn rootworm (WCR), *Diabrotica virgifera.* Bioassays performed with separately produced and biochemically purified 14 (Cry34) and 44 (Cry35) -kDa polypeptides have shown that both proteins are required for the lethal effects on WCR (Ellis et al 2002). Because both proteins are required for effective mortality of the insects, they have been referred to as binary insecticidal proteins. Sequence comparisons with other known *B. thuringiensis* insecticidal proteins have not revealed homology with previously described Cry, Cyt, or Vip proteins (Schnepf et al. 2005). There is, however, evidence that the 44-kDa polypeptide and the 41.9- and 51.4-kDa binary dipteran insecticidal proteins from *Bacillus sphaericus* are evolutionarily related (Ellis et al 2002), and that the 14-kDa-protein is similar to proteins in *Photorhabdus luminescens* and *Dictyostelium discoidium*

(Schnepf et al 2005). A study on the binding site of Cry34Ab1 and Cry35Ab1 to the western corn rootworm midgut brush border membrane vesicles (BBMV) showed that the interaction between the proteins and the binding site was highly specific (Li et al 2013). The study also showed that the competitive binding of Cry35Ab1 to the vesicles was enhanced by Cry34Ab1, and that no competitive binding to the vesicles was observed for other putative competitor Cry-proteins used in excess.

Cry proteins originating from *Bacillus thuringiensis* have not been found to have harmful effects on the health of humans and animals (US EPA, 2005, 2010; McClintock *et al.*, 1995). In addition, there is no evidence for the presence of specific receptors in mammalian tissues for related Cry proteins such as Cry1Ab (Noteborn & Kuiper 1995; Kuiper et al. 2001).

4.3.1.2 Acute toxicity testing

A 15 day study on acute intravenous exposure to PAT protein in mice
In application EFSA/GMO/NL/23005/12 Pioneer Hi-Bred Int. refers to Dow AgroSciences LLC for evaluation of potential toxicity of the PAT-protein.

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 practice (Organisation for Economic Cooperation and Development) Principles of Good Laboratory Practice, 1997, European Commission Directive 1999/1 I/EC, 1999, French decree n°98-1312, regarding Good Laboratory Practice, December 31, 1998, E.P.A. (Environmental Protection Agency) 40 CFR part 160 Federal Insecticide, Fungicide and Rodenticide Act (F1FRA): Good Laboratory Practice Standards: Final Rule, August 17, 1989, and Good Laboratory Practice Standards for Toxicology studies on Agricultural Chemicals, Ministry of Agriculture, Forestry and Fisheries (M.A.F.F.), notification 12 NohSan n°8628, (December 06 2000).

The objective of the study was to assess the acute intravenous toxicity of PAT (>95% purity) in OF1 mice. Acute intravenous toxicity of melittin was used as positive control and aprotinin as negative control. Groups of 5 female OF1 mice were administered either PAT, melittin or aprotinin in physiological saline at dose levels of 1 and 10 mg/kg body weight (bw).

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 treated animals or in control groups throughout the study period. Body weight development was unaffected by the treatment with either PAT at 1 or 10 mg/kg bw, or control substances up to Day 15. At termination of the study period, animals were subjected to a necropsy including a macroscopic examination. No treatment related macroscopic abnormalities were detected in animals treated with PAT at 1 or 10 mg/kg bw or control substances. The positive control melittin induced 100% mortality at 10 mg/kg bw. Animals treated with 1 mg/kg bw of melittin or the negative control aprotinin at 1 or 10 mg/kg bw showed no visible signs of systemic toxicity (Hérouet et al. 2005).

Observations for mortality and/or clinical or behavioural signs of pathology as well as body weights were made during the course of the study, and gross necropsies were conducted at the end of the study. Apart from the positive control groups treated with 10 mg/kg bw of melittin, no mortality occurred during the course of the study. No adverse clinical signs were observed during the study and no adverse findings were noted at necropsy. The dose range tested in this study did not give rise to any toxicity and therefore the acute LD_{50} for PAT protein could not be determined.

A 14 day study on acute oral exposure to PAT protein in mice
An acute oral toxicity study in mice was conducted with microbially derived PAT protein (84% purity) prepared as a reference standard also to be used in other analyses (Brooks 2000, Dow AgroSciences LLC). 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. All mice, except for one female, gained weight over the duration of the study. There were no gross pathological lesions found in any of the animals. The results show that the acute oral LD_{50} of microbially derived PAT protein in mice is greater than 5000 mg/kg bw.

The potential toxicity of the Cry34Ab1 and Cry35Ab1 proteins to humans and animals was examined in acute oral toxicology studies. The microbially derived Cry34Ab1 and Cry35Ab1 proteins were evaluated either separately or as a Cry34Ab1/Cry35Ab1 protein mixture for acute toxicity potential in mice (Brooks and DeWildt, 2000a; Brooks and DeWildt, 2000b; Brooks and DeWildt, 2000c. Unpublished technical reports, Annex 11-13).

A 14 day study on acute oral exposure to Cry34Ab1 protein in mice
The Cry34Ab1 protein was evaluated for acute oral toxicity with a tested dose of 5000 mg/kg bw of test material. When adjusted for purity (54% pure; Brooks and DeWildt, 2000a), the actual administered dose of Cry34Ab1was 2700 mg/kg bw. 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. 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 was estimated to be higher than 2700 mg/kg bw.

A 14 day study on acute oral exposure to Cry35Ab1 protein in mice
The Cry35Ab1 protein was evaluated for acute oral toxicity with a tested dose of 5000 mg/kg bw of test material. When adjusted for purity (37% pure; Brooks & DeWildt 2000b), the actual administered dose of Cry35Ab1 was 1850 mg/kg bw. 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. 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 was estimated to be higher than 1850 mg/kg bw.

A 14 day study on acute oral exposure to a mixture of Cry34Ab1 and Cry35Ab1 protein in mice
A mixture of Cry34Ab1 and Cry35Ab1 proteins was evaluated for acute oral toxicity in mice with a tested dose of 5000 mg/kg bw of test material. When adjusted for purity (54% pure for Cry34Ab1 protein and 37% pure for the Cry35Ab1 protein; Brooks & DeWildt 2000c), the actual administered doses were 482 mg/kg bw Cry34Ab1, and 1520 mg/kg bw Cry35Ab1. 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. No treatment related adverse clinical signs were observed during the study 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 was estimated to be higher than 2000 mg/kg bw of an equimolar mixture of Cry34Ab1 and Cry35Ab1 proteins.

4.3.1.3 Repeated dose oral toxicity testing

A 14-day repeated dose toxicity study with PAT protein in rats
In this 14 day repeated dose toxicity study the PAT protein was administered by feed admixture to male and female Wistar rats (Hoechst Schering AgrEvo GmbH, RCC Project 616307, (Pfister et al. 1996, unpublished report)). The study was performed in accordance with the principles of Good

Laboratory Practice (GLP) in Switzerland, Procedures and Principles of 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.

The study procedures mostly conformed to the 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 these 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 purity of the PAT protein was assessed by SDS-PAGE and estimated to be 98%. The animals were divided into four different groups with different diets, with five male and five females in each group. One group received a standard diet (group 1), whereas the three remaining groups received a low-protein diet adjusted with either soy protein (soyamin) and/or PAT protein to match the protein content of group 1. The PAT protein content (ppm) in the diets were 0 (group 1), 5000 (group 2) and 50000 (group 3) and 0 (group 4). The protein contents of the diets in groups 2 and 4 were adjusted with the addition of 45000 and 50000 ppm of soyamin, respectively. The average intake of PAT protein in groups 2 and 3 were 712 and 7619 mg/kg/day for male rats and 703 and 7965 mg/kg/day for female rats.

The results showed that food consumption and body weight were not influenced by the PAT protein. There were no treatment related mortality or behavioural changes observed in comparison to the control. Organ weights, gross pathology and histopathology findings did not indicate differences between treated and control animals. No changes were found in hematology or urine analyses. Immunological screening parameters indicated that the PAT protein did not induce immunological effects. Overall, the study gave no indications of adverse effects attributable to the PAT protein up to the highest dose tested.

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

Groups of five male and five female CD-1 mice were fed PMI 5002 diets with different concentrations of purified Cry34Ab1 and Cry35Ab1 proteins. The highest dose administered was formulated to represent theoretical 1000-fold greater concentrations of the cry proteins than in an estimated “worst case” scenario for human exposure. The Cry34Ab1/Cry35Ab1 protein contents of the diets were given at a rate of 0/0 (diet 1; control), 1.97/0.078 (diet 2), 19.7/0.78 (diet 3), and 197/7.8 (diet 4) mg/kg bw/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. Actual concentrations of Cry34Ab1/Cry35Ab1 proteins were higher in all dose groups based on analytical results, with the exception of a lower concentration of Cry35Ab1 in the low-dose group. Additional groups of five male and five female mice were fed diets containing bovine serum albumin (BSA) at the rate of 204.8 mg/kg bw/day to serve as a protein control group for the animals that received diet 4 (197 + 0.78 = 204.8). The nominal time-weighted average concentrations of BSA were 189.3 and 202.1 mg/kg/day for males and females, respectively.

The Cry34Ab1/Cry35Ab1 protein treatment groups were statistically compared to the BSA-control group. Parameters evaluated were daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, body weights, feed consumption, hematology, clinical chemistry, selected organ weights, and gross and histopathologic examinations. Overall the study showed no treatment-related effects of the Cry34Ab1 and Cry35Ab1 proteins on any parameter (Juberg et al. 2009).

The acute and repeated dose toxicity tests performed on rats and mice have not indicated toxic effects of the PAT or Cry34Ab1 and Cry35Ab1 proteins. However, these tests do not provide enough information to conclude on possible adverse health effects of maize 59122. In whole food the concentrations of these proteins are low, and acute toxic effects in humans and animals will most probably be negligible. Acute toxicity testing of the newly expressed proteins is of little additional

value to the risk assessment of the repeated human and animal consumption of food and feed derived from GM plants and is therefore not taken into account in this risk assessment. EFSA discourages the use of acute toxicity studies in risk assessments of GMOs (EFSA 2011a).

4.3.2 Toxicological assessment of the whole GM food and feed

4.3.2.1 Subchronic feeding studies in rats

Two 90-day subchronic feeding studies with maize 59122 have been peer reviewed and published in Food and Chemical Toxicology (Malley et al. 2007; He et al. 2008).

90-day subchronic feeding study in rats (Malley et al. 2007)
A 90-day feeding study was performed on male and female Sprague Dawley rats in compliance with U.S. EPA Health Effects Test Guidelines OPPTS 870.3100, 90-Day Oral Toxicity in Rodents (1998), and OECD Guidelines for the Testing of Chemicals Section 4: Health Effects, Number 408 (1998) guidelines.

Adult male and female rats (12/sex/group) were fed diets with 35% maize grain from either 59122 maize, a non-GM control (091) with a comparable genetic background to 59122 maize, a non-GM commercial hybrid maize (33R77), or one of two separate lots of rodent feed (5002A and 5002B) prepared with 35% commercially available grain.

