



Vitenskapskomiteen for mattrygghet  
Norwegian Scientific Committee for Food Safety

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

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

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## Summary

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Scientific Committee for Food Safety (VKM) has been requested by the Norwegian Environment Agency (former Norwegian Directorate for Nature Management) 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 VKM already has conducted its final risk assessments on. However, the Agency requests VKM to consider whether updates or other changes to earlier submitted assessments are necessary.

The insect-resistant and herbicide-tolerant genetically modified maize 1507 x 59122 from Dow AgroSciences and Pioneer Hi-Bred International, Inc. (Unique Identifier DAS-Ø15Ø7-1 x DAS-59122-7) is approved under Regulation (EC) No 1829/2003 for food and feed uses, import and processing since 28 July 2010 (Commission Decision 2010/432/EC).

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

The environmental risk assessment of the maize 1507 x 59122 is based on information provided by the applicant in the applications EFSA/GMO/NL/2005/15 and EFSA/GMO/NL/2005/28, and scientific comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment also considered other peer-reviewed scientific literature as relevant.

The VKM GMO Panel has evaluated 1507 x 59122 with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed. The Norwegian Scientific Committee for Food Safety has also decided to take account of the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2011a), the environmental risk assessment of GM plants (EFSA 2010), 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 scientific risk assessment of maize 1507 x 59122 include molecular characterisation of the inserted DNA and expression of novel proteins, comparative assessment of agronomic and phenotypic characteristics, unintended effects on plant fitness, potential for gene transfer, interactions between the GM plant and target and non-target organisms, effects on biogeochemical processes and evaluations of the post-market environmental plan.

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

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

### **Molecular characterisation**

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

The characterisation of the recombinant insert and the physical, chemical and functional characteristics of the single events maize 1507 (VKM 2004) and maize 59122 (VKM 2005a, 2008b), have previously been evaluated by the VKM GMO Panel and considered adequate.

### **Comparative assessment**

Comparative analyses of data from field trials located at representative sites and environments in the USA, Canada and Europe indicate that maize 1507 x 59122 is agronomically and phenotypically equivalent to the conventional counterpart, with the exception of the lepidopteran and coleopteran-protection traits and herbicide tolerance, conferred by the expression of the Cry1F, Cry34Ab1/Cry35Ab1 and PAT proteins. The field evaluations support the applicant's conclusion of no other phenotypic changes indicative of increased plant weed/pest potential of 1507 x 59122 compared to conventional maize.

The VKM GMO Panel has previously assessed these data and concluded that maize 1507 x 59122 is agronomically and phenotypically equivalent to the conventional comparators, except for the newly introduced traits (VKM 2007a, 2008a).

### **Environmental assessment**

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

The available data indicate that 1507 x 59122 has no altered survival, multiplication or dissemination characteristics, and there are no indications of an increased likelihood of spread and establishment of feral maize plants in the case of accidental release into the environment of seeds from maize 1507 x 59122. Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation. The VKM GMO Panel considers the risk of gene flow from occasional feral GM maize plants to conventional maize varieties to be negligible in Norway. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered by the GMO Panel to be an issue.

### **Overall conclusion**

The VKM GMO Panel concludes that maize 1507 x 59122, based on current knowledge, is comparable to conventional maize varieties concerning environmental risk in Norway with the intended usage.

## Keywords

Maize, *Zea mays* L., genetically modified maize 1507 x 59122, EFSA/GMO/NL/2005/15, insect-resistance, herbicide-tolerance, Cry proteins, *cry34Ab1*, *cry35Ab1*, *cry1F*, PAT, glufosinate-ammonium, 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 naturforvaltning [DN]) om å utarbeide endelige miljørisikovurderinger av alle genmodifiserte organismer (GMOer) og avledete produkter som inneholder eller består av GMOer som er godkjent i EU under forordning 1829/2003 eller direktiv 2001/18, og som er godkjent for ett eller flere bruksområder som omfattes av genteknologiloven. Miljødirektoratet har bedt VKM om endelige risikovurderinger for EU-godkjente søknader hvor VKM ikke har avgitt endelig miljørisikovurdering. I tillegg har Direktoratet bedt VKM vurdere hvorvidt det er nødvendig med oppdatering eller annen endring av de endelige miljørisikovurderingene som VKM tidligere har levert.

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

Maishybriden har tidligere vært vurdert av VKMs faggruppe for genmodifiserte organismer med hensyn på mulig helserisiko i forbindelse med EFSAAs offentlige høring av søknaden i 2007 (VKM 2007a). En søknad om godkjenning av maishybrid 1507 x 59122 til dyrking (EFSA/GMO/NL/2005/28), som var på offentlig høring høsten 2007, er også vurdert av faggruppen med hensyn på mulig miljørisiko (VKM 2008a). Foreldrelinjene 1507 og 59122 er også tidligere risikovurdert av VKM, både som enkelt-eventer og i en rekke andre hybrider (VKM 2004, VKM 2005a,b, VKM 2007b,c, VKM 2008b,c, VKM 2009a,b, VKM 2012).

Risikovurderingen av den genmodifiserte maislinjen er basert på uavhengige vitenskapelige publikasjoner og dokumentasjon som er gjort tilgjengelig på EFSAAs 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, utsetningsdirektiv 2001/18/EF (vedlegg 2,3 og 3B) og veiledende notat til Annex II (2002/623/EF), samt prinsippene i EFSAAs retningslinjer for risikovurdering av genmodifiserte planter og avledete næringsmidler (EFSA 2006, 2010, 2011a,b,c) lagt til grunn for vurderingen.

Den vitenskapelige vurderingen omfatter transformeringsprosess og vektorkonstruksjon, karakterisering og nedarving av genkonstruksjonen. Videre er agronomiske egenskaper, potensiale for utilsiktede effekter på fitness, genoverføring, mulige effekter på mål- og ikke-målorganismer, biogeokjemiske prosesser, samt søkers overvåkingsplaner vurdert.

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

F<sub>1</sub>-hybriden 1507 x 59122 er resultat av konvensjonelle kryssinger mellom de genmodifiserte maislinjene 1507 og 59122. Kryssingene er utført for å utvikle en maishybrid med resistens mot visse skadegjørere i sommerfuglordenen Lepidoptera og billeslekten *Diabrotica*, samt toleranse mot herbicider med virkestoff glufosinat-ammonium.

Foreldrelinjen 1507 har fått innsatt et *cryIF*-gen fra bakterien *Bacillus thuringiensis* var. *aizawai* og et *pat*-gen, som er isolert fra *Streptomyces viridochromogenes*. *CryIF*-genet koder for et  $\delta$ -endotoksin og gir resistens mot enkelte arter i sommerfuglordenen Lepidoptera, eksempelvis maispyralide (*Ostrinia nubilalis*) og nattflyarten *Sesamia nonagrioides*. *Pat*-genet koder for enzymet fosfinotricin

acetyltransferase (PAT), som acetylerer og inaktiverer glufosinat-ammonium, virkestoffet i fosfinotricin-herbicerer av typen Finale. Fosfinotricin er et ikke-selektivt kontaktherbicide som hemmer glutaminsyntetase. Enzymet deltar i assimilasjonen av nitrogen og katalyserer omdanning av glutamat og ammonium til aminosyren glutamin. Hemming av glutaminsyntetase fører til akkumulasjon av ammoniakk, og til celledød i planten. De transgene maisplantene vil derfor tolerere høyere doser av sprøytemiddelet glufosinat sammenlignet med konkurrerende ugras.

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

### Molekylær karakterisering

Maishybriden 1507 x 59122 er dannet ved konvensjonell kryssing mellom maislinjene 59122 og 1507. Spaltingsdata og PCR-analyser indikerer at de innsatte strukturer nedarves stabilt, og at antall, struktur og organisering av disse genkonstruksjonene er ekvivalent med de som finnes i foreldrelinjene. Nivåene av Cry1F-, Cry34Ab1-, Cry35Ab1- og PAT-proteiner i vegetativt vev og frø er sammenlignbare med uttrykk av tilsvarende proteinprodukter i foreldrelinjene.

### Komparative analyser

Feltforsøk over en vekstsesong i henholdsvis Nord-Amerika og Europa viser små eller ingen signifikante forskjeller mellom den transgene maishybriden 1507 x 59122 og korresponderende, nær-isogene kontrollhybrider med hensyn på morfologiske og agronomiske karakterer. Resultatene indikerer agronomisk og fenotypisk ekvivalens mellom 1507 x 59122 og umodifisert kontroll, og at de innsatte genene i 1507 x 59122 ikke har medført utilsiktede endringer i egenskaper knyttet til vekst og utvikling hos maisplantene.

