Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Food/feed and environmental risk assessment of insect-resistant and herbicide-tolerant genetically modified maize GA21 from Syngenta Seeds for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (EFSA/GMO/UK/2005/19)

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

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Contributors

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

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Summary

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

The herbicide-tolerant genetically modified maize GA21 from Syngenta Seeds (Unique Identifier MON-ØØØ21-9) is authorised for the import and placing on the market as food or feed in the EU pursuant to Regulation (EC) 1829/2003 by the Commission Decision 2008/280/EC. An application for granting consent to all uses of GA21 maize including the cultivation was submitted by Syngenta in accordance with articles 5 and 17 of the Regulation (EC) No. 1829/2003 on June 30 2008.

Maize GA21 has previously been assessed as food and feed by the VKM GMO Panel commissioned by the Norwegian Food Safety Authority in connection with the national finalisation of the procedure of the notification C/ES/98/01 in 2005 (VKM 2005a). VKM also participated in the 90 days public consultation of the application for placing on the market of maize GA21 for food and feed uses, import, processing and cultivation (EFSA/GMO/UK/2008/60) in 2009, and submitted a preliminary opinion in April 2010 (VKM 2010). GA21 has also been evaluated by the VKM GMO Panel as a component of several stacked GM maize events under and Regulation (EC) 1829/2003 (VKM 2008, VKM 2009a,b,c,d, VKM 2012a,b, VKM 2013a,b,c). Due to the publication of new scientific literature and updated guidelines for risk assessment of genetically modified plants, the VKM GMO Panel has decided to deliver an updated food/feed and environmental risk assessment of GA21.

The updated food/feed and environmental risk assessment of the maize GA21 is based on information provided by the applicant in the applications EFSA/GMO/UK/2005/19, EFSA/GMO/UK/2008/60 and EFSA/GMO/RX/GA21 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 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 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.

Maize GA21 expresses a modified version of 5-enolpyruvylshikimate-3-phosphate synthase (mEPSPS), which is derived from maize EPSPS, and renders maize GA21 tolerant to the herbicidal active substance glyphosate.

Molecular characterisation

The molecular characterisation data indicate that several copies of the GA21 construct are integrated at a single locus in the DNA, and that they are inherited as a dominant, single locus trait. Appropriate analyses of the integration site, inserted DNA sequence, flanking regions, and bioinformatics have been performed. The VKM GMO Panel considers the molecular characterisation of maize GA21 as adequate.

Comparative assessment

Comparative analyses of maize event GA21 to its conventional counterpart) have been performed during multiple field trials located at representative sites and environments in North America (1997, 2004, and 2005), Europe (1996, 1997, and 2006) and Brazil (2003). With the exception of small intermittent variations, no biologically significant differences were found between maize GA21 and controls. Based on the assessment of available data, the VKM GMO Panel concludes that maize GA21 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, except for the mEPSPS protein, and that its composition fell within the range observed among non-GM varieties.

Food and feed risk assessment

Whole food feeding studies in rats, broilers and cattles have not indicated any adverse health effects of maize GA21. These studies also indicate that maize GA21 is nutritionally equivalent to conventional maize. The mEPSPS protein does not show sequence resemblance to other known toxins or IgE allergens, nor has mEPSPS been reported to cause IgE mediated allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize GA21 is nutritionally equivalent to conventional maize varieties. It is unlikely that the mESPSPS protein will introduce a toxic or allergenic potential in food or feed based on maize GA21 compared to conventional maize.

Environmental risk assessment

The scope of the application EFSA/GMO/UK/2005/19 includes import and processing of maize GA21 for food and feed uses. Considering the intended uses of maize 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 GA21.

Maize 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 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 GA21 is nutritionally equivalent to conventional maize varieties. It is unlikely that the mEPSPS protein will introduce a toxic or allergenic potential in food derived from maize GA21 compared to conventional maize.

The VKM GMO Panel likewise concludes that maize 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 GA21, EFSA/GMO/UK/2005/19, herbicide-tolerance, glyphosate, *mepsps* gene, mEPSPS protein, 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, er Vitenskapskomiteen for mattrygghet (VKM) bedt av Miljødirektoratet (tidligere Direktoratet for naturforvalting (DN)) og Mattilsynet om å utarbeide endelige helse- og miljørisikovurderinger av alle genmodifiserte organismer (GMOer) og avledete produkter som inneholder eller består av GMOer som er godkjent under forordning 1829/2003 eller direktiv 2001/18, og som er godkjent for ett eller flere bruksområder som omfattes av genteknologiloven. Miljødirektoratet og Mattilsynet har bedt VKM om endelige risikovurderinger for de EU-godkjente søknader hvor VKM ikke har avgitt endelige risikovurderinger. I tillegg er VKM bedt om å vurdere hvorvidt det er nødvendig med oppdatering eller annen endring av de endelige helse- og miljørisikovurderingene som VKM tidligere har levert.

Den herbicidtolerante maislinjen GA21 (unik kode MON-ØØØ21-9) fra Syngenta Seeds er godkjent til import, videreforedling og til bruk som mat og fôr under EU-forordning 1829/2003 (søknad EFSA/GMO/UK/2005/19, Kommisjonsbeslutning 2008/280/EU).

Maislinjen har tidligere vært vurdert av VKM med hensyn på mulige helseeffekter ved bruk som mat og fôr (VKM 2005a). Risikovurderingen ble utarbeidet på oppdrag fra Mattilsynet i forbindelse med vurdering av markedsadgang i Norge. I forbindelse med EFSAs offentlige høring av søknad EFSA/GMO/UK/2008/60) i 2009 utarbeidet VKMs faggruppe for GMO en foreløpig helse- og miljørisikovurdering av GA21 for alle bruksområder, inkludert dyrking (VKM 2010). VKMs faggruppe for GMO har også risikovurdert en rekke maishybrider der GA21 inngår som en av foreldrelinjene (VKM 2008, VKM 2009a,b,c,d, VKM 2012a,b, VKM 2013a,b,c). Etablering av nye, reviderte retningslinjer for helse- og miljørisikovurderinger av genmodifiserte planter og publisering av ny vitenskapelig litteratur har medført at VKM har valgt å utarbeide en ny, oppdatert helse- og miljørisikovurdering av mais GA21.

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.

Maislinje 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 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. De transgene plantene vil derfor tolerere høyere doser av herbicider med virkestoff glyfosat sammenlignet med konkurrerende ugras. GA21 inneholder ingen markørgener for antibiotikaresistens.

Molekylær karakterisering
Data fra den molekylære karakteriseringen indikerer at det er integrert flere kopier av *mEPSPS* genet i genomet til mais GA21, og at genene og egenskapene er dominant og stabilt nedarvet. Bioinformatikk og sekvensanalyser er utført av integreringssete i plantens genom, og innsatt og flankerende DNA. VKMs faggruppe for genmodifiserte organismer vurderer den molekylære karakteriseringen av mais GA21 som tilfredsstillende

Komparative analyser
Feltforsøk i Nord-Amerika og Europa viser, med unntak av herbicidtoleranse, små eller ingen signifikante forskjeller mellom den transgene maislinjen GA21 og korresponderende, nær-isogene kontrollhybrider med hensyn på næringsmessige, morfologiske og agronomiske karakterer. Resultatene viser ingen indikasjon på at det innsatte genet i GA21 har medført utilsiktede endringer i egenskaper knyttet til vekst og utvikling hos maisplantene

Helserisiko
Fôringsstudier utført på rotter, broiler og kyr har ikke indikert helseskadelige effekter av mais GA21. mEPSPS– proteinet viser ingen likhet til kjente toksiner eller allergener, og er heller ikke rapportert å ha forårsaket IgE-medierte allergiske reaksjoner. Det er heller ikke dokumentert at noen av proteinene kan utløse IgE-medierte allergiske reaksjoner.

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at mais GA21 er næringsmessig vesentlig lik konvensjonell mais, og at det er lite trolig at de nye proteinene vil introdusere et toksisk eller allergent potensiale i mat og fôr basert på mais GA21 sammenliknet med konvensjonelle maissorter.

Miljørisiko
Søknaden EFSA/GMO/UK/2005/19 gjelder godkjenning av maislinje 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 av GMO panelet til å være ubetydelig. Ved foreskreven bruk av maislinjen GA21 antas det ikke å være risiko for utilsiktede effekter på målorganismer, ikke-målorganismer eller på abiotisk miljø i Norge.

Samlet vurdering
VKMs faggruppe for GMO har ikke identifisert toksiske eller endrede ernæringsmessige egenskaper ved mais GA21 eller dens avledete produkter sammenlignet med konvensjonell mais. Faggruppen finner også at mais 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).
CTP	Chloroplast transit peptide
DAP	Days after planting
DNA	Deoxyribonucleic acid
DT50	Time to 50% dissipation of a protein in soil
DT90	Time to 90% dissipation of a protein in soil
dw	Dry weight
dwt	Dry weight tissue
EC	European Commission
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	Environmental risk assessment

E-score	Expectation score
EU	European Union
fa	Fatty acid
FAO	Food and Agriculture Organisation
FIFRA	US EPA Federal Insecticide, Fungicide and Rodenticide Act
Fitness	Describes an individual's ability to reproduce successfully relative to that of other members of its population.
fw	Fresh weight
fwt	Fresh weight tissue
GAT	Glyphosate N-acetyltransferase
GLP	Good Laboratory Practice
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
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 8 August 2005, the European Food Safety Authority (EFSA) received from the Competent Authority of the United Kingdom an application (Reference EFSA/GMO/UK/2005/19) for authorisation of the herbicide tolerant genetically modified (GM) maize GA21 (Unique Identifier 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 GA21
- GM plants for food and feed use
- Food and feed, containing or consisting of maize GA21
- Food and feed produced from maize GA21
- Food containing ingredients produced from maize GA21

After receiving the application EFSA/GMO/NL/2005/15 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the EU- and EFTA Member States (MS) and the European Commission and made the summary of the dossier publicity available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of regulation (EC) No 1829/2003. On 2 April 2006, 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.

An application for renewal of the authorisation of existing feed materials and food and feed additives produced from maize GA21, notified as existing products under Regulation (EC) 1829/2003 (EFSA/GMO/RX/GA21) was submitted by Syngenta Seeds in June 2007. The EFSA GMO Panel assessed these two applications together, and published its scientific opinion in September 2007 (EFSA 2007). The Commission Decision 2008/280/EC authorised the placing on the market of products containing, consisting of, or produced from maize GA21 pursuant to Regulation (EC) No 1829/2003 (EC 2008) on 28 March 2008.

Previously, the use of food and feed ingredients produced from maize GA21 has been evaluated by the Scientific Committee on Food (SCF) (SCF 2002), and approved under Regulation 258/97/EC on novel foods and novel food ingredients in 2006 (Commission Decision 2006/69/EC). Maize GA21 has also been evaluated by the Scientific Committee on Plants (SCP) in 2000 for other commercial uses (import, processing and feed) under Directive 2001/18/EC (Notification C/ES/98/01) (SCP 2000).

An application for authorisation of maize GA21 for food and feed uses, import and processing and cultivation in the EU was submitted by Syngenta Seeds in July 2008 (EFSA/GMO/UK/2008/60). EFSA stopped the application process in February 2009 requesting additional data from Syngenta. The clock was restartet in December 2010 and the EFSA GMO Panel adopted its scientific opinion on maize GA21 in June 2011 (EFSA 2011d).

Maize GA21 has previously been assessed as food and feed by the VKM GMO Panel commissioned by the Norwegian Food Safety Authority in connection with the national finalisation of the procedure of the notification C/ES/98/01 in 2005 (VKM 2005a). VKM also participated in the 90 days public consultation of the application for placing on the market of maize GA21 for food and feed uses, import, processing and cultivation (EFSA/GMO/UK/2008/60) in January 2009, and submitted a preliminary opinion in April 2010 (VKM 2010). Due to the publication of new scientific literature and updated guidelines for risk assessment of genetically modified plants, the VKM GMO Panel has decided to deliver an updated food/feed and environmental risk assessment of GA21.

GA21 has also been evaluated by the VKM GMO Panel as a component of several stacked GM maize events under and Regulation (EC) 1829/2003 (VKM 2008, VKM 2009a,b,c,d, VKM 2012a,b, VKM 2013a,b,c). VKM did not participate in the EFSA public consultations of the applications EFSA/GMO/UK/2005/19 and EFSA/GMORX/GA21 in 2006 and 2007, respectively.

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 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 (former Norwegian Directorate for Nature Management) has the overall responsibility for processing applications for the deliberate release of genetically modified organisms (GMOs). This entails inter alia coordinating the approval process, and to make a holistic assessment and recommendation to the Ministry of the Environment regarding the final authorization process in Norway. The 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.

The Norwegian Environment Agency

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

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

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

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

The Norwegian Food Safety Authority

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Environment Agency has requested the Norwegian Food Safety Authority (NFSA) to give final opinions on all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are

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

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

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

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

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

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

Assessment

1 Introduction

Genetically modified maize 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 (ref. EFSA 2011). The disruption of this pathway and the resulting inability to produce key amino acids prevents growth and ultimately leads to plant death. However, in 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 (Green 2009; Dill et al. 2010)

The genetic modification in maize GA21 is intended to improve agronomic performance only and is not intended to influence the nutritional properties, the processing characteristics and the overall use of maize as a crop.

Maize GA21 (Unique Identifier 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).