Prior to initiating the study, the maize were analysed for the protein content of Cry34Ab1, Cry35Ab1, and PAT with antibody specific ELISA. Anti-nutrients (trypsin inhibitor, phytic acid, and raffinose) and secondary metabolites (furfural, ferulic acid, p-coumaric acid, and inositol), pesticide residues of chlorinated hydrocarbons (aldrin, benzene hexachloride-alpha [BHC-alpha], BHC-beta, BHC-delta, chlordane, dichlorodiphenyltrichloroethane [DDT]-related substances, dieldrin, endrin, hexachlorobiphenyl [HCB], heptachlor, heptachlor epoxide, lindane, methoxychlor, mirex, polychlorinated biphenyls [PCB],organophosphates (diazinon, disulfoton, ethion, malathion, methyl parathion, parathion [ethyl], thimet, thiodan, and trithion) were determined. Mycotoxin concentrations (aflatoxin B1 [AFB1], B2, G1, G2, and M2, zearalenone, oosporein, ergosine, ergotamine, ergocornine, ergocryptine, ergocristine, deoxynivalenol, 13-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol, cyclopiazonic acid, fumonisin B1 [FB1], B2, and B3, moniliformin, and T-2 toxin) were also determined. All maize grain samples contained measurable concentrations of AFB1 and FB1. Based on the dietary incorporation rates for the maize grains, the concentration of AFB1 was lower than the US FDA action level for animal feed (20–300 ppb; US FDA, 2000) and the concentration of FB1 was lower than a published no observed adverse effect level (NOAEL) found for B6C3F1 Mice and Fischer 344 Rats (Voss et al. 1995).

Prior to diet preparation the concentrations of Cry34Ab1 (73.8 ±60.1 µg/g grain), Cry35Ab1 (2.2 ± 0.2 µg/g grain), and PAT (0.03 ± 0.02 µg/g grain) proteins were measured. These proteins were not detected in either of the non-GM maize grains. The average concentration of Cry34Ab1 and Cry35Ab1 proteins in the 59122 maize grain diet was 11.9 µg/g and 0.55 µg/g, respectively. The presence of the Cry - proteins in the diet was stable over the course of the study. The concentration of PAT protein was below the limit of quantitation (LLOQ = 0.02 µg/g diet). The transgenic proteins were not detected in any of the other diets. All diets possessed similar nutritional and contaminant profiles.

At the end of the feeding period the following organs were excised and weighed: liver, kidneys, adrenal glands, thymus, brain, spleen, heart, ovaries and uterus (female) or testes and epididymides (male). Relative organ weights (percent of final body weight; ratio to brain weight) were calculated. Tissues from the following organ systems were processed to slides, stained with hematoxylin and eosin, and examined microscopically by a pathologist: digestive system (liver, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, salivary glands, and pancreas), urinary system (kidneys and urinary bladder), respiratory system (lungs, trachea, nose, larynx, and pharynx),

cardiovascular system (heart and aorta), hematopoietic system (spleen, thymus, mandibular lymph node, mesenteric lymph node, and bone marrow), endocrine system (pituitary gland, thyroid gland, parathyroid glands, and adrenal glands), nervous system (brain [including cerebrum, cerebellum, and medulla/pons], spinal cord [cervical, mid-thoracic, and lumbar] and sciatic nerve), musculoskeletal system (skeletal muscle, femur/knee joint, sternum), reproductive system of males (testes, epididymides, prostate, and seminal vesicles) and females (ovaries, uterus, mammary glands, and vagina), skin, and eyes (including retina and optic nerve).

With regard to clinical pathology, the following parameters were measured: red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, white blood cell count, absolute neutrophils, absolute lymphocytes, absolute eosinophils, absolute basophils, absolute large unstained cells, prothrombin time, aspartate aminotransferase, alanine aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, total bilirubin, urea nitrogen, creatinin, cholesterol, triglycerides, glucose, globulin, inorganic phosphorous, sodium, chloride, urine osmolality, urinalysis specific gravity, urinalysis pH, urobilinogen and urinalysis protein.

No statistically significant differences were observed when the rats fed the 59122 maize diet were compared to the combined control groups (i.e. the rats fed the diet with the non-GM control maize with comparable genetic background, the rats fed the non-GM commercial maize diet, and the rats fed the two standard lab diets).

Compared to control groups, no adverse diet-related differences were observed in rats fed diets formulated with 59122 maize grain with respect to body weight/gain, food consumption/efficiency, clinical signs of toxicity, mortality, ophthalmology, neurobehavioral (FOB and motor activity) assessments, clinical pathology (hematology, clinical chemistry, coagulation, and urinalysis), and pathology (organ weights and gross and microscopic pathology). Results from this study indicate that 59122 maize grain is nutritionally equivalent to and as safe as conventional maize grain.

No biologically significant, diet-related differences were observed among the groups fed with any of the different diets with respect to organ weights and gross and microscopic pathology. The statistically significant increase in uterine weight that was observed for female rats fed the 59122 maize diet was a reflection of the variations in uterine weight that occur as a result of normal estrus cycling in female rats and was therefore not considered to be due to consumption of the 59122 maize diet.

Overall the results of the study show no significant or biologically relevant differences between 59122 maize grain and conventional maize grain when evaluated in a subchronic feeding study in rats.

90-day subchronic feeding study in rats (He et al. 2008)

A 90-day feeding study was performed on male and female Sprague Dawley rats in compliance with Chinese Toxicology Assessment Procedures and Methods for Food Safety (Chinese standard GB15193.13-2003) which is similar to OECD 408 Guidelines (OECD, 1998). Maize grain from 59122 maize and the near isogenic non-GM control maize line 091 (mentioned in the study above) were supplied by Pioneer Hi-Bred International., Inc. (Johnston, IA, USA; a DuPont Company).

Concentrations of nutritional proximates (moisture, ash, fat, crude fiber, crude protein, and carbohydrates) in 59122 maize and control were measured in maize grain, whereas the other compositional analyses were conducted on ground maize flour before preparation of the rat diets. The nutritional proximates were similar in both maize lines and within the normal ranges reported for maize (OECD 2002; ILSI 2006).

Diets were prepared from the flours of 59122 maize and 091 control maize in two different concentrations each: 50% and 70% wt/wt of 59122 or control (compared with 35% used in the study by Malley, 2007). The two diets had similar nutrient compositions as the commercial diet AIN93G (total energy 3766 kal/kg; moisture 66.0 g/kg; total fat 70.0 g/kg; total carbohydrate 643.7 g/kg; total protein 178.6 g/kg; total ash 41.7 g/kg (Reeves et al. 1993)).

AIN93G diet containing 43.3% maize flour was included as an additional negative control. The identity of the maize used in the diet was unclear. The composition of the diet was consistent with the nutrient requirements of AIN93G diets. All experimental diets were produced by Ke Ao Xie Li Feed Co. Ltd. (Beijing, China).

Sprague-Dawley male and female rats were approximately 4 weeks old upon arrival. The average body weight was between 40–60 g. Following a 5 day acclimatisation the rats were divided randomly into groups of 20 animals, 10 male and 10 female. Rats in the experimental groups were fed diets formulated with either 50% or 70% (wt/wt) 59122 maize flour, while control groups were fed diets with 50% or 70% of 091 maize flour. Rats in the negative control group were fed AIN93G diet containing 43.3% maize. All animals were housed individually with ad libitum access to water and diets.

Rats were observed daily for mortality and signs of toxicity. Body weights were measured twice a week and feed consumption was measured once a week. At the end of the study, total body weights, weight gain, and total feed consumption were determined.

With regard to clinical pathology, the following parameters were measured: red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell volume distribution (RDW), blood platelet count (PLT), white blood cell count (WBC), lymphocyte count (LYMPH), mean platelet volume (MPV) and platelet distribution width (PDW), levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKP), blood urea nitrogen (BUN), creatinine (CREA), total cholesterol (CHOL), high-density lipoproteins cholesterol (HDLC), low-density lipoproteins cholesterol (LDLC), triglycerides (TRIG), glucose (GLUC), total protein (TP), albumin (ALB), calcium (CALC), and phosphorus (IPHS).

Gross necropsy of major organs was conducted by visual inspection. Brain, heart, liver, lung, spleen, kidney, thymus, testes or ovaries weight were determined and the relative weight of each organ (or paired organ weights) was determined based on final individual body weights. Tissue sections from liver, spleen, kidney, stomach and intestine, testes and ovaries were fixed with buffered formalin and embedded in paraffin and stained with hematoxylin and eosin for histopathological examinations.

The data generated during the study was subjected to following statistical tests. Combined group data variances were analysed with Levene's test. Homogeneity variance was analysed by one-way analysis of variance (ANOVA) and then a least squared differences (LSD) model with a statistical software Statistical Product and Service Solutions (SPSS) v12.0 (SPSS Inc., Chicago, IL, USA). Differences were considered significant when $p < 0.05$. Data obtained from each treatment group was first compared individually with the values from the AIN93G diet group (AIN93G diet). Data from groups of rats consuming diets formulated with 59122 maize flour were compared to the corresponding non-GM control maize groups (50% vs 50% and 70% vs 70%).

Statistically significant differences ($p < 0.05$) were observed for several measured endpoints between the different diet groups. None of the observed differences between animals fed different diets were however attributed specifically to 59122 maize, rather they were associated with the higher concentrations of maize flour in the 091 control and 59122 diets compared to AIN93G. This was observed e.g. for certain haematology and serum chemistry response variables that were statistically significantly different between rats consuming diets formulated with either 59122 maize or 091 control compared to rats fed AIN93G. The mean values of these response variables in rats fed the 59122 maize diets were not statistically different from those observed in rats fed correspondingly high (70%) and low (50%) concentrations of 091 control maize flour.

The overall results of the study indicates no adverse health effects associated with 59122 maize or dietary related differences between 59122 maize and the non-GM control.

Additional whole food feeding studies that also consider health effects of 59122 maize
The applicant has performed a 42-day broiler feeding study with emphasis on nutritional properties of 59122 maize, which also considers health effects. Additional feeding studies with maize 59122 include studies on hens, dairy cows, steers, and pigs. The studies are described under section 4.5.2.

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 2010b). Most food allergies are mediated by IgE (type-I reaction).

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

4.4.1 Assessment of allergenicity of the newly expressed protein

The applicant has performed a weight-of-evidence approach (FAO/WHO 2001; Codex, 2003) for an overall assessment of the IgE allergenic potential of the Cry34Ab1, Cry35Ab1 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

The assessment has previously been described by the applicant for the single maize events 59122 (EFSA-GMO-NL-2005-12, EFSA-GMO-NL-2005-23), and were based on the following aspects:

i) The sources of the transgenes: *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 (US EPA 2005, 2010), no indications of Cry proteins originating from *Bacillus thuringiensis* having harmful effects on the health of humans and animals
iii) The Cry34Ab1 and Cry35Ab1 proteins do not show significant amino acid sequence similarity to known protein toxins, and do not share immunologically relevant sequence similarity with known allergens (US EPA 2005, 2010)
iv) The Cry34Ab1 and Cry35Ab1 proteins are rapidly degraded, as shown by SDS-PAGE, under simulated gastric fluid digestive conditions (Herman et al. 2003)
v) The Cry34Ab1 and Cry35Ab1 proteins have been considered as heat labile since the biological activity of Cry34Ab1 and Cry35Ab1 proteins was lost after exposure at 60^{o}C for 30 minutes (Herman et al. 2003)

vi) The proteins Cry34Ab1, Cry35Ab1 and PAT are not glycosylated (US EPA 2010, Hérouet et al. 2005)
vii) The PAT protein has been the subject of previous safety assessments for genetically modified plants and found to have no potential for allergenicity (Hérouet et al 2005)
viii) PAT do not resemble any characteristics of known IgE-allergens, and no significant homologies between the amino acid sequences of the PAT protein and IgE-allergenic proteins have been found (Herouet et al. 2005, US EPA, 2010).
ix) The PAT protein lacks homology to known toxic proteins (Hérouet et al. 2005)
x) Rapid degradation of the PAT protein in simulated gastric fluids (Hérouet et al 2005)

The information listed above indicates that the newly expressed proteins in maize 59122 lack IgE allergenic potential with regard to human and animal health. However, it does not cover possible allergic reactions (e.g. enteropathies) that are not IgE mediated.

