Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Food/feed and environmental risk assessment of insect-resistant and herbicide-tolerant genetically modified maize Bt11 x MIR604 in the European Union under Regulation (EC) No 1829/2003 (EFSA/GMO/UK/2007/50)

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

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EFSA/GMO/UK/2007/50 – Genetically modified maize Bt11 x MIR604

Contributors

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

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Summary

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Environment Agency (former Norwegian Directorate for Nature Management) has requested the Norwegian Food Safety Authority (NFSA) to give final opinions on all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are authorised in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC within the Authority's sectoral responsibility. The Norwegian Food Safety Authority has therefore, by letter dated 13 February 2013 (ref. 2012/150202), requested the Norwegian Scientific Committee for Food Safety (VKM) to carry out scientific risk assessments of 39 GMOs and products containing or consisting of GMOs that are authorised in the European Union. The request covers scope(s) relevant to the Gene Technology Act. The request does not cover GMOs that VKM already has conducted its final risk assessments on. However, the Agency requests VKM to consider whether updates or other changes to earlier submitted assessments are necessary.

The insect-resistant and herbicide-tolerant genetically modified maize Bt11 x MIR604 (Unique Identifier SYN-BTØ11-1 x SYN-IR6Ø4-5) from Syngenta Seeds is approved under Regulation (EC) No 1829/2003 for food and feed uses, import and processing since 21 December 2011 (Commission Decision 2011/893/EC).

The genetically modified maize Bt11 x MIR604 has previously been risk assessed by the VKM Panel on Genetically Modified Organisms (GMO), commissioned by the Norwegian Food Safety Authority and the Norwegian Environment Agency related to the EFSAs public hearing of the application EFSA/GMO/UK/2007/50 in 2008 (VKM 2009a). In addition, Bt11 and MIR604 have been evaluated by the VKM GMO Panel as single events and as components of several stacked GM maize events (VKM 2005a,b, 2007, 2008, 2009b,c,d,e, 2012a,b, 2013a,b,c).

The food/feed and environmental risk assessment of the maize Bt11 x MIR604 is based on information provided by the applicant in the application EFSA/GMO/UK/2007/50, 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 Bt11 x MIR604 with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed. The Norwegian Scientific Committee for Food Safety has also decided to take account of the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2011a), the environmental risk assessment of GM plants (EFSA 2010), selection of comparators for the risk assessment of GM plants (EFSA 2011b) and for the post-market environmental monitoring of GM plants (EFSA 2011c).

The scientific risk assessment of maize Bt11 x MIR604 include molecular characterisation of the inserted DNA and expression of novel proteins, comparative assessment of agronomic and phenotypic characteristics, nutritional assessments, toxicology and allergenicity, unintended effects on plant fitness, potential for gene transfer, interactions between the GM plant and target and non-target organisms and effects on biogeochemical processes.

It is emphasised that the VKM mandate does not include assessments of contribution to sustainable development, societal utility and ethical considerations, according to the Norwegian Gene Technology Act and Regulations relating to impact assessment pursuant to the Gene Technology Act. These

considerations are therefore not part of the risk assessment provided by the VKM Panel on Genetically Modified Organisms.

The genetically modified maize stack Bt11 x MIR604 has been produced by conventional crossing between GM inbred lines of maize containing the single events Bt11 and MIR604. The maize hybrid was developed to provide protection against certain lepidopteran and coleopteran target pests, and to confer tolerance to glufosinate-ammonium based herbicides.

Molecular characterisation

Conventional crossing methods were used to produce the stacked maize Bt11 x MIR604. Southern blot analyses have indicated that the recombinant inserts in the parental maize lines Bt11 and MIR604 are retained in the stacked maize Bt11 x MIR604. Genetic stability of the inserts has previously been demonstrated in the parental events. Protein measurements show comparable levels of the Cry1Ab, mCry3A, PAT and PMI proteins between the stacked and single maize lines.

The VKM GMO Panel considers the molecular characterisation of maize Bt11 x MIR604 and the single maize events Bt11 and MIR604 as adequate.

Comparative assessment

Comparative analyses of agronomic and phenotypic data from field trials located at representative sites and environments in USA in 2005 indicate that maize stack Bt11 x MIR604 is equivalent to its conventional counterpart, with the exception of the insect resistance and the herbicide tolerance, conferred by the Cry1Ab, mCry3A and PAT proteins. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of maize Bt11 x MIR604 compared to conventional maize varieties.

The applicant has performed a compositional analysis on the triple-stack Bt11 x MIR604 x GA21 instead of maize Bt11 x MIR604. The analysis was performed on plant materials from maize Bt11 x MIR604 x GA21 and a near-isogenic control hybrid from field trials in USA in 2006. With the exception of small intermittent variations, no biologically significant compositional differences were found between the triple-stack and the near-isogenic control. The results of the study are considered valid by EFSA also for maize Bt11 x MIR604, since maize Bt11 x MIR604 x GA21 encompasses the transgenic properties of maize Bt11 x MIR604. This is in accordance with the EFSA guidance document for the risk assessment of genetically modified plants containing stacked transformation events (EFSA, 2007b).

The VKM GMO Panel is of the opinion that the applicant should have performed a compositional analysis of maize Bt11 x MIR604 and not only referred to analyses of the triple- stack Bt11 x MIR604 x GA21. However, based on all information available, including agronomic and phenotypic data from field trials with maize Bt11 x MIR604, a feeding study on broilers showing nutritional equivalence to non-GM maize, and assessments of the single events Bt11 and MIR604, the VKM GMO Panel concludes that forage and grain from maize Bt11 x MIR604 are compositionally equivalent to its conventional counterpart.

Food and feed risk assessment

A whole food feeding study on broilers has not indicated any adverse effects of maize Bt11 x MIR604, and shows that maize Bt11 x MIR604 is nutritionally equivalent to conventional maize. The Cry1Ab, PAT, mCry3A, and PMI proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE-mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize Bt11 x MIR604 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1Ab, PAT, mCry3A, or PMI proteins will introduce a toxic or allergenic potential in food or feed based on maize Bt11 x MIR604 compared to conventional maize.

Environmental risk assessment

The scope of the application EFSA/GMO/UK/2007/50 includes import and processing of maize stack Bt11x MIR604 for food and feed uses. Considering the intended uses of maize Bt11 x MIR604, excluding cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize Bt11 x MIR604.

Maize Bt11 x MIR604 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 Bt11 x MIR604. 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

Based on current knowledge, the VKM GMO Panel concludes that maize Bt11 x MIR604 is nutritionally equivalent to its conventional counterpart, except for the presence of the newly expressed proteins. It is unlikely that the Cry1Ab, PAT, mCry3A, or PMI proteins will introduce a toxic or allergenic potential in food or feed derived from maize Bt11 x MIR604 compared to conventional maize.

The VKM GMO Panel likewise concludes that maize Bt11 x MIR604, 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 Bt11 x MIR604, EFSA/GMO/UK/2007/50, insect-resistance, herbicide-tolerance, Cry proteins, *cry1Ab, mcry3Ab, pat, pmi,* glufosinate-ammonium, food and feed risk assessment, environmental risk assessment, Regulation (EC) No 1829/2003

Norsk sammendrag

I forbindelse med forberedelse til implementering av EU-forordning 1829/2003 i norsk rett har Miljødirektoratet (tidligere Direktoratet for Naturforvalting) bedt Mattilsynet om vurderinger av alle genmodifiserte organismer (GMOer) og avledete produkter som inneholder eller består av GMOer som er godkjent under forordning 1829/2003 eller direktiv 2001/18, og som er godkjent for ett eller flere bruksområder som omfattes av genteknologiloven. På den bakgrunnen har Mattilsynet, i brev av 13. februar 2013 (ref. 2012/150202), bedt Vitenskapskomiteen for mattrygghet (VKM) om å utarbeide endelige vitenskapelige risikovurderinger av 39 GMOer og avledete produkter som inneholder eller består av genmodifiserte organismer, innen Mattilsynets sektoransvar. VKM er bedt om endelige risikovurderinger for de EU-godkjente søknader hvor VKM ikke har avgitt endelig risikovurdering. I tillegg er VKM bedt om å vurdere hvorvidt det er nødvendig med oppdatering eller annen endring av de endelige risikovurderingene som VKM tidligere har levert.

Den genmodifiserte maishybriden Bt11 x MIR604 (Unik kode SYNBTØ11-1 x SYN-IR6Ø4-5) fra Syngenta Seeds Inc. ble godkjent til import, videreforedling og bruk som mat og fôr under EU-forordning 1829/2003 i 21. desember 2010 (søknad EFSA/GMO/UK/2007/50).

Maishybrid Bt11 x MIR604 er tidligere vurdert av VKMs faggruppe for genmodifiserte organismer med hensyn på mulig helse- og miljørisiko i forbindelse med EFSAs offentlige høring av søknaden i 2008 (VKM 2009a). Foreldrelinjene Bt11 og MIR604 er også tidligere risikovurdert av VKM, både som enkelteventer og i en rekke andre hybrider (VKM 2005a,b, 2007, 2008, 2009b,c,d,e, 2012a,b, 2013a,b,c).

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

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

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

F_1-hybriden Bt11 x MIR604 er resultat av konvensjonelle krysninger mellom innavlede maislinjer med eventene Bt11 og MIR604. Krysningene er utført for å utvikle en maishybrid med resistens mot visse skadegjørere i sommerfuglordenen Lepidoptera og billeslekten *Diabroticia,* samt toleranse mot herbicider med virkestoff glufosinat-ammonium.

Foreldrelinjen Bt11 inneholder de bakterielle genene *cry1Ab* og *pat*, fra henholdsvis *Bacillus thuringiensis* subsp. *kurstaki* og *Streptomyces viridochromogenes* strain Tu494. *Cry1Ab*-genet koder for et δ-endotoksin, som gir plantene toleranse mot enkelte arter i ordenen Lepidoptera. *Pat*-genet koder for enzymet phosphinothricin acetyl transferase (PAT), som acetylerer og inaktiverer glufosinat-

ammonium, virkestoffet i fosfinotricin-herbicider av typen Finale. Fosfinotricin er et ikke-selektivt kontaktherbicid som hemmer glutaminsyntetase. Enzymet deltar i assimilasjonen av nitrogen og katalyserer omdanning av glutamat og ammonium til aminosyren glutamin. Hemming av glutaminsyntetase fører til akkumulasjon av ammoniakk, og til celledød i planten. Bt11-plantene vil derfor tolerere høyere doser av sprøytemiddelet glufosinat sammenlignet med konkurrerende ugras.

Foreldrelinjen MIR604 har fått innsatt et modifisert *cry3A*-gen (*mcry3A*) fra *Bacillius thuringiensis* subsp. *tenebrionis* og genet *pmi* fra *E. coli*. *mCry3A* genet uttrykker δ-endotoksinet mCry3A, som gir plantene toleranse mot angrep fra bladbiller i slekten *Diabrotica*. *Pmi*-genet uttrykker enzymet fosfomannose isomerase, som gir toleranse overfor sukkerarten mannose.

Molekylær karakterisering
Maishybriden Bt11 x MIR604 er dannet ved konvensjonelle krysninger mellom maislinjene Bt11 og MIR604. Spaltingsdata, Southern blot og PCR-analyser indikerer at de rekombinante innskuddene fra mais Bt11 og MIR604 er stabilt nedarvet i mais Bt11 x MIR604, og at antall innsatte gener, struktur og organiseringen av disse er ekvivalent med de som finnes i mais Bt11 og MIR604. Nivåene av Cry1Ab, mCry3A, PAT og PMI -proteiner i vegetativt vev og korn fra mais Bt11 x MIR604 er også sammenlignbare med nivåene i henholdsvis mais Bt11 og MIR604. VKMs faggruppe for GMO anser den molekylære karakteriseringen av mais Bt11 x MIR604 som adekvat.

Komparative analyser
Data fra feltforsøk i Nord-Amerika vekstsesongen 2005 indikerer, med unntak av insektsresistens og herbicidtoleranse, agronomisk og fenotypisk ekvivalens mellom maishybriden Bt11 x MIR604 og korresponderende nær-isogen kontrollhybrid. Feltforsøkene understøtter konklusjonen om uendret sannsynlighet for spredning, etablering og invasjon av mais Bt11 x MIR604 sammenliknet med konvensjonelle maissorter.