### Miljørisiko

Søknaden gjelder godkjenning av maishybrid 1507 x 59122 for import, prosessering og til bruk i næringsmidler og fôrvarer, og omfatter ikke dyrking. Med bakgrunn i tiltenkt bruksområde er miljørisikovurderingen avgrenset til mulige effekter av utilsiktet frøspredning i forbindelse med transport og prosessering, samt indirekte eksponering gjennom gjødsel fra husdyr fôret med genmodifisert mais.

Tilgjengelig dokumentasjon indikere ingen 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 foreskrevet bruk av maislinjen 1507 x 59122 antas det ikke å være risiko for utilsiktede effekter på målorganismer, ikke-målorganismer eller på abiotisk miljø i Norge.

### Samlet vurdering

VKMs faggruppe for genmodifiserte organismer finner at maishybriden 1507 x 59122, ut fra dagens kunnskap, er sammenlignbar med konvensjonell mais når det gjelder mulig miljørisiko ved den omsøkte bruken.



## Abbreviations and explanations

ALS	Acetolactate synthase, an enzyme that catalyses the first step in the synthesis of the branched-chain amino acids, valine, leucine, and isoleucine
AMPA	Aminomethylphosphonic acid, one of the primary degradation products of glyphosate
ARMG	Antibiotic resistance marker gene
BC	Backcross. Backcross breeding in maize is extensively used to move a single trait of interest (e.g. disease resistance gene) from a donor line into the genome of a preferred or “elite” line without losing any part of the preferred lines existing genome. The plant with the gene of interest is the donor parent, while the elite line is the recurrent parent. BC <sub>1</sub> , BC <sub>2</sub> 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	<i>Bacillus thuringiensis</i>
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 <i>Bacillus thuringiensis</i> . Activated by enzymes in the insects midgut, these proteins attack the cells lining the gut, and subsequently kill the insect
Cry1F	Cry1 class crystal protein from <i>Bacillus thuringiensis</i> var. <i>aizawai</i>
Cry34/35Ab1	Binary crystal protein containing of Cry34Ab1 and Cry35Ab1.
Cry34Ab1	Cry34 class crystal protein from <i>Bacillus thuringiensis</i> stamme 149B1.
Cry35Ab1	Cry35 class crystal protein from <i>Bacillus thuringiensis</i> stamme 149B1.
CTP	Chloroplast transit peptide
CTP	Chloroplast transit peptide
DAP	Days after planting
DN	Norwegian Directorate for Nature Management (Direktoratet for naturforvaltning)
DNA	Deoxyribonucleic acid
DT50	Time to 50% dissipation of a protein in soil
DT90	Time to 90% dissipation of a protein in soil
dw	Dry weight
dwt	Dry weight tissue
EC	European Commission/Community
ECB	European corn borer, <i>Ostrinia nubilalis</i>
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	Environmental risk assessment
E-score	Expectation score
EU	European Union
fa	Fatty acid
FAO	Food and Agriculture Organisation



FIFRA	US EPA Federal Insecticide, Fungicide and Rodenticide Act
Fitness	Describes an individual's ability to reproduce successfully relative to that of other members of its population
fw	Fresh weight
fwt	Fresh weight tissue
GAT	Glyphosate N-acetyltransferase
GLP	Good Laboratory Practices
Glufosinate-ammonium	Broad-spectrum systemic herbicide
Glyphosate	Broad-spectrum systemic herbicide
GM	Genetically modified
GMO	Genetically modified organism
GMP	Genetically modified plant
H	hybrid
ha	Hectare
ILSI	International Life Sciences Institute
IPM	Integrated Pest Management
IRM	Insect resistance management
Locus	The position that a given gene occupies on a chromosome
LOD	Limit of detection
LOQ	Limit of quantitation
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization-Time Of Flight. A mass spectrometry method used for detection and characterisation of biomolecules, such as proteins, peptides, oligosaccharides and oligonucleotides, with molecular masses between 400 and 350,000 Da
MCB	Mediterranean corn borer, <i>Sesamia nonagrioides</i>
mRNA	Messenger RNA
MT	Norwegian Food Safety Authority (Mattilsynet)
NDF	Neutral detergent fibre, measure of fibre used for animal feed analysis. NDF measures most of the structural components in plant cells (i.e. lignin, hemicellulose and cellulose), but not pectin
Northern blot	Northern blot is a technique used in molecular biology research to study gene expression by detection of RNA or isolated mRNA in a sample
NTO	Non-target organism
Nicosulfuron	Herbicide for maize that inhibits the activity of acetolactate synthase
Near-isogenic lines	Term used in genetics, defined as lines of genetic codes that are identical except for differences at a few specific locations or genetic loci
OECD	Organisation for Economic Co-operation and Development
ORF	Open Reading Frame, in molecular genetics defined as the part of a reading frame that contains no stop codons
OSL	Overseason leaf
OSR	Overseason root
OSWP	Overseason whole plant
<i>pat</i>	Phosphinothricin-Acetyl-Transferase gene
PAT	Phosphinothricin-Acetyl-Transferase protein
PCR	Polymerase chain reaction, a biochemical technology in molecular biology to amplify a single or a few copies of a piece of DNA
R0	Transformed parent
Rimsulfuron	Herbicide, inhibits acetolactate synthase
RNA	Ribonucleic acid
RP	Recurrent parent
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis. Technique to separate proteins according to their approximate size
SAS	Statistical Analysis System
SD	Standard deviation

Southern blot	Method used for detection of DNA sequences in DNA samples. Combines transfer of electrophoresis-separated DNA fragments to a filter membrane and subsequent fragment detection by probe hybridisation
T-DNA	Transfer DNA, the transferred DNA of the tumour-inducing (Ti) plasmid of some species of bacteria such as <i>Agrobacterium tumefaciens</i> and <i>A. rhizogenes</i> . The bacterium transfers this DNA fragment into the host plant's nuclear genome. The T-DNA is bordered by 25-base-pair repeats on each end. Transfer is initiated at the left border and terminated at the right border and requires the <i>vir</i> genes of the Ti plasmid.
TI	Trait integration
U.S. EPA	United States Environmental Protection Agency.
Maize growth stages:	<p><i>Vegetative</i></p> <p>VE: emergence from soil surface</p> <p>V1: collar of the first leaf is visible</p> <p>V2: collar of the second leaf is visible</p> <p>Vn: collar of the leaf number 'n' is visible</p> <p>VT: last branch of the tassel is completely visible</p> <p><i>Reproductive</i></p> <p>R0: Anthesis or male flowering. Pollen shed begins</p> <p>R1: Silks are visible</p> <p>R2: Blister stage, Kernels are filled with clear fluid and the embryo can be seen</p> <p>R3: Milk stage. Kernels are filled with a white, milky fluid.</p> <p>R4: Dough stage. Kernels are filled with a white paste</p> <p>R5: Dent stage. If the genotype is a dent type, the grains are dented</p> <p>R6: Physiological maturity</p> <p>Seedling growth (stages VE and V1); Vegetative growth (stages V2, V3... Vn); Flowering and fertilization (stages VT, R0, and R1); Grain filling and maturity (stages R2 to R6)</p>
Western blot	Analytical technique used to detect specific proteins in the given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native proteins by 3-D structure or denatured proteins by the length of the polypeptide. The proteins are then transferred to a membrane where they are stained with antibodies specific to the target protein.
WHO	World Health Organisation.
ZM	<i>Zea maize</i> L.
ZM-HRA	A modified version of the native acetolactate synthase protein from maize. Confers tolerance to the ALS-inhibiting class of herbicides

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## Background

On 30 May 2005, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands an application (Reference EFSA/GMO/NL/2005/15) for authorisation of the insect-resistant and herbicide tolerant genetically modified (GM) maize 1507 x 59122 (Unique Identifier DAS-Ø15Ø7-1 x DAS-59122-7), submitted by Dow AgroScience and Pioneer Hi-Bred International, Inc. within the framework of Regulation (EC) No 1829/2003.

The scope of the application covers:

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

After receiving the application EFSA/GMO/NL/2005/15 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the EU- and EFTA Member States (MS) and the European Commission and made the summary of the dossier publicly available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of regulation (EC) No 1829/2003. On 6 June 2007, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the EC and consulted nominated risk assessment bodies of the MS, including the Competent Authorities within the meaning of Directive 2001/18/EC (EC 2001), following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Within three months following the date of validity, all MS could submit via the EFSA GMO Extranet to EFSA comments or questions on the valid application under assessment. The VKM GMO Panel assessed the application in connection with the EFSA official hearing, and submitted a preliminary opinion in September 2007 (VKM 2007a). EFSA published its scientific opinion 21 April 2009 (EFSA 2009b), and maize 1507 x 59122 was approved for food and feed uses, import and processing in 28 July 2010 (Commission Decision 2010/432/EC).