4.4.2 Assessment of the allergenicity of the whole GM plant

Allergenicity of 59122 maize could be increased as an unintended effect of the random insertion of the transgenes 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 59122 maize with the exception of the introduced traits, no increased allergenicity is anticipated for 59122 maize. Moreover, maize is not considered a common allergenic food.

4.4.3 Assessment of the IgE-mediated allergenicity of proteins from the GM plant

It is the opinion of the VKM GMO Panel that a possible over-expression of any endogenous protein, which is not known to be allergenic, in 59122 maize would be unlikely to alter the overall allergenicity of the whole plant or the allergy risk for consumers.

4.4.4 Adjuvanticity

According to the EFSA Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed (EFSA 2010b) adjuvants are substances that, when co-administered with an antigen increases the immune response to the antigen and therefore might increase the allergic response. In cases when known functional aspects of the newly expressed protein or structural similarity to known strong adjuvants may indicate possible adjuvant activity, the potential role of these proteins as adjuvants should be considered. As for allergens, interactions with other constituents of the food matrix and/or processing may alter the structure and bioavailability of an adjuvant and thus modify its biological activity.

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

Studies with immunological mapping of the systemic and mucosal immune responses to Cry1Ac have shown that mice produce both systemic IgM and IgG and secretory IgA following intraperitonal (i.p.), intragastric (i.g.) or intranasal (i.n.) immunisation, and that the adjuvant effects of Cry1Ac is comparable to that of cholera toxin (CT) (Guerrero et al. 2004; Vazquez-Padron et al. 1999a,b, 2000; Moreno-Fierros et al, 2003). It is uncertain whether this applies to the same extent to other Cry proteins. A possible immunogenicity and adjuvanticity of Cry proteins has been considered by EFSA and VKM (EFSA 2009b; EFSA 2010b; VKM 2012d).

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

Both in vitro and in vivo experiments have demonstrated that when an IgG response which can result in a complement activation (among other) is not balanced by an IgA response, the epithelial barrier may become leaky, allowing unwanted proteins to enter the body (bystander-penetration) and possibly lead to allergic sensitisation (Brandtzaeg & Tolo 1977; Lim & Rowley 1982).

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

4.5 Nutritional assessment of GM food/feed

Compositional analyses of maize 59122 indicate nutritional equivalence to the non-GM control maize lines with comparable genetic backgrounds and to the reported range of literature values. The nutritional equivalence between 59122 maize and non-GM control maize has also been shown by a poultry feeding study, and other feeding studies described in 4.5.2.

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 of 0.6%. The estimated median daily intake of sweet maize is 3.25 g/day, with a 97.5% percentile of 17.5 g/day. The production of maize porridge for children in 2007 was about 37.5 tons, corresponding to a daily intake of 1.7 g/day or an estimated daily energy intake of 0.6% for a 6 month child (Vikse 2009, unpublished).

The VKM GMO Panel has calculated a Theoretical Maximum Daily Intake (TMDI) for acute dietary consumption of Cry34Ab1 and Cry35Ab1 protein in maize and maize products. The TMDI is 174 µg of Cry34Ab1 and 8 µg of Cry35Ab1 protein per adults per day, and 67 µg and 3.4 µg, respectively, per child per day. These exposure estimates are based on the mean protein levels of Cry34Ab1 39.6 ± 8.8 µg/g dry weight and Cry35Ab1 1.98 ± 0.1 µg/g dry weight levels reported for 59122 maize grain in the application EFSA/GMO/NL/2005/12.

These levels are several orders of magnitude below the levels shown to have no effect in laboratory toxicological tests. Also, these levels are considerably below the proposed threshold of toxicological concern (TTC) level of 1800 µg/person/day (Class 1, oral exposure) for chemicals considered to have a low potential for toxicity based on metabolism and mechanistic data (Vermeire et al., 2010). Transgenic proteins produced by genetically modified plants are generally considered non-toxic to humans. This dietary exposure assessment is also very conservative, as it assumes that all consumed maize consists of maize 59122 and that protein levels are not reduced by processing.

The VKM GMO Panel notes that farm (production) animals e.g. pigs and poultry often are fed diets with a substantial inclusion of unprocessed maize grain, and that the exposure to transgenic proteins from maize 59122 may be higher for these animals.

4.5.2 Nutritional studies

A 42-day feeding study on broiler chickens

A poultry feeding study over a period of 42 days was carried out to assess the nutritional equivalence of 59122 maize to its non-GM control (Pioneer Hi-Bred Study ID PHI-2004-033, (McNaughton et al 2007)). The feeding study was not conducted under Good Laboratory Practice Standards, 40 CFR160 (FIFRA (EPA-FIFRA 1989; OECD 1998). According to the applicant comprehensive documentation of all critical data and quality control measures were used to ensure the integrity of the study.

Fumonisin B1 (FB1) was detected in all maize grain samples, except in the commercial reference grain 33P66, at concentrations ranging from 0.1 to 0.5 mg/kg. All concentrations were below the US FDA guideline values for incorporation into broiler feed (U.S. FDA, 2000, 2001). The proximates, essential amino acids (lysine, methionine, cystine, threonine, tryptophan, and arginine), calcium, phosphorous, and gross energy contents of all maize grains were similar. Compositional analyses of the nutrients in all diets demonstrated that there were no significant differences between diets produced with 59122 maize grain compared to diets produced with the near-isogenic non-GM control (091 maize) or commercial reference grains (33P66, 33J56, and 33R77).

According to the applicant and McNaughton et al. (2007) data generated during this feeding study was analysed with a linear mixed model to obtain the estimates of the variance components (PROC MIXED, SAS® version 8.2 software, SAS Institute Inc., Cary NC, USA) that were used to provide estimates of the treatment means (Appendix / Statistics).

Data from commercial reference maize grain groups (33P66, 33J56, and 33R77) were not included in the statistical analysis; instead, these data were used to construct a 95% tolerance interval on 99% of the population for each trait as described by Graybill, 1976 (not included in the reference list). Data from the 59122 maize and Control treatment groups were then graphically evaluated to determine whether or not the observed values were contained within this interval. If all observations for a treatment were contained within the interval, the treatment was considered to be consistent with how a broiler would perform when fed a diet containing similar levels of commercial reference maize with respect to the desired response variable. Tolerance intervals for organ and carcass response variables were created by gender due to the expected yield differences between male and female broilers. The feeding study was carried out on 600 broiler chickens (50% males and 50% females) randomly distributed into five treatment groups (59122, 091, and the references 33P66, 33J56, and 33R77). There were 12 pens per treatment group with 10 broilers per pen (5 males and 5 females).

Broilers were fed their respective dietary treatments from time of hatching (Trial Day 0) to 42 days of age; day 0-21 a starter diet (53% maize), day 22 to 35 a grower diet (58% maize), and day 36 to 42 a finisher diet (70% maize). Homogeneity and stability of the Cry34Ab1, Cry35Ab1 and PAT proteins in the diet were evaluated with specific ELISA. Actual concentrations were : 13 ± 1.27, 12.4 ± 0.63 and 18.3 ± 0.75 µg Cry34Ab1 /g diet; 0.68 ± 0.08, 0.75 ± 0.04 and 1 ± 0.04 µg Cry35Ab1 /g diet in the starter, grower and finisher diet respectively. In all diets, the concentration of PAT protein was below the limit of detection (0.034 µg/g diet).

Body weights and feed weights (including amount of feed added and amount remaining) were determined every 7 days. Body weight gain, feed intake, and mortality-corrected feed efficiency (weight of feed consumed per weight of gain) were calculated for Trial Days 0 through 42. A growth curve was prepared from weekly body weights.

All birds were humanely euthanized on trial day 42 by cervical dislocation, and each bird received a gross necropsy. Carcass and carcass-parts yield data were collected from 480 broilers (four males and four females per pen per treatment). Carcass-parts yield data included: carcass dry yield (post-chill), thighs, breasts, wings, legs, abdominal fat (including fat around gizzard), kidneys and whole liver. Combined total mass was recorded for all parts (i.e. legs, thighs, both sides of the breast). Kidney and liver weights were expressed as g/kg of whole live bird weight. Carcass dry yield was expressed as

g/kg of whole live bird weight, and part yields were expressed as g/kg of post-chilled dressed carcass weight.

Regarding growth performance (body weight and gain, mortality, and feed efficiency) there was no observable effect of the different dietary exposure groups particularly between the non-GM control maize and maize 59122 groups.

No statistically significant difference in carcass yields and organ weights were observed between the non-GM control maize group and the maize 59122 group except for liver weight in females which was higher for broilers fed maize 59122 diet than those fed the control diet. After consideration of the multiplicity of the tests performed (McNaughton, 2007) and the variability calculated from data relating to the non-GM commercial maize varieties, the VKM GMO Panel considers this difference unlikely to be of any biological significance.

The results of the study showed no consistent differences between dietary treatments with the maize 59122 and the non-GM control maize which is in line with the absence of significant differences observed in the compositional analysis of the diets.

12-weeks nutritional feeding study on laying hens

A 12 week feeding study with 216 Hy-Line W-36 hens was conducted to evaluate the nutritional value of transgenic 59122-maize grain (Jacobs et al. 2008). Hens (20 weeks of age) were placed in cage lots (3 hens/cage, 2 cages/lot) and were randomly assigned to 1 of 3 maize-soybean meal dietary treatments (12 lots/treatment), for a total of 72 hens per treatment. Before the initiation of the feeding period the hens were acclimatised for a 4-week period.

The experiment started when the hens were 24-weeks of age. The diets were formulated with the following maize grains: near-isogenic non-GM control (control), conventional maize (Pioneer maize 3394), and maize 59122. Maize grains were included in the diets at a 65% inclusion level.

The experiment was divided into three 4-weeks phases: phase 1, weeks 24 - 28; phase 2, weeks 28 - 32, and phase 3, weeks 32 - 36. Eggs were collected daily, and egg production and egg mass (grams of egg produced per day) were determined weekly for each phase. Egg weight, number of cracked eggs, and egg grade measures were determined on eggs collected on day 2 of the egg production during the last week of each phase. Egg component weights (albumen, yolk, and wet shell) and Haugh units (a measure of egg protein quality based on the height of its egg white (albumen)) were determined on 4 eggs/cage-lot during the last week of each phase.

