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 GA21 in the European Union under Regulation (EC) No 1829/2003 (EFSA/GMO/UK/2007/49)

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

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

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Summary

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Environment Agency (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 authorized 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 authorized in the European Union. The request covers scope(s) relevant to the Gene Technology Act. The request does not cover GMOs that VKM already has conducted its final risk assessments on. However, the Agency requests VKM to consider whether updates or other changes to earlier submitted assessments are necessary.

The insect-resistant and herbicide-tolerant genetically modified maize Bt11 x GA21 (Unique Identifier SYN-BTØ11-1 x MON-ØØØ21-9) from Syngenta Seeds is approved under Regulation (EC) No 1829/2003 for food and feed uses, import and processing since 28 July 2010 (Commission Decision 2010/4263/EC). Genetically modified maize Bt11 x GA21 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/2007/49 in 2008 (VKM 2009a). In addition, Bt11 and GA21 has 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, 2010, 2012a,b).

The food/feed and environmental risk assessment of the maize Bt11x GA21 is based on information provided by the applicant in the application EFSA/GMO/UK/2007/49, 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 GA21 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 Bt11x GA21 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 emphasized that the VKM mandate does not include assessments of contribution to sustainable development, societal utility and ethical considerations, according to the Norwegian Gene Technology Act and Regulations relating to impact assessment pursuant to the Gene Technology Act. These

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

The genetically modified maize stack Bt11 x GA21 has been produced by conventional crossing between inbred lines of maize containing the single events Bt11 and GA21. The F_1 hybrid was developed to provide protection against certain lepidopteran target pests, and to confer tolerance to glufosinate-ammonium and glyphosate-based herbicides.

Molecular characterisation

Southern blot and PCR analyses have indicated that the recombinant inserts in the parental maize lines Bt11 and GA21 are retained in the stacked maize Bt11 x GA21. Genetic stability of the inserts has previously been demonstrated in the parental maize lines. Protein measurements show comparable levels of the Cry1Ab, PAT and mEPSPS proteins between the stacked and single maize lines. Phenotypic analyses also indicate stability of the insect resistance and herbicide tolerance traits in the stacked maize. The VKM Panel on GMO considers the molecular characterisation of maize Bt11 x GA21 and its parental events Bt11 and GA21 as adequate.

Comparative assessment

Comparative analyses of data from field trials located at representative sites and environments in North America during the 2005 growing season indicate that maize stack Bt11 x GA21 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, with the exception of the insect resistance and the herbicide tolerance, conferred by the expression of Cry1Ab, PAT and mEPSPS proteins.

Based on the assessment of available data, the VKM GMO Panel is of the opinion that conventional crossing of maize Bt11 and GA21 to produce the hybrid Bt11 x GA21 does not result in interactions between the newly expressed proteins affecting composition and agronomic characteristics.

Food and feed risk assessment

A whole food feeding study on broilers has not indicated any adverse health effects of maize Bt11 x GA21, and shows that maize Bt11 x GA21 is nutritionally equivalent to conventional maize. The Cry1Ab, PAT or mEPSPS 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 GA21 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1Ab, PAT or mEPSPS proteins will introduce a toxic or allergenic potential in food or feed based on maize Bt11 x GA21 compared to conventional maize.

Environmental risk assessment

The scope of the application EFSA/GMO/UK/2007/49 includes import and processing of maize stack Bt11x GA21 for food and feed uses. Considering the intended uses of maize Bt11 x GA21, 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 GA21.

Maize Bt11 x GA21 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 GA21. 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 GA21 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1Ab, PAT or mEPSPS proteins will introduce a toxic or allergenic potential in food or feed based on maize Bt11 x GA21 compared to conventional maize.

The VKM GMO Panel likewise concludes that maize Bt11 x GA21, 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 GA21, EFSA/GMO/UK/2007/49, insect-resistance, herbicide-tolerance, Cry protein, *cryAb1, mepsps,* PAT, mCP4 EPSPS, glufosinate-ammonium, glyphosate, food and feed risk assessment, environmental risk assessment, Regulation (EC) No 1829/2003

Norsk sammendrag

I forbindelse med forberedelse til implementering av EU-forordning 1829/2003 i norsk rett 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 GA21 (Unik kode SYN-BTØ11-1 x MON-ØØØ21-9) fra Syngenta Seeds Inc. ble godkjent til import, videreforedling og bruk som mat og fôr under EU-forordning 1829/2003 i 2010 (søknad EFSA/GMO/UK/2007/49). Bt11 x GA21 er resultat av konvensjonelle kryssinger mellom innavlede maislinjer med eventene Bt11 og GA21. Kryssingene er utført for å utvikle en maishybrid med resistens mot visse skadegjørere i sommerfuglordenen Lepidoptera og toleranse mot herbicider med virkestoff glyfosat og glufosinat-ammonium. Maishybrid Bt11 x GA21 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 GA21 er også tidligere risikovurdert av VKM, både som enkelteventer og i en rekke andre hybrider (VKM 2005a,b, 2007, 2008, 2009b,c,d, 2010, 2012a,b).

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.

Foreldrelinjen Bt11 inneholder de bakterielle genene *cry1Ab* og *pat*, fra henholdsvis *Bacillius 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 GA21 er fremkommet ved biolistisk transformasjon av embryonale maisceller fra en ikke navngitt maislinje. Den innsatte genkonstruksjonen inneholder et endogent 5-enolpyruvylsikimat-3-fosfatsyntetase (*mepsps*)-gen, som er modifisert ved hjelp av *in vitro*-mutagenese. *Mepsps*-genet koder for enzymet 5-enolpyruvylsikimat-3-fosfatsyntetase (mEPSPS), som omdanner fosfoenolpyruvat og sikimat-3-fosfat til 5-enolpyruvylsikimat-3-fosfat, viktige metabolitter i syntesen av aromatiske aminosyrer. N-fosfonometylglycin er et systemisk, ikke selektivt herbicid som hemmer EPSPS-enzymer og derved blokkerer biosyntesen av aromatiske aminosyrer i planter. I motsetning til plantens EPSPS-enzym er det modifiserte mEPSPS-enzymet fra mais også aktivt ved nærvær av glyfosat.

Molekylær karakterisering
Maishybriden Bt11 x GA21 er dannet ved konvensjonelle kryssinger mellom maislinjene Bt11 og GA21. Spaltingsdata, Southern blot og PCR-analyser indikerer at de rekombinante innskuddene fra mais Bt11 og GA21 er stabilt nedarvet i mais Bt11 x GA21, og at antall innsatte gener, struktur og organiseringen av disse er ekvivalent med de som finnes i mais Bt11 og GA21. Nivåene av Cry1Ab, PAT og mEPSPS-proteiner i vegetativt vev og korn fra mais Bt11 x GA21 er også sammenlignbare med nivåene i henholdsvis mais Bt11 og GA21.

Komparative analyser
Data fra feltforsøk i Nord Amerika vekstsesongen 2005 indikerer, med unntak av insektsresistens og herbicidtoleranse, ekvivalens mellom maishybrid Bt11 x GA21 og korresponderende, nær-isogen kontrollhybrid med hensyn på ernæringsmessige, agronomiske og fenotypiske karakterer.

