Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Food/feed and environmental risk assessment of insect-resistant and herbicide-tolerant genetically modified maize Bt11 from Syngenta Seeds for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (EFSA/GMO/RX/Bt11)

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

Date: 21 January 2014

Doc. no.: 13/330- final

ISBN: 978-978-82-8259-123-2

VKM Report 2014: 32

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.

Acknowledgements

Monica Sanden, The National Institute of Nutrition and Seafood Research, is acknowledged for her valuable work on this opinion.

Assessed by

Panel on Genetically Modified Organisms

Åshild K. Andreassen (Chair), Per Brandtzæg, Hilde-Gunn Hoen-Sorteberg, Askild Holck, Olavi Junttila, Heidi Sjursen Konestabo, Richard Meadow, Kåre M. Nielsen, Rose Vikse

Scientific coordinators from the secretariat

Merethe Aasmo Finne, Anne-Marthe Jevnaker, Arne Mikalsen

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 insect-resistant and herbicide-tolerant genetically modified maize Bt11 from Syngenta Seeds (Unique Idientifier SYN-BT Ø11-1) is approved under Regulation (EC) No 1829/2003 for food and feed uses, import and processing (Commission Decision 2010/419/EC).

Genetically modified maize Bt11 has previously been risk assessed by the VKM Panel on Genetically Modified Organisms (GMO) as sweet maize in 2005 (Notification xx) and fodder/field maize in 2007 (Notification C/F/96/05.10) (VKM 2005, VKM 2007). Bt11 has also been evaluated by the VKM GMO Panel as a component of several stacked GM maize events (VKM 2008, VKM 2009a,b,c,d,e VKM 2012a,b, 2013a,b,c).

The food/feed and environmental risk assessment of the maize Bt11 is based on information provided by the applicant in the application EFSA/GMO/RX/Bt11, and scientific comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment also considered other peer-reviewed scientific literature as relevant.

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

The scientific risk assessment of maize Bt11 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.

Molecular characterisation

The molecular characterisation data indicate that a single copy of the transgenic insert with the *cry1Ab* and *pat* genes is integrated in the nuclear genome of maize Bt11, and that it is 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 Bt11 as adequate.

Comparative assessment

Comparative analyses of data from field trials located at representative sites and environments in North America and Europe indicate that maize Bt11 is compositionally equivalent to its conventional counterpart, with the exception of the herbicide tolerance and insect resistance traits, conferred by the expression of the Cry1Ab and PAT proteins. However, data on the amino acid tryptophan, is only given in one out of six studies. Based on current knowledge, the VKM GMO panel concludes that maize Bt11 is compositionally equivalent to conventional maize.

The data provided by the applicant are not sufficient to show that Bt11 maize is phenotypically and agronomically equivalent to conventional near-isogenic maize lines. The agronomic assessment data are provided from one growing season in the North America and one growing season in France. This is not considered to be sufficient for representative testing of agricultural environments.

Food and feed risk assessment

Whole food feeding studies have not indicated any adverse health effects of maize Bt11. These studies also indicate that maize Bt11 is nutritionally equivalent to conventional maize. The Cry1Ab and PAT proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

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

Environmental risk assessment

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

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

Overall conclusion

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

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

Keywords

Maize, *Zea mays* L., genetically modified maize Bt11, EFSA/GMO/RX/Bt11, insect- resistance, herbicide-tolerance, Cry proteins, *cry1Ab, pat,* PAT, glufosinate-ammonium, food and feed risk assessment, environmental risk assessment, Regulation (EC) No 1829/2003

Norsk sammendrag

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

Den insektsresistente og herbicidtolerante maislinjen Bt11 (unik kode SYN-BT Ø11-1) fra Syngenta Seeds er godkjent til import, videreforedling og til bruk som mat og fôr under EU-forordning 1829/2003 (søknad EFSA/GMO/RX/Bt11, Kommisjonsbeslutning 2010/419/EU).

Maislinjen Bt11 har tidligere vært vurdert av VKMs faggruppe for genmodifiserte organismer med hensyn på mulig helserisiko. Maislinjen ble risikovurdert som sukkermais i 2005 (Notifisering xx) (VKM 2005) og som fôrmais i 2007 (Notifisering C/F/96/05.10) (VKM 2007). Bt11 også tidligere risikovurdert av VKM i en rekke hybrider, der Bt11 inngår som en av foreldrelinjene (VKM 2008, VKM 2009a,b,c,d,e VKM 2012a,b, 2013a,b,c).

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

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

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

Molekylær karakterisering
Data fra den molekylære karakteriseringen indikerer at det kun er integrert ett eksemplar av ekspresjonskassetten med *Cry1Ab* og *pat* i genomet til mais Bt11, 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 Bt11 som tilfredsstillende.