Differences between 59122 and control group means were evaluated with statistical significance at $P < 0.05$. Body weight and gain, egg production, egg mass, and feed efficiency for hens fed the 59122 maize were not significantly different from the respective values for hens fed diets formulated with control maize grain. Egg component weights, Haugh unit measures, and egg weight class distribution were similar regardless of the maize source.

The results indicate that performance of hens fed diets containing 59122 maize grain, as measured by egg production and egg quality, was similar to that of hens fed diets formulated with the near isogenic non-GM maize grain. No statistically significant differences were noted between dietary treatments with maize 59122, the control and the conventional maize.

Six weeks nutritional feeding study on lactating dairy cows

The nutritional equivalency of grain and whole plant silage from 59122 maize plants to grain and whole plant silage from a near-isogenic non-GM control maize was assessed with lactating dairy cows (Brouk et al 2011). Effects on feed intake, milk production, and milk composition were determined. The 59122 grain and the control grain were produced in 2005 from isolated plots in Richland, Iowa. Whole plant maize silage for the 59122 and control treatments were grown in isolated plots at the Kansas State University Dairy Center and ensiled in Ag-Bags (storage-bags used for proper storage of

e.g. grass, maize, whole crop and grain). Thirty lactating Holstein cows blocked by lactation number, day of lactation, and previous energy-corrected milk production were used in a switchback design. All cows were fed diets that contained 22.7% grain plus 21.3% whole plant silage from either 59122 maize or control, in addition to 21% wet maize gluten feed, 12.3% protein mix, 8.0% whole cottonseed, and 14.7% alfalfa hay. Each period of the switchback trial included 2 weeks of diet adjustment followed by 4 weeks of data and sample collection. Milk samples (a.m. and p.m.) collected from 2 consecutive milkings of each collection week were analysed for fat, protein, lactose, solids-not-fat, milk urea nitrogen, and somatic cell count. Percentages of milk fat, protein, lactose, and solids-not-fat were not affected by dietary treatment. Yields of milk, 4% fat-corrected milk, energy-corrected milk, solids-corrected milk, and the concentrations and yields of milk fat, milk protein, milk solids, and milk lactose were not significantly different between treatments. Efficiencies of milk production (fat-corrected milk, energy-corrected milk, and solids-corrected milk) were also not different when cows were fed 59122 maize than when they were fed the control hybrid. Milk production efficiency averaged 1.48 and 1.50 kg/kg of dry matter intake for cows fed diets containing the control and 59122 maize, respectively. These data indicate that the nutritional value for milk production was not different between a diet containing grain plus whole plant maize silage produced from a 59122 maize hybrid versus a diet containing grain and maize silage from its near-isogenic control maize hybrid.

Sixteen weeks nutritional feeding study on steers

Huls et al. (2008) reported a steer feeding study in which groups of 60 cross-bred steers (396 kg) were individually fed either 59122 maize (82% dry-rolled grain), a near-isogenic conventional counterpart or a non-GM commercial maize, for 109 days. The composition of the feed was analysed and animal performance and various carcass characteristics were measured. When adjusted with the statistical analysis of false discovery rate, DMI (dry matter intake), ADG (average daily gain), and G:F (feed efficiency) were not different between control and 59122 ($P > 0.33$). No differences were observed between control and 59122 for HCW (hot carcass weights), marbling score (meat contains various amounts of intramuscular fat, giving it an appearance similar to a marble), LM (lean mass) area, fat depth, or calculated USDA YG (yield grade) ($p> 0.12$). Feeding on Maize 59122 grain did not influence steer performance or carcass quality. No statistically significant differences were identified between the animals fed maize 59122 and those fed the conventional counterpart. The results show that maize 59122 is nutritionally equivalent to commercial non-GM maize grain when fed to finishing steers.

Thirteen weeks nutritional feeding study on growing–finishing pigs

Stein et al. (2009) reported a growing–finishing pig feeding study from February to May 2006 with diets containing maize 59122, a conventional counterpart (not specified) and a commercial maize hybrid (35P12). All maize sources were grown in 2005 by Pioneer Hi-Bred International Inc. in field production plots. The 59122 maize plants received an application of glufosinate -ammonium herbicide (Liberty, Bayer AG) at the V4 and V7 growth stages (0.41 and 0.50 kg of active ingredient/ha, respectively); no application occurred beyond the V7 growth stage. The absence or presence of the Cry34Ab1 and Cry35Ab1 proteins in the non-transgenic and transgenic grains was confirmed with ELISA methods specific for each protein. The experimental diets were formulated based on maize and soybean meal. The Cry34Ab1 and the Cry35Ab1 proteins were present in the 59122 maize in quantities of 310 and 9.6 µg/g, respectively. No traces of these proteins were in the other 2 maize hybrids or in the soybean meal. Each source of maize and the soybean meal was also analysed for mycotoxins with ELISA. Results of the chemical analyses did not reveal the presence of mycotoxins in any of the grains that were used. Energy and nutrients differed only marginally among the 3 maize hybrids and were close to expected values (NRC, 1998). Likewise, all diets contained the expected energy and nutrient levels.

Data were analysed as described by Jacobs et al. (2008), with the false discovery rate as described by Benjamini and Hochberg (1995), to minimise the chance of falsely declaring a difference for a measured trait as significant when the difference was only a chance event. Data were analysed with a mixed model ANOVA (PROC MIXED, SAS Institute Inc., Cary, NC). Maize, sex, and the maize × sex interaction were fixed effects in the analysis of performance and carcass data. Start date and the

start date × maize interaction were random effects for performance data, and start date, the start date × maize interaction, and pen nested within start date and type of maize were random effects for carcass data.

A total of 108 pigs of SP-1 boars were randomly allotted to 3 dietary treatments based on BW (body weight) and sex in a complete randomised block design. The treatment groups consisted of 36 pigs each, which were kept in 12 replicate pens with three animals per pen, with starting and final weights at approximately 37 and 127 kg, respectively. The inclusion level of maize in the diets ranged from 69% in starter diets to 82% in finishing diets. Feeds were analysed for their composition. During and after the experiment the animals were analysed for performance (weight, feed intake) and various carcass characteristics. No statistically significant differences were observed between the groups fed the maize 59122 and the conventional counterpart.

The results of the study indicate that grain of maize 59122 have a feeding value that is not different from commercial maize.

4.6 Conclusion

A 90-day subchronic feeding study in rats, as well as whole food feeding studies on broilers, laying hens, lactating dairy cows, feedlot steers, and growing-finishing pigs, have not indicated any adverse effects of maize 59122, and shows that maize 59122 is nutritionally equivalent to conventional maize. The PAT, Cry34Ab1 and Cry35Ab1 proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE-mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize 59122 is nutritionally equivalent to conventional maize varieties. It is unlikely that the PAT, Cry34Ab1 and Cry35Ab1 proteins will introduce a toxic or allergenic potential in food or feed based on maize 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 by seed produced predominantly by cross-pollination (OECD 2003). In contrast to weedy plants, maize has a pistillate inflorescence (ear) with a cob enclosed with husks. Due to the structure of the cob, the seeds remain on the cob after ripening and natural dissemination of the kernels rarely occurs.

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

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

It is considered very unlikely that the establishment, spread and survival of maize 59122 would be increased due to the insect resistance and herbicide tolerance trait. The herbicide tolerant trait can only be regarded as providing a selective advantage for the GM maize plant where and when glufosinate-based herbicides are applied. It is considered very unlikely that maize 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.

A series of field trials with maize 59122 was conducted by the applicant at several maize growing locations in Chile, North America and Europe during the 2002-2004 growing seasons to compare the agronomic performance and field characteristics of maize 59122 with its comparators (see section 3.3).

The agronomic and phenotypic field trial data did not show major changes in plant characteristics indicating altered fitness, persistence and invasiveness of maize 59122 plants. A number of endpoints (i.e., plant height, ear height, mean early population and final population) showed statistically significant differences in the acrosslocation comparisons between maize 59122 and its near-isogenic lines in the European field trials in 2004. These differences were, however, numerically small and did not show any consist trend across trials. Moreover, the range of values for agronomic and phenotypic characteristics was shown to fall within the range of values observed for conventional maize hybrids. No visually observable response to naturally occurring insects, diseases and/or abiotic stressors

recorded during the growing season provided any indication of altered stress responses of maize 59122 as compared with its conventional counterpart.

In addition to the data presented by the applicant, the VKM GMO Panel is not aware of any scientific reports indicative of increased establishment or spread of maize 59122, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of maize 59122 are unchanged, the insect resistance and herbicide tolerance are not likely to provide a selective advantage outside of cultivation in Norway. The VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects based on establishment and survival of maize 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 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 2004, 2009a; Bensasson et al. 2004; VKM 2005b).

Based on established scientific knowledge of the barriers for gene transfer between unrelated species and the experimental research on horizontal transfer of genetic material from plants to microorganisms, there is today little evidence pointing to a likelihood of random transfer of the transgenes present in maize 59122 to unrelated species such as bacteria.

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

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

GM DNA was detected in the faeces from the control group. Rizzi et al. (2012) provides an extensive review of the fate of feed-derived DNA in the gastrointestinal system of mammals.

In conclusion, the VKM GMO Panel consider it is unlikely that the introduced gene from maize 59122 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 *cry34Ab1, cry35Ab2* and *pat* gene from 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 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 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

Maize 59122 was transformed to co-express Cry34Ab1 and Cry35Ab1 proteins from *Bacillus thuringiensis*. This binary insecticidal toxin is made of two components, the Cry34Ab1 and Cry35Ab1 proteins, acting together in the control of certain 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 2013a). *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. There have been no reports of *D. virgifera virgifera* in Norway (http://www.faunaeur.org/distribution.php).

Considering the intended uses of maize 59122, excluding cultivation, the environmental exposure is limited to exposure through manure and faeces from the gastrointestinal tract mainly of animals fed on the GM maize as well as to the accidental release into the environment of GM seeds during transportation and processing and subsequently to potential occurrence of sporadic feral plants. Thus the level of exposure of target organisms to the Cry34Ab1/Cry35Ab1 protein is likely to be extremely low and of no ecological relevance.

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

Considering the intended uses of maize 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 2008).

Data supplied by the applicant indicate that a limited amount of the Cry34Ab1 and Cry35Ab1 protein enters the environment due to expression in the grains (mean value of 36.4 and 8.9 ng/mg d.w, respectively). In addition, the data show that at least 99% of microbially produced Cry35Ab1/Cry35Ab1 protein was rapidly degraded in simulated gastric fluid. In conclusion, the VKM GMO Panel considers that the exposure of potentially non-target organisms to the Cry35Ab1/Cry35Ab1 protein is likely to be very low and of no ecological relevance.

5.5 Potential interactions with the abiotic environment and biochemical cycles

Considering the intended uses of maize 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.

5.7 Conclusion

Considering the intended uses of maize 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 59122.

Maize 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 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.

6 Post-market environmental monitoring

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

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

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

No specific environmental impact of genetically modified maize 59122 was indicated by the environmental risk assessment and thus no case specific monitoring is required. The VKM GMO Panel is of the opinion that the monitoring plan provided by the applicant is in line with the intended uses of maize 59122.