Basert på tilgjengelig dokumentasjon, konkluderer VKMs GMO-panel med at konvensjonelle kryssinger mellom de genmodifiserte maislinjene Bt11 og GA21 ikke resulterer i nye interaksjoner mellom genproduktene fra de genmodifiserte foreldrelinjene som påvirker ernæringsmessige og agronomiske karakterer i hybriden Bt11 x GA21.

Helserisiko
I en fôringsstudie utført på broilere ble det vist at mais Bt11 x GA21 ikke førte til negative helseeffekter blant dyrene, og at maisen var ernæringsmessig ekvivalent konvensjonell mais. De introduserte proteinene Cry1Ab, PAT og mEPSPS 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 GA21 er ernæringsmessig ekvivalent konvensjonell mais. Det er lite sannsynlig at proteinene Cry1Ab, PAT eller mEPSPS vil introdusere et toksisk eller allergent potensiale i mat eller fôr basert på mais Bt11 x GA21 sammenliknet med konvensjonelle maissorter.

Miljørisiko
Søknaden EFSA/GMO/UK/2007/49 gjelder godkjenning av maislinjen Bt11 x GA21 for import, prosessering og til bruk i næringsmidler og fôrvarer, og omfatter ikke dyrking. Med bakgrunn i tiltenkt bruksområde er miljørisikovurderingen avgrenset til mulige effekter av utilsiktet frøspredning i forbindelse med transport og prosessering, samt indirekte eksponering gjennom gjødsel fra husdyr fôret med genmodifisert mais.

Det er ingen indikasjoner på økt sannsynlighet for spredning, etablering og invasjon av maislinjen i naturlige habitater eller andre arealer utenfor jordbruksområder som resultat av frøspill i forbindelse

med transport og prosessering. Risiko for utkryssing med dyrkede sorter vurderes til å være ubetydelig. Ved foreskreven bruk av maislinjen Bt11 x GA21 antas det ikke å være risiko for negative 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 GA21 er ernæringsmessig ekvivalent konvensjonell mais. Det er lite sannsynlig at proteinene Cry1Ab, PAT eller mEPSPS vil introdusere et toksisk eller allergent potensiale i mat eller fôr basert på mais Bt11 x GA21 sammenliknet med konvensjonelle maissorter.

Faggruppen finner at maishybrid Bt11 x GA21, 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*
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

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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/2007/49) for authorisation of the insect-resistant and herbicide-tolerant genetically modified (GM) maize Bt11 x GA21 (Unique Identifier SYNBTØ11-1 x MON-ØØØ21-9), 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 GA21
- GM plants for food and feed use
- Food and feed, containing or consisting of maize Bt11 x GA21
- Food and feed produced from maize Bt11 x GA21
- Food containing ingredients produced from maize Bt11 x GA21

After receiving the application EFSA/GMO/UK/2007/49 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 19 February 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 VKM GMO Panel assessed the application in connection with the EFSA official hearing, and submitted a preliminary opinion in April 2009 (VKM 2009a). The EFSA GMO Panel published its scientific opinion in September 2009 (EFSA 2009). The Commission Decision 2010/4263/EC authorised the placing on the market of products containing, consisting of, or produced from maize Bt11 x GA21 pursuant to Regulation (EC) No 1829/2003 (EC 2008) on 28 July 2010.

Genetically modified maize Bt11 x GA21 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/2007/49 in 2008 (VKM 2009). In addition, Bt11 and GA21 has been evaluated by the VKM GMO Panel as single events and as a component of several stacked GM maize events (VKM 2005a,b,c, 2007, 2009b,c,d,e, 2010, 2011, 2012a,b,).

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 and GA21. 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 authorized in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC within the Authority's sectoral responsibility. The request covers scope(s) relevant to the Gene Technology Act.

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

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

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

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

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

Assessment

1 Introduction

Maize Bt11 x GA21 has been obtained from traditional breeding methods between progeny (inbred lines) of the genetically modified maize lines Bt11 and GA21.

The parental line Bt11 has been developed to provide protection against certain lepidopteran target pests, such as the European corn borer (*Ostrinia nubilalis*), and species belonging to the genus *Sesamia* (in particular the Mediterranean corn borer (*Sesamia nonagrioides*)), by the introduction of a part of a *Bacillus thuringiensis* (*Bt*) gene encoding the insecticidal Cry1Ab protein. None of the target pests for maize Bt11 are present in the Norwegian agriculture. Maize Bt11 also expresses the phosphinothricin-N-acetyltransferase (PAT) protein from *Streptomyces viridochromogenes*, which confers tolerance to the herbicidal active substance glufosinate-ammonium. The PAT protein expressed in maize Bt11 has been used as selectable marker to facilitate the selection process of transformed plant cells, and is not intended for weed management purposes.

The parental line GA21 was developed to provide tolerance to the herbicidal active substance glyphosate by the introduction of a gene coding for the modified enzyme 5-enolpyruvylshikimate-3-phosphate synthase (mEPSPS). Glyphosate is normally phytotoxic to a broad range of plants. Its mode of action is to bind to and competitively inhibit the EPSPS protein, which is the key enzyme in the shikimate pathway that leads to the biosynthesis of the aromatic amino acids tyrosine, tryptophan and phenylalanine. The disruption of this pathway and the resulting inability to produce key amino acids prevents growth and ultimately leads to plant death. In the case of maize GA21, a gene has been introduced that codes for the expression of the mEPSPS protein, which is insensitive towards inhibition by glyphosate. This protein is similar to the native EPSPS in maize, but it is not inhibited by glyphosate thus allowing the crop to be protected from the recommended dosages of glyphosate.

Maize stack Bt11 x GA21 (Unique Identifier SYN-BT Ø11-1 x MON-ØØØ21-9) has been evaluated with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed.

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

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

2 Molecular characterisation

2.1 Evaluation of relevant scientific data

2.1.1 Method of production of maize Bt11 x GA21

Conventional breeding methods were used to produce maize Bt11xGA21 and no new genetic modification was involved. The two inserts that are present in maize Bt11xGA21 were derived from maize lines containing two independent events: Bt11 and GA21. Each of these single maize events was the subject of earlier safety evaluation and opinions (VKM 2014a,b). Maize Bt11 x GA21 combines insect resistance and glufosinate-ammonium tolerance traits from maize Bt11, and glyphosate tolerance from maize GA21.

2.1.2 Summary of evaluation of the single events

Maize Bt11

Maize Bt11 was generated by transformation of a proprietary inbred maize line, H8540 *(Zea mays)*, using 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 using 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.