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

Scientific opinions on the parental lines of the stack 1507 x 59122 have previously been submitted by the VKM GMO Panel (VKM 2004, 2005a, 2008b). In addition, maize 1507 and 59122 have been evaluated by the VKM GMO Panel as a component of several other stacked GM maize events under Directive 2001/18/EC and Regulation (EC) 1829/2003 (VKM 2005b, VKM 2007b,c, VKM 2008c, VKM 2009a,b, VKM 2012).

## Terms of reference

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

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

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

The risk assessments' primary geographical focus should be Norway, and the risk assessments should include the potential environmental risks of the product 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.

# Assessment

## 1 Introduction

Maize 1507 x 59122 has been obtained from traditional breeding methods between progeny (inbred lines) of the genetically modified maize lines 1507 and 59122.

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

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

None of the target pests for maize 1507 and maize 59122 are present in the Norwegian agriculture. The PAT protein expressed in maize 1507 and maize 59122 has been used as selectable markers to facilitate the selection process of transformed plant cells and is not intended for weed management purposes.

Maize stack 1507 x 59122 has been evaluated with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed.

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

The environmental risk assessment of the genetically modified maize 1507 x 59122 is based on information provided by the applicant in the applications EFSA/GMO/NL/2005/15 and EFSA/GMO/NL/2005/28, and scientific opinions and comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment is also based on a review and assessment of relevant peer-reviewed scientific literature.

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

## 2 Molecular characterisation

### 2.1 Evaluation of relevant scientific data

#### 2.1.1 Method of production of maize 1507 x 59122

Conventional breeding methods were used to develop the insect-resistant and herbicide-tolerant maize 1507 x 59122, and no genetic modification was involved. The two inserts present in maize 1507 x 59122 were derived from two independent events: 1507 and 59122, and combines resistance to certain lepidopteran and coleopteran pests, and tolerance to glufosinate-ammonium based herbicides. The genetically modified maize 1507 and 59122 have been subjects of earlier safety assessments from the Norwegian Scientific Committee (VKM 2004, VKM 2005a, VKM 2008b).

#### 2.1.2 Summary of evaluation of the single events

##### 2.1.2.1 Maize 1507

Maize 1507 have been developed to provide protection against certain lepidopteran target pests (such as the European corn borer, *Ostrinia nubilalis*, and species belonging to the genus *Sesamia*) by the introduction of a part of a *Bacillus thuringiensis* gene encoding the insecticidal Cry1F protein. The bacteria produce the intracellular crystal protein which has entomopathogenic effect. The base sequence of the *cry1F* gene is modified so it can successfully be expressed in plants, while the amino acid sequence of the translated Cry1F protein remains identical to the protein expressed by the bacteria. The expression of *cry1F* is regulated by the maize promoter *ubiZM1*. Termination of expression is controlled by the terminator *mas1* from *Agrobacterium tumefaciens*.

Maize 1507 also express the phosphinothricin-N-acetyltransferase (PAT) protein from *Streptomyces viridochromogenes*, which confers tolerance to the herbicidal active substance glufosinate-ammonium. PAT inactivates phosphinothricin through N-acetylation, thereby protecting the plant in a phosphinothricin containing environment. The PAT protein expressed in maize 1507 has been used as selectable marker to facilitate the selection process of transformed plant cells. The promoter CaMV 35S Pro guides the expression of *pat* while termination of expression is directed by CaMV 35S Term. The promoter (*Pro*) and terminator (*Term*) 35S are originated from the Cauliflower Mosaic Virus (CaMV).

No vector was used in the transformation of 1507 maize. The intended insert in 1507 maize consisted of a linear DNA fragment, containing the *cry1F* and *pat* coding sequences together with the necessary regulatory components. Transformation of 1507 resulted in the stable insertion of the T-DNA region from binary vector PHI8999. No additional DNA sequences were used in the introduction of the respective inserts into 1507 maize.

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

Western blot and detection with polyclonal antibodies showed that both the Cry1F and PAT proteins had the expected molecular weights. Cry1F exists as a doublet of 65 kb and 68 kb, respectively. This



is explained by plant proteases that cleave off an N-terminal fragment, since trypsin treatment of Cry1F also yields a protein of 65 kb. There are no indications of fusion proteins.

A detailed study was performed to detect open reading frames. Five ORFs were detected: ORF1, ORF2, ORF3, ORF4 and ORF25PolyA. ORF25PolyA is part of the CaMV 35S promoter and terminator. ORF4 lies within ORF25PolyA. ORF1 and 2 are parts of the 1507 transcript and originate from the maize genome. These ORFs were also detected in unmodified maize, but do not share homology to described sequences in the maize genome, and do not contain regulatory elements that can lead to transcription. ORF3 and ORF4 are located at the border of and inside the 1507 fragment, respectively. No transcripts of ORF3 are detected by Northern blot or RT-PCR. Neither do analyses of ORF4 with Northern blot and RT-PCR indicate that ORF4 is capable of transcription even though it resides within ORF25PolyA.

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

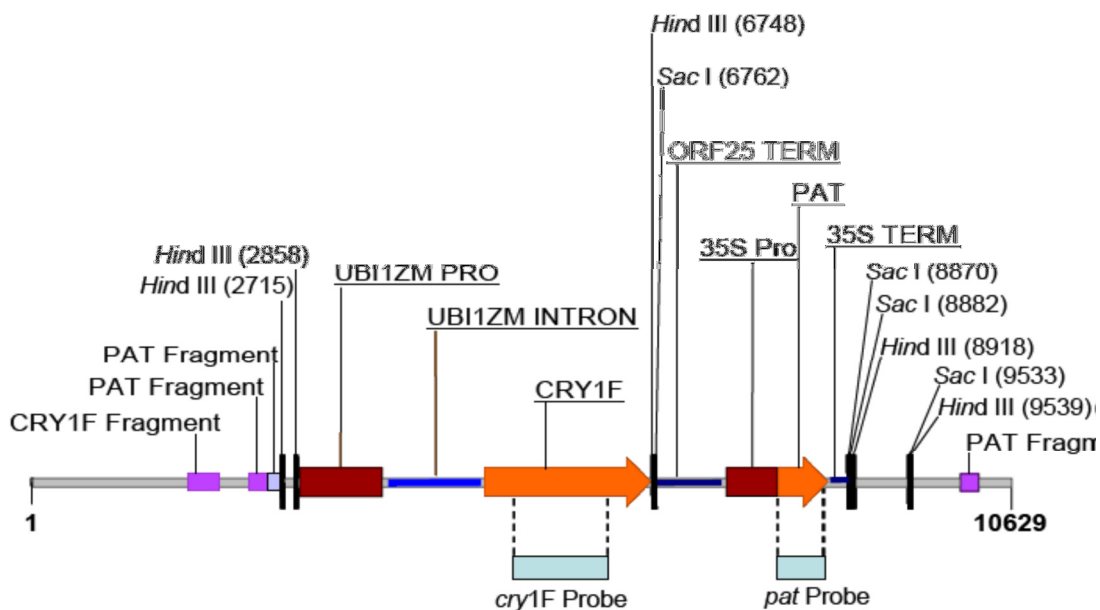


Figure 1. Restriction map of the various gene elements of the recombinant DNA fragment inserted in the genome of the maize strain 1507.

### 2.1.2.2 Maize 59122

The gene modified maize strain 59122 expresses herbicide and insect tolerance through *Agrobacterium tumefaciens* mediated transformation of maize cells, with the insertion of a linear DNA fragment of 7390 bp from the binary vector PHP17662 into the maize genome. The DNA fragment does not contain an antibiotic resistance gene. Transformation of 59122 maize resulted in the stable insertion of the T-DNA region into the maize genome. The T-DNA region in PHP17662 contained the *cry34Ab1*, *cry35Ab1* and *pat* coding sequences and the necessary regulatory components to regulate gene expression.

The maize-optimised *cry34Ab1* gene is derived from *Bacillus thuringiensis* strain PS149B1. *Cry34Ab1* encodes a protein comprising 123 amino acids. The amino acid sequence of the Cry34Ab1 protein (14 kDa) encoded by the maize-optimised *cry34Ab1* gene is identical to the Cry34Ab1 protein (14 kDa) expressed in the bacteria. Expression of the maize-optimised *cry34Ab1* gene is regulated by the ubiquitin promoter from *Zea mays* (*ubi1ZM*). Termination of transcription for the maize-optimised *cry34Ab1* gene is controlled by the terminator sequence from the *Solanum tuberosum* proteinase inhibitor II gene (*pinII*).