7 Data gaps

Adjuvanticity
There are many knowledge gaps related to assessment of adjuvants. Most of the immunologic adjuvant experiments have been performed with 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.

8 Conclusions

Molecular characterisation
Appropriate analyses of the transgenic DNA insert, its integration site, number of inserts and flanking sequences in the maize genome, have been performed. The results show that only one copy of the insert is present in maize 59122. Homology searches with databases of known toxins and allergens have not indicated any potential production of harmful proteins or polypeptides caused by the genetic modification in maize 59122. Southern blot analyses and segregation studies show that the introduced genes *cry34Ab1, cry35Ab1* and *pat* are stably inherited and expressed over several generations along with the phenotypic characteristics of maize 59122. The VKM GMO Panel considers the molecular characterisation of maize 59122 satisfactory.

Comparative assessment
Comparative analyses of maize 59122 to its non-GM conventional counterpart have been performed during multiple field trials in representative areas for maize cultivation in Chile (2002/2003), North America (2003, 2004) and Europe (2003, 2004). With the exception of small intermittent variations, no biologically significant differences were found between maize 59122 and the conventional non-GM control. Based on the assessment of available data, the VKM GMO Panel concludes that maize 59122 is compositionally, agronomical and phenotypically equivalent to its conventional counterpart, except for the introduced characteristics.

Food and feed safety assessment
A 90-day subchronic feeding study in rats, as well as whole food feeding studies on broilers, laying hens, lactating dairy cows, feedlot steers, and growing-finishing pigs, have not indicated any adverse effects of maize 59122, and shows that maize 59122 is nutritionally equivalent to conventional maize. The PAT, Cry34Ab1 and Cry35Ab1 proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE-mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize 59122 is nutritionally equivalent to conventional maize varieties. It is unlikely that the PAT, Cry34Ab1 and Cry35Ab1 proteins will introduce a toxic or allergenic potential in food or feed based on maize 59122 compared to conventional maize.

Environmental risk assessment
Considering the intended uses of maize 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 59122.

Maize 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 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.

Overall conclusion
Based on current knowledge, the VKM GMO Panel concludes that maize 59122 is nutritionally equivalent to conventional maize varieties. It is unlikely that the PAT, Cry34Ab1 and Cry35Ab1 proteins will introduce a toxic or allergenic potential in food or feed based on maize 59122 compared to conventional maize.

The VKM GMO Panel likewise concludes that maize 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

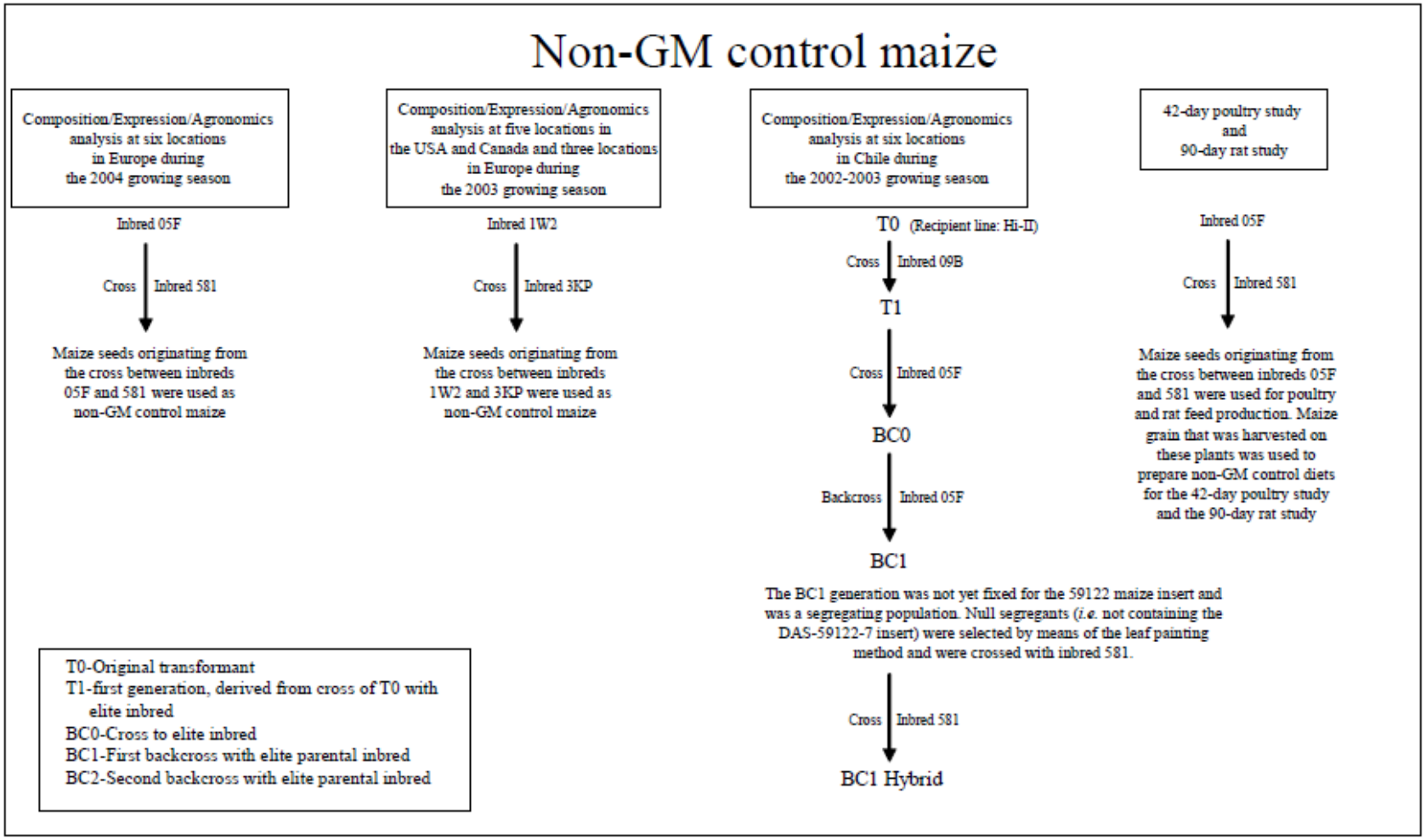

Figure 1. Breeding diagram of the 59122 maize and non-GM maize used in compositional analysis and animal studies.

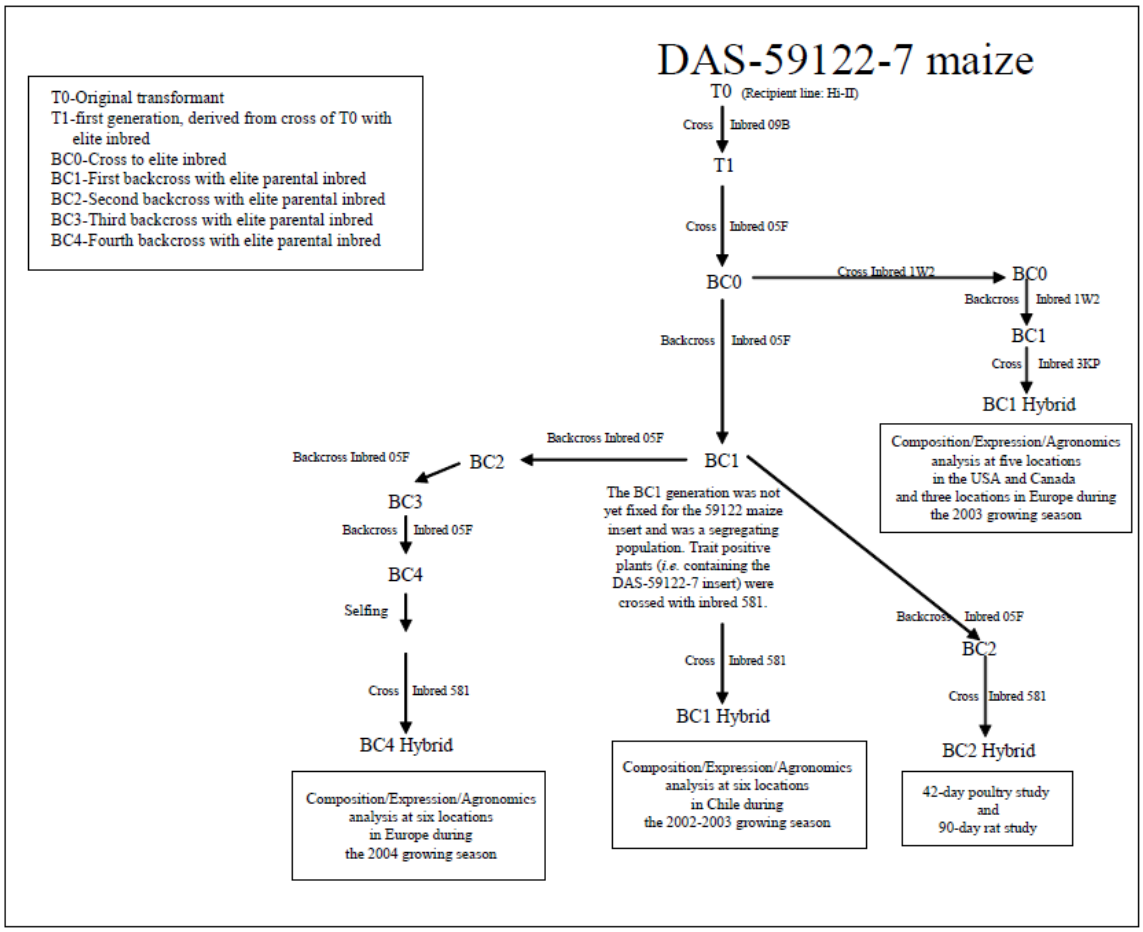
DAS-59122-7 maize
T0-Original transformant
T1-first generation, derived from cross of T0 with elite inbred
BC0-Cross to elite inbred
BC1-First backcross with elite parental inbred
BC2-Second backcross with elite parental inbred
BC3-Third backcross with elite parental inbred
BC4-Fourth backcross with elite parental inbred
T0 (Recipient line: Hi-II)
Cross Inbred 09B
T1
Cross Inbred 05F
BC0
Cross Inbred 1W2
BC0
Backcross Inbred 1W2
BC1
Cross Inbred 3KP
BC1 Hybrid
Composition/Expression/Agronomics analysis at five locations in the USA and Canada and three locations in Europe during the 2003 growing season
Backcross Inbred 05F
BC1
Backcross Inbred 05F
BC2
BC3
Backcross Inbred 05F
BC4
Selfing
Cross Inbred 581
BC4 Hybrid
Composition/Expression/Agronomics analysis at six locations in Europe during the 2004 growing season
The BC1 generation was not yet fixed for the 59122 maize insert and was a segregating population. Trait positive plants (i.e. containing the DAS-59122-7 insert) were crossed with inbred 581.
Cross Inbred 581
BC1 Hybrid
Composition/Expression/Agronomics analysis at six locations in Chile during the 2002-2003 growing season
Backcross Inbred 05F
BC2
Cross Inbred 581
BC2 Hybrid
42-day poultry study and 90-day rat study

Figure 1. continued.