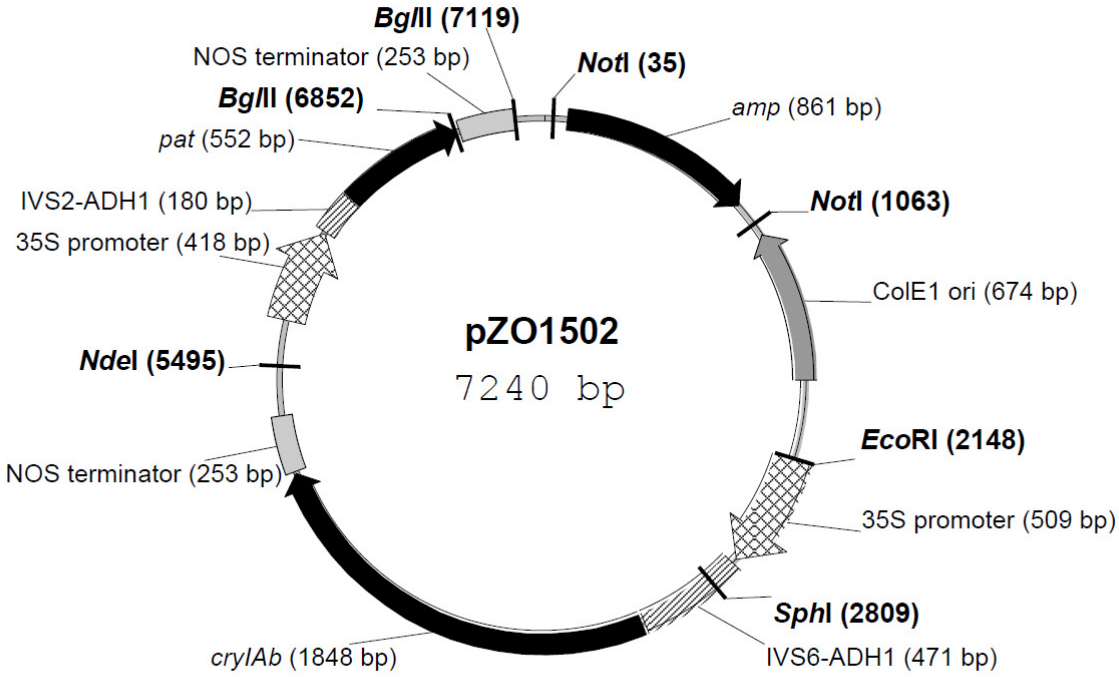

Figure 1. Various gene elements of t transformation vector pZO1502 used for generation of the maize strain Bt11.

Maize GA21

Maize GA21 was generated by microprojectile bombardment transformation with a 3.49 kb *Not*I restriction fragment of the plasmid pDPG434 (derived from pUC19). The plasmid was derived from a pSK- vector, commonly used in molecular biology and is derived from pUC19. The DNA fragment used for transformation consisted of the following m*epsps* cassette: the rice actin promoter (5‟ region of the rice actin 1 gene containing the promoter and first non-coding exon and intron), an optimised transit peptide containing sequences from maize and sunflower, a modified maize *epsps* coding sequence (m*epsps*), and the 3‟ nos terminator from *Agrobacterium tumefaciens*. The mutations in the

coding sequence of the maize *epsps* gene led to amino acid changes at positions 102 (threonine to isoleucine) and 106 (proline to serine). As a result of these mutations, the m*epsps* containing maize line GA21 is tolerant to glyphosate-based herbicides. The vector backbone contained the origin of replication (*ori* ColE1), the *lac* sequence as present in pUC19, and the bacterial *bla* gene conferring resistance to ampicillin in bacteria. The mEPSPS is only different from the naturally present EPSPS protein by two amino acids.

Southern analyses showed that the insert in maize GA21 consists of six contiguous complete or truncated versions (fragments 1 to 6) of the 3.49 kb *Not*I restriction fragment. The insertions are located at a single locus. The absence of vector backbone sequences in GA21 plants has been demonstrated using a probe specific for the pDPG434 vector backbone. Therefore, the *bla* gene has not been transferred to maize GA21.

The nucleotide sequence of the insert introduced into maize GA21 has been determined in its entirety. Fragment 1 contains the rice actin promoter with a deletion of 696 bp at the 5‟ end, the actin first exon and intron, the optimized transit peptide, the *mepsps* gene and *nos* terminator. Fragments 2, 3 and 4 are complete versions of the 3.49 kb NotI fragment. Fragment 5 contains the complete rice actin promoter, the actin first exon and intron, the optimized transit peptide, and 288 bp of the *mepsps* gene which ends in a stop codon. Fragment 6 contains the rice actin promoter and the actin first exon truncated but no other elements. A single base pair change was observed in the *nos* terminator in fragments 1 and 2 (nucleotide C instead of G). In addition, a single base pair deletion is observed in the actin promoter of fragment 6. The observed mutations do not have an impact on the amino acid sequence of the newly expressed protein.

The sequences of 1 kb of the plant genome adjacent to the 3‟ and 4.2 kb at the 5‟ end were also determined and bioinformatic analysis gave no indication that the sequence was inserted in a functional maize gene. The 3‟ sequence shows homology to repetitive sequences in the maize genome. The 5‟ flanking sequence was shown to be of chloroplast origin. The five putative ORFs found at the junction between the insert and the plant DNA show no significant sequence homology to any known toxic proteins and allergens. One potential new ORF was apparently created at the junction between fragment 5 and 6 but lacked the necessary components to be transcribed. This ORF does not show homology to known or putative allergens or toxic proteins. Updated (2008) bioinformatic analysis of the 5‟ and 3‟ flanking regions of the GA21 insert provided data which were similar to that previously reported and 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 concentrations of the mEPSPS protein in maize plants derived from GA21were examined by ELISA in several plant tissues and whole plants at four growth stages (whorl, anthesis, seed maturity and senescence) in two maize hybrids. Across all growth stages, mean mEPSPS concentrations measured in leaves, roots and whole plants ranged from below the limit of quantification (<0.2 µg/g fw) to 15 µg/gfw (<0.4—71 µg/g dw). Mean mEPSPS concentrations measured in grain ranged from 4—7 µg/g fw (5—10 µg/gdw) and in pollen averaged 168 µg/g fw.

The inheritance of the introduced glyphosate tolerant phenotype follows a Mendelian segregation pattern and the mEPSPS protein is stably expressed in maize GA21 across multiple generations. Southern analysis demonstrated that the insert in maize GA21 is stably inherited over three backcross generations.

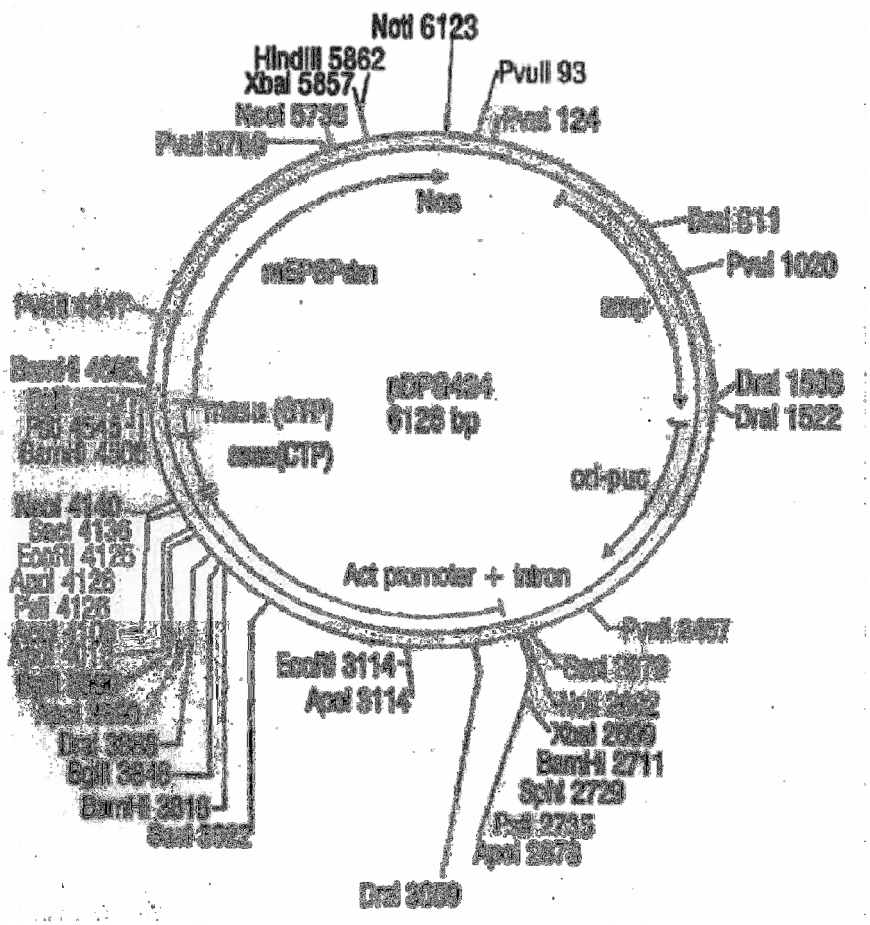

Figure 2. Various gene elements of t transformation vector pDPG434 used for generation of the maize strain GA21.