The food/feed and environmental risk assessment of the genetically modified maize GA21 is based on information provided by the applicant in the applications EFSA/GMO/2005/19, EFSA/GMO/UK/2008/60 and EFSA/GMO/RX/GA21, and scientific opinions and comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment is also based on a risk analysis report of GA21 from the Australia New Zealand Food Authority (ANZFA 2000) and a review and assessment of relevant peer-reviewed scientific literature.

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

2 Molecular characterisation

2.1 Information related to the genetic modification

2.1.1 Description of the methods used for the genetic modification

Event GA21 was produced via microprojectile bombardment of maize suspension culture cells. The method is described in the International Patent PCT/US98/06640 (Spencer et al. 1998a, Spencer et al. 1998b).

2.1.2 Nature and source of vector used

Plasmid pDPG434, was used to generate Event GA21 via microprojectile bombardment transformation. The plasmid was derived from a pSK- vector, commonly used in molecular biology and is derived from pUC19 (Short *et al.*, 1988). A vector map of pDPG434 is shown in Figure 1. The *Not*I restriction fragment containing the expression cassette was used for the transformation. The components are shown in Table 1 and 2. The *Not*I restriction fragment contains the modified 5-enolpyruvylshikimate-3-phosphate synthase (*mepsps*) expression cassette but does not contain the origin of replication, the *bla* gene or the partial *lacZ* sequence. The size and intended function of each constituents intended for insertion in GA21 maize are summarised in Table 3.

According to the applicant, a molecular characterization and sequencing analysis of Event GA21 has been performed using Southern Blot and PCR analyses. Information relating to the restriction sites for generation of probes and the position of primers used in the PCR analysis is described by the applicant (conf.).

Table 1. Vector backbone components of pDPG434

Vector Component	Description
lac	A partial lacI coding sequence, the promoter plac, and a partial coding sequence for β-galactosidase or LacZ proteins (Yanisch-Perron *et al.*, 1985)
bla	The TEM type β-lactamase gene from *E. coli* plasmid pBR322 confers resistance on bacterial cells to ampicillin and other penicillins (Sutcliffe, 1978). The gene is under the control of its native bacterial promoter.
ColE1ori	The origin of DNA replication from the *E.Coli* high copy plasmid pUC19 (Yanisch-Perron *et al.*, 1985)

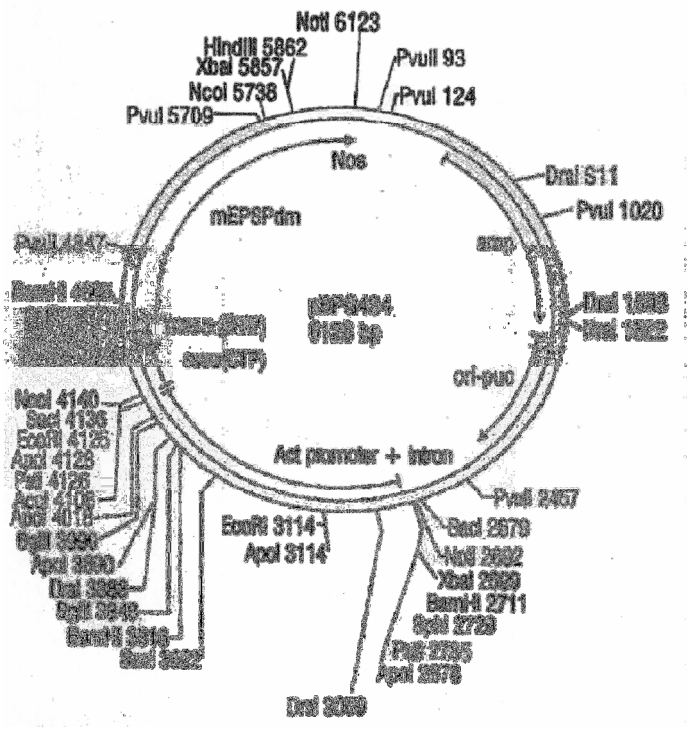
Table 2. Vector region intended for insertion from pDPG434

Vector Component	Description
Rice actin promoter and intron	5' region of the rice actin 1 gene containing the promoter and first exon and intron (McElroy *et al.,* 1990) Note: this is described as "Act promoter +intron" in the vector shown in Fig.1
Optimised transit peptide	N-terminal optimised transit peptide sequence constructed based on transit peptide sequences from maize and sunflower ribulose-1,5-bis phosphate carboxylase oxygenase (RuBisCo) genes (Lebrun *et al.,* 1996) Note: this is described as "mssu (CTP) and sssu (CTP)" in the vector shown in Fig.1
Mutant maize *epsps* gene	Wild-type maize *epsps* gene (Genbank Accession X63374) containing mutations at amino acid position 102 (threonine to isoleucine and 106 (proline to serine). Note: this is described as "mEPSPdm" in the vector shown in Fig.1
Nos 3' end	3' nontranslated region from the nopaline synthase gene from *Agrobacterium tumefaciens* (Bevan, 1984)

Table 3. Source, size and intended function of constituents intended for insertion in GA21 maize

Vector Component	Approx. Size (Kb)	Description of intended function
Rice actin promoter and intron	1.4	Provides constitutive expression of the *mepsps* gene in maize. Note: this is described as "Act promoter +intron" in the vector shown in Fig.1
Optimised transit peptide	0.4	Directs the modified 5-enolpyruvylshikimate-3-phosphate synthase (mEPSPS) protein to the chloroplast (Lebrun *et al.,* 1996) Note: this is described as "mssu (CTP) and sssu (CTP)" in the vector shown in Fig.1
Mutant maize *epsps* gene	1.3	Sequence encoding the modified maize (*Zea mays*), EPSPS protein (mEPSPS), which imparts tolerance to glyphosate Note: this is described as "mEPSPdm" in the vector shown in Fig.1
Nos 3' end	0.3	Ends transcription and directs polyadenylation of the mRNA

Figure 1. Plasmid map of transformation vector pDPG434



2.2 Information relating to the GM plant

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

Maize GA21 is genetically modified (GM) maize, which expresses a mutated maize 5-enolpyruvylshikimate-3-phosphate synthase enzyme (mEPSPS). EPSPS is a key enzyme in the shikimic acid pathway, involved in the biosynthesis of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) and is naturally found in all plants, fungi, and bacteria but absent in animals. EPSPS is highly sensitive to herbicide products containing glyphosate. Maize plants transformed with the mutated *epsps* (*mepsps*) gene synthesize mEPSPS protein that confers tolerance to herbicide products containing glyphosate (Spencer et al, 2000; Lebrun et al. 2003). The mutation has been introduced to confer resistance to herbicide products containing glyphosate, and results in two specific changes to the wild type maize EPSPS. These changes are at amino acid position 102 (threonine to isoleucine) and 106 (proline to serine).

2.2.2 Information on the sequences actually inserted or deleted

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

According to the applicant, the entire Event GA21 insert and flanking regions have been sequenced. Sequence analysis of the Event GA21 insert indicate that the insert is comprised of six contiguous regions derived from the 3.4 kb *Not*I restriction fragment from pDPG434 employed in the generation of Event GA21 (copies 1-6). Copy 1 contains the rice actin promoter that has a 5' deletion of 696 bp, the actin first exon and intron, the optimized transit peptide, the *mepsps* gene and the NOS terminator. Copies 2, 3 and 4 are intact versions of the 3.4 kb *Not*I restriction fragment from pDPG434. Copy 5 contains a complete rice actin promoter, the actin first exon and intron, the optimized transit peptide and the first 288 bp of the *mepsps* gene which ends in a stop codon and does not contain the NOS terminator. Copy 6 contains the rice actin promoter and a truncated actin first exon; it contains no other elements from pDPG434. According to the applicant, a single base-pair change was noted in the NOS terminator in Copies 1 and 2. The nucleotide at this location in Copies 1 and 2 is a "C" whereas in Copies 3 and 4 it is a "G", which is the intended nucleotide at this location. Northern and Western analyses have been performed to investigate the possibility of transcription of the truncated *mepsps* gene and subsequent translation. Results presented by the applicant indicate no evidence for transcripts or truncated protein relating to the truncated *mepsps* gene.

In addition to sequencing, Southern analysis has been performed to determine the absence of further copies of the insert elsewhere in the genome.

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

The entire GA21 insert and the 5' and 3' flanking regions have been sequenced. According to the applicant, the mEPSPS shows greater than 99.3% identity to the native EPSPS.The maize genomic sequence 5' of the GA21 insert was determined during the sequencing of the 20.5 kb *Eco*RV fragment. BLAST analysis of this sequence showed homology to maize chloroplast DNA (accession number X86563.2). According to the applicant, the presence of organelle sequences in the nuclear genome is not without precedent as this observation has been made previously in several conventional (non-GM) plant species, including maize (Figueroa et al., 1999a and 1999b; Fukuchi et al., 1991; Goff et al., 2002; Kemble *et al*., 1983). An *in silico* screen for putative ORFs at the junction between the maize genome and the GA21 insert was performed. The assessment defined an ORF as beginning with an ATG and ending with any of the three stop codons (TAG, TAA or TGA) and with a minimum size of 50 amino acids. Employing these criteria, two putative ORFs were identified at the 5' end of the insert. One putative ORF was identified which originates in the maize 5' sequence and which continues into the GA21 insert. In addition, a second ORF was identified in which the first 17 amino acids correspond to the hypothetical Cytochrome C biogenesis protein (accession number CAA60348) found in the maize chloroplast DNA. The GA21 insert, therefore, appears to have disrupted an identified ORF contained within the maize chloroplast DNA, which has resulted in a putative fusion protein. It is highly likely that the presence of a functional cytochrome C biosynthesis gene in the maize chloroplast genome of GA21 would compensate for the disrupted version seen in the nuclear genome. According to the applicant, henotypic and compositional measurements could find evidence for disruption of cytochrome C activity which suggest that GA21 is substantially equivalent to conventional maize. None of the putative ORFs identified in the 5' flanking sequence to the GA21 insert demonstrated homology to proteins known to be toxins or allergens.

Sequence data of the flanking 3' region

BLAST analysis of the maize sequence 3' of the GA21 insert showed homology to several maize entries within the National Center for Biotechnology Information nucleotide database (GenBank). The

regions of homology within these maize sequences appear to be repetitive sequence elements common to these entries. An *in silico* screen for putative ORFs at the junction between the maize genome and the GA21 insert was performed as described for the 5' flanking region. Employing these criteria, two putative ORFs were identified at the 3' end of the insert. The two ORFs are wholly contained within the maize sequence 3' of the GA21 insert. While these putative ORFs are comprised entirely of maize sequence, due to their proximity to the truncated actin promoter at the 3' end of the insert they were examined further. None of the putative ORFs identified in the 3' flanking sequence to the GA21 insert indicated homology to proteins known to be toxins or allergens.

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

Not applicable.

2.2.2.4 Chromosomal location(s) of insert(s)

The inheritance pattern of the insert derived from pDPG434 in GA21 maize was investigated; the results indicate that insertion has taken place in the nucleus. Statistical analysis show Mendelian inheritance ratio for herbicide tolerance trait.

2.3 Information on the expression of the insert

2.3.1 Part of the plant where the insert is expressed

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. According to the applicant, measureable concentrations of mEPSPS protein were detected in most GA21-derived plant tissues. 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 concentrations of mEPSPS were generally similar between hybrids for each tissue type at each time point (results – conf.). Such constitutive expression is anticipated from the rice actin promoter (Zhong et al. 1996).

2.3.2 Expression of potential fusion proteins

According to the applicant, BLAST analysis of the 5' and 3' junctions of the GA21 insert has been performed. An *in silico* screen for putative ORF's at the junction between the maize genome and the GA21 insert was also performed. The assessment defined an ORF as beginning with an ATG and ending with any of the three stop codons (TAG, TAA or TGA) and with a minimum size of 50 amino acids. Employing these criteria, two putative ORF's were identified which spanned the 5' maize-insert junction. These putative ORF's were examined for sequence homology to known toxin and allergens. According to the applicant, none of the putative ORF's identified in GA21 demonstrated homology to proteins known to be toxins or allergens.

Sequence analysis of the GA21 insert revealed the presence of a truncated *mepsps* within the insert, which ends in a stop codon. The presence of this stop codon makes it unlikely that a potential fusion protein would arise as a result of the truncated *mepsps*. Despite this, northern analysis of GA21 polyA+ RNA employing a *mepsps*-specific probe was performed to investigate if a truncated *mepsps* transcript was detectable. According to the applicant, no truncated *mepsps* transcript was detected. In

addition, Western blot analysis with anti-mEPSPS antibodies was unable to detect any protein products except the full-length mEPSPS protein.

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

2.4.1 Genetic stability of the insert

Southern analysis on DNA derived from several generations of GA21 plants indicates presence of a single insertion site of the GA21 insert. Seed from DNA representing three generations of GA21 plants (BC1, BC2 and BC3) were digested with the restriction enzyme *Hin*dIII and subsequently hybridized with the *mepsps*-specific probe. The hybridization data indicate that the insert incorporated into GA21 is stable over several generations. No changes in reproduction, dissemination or survivability were observed compared to non-GM maize in field trials with GA21.

2.4.2 Phenotypic stability of the GM plant

The stability of mEPSPS protein expression over multiple generations was evaluated. Seed from three backcross generations was grown under greenhouse conditions and leaf material was collected at anthesis for analysis of mEPSPS protein concentrations. Mean mEPSPS concentrations measured across all backcross generations were 13—14 µg/g fw (82—96 µg/g dw). Overall, mEPSPS concentrations were similar across the three generations analyzed, indicating stable expression of mEPSPS protein across multiple generations.