Søker har utført en ernæringsmessig komponentanalyse av trippel-maishybriden Bt11 x MIR604 x GA21 istedenfor mais Bt11 x MIR604. Analysen ble utført på plantemateriale fra mais Bt11 x MIR604 x GA21 og korresponderende nær-isogen kontroll, fra feltforsøk i Nord-Amerika i 2006. Med unntak av små tilfeldige avvik ble det ikke avdekket forskjeller av biologisk betydning mellom mais Bt11 x MIR604 x GA21 og kontrollen. Ettersom de genetiske modifiseringene i Bt11 x MIR604 er representert i Bt11 x MIR604 x GA21, anser EFSA resultatene som gyldige også for mais Bt11 x MIR604. Dette er i tråd med EFSAs veiledende dokument for risikovurdering av genmodifiserte planter som inneholder stablete genmodifiserte egenskaper (EFSA 2007b). VKMs faggruppe for GMO mener søker heller burde ha utført en ernæringsmessig analyse av mais Bt11 x MIR604 og ikke bare referert til analysene av trippel-maisen. Basert på tilgjengelig informasjon, inkludert feltforsøkene vedrørende agronomiske og fenotypiske egenskaper, fôringsforsøk med broilere, og tidligere vurderinger av maislinjene Bt11 og MIR604, konkluderer VKMs faggruppe for GMO at mais Bt11 x MIR604 er ernæringsmessig ekvivalent dens konvensjonelle motpart.

Helserisiko
I en fôringsstudie utført på broilere ble det vist at mais Bt11 x MIR604 ikke førte til negative helseeffekter blant dyrene, og at maisen var ernæringsmessig ekvivalent konvensjonell mais. De introduserte proteinene Cry1Ab, PAT, mCry3A, og PMI viser ingen sekvenslikhet til kjente toksiner eller IgE-allergener. Det er heller ikke dokumentert at noen av disse proteinene kan utløse IgE-medierte allergiske reaksjoner. Enkelte studier har derimot indikert at noen typer Cry-proteiner potensielt kan forsterke andre allergiske reaksjoner (virke som adjuvans).

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at mais Bt11 x MIR604 er ernæringsmessig ekvivalent konvensjonell mais. Det er lite sannsynlig at proteinene Cry1Ab, PAT, mCry3A, eller PMI vil introdusere et toksisk eller allergent potensiale i mat eller fôr basert på mais Bt11 x MIR604 sammenliknet med konvensjonelle maissorter.

Miljørisiko
Søknaden EFSA/GMO/UK/2007/50 gjelder godkjenning av maishybrid Bt11 x MIR604 for import, prosessering og til bruk i næringsmidler og fôrvarer, og omfatter ikke dyrking. Med bakgrunn i tiltenkt bruksområde er miljørisikovurderingen avgrenset til mulige effekter av utilsiktet frøspredning i forbindelse med transport og prosessering, samt indirekte eksponering gjennom gjødsel fra husdyr fôret med genmodifisert mais.

Det er ingen indikasjoner på økt sannsynlighet for spredning, etablering og invasjon av maislinjen i naturlige habitater eller andre arealer utenfor jordbruksområder som resultat av frøspill i forbindelse med transport og prosessering. Risiko for utkrysning med dyrkede sorter vurderes av GMO panelet til å være ubetydelig. Ved foreskreven bruk av maishybriden Bt11 x MIR604 antas det ikke å være risiko for utilsiktede effekter på målorganismer, ikke-målorganismer eller på abiotisk miljø i Norge.

Samlet vurdering
Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at mais Bt11 x MIR604 er ernæringsmessig ekvivalent konvensjonell mais. Det er lite sannsynlig at proteinene Cry1Ab, PAT, mCry3A, eller PMI vil introdusere et toksisk eller allergent potensiale i mat eller fôr basert på mais Bt11 x MIR604 sammenliknet med konvensjonelle maissorter.

Faggruppen finner at maishybrid Bt11 x MIR604, ut fra dagens kunnskap og omsøkt bruk, er sammenlignbar med konvensjonell mais når det gjelder mulig miljørisiko i Norge.

Abbreviations and explanations

ALS	Acetolactate synthase, an enzyme that catalyses the first step in the synthesis of the branched-chain amino acids, valine, leucine, and isoleucine
AMPA	Aminomethylphosphonic acid, one of the primary degradation products of glyphosate
ARMG	Antibiotic resistance marker gene
BC	Backcross. Backcross breeding in maize is extensively used to move a single trait of interest (e.g. disease resistance gene) from a donor line into the genome of a preferred or "elite" line without losing any part of the preferred lines existing genome. The plant with the gene of interest is the donor parent, while the elite line is the recurrent parent. BC_1, BC_2 etc. designates the backcross generation number.
BLAST	Basic Local Alignment Search Tool. Software that is used to compare nucleotide (BLASTn) or protein (BLASTp) sequences to sequence databases and calculate the statistical significance of matches, or to find potential translations of an unknown nucleotide sequence (BLASTx). BLAST can be used to understand functional and evolutionary relationships between sequences and help identify members of gene families.
bp	Basepair
Bt	*Bacillus thuringiensis*
CaMV	Cauliflower mosaic virus
Codex	Set by The Codex Alimentarius Commission (CAC), an intergovernmental body to implement the Joint FAO/WHO Food Standards Programme. Its principle objective is to protect the health of consumers and to facilitate the trade of food by setting international standards on foods (i.e. Codex Standards).
Cry	Any of several proteins that comprise the crystal found in spores of *Bacillus thuringiensis*. Activated by enzymes in the insects midgut, these proteins attack the cells lining the gut, and subsequently kill the insect.
Cry1Ab	Cry1 class crystal protein from *Bacillus thuringiensis* subsp. *kurstaki.* Provide protection against certain lepidopteran target pests, such as the European maize borer (*Ostrinia nubilalis*), and species belonging to the genus *Sesamia.*
Cry3A	Cry3 class crystal protein from *Bacillus thuringiensis* subsp. *tenebrionis.* Provide protection against certain coleopteran target pests
mCry3A	Modified Cry3A protein optimized for maize
CTP	Chloroplast transit peptide
DAP	Days after planting
DNA	Deoxyribonucleic acid
DT50	Time to 50% dissipation of a protein in soil
DT90	Time to 90% dissipation of a protein in soil
dw	Dry weight
dwt	Dry weight tissue
EC	European Commission

ECB	European corn borer, *Ostrinia nubilalis*
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	Environmental risk assessment
E-score	Expectation score
EU	European Union
fa	Fatty acid
FAO	Food and Agriculture Organisation
FIFRA	US EPA Federal Insecticide, Fungicide and Rodenticide Act
Fitness	Describes an individual's ability to reproduce successfully relative to that of other members of its population.
fw	Fresh weight
fwt	Fresh weight tissue
GAT	Glyphosate N-acetyltransferase
GLP	Good Laboratory Practice
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/area that a given gene occupies on a chromosome
LOD	Limit of detection
LOQ	Limit of quantification
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization-Time Of Flight. A mass spectrometry method used for detection and characterisation of biomolecules, such as proteins, peptides, oligosaccharides and oligonucleotides, with molecular masses between 400 and 350,000 Da.
MCB	Mediterranean corn borer, *Sesamia nonagrioides*
mEPSPS	Modified 5-enolpyruvylshikimate-3-phosphate synthase
mRNA	Messenger RNA
MT	Norwegian Food Safety Authority (Mattilsynet)

NDF	Neutral detergent fibre, measure of fibre used for animal feed analysis. NDF measures most of the structural components in plant cells (i.e. lignin, hemicellulose and cellulose), but not pectin.
Northern blot	Northern blot is a technique used to study gene expression by detection of RNA or mRNA separated in a gel according to size.
NTO	Non-target organism
Nicosulfuron	Herbicide for maize that inhibits the activity of acetolactate synthase
Near-isogenic lines	Term used in genetics/plant breeding, and defined genetic lines that are identical except for differences at a few specific locations or genetic loci.
OECD	Organisation for Economic Co-operation and Development
ORF	Open Reading Frame, in molecular genetics defined as a reading frame that can code for amino acids between two stop codons (without stop codons).
OSL	Over season leaf
OSR	Over season root
OSWP	Over season whole plant
pat	*Phosphinothricin-Acetyl-Transferase* gene
PAT	Phosphinothricin-Acetyl-Transferase protein
PCR	Polymerase chain reaction, a technique to amplify DNA by copying it
PMI	Phosphomannose Isomerase enzyme. Metabolizes mannose and allows positive selection for recovery of transformed plants
R0	First transformed generation, parent
Rimsulferon	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 transfer of electrophoresis-separated DNA fragments to a filter membrane and possible subsequent fragment detection by probe hybridisation
T-DNA	Transfer DNA, the transferred DNA of the tumour-inducing (Ti) plasmid of some species of bacteria such as *Agrobacterium tumefaciens* and *A. rhizogenes*, into plant's nuclear genome. The T-DNA is bordered by 25-base-pair repeats on each end. Transfer is initiated at the left border and terminated at the right border and requires the *vir* genes of the Ti plasmid.
TI	Trait integrated
TMDI	Theoretical Maximum Daily Intake
U.S. EPA	United States Environmental Protection Agency.

Maize growth stages	*Vegetative*
	VE: emergence from soil surface
	V1: collar of the first leaf is visible
	V2: collar of the second leaf is visible
	Vn: collar of the leaf number 'n' is visible
	VT: last branch of the tassel is completely visible
	Reproductive
	R0: Anthesis or male flowering. Pollen shed begins
	R1: Silks are visible
	R2: Blister stage. The kernels are filled with a clear nourishing endosperm fluid and the embryo can be seen
	R3: Milk stage. The kernels endosperm is milky white.
	R4: Dough stage. The kernels endosperm has developed to a white paste
	R5: Dent stage. If the genotype is a dent type, the grains are dented
	R6: Physiological maturity
Western blot	Technique used to transfer proteins separated by gel electrophoresis by 3-D structure or denatured proteins by the length of the polypeptide to a membrane, where they might be identified by antibody labelling.
WHO	World Health Organisation
ZM	*Zea maize* L.
ZM-HRA	A modified version of the native acetolactate synthase protein from maize. Confers tolerance to the ALS-inhibiting class of herbicides

Table of contents

Background

On 14 November 2007, the European Food Safety Authority (EFSA) received from the Competent Authority of the United Kingdom an application (Reference EFSA/GMO/UK/2007/50) for authorisation of the insect-resistant and herbicide-tolerant genetically modified (GM) maize Bt11 x MIR604 (Unique Identifier SYN-BTØ11-1 x SYN-IR6Ø4-5), submitted by Syngenta Seeds S.A.S. within the framework of Regulation (EC) No 1829/2003.

The scope of the application covers:

- Import and processing of maize Bt11 x MIR604
- GM plants for food and feed use
- Food and feed, containing or consisting of maize Bt11 x MIR604
- Food and feed produced from maize Bt11 x MIR604
- Food containing ingredients produced from maize Bt11 x MIR604

After receiving the application EFSA/GMO/UK/2007/50 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the EU- and EFTA Member States (MS) and the European Commission and made the summary of the dossier publicity available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of regulation (EC) No 1829/2003. On 11 March 2008, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the EC and consulted nominated risk assessment bodies of the MS, including the Competent Authorities within the meaning of Directive 2001/18/EC (EC 2001), following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1929/2003, to request their scientific opinion. Within three months following the date of validity, all MS could submit via the EFSA GMO Extranet to EFSA comments or questions on the valid application under assessment.

The EFSA GMO Panels scientific opinion was published in 29 April 2010 (EFSA 2010b). The Commission Decision 2011/893/EC authorised the placing on the market of products containing, consisting of, or produced from maize Bt11 x MIR604 pursuant to Regulation (EC) No 1829/2003 (EC 2008) on 21 December 2011.

Genetically modified maize Bt11 x MIR604 has previously been risk assessed by the VKM Panel on Genetically Modified Organisms (GMO), commissioned by the Norwegian Food Safety Authority and the Norwegian Environment Agency related to the EFSAs public hearing of the application EFSA/GMO/UK/2007/48 in 2008 (VKM 2009a). In addition, Bt11 and MIR604 have been evaluated by the VKM GMO Panel as single events and as a component of several stacked GM maize events (VKM 2005a,b, 2007, 2008, 2009b,c,d,e, 2012a,b, 2013a,b,c).

Exemption of the authorisation requirements of 19 existing products in Norway

Through the Agreement of the European Economic Area (EEA), Norway is obliged to implement the EU regulations on GM food and feed (regulations 1829/2003, 1830/2003 et al). Until implementation of these regulations, Norway has a national legislation concerning processed GM food and feed products that are harmonised with the EU legislation. These national regulations entered into force 15 September 2005. For genetically modified feed and some categories of genetically modified food, no requirements of authorisation were required before this date. Such products that were lawfully placed on the Norwegian marked before the GM regulations entered into force, the so-called existing products, could be sold in a transitional period of three years when specific notifications were sent to the Norwegian Food Safety Authority. Within three years after 15. September 2005, applications for

authorisation should be sent to the Authority before further marketing. Four fish feed producing companies have once a year since 2008, applied for an exemption of the authorisation requirements of 19 existing products, including maize Bt11. These 19 GM events are all authorised in the EU, and the Norwegian Food Safety Authority has granted exemption for a period of one year each time.
http://www.mattilsynet.no/planter_og_dyrking/genmodifisering/fire_virksomheter_har_faatt_dispensasjon_fra_kravet_om_godkjenning_av_genmodifisert_fiskefor.10951

Terms of reference

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

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

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

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

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

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

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

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

Assessment

1 Introduction

The genetically modified maize stack Bt11 x MIR604 was produced by conventional crossing between inbred lines of maize containing the single events Bt11 and MIR604. The F_1 hybrid was developed to provide protection against certain lepidopteran and coleopteran target pests, and to confer tolerance to glufosinate-ammonium-based herbicides.