Table 1. Summary Analysis of Proximates and Fiber in Forage for the 59122 **sprayed** with Glufosinate and Control hybrids, Europe 2003, (table 39 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (% Dry weight)		Standard Error
		59122 + Glufosinate	Control	
Crude Protein	3.14 - 15.9	9.96*	7.78	0.211
Crude Fat	0.373 - 6.7	2.47	2.54	0.0701
Crude Fiber	19 - 42	24.7	24.3	0.247
ADF	16.1 - 41.9	32.2*	30.8	0.556
NDF	10.3 - 63.7	54.7*	52.4	0.525
Ash	1.3 - 10.5	6.27*	5.16	0.189
Carbohydrates	66.9 - 94.5	81.3*	84.5	0.369

[1]Combined ranges, see Appendix 5
*P-value<0.05 between the 59122 + glufosinate and control hybrids

Table 2. Summary Analysis of Proximates and Fiber in Forage for the 59122 **not sprayed** with Glufosinate and Control hybrids, Europe 2003, (table 21 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (% Dry weight)		Standard Error
		59122 - Glufosinate	Control	
Crude Protein	3.14 - 15.9	8.05	7.78	0.308
Crude Fat	0.373 - 6.7	2.64	2.54	0.0864
Crude Fiber	19 - 42	25.0	24.3	0.378
ADF	16.1 - 41.9	31.9	30.8	0.678
NDF	10.3 - 63.7	53.1	52.4	0.515
Ash	1.3 - 10.5	5.69*	5.16	0.200
Carbohydrates	66.9 - 94.5	83.6*	84.5	0.519

[1]Combined ranges, see Appendix 5
*P-value<0.05 between the 59122 - glufosinate and control hybrids

Table 3. Summary Analysis of Minerals in Forage for the 59122 **sprayed** with Glufosinate and Control hybrids, Europe 2003, (table 40 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (% Dry weight)		Standard Error
		59122 + Glufosinate	Control	
Calcium	0.0969 - 0.6	0.260	0.243	0.0115
Phosphorus	0.118 - 0.55	0.279*	0.217	0.00372

[1]Combined ranges, see Appendix 5
*P-value<0.05 between the 59122 + glufosinate and control hybrids

Table 4. Summary Analysis of Minerals in Forage for the 59122 **not sprayed** with Glufosinate and Control hybrids, Europe 2003, (table 22 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (% Dry weight)		Standard Error
		59122 - Glufosinate	Control	
Calcium	0.0969 - 0.6	0.259	0.243	0.0104
Phosphorus	0.128 - 0.55	0.214	0.217	0.00384

[1]Combined ranges, see Appendix 5

Table 5. Summary Analysis of Proximates and Fiber in Grain for the 59122 **sprayed** with Glufosinate and Control hybrids, Europe 2003, (table 41 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (% Dry weight)		Standard Error
		59122 + Glufosinate	Control	
Crude Protein	6 - 15.0	10.3*	9.62	0.179
Crude Fat	1.2 - 18.8	3.80*	3.97	0.116
ADF	1.82 - 11.3	2.92*	2.38	0.141
Crude Fiber	1.60 - 5.5	2.12*	1.83	0.0503
NDF	5.59 - 22.6	9.82*	8.09	0.267
Ash	0.616 - 6.28	1.68*	1.50	0.0505
Carbohydrates	63.3 - 89.8	84.3*	84.9	0.194

[1]Combined ranges, see Appendix 5
*P-value<0.05 between the 59122 + glufosinate and control hybrids

Table 6. Summary Analysis of Proximates and Fiber in Grain for the 59122 **not sprayed** with Glufosinate and Control hybrids, Europe 2003, (table 23 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (% Dry weight)		Standard Error
		59122 - Glufosinate	Control	
Crude Protein	6 - 15.0	9.77	9.62	0.259
Crude Fat	1.2 - 18.8	3.86	3.97	0.115
ADF	1.82 - 11.3	2.75	2.38	0.147
Crude Fiber	1.60 - 5.5	2.17*	1.83	0.0572
NDF	5.59 - 22.6	9.75*	8.09	0.238
Ash	0.616 - 6.28	1.56	1.50	0.0273
Carbohydrates	63.3 - 89.8	84.8	84.9	0.301

[1]Combined ranges, see Appendix 5
*P-value<0.05 between the 59122 - glufosinate and control hybrids

Table 7. Summary Analysis of Fatty Acids in Grain for the 59122 **sprayed** with Glufosinate and Control hybrids, Europe 2003, (table 42 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (% Total fatty acids)		Standard Error
		59122 + Glufosinate	Control	
Palmitic acid	7 - 19	11.8	11.7	0.0948
Stearic acid	0 - 4.0	1.49	1.51	0.0200
Oleic acid	18.6 - 50	26.0*	27.7	0.261
Linoleic acid	34.0 - 70	59.1*	57.6	0.293
Linolenic acid	0 - 2.0	1.23*	1.21	0.0114

[1]Combined ranges, see Appendix 5
*P-value<0.05 between the 59122 + glufosinate and control hybrids

Table 8. Summary Analysis of Fatty Acids in Grain for the 59122 **not sprayed** with Glufosinate and Control hybrids, Europe 2003, (table 24 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (% Total fatty acids)		Standard Error
		59122 - Glufosinate	Control	
Palmitic acid	7 - 19	12.0*	11.7	0.0916
Stearic acid	0 - 4.0	1.55	1.51	0.0200
Oleic acid	18.6 - 50	26.9	27.7	0.339
Linoleic acid	34.0 - 70	58.0	57.6	0.362
Linolenic acid	0 - 2.0	1.21	1.21	0.0130

[1]Combined ranges, see Appendix 5
*P-value<0.05 between the 59122 - glufosinate and control hybrids

Table 9. Summary Analysis of Amino Acids in Grain for the 59122 **sprayed** with Glufosinate and Control hybrids, Europe 2003, (table 43 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (% Dry weight)		Standard Error
		59122 + Glufosinate	Control	
Methionine	0.10 - 0.46	0.265	0.254	0.00698
Cystine	0.08 - 0.32	0.249	0.227	0.00846
Lysine	0.05 - 0.56	0.349*	0.328	0.00656
Tryptophan	0.04 - 0.13	0.0737*	0.0696	0.00126
Threonine	0.22 - 0.65	0.464*	0.441	0.0106
Isoleucine	0.20- 0.71	0.349	0.327	0.00722
Histidine	0.15 - 0.42	0.298	0.287	0.00577
Valine	0.21 - 0.85	0.436	0.412	0.00900
Leucine	0.64 - 2.41	1.27*	1.14	0.0320
Arginine	0.22 - 0.64	0.367*	0.328	0.0114
Phenylalanine	0.26 - 0.83	0.512*	0.468	0.0126
Glycine	0.26 - 0.50	0.400*	0.379	0.0107
Alanine	0.44 - 1.20	0.849	0.797	0.0175
Aspartic Acid	0.40 - 0.95	0.729*	0.677	0.0165
Glutamic Acid	1.04 - 3.04	2.04*	1.85	0.0456
Proline	0.53 - 1.46	0.984*	0.911	0.0200
Serine	0.24 - 0.91	0.541	0.507	0.0141
Tyrosine	0.11 - 0.79	0.296*	0.245	0.00895

[1]Combined ranges, see Appendix 5
*P-value<0.05 between the 59122 + glufosinate and control hybrids

Table 10. Summary Analysis of Amino Acids in Grain for the 59122 **not sprayed** with Glufosinate and Control hybrids, Europe 2003, (table 25 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (% Dry weight)		Standard Error
		59122 - Glufosinate	Control	
Methionine	0.10 - 0.46	0.268	0.254	0.0115
Cystine	0.08 - 0.32	0.239	0.227	0.00972
Lysine	0.05 - 0.56	0.327	0.328	0.00664
Tryptophan	0.04 - 0.13	0.0702	0.0696	0.00155
Threonine	0.22 - 0.65	0.447	0.441	0.0112
Isoleucine	0.20- 0.71	0.325	0.327	0.00923
Histidine	0.15 - 0.42	0.288	0.287	0.00803
Valine	0.21 - 0.85	0.413	0.412	0.0115
Leucine	0.64 - 2.41	1.17	1.14	0.0411
Arginine	0.22 - 0.64	0.325	0.328	0.00861
Phenylalanine	0.26 - 0.83	0.473	0.468	0.0154
Glycine	0.26 - 0.50	0.376	0.379	0.0110
Alanine	0.44 - 1.20	0.810	0.797	0.0262
Aspartic Acid	0.40 - 0.95	0.700	0.677	0.0199
Glutamic Acid	1.04 - 3.04	1.88	1.85	0.0634
Proline	0.53 - 1.46	0.928	0.911	0.0235
Serine	0.24 - 0.91	0.508	0.507	0.0147
Tyrosine	0.11 - 0.79	0.256	0.245	0.00995

[1]Combined ranges, see Appendix 5

Table 11. Summary Analysis of Minerals in Grain for the 59122 **sprayed** with Glufosinate and Control hybrids, Europe 2003, (table 44 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (% Dry weight)		Standard Error
		59122 + Glufosinate	Control	
Calcium	0.00216 - 0.1	0.00473*	0.00515	0.000177
Copper	0.000073 - 0.001	0.000149*	0.000134	0.00000361
Iron	0.0001 - 0.01	0.00214*	0.00196	0.0000178
Magnesium	0.0788 - 1.0	0.123	0.123	0.00158
Manganese	0.00007 - 0.0054	0.000552*	0.000503	0.0000127
Phosphorus	0.208 - 0.75	0.322*	0.304	0.00331
Potassium	0.271 - 0.72	0.414*	0.388	0.00493
Sodium	0.0 - 0.15	0.000364	0.000211	0.000104
Zinc	0.00065 - 0.0037	0.00223*	0.00207	0.0000357

[1]Combined ranges, see Appendix 5
*P-value<0.05 between the 59122 + glufosinate and control hybrids

Table 12. Summary Analysis of Minerals in Grain for the 59122 **not sprayed** with Glufosinate and Control hybrids, Europe 2003, (table 26 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (% Dry weight)		Standard Error
		59122 - Glufosinate	Control	
Calcium	0.00216 - 0.1	0.00548	0.00515	0.000145
Copper	0.000073 - 0.001	0.000196	0.000134	0.0000397
Iron	0.0001 - 0.01	0.00205	0.00196	0.0000292
Magnesium	0.0788 - 1.0	0.120	0.123	0.00173
Manganese	0.00007 - 0.0054	0.000537*	0.000503	0.00000881
Phosphorus	0.208 - 0.75	0.306	0.304	0.00622
Potassium	0.271 - 0.72	0.415*	0.388	0.00672
Sodium	0.0 - 0.15	0.000300	0.000211	0.0000775
Zinc	0.00065 - 0.0037	0.00211	0.00207	0.0000387

[1]Combined ranges, see Appendix 5
*P-value<0.05 between the 59122 - glufosinate and control hybrids