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

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

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

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

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

Further, the results of the molecular characterization support the conclusion that the 59122 maize contains a single intact copy of the T-DNA region from binary vector PHP17662. Southern blot and sequence analysis shows that nearly a full length copy of the PHP17662 recombinant DNA fragment (7343 bp out of the 7390 bp fragment) is inserted in the maize genome. The 59122 maize does not contain fragments from the vector backbone portion of binary vector PHP17662, in particular the tetracycline and spectinomycin resistance genes, the *virG* gene and other backbone sequences not intended for transformation. In addition, PCR amplification and sequence analysis have confirmed that the 5' and 3' regions flanking the 59122 maize insert are of maize genomic origin. A 22 bp are missing from the 5' end and 25 bp from the 3' end of the fragment. The fragment contains all genes (*pat*, *cry34Ab1* and *cry35Ab1*) and respective regulatory sequences of the insert. Two base modifications have also been identified in the non-coding region of the fragment, but none of these affect the ORFs of the fragment. A 2593 bp of the 5'-, and 1986 bp of the 3' - flanking sequences have also been sequenced, where small regions display homology to e.g. chromosomal sequences and various expressed sequence tags, ESTs. The longest region of these is 179 bp. None of the flanking sequences contain coding regions to known proteins. The contents of genes and regulatory elements in the recombinant DNA fragment are outlined in Figure 2.

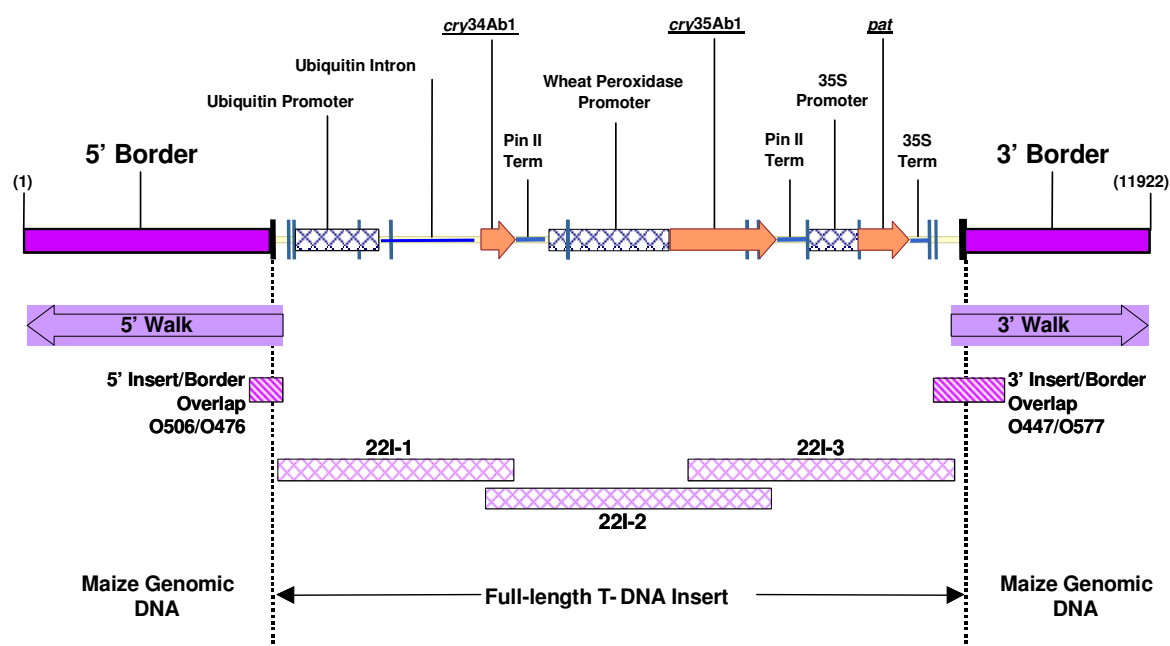


Figure 2. Restriction map of the various gene elements of the recombinant DNA fragment inserted in the genome of the maize strain 59122.

### 2.1.3 Transgene constructs in maize 1507 x 59122

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

In order to confirm the intactness and stability of the insert present in 1507 x 59122 maize compared to the insert in the individual parental events of 1507 maize and 59122 maize, a complete and detailed analysis was performed by Southern Blot. Using the *cry1F*, *cry34Ab1*, *cry35Ab1* and *pat* probes, southern blot hybridization showed intactness of the inserts, including their flanking sequences, present in 1507x59122 maize compared to the inserts in the 1507 and the 59122 maize. These Southern analyses with the inserted gene probes showed that the insertions in the 1507 maize and the 59122 maize were equivalent to that of 1507 x 59122 maize indicating that it was a successful cross of the two lines: the 1507 maize and the 59122 maize.

*Hind* III digestion was selected for comparing the 1507 x 59122 maize to 1507 maize. *Hind* III sites are indicated on the 1507 maize insertion map in Figure 1. Two bands were expected to hybridize to the *cry1F* probe based on the insertion map, a single band of 3890 bp and one greater than 2715 bp (Figure 1). Consistent with the insertion map, two fragments, one of 3890 bp and one of 4200 bp, were observed in all of the samples of the 1507 maize and the 1507x59122 maize. Therefore, the inserts in 1507 maize and 1507 x 59122 maize were equivalent to each other. Using the *pat* probe and *Hind* III digestion, three bands were expected to hybridize to the 1507 maize insert, a single band of 2170 bp, one of approximately greater than 2715 bp, and a third band of approximately greater than 1090 based on the 1507 maize insertion map (Figure 1). In addition, the T-DNA of PHP17662 was also expected to hybridize to the *pat* probe, resulting in an internal fragment of 6963 bp (Figure 1). Consistent with the event insertion map, three bands were observed in 1507 maize, one of 2170 bp, one of approximately 2300 bp and a faintly hybridizing band of approximately 4100 bp. A single band of 6963 bp was observed in the 59122 maize, as expected.

*Sac* I digestion was selected for comparing 1507x59122 maize to 59122 maize. *Sac* I sites are indicated on the T-DNA insertion in 59122 maize in Figure 2. Hybridization of the *cry34Ab1* probe with individual plants containing the DAS-59122-7 insertion was expected to result in a border fragment of approximately 3400 bp based on the T-DNA insertion map (Figure 2). This fragment was observed in both the 59122 maize and the 1507x59122 maize. The 59122 maize and the 1507x59122 maize exhibited the same hybridization pattern with the *cry34Ab1* probe, indicating that the insert present in the 59122 maize was equivalent to that found in the 1507x59122 maize. Using the *cry35Ab1* probe, three internal bands, one of 1855 bp, one of 1941 bp and one of 123 bp, were expected to hybridize in the *Sac* I digestion based on the T-DNA map derived from binary vector PHP17662 and consistent with the T-DNA insertion in 59122 maize. The 1855 bp and 1941 bp fragments were observed in both the 59122 maize and the 1507x59122 maize, indicating that the 1507x59122 maize contained the same insertion as the 59122 maize. The predicted 123 bp fragment was not detected, as fragments below approximately 1000 bp ran off the gel during electrophoresis and were not transferred to the nylon membrane.

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

None of the gene probes, *cry1F*, *pat*, *cry34Ab1*, or *cry35Ab1* hybridized to control samples analyzed in Southern analysis. As expected, *cry1F* did not hybridize to 59122 maize or PHP17662 plasmid control nor did *cry34Ab1* and *cry35Ab1* hybridize to 1507 maize or PHP8999 plasmid control.

These Southern analyses with the inserted gene probes showed that the insertions in 1507 maize and 59122 maize were equivalent to that of the 1507 x 59122 maize.

#### **2.1.4 Information on the expression of insert**

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

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

##### **Cry1F**

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

##### **Cry34Ab1**

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

ranging from 42.9 up to 45.7 ng/mg dry weight, results which are comparable to the levels of the Cry34Ab1 protein in the 59122 maize, which ranged from 39.6 up to 49.7 ng/mg dry weight.

### **Cry35Ab1**

In the European study, the Cry35Ab1 protein was detected, in forage at levels from 30.5 up to 58.0 ng/mg dry weight and in grain, from 0.29 up to 1.50 ng/mg dry weight, which are in the same order of magnitude as expression levels in 59122 maize, which ranged in forage from 41.3 up to 52.5 ng/mg dry weight (mean range across EU sites in 2003-2004) and in grain from 1.05 up to 1.11 ng/mg dry weight. In the U.S. and Canadian study, the Cry35Ab1 protein was detected (mean level) at levels from 1.41 up to 1.61 ng/mg dry weight, which are in the same order of magnitude as expression levels in 59122 maize, which ranged from 0.99 up to 2.00 ng/mg dry weight.