None of the target pests for maize Bt11 x MIR604 are present in the Norwegian agriculture. The PAT protein expressed in maize Bt11 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 Bt11 x MIR604 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 into account the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2011a), the environmental risk assessment of GM plants (EFSA 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 food/feed and environmental risk assessment of the genetically modified maize Bt11 x MIR604 is based on information provided by the applicant in the applications EFSA/GMO/UK/2007/50, and scientific opinions and comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment is also based on a review and assessment of relevant peer-reviewed scientific literature.

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

2 Molecular characterisation

2.1 Evaluation of relevant scientific data

2.1.1 Method of production of maize Bt11 x MIR604

The stacked maize Bt11 x MIR604 was developed through conventional crossing of the single maize events Bt11 and MIR604. Maize Bt11 x MIR604 combines the insect resistance of both Bt11 and MIR604 with the tolerance to glufosinate ammonium-based herbicides of maize Bt11. These traits are conferred through the expression of the *cry1Ab, mcry3A* and *pat* genes. In addition, the stacked maize contains the selectable marker gene *pmi,* used in the development of maize MIR604.

2.1.2 Summary of evaluation of the single events

2.1.2.2 Maize Bt11

Maize Bt11 was generated by transformation of a proprietary inbred maize line, H8540 *(Zea mays)*, with a DNA fragment obtained by a restriction digest of the plasmid pZO1502 with the enzyme *Not*I. Regenerated plants were backcrossed to a selected line resulting in maize Bt11. The DNA fragment used for transformation carried two expression cassettes; a selectable marker gene *pat,* encoding phosphinothricin-N-acetyl transferase and a trait gene encoding a variant *Bacillus thuringiensis cry1Ab* gene encoding Bt endotoxin. Both the *cry1Ab* and *pat* gene cassette are controlled by the 35S promoter from the *Cauliflower mosaic virus* (CaMV), supplemented with the intron sequences to enhance gene expression. The polyadenylation signals are derived from the nopaline synthase (*nos*) gene from *Agrobacterium tumefaciens* (Fig.1).

Southern analyses of the single maize event Bt11 used a variety of DNA probes that included the *pat* and *cry1Ab* genes as probes for the genes intended to be inserted and the *amp* gene and the entire plasmid as probes to detect genome wide unintended insertions. The data obtained indicated that maize Bt11 contains a single DNA insertion with one copy of both the *cry1Ab* and the *pat* cassettes.

The entire Bt11 maize insert and flanking regions was sequenced. The maize sequences flanking the Bt11 maize insert were also identified. A blast analysis of the sequences flanking the Bt11 maize insert was carried out against publicly available nucleotide databases. DNA sequences at the junctions between the insert and the parent genome were determined. At the 5' flank, approximately 350 bp of the plant DNA adjacent to the insert was sequenced. At the 3' flank, approximately 540 bp of the plant DNA adjacent to the insert was sequenced. The 5' and 3' flanking sequences were screened for homologies with sequences found in public databases. BLAST analysis of both the 5' and 3' regions of the Bt11 maize insert revealed homology primarily to the *Zea mays* 180 bp knob-associated tandem repeat. The data do not indicate any safety concerns with regard to the interruption of known genes or from the potential production of new toxins or allergens.

The range of expression of Cry1Ab and PAT proteins in Bt11 maize plants were determined by ELISA in several plant tissues and whole plants at various growth stages from different hybrids of field and sweet maize. The Cry1Ab protein was found in all tissues examined, with a decrease in concentration at the time of plant maturation and senescence.

Levels in pollen were below the lower limit of quantification, < 0.08 µg/g fresh wt. pollen and < 0.15 µg/g dry wt. pollen. Across all plant stages, mean Cry1Ab levels measured in leaves, roots and whole plants ranged from *ca.* 10 - 22 µg/g fresh wt. (12 – 154 µg/g dry wt.), 2 – 4 µg/g fresh wt. (9 – 22 µg/g

dry wt.), and 4 – 9 µg/g fresh wt. (6 – 70 µg/g dry wt.), respectively. Mean Cry1Ab levels measured in grain at seed maturity and senescence were 1 – 2 µg/g fresh wt (2 µg/g dry wt.).

The level of the Cry1Ab protein was present at low levels in Bt11 sweet maize hybrids. Cry1Ab protein was not detectable in any of the canned maize samples tested. The level of the PAT protein was determined with Bt11 field maize plants; measurable levels (ng/g) were only found in leaves, silk and tassel. For grain, pollen, root and stalk concentrations were below the limits of detection. The PAT protein is present at less than 0.000008% fresh weight and 0.00016% of the total maize grain protein.

The genetic stability of the inserted DNA in maize Bt11 was demonstrated over several generations by Southern analysis. Segregation data for glufosinate-ammonium tolerance and insect resistance also demonstrated the traits are stable and inherited according to Mendel's laws of genetics. These data also support the presence of a single insertion locus.

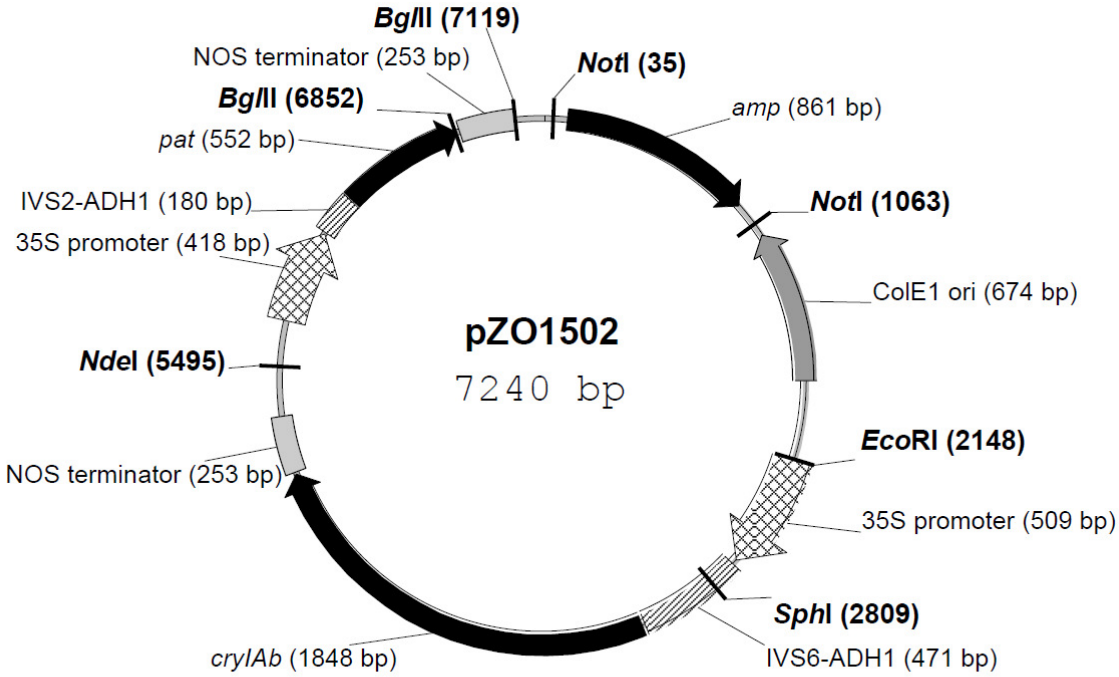

Figure 1. Various gene elements of t transformation vector pZO1502 used for generation of the maize strain Bt11.

2.1.2.1 Maize MIR604

Maize MIR604 was developed by transforming immature maize embryos derived from a proprietary *Zea mays* line (A188) via *Agrobacterium*-mediated transformation, with the binary transformation vector pZM26. By this method, genetic elements within the left and right border regions (the T-DNA) of the transformation vector, are transferred and integrated into the genome of the plant cell, while genetic elements outside these border regions are (generally) not. The T-DNA genetic elements transferred to produce maize MIR604 are shown in Table 1 and Figure 2.

Maize MIR604 expresses the *mcry3A* gene, which is a modified version of the *cry3A* gene from *Bacillus thuringiensis* subsp. *tenebrionis.* The *mcry3A* gene encodes the mCry3A protein that confers resistance to the Western Corn rootworm (*Diabrotica virgifera virgifera*) and other related coleopteran

pests of maize. The native *cry3A* gene was modified to incorporate a cathepsin-G serine protease recognition site within the expressed protein. The original N-terminal region of this protein has been removed and the mCry3A protein commences at a methionine residue in position 48 of the native protein. The *mcry3A* gene is regulated by the promoter from the metallothionein-like gene from *Zea mays*, which is preferentially expressed in root tissue, and the nopaline synthase (NOS) terminator from *Agrobacterium tumefaciens.*

MIR604 also expresses the *pmi* (*manA*) gene from *Escherichia coli*, which encodes the enzyme phosphomannose isomerase (PMI). The gene was introduced as a selectable marker for the development of maize MIR604. Mannose is taken up by plants and converted to mannose-6-phosphate by hexokinase. Usually this product cannot be further utilised in maize plants as they lack the PMI enzyme. The accumulation of mannose-6-phosphate inhibits phosphoglucose isomerase, causing a block in glycolysis. It also depletes cells of orthophosphate required for the production of ATP. Therefore, while mannose has no direct toxicity on plant cells, it causes growth inhibition. This does not occur in plants transformed with the *pmi* gene as they can utilise mannose as a source of carbon. The *pmi* gene is regulated by the polyubiquitin promoter (ZmUbiInt) from Zea mays and the NOS terminator from *A. tumefaciens*.

Table 1. T-DNA genetic elements

Component	Size (bp)	Function and origin of the sequence
Right border	25	T-DNA right border region
MTL promoter	2556	Promoter derived from the metallothionein-like gene from Zea mays. Provides preferential expression in roots of Zea mays
mcry3A	1797	Modified version of the native *cry3A* gene (maize optimised)
NOS	253	Terminator sequence from nopaline synthase gene from *A. tumefaciens*
ZmUbiInt	1993	Promoter region and intron from the Zea mays polyubiquitin gene. Provides constitutive expression
pmi	1176	Phosphomannose isomerase gene from *E. coli*. Selectable marker gene
NOS	253	Terminator sequence from nopaline synthase gene from *A. tumefaciens*
Left border	25	T-DNA left border region

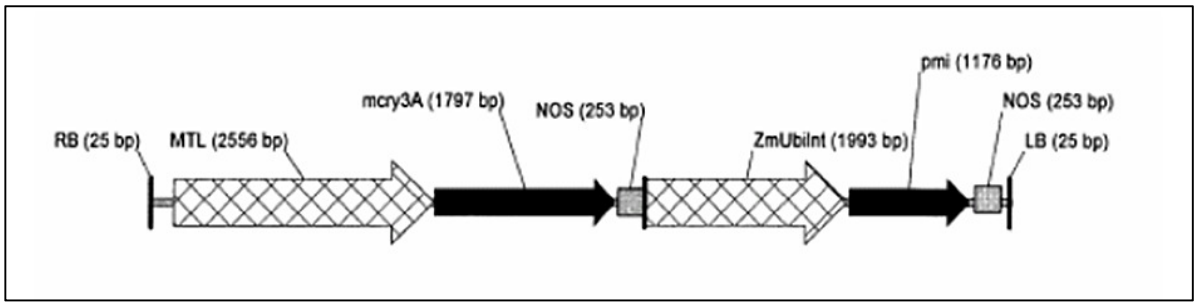

Figure 2. Genes and regulatory elements inserted in MIR604

Southern blot analyses have indicated that the maize event MIR604 occurred as an integration of a single intact T-DNA from plasmid pZM26 into the proprietary maize line genome, and that plasmid backbone DNA is not present in maize MIR604.

Sequence analyses of the entire T-DNA insert and flanking regions have shown that a total of 8416 bp of T-DNA was inserted in the maize genome, and that a 44bp segment was missing from the Right

border region, as well as 43bp at the Left border region. Three base pair changes were found within the insert in MIR604: one within the MTL promoter, and two within the *pmi* gene. These modifications have resulted in two amino acid substitutions, however without affecting the functions of the inserted elements in MIR604. The sequence analyses indicated that the overall integrity of the insert and the contiguousness of the functional elements from pZM26 are maintained.