2.1.3 Transgene constructs in Bt11 x GA21 maize

Bt11 x GA21 maize was produced by combining Bt11 maize and GA21 maize through conventional breeding. Therefore, Bt11 x GA21 maize express the three transgenic proteins present in Bt11 maize and GA21 maize plants: Cry1Ab, PAT and mEPSPS.

Comparative Southern blot analysis of Bt11 x GA21 maize with the individual Bt11 and GA21 maize events was conducted to determine the hybridization patterns following stacking of the genes by traditional breeding methods. Southern blot analysis comparing Bt11 x GA21 maize with individual parental Bt11 and GA21 lines indicate that Bt11 x GA21 maize has stably inherited the *cry1Ab* and *pat* genes from the parent Bt11 maize and the *mepsps* gene from the parent GA21 maize.

For each Southern analysis performed, genomic DNA samples from the single events, the hybrid and non-transgenic control were analyzed *via* three restriction enzyme digestion strategies. A comparison of hybridization patterns for Bt11 maize and the Bt11 x GA21 hybrid was performed employing *cry1Ab*- and *pat*-specific probes. A comparison of hybridization patterns for GA21 maize and the Bt11 x GA21 hybrid was performed employing the *mepsps*-specific probe. A non-transgenic control was included in each Southern analysis to identify any endogenous *Zea mays* sequences that cross-hybridize with the element-specific probe.

***cry1Ab* specific probe**
According to the applicant, genomic DNA from Bt11 maize and the Bt11 x GA21 hybrid digested with *Nde* I produced a single hybridization signal of approximately 4.6 kb corresponding to a single copy of the *cry1Ab* gene present in Bt11 maize and the Bt11 x GA21 hybrid. Genomic DNA from Bt11 maize and the Bt11 x GA21 hybrid digested with *Sph*I produced a single hybridization signal of approximately 20 kb corresponding to a single copy of the *cry1Ab* gene present in Bt11 maize and the Bt11 x GA21 hybrid. Genomic DNA from Bt11 maize and the Bt11 x GA21 hybrid digested with *Bgl*II + *Eco*RI produced a single hybridization signal of approximately 4.7 kb corresponding to a single copy of the *cry1Ab* gene present in Bt11 maize and the Bt11 x GA21 hybrid and indicating the intactness of the insert.

According to the applicant, the hybridization pattern was identical between Bt11 maize and the Bt11 x GA21 hybrid. GA21 maize and non-transgenic maize showed no hybridization signal. The hybridization patterns for Bt11 maize and the Bt11 x GA21 hybrid in the Southern analysis were identical, showing the integrity of the *cry1Ab* cassette during conventional breeding.

***pat* specific probe**
According to the applicant, genomic DNA from Bt11 maize and the Bt11 x GA21 hybrid digested with *Nde*I produced a single hybridization signal of approximately 1.9 kb corresponding to a single copy of the *pat* gene present in Bt11 maize and the Bt11 x GA21 hybrid. Bt11 x GA21 hybrid digested with *Sph*I produced a single hybridization signal of approximately 20 kb corresponding to a single copy of the *pat* gene present in Bt11 maize and the Bt11 x GA21 hybrid. Genomic DNA from Bt11 maize and the Bt11 x GA21 hybrid digested with *Bgl*II + *Eco*RI produced a single hybridization signal of approximately 4.7 kb corresponding to a single copy of the *pat* gene present in Bt11 maize and the Bt11 x GA21 hybrid and indicating the intactness of the insert.

In all digests, the hybridization pattern was identical between Bt11 maize and the Bt11 x GA21 hybrid. GA21 maize and the non-transgenic maize showed no hybridization signal. The hybridization patterns for Bt11 maize and the Bt11 x GA21 hybrid in the Southern analysis were identical, showing the integrity of the *pat* cassette during conventional breeding.

***mepsps* specific probe**
According to the applicant, genomic DNA from GA21 maize and the Bt11 x GA21 hybrid digested with *Hin*dIII produced three unique hybridization bands of approximately 3.5 kb, 4.7 kb and 6.7 kb corresponding to the multiple copies of the *mepsps* gene present in GA21 maize and the Bt11 x GA21 hybrid. There were also a hybridization band representing an endogenous maize sequence at approximately 16 kb present in Bt11 maize, GA21 maize, the Bt11 x GA21 hybrid and the non-transgenic control.

Genomic DNA from GA21 maize and the Bt11 x GA21 hybrid digested with *Sph*I produced three unique hybridization bands of approximately 2.1 kb, 3.5 kb and 16 kb corresponding to the multiple copies of the *mepsps* gene present in GA21 maize and the Bt11 x GA21 hybrid. There were also a hybridization band representing an endogenous maize sequence at 8 kb present in Bt11 maize, GA21 maize, the Bt11 x GA21 hybrid and the non-transgenic control.

Genomic DNA from GA21 maize and the Bt11 x GA21 hybrid digested with *Sac*I and produced two unique hybridization bands of approximately 3.5 kb and 2.1 kb corresponding to the multiple copies of the *mepsps* gene present in GA21 maize and the Bt11 x GA21 hybrid. There were also a hybridization band representing an endogenous maize sequence at approximately 5.5 kb present in Bt11 maize, GA21 maize, the Bt11 x GA21 hybrid and the non-transgenic control.

According to the applicant, the hybridization pattern was identical between GA21 maize and the Bt11 x GA21 hybrid. The Bt11 maize and the non-transgenic control showed no hybridization other than that observed with an endogenous maize sequence.

2.1.3.1 Information on the expression of insert

ELISA was used to compare the concentrations of Cry1Ab, PAT and mEPSPS proteins produced in the plants of maize Bt11xGA21 grown alongside the single maize events Bt11 and GA21 in a single field trial in 2005 in the United States. The concentrations of the proteins were determined in several plant tissues (leaf, root, kernel, pollen) at three different growth stages from Bt11 maize, GA21 maize and Bt11 x GA21.To control for background effects, the corresponding tissues from near-isogenic control maize were also analyzed.