2.5 Conclusion

The molecular characterisation data indicate that several copies of the GA21 construct are integrated at a single locus in the DNA, and that they are inherited as a dominant, single locus trait. Appropriate analyses of the integration site, inserted DNA sequence, flanking regions, and bioinformatics have been performed. The VKM GMO Panel considers the molecular characterisation of maize GA21 as adequate.

3 Comparative assessment

3.1 Production of material for the comparative assessment

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.

3.1.1 Experimental design and statistical analysis

Applications EFSA-GMO-UK-2005-19 and EFSA-GMO-RX-GA21

North American field trails 2004
In the applications EFSA-GMO-UK-2005-19 and EFSA-GMO-RX-GA21 for import and use of genetically modified herbicide tolerant maize event GA21 the applicant presents compositional analysis from grain and forage material collected in field trails in North America during the 2004 growing season. Replicate trials of transgenic GA21 maize and corresponding isogenic controls were planted in 6 locations. According to the applicant, the locations of the trial sites were selected to be representative of the range of environmental conditions under which the hybrid varieties are expected to be grown.

Location Code	City/State
L1	Bloomington, IL
L2	Shirley, IL
L3	Bondville, IL
L4	Glidden, IA
L5	Stanton, MN
L6	Faribault, MN

At each location, three replicate plots for each entry were planted in randomized complete blocks. The transgenic hybrid was sprayed at the V3-V4 growth stage with Touchdown glyphosate herbicide at the labeled rate (E2), or treated with conventional herbicides (E1). The non-transgenic control hybrid was also treated with conventional herbicides (E3).

Entry	Genotype	Description
E1 (+)	115TT-189	GA21
E2 (+ TD)	115TT-189	GA21 + Touchdown
E3 (-)	115-083	non-transgenic control

Levels of various nutritive components were compared in maize grain and forage produced from GA21 maize plants sprayed with conventional herbicides (E1), or Touchdown glyphosate herbicide (E2), and simultaneously grown near-isogenic control plants sprayed with conventional herbicides (E3). Data for each genotype were subjected to analysis of variance.

The statistical significance of any differences in the quantities were measured between genotypes E1 (+) and E3 (-), and between E2 (+ TD) and E3 (-). For each analyte the statistical significance of the genotype effect was determined using a standard F-test at the 5% probability. An F-test probability of <5% 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 x genotype) interaction. A significant outcome (F-test probability <5%) indicated that the effect of genotype was not consistent across all locations, and reduces to some extent the relevance of the comparison of genotypes averaged across all locations. In datasets where some but not all of the values were less than the limit of quantification (<LOQ), the average is represented as less than (<) the mean of the quantifiable values and the known LOQ value for that analyte. Those data were not suitable for additional statistical analysis.The results were compared to compositional analysis data for grain and forage published in the literature and in compositional analysis databases.

The statistical analysis also includes the standard deviation and coefficient of variation for each analyte. Their derivation takes into account all data from all entries and all locations. Both are measures of random variation, and while both are informative in showing the level of variation present in the data, neither is used directly in the comparison of genotypes. In conducting statistical tests at the customary 5% level, *approximately* one of 20 analyses will result in a statistically significant outcome simply because of random variation alone (*i.e.* there is a one in 20 risk of observing a "false positive"). According to the applicant, in the current study, over 3500 individual data points were assembled into *approximately* 124 pair-wise comparisons of transgenic and control values for each analyte, across locations, and these data points were subject to analysis of variance.

North American and European field trials 1996-1997

In addition to the North American field trials in 2004, the applicant presents compositional analysis from grain and forage material collected in field trails in North America during the 1996 growing season and in North America and Europe during the 1997 growing season (Sidhu et al. 2000).

In the 1996 field trials, maize plants were grown at five sites in the United States. A population of negative segregant plants of maize line GA21 (i.e., those lacking the mEPSPS gene) was utilized as the control. The VKM´s, as well as, EFSA´s GMO Panels do not consider negative segregants derived from GM organisms as appropriate conventional counterparts with a history of safe use (EFSA 2006; EFSA 2011a). Data obtained from field trials with negative segregants are considered as supplementary information only. Further, only limited data from treated plots were available in the 1996 field trails, therefore, only data from the untreated grain samples are reported. Forage was collected at the late dough/early dent stage and grain at normal grain maturity.

In 1997, grain and forage samples were collected from three field studies: a US single-site replicated trial, a U.S. multisite non-replicated trial, and an EU multisite trial. The parental line, DK626, was the control line in these trials. In addition, five to six conventional commercial lines were planted at each site as reference lines. The USA single-site replicated trial was based on a randomized complete block design to allow for a within-site statistical evaluation of composition data. Roundup Ultra herbicide was applied to plots containing Roundup Ready plants. The genetic purity of plants was maintained, and forage and grain samples were collected as described for the 1996 field trials.

Statistical analyses of the composition data were performed using the SAS statistical program (SAS Institute, 1990). For the statistical analysis of the 1996 data, least squares means and ranges for each combination of tissue, component, and sample type (i.e. Roundup Ready maize line GA21 or the control) were computed across all sites. For a particular component/tissue combination, the difference between the mean of the control and the mean of GA21 was considered to be statistically significant at the 5% level if the p value was found to be <0.05 (the p value is the observed significance level for a two-sided t test of zero difference). The lower and upper 95% confidence intervals for the mean difference of GA21 from control maize were also calculated.

Statistical analysis of the 1997 data was conducted separately for the three studies as well as combined. For the analysis of all three studies combined, the U.S. single-site replicated study was treated as an additional non-replicated site with only the line means being used. The sites for all three studies were then simply treated as a single composite study containing 11 sites (1 + 6 + 4). For comparison of GA21 with the conventional control, all lines were treated as fixed effects. The comparison to the control was by means of a simple pooled-variance *t* statistic. For each compositional measure, the *p* value for a test of GA21 equal to the control, the observed difference of GA21 from the control, and lower and upper 95% confidence intervals for the mean difference of GA21 from the control were calculated. For comparing GA21 with the population of commercial reference lines, the analysis was similar to that described above. In this case, however, all conventional lines (control and reference) were treated as another level of random effects in the mixed linear model. In this case, the *t* statistic compares the means of the two populations of lines from which (1) GA21 and (2) the other commercial lines are considered samples. The *p* values and 95% confidence intervals for this comparison were also computed in the mixed linear model procedure of SAS.

EFSA-GMO-UK-2008-60

In the application EFSA-GMO-UK-2008-60 for import and use (including cultivation) of genetically modified herbicide tolerant maize GA21, the applicant presents compositional analysis from grain and forage material collected in field trails in North America during the 2004 and 2005 growing season and in Europe during the 2006 growing season. The compositional analysis from material collected in the North American field trial in 2004 is the same analysis as presented in application EFSA-GMO-UK-2005-19/EFSA-GMO-RX-GA21.

The following maize hybrids were used in the 2006 study:

Description	Entry	Genotype
Non-transgenic maize	E1	NP2673/NP2846
GA21 maize	E2	NP2673(GA21)/NP2846
GA21 maize treated with Touchdown®	E3	NP2673(GA21)/NP2846

During 2006, hybrid maize plants were grown according to local agronomic practices at the following six locations in Europe, representing agricultural regions where the hybrid varieties typically would be cultivated (Field Trial GA21-06-101):

Location Code	City and Country	Location Identifier
L6	Dâlga, Romania	RR01
L7	Ramnicu Sarat, Romania	RR02
L8	Lovrin, Romania	RR03
L9	Barbens, Spain	RS01
L10	Espuñes, Spain	RS02
L11	Bellpuig, Spain	RS03

Replicate trials of transgenic GA21 maize and corresponding isogenic controls were planted in 6 locations in all trials. According to the applicant, the locations of the trial sites were selected to be representative of the range of environmental conditions under which the hybrid varieties are expected to be grown. At each location, three replicate plots of each genotype were planted. The field trials were grown following a randomised complete block design with three replicate plots of each genotype.

The levels of nutritional and anti-nutritional components were compared in maize grain and forage produced from GA21 maize plants and concurrently grown non-transgenic near-isogenic control plants. The mean values were also compared with the range of data published in the literature, where data was available.

Compositional data were subjected to analysis of variance. For each component, the statistical significance of the genotype effect and the location-by-genotype interaction were determined using a standard F-test at the 5% probability level. F tests were used to assess the statistical significance of the genotype effect between the non-transgenic and GA21 maize treated with Touchdown®, and between the non-transgenic and the GA21 maize not treated with Touchdown®. The results were compared to compositional analysis data for grain and forage from conventional maize hybrids published in the literature and in compositional analysis databases. Moisture content of forage and grain was not statistically analysed, because both had been mechanically dried.

Details on the field trials and compositional analysis can be found in the technical dossiers from the applicant, considered confidential by Syngenta.

3.2 Compositional Analysis

EFSA-GMO-UK-2005-19/EFSA-GMO-RX-GA21
Key nutritional components in maize grain and forage derived from transgenic maize GA21 and near-isogenic non-transgenic control plants were compared. The forage and grain analysed were from a GA21-derived maize hybrid that was treated with either conventional herbicides (selective herbicides appropriate to local agronomic conditions) or herbicide products containing glyphosate, and from a non-transgenic isogenic control treated with conventional herbicides.

Compositional analyses were conducted to measure proximate (protein, fat, ash, carbohydrate, and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), amino acid, fatty acid, calcium, and phosphorus contents of grain; and proximate, ADF, NDF, calcium, and phosphorus contents of forage.

Proximates and minerals
Relative to the non-transgenic control hybrid E3 (-), statistically significant differences were observed for ash and fat composition in E1 (+) and E2 (+ TD) maize grain, respectively, and NDF levels in E1 (+) forage. According to the applicant , these differences are not consistently associated with the presence of the transgene (observed in only one transgenic sample set) and levels of these analytes as well as the other proximates are within the range of values reported in the literature.

Statistically significant differences were observed for phosphorus in the comparison of E1 (+) grain samples and E3 (-) grain samples, with phosphorous in the E1 (+) grain slightly higher. Statistically significant differences were also observed for phosphorous in E2 (+ TD) vs. E3 (-) forage samples, although variability at different locations undermines the statistical significance. Average calcium levels measured in E2 (+ TD) grain were significantly lower than in the non-transgenic samples. However, for both calcium and phosphorous, the observed differences were not consistently associated with the presence of the transgene and levels of these and all the other minerals measured were within normal variation as reported in the literature. Sodium levels were below the limit of quantitation (LOQ) in all the samples and in many of the samples selenium levels were also below LOQ.

Compositional analysis results for forage and grain from North American and European field trials in 1996 and 1997 are presented in the Appendix, Tables 1 and 2, respectively. The results for forage and grain from North American and European field trials show that the levels of proximate components (protein, fat, ash, carbohydrate, and moisture), fiber (ADF and NDF), and phosphorus in the grain and forage of GA21 maize were comparable to those in the grain and forage of the control line. In

addition, these values were either within published literature ranges, within the range determined for commercial varieties evaluated in the 1997 field trials, or within the range of historical conventional control values determined from previous studies.

The contents of calcium in the grain of GA21maize and the control maize were ~2-4- fold lower than the values reported in the literature. According to the applicant, the results may be attributed to differences in analytical methods with older procedures subject to interferences from elements such as phosphorus. The range of values found for calcium content was approximately the same as those reported for the reference material (five to six conventional commercial lines). No statistically significant differences were observed for the content of calcium in forage in data from either 1996 or 1997 field trials. Similarly, the content of calcium in the grain of GA21 maize was not statistically significantly different from the control line in data from 1996 field trials. However, the content of calcium in the grain of GA21 maize was statistically significantly lower (~9%) than the control line in data from 1997 field trials. According to the applicant, the small difference is unlikely to be of biological significance as there were no statistically significant differences observed for the content of calcium in grain in data from 1996 field trials and in comparisons of GA21 maize with commercial lines in data from 1997 field trials.

Amino Acids
Aspartic acid levels were higher in the transgenic E1 (+) samples, although this difference was not observed consistently at all locations and was not seen in the comparison between E2 (+ TD) and E3 (-). No other statistically significant differences in amino acid levels were observed, and all levels of amino acids, including aspartic acid, were within published ranges.

The amino acids composition of GA21 maize grain from North American and European field trials in 1996 and 1997 were comparable to those in the grain of the control line (Table 3 – Appendix). In addition, these values were either within published literature ranges, within the range determined for commercial varieties evaluated in 1997 field trials, or within the range of historical conventional control values determined from previous studies. Statistically significant differences between GA21 and control grain were noted for serine and tyrosine in the data from 1996 field trials; however, there were no statistically significant differences in amino acid content between GA21 and control grain in the data from 1997 field trials. The contents of serine and tyrosine in maize line GA21 were 1.1% higher and 3.5% lower, respectively, than the control line in the data from 1996 field trials. However, these small differences are unlikely to be of biological significance as these statistically significant differences were not observed in data from 1997 field trials. The data from GA21 maize grown at 16 sites over two years establish that there were no statistically significant differences in the levels of the aromatic amino acids phenylalanine and tryptophan in maize line GA21 compared to the control line. The statistically significant decrease in tyrosine levels in the grain of maize line GA21 versus the control line in data from the 1996 field trials is unlikely to be of biological significance as no statistically significant difference in tyrosine levels was observed in data from 1997 field trials.