According to the applicant, BLAST analyses show that the insertion of the T-DNA in MIR604 occurred in a region of the *Zea mays* genome that was not well annotated and that the insert did not appear to disrupt any identified endogenous *Zea mays* genes. Analyses of six potential reading frames at both the 5' and 3' T-DNA to genome junctions did not show the presence of any novel ORF's.

Segregation analyses of trait negative and trait positive plants, determined by ELISA and PCR, from a selected generation of maize (T5), have shown that the introduced traits in MIR604 are stably inherited in a Mendelian fashion, as analysed by Chi square analysis.

The levels of mCry3A and PMI proteins in maize MIR604 were determined by ELISA at the four growth stages: whorl, anthesis, seed maturity and senescence.
Across all growth stages, mean mCry3A levels measured ranged from 4 – 94 µg/g dry weight (dw) in leaves, 7 – 62 µg/g dw in roots, and 3 - 28 µg/g dw in whole plants. Mean mCry3A levels measured in grain at seed maturity and senescence ranged from 0.8 – 2.0 µg/g dw. Mean mCry3A levels measured in silk tissue at anthesis were below the lower limit of quantification (LOQ), <1.0 µg/g dw. Mean mCry3A levels measured in silk tissue at seed maturity ranged from 1 – 3 µg/g dw. No mCry3A protein was detectable in pollen.

PMI protein was detected in most maize MIR604 plant tissues, although at low levels. Across all plant stages, mean PMI levels ranged from not detectable (ND) to 2.1 µg/g dw in leaves, below the LOQ (<0.04 µg/g dw) to 2 µg/g dw in roots, and below the LOQ (<0.1 µg/g dw) to 1.0 µg/g dw in whole plants. Mean PMI levels measured in grain at seed maturity and senescence ranged from below the LOQ (<0.07 µg/g dw) to 0.5 µg/g dw. Mean PMI levels measured in silk tissue at anthesis and seed maturity ranged from below the LOQ (<0.2 µg/g dw.) to 6.8 µg/g dw. PMI in pollen ranged from 3.9 – 5.2 µg/g dw.

Overall levels of mCry3a protein were measured to be similar across four generations analysed without any significant trend either up or down, indicating that the expression of *mcry3A* in MIR604 is stable. A similar result was obtained for the PMI protein. Since no novel ORF's were identified that spanned either the 5' or 3' junctions between the MIR604 T-DNA and *Zea mays* genomic sequence, no fusion proteins are expected.

In summary, the molecular characterisation of maize MIR604 indicates the presence of only single copies of the *mcry3A* and *pmi* genes, and that the T-DNA insert and phenotypic traits are stably inherited over several generations. The VKM GMO Panel considers the molecular characterisation of maize MIR604 as adequate.

2.1.3 Transgene constructs in maize Bt11 x MIR604

Maize Bt11 x MIR604 was produced by combining Bt11 and MIR604 maize through conventional breeding, and therefore expresses the four transgenic genes *cry1Ab*, *pat*, *mcry3A* and *pmi*.

The applicant has performed a comparative Southern blot analysis of maize Bt11 x MIR604 with the parental maize lines Bt11 and MIR604, to investigate if the *cry1Ab*, *pat*, *mcry3A* and *pmi* genes are intact and stably inherited by maize Bt11 x MIR604. Detailed information in applicant Appendix 1, considered confidential by Syngenta.

cry1Ab specific probe
Genomic DNA from Bt11 and Bt11 x MIR604, were digested with the restriction enzymes *Nde*I, *Sph*I, and *Bgl*II + *Eco*RI, and then hybridised with the *cry1Ab* specific probe. This produced single hybridisation bands of approximately 4.6 kb, 20 kb and 4.7 kb, respectively, in both maize lines, corresponding to single copies of the *cry1Ab* gene. These results together with negative (inbred hybrid and MIR604) and positive control (plasmid pZO1502) indicate that the *cry1Ab* gene is intact in Bt11 x MIR604, and equivalent to the *cry1Ab* gene in Bt11.

pat specific probe
Genomic DNA from Bt11 and Bt11 x MIR604, were digested with the restriction enzymes *Nde*I, *Sph*I, and *Bgl*II + *Eco*RI, and then hybridised with the *pat* specific probe. This produced single hybridisation bands of approximately 1.9 kb, 20 kb and 4.7 kb, respectively, in both maize lines, corresponding to single copies of the *pat* gene. These results together with negative (inbred hybrid and MIR604) and positive control (plasmid pZO1502) indicate that the *pat* gene is intact in Bt11 x MIR604, and equivalent to the *pat* gene in Bt11.

***mcry3A* specific probe**
Genomic DNA from Bt11 and Bt11 x MIR604, were digested with the restriction enzymes *Kpn*I, *Hin*dIII, and *Asc*I + *Xma*I, and then hybridised with the *mcry3A* specific probe. This produced single hybridisation bands of approximately 5.6 kb, 10 kb and 8.2 kb, respectively, in both maize lines, corresponding to single copies of the *mcry3A* gene. These results together with negative (inbred hybrid and Bt11) and positive control (plasmid pZM26) indicate that the *mcry3A* gene is intact in Bt11 x MIR604, and equivalent to the *mcry3A* gene in Bt11. Expected and observed band sizes for the different restriction enzymes are shown in Table 4.

***pmi* specific probe**
Genomic DNA from MIR604 and Bt11 x MIR604 were digested with the restriction enzymes *Kpn*I, *Bam*HI, and *Asc*I + *Xma*I, and then hybridised with the *pmi* specific probe. This produced single hybridisation bands of approximately 5.2 kb, 2.7 kb and 8.2 kb, respectively, in both maize lines, corresponding to single copies of the *pmi* gene. These results together with negative (inbred hybrid and Bt11) and positive control (plasmid pZM26) indicate that the *pmi* gene is intact in Bt11 x MIR604, and equivalent to the *pmi* gene in Bt11. Expected and observed band sizes for the different restriction enzymes are shown in Table 5.

In summary, the results from the comparative Southern blot analysis show that *cry1Ab, pat, mcry3A, and pmi* genes are intact and stably inherited by maize Bt11 x MIR604.

2.1.3.1 Information on the expression of the inserts
Plants used in this study were derived from four near-isogenic field maize hybrids (Bt11, MIR604, Bt11 x MIR604, and nontransgenic control) grown in 2005 according to standard local agronomic practices at a Syngenta Seeds research station in Bloomington, IL, USA. Detailed information in applicant Appendix 2, considered confidential by Syngenta!

The pollen used in this study was produced from plants derived from four near-isogenic field maize hybrids (listed above) grown in 2006 according to standard local agronomic practices collected from three Syngenta Seeds research stations in Seward, NE, Monroeville, IN and Mackinaw, IL, USA.

Five plants per transgenic genotype and two plants from the near-isogenic, nontransgenic control genotype were harvested at each of three developmental time points and separated into the following plant parts for analysis:

Stage	Tissues sampled
V9-V12 stage (ca. 8 weeks after planting)	Leaves, Roots
Anthesis (pollen shed)	Leaves, Roots, Pollen
Seed maturity (black layer)	Leaves, Roots, Kernels

Each individual dataset (consisting of the data from either Bt11 or MIR604 and Bt11 x MIR604) was subjected to two-tailed analysis of variance, in which the effect of the genotype was assessed with an F-test. Additionally, mean pollen concentrations were analysed across the three collection locations. The F-test results in a probability level or "p-value", expressed on the 0-1 scale. An F-test probability <0.05 indicates that the genotypes are significantly different at the customary 5% level. Only the dry weight data was statistically analysed.

RESULTS

Cry1Ab

Maize Bt11

Mean Cry1Ab levels in maize Bt11 across the three plant stages sampled, ranged from 16.8 - 25.9 µg/g dw in leaves and 4.3 - 10.0 µg/g dw in roots. Mean Cry1Ab levels in grain (kernels) were 1.5 µg/g dw. Mean levels in pollen were <LOQ (<0.037 µg/gdw), 0.04 and 0.06 µg/g dw, in samples from the three separate field locations, respectively.

Maize Bt11 x MIR604

Mean Cry1Ab levels in maize Bt11 x MIR604 across the three plant stages sampled, ranged from 19.7 - 27.7 µg/g dw in leaves and 5.6 to 10.0 µg/g dw in roots. Mean Cry1Ab levels in grain (kernels) were 1.7 µg/g dw. Mean levels in pollen were <LOQ (<0.037 µg/gdw), 0.04 and 0.06 µg/g dw, in samples from the three separate field locations, respectively.

mCry3A

Maize MIR604

Mean mCry3A levels in maize MIR604 across the three plant stages sampled, ranged from 23.7 - 41.5 µg/g dw in leaves and 18.0 - 21.3µg/g dw in roots. Mean mCry3A levels in grain (kernels) were 0.7 µg/g dw. Mean levels in pollen were 0.004, 0.030 µg/g dw and <LOQ (<0.053 µg/g dw), in samples from the three separate field locations, respectively.

Maize Bt11 x MIR604

Mean mCry3A levels in maize Bt11 x MIR604 across the three plant stages sampled, ranged from 33.4 - 46.3 µg/g dw in leaves and 18.9 - 23.9µg/g dw in roots. Mean mCry3A levels in grain (kernels) were 0.7 µg/g dw. Mean levels in pollen were 0.004, 0.030 µg/g dw and <LOQ (<0.053 µg/g dw), in samples from the three separate field locations, respectively.

PAT

Maize Bt11

Mean PAT levels in maize Bt11 across the three plant stages sampled, ranged from < 0.05 - 0.15 µg/g dw in leaves and <0.07 - 0.17µg/g dw in roots. Mean PAT levels in grain (kernels) were <LOQ (<0.04 µg/g dw). Mean levels in pollen were <LOQ (<0.024 µg/g dw), <LOQ (<0.022 µg/g dw) and <LOQ (<0.034 µg/g dw), in samples from the three separate field locations, respectively.

Maize Bt11 x MIR604

Mean PAT levels in maize Bt11 x MIR604 across the three plant stages sampled, ranged from <0.05

- 0.17 µg/g dw in leaves and 0.08 - 0.19 µg/g dw in roots. Mean PAT levels in grain (kernels) were <LOQ (<0.04 µg/g dw). Mean levels in pollen were <LOQ (<0.024 µg/g dw), <LOQ (<0.022 µg/g dw) and <LOQ (<0.034 µg/g dw), in samples from the three separate field locations, respectively.

PMI

Maize MIR604
Mean PMI levels in maize MIR604 across the three plant stages sampled, ranged from 4.7 to 10.6 µg/g dw in leaves and 2.6 to 6.0 µg/g dw in roots. Mean PMI levels in grain (kernels) were 1.8 µg/g dw. Mean levels in pollen were 43.4, 60.1 and 37.4 µg/g dw, in samples from the three separate field locations, respectively.

Maize Bt11 x MIR604
Mean PMI levels in maize Bt11 x MIR604 across the three plant stages sampled, ranged from 5.7 to 10.4 µg/g dw in leaves and 2.3 to 6.0 µg/g dw in roots. Mean PMI levels in grain (kernels) were 1.9 µg/g dw. Mean levels in pollen were 46.6, 56.9 and 39.3 µg/g dw, in samples from the three separate field locations, respectively.

Overall results
Except for minor statistically significant differences that were not consistent across the growing season, overall concentrations and expression patterns of the transgenic proteins Cry1Ab, mCry3A, PAT and PMI are similar between the parental maize lines Bt11, MIR604 and maize Bt11 x MIR604.

2.1.3.2 Parts of the plant where the insert is expressed
The range of expression of Cry1Ab, mCry3A, PAT and PMI proteins in maize Bt11 x MIR604, were determined by ELISA in samples from leaves, roots, grain (kernels) and pollen, as described above.

2.1.3.3 Potential fusion proteins
Maize Bt11 x MIR604 was produced by combining maize Bt11 and maize MIR604 through conventional breeding. An Open Reading Frame (ORFs) analyses have been performed for each of the parental lines. No novel ORFs were identified that spanned either the 5' or the 3' junctions between Bt11 maize T-DNA and Zea mays genomic sequences. Likewise, no novel ORF's were identified that spanned either the 5' or 3' junctions between MIR604 maize T-DNA and Zea mays genomic sequences. No expression of potential fusion proteins are therefore expected in maize Bt11 x MIR604.

2.1.3.4 Inheritance and genetic stability of inserted DNA
Genetic stability of the inserts has previously been demonstrated in the parental maize lines Bt11 and MIR604. Comparative Southern blot analyses have indicated that the recombinant inserts in the parental maize lines are retained in the stacked maize Bt11 x MIR604, and protein measurements with ELISA show comparable levels of the Cry1Ab, mCry3A, PAT and PMI proteins between the stacked and single maize lines.