For Cry1Ab and PAT, the overall concentrations were generally comparable between the Bt11 x GA21 hybrid and the Bt11 hybrid. Similarly, for the mEPSPS protein, the overall concentrations were also generally comparable between the Bt11 x GA21 hybrid and the GA21 hybrid. The Cry1Ab, PAT or mEPSPS proteins were not detected in the near iso-genic control samples. Although some statistically significant differences were seen, these differences were small or not consistent across the growing season. According to the applicant, the results support the conclusion that transgenic protein expression in Bt11 x GA21 maize is not substantially different from that of the Bt11 and GA21 maize.

Cry1Ab protein
According to the applicant all Cry1Ab expression data are calculated on a dry-weight (dw) and fresh-weight (fw) basis. The Cry1Ab concentrations are presented for grains on a dry-weight basis in Bt11 and Bt11 x GA21. At the seed maturity stage the concentration in Bt 11 and Bt11 x GA21 was 1.24 ± 0.32 (range 0.84-1.60) µg/gdw and 0.99 ± 0.15 (range: 0.86-1.18) µg/gdw, repectively. According to the applicant there were no statistically significant differences between the mean concentrations of protein in maize Bt11 and Bt11xGA21 plant tissues, except for roots at the anthesis stage.

No statistical analysis of Cry1Ab concentrations in pollen was possible because the pollen samples were collected as pooled samples yielding a single sample for each hybrid. However, the Cry1Ab concentration in the maize Bt11 x GA21 pooled pollen sample (0.12 µg/g dry weight) was very similar to that of the maize Bt11 pooled pollen sample (0.10 µg/g dry weight). Data for all non-GM maize samples were below the limit of detection.

PAT protein
According to the applicant, all PAT expression data are calculated on a dry-weight (dw) and fresh-weight (fw) basis. In grain the PAT concentrations in Bt11 and Bt11 x GA21 were below the limit of detection, which is <0.021 µg PAT/gdw. According to the applicant there were no statistically significant differences between the mean concentrations of the protein in maize Bt11 and Bt11xGA21 plant tissues. No statistical analysis of PAT concentrations expressed in leaves and grain at seed maturity and pollen collected at anthesis was possible due to the low levels of the PAT (below detection limits). Data for all non-GM maize samples were below the limit of detection for PAT.

mEPSPS protein
According to the applicant, the endogenous maize EPSPS protein is expressed at a significantly lower concentration than the mEPSPS protein in maize GA21. Although the antibodies used in the ELISA are capable of detecting the endogenous EPSPS, the EPSPS concentrations in all non-GM maize samples were below the limit of detection. There were no statistically significant differences between the mean concentrations of protein in maize GA21 and Bt11 x GA21 plant tissues, except for grains at the seed maturity stage. In this case, the difference between the two means (6.08 µg (range: 5.78-6.41)

mEPSPS/gdw for maize GA21 grain and 5.35 µg (range: 4.77-5.98) mEPSPS/gdw for maize Bt11xGA21 grain) is small (~ 12%).

No statistical analysis of pollen mEPSPS concentrations was possible because the pollen samples were collected as pooled samples yielding a single sample for each hybrid. However, mEPSPS concentration for the maize GA21 pooled pollen sample (65.32 µg/g dry weight) was approximately 20% different from that of the maize Bt11xGA21 pooled pollen sample (80.53 µg/g dry weight). Data for all non-GM maize samples were below the limit of detection.

2.1.3.2 Parts of the plant where the insert is expressed

Quantifiable concentrations of Cry1Ab protein were detected in leaves, roots and grain derived from Bt11 maize and Bt11 x GA21 maize. Very low levels of Cry1Ab expression were detected in the pollen of Bt11 maize and Bt11 x GA21 maize (0.1 and 0.12 ug/gdw respectively). Quantifiable concentrations of PAT protein were detected in leaves and roots derived from Bt11 maize and Bt11 x GA21 maize at most stages of development, however, no quantifiable levels could be detected in grain or pollen.

2.1.3.3 Potential fusion proteins

Bt11 x GA21 maize was produced by combining Bt11 maize and GA21 maize through conventional breeding. An Open Reading Frame (ORFs) analysis was performed for each of the parental lines. No expression of potential fusion proteins are, therefore, expected in Bt11 x GA21 maize.

2.1.3.4 Inheritance and genetic stability of inserted DNA

According to the data from the applicant, the parental maize lines Bt11 and GA21 have both incorporated a single DNA insert containing a single copy of their respective DNA fragments, and that these are located at different loci in the maize genome. Interactions of the transgene inserts are, therefore, expected to be minimal during conventional breeding of the genetically modified maize lines Bt11 and GA21.

2.2 Conclusion

Southern blot and PCR analyses have indicated that the recombinant inserts in the parental maize lines Bt11 and GA21 are retained in the stacked maize Bt11 x GA21. Genetic stability of the inserts has previously been demonstrated in the parental maize lines. Protein measurements show comparable levels of the Cry1Ab, PAT and mEPSPS proteins between the stacked and single maize lines. Phenotypic analyses also indicate stability of the insect resistance and herbicide tolerance traits in the stacked maize. The VKM Panel on GMO considers the molecular characterisation of maize Bt11 x GA21 and its parental events Bt11 and GA21 as adequate.

3 Comparative assessment

3.1. Summary of the previous evaluation of the single events

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).

Maize GA21

Maize GA21 was compared to non-transgenic maize with a comparable genetic background (near-isogenic control) during field trials at multiple locations and over several seasons: five locations in USA in 1996, seven locations in USA in 1997, four locations in Europe in 1997 and six locations during two seasons in USA in 2004 and 2005. Maize GA21 plants treated with glyphosate-based herbicides as well as plants untreated with the target herbicides were included in these field trials. No consistent compositional differences were observed between maize GA21 and non-transgenic maize. Agronomic traits were assessed during multiple field trials and seasons in USA in 2004, Brazil in 2003 and Europe in 2007 and 2008. Results from these field trials did not indicate consistent differences between maize GA21 and its comparators with respect to agronomical and phenotypical characteristics, except herbicide tolerance.

In the latest risk assessment of maize GA21 the VKM GMO Panel concludes that maize GA21 is compositionally, agronomically and phenotypically equivalent to conventional maize varieties, except for the herbicide tolerance conferred by the mEPSPS protein (VKM 2014b).

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

3.2.1. Experimental design & statistical analysis

Maize Bt11 x GA21 was compared with a non-GM maize counterpart (the corresponding non-transgenic, near-isogenic hybrid. Pedigree chart shown in Figure 1 - Appendix during field trials in six locations in the USA in 2005. At each location, one hybrid pair, composed of a Bt11 x GA21 maize hybrid and the corresponding non-transgenic hybrid, was grown in a randomized complete block design, with three replicates for each genotype. Maize Bt11xGA21 plants were treated with both glyphosate- and glufosinate-ammonium-based herbicides. The non-transgenic hybrid was not treated with glufosinate and glyphosate herbicides. Both the Bt11 x GA21 and the non-transgenic hybrids were treated with conventional pesticides as needed. Plants were self-pollinated by hand and the developing ears were bagged to avoid crosspollination. Forage and grain derived from maize

Bt11xGA21 and the non-GM maize counterpart were collected from the field trials for compositional analysis.

For each analyte, the statistical significance of the genotype effect was determined using a standard F-test. An F-test probability of <0.05 indicates that the difference between the genotypes was statistically significant at the customary 5% level.

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.