Fatty Acids
Five fatty acids account for 90% of the total lipids in maize grain. The two most abundant fatty acids are 18:2 (linoleic) and 18:1 (oleic). Less abundant are 16:0 (palmitic), 18:0 (stearic) and 18:3 (linolenic) fatty acids (EuropaBio, 2003). Statistically significant differences were observed in palmitic, stearic, linoleic and linolenic fatty acid levels for E2 (+ TD). These differences are consistent with the difference observed in the total fat levels in E2 as described in the section on proximates above. This difference was not seen for the E1 (+) vs. E3 (-) comparison and therefore not linked to the presence of the transgene. All values are within the ranges reported in the literature and the observed differences are unlikely to be of biological significance.

The fatty acids composition of GA21 maize grain from North American and European field trials in 1996 and 1997 were comparable to those in the grain of the control line (Table 4- Appendix).

According to the applicant, these values were either within published literature ranges, within the range determined for commercial varieties evaluated in 1997 field trials, or within the range of historical conventional control values determined from previous studies. No statistically significant differences in the levels of fatty acids between GA21 maize and the control maize were observed in the data from either the 1996 or 1997 field trials. Also, no statistically significant differences were observed in comparisons of maize line GA21 with commercial lines in the data from 1997 field trials.

Vitamins
In the transgenic grain from plants treated either with conventional herbicides or with glyphosate, β-carotene levels were statistically significantly higher than the non-transgenic control. However, this was not consistent across all sites and the values obtained for β-carotene for all samples were within the range of natural variation reported, so this difference is unlikely to be of biological significance. No other statistically significant differences in vitamin levels were observed, and the values for all other vitamins, were also within the range of natural variation reported in the literature. Vitamin C, beta-tocopherol and gamma-tocopherol were not quantifiable (<LOQ) in any of the samples.

The transgenic grain samples (treated with either conventional herbicides or glyphosate) did indicate statistically significantly higher concentrations of β-carotene than the non-transgenic control samples. However, all β-carotene concentrations were within the range of natural variation for maize as published in the literature. In fact, the levels of all components evaluated in this study were within the ranges of reported literature values for maize, except for inositol, for which the values of all three entries (both transgenic and non-transgenic) were slightly above the maximum values reported in the literature.

Secondary Metabolites and Anti-Nutrients
Analytical data for the following secondary metabolites and anti-nutrients are provided: furfural, phytic acid, inositol, raffinose, trypsin inhibitor, ferulic acid and *p*-coumaric acid. Furfural was below the limit of quantitation in all samples. Phytic acid is present in maize germ and binds 60-75% of phosphorus, decreasing the bioavailability of phosphorus in maize for non-ruminant animals (OECD 2002). The levels of raffinose were <LOQ in all the samples.

Maize contains low levels of trypsin inhibitors (OECD 2002), but no significant differences were detected in trypsin inhibitor levels between the transgenic and non-transgenic samples, although there was a strong indication of location effect.

Statistically significant differences between E1 (+) and E3 (-) ferulic and *p*-coumaric acid levels are indicated in Table 22 - appendix, but the difference was not consistently observed at all locations or in the E2 (+ TD) and E3 (-) comparisons, and is therefore not consistently associated with the presence of the transgene.

Levels of all the anti-nutrients and secondary metabolites were within the ranges of natural variation as reported in the literature, except for inositol, which was slightly above the reported ranges. However there were no statistically significant differences in inositol levels between the two transgenic entries (E1, E2) and the control (E3).

EFSA-GMO-UK-2008-60

Key nutritional components in maize grain and forage derived from GA21 maize and non-transgenic near-isogenic control plants were compared. Based on guidance of the OECD (OECD 2002) the components measured in grain were proximates (including starch and fiber), minerals, amino acids and selected fatty acids, vitamins, anti-nutrients and secondary metabolites. Forage was analysed for proximates (including fiber), calcium and phosphorus.

Proximates
In the 2005 study, forage from GA21 maize plants and the corresponding non-transgenic maize plants was analyzed for proximates including acid detergent fiber [ADF] and neutral detergent fiber [NDF], for calcium and phosphorus. No statistically significant differences were observed that could be confidently attributed to the transgene for any forage analytes measured. In general, fat levels measured in the compositional analyses of maize forage from the North American in 2005 are low, and may fall below the limit of quantitation (<LOQ). Data on fat levels in forage were not suitable for statistical analysis as one value from the GA21 hybrid was reported as <LOQ. An estimated overall average for fat content in the GA21 forage was calculated by converting the LOQ for the analytical method for fat (in FW units) to DW units, based on the moisture content of the individual sample for which the fat level was reported as <LOQ. This calculated DW value of the LOQ for the analytical method was substituted for the <LOQ data point and used to further calculate the estimated overall average for fat content in the GA21 forage. The calculated estimated average level of fat in the GA21 forage and the average value of fat in the non-transgenic forage were both within the ranges reported in the literature. The only statistically significant difference observed in proximates in forage was a slightly higher mean level of carbohydrates in the GA21 hybrid. The average values for all proximates in forage were within the ranges reported in the literature.

No statistically significant differences were observed for any of the analytes measured. Average values for all proximates measured in grain were within the ranges reported in the literature.

In the 2006 study levels of protein, ash, carbohydrates, ADF and NDF did not differ significantly between the genotypes. A statistically significant location-by genotype interaction was observed for total fat levels in the comparison between the non-transgenic maize and the GA21 maize treated with Touchdown®. All mean levels across locations and for each location were within the ranges reported in the ILSI database.

Levels of protein, ash, carbohydrates, and starch in grain did not differ significantly between the genotypes. A statistically significant difference was observed for ADF levels in the comparison between the non-transgenic maize and the GA21 maize not treated with Touchdown®. Significant location-by-genotype interactions were observed for fat, NDF, and TDF levels in the comparison between the non-transgenic maize and the GA21 maize not treated with Touchdown®. Significant location-by-genotype interactions were observed for ADF, NDF and TDF levels in the comparison between the non-transgenic maize and the GA21 maize treated with Touchdown®. For NDF and TDF, the observed differences at location 8 (L8) were not seen at the other five locations, and it is this inconsistency that has resulted in the significant location-by-genotype interactions in the comparisons between the non-transgenic maize and GA21 maize (not treated with Touchdown® and treated with Touchdown®). When NDF and TDF data from location 8 were excluded and statistical analysis was performed on the data from the remaining five locations, no significant genotype effects or location-by-genotype interactions were observed. All mean levels across locations and for each location were within the ranges reported in the ILSI database.

Minerals
In the 2005 study, no statistically significant differences were observed in levels of any mineral analyzed in grain or forage. For all minerals statistically analyzed, average values were within the ranges reported in the literature for forage. In grain, levels of both sodium and selenium were <LOQ in many samples and, therefore, the data sets were not suitable for statistical analysis. The quantifiable data points for sodium in the GA21 samples (ranging from 163 to 192 ppm DW) and for the non-transgenic hybrid (ranging from 114 to 186 ppm DW) were all within the ranges reported in the literature. The quantifiable data points for selenium in the GA21 samples (ranging from 83 to 337 ppb DW) and the non-transgenic hybrid (ranging from 40 to 415 ppb DW) were also within the ranges

reported in the literature. In the 2006 study, calcium and phosphorus levels in forage did not differ significantly between the genotypes and no significant location-by-genotype interactions were observed (Table 34). As shown in Table 35, none of the mineral levels in grain differed significantly between the genotypes. For selenium and sodium, levels that were below the limit of quantitation (LOQ) precluded statistical analysis. No significant location-by-genotype interactions were observed. All mean levels across locations and for each location were within the ranges reported in the ILSI database

Vitamins
In the 2005 study, no statistically significant differences were seen in levels of vitamins B1, B2, B3, B6 or vitamin E, and all average levels were within the ranges reported in the literature Table 5 - appendix). Statistically, the levels of both β-carotene and folic acid were significantly different between the GA21 hybrid and the non-transgenic hybrid. For β-carotene, at all locations, the GA21 transgenic hybrid consistently showed higher levels than the non-transgenic hybrid. Folic acid levels were very consistent for each genotype across locations, and the mean values were almost identical (0.029 vs 0.030 mg/100 g DW). All average values for both β- carotene and folic acid fell within the ranges reported in the literature.

In the 2006 study, levels of vitamins A, B1, B2, B3, B6, and B9 did not differ significantly between the genotypes. A statistically significant difference was observed for vitamin E levels only in the comparison between the non-transgenic maize and the GA21 maize not treated with Touchdown®. No significant location-by-genotype interactions were observed. All mean levels across locations and for each location were within the ranges reported in the ILSI database.

Amino Acids
In the 2005 study, no significant differences were noted for any of the 18 amino acids measured (Table 39). Average levels of all amino acids were within the ranges reported in the literature (Table 6-appendix).

In the 2006 study, levels of seventeen of the eighteen amino acids analyzed did not differ significantly between the genotypes. A significant location-by genotype interaction was observed for tryptophan levels only in the comparison between the non-transgenic maize and the GA21 maize treated with Touchdown®. All mean levels across locations and for each location were within the ranges reported in the ILSI database.

Fatty Acids
In the 2005 study, a statistically significant difference was observed only for 18:2 linoleic acid and not for the other four fatty acids measured. Average levels of all five measured fatty acids, including 18:2 linoleic acid, were within the ranges reported in the literature (Table 7 - appendix).

In the 2006 study, the proportions of 16:0 palmitic, 18:1 oleic acid, 18:2 linoleic, and 18:3 linolenic acid did not differ significantly between the genotypes. A significant location-by-genotype interaction was observed for 18:0 stearic acid levels only in the comparison between the non-transgenic maize and the GA21 maize treated with Touchdown®. All mean levels across locations and for each location were within the ranges reported in the ILSI database.

Secondary Metabolites and Anti-nutrients
There are no generally recognised anti-nutrients in maize at levels considered to be harmful, but for the purposes of assessment of substantial equivalence, the OECD has asked for analytical data for the following secondary metabolites in maize: ferulic acid, p-coumaric acid, furfural, inositol, phytic acid, raffinose and trypsin inhibitor. In the 2005 study, no significant differences were noted for levels of

inositol, phytic acid, ferulic acid or ρ-coumaric acid and all average values for these analytes were within the ranges reported in the literature (Table 8 - appendix).

Trypsin inhibitor data were not suitable for statistical analysis as three samples were reported as below the limit of quantitation (<LOQ). An estimated overall average for trypsin inhibitor content in grain was calculated by converting the LOQ for the analytical method for trypsin inhibitor (in FW units) to DW units, based on the moisture content of the three individual samples for which trypsin inhibitor levels were reported as <LOQ. The calculated DW values of the LOQ for the analytical method were substituted for the <LOQ data points and used to further calculate the estimated overall averages for trypsin inhibitor content in both GA21 grain and non-transgenic grain. These estimated overall average levels of trypsin inhibitor in grain of both hybrids were within ranges reported in the literature.

Furfural levels were <LOQ in all grain samples and levels of raffinose were <LOQ in all samples except one GA21 sample.

In the 2006 study, levels of *p*-coumaric acid, phytic acid, and trypsin inhibitor did not differ significantly between the genotypes. Statistically significant differences were observed for ferulic acid and inositol levels only in the comparison between the non-transgenic maize and the GA21 maize treated with Touchdown®. For raffinose and furfural, levels that were below the limit of quantitation precluded statistical analysis. No significant location-by-genotype interactions were observed. Mean levels across all locations and for each location were within the ranges reported in the ILSI database for all components, except inositol. The average inositol levels across all locations, for all entries, were within the ranges reported in the ILSI database. At one location in the GA21 maize not treated with Touchdown® and two locations in the GA21 maize treated with Touchdown® mean levels of inositol were above the range reported in the literature.

3.3 Agronomic and phenotypic characters

Application EFSA-GMO-UK-2005-19
In the application EFSA/GMO/UK/2005/19 for food and feed uses, import and processing of maize GA21 within the European Union, the applicant present agronomic and phenotypic data from field trials in the USA in 2004 and in Brazil in 2003. The field trials in the USA were performed at eight sites in commercial maize-growing regions in Iowa, Illinois and Indiana. The trials included two GA21 hybrids (115-GA21 and 47-GA21) and their corresponding near-isogenic, non-transgenic hybrids. Details on the experimental field trials are, however, missing in the dossier (Appendix 10). The agronomic assessment of maize Event GA21 contains no specifications on field design, agronomic practices or whether the plants used for the agronomic assessment were treated with the target herbicide.

During the field trial extensive agronomic data (e.g. grain yield, number of emerged plants, plant population at harvest, ear height, plant height, percent snapped plants, stalk and rood lodging) and data related to disease susceptibility were collected. In addition, the applicant assessed the efficacy and selectivity of herbicide treatments. Twenty agronomic and phenotypic traits and two disease traits were evaluates in these trials, although not all traits were recorded at all locations.

Analyses of variance across trial locations showed statistically significant differences between maize GA21 and the corresponding non-GM comparator for grain yield ($p<0.05$). The effect of genotype within both of the two hybrids was significant indicating that the transgenic and non-transgenic versions of each of the hybrids did not yield equivalently. For Hybrid 47, the GA21 version yielded 8.11 bushels/acre more than the corresponding near-isogenic hybrid whereas for Hybrid 115, the

converse was true with the GA21 version yielding 10.25 bushels/acre less than the corresponding near-isogenic version (data not shown). According to the applicant, poor seed quality of GA21 may have influence the yield data. Given that the number of emerged plants for the GA21 version of Hybrid 115 was less than that of its near-isogenic version and that it yielded less than its near-isogenic version at seven out of the eight locations, it is possible that seed quality of the GA21 version of hybrid 115 may have been inferior resulting in poorer grain yield. However, all yield data fell within the normal range of variation reported for conventional maize varieties.