2.2 Conclusion

Conventional crossing methods were used to produce the stacked maize Bt11 x MIR604. Southern blot analyses have indicated that the recombinant inserts in the parental maize lines Bt11 and MIR604 are retained in the stacked maize Bt11 x MIR604. Genetic stability of the inserts has previously been demonstrated in the parental events. Protein measurements show comparable levels of the Cry1Ab, mCry3A, PAT and PMI proteins between the stacked and single maize lines. The VKM Panel on GMO considers the molecular characterisation of maize Bt11 x MIR604 and the stack maize events Bt11 and MIR604 as adequate.

3 Comparative assessment

3.1. Summary of the previous evaluation of the single events

3.1.1 Maize Bt11

Maize Bt11 was compared to non-transgenic maize with a comparable genetic background. Forage and grain samples were collected for compositional analysis from field trials conducted in USA (studies involving 3-6 sites in 1995) and Europe (two locations in 1998). No consistent compositional differences were observed between maize Bt11 and non-transgenic maize. In addition, field trials over several seasons at different locations in Europe did not indicate significant differences between maize Bt11 and its comparators with respect to agronomical and phenotypical characteristics, except for herbicide tolerance and insect resistance.

Maize Bt11 has a long history of use and has been evaluated extensively by The VKM GMO Panel. In the latest risk assessment, it was concluded that maize Bt11 is compositionally, agronomically and phenotypically equivalent to conventional maize varieties, except for the herbicide tolerance and insect resistance traits conferred by the transgenic proteins Cry1Ab and PAT (VKM 2014a)

3.1.2 Maize MIR604

Maize MIR604 was compared to non-transgenic maize with comparable genetic background (near-isogenic control) during field trials at multiple locations in USA in 2002 and 2003. The composition of forage and grain samples were analysed in line with recommendations from the OECD consensus document on key nutrients, anti-nutrients, and secondary plant metabolites of maize (OECD 2002). No consistent compositional differences were observed between maize MIR604 and non-transgenic maize. Agronomic traits were assessed during field trials (and greenhouse trials) at 22 locations in 8 states in USA in 2002 and 2003. The results did not indicate consistent differences between maize MIR604 and its comparators with respect to agronomical and phenotypical characteristics, except for insect resistance.

Analyses of mono- and disaccharides, including phosphorylated forms of these saccharides, in maize MIR604 and near-isogenic control, were performed by the applicant at six locations in USA in 2006 at the request of the EFSA GMO Panel. In compounds that could theoretically be linked to PMI activity (e.g., starch and other carbohydrates), no consistent compositional differences were observed in the comparison between maize MIR604 and control.

In the latest risk assessment of maize MIR604 the VKM GMO Panel concludes that maize MIR604 is compositionally, agronomically and phenotypically equivalent to conventional maize varieties, except for the presence of the transgenic proteins and the insect resistance traits conferred by the mCry3A protein (VKM 2014)

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

The applicant has measured key nutritional components in forage and grain from the triple stacked maize event Bt11 x MIR604 x GA21, instead of Bt11 x MIR604. This is in accordence with the EFSA guidance document for the risk assessment of genetically modified plants containing stacked transformation events (EFSA 2007b). Maize Bt11 x MIR604 x GA21 expresses the *mepsps* gene from GA21 in addition to the *cry1Ab*, *pat, mcry3A* and *pmi* from Bt11 x MIR604. Forage and grain from

maize Bt11 x MIR604 x GA21 were measured and compared with those from a near-isogenic control maize. No other control/reference maize was used in the study. The mazie were grown at six locations in USA in 2006. The VKM GMO Panel commented the use of the triple stacked maize instead of maize Bt11 x MIR604 during the EFSA official hearing in 2007.

All plant materials used in the study were from the two hybrids:

1. **Bt11 x MIR604 x GA21, [E1 (+)],** **Genotype: NP2673(GA2l)/NP2171(Bt11 + MIR604)**
2. **Nontransgenic hybrid, [E3 (-)],** **Genotype: NP2673/NP2171**

A pedigree chart of the two maize hybrids is shown in Figure 1 in Appendix of this document.

According to the updated EFSA guidance on risk assessment of food and feed from genetically modified plants (EFSA 2011a), there should be at least three appropriate non-GM reference varieties of the crop that have a known history of safe use at each site. The test of equivalence is used to verify whether the agronomic, phenotypic and compositional characteristics of the GM plant fall within the normal range of natural variation. Such a range of natural variation is estimated from a set of non-GM reference varieties with a history of safe use (EFSA 2011b) and therefore allows comparisons of the GM plant with a similar food or feed produced without the help of genetic modification and for which there is a well-established history of safe use. These requirements were however not in place at the time of submission.

3.2.1. Experimental design & statistical analysis

The Bt11 x MIR604 x GA2l maize and corresponding near-isogenic hybrid were grown at six locations in USA in 2006.

At each location, the hybrids were grown in a randomised complete block design, with three replicates per genotype. Forage and grain were harvested and analysed for key food and feed nutrients, antinutrients, and secondary plant metabolites chosen based on recommendations of OECD for comparative assessment of the composition of new varieties of maize (OECD 2002). Forage was analysed for proximates, calcium, and phosphorus (a total of 9 analytes), and grain was analysed for proximates, minerals, amino acids, fatty acids, vitamins, secondary metabolites, and antinutrients (a total of 56 analytes). Analysis of variance was used to test for genotype effects and location-by-genotype interactions. In addition, mean levels of nutritional components were compared with the ranges of variation for conventional maize hybrids published in the International Life Sciences Institute Crop Composition Database (ILSI 2006).

The Bt11 x MIR604 x GA21 maize was treated with glyphosate and glufosinate herbicides. Both the Bt11 x MIR604 x GA21 and the near-isogenic maize were treated with other conventional pesticides as needed to maintain optimal plant health. Plants were self-pollinated by hand, and the developing ears were bagged to prevent crosspollination.

According to the applicant, all compositional analyses were conducted by Covance Laboratories, Inc., according to methods published and approved by AOAC International, or other industry-standard analytical methods. Component levels were converted to equivalent units of dry weight (DW) based on the moisture content of each sample.

The data for each component were subjected to analysis of variance with the model

$$Y_{ijk} = U + T_i + L_j + B(L)_{jk} + LT_{ij} + e_{ijk}$$

where *Yijk* is the observed response for genotype *i* at location *j* block *k*, *U* is the overall mean, T_i is the genotype effect, L_j is the location effect, $B(L)_{jk}$ is the effect of block within location, LT_{ij} is the location x genotype interaction effect and e_{ijk} is the residual error.

For each quantifiable component, *F*-tests were used to assess the statistical significance of the genotype effect, with an alpha level of 0.05. An *F*-test was also used to assess the significance of the location by genotype interaction. An *F*-test probability of <0.05 suggests that the effect of genotype was not consistent across locations and that the comparison of genotypes averaged across locations may not be valid. Moisture content of grain was not statistically analysed, because the grain had been mechanically dried.

Details of the study can be found in Appendix 4 of Technical Dossier.

3.3. Compositional Analysis

3.3.1 Forage composition

Proximates and fibres
No statistically significant differences were found for moisture, protein, ash, carbohydrates, ADF and NDF. A significant location-by-genotype interaction was observed for fat. The mean levels of all proximates across locations and for each location were within the ranges reported in the ILSI database.

Phosphorus and calcium
Phosphorus levels did not differ significantly between the two genotypes. A significant location-by-genotype interaction was observed for calcium. The mean levels of both minerals across locations and for each location were within the ranges reported in the ILSI database.

3.3.2 Grain composition

Proximates
No statistically significant differences were found for fat, carbohydrates, ADF, NDF, TDF and starch. A statistically significant difference in protein levels between the genotypes was observed, in addition to a significant location-by-genotype interaction for ash. The mean levels of all proximates across locations and for each location were within the ranges reported in the ILSI database.

Minerals
Zinc levels were significantly different between the two genotypes and a significant location-by-genotype interaction was observed for calcium. Some sodium levels below the limit of quantitation (LOQ) precluded statistical analysis. No statistically significant differences were found for copper, iron, magnesium, manganese, phosphorus, potassium, or selenium between the two genotypes, and all Mean levels of all minerals across locations and for each location were within the ranges reported in the ILSI database.

Vitamins
A statistically significant difference was observed for vitamin B_1. For vitamin E, levels below the limit of quantitation (LOQ) precluded statistical analysis. All mean levels across locations and for each location were within the ranges reported in the ILSI database except for vitamin B_2 levels, which were slightly higher in the transgenic grain at location 8 and in control grain at location l, and the vitamin E levels that were <LOQ in both transgenic and control grain at some locations. Below LOQ values for vitamin E are not represented in the ILSI database. Vitamins A, B_2, B_3, B_6, and B_9 did not differ significantly between the genotypes.

Amino acids
Most of the amino acid levels differed significantly between the genotypes. All mean amino acid levels across locations and for each location were within the ranges reported in the ILSI database.

Fatty acids
The proportion of the five most abundant fatty acids, as a fraction of total fatty acids did not differ significantly between the genotypes. All mean levels across locations and for each location were within the ranges reported in the ILSI database.

Secondary metabolites and anti-nutrients
There are no generally recognised anti-nutrients in maize at levels considered to be harmful, but for the purposes of assessment of substantial equivalence, the OECD has asked for analytical data for the following secondary metabolites in maize: ferulic acid, p-coumaric acid, furfural, inositol, phytic acid, raffinose and trypsin inhibitor.

Levels of ferulic acid, *p*-coumaric acid, inositol, phytic acid, and trypsin inhibitor did not differ significantly between the genotypes. Levels of raffinose and furfuralbelow the LOQ precluded statistical analysis. All mean levels across locations and for each location were within the ranges reported in the ILSI database.

3.4 Agronomic and phenotypic characters

During field trials at ten locations in USA in the 2005 growth season, data on phenotypic characteristics, agronomic performance and disease susceptibility were collected for the maize stack Bt11 x MIR604 and its conventional counterpart (near-isogenic conventional maize). Up to 20 separate agronomic parameters and three disease traits were assessed at each location, although not all parameters were recorded at all locations. Early growth, leaf color, gray leaf spot, northern corn leaf blight, southern corn leaf blight, and intactness were evaluated and recorded in qualitative scores on a scale of 1-9, where 1 is a good rating and 9 is a bad rating. Grain moisture, lodging, green snap, barrenness, dropped ears and stay green were recorded in percentages. Flowering data were recorded as heat units accumulated from date of planting.

The agronomic equivalence trials were conducted using one Bt11 x MIR604 maize (field maize) hybrid, known as NP2276Bt11/NP2391MIR604. The corresponding near-isogenic non-transgenic hybrid is known as NP2276/NP2391.

According to the applicant, the test locations were selected to be representative of the range of environmental conditions under which the tested hybrid varieties would typically be grown. Each of the agronomic trials was conducted as a randomized complete block design with four replications per location. For each agronomic or disease trait suitable for formal analysis, data were subjected to analysis of variance across locations. The statistical significance of the genotype effect (Bt11 x MIR604 vs. the near-isogenic control) was determined using a standard F-test at the 5% probability. Several traits could not be analysed formally using analyses of variance because there was either little or no variation in the data or that the distribution of the data deviated from normality. Results of such variates are presented as means.

Analyses of variance across trial locations showed statistically significant differences between maize Bt11 x MIR604 and the near-isogenic control hybrid for grain moisture, heat units to 50% of plants extruding silks and shedding pollen, respectively ($p<0.05$) (data not shown). For the other agronomic

and phenotypic characters recorded, including grain yield, the effect of genotype was not significant across locations. The Bt11 x MIR604 hybrid tended to flower a little earlier and had slight less grain moisture at harvest than the corresponding near-isogenic hybrid. However, these differences were relatively small and not always consistent across all sites.

Both maize Bt11 and maize MIR604 have previously been compared to conventional near-isogenic maize lines for several agronomic characteristics. According to the applicant, the data generated from several such field trials show that Bt11 and MIR604 maize are no different to conventional maize. Additional studies have been performed by the applicant to show that the stacked Bt11 x MIR604 maize does not differ in agronomic traits compared to conventional maize.

Details on the field trials conducted with Bt11 x MIR604 maize can be found in the technical dossier from the applicant (Appendix 3), considered confidential by Syngenta.

3.5 Conclusion

Comparative analyses of agronomic and phenotypic data from field trials located at representative sites and environments in USA in 2005 indicate that maize stack Bt11 x MIR604 is equivalent to its conventional counterpart, with the exception of the insect resistance and the herbicide tolerance, conferred by the Cry1Ab, mCry3A and PAT proteins. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of maize Bt11 x MIR604 compared to conventional maize varieties.