In conducting statistical tests at the customary 5% level, 1 in 20 analyses could result in a statistically significant outcome due to random variation alone. The analyte composition tables for forage and grain include the overall averages of each analyte across locations in both the Bt11 x GA21 maize and the non-transgenic maize hybrids. Also included are the F-test probabilities for both the genotype comparisons and the location by genotype interactions. F-test probabilities that were statistically significant ($p<0.05$) are indicated in italics.

Moisture levels in grain were not subjected to analysis of variance since the moisture analysis was performed on grain that had been mechanically dried, thus altering the original moisture content of the harvested grain. Mechanical drying after harvest is a standard agronomic practice for improving storage characteristics of maize grain.

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.

3.3 Compositional Analysis

Analytes measured in maize forage and grain was based on recommendations of the Organization for Economic Co-Operation and Development (OECD 2002). Forage was analyzed for proximates and the minerals calcium and phosphorus. Grain was analyzed for major constituents (proximates, including starch, ADF, NDF and TDF), minerals, amino acids, fatty acids, vitamins and selected anti-nutrients and secondary metabolites.

All compositional analyses were conducted by Covance Laboratories, Inc. using methods published and approved by AOAC International, or other industry-standard analytical methods. Based on the moisture content of each sample, analyte levels were converted to equivalent units of dry weight.

Key nutritional components in grain and forage from Bt11 x GA21 hybrid maize plants were measured and compared to grain and forage from non-transgenic, near-isogenic hybrid maize plants. Grain and forage from one hybrid pair, consisting of the Bt11 x GA21 hybrid and the corresponding non-transgenic, near-isogenic hybrid, were harvested from six locations in the USA in 2005. Sixty-five analytes were measured in this study.

Forage composition

For the proximate composition of the Bt11 x GA21 forage and the non-transgenic forage no statistically significant differences were observed and average values were within the ranges reported in the literature. For the calcium and phosphorus composition of the Bt11 x GA21 forage and the non-transgenic forage there were no statistically significant differences observed and average values were within the ranges reported in the literature.

Grain composition

Proximates and fibers

No statistically significant differences between the Bt11 x GA21 and non-transgenic grain were observed for protein, ash, carbohydrates, NDF, or starch. Statistically significant differences were detected for fat and TDF. A statistically significant location by genotype interaction was observed for ADF. All average values fell within the ranges reported in the literature, including those analytes with significant differences between the Bt11 x GA21 grain and the non-transgenic grain (fat and TDF) and the single analyte with a significant location by genotype interaction (ADF).

Minerals

For the mineral composition of the Bt11 x GA21 grain and the non-transgenic grain no statistically significant differences were observed between Bt11 x GA21 grain and the non-transgenic grain. Selenium was <LOQ at one location for both the Bt11 x GA21 grain and the non-transgenic grain; since analytes with values <LOQ are not suitable for statistical analysis, the statistical comparison was based on the quantifiable values from the remaining five locations. For sodium, values that were <LOQ were distributed across all locations for both the Bt11 x GA21 hybrid and the non-transgenic hybrid, with 35 out of 36 total values <LOQ. The single quantifiable level of sodium observed in the Bt11 x GA21 samples (118.00 mg/kg DW) was within ranges reported in the literature. All average values for minerals that were statistically analyzed fell within the ranges reported in the literature.

Amino acids

For the amino acid composition of Bt11 x GA21 grain and the non-transgenic grain no statistically significant differences were noted for any of the 18 amino acids analyzed. Average levels of all amino acids were within the ranges reported in the literature.

Fatty acids

For the five most abundant fatty acids in Bt11 x GA21 grain and the non-transgenic grain statistically significant differences were observed for 16:0 palmitic acid, 18:1 oleic acid, and 18:2 linoleic acid. No significant differences were seen in levels of 18:0 stearic acid and 18:3 linolenic acid. Statistically significant location by genotype interactions were observed for 16:0 palmitic acid and 18:1 oleic acid. Average levels of all five measured fatty acids, including 16:0 palmitic acid, 18:1 oleic acid, and 18:2 linoleic acid, were within the ranges reported in the literature.

Vitamins

No statistically significant differences were seen in levels of vitamins B1, B2, B3 and B6. A statistically significant difference was noted in vitamin E levels when comparing the Bt11 x GA21 hybrid and the non-transgenic hybrid. Statistically significant location by genotype interactions were observed for vitamins A and folic acid. All average values fell within ranges reported in the literature, including those with statistically significant differences between Bt11 x GA21 and non-transgenic hybrids (vitamin E) and statistically significant location by genotype interactions (vitamins A and folic acid).

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.

The furfural levels in all grain samples, both Bt11 x GA21 grain and non-transgenic grain, were <LOQ. For raffinose, 7 of 18 Bt11 x GA21 samples were <LOQ and 7 of 18 non-transgenic samples were <LOQ. The raffinose values that were <LOQ were distributed across all locations for both Bt11 x GA21 hybrid and the non-transgenic hybrid. Analytes with values <LOQ are not suitable for statistical analysis but the ranges of quantifiable levels of raffinose in the Bt11 x GA21 samples (0.113 to 0.164 mg/kg DW) and the non-transgenic samples (0.118 to 0.155 mg/kg DW) were all within ranges reported in the literature. Statistically significant location by genotype interactions were observed for both inositol and trypsin inhibitor. No other statistically significant effects were detected. Average levels of all secondary metabolites and anti-nutrients were within the ranges reported in the literature.

3.4 Agronomic and phenotypic characters

During field trials at nine locations in the USA in the 2006 growing season, data on phenotypic characteristics, agronomic performance and disease susceptibility were collected for the maize stack Bt11 x GA21 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. 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. A list of the agronomic characteristics assessed and their descriptions are found in Appendix 3 of the Technical Dossier.

The agronomic equivalence trials were conducted using two Bt11 x GA21 Maize (field corn) hybrids. The Bt11 x GA21 maize hybrids are known as NP2672GT21/NP2171Bt11 (early maturity) and NP2673GT21/NP982Bt11 (mid maturity). The two corresponding near-isogenic non-transgenic hybrids are known as NP2672/NP2171 (early maturity) and NP2673/NP982 (mid maturity).

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 five 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 x GA21 vs. the near-isogenic control) was determined using a standard F-test at the 5% probability.

Analyses of variance across trial locations showed no statistically significant differences between maize Bt11 x GA21 and the near-isogenic control hybrid for the agronomic and phenotypic characters recorded. Although there were minor differences in means of these traits for genotypes within hybrid pairs, there did not appear to be a consistent trend indicating that the Bt11 x GA21 maize hybrids behaved differently from the corresponding near-isogenic control maize hybrids. For grain yield, the effect of genotype within each of the two hybrids was not significant across locations. The significant yield difference at two of the locations has been explained by a high occurrence of stalk lodging and a lower stand in the control, respectively. These results suggest that the two sets of hybrids essentially yielded equivalently in these trials.

3.5 Conclusion

Comparative analyses of data from field trials located at representative sites and environments in North America during the 2005 growing season indicate that maize stack Bt11 x GA21 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, with the exception of the insect resistance and the herbicide tolerance, conferred by the expression of Cry1Ab, PAT and mEPSPS proteins.

Based on the assessment of available data, the VKM GMO Panel is of the opinion that conventional crossing of maize Bt11 and GA21 to produce the hybrid Bt11 x GA21 does not result in interactions between the newly expressed proteins affecting composition and agronomic characteristics.