No statistically significant differences between the transgenic maize GA21 and the comparator were detected for any of the other assessed phenotypic characteristics in the across location analysis ($p>0.05$). Analyses within field locations showed differences in the number of emerged plants, plant height, and percent snapped plants in 2004 at some of the trial sites. No differences in general appearance of the plants or any other phenotypic differences that could indicated unintended effects of the genetic modification were found.

Specific disease trials were also conducted in 2004. The disease susceptibility of GA21-derived hybrids and non-transformed isogenic controls the maize pathogens Southern maize leaf blight (*Helminthosporium maydis*) and Gray leaf spot (*Cercospora zeae-maydis*) was assessed. No indication of differential disease susceptibility was observed between conventional and GA21-derived hybrids (Appendix 10).

In addition to the agronomic assessment trials performed in 2004, agronomic, efficacy and selectivity data generated by Bayer Crop Science from Event GA21 grown at 3 locations in Brazil in 2003 (Appendix 11). In this trials, glyphosate treatments on the transgenic plants resulted in phytotoxicity in up to 30 % of the plants at one of the three field sites. It is reported in the application that there was a high incidence of fungal disease in both GA21 maize and conventional maize in this tropical region of Brazil. Phytotoxicity was also observed in up to 50% of the non-GM control plants treated with conventional herbicides, and may result from the high incidence of fungi at this location.

Application EFSA-GMO-UK-2008-60

The application EFSA/GMO/UK/2008/60, covering authorisation of maize GA21 for all food and feed uses, including cultivation, include results from field trials with maize GA21 in Europe in 2007 and 2008. The European study was conducted at eight separate field locations in Spain, Romania and Czech Republic during the 2007 growing season and six locations in Spain and Romania during the 2008 growing season. Each agronomic trial consisted of one near-isogenic hybrid pair representing Event GA21 and non-transgenic control: H8124GT/H8123 in Spain and Romania and EX56317GT/NX27026 in Czech Republic. At each trial site, maize GA21 and the conventional counterpart were planted following a randomized complete block design containing three blocks. Depending on location, plot size ranged from 14.8 m^2 up to 16.8 m^2 and included 4 rows. Harvest measurements were taken in the central two rows of the plot. Plots of the GA21 maize either was left untreated or was treated with two applications of an herbicide containing the active ingredient glyphosate.

Extensive data on phenotypic characteristics, agronomic performance and disease susceptibility (e.g., grain yield, number of emerged plants, plant population at harvest, ear height, percent snapped plants, root lodging) were collected. When analysed across all locations, statistically significant differences were observed for grain weight in Europe during the 2007 growing season. In 2008, plant height, ear height, harvest population and grain yield were statistically significantly different between maize GA21 and the conventional counterpart. Some additional differences in agronomic data were detected at individual field trial sites only. The described differences are considered to be of small magnitude and not biologically relevant. No consistent trend was observed across locations and years.

The use of the target herbicide on Event GA21 derived hybrids does not show any impact on agronomic performance when compared to either the near-isogenic non-transgenic hybrids or GA21 hybrids not sprayed.

3.4 Conclusion

Comparative analyses of maize event GA21 to its conventional counterpart) have been performed during multiple field trials located at representative sites and environments in North America (1997, 2004, and 2005), Europe (1996, 1997, and 2006) and Brazil (2003). With the exception of small intermittent variations, no biologically significant differences were found between maize GA21 and controls. Based on the assessment of available data, the VKM GMO Panel concludes that maize GA21 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, except for the mEPSPS protein, and that its composition fell within the range observed among non-GM varieties.

4 Food /feed risk assessment

4.1 Product description and intended uses

The genetic modification in GA21 field maize will not have any impact on the existing production processes used for maize. All GA21 maize products will be produced and processed for use in food, animal feed and industrial products in the same way as other commercial maize. The GA21 field maize and all food, feed and processed products derived from GA21 field maize are expected to replace a portion of similar products from commercial maize, with total consumption of maize products remaining unchanged. The total anticipated intake/extent of use of maize and all food, feed and processed products derived from maize will remain the same.

4.2 Processing of maize

Food manufacturing of GA21 field maize includes many harsh processing steps, e.g. cooking, heating, high pressures, pH treatments, physical shearing, extrusion at high temperatures etc. under which the majority of proteins are denatured, including the mEPSPS protein (Hammond & Jez 2011).

4.3 Toxicological assessment

4.3.1. Toxicological assessment of the newly expressed protein

The *epsps*-gene was originally obtained from maize (*Zea mays*). Two intended mutations were introduced in the maize *epsps* gene, making two amino acid changes. These two changes were introduced specifically to confer tolerance to herbicide products containing glyphosate. The maize EPSPS protein has no known toxic or pathogenic potential. The mEPSPS protein is enzymatically active and it has high substrate specificity to the active ingredient glyphosate. The mEPSPS protein has already been found safe to human health during the assessment of glyphosate tolerant maize (EFSA 2007).

4.3.2. Acute toxicity testing

15- days single dose oral exposure of mEPSPS protein in mice
Syngenta has performed a single dose oral toxicity study in mice. Groups of five male and five female Alpk:APfCD-1 mice (8-12 weeks old) were dosed orally by gavage with zero (control) or 2000 mg mEPSPS protein (adjusted for purity)/kg bodyweight as a single dose on day one using deionized water as the control substance and vehicle. The purity of the mEPSPS protein before adjustment was 83 % by weight. The mEPSPS protein was the primary component of the test substance GA21-0104.
The study (AM7513) was conducted in compliance with the UK Principles of Good Laboratory Practice (The United Kingdom GLP Regulations 1999, Statutory Instrument No. 3106, as amended 2004, Statutory Instrument No. 994) except for the deviation listed below. These Principles are in accordance with the OECD Principles of Good Laboratory Practice, revised 1997 (ENV/MC/CHEM(98)17).

There was a single incidence of an intercurrent death in the study. On day one a male in the 2000 mg/kg group had decreased activity, and was found dead on day two. At *post mortem*, this animal had a white substance in the lungs which was considered to be test substance indicating that there had been a problem during dose administration. The clinical signs and mortality, therefore, are considered to be incidental to the test substance.

All remaining animals survived to scheduled termination. The health of the mice was satisfactory and there was no evidence of disease or infection, which might have compromised the interpretation of the findings. There were no effects on clinical condition, bodyweight, food consumption, clinical pathology, organ weights, macroscopic or microscopic pathology that were considered to be related to the administration of the mEPSPS protein to male and female mice. The applicant concluded with that the oral administration of 2000 mg mEPSPS protein/kg bodyweight as a single dose resulted in no treatment related effects.

The acute oral toxicity test performed on mice did not indicate toxic effects of *E. coli* produced mEPSPS protein. However, acute tests do not provide enough information to conclude on possible adverse health effects of maize GA21. In whole food the concentrations of the protein is low and acute toxic effects in humans and animals will most probably be negligible. Acute toxicity testing of the newly expressed protein is of little additional value to the risk assessment of the repeated human and animal consumption of food and feed derived from GM plants and is therefore not taken into account in this risk assessment. EFSA discourages the use of acute studies in risk assessments of GMO (EFSA Journal 2011, 9(5):2150).

4.3.3 Repeated dose toxicity testing

No repeated dose 14-day oral toxicity study of mEPSPS protein in rodents has been performed by the applicant.

4.3.4 Toxicological assessment of the whole GM food/feed

90-day subchronic feeding study in rats
The applicant has provided a subchronic (90-day) feeding study in rats using grain of maize GA21 as a component of the diet. This study design complied with the following Regulatory Guidelines: a) OECD guideline reference 408 (1998): Repeated dose 90 day oral toxicity study in rodents. b) United States Environmental Protection Agency, Health Effects Test Guidelines, OPPTS. 870.3100 (August 1998): 90-Day Oral Toxicity in Rodents. c) United States Food and Drug Administration, Office of Food Additive Safety, Redbook 2000, Toxicological Principles for the Safety Assessment of Food Ingredients (2003): IV.C.4.a. Subchronic Toxicity Studies with Rodents.

Groups of 12 male and 12 female Wistar-derived rats (Alpk:APfSD) were fed diets containing 10% or 41.5% (w/w) kernels from maize GA21 sprayed with glyphosate (treated), 10% or 41.5% kernels from maize GA21 sprayed with other herbicides (untreated) or 10% or 41.5% kernels from near isogenic non GM control maize treated with other selective herbicides. The diets were fed at least for 90 consecutive days.

Clinical observations, bodyweights and food consumption were measured throughout the study. A functional observation battery of tests and locomotor activity monitoring were performed during week 11. An ophthalmoscopic examination was performed on all animals pre-study and in week 13. At the end of the scheduled period, the animals were killed and examined post mortem. Cardiac blood samples were taken for evaluation of clinical pathology. Selected organs were weighed and specified tissues were taken for subsequent histo-pathological examination.

No clinically relevant reactions were noted in the regular observations of the animals. In detailed examinations of the animals and quantitative assessments of body functions (including landing foot splay, grip strength and motor activity measurements), there were no biologically relevant differences between groups. Ophthalmoscopic examinations did not reveal relevant effects.

Food consumption was comparable in all groups and there were no relevant differences in food utilisation. Males receiving diets with 41.5% kernels from maize GA21 treated with glyphosate showed a reduced bodyweight compared with the controls in weeks 6, 10, 12, 13 and 14. These differences were not observed in males receiving diets with 41.5% kernels from untreated maize GA21. However, all values fell within the historical control ranges.

Several statistically significant differences in haematology and clinical chemistry parameters compared with the controls were noted: reduced mean red blood cell volume in males of the low-dose groups (maize GA21 treated and untreated); reduced monocyte counts in males of the high-dose group (maize GA21 untreated); reduced neutrophil counts and plasma γ-glutamyl transferase in females of the low-dose group (maize GA21 untreated); reduced plasma phosphorous levels in males of the high-dose group (maize GA21 treated); reduced plasma creatinine in females of the low-dose groups (maize GA21 treated and untreated); reduced plasma glucose in females of the high-dose group (maize GA21 treated); reduced plasma chloride in females of the low-dose group (maize GA21 untreated). Single differences in organ weights were observed compared with the controls. In males, relative brain, heart and kidney weights were increased in the high dose group (maize GA21 treated). Relative testes weights were increased in the low-dose group (maize GA21 treated). In females of the low-dose group (maize GA21 treated) the adrenal gland weights (relative and absolute) were reduced and brain weights (absolute and relative) and liver weights (relative) were increased. Liver weights (absolute) were increased in the low-dose group (maize GA21 untreated). These findings were generally not dose related, limited to one sex and/or no consistent pattern was identified when the herbicide-treatment of the plants was considered. Since, in addition, the findings were not accompanied by histopathological changes in the respective organs or tissues; the applicant does not consider the observed statistical differences as toxicologically relevant.

49-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 GA21 with its non-GM commercial maize equivalent (Appendix 26, applicant dossier). A total of 1200 birds, commercial strain of Ross344 males and Ross 308 females, were randomly distributed into 48 pens of a curtain-sided house at one day of age. Each pen contained 25 birds of the same sex. Birds were identified by neck tag indicating animal number.

Broilers were fed over a 49-day period. Four kinds of maize grain were used in this study: “Event GA21 Positive Sprayed” (grain from Event GA21 plants treated with glyphosate herbicide), “Event GA21 Positive Unsprayed” (grain from Event GA21 plants untreated with glyphosate herbicide, but treated with conventional herbicides), “Event GA21 Negative” (grain from near isogenic, non-transgenic control plants), and “NC 2004” (a commercially available lot of North Carolina grain grown during the 2004 season). Samples of starting grain, starter diet (51 % maize), grower diet (56 % maize), and finisher diet (64 % maize) were analysed for the concentration of mEPSPS protein. The protein was not detected in Event GA21 Negative grain or diet samples. Samples of grain and diets designated Event GA21 Positive Sprayed showed concentrations of mEPSPS ranging from 1.22 ± 0.05 to 3.96 ± 0.21 µg/g fresh weight while samples of Event GA21 Positive Unsprayed showed concentrations ranging from 1.25 ± 0.10 to 4.15 ± 0.14 µg/g fresh weight.

Samples of maize grain lots were analysed for proximates, amino acids and mycotoxins (aflatoxin, deoxynivalenol, fumonisin, T2 toxin and zearalenone, all well below regulatory limits).

Body weight, feed conversion, and survival data were analyzed in order to determine statistical differences between maize grain diets and gender. Statistical analyses were performed using the

General Linear Model (GLM) procedure of SAS Institute. Gender and source of maize grain were independent variables in a two-way analysis of variance within a randomized complete block design, with random error (between-pen variation) as the error term.

No significant overt clinical findings were observed during the study. Consistent with historical data for this facility and study type, a low incidence of mortality occurred among all study groups with mortality slightly higher in the males.

The results showed that at placement (Day 1, start of feeding) there was no overall difference in the mean body weight of chickens in the different treatment groups, although males were slightly heavier than females. Males continued to weigh significantly more than females throughout the study, but there were no other statistically significant effects on body weight.