### **PAT**

In the European study, levels of combined expression, from 1507 maize and 59122 maize, of the PAT protein in 1507x59122 maize, ranged, in forage, from 1.87 up to 6.15 ng/mg dry weight and in grain from 0.00 up to 0.210 ng/mg dry weight which is consistent with the result of expression levels of PAT protein in 1507 maize and 59122 maize, which were generally below their limit of detection. In the USA and Canadian study, levels of combined mean expression of the PAT protein, from 1507 maize and 59122 maize, in 1507x59122 maize, ranged from N.D. up to 0.44 ng/mg dry weight which is consistent with the result of levels of PAT protein in 1507 maize and 59122 maize, which were generally below their limit of detection.



**Table 1. Levels of the Cry1F, Cty34Ab1, Cry35Ab1 and PAT proteins in grain and forage from 1507 x 59122 maize plants sprayed with glufosinate and unsprayed (EU 2004).**

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

<sup>1</sup>Plots treated with glufosinate-ammonium (GA)



**Table 2. Expression of Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in grain from 1507x59122 maize plants sprayed with glufosinate and unsprayed (USA and Canada 2003).**

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

<sup>1</sup> Plots treated with glufosinate-ammonium (GA)

#### *ORF sequence comparisons*

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

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

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

there are no potential fusion proteins in the 59122 maize that could be harmful to human or animal health.

### **2.1.5 Inheritance and stability of inserted DNA**

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

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

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

Genetic stability of the inserted gene construct was evaluated through Southern blot and segregation analysis of four different generations (T1S1, T1S2, BC1 and BC2S1). The breeding strain Hi-II with the 59122 event (T0) was crossbred with the inbred elite strain PH098B to make the F<sub>1</sub> generation. The F<sub>1</sub> plants were self-pollinated to generate the T1S and T1S2 generations. To produce the BC1-hybride the F1-plants were crossed and backcrossed with the inbred strain 05F, and then crossed with yet another inbred strain, 581. To produce the BC2S1 generation, F1 plants were crossed and backcrossed twice with the inbred strain 581, and finally self-pollinated. Analysis of the progeny from the BC2S1 generation displayed the expected Mendelian inheritance of herbicide tolerance and expression of Cry34Ab1. Analyses of Cry34Ab1/35Ab1 and PAT expression data from field studies spanning two growth seasons in Europe, North- and South- America indicate phenotypic stability.

## **2.2 Conclusion**

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

The characterisation of the recombinant insert and the physical, chemical and functional characteristics of the single maize events 1507 (VKM 2004) and 59122 (VKM 2005a, 2008b), have previously been evaluated by the VKM Panel on GMO and considered adequate.

## 3 Comparative assessment

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

#### 3.1.1 Experimental design & statistical analysis

##### **Application EFSA/GMO/NL/2005/15**

In the application EFSA/GMO/NL/2005/15 for food and feed uses, import and processing of maize 1507 x 59122 within the European Union, the applicant present compositional data from seed and forage material collected in field trials in the North America during the 2003 growth season. In addition, agronomic data derived from material obtained from field trials with the single events and the respective comparators were provided by the applicant.

The field trials in North America were performed at five separate sites in commercial maize-growing regions of the USA (Iowa, Indiana and Nebraska) and two field sites in Ontario, Canada. These trials compared the composition of maize 1507 x 59122 with a conventional counterpart having a genetic background representative of the test entry 1507 x 59122 (near-isogenic hybrid, Pioneer brand commercial hybrid 36B08). Upon request of the EFSA GMO Panel, the applicant provided additional information on the breeding scheme used to produce the conventional control maize. According to EFSA, the pedigree information on the control, non-GM maize showed that the control had a genetic background comparable with that of maize 1507 x 59122 and thus represented an appropriate comparator for the F<sub>1</sub> hybrid 1507 x 59122 in the field trials (EFSA 2009b).

No conventional commercial reference varieties were included in the field trials and the comparative assessments. However, comparisons with baseline data on commercial maize, compiled from publicly available literature, have been used in the comparisons with maize 1507 x 59122 for consideration of natural variations.

At each trial site, maize 1507 x 59122 and the conventional counterpart were planted following a randomized complete block design containing four blocks with test and control entries planted in 2-row plots located randomly within each block. Each plot was bordered by a single row of non-transgenic, commercial maize in order to limit edge effects. Prior to planting, each site prepared a proper seed bed according to local agronomic practices which could include tillage, fertility and pest managements practices. Each field location was scouted for agronomic and pest management needs including pest arthropods, diseases and weeds. Fertilizer, irrigation, agricultural chemicals and other management practices were applied as necessary. All maintenance operations were performed uniformly across the entire study area. Plots of the test entry 1507 x 59122 maize either received two sequential applications of herbicide containing glufosinate-ammonium or were unsprayed. The first application was applied at a rate that ranged from 0.36 to 0.38 lb ai/A (pounds of active ingredients per acre) at the V4 growth stage. The second application, at V7 growth stage, was applied at a rate ranging from 0.44 to 0.45 lb ai/A. The agronomic/phenotypic analyses were carried out from the same fields as the compositional analyses, but only from the control entry and plots with the test entry treated with glufosinate.

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

**Application EFSA/GMO/NL/2005/28**

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

**3.2 Agronomic and phenotypic characters**

During field trials over at six different locations in North America in the growth season 2003, phenotypic and agronomic data related to dormancy and germination, emergence and vegetative growth, reproductive growth, seed retention, and stress (i.e. disease and biotic stress responses) were collected. Both in the field trials in USA and Canada, the early population/germination, seeding vigour, number of accumulated heat units (GDU) when approximately 50 % of the plants are silking or shedding pollen, plant height, ear height, number of stalk and root lodged plants, final stand count, stay green, pollen shape, disease incidence and insect damage, were measured.

Analyses of variance across trial locations showed statistically significant differences between maize 1507 x 59122 (treated with glufosinate ammonium) and the corresponding conventional counterpart for early population, plant height and final population (number of viable plants remaining at maturity) ( $p < 0.05$ ) (Table 22). None of these differences were consistently observed over locations.

In 2004, corresponding agronomic and phenotypic characters were measured for maize stack 1507 x 59122 and the non-GM control maize in field trials at five locations in Europe. Analyses of variance across trial locations showed statistically significant differences between the transgenic maize 59122 x 1507 x NK603 (untreated) and the comparator for plant height and number of accumulated heat units to 50 % silking ( $p < 0.05$ ) (Table 23). On average 1507 x 59122 maize plants had a higher number of accumulated heat units before 50 % of the plants were silking (865 vs. 838 GDU) and was significant lower (235 vs. 236 cm) compared with the conventional counterpart. Significant differences for time to silking and plant height were observed at one and three of the five locations, respectively. No statistically significant differences between the transgenic maize 1507 x 59122 and the comparator were observed for the characteristics mean early population, final population, time to pollen shed, ear height, stalk lodging, root lodging, seedling vigour, stay green, disease incidence, insect damage and pollen viability values in the across location analysis ( $p > 0.05$ ). The VKM GMO Panel is of the opinion that the observed differences are not biologically relevant.