4 Food /feed risk assessment

4.1. Summary of the previous evaluation of the single events

Both single maize events, Bt11 and GA21, have previously been evaluated by the VKM GMO Panel, and updated risk assessments were finalised in January 2014 (VKM 2014a,b).

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 2014) 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 GA21

In the latest risk assessment of maize GA21 the VKM GMO Panel concluded, based in part on data from whole food feeding studies on rats, feedlot cattle and broilers, that maize GA21 is nutritionally equivalent to conventional maize varieties. It is unlikely that the mEPSPS protein will introduce a toxic or allergenic potential in food or feed based on maize GA21 compared to conventional maize.

4.2 Product description and intended uses

The scope of application EFSA/GMO/UK/2007/49 includes the import and processing of maize Bt11 x GA21 and its derived products for use as food and feed. The possible uses of maize Bt11xGA21 include the production of animal feed and food products, such as starch, syrups and oils. The genetic modification of maize Bt11xGA21 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 GA21 as a food/feed plant.

4.3 Effects of processing

There are two basic methods employed in processing field maize 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 GA21 will be produced and processed in the same way as any field maize.

The food manufacturing of Bt11 x GA21 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 Cry1Ab1, PAT and mEPSPS 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 varieties have been introduced in maize Bt11 x GA21. The VKM GMO Panel has considered all the data available for maize Bt11xGA21, and also the newly expressed proteins Cry1Ab, PAT and mEPSPS. 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 GA21 are unlikely.

No new constituents other than the Cry1Ab, PAT and mEPSPS proteins are expressed in maize Bt11 x GA21 and no relevant changes in the composition of maize Bt11xGA21 were detected by the compositional analysis.

4.4.2 Toxicological assessment of the whole GM food/feed

44-day feeding study on broiler chickens
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 GA21 (lot WN-R53149) with its near-isogenic control (lot WNR53239), and a locally grown commercial maize NC 2006 (North Carolina, growing season 2005). 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 1).

Table 1. Composition of Starter, Grower and Finisher diets for the three maize lines tested.

	NC 2006			Isogenic control			Bt11 x GA21		
Ingredients	**Starter**	**Grower**	**Finisher**	**Starter**	**Grower**	**Finisher**	**Starter**	**Grower**	**Finisher**
Maize grain, %	51.60	58.26	64.11	50.56	57.05	62.79	51.00	57.56	62.34
Soybean oil cake (48%), %	38.72	31.50	26.46	37.59	30.23	25.06	37.22	29.81	24.60
Other, %	9.68	10.24	9.43	11.85	12.72	12.15	11.78	12.63	13.06
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.

Starter contained 0.24 ± 0.04 µg Cry1Ab/g fresh weight and 51 % maize, grower 0.35 ± 0.04 µg Cry1Ab/g fresh weight and 58 % maize, and finisher diets contained 0.40 ± 0.07 µg Cry1Ab/g fresh weight and 63.0 % maize. These diets also had concentrations of mEPSPS as follows: (a) starter: 0.63 ± 0.03 µg/g fresh weight; (b) grower: 0.73 ± 0.08 µg/g fresh weight; (c) finisher: 0.77 ± 0.08 µg/g fresh weight. Concentrations of PAT were less than the limit of detection for starter, grower, and finisher diets.

Growth was excellent for birds in all treatment groups, with the average male reaching 3,059 grams and the average female reaching 2,470 grams at 44 days of age. Final overall mean body weights in the Bt11 x GA21 and Isogenic control groups were not significantly different from one another or from the NC 2006 control group. Males pooled across all groups had an improved feed conversion ratio (significantly lower unadjusted and adjusted feed conversion ratios) to 44 days of age relative to females, as expected. Feed conversion to 44 days of age did not differ among the groups fed diets containing the three different lots of maize grain for males and females combined. However, there was a maize source by sex interaction.

Birds fed with Bt11xGA21 maize grain had slightly, but statistically significant, increased feed conversion ratios compared to the control groups fed diets containing either isogenic control maize grain or the NC 2006 maize grain. No statistically significant differences were noted for body weight. Further, consumption of diets containing Bt11 x GA21 maize grain had no effect on carcass yield for males or females. Therefore, the slight differences in feed conversion ratios are not considered adverse. There was no overall effect of maize source or sex on mortality to 44 days.

At the end of the feeding period, samples of starting grain, starter diet, grower diet, and finisher diet were analyzed for the concentrations of Cry1Ab, PAT, and mEPSPS. Cry1Ab, PAT, and mEPSPS were not detected in the negative control or NC2006 diet samples.

Broilers fed diets prepared with Bt11 x GA21 grain indicated no adverse effects compared to broilers consuming diets made with isogenic control or commercially available control maize grain.

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 using an array of bioinformatics tools. Sequence homology searches comparing the structure of novel proteins to known IgE-allergens in a database are conducted using various algorithms such as FASTA to predict overall structural similarities. According to FAO/WHO (2001) in cases where a novel protein and a known IgE-allergen have more than 35% identity over a segment of 80 or greater amino acids, IgE cross-reactivity between the novel protein and the allergen should be considered a possibility.

4.5.1 Assessment of 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 comprise sequence homology to known allergens, specific or targeted serum screens for IgE cross-reactions to known allergens, digestability 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 and mEPSPS present in maize Bt11xGA21 have been evaluated previously and it was found unlikely that they are allergenic.

These assessments have previously been described by the applicant for the single maize events Bt11 (EFSA-GMO-RX-Bt11) and GA21 (EFSA-GMO-2005-19 and EFSA-GMO-RX-GA21), and were based on the following aspects:

The proteins expressed by the transgenes in maize (*Zea mays*) are not considered common food allergens.

<u>Cry1Ab and PAT</u>

i) The sources of the transgene genes: *B. thuringiensis* (*cry-genes*) and *S. viridochromogenes* (*pat*) have no history of causing allergy
ii) History of safe use of Cry proteins as microbial pesticides (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 IgE-allergenic proteins
v) The microbially produced Cry1Ab and PAT proteins were rapid 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 are not glycosylated (Raybold et al, 2013, US EPA, 2010)

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

mEPSPS

ix) The sources of the transgene gene is maize (*Zea mays*), which is not considered a common food allergen.

x) EPSPS enzymes are ubiquitous in plants and microorganisms

xi) A gene coding for the mEPSPS was expressed in bacteria and the resulting enzyme compared to the plant derived mEPSPS by Western blot. The enzymes expressed from the two sources were shown to be identical (Raybould et al. 2013).

xii) The mEPSPS is functionally equivalent to other food derived EPSPS enzymes except for its tolerance to Roundup® herbicides.

xiii) The EPSPS proteins have been previously assessed for genetically modified plants and found to have no potential for allergenicity by EPA, Canadian Food Inspection Agency and OECD.

xiv) The expressed mEPSPS protein is a single polypeptide with a 99.3 % sequence identity to the wild type.

xv) The mEPSPS protein lacks homology to known toxins or allergenic proteins (Meyer, 1999; Cressman, 2003).

xvi) Immunoblot glycosylation analyses of mEPSPS derived from recombinant E.coli and from extracts of leaf material from transgenic GA21 maize, indicate that both mEPSPS proteins are not glycosylated (Raybould et al. 2013).

xvii) Rapid degradation of the mEPSPS protein in simulated gastric fluids *in vitro* (OECD, 1999). No degradation assay in gastrointestinal fluids has been performed by the applicant.

xviii) The sources of the transgene genes: *B. thuringiensis* (*cry-genes*), *E. coli* (*pmi*), and *Zea mays (mepsps)* have no history of causing allergy

4.4.2 Assessment of the IgE-mediated 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 x GA21 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.