The applicant has performed a compositional analysis on the triple-stack Bt11 x MIR604 x GA21 instead of maize Bt11 x MIR604. The analysis was performed on plant materials from maize Bt11 x MIR604 x GA21 and a near-isogenic control hybrid from field trials in USA in 2006. With the exception of small intermittent variations, no biologically significant compositional differences were found between the triple-stack and the near-isogenic control. The results of the study are considered valid by EFSA also for maize Bt11 x MIR604, since maize Bt11 x MIR604 x GA21 encompasses the transgenic properties of maize Bt11 x MIR604. This is in accordance with the EFSA guidance document for the risk assessment of genetically modified plants containing stacked transformation events (EFSA, 2007b).

The VKM GMO Panel is of the opinion that the applicant should have performed a compositional analysis of maize Bt11 x MIR604 and not only referred to analyses of the triple- stack Bt11 x MIR604 x GA21. However, based on all information available, including agronomic and phenotypic data from field trials with maize Bt11 x MIR604, a feeding study on broilers showing nutritional equivalence to non-GM maize, and assessments of the single events Bt11 and MIR604, the VKM GMO Panel concludes that forage and grain from maize Bt11 x MIR604 are compositionally equivalent to its conventional counterpart.

4 Food /feed risk assessment

Both single maize events, Bt11 and MIR604, have previously been evaluated by the VKM GMO Panel, and updated risk assessments were finalised in january 2014 (VKM 2014a,b)

4.1. Summary of the previous evaluation of the single events

Maize Bt11
Maize Bt11 has a long history of use, and has been evaluated extensively by The VKM GMO Panel. In the latest risk assessment (VKM 2014a) it was concluded that Bt11 is nutritionally equivalent to conventional maize varieties and that it is unlikely that the Cry1Ab or PAT proteins will introduce a toxic or allergenic potential in food or feed based on maize Bt11 compared to conventional maize.
With regard to animal studies with the whole product, feeding studies with maize Bt11 grain with different target animals, such as rats (Hammond et al. 2006), broilers (Brake et al. 2003a) and laying hens, mice (Brake et al. 2004), dairy cows (Folmer et al. 2002) and beef cattle fed silage (Folmer et al. 2002), have all indicated nutritional equivalence between maize Bt11 and its non-GM maize counterpart and to conventional maize (Chowdhury et al. 2003 a,b; 2004; Shimada et al 2006 a,b,c; 2008).

Furthermore, in a multi-generation study (5 generations) with ICR mice, performance and life span was investigated on mice fed diets containing 68% of either Bt11 maize or isogenic non-Bt maize. Multiple parameters were measured e.g. feed intake and growth, mating, gestation, milking periods, reproduction, longivety and pathology. No significant differences were found between the Bt11- and non-Bt -fed mice in any of the generations (Haryu et al. 2009).

Maize MIR604
In the latest risk assessment of maize MIR604 the VKM GMO Panel concluded, based in part on data from whole food feeding studies on rats, rainbow trout and broilers, that maize MIR604 is nutritionally equivalent to conventional maize varieties. It is unlikely that the mCry3A or PMI proteins will introduce a toxic or allergenic potential in food or feed based on maize MIR604 compared to conventional maize (VKM 2014b).

4.2 Product description and intended uses

The scope of application EFSA/GMO/UK/2007/50 includes the import and processing of maize Bt11 x MIR604 and its derived products for use as food and feed. The possible uses of maize Bt11 x MIR604 include the production of animal feed and food products, such as starch, syrups and oils. The genetic modification of maize Bt11 x MIR604 is intended to improve agronomic performance only and is not intended to influence the nutritional properties, processing characteristics and overall use of maize Bt11 x MIR604 as a food/feed plant.

4.3 Effects of processing

There are two basic methods employed in processing field maize grain (kernels), dry milling and wet milling. In dry milling, maize is separated into flour, maize-meal, grits and other products. Wet milling is the process by which maize is separated into starch, germ to produce oil and fiber, and gluten for animal feed. Bt11 x MIR604 will be produced and processed in the same way as any field maize.

The food manufacturing of Bt11 x MIR604 field maize includes processing steps that are harsh, e.g. cooking, heating, high pressures, pH treatments, physical shearing, extrusion at high temperatures etc.

under which the majority of proteins are denatured, which also applies to mCry3A1 and PMI, Cry1Ab and PAT proteins (Hammond & Jez 2011). Concentrations of these proteins will be below the limit of detection in wet-milled fractions, in maize chips and maize oil. In the unprocessed kernel, and all of dry-milled fractions these protein will probably been found in quantifiable amounts.

4.4 Toxicological assessment

In assessing the potential risks of GM foods, it is important to consider both adverse health effects that may arise from substances that are intentionally introduced or modified in food crops, and adverse effects that may be produced unexpectedly as a result of the genetic modification process (Chao &Krewski, 2008)

4.4.1 Toxicological assessment of the newly expressed protein

No new genes in addition to those present in the parental maize lines have been introduced in maize Bt11 x MIR604. The VKM GMO Panel has considered the data available for maize Bt11 x MIR604, and also the newly expressed proteins mCry3A and PMI, Cr1Ab and PAT. The Panel is of the opinion that interactions between the single maize events that might impact on the food and feed safety of maize Bt11 x MIR604 are unlikely.

No new constituents other than the mCry3A and PMI, Cry1Ab and PAT proteins are expressed in maize Bt11 x MIR604 and no relevant changes in the composition of maize Bt11 x MIR604 were detected by the compositional analysis.

4.4.2 Toxicological assessment of the whole GM food/feed

43-day feeding study on broilers
Poultry studies are considered useful because chickens are fast growing organism that can consume large quantities of maize in the diet and thus are sensitive to potentially toxic effects of maize dietary components (OECD 2003).

A broiler feeding study was conducted to compare the nutritional properties of maize Bt11 x MIR604 (NP2276(Bt11)/NP2391(MIR604) with its near-isogenic control (NP2276/NP2391), and a locally grown commercial maize NC 2006 (North Carolina, growing season 2006) (Applicant Appendix 5). Prior to the study, grain samples were analysed for proximates, amino acids and mycotoxins. The mycotoxin determinations showed low contamination by aflatoxins, fumonisin, T2 toxin, zearalenone, and deoxynivalenol (vomitoxin) in grain from all three maize lines.

Three different diets: 1) Starter, 2) Grower, and 3) Finisher, were prepared for each of the three maize lines. Maize grain was mixed with soybean oil cake (48%) and other nutrients with an increasing inclusion of maize from starter to finisher diets (Table 10).

Table 10. Composition of Starter, Grower and Finisher diets for the three maize lines tested.

	NC 2006			Isogenic control			Bt11 x MIR604		
Ingredients	**Starter**	**Grower**	**Finisher**	**Starter**	**Grower**	**Finisher**	**Starter**	**Grower**	**Finisher**
Maize grain, %	51.36	58.07	63.94	50.00	56.56	62.33	49.77	56.30	62.04
Soybean oil cake (48%), %	38.77	31.55	26.49	37.82	30.44	25.26	37.60	30.19	24.98
Other, %	9.87	10.38	9.57	12.18	13.00	12.413	12.63	13.51	12.98
Total, %	100	100	100	100	100	100	100	100	100

One day old male (commercial strain Ross344) and female (commercial strain Ross 508) birds were distributed into 36 pens assigned in a randomised complete block design. Male and female birds were housed separately. Each test group (GM, control, and reference) consisted of six replicated pens of 15 birds/gender, - a total of 540 birds. The birds were fed *ad libitum* the Starter diets from day 0 - 16, Grower diets from day 17 – 35, and Finisher diets from day 35-44.

The mean Cry1Ab concentrations in the diets were 0.52 µg/g in Starter, 0.54 µg/g in Grower, and 0.67 µg/g in the Finisher. The concentrations of PAT and mCry3A in both maize Bt11 x MIR604 grain and diets prepared with this grain were below the limit of quantitation (LOQ). The PAT and mCry3A LOQs were 0.55 µg/g diet and 0.50 µg/g diet, respectively. Limit of detection (LOD) was 0.019 µg/g diets for PAT and 0.006 µg/g diet for mCry3A. The mean PMI concentrations in the diets prepared from Bt11 x MIR604 maize grain were 0.40 µg/g in Starter, 0.49 µg/g in Grower and 0.33 µg/g in the Finisher diet. The values reported above were not corrected for extraction efficiency.

At day 21, broilers fed the NC 2006 diet were significantly smaller in body weight than broilers fed either the Bt11 x MIR604 or non-GM control diet, but not at any other measured time point. Overall mean body weights were comparable in all treatment groups on day 43 with the average male reaching 2,434 grams and the average female reaching 1,995 grams.

According to the applicant overall survival was good (>93 %), despite a heat-stress-related mortality at 30 - 43 days, caused by an increase in ambient temperature. The highest mortality was observed in broilers fed NC 2006 maize; however, neither mortality nor other differences were attributed to the different diets by the applicant.

Males pooled across all groups showed significantly improved adjusted feed conversion relative to females, except for the 30 to 43 day finisher period when the ambient temperature was elevated. Broilers fed NC 2006 diets had slightly improved adjusted feed conversion efficiency at 43 days of age relative to broilers fed either the Bt11 x MIR604 or control diets. There were no differences between maize grain sources at any other time. According to the applicant there were no obvious deleterious effects associated with broilers' consumption of Bt11 x MIR604 maize grain when compared with consumption of control maize grain. A slight difference in feed conversion ratios were not considered adverse by the applicant. Consumption of diets containing Bt11 x MIR604 had no effect on carcass yield for males or females, and there was no overall effect of diets or gender on mortality.

At the end of the feeding period of the study, samples of Starting maize grain, Starter diet, Grower diet, and Finisher diet were again analysed for the concentrations of Cry1Ab, PAT, mCry3A, and PMI by ELISA.

In the EFSA Scientific Opinion adopted in 2010 this study was not considered by the EFSA GMO Panel.

4.5 Allergenicity assessment

Most food allergies are mediated by immunoglobulin E (IgE, type-I reactions). The strategies used when assessing the potential allergenic risk focuses on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation, or to elicit allergic reactions in already sensitised individuals and whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (EFSA 2010).

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

4.5.1 Assessment of IgE-mediated allergenicity of the newly expressed protein

A weighted risk analysis based on the decision tree approach has been performed by the applicant. The individual steps of this analysis starts by analysing the primary amino sequence of the novel protein and looking for similarity with sequences to known allergens, followed by specific or targeted serum screens for IgE cross-reactions to known allergens, digestibility studies of the proteins in simulated gastric and/or intestinal fluids, and animal studies (FAO/WHO, 2001, Codex Alimentarius, 2003, König et al., 2004, Poulsen 2004).

The proteins Cry1Ab, PAT, mCry3A and PMI, present in maize Bt11 x MIR604 have previously been evaluated and found unlikely to be allergenic.

These assessments have previously been described by the applicant for the single maize events MIR604 (EFSA-GMO-UK-2005-11) and Bt11 (Notification C/F/96/05.10 and EFSA-GMO-RX-Bt11), and were based on the following aspects:

PMI:

i) PMI enzymes are found in various plants and microorganisms.

ii) The *pm*i (*manA*) gene came from *Escherichia coli*,

iii) The manA protein is a member of the superfamily of "cupins," which are proteins with a specific 3-D structure. Some members of this super family are known IgE allergens.

iv) The gene coding for the PMI in the MIR604 was expressed in bacteria and the resulting enzyme compared to the MIR604 derived PMI by Western blot. The enzymes expressed from the two sources were shown not to be identical, two amino acids were changed, valine-61 was substituted by alanine, and glutamine-210 by histidine.

v) Bioinformatic analysis did not reveal any relevant sequence homology between the PMI expressed in maize MIR604 and known IgE allergens of the cupin superfamily.

vi) No significant similarity was found between any of the PMI 80-amino acid peptides and any entries in the SBI Allergen Database.

vii) In the eight or more contiguous amino acids homology search, there was an alignment between the PMI protein and a recently identified allergen, α-parvalbumin from Rana species CH2001 (a frog of Indonesian origin).

viii) Serum screening with serum IgE obtained from an allergic individual who displayed food-induce anaphylaxis against α-parvalbumin showed no cross-reactivity with PMI.

ix) The *E. coli* expressed PMI protein is also found in human intestinal microbiota, e.g. *E. coli*

x) There has always been a background of human exposure and a low quantity of PMI found in the human diet.

xi) The PMI-protein has previously been assessed for genetically modified plants and found to have no potential for IgE allergenicity (EFSA 2009; Delany et al. 2008,).

mCry3A:

i) The Cry3A protein from *Bacillus thuringiensis* subsp.*tenebrionis* is not considered a common food allergen.

ii) The expressed mCry3A protein is a single polypeptide with a 92.9 % sequence identity to the wild type.

iii) Immunoblot and glycosylation analysis of mCry3A derived from recombinant *E.coli* and from extracts of leaf material from transgenic MIR604 maize, indicate that post-translational glycosylation of mCry3A protein has not occurred.

iv) A comparison of amino acid sequence to known allergens indicated no homology between mCry3A and known allergens at the level of 8 contiguous amino acids.

v) The mCry3A protein is rapidly degraded by simulated gastric fluids *in vitro*. No assay for degradation in gastrointestinal fluids has been performed by the applicant.

vi) At 4°C, 25°C, and 37° C there was little or no effect on mCry3A bioactivity, while at 65°C there was some reduction in the bioactivity. At 95°C mCry3A protein was completely inactivated (US EPA 2010).