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

**Table 22. Mean agronomic data from maize stack 1507 x 59122, sprayed with glufosinate-ammonium and non-GM control with comparable genetic background from field trials at five locations in the USA and Canada in 2003.**

Entry	Maize 1507 x 59122	Non-GM control hybrid
Germination/early population <sup>1</sup>	54*	54
Seedling vigour (1-9)	7	7
GDU 50% silking <sup>2</sup>	1264	1240
GDU 50 % pollen shed <sup>3</sup>	1289	1276
Plant height (in)	98*	96
Ear height (in)	37	36
Stalk lodging (%)	0	1
Root lodging (%)	0	0
Final population <sup>4</sup>	52*	52
Stay green <sup>5</sup> (1-9)	4	3
Disease incidence	8	8
Insect damage	8	7
Pollen shape	86	83

\* p<0.05

<sup>1</sup> Number of plants emerged per 60 seed planted

<sup>2</sup> Number of accumulated heat units when approximately 50% of the plants are silking

<sup>3</sup> Number of accumulated heat units when approximately 50% of the plants are shedding pollen

<sup>4</sup> Total number of viable plants (per plot) remaining at maturity

<sup>5</sup> Overall plant height at maturity evaluated on a 1 to 9 scale when 1 is completely dead and 9 is very green

**Table 23. Mean agronomic data from maize stack 1507 x 59122, untreated, and non-GM control with comparable genetic background, collected from field trials at five locations in the EU in 2004.**

Entry	Maize 1507 x 59122	Non-GM control hybrid
Germination/early population <sup>1</sup>	52 (45-57)	52 (40-59)
Seedling vigour (1-9)	6	7
GDU 50% silking <sup>2</sup>	865*	838
GDU 50 % pollen shed <sup>3</sup>	841	823
Plant height (in)	235*	236
Ear height (in)	95.1	95.8
Stalk lodging (%)	0.13	2.7
Root lodging (%)	0.13	2
Final population <sup>4</sup>	51	50
Stay green <sup>5</sup> (1-9)	3	3
Disease incidence	6	6
Insect damage	7	6
Pollen shape	87	83
Pollen colour	71	74

\* p<0.05

<sup>1</sup> Number of plants emerged per 60 seed planted

<sup>2</sup> Number of accumulated heat units when approximately 50% of the plants are silking

<sup>3</sup> Number of accumulated heat units when approximately 50% of the plants are shedding pollen

<sup>4</sup> Total number of viable plants (per plot) remaining at maturity

<sup>5</sup> Overall plant height at maturity evaluated on a 1 to 9 scale when 1 is completely dead and 9 is very green

### 3.3 Conclusion

Comparative analyses of data from field trials located at representative sites and environments in the USA, Canada and Europe indicate that maize 1507 x 59122 is agronomically and phenotypically equivalent to the conventional counterpart, with the exception of the lepidopteran and coleopteran-protection traits and herbicide tolerance, conferred by the expression of the Cry1F, Cry34Ab1/Cry35Ab1 and PAT proteins. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of 1507 x 59122 compared to conventional maize.

The VKM GMO Panel has previously assessed these data and concluded that maize 1507 x 59122 is agronomically and phenotypically equivalent to the conventional comparators, except for the newly introduced traits (VKM 2007a, 2008a).

## 4 Environmental risk assessment

### 4.1 Potential unintended effects on plant fitness due to the genetic modification

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

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

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

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

Field trials carried out by the applicant do not indicate altered fitness of maize 1507 x 59122 relative to its conventional counterpart. A series of field trials with maize 1507 x 59122 were carried out across five locations in the USA and Canada in 2003 (application EFSA/GMO/NL/2005/15). In addition, agronomic observations performed in field trials in the EU in 2004 (Spain, Hungary and Bulgaria) have been provided by the applicant in application EFSA/GMO/NL/2005/28. Information on phenotypic (e.g. crop physiology, morphology, development) and agronomic characteristics was provided to assess the agronomic performance of maize 1507 x 59122 in comparison with its



conventional counterpart (see section 3.1). Data from the field trials in the USA shows some statistical significant differences at individual field sites, e.g. for plant height and early and final population count. These differences were however small in magnitude and were not consistently observed over locations. In the European field trials mean time to silking and plant height values across locations for the maize 1507 x 59122 and control maize were statistically different ( $p < 0.05$ ). The VKM GMO Panel is of the opinion that they do not raise any environmental safety concern.

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

## 4.2 Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or vertical gene flow via pollen or seed dispersal. Exposure of microorganisms to transgenic DNA occurs during decomposition of plant material remaining in the field after harvest or comes from pollen deposited on cultivated areas or the field margins. Transgenic DNA is also a component of a variety of food and feed products derived from maize 1507 x 59122. This means that micro-organisms in the digestive tract in humans and animals (both domesticated animals and other animals feeding on fresh or decaying plant material from the transgenic maize line) may be exposed to transgenic DNA.

Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation with which maize can hybridise and form backcross progeny (Eastham & Sweet 2002; OECD 2003). Vertical gene transfer in maize therefore depends on cross-pollination with other conventional or organic maize varieties. All maize varieties which are cultivated in Europe can interbreed. In addition, unintended admixture/adventitious presences of genetically modified material/transgenes in seeds represent a possible way for gene flow between different production systems.

### 4.2.1 Plant to micro-organisms gene transfer

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

Based on established scientific knowledge of the barriers for gene transfer between unrelated species and the experimental research on horizontal transfer of genetic material from plants to microorganisms, there is today little evidence pointing to a likelihood of random transfer of the transgenes present in maize 1507 x 59122 to unrelated species such as bacteria.

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

Experiments have been performed to study the stability and uptake of DNA from the intestinal tract in mice after M13 DNA was administered orally. The DNA introduced was detected in stool samples up

to seven hours after feeding. Small amounts (<0.1%) could be traced in the blood vessels for a period of maximum 24 hours, and M13 DNA was found in the liver and spleen for up to 24 hours (Schubbert et al. 1994). By oral intake of genetically modified soybean it has been shown that DNA is more stable in the intestine of persons with colostomy compared to a control group (Netherwood et al. 2004). No GM DNA was detected in the feces from the control group. Rizzi et al. (2012) provides an extensive review of the fate of feed-derived DNA in the gastrointestinal system of mammals.

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

#### 4.2.2 Plant to plant gene flow

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

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

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

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

As maize 1507 x 59122 has no altered survival, multiplication or dissemination characteristics, the VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this GM maize in Norway will not differ from that of conventional maize varieties. The likelihood of cross-pollination between cultivated maize and the occasional feral maize plants resulting from grain spillage is considered extremely low.

### 4.3 Potential interactions between the GM plant and target organisms

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

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

Maize 59122 expresses the *cry34Ab1* and *cry35Ab1* genes from *Bacillus thuringiensis*, conferring resistance to coleopteran insect pests belonging to the genus *Diabrotica*, such as larvae of western corn rootworm (WCR; *D. virgifera virgifera*) and the northern corn rootworm (NCR; *D. barberi*). WCR has been introduced to Europe from North America, where it is native and widespread (Miller et al. 2005, ref. EFSA 2013). *D. virgifera virgifera* was first detected in Serbia in 1992, but has since spread across the continent, resulting in well-established populations in approximately 19 European countries (EC 2012). There have been no reports of *D. virgifera virgifera* in Norway (<http://www.faunaeur.org/distribution.php>)

Considering the intended uses of maize 1507 x 59122, excluding cultivation, the environmental exposure is limited to exposure through manure and feces from the gastrointestinal tract mainly of animals fed on the GM maize as well as to the accidental release into the environment of GM seeds during transportation and processing and subsequently to potential occurrence of sporadic feral plants. Thus the level of exposure of target organisms to Cry1F, Cry34Ab and Cry35Ab1 proteins is likely to be extremely low and of no ecological relevance.

#### **4.4 Potential interactions between the GM plant and non-target organisms (NTOs)**

Considering the intended uses of maize stack 1507 x 59122, excluding cultivation, the environmental risk assessment is concerned with accidental release of GM maize viable grains into the environment during transportation and processing, and exposure through manure and faeces from the gastrointestinal tracts of animals fed the GM maize.

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

Data supplied by the applicant indicate that a limited amount of the Cry1F, Cry34Ab1 and Cry35Ab1 proteins enters the environment due to expression in the grains (mean value of 2.04, 45.7 and 1.61 µg/g d.w., respectively). In addition, the data show that at least 99% of microbially produced Cry1F and Cry34Ab1/Cry35Ab1 proteins were rapidly degraded in simulated gastric fluid.

In conclusion, the VKM GMO Panel considers that the exposure of potentially non-target organisms to the Cry1F and the binary Cry34Ab1 and Cry35Ab1 proteins is likely to be very low and of no biological relevance.

#### **4.5 Potential interactions with the abiotic environment and biochemical cycles**

Considering the intended uses of maize 1507 x 59122, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with the abiotic environment and biogeochemical cycles were not considered an issue by the VKM GMO Panel.

#### **4.6 Conclusion**

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

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

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

### **5 Post-market environmental monitoring**

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

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

relate observed changes to specific risks. It is triggered by scientific uncertainty that was identified in the ERA.

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

No specific environmental impact of genetically modified maize 1507 x 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 scope of the monitoring plan provided by the applicant is in line with the intended uses of maize 1507 x 59122 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects.

## **6 Data gaps**

No significant data gaps reported.

## 7 Conclusions

### Molecular characterisation

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

The characterisation of the recombinant insert and the physical, chemical and functional characteristics of the single events 1507 (VKM 2004) and maize 59122 (VKM 2005a, 2008b) have previously been evaluated by the VKM GMO Panel and considered adequate.