However, an assessment of endogenous allergens in maize, ie 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).

4.4.3 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)

4.5 Nutritional assessment of GM food/feed

The compositional analyses indicate nutritional equivalence between maize Bt11 x GA21 and near isogenic control. This nutritional equivalence is further supported by the broiler feeding study.

4.5.1 Intake information/exposure assessment

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

This dietary exposure assessment is very conservative. It assumes that all maize consumed consists of maize and that protein levels are not reduced by processing. The comparable composition and nutritional value of the maize, together with the results of the assessment of dietary intake and nutritional impact, indicate that food products derived from Bt11 maize are nutritionally equivalent to food products derived from commercial maize. Hence, anticipated dietary intake is not expected to change.

Since most foods and foodstuffs from maize are derived from field maize grains, an estimated maximum daily intake for a Norwegian adult of Cry1Ab protein from maize grain (0.99 ±0.15 µg/gdw) is calculated to be 4.4 µg, based on grain dry weight and mEPSPS protein from maize grain (5.35 ±0.50 µg/gdw) is calculated to be 23.5 µg, based on grain dry-weight. The PAT protein was below detection level which is less than 0.021 µg/gdw, accordingly, no calculation of the possible consumption of PAT protein is possible. The levels of these three proteins 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).

Based on feed conversion ratio (FCR) of broilers at 21-35 days and at 35-44 days, and body weights at 35 day and 44 day, VKM has calculated the intake of Cry1Ab- and mEPSPS-protein per broiler chicken. The feed intake from 21-35 day (grower diet) is 141 g/day for males and 116 g/day for females, at 35-44 days (finisher diet) it is 192 g/day for males and 154 g/day for females.

Based on these feed intakes an estimated daily intake per broiler per day of Cry1Ab from grower diets (21-35 days) is calculated to be 49 µg for males and 41 µg for females, and at 35-44 days (finisher diet) 77 µg for males and 62 µg for females. The estimated daily intake of mEPSPS protein at 21-35 day is per day 103 µg for males and 85 µg for females, and at 35-44 days is per day 148 µg for males and 119 µg for females. The levels of these two proteins are several orders of magnitude below the levels established to give no effect in laboratory toxicology testing, and are also considerably below the proposed TTC level. 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 GA21 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 GA21 and that the transgenic proteins are not denatured by processing.

4.5.2 Nutritional assessment of feed derived from the GM plant

Based on the compositional analyses comprising proximates, minerals, fatty acids, amino acids, vitamins, secondary metabolites and anti-nutrients of forage and grain samples from Bt11 x GA21 maize; nutritional equivalence shown in a poultry feeding study; and, safety evaluation of the Cry1Ab,

PAT and mEPSPS proteins expressed in Bt11 x GA21 maize, the Bt11 x GA21 maize and derived feed products seem to be substantially and, nutritionally equivalent, and as safe as commercial maize grain and derived feed products.

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

4.6 Conclusion

A whole food feeding study on broilers has not indicated any adverse health effects of maize Bt11 x GA21, and shows that maize Bt11 x GA21 is nutritionally equivalent to conventional maize. The Cry1Ab, PAT or mEPSPS 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 GA21 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1Ab, PAT or mEPSPS proteins will introduce a toxic or allergenic potential in food or feed based on maize Bt11 x GA21 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 GA21 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 and glyphosate 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 pests provides a potential advantage in cultivation of Bt11 under infestation conditions. It is considered very unlikely that maize Bt11 x GA21 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 GA21 relative to its conventional counterpart. A series of field trials with maize Bt11x GA21 were carried out across 9 locations in the USA in 2006 (application EFSA/GMO/UK/2007/49). 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 GA21 in comparison with its

conventional counterpart (see section 3.1). Data from the field trials shows no statistical significant differences for the parameters assessed.

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 GA21, 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 GA21 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 GA21. 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 GA21 to unrelated species such as bacteria.

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

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

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

In conclusion, the VKM GMO Panel consider it is unlikely that the introduced gene from maize Bt11 x GA21 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* and *mepsps* genes from Bt11 x GA21 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 GA21 (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 GA21 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 *A. 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.

Considering the intended uses of maize Bt11 x GA21, 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 GA21, 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 protein enters the environment due to expression in the grains (mean value of 45.7 and 1.61µg/g d.w., respectively). In addition, the data show that at least 99% of microbially produced 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 protein is likely to be very low and of no ecological relevance.

5.5 Potential interactions with the abiotic environment and biochemical cycles

Considering the intended uses of maize Bt11 x GA21, 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/2007/49 includes import and processing of maize Bt11x GA21 for food and feed uses. Considering the intended uses of maize Bt11 x GA21, 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 GA21.

Maize Bt11 x GA21 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 using Cry1Ac. Whether the other Cry proteins have similar adjuvant properties is unknown.

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

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

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

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

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

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

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

7 Conclusions

Molecular characterisation
Southern blot and PCR analyses have indicated that the recombinant inserts in the parental maize lines Bt11 and GA21 are retained in the stacked maize Bt11 x GA21. Genetic stability of the inserts has previously been demonstrated in the parental maize lines. Protein measurements show comparable levels of the Cry1Ab, PAT and mEPSPS proteins between the stacked and single maize lines. Phenotypic analyses also indicate stability of the insect resistance and herbicide tolerance traits in the stacked maize. The VKM Panel on GMO considers the molecular characterisation of maize Bt11 x GA21 and its parental events Bt11 and GA21 as adequate.

Comparative assessment
Comparative analyses of data from field trials located at representative sites and environments in North America during the 2005 growing season indicate that maize stack Bt11 x GA21 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, with the exception of the insect resistance and the herbicide tolerance, conferred by the expression of Cry1Ab, PAT and mEPSPS proteins.

Based on the assessment of available data, the VKM GMO Panel is of the opinion that conventional crossing of maize Bt11 and GA21 to produce the hybrid Bt11 x GA21 does not result in interactions between the newly expressed proteins affecting composition and agronomic characteristics.

Food and feed risk assessment
A whole food feeding study on broilers has not indicated any adverse health effects of maize Bt11 x GA21, and shows that maize Bt11 x GA21 is nutritionally equivalent to conventional maize. The Cry1Ab, PAT or mEPSPS 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 GA21 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1Ab, PAT or mEPSPS proteins will introduce a toxic or allergenic potential in food or feed based on maize Bt11 x GA21 compared to conventional maize.

Environmental risk assessment
The scope of the application EFSA/GMO/UK/2007/49 includes import and processing of maize stack Bt11x GA21 for food and feed uses. Considering the intended uses of maize Bt11 x GA21, 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 GA21.

Maize Bt11 x GA21 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 GA21. 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 GA21 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1Ab, PAT or mEPSPS proteins will introduce a toxic or allergenic potential in food or feed based on maize Bt11 x GA21 compared to conventional maize.

The VKM GMO Panel likewise concludes that maize Bt11 x GA21, based on current knowledge, is comparable to conventional maize varieties concerning environmental risk in Norway with the intended usage.

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