Cry1Ab and PAT

i) The sources of the transgenes: *B. thuringiensis* (*cry-genes*) and *S. viridochromogenes* (*pat*) have no history of causing allergy

ii) History of safe use of Cry proteins as microbial pesticides (US EPA, 1998), no indications of Cry proteins originating from *Bacillus thuringiensis* having harmful effects on the health of humans and animals (US EPA, 1996).

iii) The PAT protein has been subjected to previous safety assessments for genetically modified plants and found to have no allergenic potential

iv) The PAT protein has no homology to known toxins or allergenic proteins

v) The microbially produced Cry1Ab and PAT proteins were rapidly degraded in simulated gastric fluids *in vitro*. No degradation assay in gastrointestinal fluids has been performed by the applicant.

vi) PAT and Cry1Ab do not resemble any characteristics of known IgE-allergens, and no significant homologies between the amino acid sequences of the PAT and Cry1Ab proteins and IgE-allergenic proteins have been found (Fard et al, 2013, Kim et al, 2010, Randhawa et al 2011, Meyer, 1999, US EPA, 2010).

vii) The PAT and Cry1Ab protein is not glycosylated (Raybold et al, 2013, US EPA, 2010)

viii) Cry1Ab and PAT are considered heat labile (US EPA 2010)

4.5.2 Assessment of the allergenicity of the whole GM plant

Food allergies to maize are of low frequency and mainly occur in populations of specific geographic areas. Rare cases of occupational allergy to maize dust have been reported. There is no reason to

expect that the use of maize Bt11 x MIR604 will significantly increase the intake and exposure to maize. According to the applicant, a possible overexpression of any endogenous protein, which is not known to be allergenic, would be unlikely to alter the overall allergenicity of the whole plant or the allergy risk for consumers.

An assessment of endogenous allergens in maize, i.e. mLTP (maize lipid transfer protein), has been carried out with immunoassays based on rabbit anti-mLPT-peptide serum (Panda et al. 2013). According to Panda et al. (2013) the intent of this study was to demonstrate that natural variation exists between varieties of commodity crops, demonstrating a 15-fold variation in mLTP concentration between nine maize varieties.

The allergenicity assessment of GM plants is not meant to address the adventitious presence of an allergen in a given food but rather to understand whether a GM plant might be more allergenic than its non-GM comparator(s) to such an extent to be of concern for human and animal health (Fernandez et al. 2013). A major concern for the allergenicity assessment of GM plants, however, is to evaluate whether the genetic modification introduces new allergens into the GM plant, and to verify that an increased expression of endogenous allergens in the GM plant has not taken place (Fernandez et al, 2013).

4.5.3 Adjuvanticity

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

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

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

“Bystander sensitisation”
"Bystander sensitisation” can occur when an adjuvant in food, or an immune response against a food antigen, results in an increased permeability of the intestinal epithelium for other components in food. Traditionally it was assumed that the epithelial cells of the intestine were permanently "glued together" by the so-called "tight junctions". Studies have however shown that these complex protein structures are dynamic and that they can be opened up by different stimuli.
Both *in vitro* and *in vivo* experiments have demonstrated that when an IgG response which can result in a complement activation (among other) is not balanced by an IgA response, the epithelial barrier

can be opened and unwanted proteins are able to enter the body (bystander-penetration) and lead to allergic sensitisation (Brandtzaeg P, Tolo K 1977; Lim PL, Rowley D1982).

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

4.6 Nutritional assessment of GM food/feed

The compositional analyses indicate nutritional equivalence between maize Bt11 x MIR604, near isogenic non-GM control and a commercial maize line. This is further supported by the broiler feeding study.

4.6.1 Intake information/exposure assessment

Net import of maize staple, e.g. flour, starch and mixed products, in Norway in 2007 was 7600 tons, corresponding to 4.4 g dry weight/person/day or an estimated daily energy intake for adults to be 0.6 % (Vikse 2009). The estimated median daily intake of sweet maize is 3.25 g/day, with a 97.5 % percentile of 17.5 g/day. The production of maize porridge for children in 2007 was about 37.5 tons, corresponding to a daily intake of 1.7 g/day or an estimated daily energy intake to be 0.6 % for a 6 month child (Vikse 2009).

Since most foods and foodstuffs from maize are derived from field maize grains, an estimated maximum daily intake for a Norwegian adult of Cry1Ab, PAT, mCry3A, and PMI proteins from maize Bt11 x MIR604 is calculated to be 8.5 µg, 0.18 µg, 3.7µg, and 12 µg, respectively, based on intake of maize staple (4.4 g/person/day) and the maximum protein levels in grain (kernel) at seed maturity in Tables 6, 8, 7 and 9. The estimated maximum daily intake of Cry1Ab, PAT, mCry3A, and PMI proteins from sweet maize is calculated to be 33.8 µg, 0.71 µg, 14.7 µg, and 47.7 µg, respectively, based on a daily intake of 17.5 g fresh sweet maize/day (97.5 % percentile). These levels are several orders of magnitude below the levels shown to have no effect in laboratory toxicology testing. Also, these levels are considerably below the proposed threshold of toxicological concern (TTC) level of 1800 µg/person/day (Class 1, oral exposure) for chemicals considered to have a low potential for toxicity based on metabolism and mechanistic data (Vermeire et al. 2010). Transgenic proteins produced by genetically modified plants are generally considered non-toxic to humans.

The VKM GMO Panel notes that farm (production) animals e.g. pigs and poultry often are fed diets with a substantial inclusion of unprocessed maize grain, and that the exposure to transgenic proteins from maize Bt11 x MIR604 may be higher for these animals.

This dietary exposure assessment is very conservative as it assumes that all maize consumed comes from maize Bt11 x MIR604 and that the transgenic proteins are not denatured by processing.

4.6.2 Nutritional assessment of feed derived from the GM plant

Based on the compositional analyses of forage and grain samples from maize Bt11 x MIR604 x GA21; nutritional equivalence of maize Bt11 xMIR604 to non-GM maize shown in a broiler feeding study; and evaluation of the transgenic proteins expressed in maize Bt11 xMIR604, maize Bt11 x MIR604 and derived feed products seem to be substantially and nutritionally comparable to conventional maize and maize products, except for the expression of the transgenic proteins.

4.7 Conclusion

A whole food feeding study on broilers has not indicated any adverse effects of maize Bt11 x MIR604, and shows that maize Bt11 x MIR604 is nutritionally equivalent to conventional maize. The Cry1Ab, PAT, mCry3A, and PMI proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE-mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize Bt11 x MIR604 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1Ab, PAT, mCry3A, or PMI proteins will introduce a toxic or allergenic potential in food or feed based on maize Bt11 x MIR604 compared to conventional maize.

5 Environmental risk assessment

5.1 Unintended effects on plant fitness due to the genetic modification

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

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

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

It is considered very unlikely that the establishment, spread and survival of maize Bt11 x MIR604 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 target pests provides a potential advantage in cultivation of Bt11x MIR604 under infestation conditions. It is considered very unlikely that maize Bt11 x MIR604 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 Bt11x MIR604 relative to its conventional counterpart. A series of field trials with maize Bt11x MIR604 were carried out across ten locations in the USA in 2005 (application EFSA/GMO/UK/2007/50). Information on phenotypic (e.g. crop physiology, morphology, development) and agronomic (e.g. grain yield) characteristics was provided to assess the agronomic performance of maize Bt11x MIR604 in comparison with its conventional counterpart (see section 3.1). Maize Bt11 x MIR604 tended to flower a little earlier and had slight less grain moisture at harvest than the corresponding near-isogenic

hybrid, but did not show changes in plant characteristics that indicate altered fitness and invasiveness of maize Bt11 x MIR604 plants.

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 Bt11 x MIR604, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of maize Bt11 are unchanged, insect resistance, glufosinate and glyphosate tolerance are not likely to provide a selective advantage outside of cultivation in Europe. The VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects based on establishment and survival of maize Bt11 x MIR604 will not differ from that of conventional maize varieties.

5.2 Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or vertical gene flow via pollen or seed dispersal. Exposure of microorganisms to transgenic DNA occurs during decomposition of plant material remaining in the field after harvest or comes from pollen deposited on cultivated areas or the field margins. Transgenic DNA is also a component of a variety of food and feed products derived from maize Bt11 x MIR604. This means that micro-organisms in the digestive tract in humans and animals (both domesticated animals and other animals feeding on fresh or decaying plant material from the transgenic maize line) may be exposed to transgenic DNA.

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

5.2.1 Plant to micro-organisms gene transfer

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

Based on established scientific knowledge of the barriers for gene transfer between unrelated species and the experimental research on horizontal transfer of genetic material from plants to microorganisms, there is today little evidence pointing to a likelihood of random transfer of the transgenes present in maize Bt11 x MIR604 to unrelated species such as bacteria.

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

Experiments have been performed to study the stability and uptake of DNA from the intestinal tract in mice after M13 DNA was administered orally. The DNA introduced was detected in stool samples up to seven hours after feeding. Small amounts (<0.1%) could be traced in the blood vessels for a period of maximum 24 hours, and M13 DNA was found in the liver and spleen for up to 24 hours (Schubbert

et al. 1994). By oral intake of genetically modified soybean it has been shown that DNA is more stable in the intestine of persons with colostomy compared to a control group (Netherwood et al. 2004). No GM DNA was detected in the faeces from the control group. Rizzi et al. (2012) provides an extensive review of the fate of feed-derived DNA in the gastrointestinal system of mammals.

In conclusion, the VKM GMO Panel consider it is unlikely that the introduced genes from maize Bt11 x MIR604 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 *cry1Ab, pat, mcry3A* and *pmi* and genes from Bt11 x MIR604 to soil bacteria, no novel property would be introduced into or expressed in the soil microbial communities; as these genes are already present in other bacteria in soil. Therefore, no positive selective advantage that would not have been conferred by natural gene transfer between bacteria is expected.

5.2.2 Plant to plant gene flow

Considering the intended uses of maize Bt11 x MIR604 (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 Bt11 x MIR604 has no altered survival, multiplication or dissemination characteristics, the VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this GM maize in Norway will not differ from that of conventional maize varieties. The likelihood of cross-pollination between cultivated maize and the occasional feral maize plants resulting from grain spillage is considered extremely low.

5.3 Interactions between the GM plant and target organisms

Genetically modified maize Bt11 was developed to provide protection against a variety of target pests of the order Lepidoptera. Protection is achieved through expression in the plant of insecticidal Cry protein Cry1Ab, derived from *Bacillus thuringiensis*, a common soil bacterium. Cry1Ab, encoded by

the *cry1Ab* gene, is derived from *B. thuringiensis* subspecies *kurstaki*. Two Lepidoptera pests are primarily targeted by Bt11; *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.

Aphids are the only pests reported on maize in Norway. Studies have shown that aphids are not affected by the Cry1Ab protein (Bourguet et al. 2002). Under the development of Bt maize expressing Cry1Ab, the noctuid *Agrotis ipsilon* was tested as a target, but there was little or no effect (Pilcher et al. 1997). This species is occasionally a pest in root crops in Norway and it is conceivable that it could become a pest of maize.

Maize MIR604 was transformed to express a modified version of the Cry3A protein from *Bacillus thuringiensis* subsp. *tenebrionis*. The insecticidal toxin is active in the control of certain coleopteran insect pests belonging to the genus *Diabrotica,* such as larvae of western corn rootworm (WCR; *D. virgifera virgifera*) and the northern corn rootworm (NCR; *D. barberi*). WCR has been introduced to Europe from North America, where it is native and widespread (Miller et al. 2005, ref. EFSA 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 Bt11 x MIR604, excluding cultivation, the environmental exposure is limited to exposure through manure and faeces from the gastrointestinal tract mainly of animals fed on the GM maize as well as to the accidental release into the environment of GM seeds during transportation and processing and subsequently to potential occurrence of sporadic feral plants. Thus the level of exposure of target organisms to the Cry1Ab protein is likely to be extremely low and of no ecological relevance.

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

Considering the intended uses of maize stack Bt11 x MIR604, 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 Cry1Ab and mCry3A protein enters the environment due to expression in the grains (mean

value of 1.74 and 0.66 µg/g d.w., respectively). In addition, the data show that at least 99% of microbially produced Cry1Ab protein was rapidly degraded in simulated gastric fluid.

In conclusion, the VKM GMO Panel considers that the exposure of potentially non-target organisms to the Cry1Ab and mCry3A proteins is likely to be very low and of no ecological relevance.