### Comparative assessment

Comparative analyses of data from field trials located at representative sites and environments in the USA, Canada and Europe indicate that maize 1507 x 59122 is agronomically and phenotypically equivalent to the conventional counterpart, with the exception of the lepidopteran and coleopteran-protection traits and herbicide tolerance, conferred by the expression of the Cry1F, Cry34Ab1/Cry35Ab1 and PAT proteins. The field evaluations support the applicant's conclusion of no other phenotypic changes indicative of increased plant weed/pest potential of 1507 x 59122 compared to conventional maize.

The VKM GMO Panel has previously assessed these data and concluded that maize 1507 x 59122 is agronomically and phenotypically equivalent to the conventional comparators, except for the newly introduced traits (VKM 2007a, 2008a).

### Environmental assessment

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

The available data indicate that 1507 x 59122 has no altered survival, multiplication or dissemination characteristics, and there are no indications of an increased likelihood of spread and establishment of feral maize plants in the case of accidental release into the environment of seeds from maize 1507 x 59122. Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation. The VKM GMO Panel considers the risk of gene flow from occasional feral GM maize plants to conventional maize varieties to be negligible in Norway. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered by the GMO Panel to be an issue.

### Overall conclusion

The VKM GMO Panel concludes that maize 1507 x 59122, based on current knowledge, is comparable to conventional maize varieties concerning environmental risk in Norway with the intended usage.



## References

- Andreadis S (2011) Origin and taxonomic status of the Palearctic population of the stem borer *Sesamia nonagriodes* (Lefèbvre) Lepidoptera: Noctuidae. *Biological Journal of the Linnean Society* 103: 904–922
- BEETLE report (2009). Long term effects of genetically modified (GM) crops on health and the environment (including biodiversity): prioritization of potential risks and delimitation of uncertainties. German Federal Office of Consumer Protection and Food Safety, BLaU-Umweltstudien and Genius GmbH.  
[http://ec.europa.eu/environment/biotechnology/pdf/beetle\\_report.pdf](http://ec.europa.eu/environment/biotechnology/pdf/beetle_report.pdf)
- Bensasson D, Boore JL, Nielsen KM (2004) Genes without frontiers. *Heredity* 92: 483-489
- de Vries J, Wackernagel W (2002) Integration of foreign DNA during natural transformation of *Acinetobacter* sp. by homology-facilitated illegitimate recombination. *The Proceedings of the National Academy of Sciences USA* 99: 2094-2099
- Devos Y, Demont M, Dillen K, Reheul D, Kaiser M, Sanvido O (2009) The coexistence of genetically modified (GM) and non-GM crops in the European Union. *Agronomy for Sustainable Development* 29: 11-30
- Eastham K, Sweet J (2002) Genetically modified organisms (GMO): The significance of gene flow through pollen transfer. Environmental issue report. No 28. European Environment Agency (EEA), Copenhagen.  
[http://reports.eea.eu.int/environmental\\_issue\\_report\\_2002\\_28/en](http://reports.eea.eu.int/environmental_issue_report_2002_28/en)
- EFSA (2004) Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. *The EFSA Journal*, 48, 1-18. [http://www.efsa.europa.eu/en/science/gmo/gmo\\_opinions/384.html](http://www.efsa.europa.eu/en/science/gmo/gmo_opinions/384.html).
- EFSA (2006) Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. ISBN: 92-9199-019-1. European Food Safety Authority, Parma, Italy. 100 p.  
[http://www.efsa.europa.eu/en/science/gmo/gmo\\_guidance/660.html](http://www.efsa.europa.eu/en/science/gmo/gmo_guidance/660.html)
- EFSA (2009a) Use of antibiotic resistance genes as marker genes in genetically modified plants. Scientific Opinion of the Panel on Genetically Modified Organisms (GMO) and the Panel on Biological Hazards (BIOHAZ). *The EFSA Journal* 1034: 1-82  
[http://www.efsa.europa.eu/cs/BlobServer/Statement/gmo\\_biohaz\\_st\\_ej1108\\_Consolidate\\_dARG\\_en.pdf?ssbinary=true](http://www.efsa.europa.eu/cs/BlobServer/Statement/gmo_biohaz_st_ej1108_Consolidate_dARG_en.pdf?ssbinary=true)
- EFSA (2009b) Application (Reference EFSA-GMO-NL-2005-15) for the placing on the market of the insect-resistant and herbicide-tolerant genetically modified maize 1507 x 59122, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Mycogen Seeds, c/o Dow AgroSciences LLC and Pioneer Hi-Bred International, Inc. as represented by Pioneer Overseas Corporation. *The EFSA Journal* 1074: 1-28  
<http://www.efsa.europa.eu/en/efsajournal/pub/1074.htm>
- EFSA (2010) Guidance on the environmental risk assessment of genetically modified plants. Scientific opinion from the EFSA Panel on Genetically Modified Organisms (GMO). *The EFSA Journal* 8 (11):1-111  
<http://www.efsa.europa.eu/en/efsajournal/doc/1879.pdf>

- EFSA (2011a) Guidance for risk assessment of food and feed from genetically modified plants. The EFSA Journal 9(5): 2150. <http://www.efsa.europa.eu/en/efsajournal/doc/2150.pdf>
- EFSA (2011b) EFSA Panel on Genetically Modified Organisms (GMO). Scientific Opinion on Guidance on selection of comparators for the risk assessment of genetically modified plants and derived food and feed. EFSA Journal 9(5):2149
- EFSA (2011c) Guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. The EFSA Journal 9(8):2316
- EFSA (2013) Scientific Opinion on an application from Pioneer Hi-Bred International and Dow AgroSciences LLC (EFSA-GMO-NL-2005-23) for placing on the market of genetically modified maize 59122 for food and feed uses, import, processing and cultivation under Regulation (EC) No 1829/20031. The EFSA Journal 11: 3135  
<http://www.efsa.europa.eu/en/search/doc/3135.pdf>
- Gruber S, Colbach N, Barbottin A, Pekrun C (2008) Post-harvest gene escape and approaches for minimizing it. *Cab reviews: Perspective in Agriculture, Veterinary Science, Nutrition and Natural Resources*, 3, No. 015, 17 pp.
- Guertler P, Lutz B, Kuehn R, Meyer HHD, Einspanier R, Killermann B, Albrecht C (2008) Fate of recombinant DNA and Cry1Ab protein after ingestion and dispersal of genetically modified maize in comparison to rapeseed by fallow deer (*Dama dama*). *European Journal of Wildlife Research* 54: 36–43
- Icoz, Stotzky G (2008) Fate and effects of insect-resistant Bt crops in soil ecosystems. *Soil Biology and Biochemistry* 40: 559–586
- Lid J, Lid DT (2005) *Norsk flora*. Det Norske Samlaget, Oslo. 7. utgave. 1230s
- Lutz B, Wiedermann S, Einspanier R, Mayer J, Albrecht C (2005) Degradation of Cry1Ab protein from genetically modified maize in the bovine gastrointestinal tract. *Journal of Agricultural and Food Chemistry* 53: 1453–1456
- Netherwood T, Martín-Orúe SM, O'Donnell AG, Gockling S, Graham J, Mathers JC, Gilbert HJ (2004) Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. *Nature Biotechnology* 22: 204-209
- Nielsen KM, van Elsas JD, Smalla K. (2000) Transformation of *Acinetobacter* sp. 13(pFG4delta*nptII*) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. *Applied Environmental Microbiology* 66: 1237-42
- Nielsen KM (2003) An assessment of factors affecting the likelihood of horizontal transfer of recombinant plant DNA to bacterial recipients in the soil and rhizosphere. *Collection of Biosafety Reviews* 1: 96-149
- Nielsen KM, Townsend J P (2004) Monitoring and modeling horizontal gene transfer. *Nature Biotechnology* 22(9):1110-1114
- OECD (2003) Consensus Document on the biology of *Zea mays* subsp. *Mays* (Maize). Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO, No. 27, 1-9
- Palauelmás M, Peñas G, Melé E, Serra J, Salvia J, Pla, M-, Nadal A, Messeguer J (2009) Effect of volunteers on maize gene flow. *Transgenic Research* 18: 583-594