Table 13. Summary Analysis of Vitamins in Grain for the 59122 sprayed with Glufosinate and Control hybrids, Europe 2003, (table 45 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (mg/kg Dry Weight)		Standard Error
		59122 + Glufosinate	Control	
Beta-carotene	0.53 - 16.4	13.3*	9.42	0.954
Vitamin B1	1.3 - 8.6	11.1	8.30	0.996
Vitamin B2	0.25 - 5.6	1.24*	1.52	0.0400
Folic Acid	0.15 - 683	0.832*	0.612	0.0380
Vitamin E[2]	1.5 - 68.7	9.40*	5.27	0.327

[1]Combined ranges, see Appendix 5
[2]Measured as α-tocopherol
*P-value<0.05 between the 59122 + glufosinate and control hybrids

Table 14. Summary Analysis of Vitamins in Grain for the 59122 **not sprayed** with Glufosinate and Control hybrids, Europe 2003, (table 27 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (mg/kg Dry weight)		Standard Error
		59122 - Glufosinate	Control	
Beta-carotene	0.53 - 16.4	10.4	9.42	0.664
Vitamin B1	1.3 - 8.6	10.1	8.30	0.576
Vitamin B2	0.25 - 5.6	1.74	1.52	0.0885
Folic Acid	0.15 - 683	0.845*	0.612	0.0203
Vitamin E[2]	1.5 - 68.7	7.20*	5.27	0.220

[1]Combined ranges, see Appendix 5
[2]Measured as α-tocopherol
*P-value<0.05 between the 59122 - glufosinate and control hybrids

Table 15. Summary Analysis of Secondary Metabolites in Grain for the 59122 **sprayed** with Glufosinate and Control hybrids, Europe 2003, (table 46 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (% Dry weight)		Standard Error
		59122 + Glufosinate	Control	
Inositol	0.0138 - 0.257	0.0325*	0.0399	0.00133
Furfural	0.0003 - 0.0005	ND[2]	ND	ND
P-Coumaric Acid	0.003 - 0.058	0.0212*	0.0184	0.000582
Ferulic Acid	0.02 - 0.373	0.174	0.165	0.00520

[1]Combined ranges, see Appendix 5
[2]ND: Not Detected
*P-value<0.05 between the 59122 + glufosinate and control hybrids

Table 16. Summary Analysis of Secondary Metabolites in Grain for the 59122 **not sprayed** with Glufosinate and Control hybrids, Europe 2003, (table 28 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (% Dry weight)		Standard Error
		59122 - Glufosinate	Control	
Inositol	0.0138 - 0.257	0.0365	0.0399	0.00138
Furfural	0.0003 - 0.0005	ND[2]	ND	ND
P-Coumaric Acid	0.003 - 0.058	0.0184	0.0184	0.000565
Ferulic Acid	0.02 - 0.373	0.180	0.165	0.00743

[1]Combined ranges, see Appendix 5
[2]ND: Not Detected

Table 17. Summary Analysis of Anti-Nutrients in Grain for the 59122 **sprayed** with Glufosinate and Control hybrids, Europe 2003, (table 47 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (% Dry weight or as indicated)		Standard Error
		59122 + Glufosinate	Control	
Raffinose	0.04 - 0.31	0.108*	0.0612	0.00280
Phytic acid	0.29 - 1.29	0.676	0.601	0.0303
Trypsin Inhibitor (TIU/g)[2]	1.10 - 7.18	3.00	3.14	0.0876

[1]Combined ranges, see Appendix 5
[2]Abbreviation: TIU, trypsin inhibitor units
*P-value<0.05 between the 59122 + glufosinate and control hybrids

Table 18. Summary Analysis of Anti-Nutrients in Grain for the 59122 **not sprayed** with Glufosinate and Control hybrids, Europe 2003, (table 29 in Annex 03, Dossier)

Analyte	Combined Ranges[1]	Least Square Means (% Dry weight or as indicated)		Standard Error
		59122 - Glufosinate	Control	
Raffinose	0.04 - 0.31	0.0744*	0.0612	0.00254
Phytic acid	0.29 - 1.29	0.586	0.601	0.0309
Trypsin Inhibitor (TIU/g)[2]	1.10 - 7.18	3.19	3.14	0.0962

[1]Combined ranges, see Appendix 5
[2]Abbreviation: TIU, trypsin inhibitor units

Table 19. Summary analysis of Nutrient Composition in Forage across Six Locations, Europe, 2004, (Table 11, Annex_04, Dossier)

Analyte	Least Square Means (Range[1])			Standard Error	Tolerance Interval[2]	FDR[3] Adjusted P-value (Non-adjusted P-value)		Combined Ranges[4]
	Control	59122 (Untreated)	59122 + Glufosinate			59122 (Untreated)	59122 + Glufosinate	
Proximates, Fiber, and Minerals Composition (% Dry Weight)								
Crude Protein	7.76 (4.09 – 11.2)	8.48[5] (4.86 – 10.5)	8.61[5] (4.22 – 10.9)	0.575	1.60 – 14.8	0.0336 (0.00126)	0.0185 (0.00410)	3.14 – 15.9
Crude Fat	2.61 (1.83 – 3.16)	2.57 (2.15 – 3.23)	2.40[5] (1.73 – 3.28)	0.108	0.998 – 3.92	0.729 (0.660)	0.0803 (0.0288)	0.37 – 6.7
Crude Fiber	22.4 (18.1 – 27.7)	22.5 (17.1 – 27.4)	21.7 (15.9 – 25.4)	0.997	10.3 – 36.7	0.861 (0.861)	0.430 (0.352)	19 – 42
ADF	30.0 (23.5 – 37.6)	29.1 (20.9 – 34.1)	28.7 (24.3 – 33.3)	1.18	12.9 – 48.7	0.473 (0.360)	0.306 (0.205)	16.1 – 41.9
NDF	51.0 (40.7 – 60.8)	50.5 (38.0 – 59.2)	50.3 (42.3 – 58.3)	1.71	21.3 – 81.9	0.776 (0.714)	0.687 (0.632)	10.3 – 63.7
Ash	4.68 (3.43 – 5.78)	5.13[5] (4.36 – 6.34)	5.00[5] (4.29 – 6.34)	0.147	0.000 – 9.97	0.0199 (0.00600)	0.114 (0.0469)	1.3 – 10.5
Carbohydrates	84.9 (80.6 – 89.7)	83.8[5] (81.4 – 87.7)	84.0[5] (80.6 – 89.8)	0.756	74.5 – 95.0	0.0163 (0.00310)	0.0385 (0.0110)	66.9 – 94.5
Calcium	0.230 (0.124 – 0.417)	0.249 (0.0966 – 0.339)	0.226 (0.133 – 0.359)	0.0283	0.000 – 0.529	0.212 (0.115)	0.784 (0.772)	0.097 – 0.6
Phosphorus	0.203 (0.122 – 0.279)	0.241[5] (0.168 – 0.300)	0.257[5] (0.165 – 0.353)	0.0162	0.000 – 0.542	0.0401 (0.0164)	0.0126 (0.00240)	0.12 – 0.55

[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 *(1, 3, 4, 5, 7,9, and 10)*.
[5]Non-adjusted P-value <0.05

Table 20. Summary analysis of Nutrient Composition in Grain across Six Locations, Europe, 2004, (Table 13, Annex_04, Dossier)

Analyte	Least Square Means (Range[1])		Standard Error	Tolerance Interval[2]	FDR[3] Adjusted P-value (Non-adjusted P-value)		Combined Ranges[4]
	Control	59122 (Untreated)	59122 + Glufosinate		59122 (Untreated)	59122 + Glufosinate	
Proximates and Fiber Composition (% Dry Weight)							
Crude Protein	9.85 (8.99 – 11.1)	10.6[9] (9.28 – 11.8)	10.4[9] (9.27 – 11.3)	0.238	4.11 – 15.6	0.00530 (0.000500)	0.0273 (0.00650)	6 – 15.0
Crude Fat	3.84 (3.23 – 4.29)	4.32[9] (3.80 – 5.06)	4.32[9] (3.82 – 5.70)	0.108	1.04 – 6.48	0.00160 (0.000100)	0.000900 (0.000100)	1.2 – 18.8
ADF	2.25 (1.63 – 2.63)	2.27 (1.97 – 2.55)	2.33 (2.05 – 2.60)	0.0667	0.958 – 6.49	0.836 (0.796)	0.348 (0.254)	1.82 – 11.3
Crude Fiber	3.65 (2.68 – 4.34)	3.57 (2.59 – 4.58)	3.57 (2.65 – 4.37)	0.181	1.18 – 3.64	0.656 (0.572)	0.636 (0.576)	1.6 – 5.5
NDF	9.60 (6.90 – 12.5)	9.96 (7.34 – 12.5)	10.1 (8.07 – 12.6)	0.395	2.34 – 20.6	0.509 (0.420)	0.382 (0.297)	5.59 – 22.6
Ash	1.35 (1.19 – 1.53)	1.49[9] (1.27 – 1.63)	1.55[9] (1.38 – 1.96)	0.0299	0.338 – 2.54	0.0168 (0.00440)	0.00210 (0.000300)	0.616 – 6.28
Carbohydrates	85.0 (83.5 – 86.2)	83.6[9] (82.8 – 84.8)	83.8[9] (82.1 – 85.1)	0.270	78.2 – 91.6	0.00160 (0.000100)	0.000900 (0.000100)	63.3 – 89.8
Fatty Acids Composition (% Total Fatty Acids)								
Palmitic acid	11.4 (10.4 – 12.1)	10.9[9] (10.3 – 11.6)	11.0 (10.2 – 12.0)	0.122	4.85 – 19.3	0.0401 (0.0172)	0.130 (0.0559)	7 – 19
Stearic acid	1.57 (1.45 – 1.69)	1.60[9] (1.44 – 1.72)	1.60[9] (1.45 – 1.76)	0.0337	0.635 – 2.04	0.0463 (0.0213)	0.0753 (0.0239)	0 – 4.0
Oleic acid	23.7 (22.0 – 25.5)	23.4 (21.7 – 25.5)	23.5 (22.2 – 25.0)	0.317	0.000 – 73.4	0.263 (0.159)	0.437 (0.368)	18.6 – 50
Linoleic acid	61.8 (59.5 – 63.0)	62.6[9] (59.6 – 65.2)	62.4[9] (60.2 – 64.5)	0.412	21.4 – 97.3	0.00690 (0.00110)	0.0385 (0.0107)	34.0 – 70
Linolenic acid	1.17 (1.07 – 1.25)	1.03[9] (0.921 – 1.17)	1.03[9] (0.902 – 1.13)	0.0146	0.000 – 2.91	0.00160 (0.000100)	0.000900 (0.000100)	0 – 2.0
Amino Acids Composition (% Dry Weight)								
Methionine	0.225 (0.196 – 0.269)	0.230 (0.213 – 0.243)	0.228 (0.167 – 0.308)	0.00562	0.0923 – 0.535	0.656 (0.543)	0.757 (0.721)	0.10 – 0.46
Cystine	0.231 (0.177 – 0.288)	0.243 (0.200 – 0.285)	0.239 (0.205 – 0.295)	0.00800	0.0831 – 0.360	0.363 (0.247)	0.488 (0.426)	0.08 – 0.32
Lysine	0.302 (0.248 – 0.350)	0.316 (0.281 – 0.348)	0.317 (0.262 – 0.346)	0.00809	0.214 – 0.537	0.363 (0.240)	0.306 (0.209)	0.05 – 0.56
Tryptophan	0.0636 (0.0514 – 0.0799)	0.0674[9] (0.0526 – 0.0812)	0.0663 (0.0512 – 0.0844)	0.00381	0.000 – 0.134	0.0296 (0.0108)	0.144 (0.0638)	0.04 – 0.13
Threonine	0.650 (0.576 – 0.780)	0.706[9] (0.545 – 0.844)	0.690[9] (0.565 – 0.811)	0.0235	0.158 – 0.660	0.0168 (0.00470)	0.0803 (0.0273)	0.22 – 0.65
Isoleucine	0.324 (0.279 – 0.370)	0.348[9] (0.290 – 0.416)	0.340 (0.297 – 0.396)	0.00987	0.121 – 0.532	0.0419 (0.0186)	0.190 (0.0966)	0.20 – 0.71
Histidine	0.265 (0.239 – 0.304)	0.281[9] (0.243 – 0.315)	0.278[9] (0.244 – 0.326)	0.00585	0.142 – 0.389	0.0278 (0.00970)	0.0915 (0.0353)	0.15 – 0.42
Valine	0.406 (0.357 – 0.468)	0.429[9] (0.373 – 0.481)	0.421 (0.368 – 0.485)	0.0110	0.179 – 0.616	0.0651 (0.0310)	0.222 (0.127)	0.21 – 0.85

Table 20. (Cont.)