The feed conversion ratio is an indicator of how efficiently a bird converts feed to live body weight. Overall, males had significantly better (lower) unadjusted and adjusted feed conversion ratios than females at 35 and 49 days of age as well as better adjusted feed conversion at 21 days, as expected. Birds fed diets containing the NC 2004 maize grain had higher adjusted and unadjusted feed conversion ratios to 21, 35, and 49 days of age. There were no significant interactions of maize grain source and gender for cumulative unadjusted and adjusted feed conversion ratios at any time.

There were significant differences due to gender, with faster-growing males exhibiting higher mortality at 21, 35, and 49 days of age, as would be expected under normal conditions. The percentage survivors to 49 days of age were slightly higher for females compared to the standards of the experimental facility, while males performed as expected. There was no effect on survival due to maize grain source nor was there a sex by maize grain source interaction. All survival rates were consistently high.

Carcass analysis demonstrated no differences due to maize grain source among females for any carcass portion. Among males there was a difference in live body weight due to maize grain source with the largest males randomly selected for processing in the NC 2004 group and the smallest males selected for processing in the Event GA21 Positive Unsprayed group; that is, random selection of two males per pen did not yield body weights that closely reflected the pen averages in this experiment. The Event GA21 Positive Sprayed and Event GA21 Negative groups were intermediate, with the former having the higher live body weight. These differences in live body weight were reflected proportionally in differences in gross dressed carcass, drums, thighs, and wings. However, there were no differences in body weights when expressed on a percentage carcass basis.

According to the applicant poultry diets prepared with transgenic GA21 maize grain from plants that were either treated or untreated with glyphosate herbicide both supported rapid broiler chicken growth at low mortality rates and good feed conversion ratios without any substantial differences in overall carcass yield. There were no deleterious effects associated with consumption of Event GA21 transgenic maize grain when compared to control (non-transgenic) maize grain.

38- and 40-day feeding study on broiler chickens

The compositional and nutritional safety of maize line GA21 was compared to that of conventional maize (Sidhu et al. 2000). Compositional analyses were conducted to measure proximate, fiber, amino acid, fatty acid, and mineral contents of grain and proximate, fiber, and mineral contents of forage collected from 16 field sites over two growing seasons. The nutritional safety of maize line GA21 was evaluated in a poultry feeding study conducted with 2-day old, rapidly growing broiler chickens, at a dietary concentration of 50-60% w/w. Compositional analysis results showed that, except for a few minor differences that are unlikely to be of biological significance, the grain and forage of GA21 maize were comparable in their composition to that of the control maize line and to conventional maize.

The termination dates for the males (38 days) and females (40 days) differed due to the limited quantities of the control line available. The average body weight/pen and for each treatment group by gender was calculated. The average feed conversion was calculated for the entire duration of the study by using the total feed consumption during the study by pen divided by the total body weight of the surviving chickens/pen. This was averaged for each group by gender. Adjusted feed conversion was calculated by using the total feed consumption/pen divided by the total body weight of the surviving chickens and body weight of chickens that died or were removed from the pen. At study termination, fat pads were collected from each chicken. The fat pads from all chickens within a pen were combined and weighed.

Statistical analyses were performed on body weight, feed efficiency, adjusted feed efficiency, and fat pad weight. Because the pens were set up as a randomized complete block experimental design with seven diet treatments (based on seven maize varieties) in each of five replicated blocks of pens, the standard randomized block analysis of variance (ANOVA) statistical model was used to analyze the data. Means were compared to each other at the 5% level of significance. An additional analysis was done to compare the fit of GA21 to the population of responses from commercial varieties to verify whether the GA21 group was consistent with the expected variation of responses of animals fed the non-transgenic commercial maize varieties. This analysis was carried out using a linear mixed model procedure in SAS, and comparisons were made at the 5% level of significance.

The results showed that there were no differences in growth, feed efficiency, adjusted feed efficiency, and fat pad weights between chickens fed with GA21 grain or with parental control grain.

4.4 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.

4.4.1 Assessment of allergenicity of the newly expressed protein

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

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

- evaluation of protein glycosylation
- assessment of protein exposure

These assessments have previously been described by the applicant for mEPSPS, and were based on the following aspects:

i) The sources of the transgene gene is maize (*Zea mays*), which is not considered a common food allergen.
ii) EPSPS enzymes are ubiquitous in plants and microorganisms and are safely consumed
iii) 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).
iv) The mEPSPS is functionally equivalent to other food derived EPSPS enzymes except for its tolerance to Roundup® herbicides.
v) The EPSPS proteins have been previously assessed for genetically modified plants and found to have no potential for allergenicity (EPA, 1995b, EPA, 1997, Canadian Food Inspection Agency, 1999, SCP, 1998 and OECD, 1999).
vi) The expressed mEPSPS protein is a single polypeptide with a 99.3 % sequence identity to the wild type.
vii) The mEPSPS protein lacks homology to known toxins or allergenic proteins (Meyer 1999; Cressman, 2003).
i) Immunoblot glycosylation analysis 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).
ii) Rapid degradation of the mEPSPS protein in simulated gastric fluids (OECD 1999).

The information listed above indicates that the newly expressed proteins in GA21 maize lack IgE allergenic potential with regard to human and animal health. However, it does not cover allergic reactions that are not IgE mediated, e.g. some gluten-sensitive enteropathies or other enteropathies that are not IgE-mediated.

4.4.2 Assessment of the IgE mediated allergenicity of the whole GM plant

Allergenicity of the maize GA21 could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, e.g. through qualitative or quantitative modifications of the expression of endogenous proteins. However, given that no biologically relevant agronomic or compositional changes have been identified in field maize GA21 with the exception of the introduced traits, no increased allergenicity is anticipated for maize GA21. Moreover, maize is not considered a common allergenic food.

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

It is the opinion of the VKM GMO Panel that a possible over-expression of any endogenous protein, which is not known to be allergenic, in maize GA21 would be unlikely to alter the overall allergenicity of the whole plant or the allergy risk for consumers.

4.4.4 Adjuvanticity

According to the EFSA guidance document for risk assessment of food and feed from GM plants (EFSA 2011b), adjuvants are substances that, when co-administered with an antigen increase the

immune response to the antigen and therefore might increase the allergic response. In cases when known functional aspects of the newly expressed protein or structural similarity to known strong adjuvants may indicate possible adjuvant activity, the possible role of these proteins as adjuvants should be considered. As for allergens, interactions with other constituents of the food matrix and/or processing may alter the structure and bioavailability of an adjuvant and thus modify its biological activity. No such associations have been reported for mEPSPS.

4.5 Nutritional assessment of GM food/feed

Compositional analyses of maize GA21 and sweet maize GA21 indicate nutritional equivalence to the non-GM control maize with comparable genetic background and to the published range of values in the literature. The nutritional equivalence between GA21 maize and non-GM control maize has been further shown by the results of a poultry feeding study, feeding study in dairy cows, beef steers, pigs and calves (see chapters 4.3.2 and 4.5.2).

4.5.1 Intake information/exposure assessment

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

The comparable composition and nutritional value of maize GA21, together with the results of the assessment of dietary intake and nutritional impact, indicate that food products derived from maize GA21 are nutritionally equivalent to food products derived from conventional maize. Hence, anticipated dietary intake is not expected to change.

Since all foods from field maize are derived from grains, the applicant has measured mEPSPS-protein in grain, the wet- and dry-milled fractions generated from standard food processing carried out on grain, flaking grits and flour produced during dry-milling. Flaking grits and flour were further processed to maize oil and maize chips, respectively. Concentrations of mEPSPS were below the limit of detection in all of the wet-milled fractions. Quantifiable amounts of mEPSPS were found in the starting grain and all of the dry-milled fractions. The concentration of mEPSPS in both the maize chip and maize oil samples were below the limit of detection.

The highest concentrations were found in the flaking grits (approximately 10 µg mEPSPS/g sample), maize hulls (approximately 8 µg mEPSPS/g sample) and coarse grits (approximately 7 µg mEPSPS/g sample) fractions. Concentrations of mEPSPS measured in the other dry-milled fractions, including fine grits, maize meal, maize cones and maize flour, were between approximately 4 and 5 µg mEPSPS/g sample.

An estimated maximum daily intake for a Norwegian adult of mEPSPS protein from maize staples is calculated to be 22 µg, based on 5 µg mEPSPS/g maize flour. These levels are several orders of magnitude below the levels shown to have no effect in laboratory toxicology testing. Also, these levels are considerably below the proposed threshold of toxicological concern (TTC) level of 1800 µg/person/day (Class 1, oral exposure) for chemicals considered to have a low potential for toxicity based on metabolism and mechanistic data (Vermeire et al. 2010). Transgenic proteins produced by genetically modified plants are generally not considered 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 GA21 may be higher for these animals.

This dietary exposure assessment is very conservative as it assumes that all maize consumed comes from maize GA21 and that the transgenic proteins are not denatured by processing.

4.5.2 Nutritional assessment of feed derived from the GM plant

28-day feeding study in dairy cattle
The effects of feeding diets containing either glyphosate-tolerant Roundup Ready maize (RR-GA21) or its non-transgenic control (RR-CON) maize was studied (Donkin et al. 2003). Feed intake, milk production, milk composition, and ruminal digestibility in lactating dairy cows were analysed.

Glyphosate-tolerant Roundup Ready maize (RR-GA21) or its control maize (RR-CON) were grown in alternating fields during one cropping season. Diets contained 42 to 60% maize silage and 20 to 34% maize grain from RR-GA21 or the appropriate nontransgenic counterpart; treatments were applied using a three period switchback design. The duration of each of the periods was 28 d, with the first 14 d used for adaptation to treatment, followed by 14 d to determine effects of treatment on feed intake, milk yield, and milk composition. Twelve non-cannulated and fourrumen cannulated multiparous midlactation Holstein cows were assigned randomly within cannulated and non-cannulated blocks to one of two treatment groups. Cows were housed in individual tie stalls and were fed a TMR once daily ad libitum. Feed offered and feed refusals were measured daily for each cow, and DMI was determined by drying samples in a convection oven at 55 C for 48 h.

Cows were fed diets containing silage and grain from either RR-GA21 for (DK626RR, Dekalb, IL) or RR-CON (DK626, Dekalb, IL) hybrids. Maize silage and grain were analyzed for nutrient composition before the initiation of the experiment and at the end of each experimental period. Intake, milk production, milk composition, BW (body weight change), and BCS (body condition scores) change were determined. Body weight was measured at the beginning and end of each period, and BW change was determined by difference for each cow within each period. Body condition scores were measured (Wildman et al., 1982) by two trained investigators using a five-point scale where 1 = thin and 5 = obese. Measurements were obtained on the day immediately before the start of the first period and on the last day of each period. Change in BCS was determined by difference for each cow within each period.

Cows were fed ad libitum and milked twice daily. There were no differences for nutrient composition between silage sources or between grain sources within an experiment. There were no differences for DMI, 4% FCM production (FCM=fat-corrected milk), and milk composition between RR-GA21 and RR-CON diets. There was no difference in ruminal degradability, determined separately for maize silage and maize grain for RR-GA21 compared with control RR-CON. These data indicate equivalence of nutritional value and production efficiency for maize containing RR-GA21 maize compared with its control.

92-day feeding study in feedlot steers
The feeding value of genetically modified maize (Roundup Ready maize GA21 and NK603) was compare to non-transgenic control hybrids (Erickson et al. 2003). The treatments included two reference hybrids, the near-isogenic control hybrid, and the genetically enhanced maize GA21, resulting in two preplanned comparisons of control hybrid vs. GA21 maize and GA21 maize vs. the average of the reference hybrids.

175 steers (BW = 427 kg) were fed in 25 pens with seven pens per maize hybrid, except the control hybrid, which contained four pens due to limited quantities of that hybrid. All experiments were

conducted as completely randomized designs and utilized maize produced at University of Illinois research farms under identity-preserved protocols.

In all experiments, DMI, ADG, and feed efficiency were similar between GA21 maize and the reference hybrid (p> 0.30). For growth performance, no difference was observed between GA21 maize and control hybrid (p>0.25). No differences were observed between GA21 maize and the control hybrid, or between GA21 maize and reference hybrid, for carcass weight, longissimus dorsi area, or marbling scores in any of the experiments. Subtle differences were observed between GA21 and either control hybrid or reference hybrid for fat depth in each experiment; however, cattle fed GA21maize were not consistently greater and varied from either the control hybrid or the reference hybrid (but not both contrasts) within an experiment.

Based on these results, insertion of glyphosate-tolerant gene had no significant effect on nutritive quality of GA21 maize. Performance and carcass characteristics were not influenced, which suggests that maize GA21 is similar to conventional, non-transgenic maize when fed to finishing feedlot cattle.

4.6 Conclusion

Whole food feeding studies in rats, broilers and cattles have not indicated any adverse health effects of maize GA21. These studies also indicate that maize GA21 is nutritionally equivalent to conventional maize. The mEPSPS protein does not show sequence resemblance to other known toxins or IgE allergens, nor has mEPSPS been reported to cause IgE mediated allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize GA21 is nutritionally equivalent to conventional maize varieties. It is unlikely that the mESPSPS protein will introduce a toxic or allergenic potential in food or feed based on maize 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 GA21 would be increased due to the herbicide tolerance trait. The herbicide tolerant trait can only be regarded as providing a selective advantage for the GM maize plant where and when glyphosate-based herbicides are applied. It is considered very unlikely that maize 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.