- Paul V, Guertler P, Wiedemann S, Meyer HH (2010) Degradation of Cry1Ab protein from genetically modified maize (MON810) in relation to total dietary feed proteins in dairy cow digestion. *Transgenic Research* 19: 863-689
- Rizzi AN, Raddadi C, Sorlini L, Nordgård L, Nielsen KM, Daffonchio D (2012) The stability and degradation of dietary DNA in the gastrointestinal tract of mammals - implications for horizontal gene transfer and the biosafety of GMOs. *Crit. Rev. Food Science Nutr.* 52:142-161
- Schubbert GW, Lettmann C, Doerfler W (1994) Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. *Molecular & general Genetics* 242: 495-504
- van de Wiel CCM, van den Brink L, Bus CB, Riemens MM, Lotz LAP (2011) Crop volunteers and climatic change. Effects of future climatic change on the occurrence of maize, sugar beet and potato in the Netherlands. COGEM report (CGM 2011-11)
- VKM (2004) Foreløpig helserisikovurdering av genmodifisert, insektresistent og herbicidtolerant mais 1507 (C/ES/01/01) til bruk som fôrvare. Uttalelse fra Faggruppe for genmodifiserte organismer 10.5. 2004 (07/316-endelig). Vitenskapskomiteen for mattrygghet, Oslo, Norge.
- VKM (2005a) Foreløpig helserisikovurdering av genmodifisert maislinje 59122 fra Pioneer Hi-Bred/Mycogen Seeds. Uttalelse fra Faggruppe for genmodifiserte organismer 10.12. 2005 (05/324-endelig). Vitenskapskomiteen for mattrygghet, Oslo, Norge.  
[http://www.vkm.no/eway/default.aspx?pid=277&trg=Main\\_6177&Main\\_6177=6187:1670842::0:6735:3:::0:0](http://www.vkm.no/eway/default.aspx?pid=277&trg=Main_6177&Main_6177=6187:1670842::0:6735:3:::0:0)
- VKM (2005b) Foreløpig helserisikovurdering av genmodifisert maishybrid 1507 x NK603 (EFSA/GMO/UK/2004/05). Uttalelse fra Faggruppe for genmodifiserte organismer 3.6. 2005 (05/312-endelig). Vitenskapskomiteen for mattrygghet, Oslo, Norge  
[http://vkm.no/eway/default.aspx?pid=277&trg=Content\\_6504&Main\\_6177=6504:0:31,2365&6563=6566:14&Content\\_6504=6187:1663891::0:6566:95:::0:0](http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&6563=6566:14&Content_6504=6187:1663891::0:6566:95:::0:0)
- VKM (2005c) Report from an *Ad Hoc* Group appointed by the Norwegian Scientific Panel on Genetically Modified Organisms and Panel on Biological Hazards – An assessment on potentially long-term health effects caused by antibiotic resistance marker genes in genetically modified organisms based on antibiotic usage and resistance patterns in Norway. Opinion 05/302-1-final. Norwegian Scientific Committee for Food Safety, Oslo, Norway. 62 p.  
[http://www.vkm.no/eway/default.aspx?pid=277&trg=Content\\_6500&Main\\_6177=6500:0:31,2296&Content\\_6500=6187:1685412::0:6295:14:::0:0](http://www.vkm.no/eway/default.aspx?pid=277&trg=Content_6500&Main_6177=6500:0:31,2296&Content_6500=6187:1685412::0:6295:14:::0:0)
- VKM (2007a) Foreløpig helserisikovurdering av genmodifiserte mais 1507 x 59122 (EFSA/GMO/NL/2005/15) fra Pioneer Hi-Bred/Mycogen Seeds. Uttalelse fra Faggruppe for genmodifiserte organismer 22.9.2007 (07/322-endelig). Vitenskapskomiteen for mattrygghet, Oslo, Norge.  
[http://vkm.no/eway/default.aspx?pid=277&trg=Main\\_6177&Main\\_6177=6187:1670907::0:6735:4:::0:0](http://vkm.no/eway/default.aspx?pid=277&trg=Main_6177&Main_6177=6187:1670907::0:6735:4:::0:0)
- VKM (2007b) Foreløpig helserisikovurdering av genmodifisert åkermais 59122 x 1507 x NK603 (EFSA/GMO/UK/2005/21) fra Pioneer Hi-Bred/Mycogen Seeds. Uttalelse fra Faggruppe for genmodifiserte organismer 22.9.2007 (07/314-endelig). Vitenskapskomiteen for mattrygghet, Oslo, Norge.

[http://vkm.no/eway/default.aspx?pid=277&trg=Main\\_6177&Main\\_6177=6187:1687145::0:6735:6::0:0](http://vkm.no/eway/default.aspx?pid=277&trg=Main_6177&Main_6177=6187:1687145::0:6735:6::0:0)

VKM (2007c) Foreløpig helserisikovurdering av genmodifisert åkermais 59122 x NK603 (EFSA/GMO/UK/2005/20) fra Pioneer Hi-Bred/Mycogen Seeds. Uttalelse fra Faggruppe for genmodifiserte organismer 22.9.2007 (07/314-endelig). Vitenskapskomiteen for mattrygghet, Oslo, Norge.

[http://vkm.no/eway/default.aspx?pid=277&trg=Main\\_6177&Main\\_6177=6187:1687138::0:6735:5::0:0](http://vkm.no/eway/default.aspx?pid=277&trg=Main_6177&Main_6177=6187:1687138::0:6735:5::0:0)

VKM (2008a) Foreløpig miljørisikovurdering av genmodifisert mais 1507 x 59122 (EFSA/GMO/NL/2005/28). Uttalelse fra Faggruppe for genmodifiserte organismer 9.5.2008 (07/316-endelig). Vitenskapskomiteen for mattrygghet, Oslo, Norge.

[http://vkm.no/eway/default.aspx?pid=277&trg=Main\\_6177&Main\\_6177=6187:1670937::0:6735:12::0:0](http://vkm.no/eway/default.aspx?pid=277&trg=Main_6177&Main_6177=6187:1670937::0:6735:12::0:0)

VKM (2008b) Endelig helse- og miljørisikovurdering av genmodifisert mais 59122 (EFSA/GMO/NL/2005/12). Uttalelse fra Faggruppe for genmodifiserte organismer 19.9.2008 (08/311). Vitenskapskomiteen for mattrygghet, Oslo, Norge.

[http://vkm.no/eway/default.aspx?pid=277&trg=Content\\_6504&Main\\_6177=6504:0:31,2365&6563=6566:7&Content\\_6504=6187:1664124::0:6566:46::0:0](http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&6563=6566:7&Content_6504=6187:1664124::0:6566:46::0:0)

VKM (2008c) Endelig helse- og miljørisikovurdering av genmodifisert maishybrid 1507 x NK603 (EFSA/GMO/UK/2004/05). Uttalelse fra Faggruppe for genmodifiserte organismer 30.5.2008 (08/312). Vitenskapskomiteen for mattrygghet, Oslo, Norge.

[http://vkm.no/eway/default.aspx?pid=277&trg=Content\\_6504&Main\\_6177=6504:0:31,2365&6563=6566:9&Content\\_6504=6187:1664118::0:6566:57::0:0](http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&6563=6566:9&Content_6504=6187:1664118::0:6566:57::0:0)

VKM (2009a) Foreløpig helse- og miljørisikovurdering av genmodifisert mais MON 89034 x 1507 x MON 88017 x 59122 (EFSA/GMO/CZ/2008/62). Uttalelse fra Faggruppe for genmodifiserte organismer 25.9.2009 (09/307). Vitenskapskomiteen for mattrygghet, Oslo, Norge.

[http://www.vkm.no/eway/default.aspx?pid=277&trg=Content\\_6504&Main\\_6177=6504:0:31,2365&6563=6566:3&Content\\_6504=6187:1685656::0:6566:21::0:0](http://www.vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&6563=6566:3&Content_6504=6187:1685656::0:6566:21::0:0)

VKM (2009b) Foreløpig helse- og miljørisikovurdering av genmodifisert mais MON 89034 x 1507 x NK603 (EFSA/GMO/NL/2009/65). Uttalelse fra Faggruppe for genmodifiserte organismer 22.10.2009 (09/312). Vitenskapskomiteen for mattrygghet, Oslo, Norge.

[http://vkm.no/eway/default.aspx?pid=277&trg=Main\\_6177&Main\\_6177=6187:1694580::0:6720:2::0:0](http://vkm.no/eway/default.aspx?pid=277&trg=Main_6177&Main_6177=6187:1694580::0:6720:2::0:0)

VKM (2012) Foreløpig helse- og miljørisikovurdering av genmodifisert mais 1507 x 59122 x NK603 x MON810 x NK603 (EFSA/GMO/NL/2009/65). Uttalelse fra Faggruppe for genmodifiserte organismer 13.6.2012 (12/307). Vitenskapskomiteen for mattrygghet, Oslo, Norge.

[http://vkm.no/eway/default.aspx?pid=277&trg=Main\\_6177&Main\\_6177=6187:1915961::0:6720:9::0:0](http://vkm.no/eway/default.aspx?pid=277&trg=Main_6177&Main_6177=6187:1915961::0:6720:9::0:0)