5.5 Potential interactions with the abiotic environment and biochemical cycles

Considering the intended uses of maize Bt11 x MIR604, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with the abiotic environment and biogeochemical cycles were not considered an issue by the VKM GMO Panel.

5.6 Conclusion

The scope of the application EFSA/GMO/UK/2007/50 includes import and processing of maize Bt11x MIR604 for food and feed uses. Considering the intended uses of maize Bt11 x MIR604, excluding cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize Bt11 x MIR604.

Maize Bt11 x MIR604 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 Bt11. Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation. The risk of gene flow from occasional feral GM maize plants to conventional maize varieties is negligible. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered to be an issue.

6 Data gaps

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

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

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

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

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

Since it is difficult to predict the toxicity of cocktails from the toxicity of the single components, there is uncertainty related to risk of confounding effects such as additive or synergistic effects between the residues in herbicide resistant plants.

The transgene technology used can possibly lead to different metabolic products of the applied herbicides from what is expected from conventional usage. The risk assessment of herbicides should take into account plants with altered metabolism.

At present the changes related to herbicide residues of stacked plants as a result of the application of plant-protection products fall outside the remit of the Norwegian VKM Panels.

7 Conclusions

Molecular characterisation

Conventional crossing methods were used to produce the stacked maize Bt11 x MIR604. Southern blot analyses have indicated that the recombinant inserts in the parental maize lines Bt11 and MIR604 are retained in the stacked maize Bt11 x MIR604. Genetic stability of the inserts has previously been demonstrated in the parental events. Protein measurements show comparable levels of the Cry1Ab, mCry3A, PAT and PMI proteins between the stacked and single maize lines.

The VKM Panel on GMO considers the molecular characterisation of maize Bt11 x MIR604 and single maize events Bt11 and MIR604 as adequate.

Comparative assessment

Comparative analyses of agronomic and phenotypic data from field trials located at representative sites and environments in USA in 2005 indicate that maize stack Bt11 x MIR604 is equivalent to its conventional counterpart, with the exception of the insect resistance and the herbicide tolerance, conferred by the Cry1Ab, mCry3A and PAT proteins. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of maize Bt11 x MIR604 compared to conventional maize varieties.

The applicant has performed a compositional analysis on the triple-stack Bt11 x MIR604 x GA21 instead of maize Bt11 x MIR604. The analysis was performed on plant materials from maize Bt11 x MIR604 x GA21 and a near-isogenic control hybrid from field trials in USA in 2006. With the exception of small intermittent variations, no biologically significant compositional differences were found between the triple-stack and the near-isogenic control. The results of the study are considered valid by EFSA also for maize Bt11 x MIR604, since maize Bt11 x MIR604 x GA21 encompasses the transgenic properties of maize Bt11 x MIR604. This is in accordance with the EFSA guidance document for the risk assessment of genetically modified plants containing stacked transformation events (EFSA, 2007b).

The VKM GMO Panel is of the opinion that the applicant should have performed a compositional analysis of maize Bt11 x MIR604 and not only referred to analyses of the triple- stack Bt11 x MIR604 x GA21. However, based on all information available, including agronomic and phenotypic data from field trials with maize Bt11 x MIR604, a feeding study on broilers showing nutritional equivalence to non-GM maize, and assessments of the single events Bt11 and MIR604, the VKM GMO Panel concludes that forage and grain from maize Bt11 x MIR604 are compositionally equivalent to its conventional counterpart.

Food and feed risk assessment

A whole food feeding study on broilers has not indicated any adverse effects of maize Bt11 x MIR604, and shows that maize Bt11 x MIR604 is nutritionally equivalent to conventional maize. The Cry1Ab, PAT, mCry3A, and PMI proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE-mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize Bt11 x MIR604 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1Ab, PAT, mCry3A, or PMI proteins will introduce a toxic or allergenic potential in food or feed based on maize Bt11 x MIR604 compared to conventional maize.

Environmental risk assessment

The scope of the application EFSA/GMO/UK/2007/50 includes import and processing of maize stack Bt11x MIR604 for food and feed uses. Considering the intended uses of maize Bt11 x MIR604, excluding cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize Bt11 x MIR604.

Maize Bt11 x MIR604 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 Bt11 x MIR604. 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

Based on current knowledge, the VKM GMO Panel concludes that maize Bt11 x MIR604 is nutritionally equivalent to its conventional counterpart, except for the presence of the newly expressed proteins. It is unlikely that the Cry1Ab, PAT, mCry3A, or PMI proteins will introduce a toxic or allergenic potential in food or feed derived from maize Bt11 x MIR604 compared to conventional maize.

The VKM GMO Panel likewise concludes that maize Bt11 x MIR604, 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 nonagiodes* (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

Bensasson D, Boore JL, Nielsen KM (2004) Genes without frontiers. Heredity 92: 483-489

Brandtzaeg P, Tolo K (1977) Mucosal penetrability enhanced by serum-derived antibodies. Nature 266: 262-263.

Brooks KJ, DeWildt PM (2000a) PS149B1 14KDa protein: Acute oral toxicity study in CD1 mice. Study number 001128. Unpublished technical report. Dow Chemical company.

Brooks KJ, DeWildt PM (2000b) PS149B1 14 kDa protein: acute oral toxicity study in CD-1 mice. Study number 001130. Unpublished technical report. Dow AgroSciences LLC

Brooks KJ, DeWildt PM (2000c) PS149B1 44 kDa protein: acute oral toxicity study in CD-1 mice. Study number 001129. Unpublished technical report. Dow AgroSciences LLC

Brooks KJ, DeWildt PM (2000d) PS149B1 14 kDa and 44 kDa proteins: acute oral toxicity study in CD-1 mice. Study number 001128. Unpublished technical report. Dow AgroSciences LLC

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

Cressman RF (2003) Evaluation of the sequence similarities of the Cry34Ab1, Cry35Ab1, and PAT proteins the public protein sequence dataset. Pioneer Hi-Bred International, Inc.

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

Dien BS, Bothast RJ,. Iten LB, Barrios L, Eckhoff SR (2002) Fate of Bt Protein and Influence of Corn Hybrid on Ethanol Production. Cereal Chem 79(4): 582-585.

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

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.

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

EFSA (2008) Scientific Opinion of the Panel on Genetically Modified Organisms on application (Reference EFSA-GMO-UK-2005-20) for the placing on the market of the insect-resistant and herbicide-tolerant genetically modified maize 59122 x NK603, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred International. The EFSA Journal 874,1-34.

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_ConsolidatedARG_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 59122 x NK603, 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 option 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.

Hammond B, Dudek R, Lemen J, Nemeth M (2004) Result of a 13 week safety assurance study with rats fed grain from glyphosate tolerant maize. Food and Chemical Toxicology, 42: 1003-1014

Hammond BG, Jez JM (2011) Impact of food processing on the safety assessment for proteins introduced into biotechnology-derived soybean and corn crops. Food Chem Tox 49(4):711-721.

Harrison, L.A., Michele, R.B., Naylor, M.W., Ream, J.E., Hammond, B.G., Nida, D.L.,Burlette, B.L., Nickson, T.E., Mitsky, T.A., Taylor, M.L., Fuchs, R.L., Padgette, S.R., (1996) The expressed protein in glyphosate-tolerant soybean, 5-enolypyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. Strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. J. Nutr: 126, 728–740.

Hérouet, C., Esdaile, D.J., Mallyon, B.A., Debruyne, E., Schulz, A., Currier, T., Hendrickx, T., van der Klis, R.-J., Rouan, D., 2005. Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the *pat* and *bar* sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants. Regul. Toxicol. Pharm. 41, 134–149.

Icoz, Stotzky G (2008) Fate and effects of insect-resistant Bt crops in soil ecosystems. Soil Biology and Biochemistry 40: 559–586

Juberg DR, Herman RA, Thomas J, Brooks, Delaney (2009) Acute and repeated dose (28 day) mouse oral toxicology studies with Cry34Ab1 and Cry35Ab1 Bt proteins used in coleopteran resistant DAS-59122-7 corn. Regul. Toxicol. Pharm. 54, 154-163

Lid J, Lid DT (2005) Norsk flora. Det Norske Samlaget, Oslo. 7. utgave. 1230s

Lim PL, Rowley D. (1982) The effect of antibody on the intestinal absorption of macromolecules and on intestinal permeability in adult mice. Int Arch Allergy Appl Immunol. 68(1):41-6.

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

Malley (2004) Thirteen-Week Feeding Study with Transgenic Maize Grain (DAS-59122-7) in Rats. Study number PHI-2003-060. Unpublished technical report. Pioneer Hi-Bred International, Inc

Metcalfe DD, Astwood DA, Townsend R, Sampson HG, Taylor SL, Fuchs RL (1996) Assessment of the allergenic potential of food derived from genetically engineered crop plants. Food Science and Nutrition 36;165-186

Meyer T (1999) Comparison of amino acid sequence similarity of Cry1F and PAT proteins to known allergen proteins. Pionner Hi-Bred Interational, Inc.

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 (2002) Consensus document on compositional considerations for new varieties of maize (*Zea mays*): Key food and feed nutrients, anti nutrients and secondary plant metabolites. Organisation for Economic Co-operation and Development, Paris. ENV/JM/MONO(2002)25

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

Palaudelmás 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

Pfister T, Schmid H, Luetkemeier H, Biedermann K, Weber K (1996) PAT protein repeated dose oral toxicity (14-day feeding) study in rats.

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

Séralini G-E, Clair E, Mesnage R, Gress S, Defarge N, Malatesta M, Hennequin D, de Vendomois JS. (2012). Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food Chem Toxicol. 50; 4221-4231.

Thomas J, Yano BL, Card TL (2006) Cry34Ab1/Cry35Ab1 proteins: 28-day dietary toxicity study in CRL: CD1(ICR) Mice. Study ID: 061128. Dow AgroSciences unpublished internal report.

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).

Vázquez RI, Moreno-Fierros L, Neri-Bazán L, De La Riva GA, López-Revilla R (1999) Bacillus thuringiensis Cry1Ac protoxin is a potent systemic and mucosal adjuvant. Scand J Immunol. 49(6):578-84.

Vermeire T, van de Bovenkamp M, de Bruin YB, Delmaar C, van Ehgelen J, Escher S, Marquart H, Meijsyer T (2010) Exposure based waiving under REACH. Regul. Toxicol. Pharmacol. 58 (3), 408–420.

Vikse R (2009) Betydning av mais i kostholdet i Norge. Unpublished report

Vijayakumar KR, Martin A, Gowda LR, Prakash V (2009) Detection of genetically modified soya and maize: Impact of heat processing. Food Chem 117: 514-521

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

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

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

VKM (2005d) Foreløpig helserisikovurdering av genmodifisert herbicidtolerant mais NK603 (C/ES/00/01) til bruk som næringsmiddel og fôrvare. Uttalelse fra Faggruppe for genmodifiserte organismer 08.3. 2005 (05/307-endelig). Vitenskapskomiteen for mattrygghet, Oslo, Norge. http://www.vkm.no/dav/83e1c98a93.pdf

VKM (2007a) Foreløpig helserisikovurdering av genmodifiserte mais 59122 x NK603 (EFSA/GMO/NL/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

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 (07/316-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

VKM (2007d) Vurdering av foreslåtte virkemidler for sameksistens mellom genmodifiserte vekster og konvensjonelt/økologisk landbruk, og rangering av spredningsrisiko av transgener fra relevante genmodifiserte planter som kan dyrkes i Norge. Uttalelse fra Faggruppe for genmodifiserte organismer 21.12.06 (06/305). Vitenskapskomiteen for mattrygghet, Oslo, Norge.
http://www.vkm.no/eway/default.aspx?pid=277&trg=Main_6177&Main_6177=6187:1663908::0:6720:4:::0:0

VKM (2008a) Foreløpig miljørisikovurdering av genmodifisert mais 59122 x NK603 (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

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

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

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

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

VKM (2012) Foreløpig helse- og miljørisikovurdering av genmodifisert mais 59122 x NK603 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

VKM (2012) Vurdering av publikasjonen "Long term toxicity of a Roundup herbicide and a Roudup-tolerant genetically modified maize" av Séralini et al. Dokument nr. 12/314. Avsluttet 20.12.2012
http://www.vkm.no/eway/default.aspx?pid=277&Main_6177=6556:0:&trg=Main_6177

VKM (2012b) Helserisikovurdering av Cry-proteiner adjuvanseffekt. Uttalelse fra Faggruppe for

genmodifiserte organismer 25.4.2012 -11/313/3-endelig. Vitenskapskomiteen for mattrygghet, Oslo, Norge.
http://www.english.vkm.no/eway/default.aspx?pid=278&trg=Content_6444&Main_6359=6582:0:31,2564&Content_6444=6393:1986886::0:6596:1:::0:0