A series of field trials with maize GA21 was conducted by the applicant at several maize growing locations in the USA during the 2004 and 2005 growth seasons and in Europe in 2007 (Spain, Romania and Czech Republic) and 2008 (Spain and Romania) to compare the agronomic performance and field characteristics of maize GA21 with its comparators (see section 3.3). Information on phenotypic and agronomic characteristics of maize GA21 and its comparators was generated to compare their growth habit, vegetative vigour and reproduction characters.

The agronomic and phenotypic field trial data did not show major changes in plant characteristics indicating altered fitness, persistence and invasiveness of maize GA21 plants. A number of endpoints (i.e., plant height, ear height, yield) showed statistically significant differences in the acrosslocation

comparisons between maize GA21 and its near-isogenic lines. These differences were, however, numerically small and did not show any consist trend across trials. Moreover, the the range of values for agronomic and phenotypic characteristics was shown to fall within the range of values observed for conventional maize hybrids. No visually observable response to naturally occurring insects, diseases and/or abiotic stressors recorded during the growing season provided any indication of altered stress responses of maize GA21 as compared with its conventional counterpart.

In addition to the data presented by the applicant, the VKM GMO Panel is not aware of any scientific reports indicative of increased establishment or spread of maize GA21, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of maize GA21 are unchanged, glyphosate tolerance are not likely to provide a selective advantage outside of cultivation in Norway. The VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects based on establishment and survival of maize 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 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, 2009; 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 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 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 *mEPSPS* gene from 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 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 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

Considering the intended uses of maize GA21, excluding cultivation, and the absence of target organisms, potential interactions of the GM plant with target organisms were not considered an issue by the VKM GMO Panel.

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

Considering the intended uses of maize GA21, excluding cultivation, potential interactions of the GM maize with non-target organisms were not considered an issue by the VKM GMO Panel.

5.5 Potential interactions with the abiotic environment and biochemical cycles

Considering the intended uses of maize 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 Post-market environmental monitoring

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

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

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

No specific environmental impact of genetically modified maize GA21 was indicated by the environmental risk assessment and thus no case specific monitoring is required. The VKM GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicant is in line with the intended uses of maize NK603 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects.

5.7 Conclusion

The scope of the application EFSA/GMO/UK/2005/19 includes import and processing of maize GA21 for food and feed uses. Considering the intended uses of maize 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 GA21.

Maize 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 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 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

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

The molecular characterisation data indicate that several copies of the GA21 construct are integrated at a single locus in the DNA, and that they are inherited as a dominant, single locus trait. Appropriate analyses of the integration site, inserted DNA sequence, flanking regions, and bioinformatics have been performed. The VKM GMO Panel considers the molecular characterisation of maize GA21 as adequate.

Comparative assessment

Comparative analyses of maize event GA21 to its conventional counterpart) have been performed during multiple field trials located at representative sites and environments in North America (1997, 2004, and 2005), Europe (1996, 1997, and 2006) and Brazil (2003). With the exception of small intermittent variations, no biologically significant differences were found between maize GA21 and controls. Based on the assessment of available data, the VKM GMO Panel concludes that maize GA21 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, except for the mEPSPS protein, and that its composition fell within the range observed among non-GM varieties.

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Food and feed risk assessment

Whole food feeding studies in rats, broilers and cattles have not indicated any adverse health effects of maize GA21. These studies also indicate that maize GA21 is nutritionally equivalent to conventional maize. The mEPSPS protein does not show sequence resemblance to other known toxins or IgE allergens, nor has mEPSPS been reported to cause IgE mediated allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize GA21 is nutritionally equivalent to conventional maize varieties. It is unlikely that the mESPSPS protein will introduce a toxic or allergenic potential in food or feed based on maize GA21 compared to conventional maize.

Environmental risk assessment

The scope of the application EFSA/GMO/UK/2005/19 includes import and processing of maize GA21 for food and feed uses. Considering the intended uses of maize 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 GA21.

Maize 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 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 GA21 is nutritionally equivalent to conventional maize varieties. It is unlikely that the mEPSPS protein will introduce a toxic or allergenic potential in food derived from maize GA21 compared to conventional maize.

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

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Appendix

Table 1. Fiber, minerals and proximate composition of GA21 maize forage from field trials in North America and Europe in 1996 and 1997 (Sidhu et al. 2000)

Table 2. Fiber, Mineral, and Proximate Composition of Forage from Roundup Ready Corn Line GA21

	1996[a]		1997[b]			
component[c]	GA21 mean (range)[h]	control[d] mean (range)[h]	GA21 mean (range)[h]	control[e] mean (range)[h]	comm lines[f] mean (range)[h]	historical[g] (range)
protein	7.91 (5.70–10.37)	7.58 (6.11–8.61)	7.49 (6.40–8.67)	7.45 (5.88–8.76)	7.20 (5.11–10.27)	(4.8–8.4)
ash	4.22 (3.20–4.67)	3.85 (2.64–5.28)	4.29 (2.12–5.29)	4.26 (2.94–5.91)	4.19 (2.00–6.60)	(2.9–5.1)
ADF[i]	25.04 (23.06–27.96)	25.89 (22.72–28.62)	23.85 (20.08–30.21)	25.55 (21.13–34.20)	25.56 (18.32–40.99)	(21.4–29.2)
NDF[i]	39.47 (35.94–44.48)	40.85 (36.97–44.31)	37.91 (31.47–46.29)	38.92 (33.99–49.28)	39.54 (26.37–54.45)	(39.9–46.6)
total fat	1.73 (1.27–2.30)	1.50 (1.24–1.93)	1.88 (0.71–2.98)	2.21 (1.16–3.22)	2.04 (0.35–3.62)	(1.4–2.1)
carbohydrates	86.14 (82.94–89.57)	87.04 (84.83–89.88)	86.35 (85.06–89.96)	86.06 (83.58–87.85)	86.62 (83.16–91.55)	(84.6–89.1)
calcium	0.1934 (0.0965–0.2488)	0.1766 (0.0866–0.2172)	0.2304 (0.1420–0.3173)	0.2177 (0.1515–0.2754)	0.1948 (0.0969–0.3184)	(not available)
phosphorus	0.2288 (0.1822–0.2622)	0.2124 (0.2016–0.2365)	0.2178 (0.1419–0.3475)	0.2179 (0.1602–0.2914)	0.1992 (0.1367–0.2914)	(not available)
moisture	72.30 (69.5–77.0)	65.52 (42.0–75.3)	68.83 (62.20–74.10)	68.73 (64.60–73.80)	68.31 (55.30–75.30)	(68.7–73.5)

[a] Data from five U.S. sites; GA21 forage harvested from plants not treated with Roundup herbicide. [b] Combined data from four nonreplicated E.U. sites, six U.S. nonreplicated sites, and one U.S. replicated site; GA21 forage harvested from plants treated with Roundup herbicide. [c] Percent dry weight of sample, except for moisture. [d] Nontransgenic negative segregant. [e] Parental control line. [f] Commercial lines; local hybrids grown at each site. [g] Range for control lines planted in Monsanto Co. field trials conducted between 1993 and 1995. [h] Range denotes the lowest and highest individual values across sites for each line. [i] ADF, acid detergent fiber; NDF, neutral detergent fiber.

Table 2. Fiber, minerals and proximate composition of GA21 maize grain from field trials in North America and Europe in 1996 and 1997 (Sidhu et al. 2000)

Table 1. Fiber, Mineral, and Proximate Composition of Grain from Roundup Ready Corn Line GA21

	1996[a]		1997[b]				
component[c]	GA21 mean (range)[h]	control[d] mean (range)[h]	GA21 mean (range)[h]	control[e] mean (range)[h]	comm lines[f] mean (range)[h]	literature (range)[h]	historical[g] (range)[h]
protein	10.05 (9.39–11.00)	10.05 (9.17–11.19)	11.05 (9.48–14.06)	10.54 (9.70–12.92)	10.87 (7.8–14.20)	(6.0–12.0)[k] (9.7–16.1)[l]	(9.0–13.6)
total fat	3.51 (2.94–3.72)	3.55 (2.76–3.93)	3.90 (3.04–4.63)	3.98 (3.30–4.81)	3.69 (2.48–4.81)	(3.1–5.7)[k] (2.9–6.1)[l]	(2.4–4.2)
ash	1.27 (1.06–1.45)	1.27 (1.21–1.40)	1.38 (1.06–1.80)	1.56 (1.07–3.09)	1.79 (0.89–6.28)	(1.1–3.9)[k]	(1.2–1.8)
ADF[i]	3.73 (3.35–3.99)	3.72 (3.52–4.05)	6.35 (2.73–9.47)	6.35 (3.00–9.33)	6.06 (2.75–11.34)	(3.3–4.3)[k]	(3.1–5.3)
NDF[i]	10.82 (10.06–11.88)	11.70 (9.40–13.58)	9.33 (7.51–11.57)	9.8 (8.03–11.58)	10.12 (7.58–15.91)	(8.3–11.9)[k]	(9.6–15.3)
carbohydrates	85.15 (84.00–86.11)	85.15 (83.71–86.14)	83.66 (80.57–84.97)	83.79 (81.69–85.26)	83.68 (77.41–87.16)	not reported in this form	(81.7–86.3)
calcium	0.0026 (0.0020–0.0031)	0.0027 (0.0024–0.0033)	0.0039[j] (0.0027–0.0056)	0.0043 (0.0033–0.0058)	0.0040 (0.0022–0.0208)	(0.01–0.1)[k]	(0.0029–0.006)
phosphorus	0.299 (0.28–0.32)	0.299 (0.28–0.31)	0.326 (0.303–0.350)	0.326 (0.292–0.349)	0.330 (0.208–0.411)	(0.26–0.75)[k]	(0.288–0.363)
moisture	14.15 (7.44–22.60)	14.40 (7.24–23.00)	16.86 (9.57–23.10)	16.21 (8.67–24.70)	16.30 (8.18–26.20)	(7–23)[k]	(9.4–15.8)

[a] Data from five U.S. sites; GA21 grain harvested from plants not treated with Roundup herbicide. [b] Combined data from four nonreplicated E.U. sites, six U.S. nonreplicated sites, and one U.S. replicated site; GA21 grain harvested from plants treated with Roundup herbicide. [c] Percent dry weight of sample, except for moisture. [d] Nontransgenic negative segregant. [e] Parental control line. [f] Commercial lines; local hybrids planted at each site. [g] Range for control lines planted in Monsanto Co. field trials conducted between 1993 and 1995. [h] Range denotes the lowest and highest individual values across sites for each line. [i] ADF, acid detergent fiber; NDF, neutral detergent fiber. [j] Statistically significantly different from the control at the 5% level ($p < 0.05$). [k] Watson (1987). [l] Jugenheimer (1976).

Table 3. Amino acid composition of GA21 maize grain from field trials in North America and Europe in 1996 and 1997 (Sidhu et al. 2000).

	1996[b]		1997[c]				
amino acid[a]	GA21 mean (range)[i]	control[d] mean (range)[i]	GA21 mean (range)[i]	control[e] mean (range)[i]	comm lines[f] mean (range)[i]	literature[g] (range)[i]	historical[h] (range)[i]
alanine	7.62 (7.34–7.81)	7.64 (7.45–7.84)	7.64 (7.49–7.86)	7.62 (7.50–7.97)	7.78 (7.44–8.98)	(6.4–9.9)	(7.2–8.8)
arginine	4.13 (3.72–4.34)	4.30 (4.05–4.51)	4.48 (3.74–4.93)	4.51 (4.11–4.90)	4.36 (3.67–5.34)	(2.9–5.9)	(3.5–5.0)
aspartic acid	6.71 (6.46–6.87)	6.78 (6.35–6.83)	6.63 (6.17–7.05)	6.65 (6.22–7.08)	6.57 (6.14–7.35)	(5.8–7.2)	(6.3–7.5)
cystine	2.10 (1.85–2.36)	2.11 (1.91–2.24)	2.22 (1.73–2.49)	2.28 (2.06–2.57)	2.19 (1.63–2.62)	(1.2–1.6)	(1.8–2.7)
glutamic acid	19.27 (18.70–19.71)	19.06 (18.61–19.64)	18.78 (18.12–19.45)	18.70 (18.04–19.43)	19.17 (17.83–20.53)	(12.4–19.6)	(18.6–22.8)
glycine	3.72 (3.44–3.95)	3.78 (3.48–3.96)	3.83 (3.44–4.27)	3.89 (3.52–4.14)	3.71 (3.05–4.29)	(2.6–4.7)	(3.2–4.2)
histidine	2.81 (2.72–2.99)	2.84 (2.75–2.93)	2.67 (2.36–2.87)	2.74 (2.46–2.86)	2.80 (2.36–3.20)	(2.0–2.8)	(2.8–3.4)
isoleucine	3.60 (3.48–3.66)	3.58 (3.44–3.70)	3.53 (3.06–3.85)	3.57 (3.13–3.92)	3.75 (3.13–4.14)	(2.6–4.0)	(3.2–4.3)
leucine	13.11 (12.32–13.71)	12.90 (12.37–13.49)	12.98 (12.33–13.96)	12.87 (12.26–13.69)	13.32 (11.99–15.19)	(7.8–15.2)	(12.0–15.8)
lysine	3.02 (2.68–3.30)	3.09 (2.69–3.27)	3.11 (2.59–4.04)	3.02 (2.66–3.33)	2.96 (2.20–3.50)	(2.0–3.8)	(2.6–3.5)
methionine	1.98 (1.78–2.24)	2.03 (1.85–2.28)	2.16 (1.80–2.34)	2.17 (1.67–2.44)	2.02 (1.53–2.44)	(1.0–2.1)	(1.3–2.6)
phenylalanine	5.15 (4.88–5.31)	5.17 (4.98–5.30)	5.31 (5.03–5.63)	5.33 (4.96–5.76)	5.36 (4.88–6.10)	(2.9–5.7)	(4.9–6.1)
proline	8.69 (8.41–8.92)	8.69 (8.49–9.10)	8.98 (8.22–9.38)	9.00 (8.62–9.23)	9.16 (8.08–9.94)	(6.6–10.3)	(8.7–10.1)
serine	5.33[j] (5.25–5.49)	5.27 (5.17–5.43)	5.17 (4.43–5.60)	5.03 (3.82–5.63)	4.64 (2.87–5.63)	(4.2–5.5)	(4.9–6.0)
threonine	3.77 (3.64–3.88)	3.73 (3.58–3.85)	3.59 (3.33–3.74)	3.54 (3.08–3.71)	3.43 (2.61–3.89)	(2.9–3.9)	(3.3–4.2)
tryptophan	0.62 (0.55–0.66)	0.57 (0.53–0.61)	0.61 (0.52–0.75)	0.61 (0.43–1.04)	0.59 (0.41–1.04)	(0.5–1.2)	(0.4–1.0)
tyrosine	3.81[j] (3.68–3.99)	3.95 (3.88–4.10)	3.73 (3.06–4.20)	3.77 (2.78–4.32)	3.48 (2.37–4.32)	(2.9–4.7)	(3.7–4.3)
valine	4.58 (4.40–4.74)	4.64 (4.45–4.73)	4.57 (4.15–5.18)	4.62 (4.00–5.00)	4.79 (3.93–5.40)	(2.1–5.2)	(4.2–5.3)