Analyte	Least Square Means (Range[1])			Standard Error	Tolerance Interval[2]	FDR[3] Adjusted P-value (Non-adjusted P-value)		Combined Ranges[4]
	Control	59122 (Untreated)	59122 + Glufosinate			59122 (Untreated)	59122 + Glufosinate	
Amino Acids Composition (% Dry Weight)								
Leucine	1.19 (0.995 – 1.36)	1.29[9] (1.03 – 1.61)	1.25 (1.06 – 1.45)	0.0418	0.333 – 2.10	0.0168 (0.00380)	0.210 (0.113)	0.64 – 2.41
Arginine	0.354 (0.294 – 0.427)	0.370 (0.326 – 0.416)	0.362 (0.327 – 0.447)	0.00869	0.162 – 0.620	0.161 (0.0844)	0.430 (0.355)	0.22 – 0.64
Phenylalanine	0.494 (0.405 – 0.579)	0.666 (0.445 – 2.96)	0.519 (0.450 – 0.600)	0.0802	0.180 – 0.774	0.257 (0.151)	0.831 (0.831)	0.26 – 0.83
Glycine	0.407 (0.349 – 0.464)	0.419 (0.360 – 0.473)	0.422 (0.353 – 0.462)	0.00736	0.205 – 0.528	0.315 (0.200)	0.202 (0.106)	0.26 – 0.50
Alanine	0.781 (0.655 – 0.928)	0.830[9] (0.708 – 1.00)	0.806 (0.651 – 0.933)	0.0266	0.298 – 1.27	0.0872 (0.0429)	0.366 (0.273)	0.44 – 1.20
Aspartic Acid	0.662 (0.593 – 0.754)	0.713[9] (0.608 – 0.789)	0.719[9] (0.623 – 0.778)	0.0151	0.332 – 1.02	0.00380 (0.000300)	0.000900 (0.000100)	0.40 – 0.95
Glutamic Acid	2.05 (1.72 – 2.36)	2.21[9] (1.74 – 2.74)	2.14 (1.86 – 2.41)	0.0653	0.742 – 3.26	0.0168 (0.00470)	0.190 (0.0953)	1.04 – 3.04
Proline	1.07 (0.958 – 1.23)	1.13[9] (0.979 – 1.24)	1.11 (0.990 – 1.22)	0.0235	0.501 – 1.84	0.0401 (0.0171)	0.227 (0.133)	0.53 – 1.46
Serine	0.501 (0.419 – 0.597)	0.538[9] (0.463 – 0.600)	0.543[9] (0.471 – 0.660)	0.0128	0.209 – 0.780	0.0168 (0.00480)	0.0120 (0.00190)	0.24 – 0.91
Tyrosine	0.228 (0.168 – 0.291)	0.260[9] (0.204 – 0.295)	0.255[9] (0.220 – 0.285)	0.00647	0.138 – 0.435	0.00540 (0.000600)	0.0150 (0.00310)	0.11 – 0.79
Minerals Composition (% Dry Weight)								
Calcium	0.00306 (0.00139 – 0.00448)	0.00402[9] (0.00283 – 0.00529)	0.00437[9] (0.00251 – 0.00590)	0.000372	0.000 – 0.00961	0.00550 (0.000700)	0.000900 (0.000100)	0.00216 – 0.1
Copper	0.000130 (0.0000962 – 0.000201)	0.000153[9] (0.000105 – 0.000272)	0.000159[9] (0.000109 – 0.000217)	0.0000126	0.000 – 0.00114	0.00690 (0.00120)	0.000900 (0.000100)	0.000073 – 0.001
Iron	0.00183 (0.000996 – 0.00269)	0.00194 (0.00127 – 0.00300)	0.00194 (0.00125 – 0.00245)	0.000149	0.000898 – 0.00274	0.363 (0.242)	0.319 (0.223)	0.0001 – 0.01
Magnesium	0.107 (0.0933 – 0.123)	0.112 (0.0951 – 0.128)	0.113 (0.0960 – 0.123)	0.00208	0.0613 – 0.193	0.212 (0.118)	0.184 (0.0849)	0.08 – 1.0
Manganese	0.000827 (0.000603 – 0.00111)	0.000870 (0.000701 – 0.00109)	0.000851 (0.000677 – 0.000999)	0.0000466	0.0000107 – 0.00102	0.230 (0.131)	0.452 (0.388)	0.00007 – 0.0054
Phosphorus	0.272 (0.237 – 0.312)	0.300[9] (0.246 – 0.343)	0.302[9] (0.256 – 0.325)	0.00740	0.103 – 0.533	0.0168 (0.00440)	0.0126 (0.00240)	0.21 – 0.75
Potassium	0.308 (0.268 – 0.338)	0.343[9] (0.305 – 0.382)	0.356[9] (0.297 – 0.389)	0.00658	0.000 – 0.835	0.00630 (0.000900)	0.000900 (0.000100)	0.27 – 0.72
Sodium	0.00265 (0.00202 – 0.00372)	0.00279 (0.00205 – 0.00381)	0.00282 (0.00205 – 0.00452)	0.000139	0.000 – 0.000999	0.483 (0.375)	0.382 (0.292)	0.0 – 0.15
Zinc	0.00168 (0.00121 – 0.00229)	0.00188[9] (0.00155 – 0.00241)	0.00200[9] (0.00151 – 0.00261)	0.000130	0.00113 – 0.00254	0.0214 (0.00680)	0.00210 (0.000300)	0.00065 – 0.0037

Table 20. (Cont.)

Analyte	Least Square Means (Range[1])			Standard Error	Tolerance Interval[2]	FDR[3] Adjusted P-value (Non-adjusted P-value)		Combined Ranges[3]
	Control	59122 (Untreated)	59122 + Glufosinate			59122 (Untreated)	59122 + Glufosinate	
Vitamins Composition (mg/kg Dry Weight)								
Beta-carotene	4.59 (3.35 – 5.83)	4.64 (2.77 – 7.29)	4.95 (2.79 – 7.71)	0.485	0.000 – 30.9	0.861 (0.860)	0.319 (0.228)	0.53 – 16.4
Vitamin B1	22.1 (11.1 – 33.3)	19.6 (9.49 – 33.0)	19.0 (9.68 – 33.3)	2.23	0.000 – 33.4	0.268 (0.166)	0.188 (0.0893)	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	0.657 (0.497 – 0.991)	0.746[9] (0.519 – 0.955)	0.696 (0.488 – 0.934)	0.0500	0.114 – 1.49	0.0264 (0.00880)	0.285 (0.181)	0.15 – 683
Vitamin E[7]	9.97 (6.36 – 12.3)	10.7 (7.66 – 14.9)	11.0 (6.62 – 13.9)	0.652	0.000 – 53.6	0.453 (0.338)	0.285 (0.181)	1.5 – 68.7
Secondary Metabolites Composition (% Dry Weight)								
Inositol	0.0115 (0.00826 – 0.0166)	0.0123 (0.00861 – 0.0182)	0.0129 (0.00898 – 0.0213)	0.000822	0.000 – 0.0437	0.489 (0.388)	0.210 (0.117)	0.0138 – 0.257
Furfural	<0.000100[95] (<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 – 0.0005
P-Coumaric Acid	0.0179 (0.0104 – 0.0270)	0.0161 (0.0108 – 0.0221)	0.0157[9] (0.0100 – 0.0254)	0.00123	0.000 – 0.0415	0.149 (0.0758)	0.0803 (0.0293)	0.003 – 0.058
Ferulic Acid	0.146 (0.0647 – 0.216)	0.156 (0.0852 – 0.203)	0.149 (0.0818 – 0.214)	0.0110	0.0585 – 0.300	0.508 (0.411)	0.783 (0.758)	0.02 – 0.373
Anti-Nutrients Composition (% Dry Weight or as Indicated)								
Raffinose	<0.160[5] (<0.160[95])	0.164 (<0.160[5] – 0.200)	0.170 (<0.160[5] – 0.310)	0.00556	0.000 – 0.495	0.656 (0.572)	0.281 (0.170)	0.04 – 0.31
Phytic acid	0.537 (0.333 – 0.629)	0.658[9] (0.506 – 0.986)	0.607[9] (0.489 – 0.759)	0.0314	0.188 – 1.29	0.00160 (0.000100)	0.0331 (0.00840)	0.29 – 1.29
Trypsin Inhibitor[8]	2.97 (2.22 – 3.61)	3.04 (2.59 – 3.00)	3.12 (2.52 – 3.58)	0.0868	1.26 – 5.05	0.363 (0.255)	0.0915 (0.0363)	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 (*1, 3, 4, 5, 7,9, and 10*).
[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]Measued as α-tocopherol
[8]Analyte reported in TIU/g (Abbreviation: TIU, trypsin inhibitor units)
[9]Non-adjusted P-value <0.05

4.3.2.2 Feeding studies in broiler chickens (additional information)

42-day feeding study on broiler chickens

Statistics

The model used for live performance data analysis was:
Yij =U+ Ti + Bj + eij where Yij = observed pen response, U= overall mean, Ti = treatment effect, Bj = random block effect, and eij = residual error. The model used for carcass data analysis was: Yij =U+ Ti + Bj + TiBj + eij where Yij = observed bird response, U= overall mean, Ti = treatment effect, Bj = random block effect, TiBj = random treatment*block effect (referred to as pen) and eij = residual error. TiBj was used as the error term for the fixed effect of treatment (Ti), which allowed within-pen variability to become the residual error. The observed P value determined whether the 59122 mean was significantly different from the Control mean for live performance and carcass data analyses; differences between means were considered significant at $P \leq 0.05$. False discovery rate (FDR; Westfall et al., 1999) method was used to account for the numerous comparisons and minimize the number of differences being declared to be significant due to chance (PROC MULTTEST, SAS® version 8.2 software, SAS Institute Inc., Cary NC, USA).