[a] Values expressed as percent of total amino acids for statistical comparisons. These values are slightly higher when expressed as percent of total protein, e.g., alanine = 7.8% for GA21 (1996). [b] Data from five U.S. sites; GA21 grain harvested from plants not treated with Roundup herbicide. [c] Combined data from four nonreplicated E.U. sites, six U.S. nonreplicated sites, and one U.S. replicated site; GA21 grain harvested from plants treated with Roundup herbicide. [d] Nontransgenic negative segregant. [e] Parental control line. [f] Commercial lines; local hybrids planted at each site. [g] Watson (1982). Values are percent of total protein [10.1% total protein (N × 6.25)]. [h] Range for control lines planted in Monsanto Co. field trials conducted between 1993 and 1995; values are percent of total protein. [i] Range denotes the lowest and highest individual values across sites. [j] Value statistically significantly different than the control at the 5% level ($p < 0.05$).

Table 4. Fatty acid composition of GA21 maize grain from field trials in North America and Europe in 1996 and 1997 (Sidhu et al. 2000)

	1996[b]		1997[c]				
fatty acid[a]	GA21 mean (range)[i]	control[d] mean (range)[i]	GA21 mean (range)[i]	control[e] mean (range)[i]	comm lines[f] mean (range)[i]	literature[g] (range)[i]	historical[h] (range)[i]
arachidic (20:0)	0.40 (0.36–0.48)	0.41 (0.39–0.46)	0.37 (0.32–0.44)	0.36 (0.33–0.41)	0.40 (0.31–0.57)	(0.1–2)	(0.3–0.5)
behenic (22:0)	0.16 (0.14–0.18)	0.17 (0.16–0.18)	0.16 (0.12–0.24)	0.15 (0.13–0.16)	0.18 (0.13–0.24)	(not reported)	(0.1–0.3)
eicosenoic (20:1)	0.28 (0.27–0.31)	0.29 (0.28–0.30)	0.30 (0.28–0.34)	0.30 (0.28–0.36)	0.30 (0.19–0.45)	(not reported)	(0.2–0.3)
linoleic (18:2)	58.56 (54.20–64.70)	58.72 (53.40–65.60)	61.40 (58.2–63.4)	61.51 (59.7–63.0)	59.18 (46.9–64.3)	(35–70)	(55.9–66.1)
linolenic (18:3)	1.10 (1.07–1.13)	1.08 (0.98–1.16)	1.14 (0.92–1.24)	1.14 (1.04–1.20)	1.11 (0.77–1.55)	(0.8–2)	(0.8–1.1)
oleic (18:1)	27.50 (22.10–31.30)	27.40 (21.40–32.40)	24.2 (22.4–26.0)	24.1 (22.9–26.0)	26.2 (21.3–39.2)	(20–46)	(20.6–27.5)
palmitic (16:0)	9.94 (9.59–10.40)	9.92 (9.60–10.40)	10.70 (10.30–11.40)	10.72 (10.40–11.40)	10.58 (8.75–13.30)	(7–19)	(9.9–12.0)
stearic (18:0)	1.87 (1.52–2.11)	1.86 (1.46–2.11)	1.68 (1.44–2.04)	1.67 (1.59–1.86)	1.88 (1.36–2.65)	(1–3)	(1.4–2.2)

[a] Value of fatty acids expressed as percent of total fatty acid. The method included the analysis of the following fatty acids, which were not detected in the majority of samples analyzed: caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), myristoleic acid (14:1), pentadecanoic acid (15:0), pentadecenoic acid (15:1), heptadecanoic acid (17:0), heptadecenoic acid (17:1), gamma linolenic (18:3), eicosadienoic acid (20:2), eicosatrienoic acid (20:3), and arachidonic acid (20:4). Palmitoleic acid (16:1) was observed at levels of ~0.17% of total fatty acids in grain samples collected in 1996 but was not detected in the majority of grain samples collected in 1997. [b] Data from five U.S. sites; GA21 grain harvested from plants not treated with Roundup herbicide. [c] Combined data from four nonreplicated E.U. sites, six U.S. nonreplicated sites, and one U.S. replicated site; GA21 grain harvested from plants treated with Roundup herbicide. [d] Nontransgenic negative segregant. [e] Parental control line. [f] Commercial lines; local hybrids planted at each site. [g] Watson (1982). Values expressed as percent of total fat except for palmitic acid (16:1), which is expressed as percent of triglyceride fatty acids. [h] Range for control lines planted in Monsanto Co. field trials conducted between 1993 and 1995; values are expressed as percent of total fatty acids. [i] Range denotes the lowest and highest individual values across sites.

Table 5. Vitamin composition of maize grain reported in the literature

Source		Vitamin A β-Carotene (DW)	Vitamin B_9 Folic Acid (DW)	Vitamin E (DW)
ILSI (2006)	average range N	0.684 mg/100 g 0.019 - 4.681 276	0.0651 mg/100 g 0.0147 - 0.1464 895	0.0103 mg/g 0.0015 - 0.0687 863
Watson *et al*. (1987)	average range	2.5 mg/kg	0.3 mg/kg	30 IU/kg[1] 17 - 47
Souci *et al*. (2000)	average range	923 µg/100 g 74 - 960	26 µg/100 g 20 - 40	2.0 mg/100 g 0.4 - 2.7

Source		Vitamin B_1 Thiamine (DW)	Vitamin B_2 Riboflavin (DW)	Vitamin B_3 Niacin (DW)	Vitamin B_6 Pyridoxine (DW)
OECD (2002)	range	2.3 - 8.6 mg/kg	0.25 - 5.6 mg/kg	9.3 - 70 mg/kg	4.6 - 9.6 mg/kg
ILSI (2006)	average range N	0.530 mg/100 g 0.126 - 4.000 894	0.125 mg/100 g 0.050 - 0.236 704	2.376 mg/100 g 1.037 - 4.694 415	0.644 mg/100 g 0.368 - 1.132 415
Reynolds *et al*. (2005)	range	0.35 - 0.50 mg/100 g	0.84 - 1.39 µg/g		
Watson *et al*. (1987)	average range	3.8 mg/kg 3.0 - 8.6	1.4 mg/kg 0.25 - 5.6	28 mg/kg 9.3 - 70	5.3 mg/kg
Souci *et al*. (2000)	average range	360 µg/100 g 200 - 600	200 µg/100 g 100 - 240	1.5 mg/100 g 1.0 - 2.0	400 µg/100 g

[1] One IU equivalent to 1 mg of standard DL-α-tocopherol.

Table 6. Amino acid composition of maize grain reported in the literature

Source		Asp (DW)	Thr (DW)	Ser (DW)	Glu (DW)	Pro (DW)	Gly (DW)	Ala (DW)	Cys (DW)	Val (DW)
OECD (2002)	range	0.48 - 0.85%	0.27 - 0.58%	0.35 - 0.91%	1.25 - 2.58%	0.63 - 1.36%	0.26 - 0.49%	0.56 - 1.04%	0.08 - 0.32%	0.21 - 0.85%
ILSI (2006)	average range N	6.88 mg/g 3.35 - 12.08 1350	3.75 mg/g 2.24 - 6.66 1350	5.12 mg/g 2.35 - 7.69 1350	20.09 mg/g 9.65 - 35.36 1350	9.51 mg/g 4.62 - 16.32 1350	3.85 mg/g 1.84 - 5.39 1350	7.90 mg/g 4.39 - 13.93 1350	2.21 mg/g 1.25 - 5.14 1350	4.90 mg/g 2.66 - 8.55 1350
Souci *et al*. (2000)	average range	620 mg/100 g 590 - 630	390 mg/100 g 320 - 510	520 mg/100 g 500 - 530	1780 mg/100 g 1740 - 1880	1020 mg/100 g 930 - 1190	430 mg/100 g 430 - 440	790 mg/100 g 770 - 830	140 mg/100 g 70 - 280	510 mg/100 g 430 - 740

Source		Met (DW)	Ile (DW)	Leu (DW)	Tyr (DW)	Phe (DW)	His (DW)	Lys (DW)	Arg (DW)	Trp (DW)
OECD (2002)	range	0.10 - 0.46%	0.22 - 0.71%	0.79 - 2.41%	0.12 - 0.79%	0.29 - 0.64%	0.15 - 0.38%	0.05 - 0.55%	0.22 - 0.64%	0.04 - 0.13%
ILSI (2006)	average range N	2.09 mg/g 1.24 - 4.68 1350	3.68 mg/g 1.79 - 6.92 1350	13.41 mg/g 6.42 - 24.92 1350	3.36 mg/g 1.03 - 6.42 1350	5.25 mg/g 2.44 - 9.30 1350	2.96 mg/g 1.37 - 4.34 1350	3.15 mg/g 1.72 - 6.68 1350	4.33 mg/g 1.19 - 6.39 1350	0.627 mg/g 0.271 - 2.150 1350
Souci *et al*. (2000)	average range	190 mg/100 g 90 - 400	430 mg/100 g 350 - 620	1220 mg/100 g 910 - 2110	380 mg/100 g 190 - 690	460 mg/100 g 320 - 510	260 mg/100 g 130 - 330	290 mg/100 g 40 - 480	420 mg/100 g 190 - 560	70 mg/100 g 40 - 110

Table7. Fatty acid composition of maize grain reported in the literature

Source		16:0 Palmitic (DW)	18:0 Stearic (DW)	18:1 Oleic (DW)	18:2 Linoleic (DW)	18:3 Linolenic (DW)
OECD (2002)	range	0.29 - 0.79%	0.04 - 0.17%	0.70 - 1.39%	0.67 - 2.81%	0.03 - 0.10%
ILSI (2006)	average range N	11.50% of total FA 7.94 - 20.71 1344	1.82% of total FA 1.02 - 3.40 1344	25.8% of total FA 17.4 - 40.2 1344	57.60% of total FA 36.2 - 66.5 1344	1.20% of total FA 0.57 - 2.25 1344
Reynolds *et al*. (2005)	range	9.16 - 15.8% of total FA	1.23 - 2.80% of total FA	18.7 - 35.2% of total FA	48.8 - 64.7% of total FA	0.92 - 2.31% of total FA
Souci *et al*. (2000)	average range	470 mg/100 g 250 - 690	90 mg/100 g 36 - 145	1100 mg/100 g	1630 mg/100 g 590 - 2460	40 mg/100 g 30 - 70

[1] Five most abundant fatty acids

Table 8. Secondary Metabolite and anti-nutrient composition of maize grain reported in the literature

Source		Inositol (DW)	Phytic Acid (DW)	Trypsin Inhibitor (DW)	Ferulic Acid (DW)	*p*-Coumaric Acid (DW)	Furfural (DW)	Raffinose (DW)
OECD (2002)	range		0.45 - 1.0%		0.02 - 0.3%	0.003 - 0.03%	<0.01 ppm	0.21 - 0.31%
ILSI[2] (2006)	average range N	1331.5 ppm 89.0 - 3765.4 504	0.745% 0.111 - 1.570 1196	2.73 TIU/mg 1.09 - 7.18 696	2201.1 mg/kg 291.9 - 3885.8 817	218.4 mg/kg 53.4 - 576.2 817	3.697 mg/kg 3.000 - 6.340 14	0.132% 0.020 - 0.320 701
EuropaBio (2003)	average		0.89%	1.9 TIU/mg				
Reynolds *et al*. (2005)	range		0.36 - 1.00%					
Souci *et al*. (2000)	average range		940 mg/100 g 890 - 990					230 mg/100 g 190 - 270

[1] Units of ppm equivalent to mg/kg and µg/g

[2] Below LOQ values are not included.