Komparative analyser
Data fra feltforsøk i Nord-Amerika og Europa indikerer, med unntak av insektsresistens og herbicidtoleranse, ekvivalens mellom genmodifisert mais Bt11 og korresponderende, nær-isogene kontrollinjer med hensyn på ernæringsmessige karakterer. Faggruppen peker imidlertid på at aminosyren tryptofan kun er analysert i ett av de seks studiene som er vedlagt søkers dokumentasjon. Faggruppen vurderer også at søkers dokumentasjon knyttet til fenotypiske og agronomiske karakterer er ufullstendig og av for dårlig kvalitet til å kunne vurdere ekvivalens med konvensjonelle, nær-isogene maislinjer.

Helserisiko
Fôringsstudier utført på rotter og broiler har ikke indikert helseskadelige effekter av mais Bt11. Cry1Ab– 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. Enkelte studier har derimot indikert at noen typer Cry-proteiner potensielt kan forsterke andre allergiske reaksjoner (virke som adjuvans).

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at mais Bt11 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 Bt11 sammenliknet med konvensjonelle maissorter.

Miljørisiko
Søknaden gjelder godkjenning av maishybrid Bt11 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 Bt11 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 ifieldifisert toksiske eller endrede ernæringsmessige egenskaper til mais Bt11 eller prosesserte produkter sammenliknet med konvensjonell mais. Basert på dagens kunnskap er det også lite trolig at Cry1Ab1- og PAT- proteinene vil øke det allergene potensialet til mat og fôr produsert fra mais Bt11 sammenliknet med konvensjonelle maissorter. Faggruppen finner at maislinje Bt11, ut fra dagens kunnskap og omsøkt bruk, er sammenlignbar med konvensjonell mais når det gjelder mulig miljørisiko i Norge.

Abbreviations and explanations

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

EC	European Commission
ECB	European corn borer, *Ostrinia nubilalis*
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
ERA	Environmental risk assessment
E-score	Expectation score
EU	European Union
fa	Fatty acid
FAO	Food and Agriculture Organisation
FIFRA	US EPA Federal Insecticide, Fungicide and Rodenticide Act
Fitness	Describes an individual's ability to reproduce successfully relative to that of other members of its population.
fw	Fresh weight
fwt	Fresh weight tissue
GAT	Glyphosate N-acetyltransferase
GLP	Good Laboratory Practice
Glufosinate-ammonium	Broad-spectrum systemic herbicide
Glyphosate	Broad-spectrum systemic herbicide
GM	Genetically Modified
GMO	Genetically Modified Organism
GMP	Genetically Modified Plant
H	Hybrid
ha	Hectare
ILSI	International Life Sciences Institute
IPM	Integrated Pest Management
IRM	Insect Resistance Management
Locus	The position/area that a given gene occupies on a chromosome
LOD	Limit of detection
LOQ	Limit of quantification
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization-Time Of Flight. A mass spectrometry method used for detection and characterisation of biomolecules, such as proteins, peptides, oligosaccharides and oligonucleotides, with molecular masses between 400 and 350,000 Da.
MCB	Mediterranean corn borer, *Sesamia nonagrioides*
mEPSPS	Modified 5-enolpyruvylshikimate-3-phosphate synthase
mRNA	Messenger RNA
MT	Norwegian Food Safety Authority (Mattilsynet)
NDF	Neutral detergent fibre, measure of fibre used for animal feed analysis.

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

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

Table of contents

Background

Application EFSA/GMO/RX/Bt11
On 17 April 2007, Syngenta Seeds, submitted to the European Commission an application, in accordance with Articles 5, 11, 17 and 23 of Regulation (EC) No 1829/2003, for renewal of the authorisation for continued marketing of existing foods and food ingredients produced from Bt11 maize (including food additives), and renewal of the authorisation for continued marketing of existing feed containing, consisting of or produced from Bt11 maize (including feed additives and feed materials) and products other than food and feed containing and consisting of Bt11 maize with the exception of cultivation which were previously notified in accordance with Article 8(1)(a)(b) and Article 20(1)(a)(b) of that Regulation. The application also covers the renewal of the authorisation for the placing on the market of foods and food ingredients which are authorised under Commission Decision 2004/657/EC of 19 May 2004 authorising the placing on the market of sweet maize from genetically modified maize line Bt11 as a novel food or novel food ingredient under Regulation (EC) No 258/97. Within its application, Syngenta Seeds also requested the authorisation of foods and food ingredients containing or consisting of Bt11 field maize which were never authorised in the EU.

In 2009, the EFSA GMO Panel delivered a scientific opinion for the continued marketing of existing products produced from maize Bt11 for import, processing for food & feed uses, but not for cultivation (EFSA 2009a). EFSA concluded that the new information provided in the application and the review of the literature that has been published since the previous scientific opinion on Bt11 maize by EFSA does not require changes and confirmed the previous conclusion that Bt11 maize is as safe as its non-genetically modified counterpart and that it is unlikely to have an adverse effect on human and animal health or the environment in the context of its proposed uses which also applies to the products which are subject of the application.

On 29 July 2010 the European Commission granted a renewing of the authorisation for continued marketing of products containing, consisting of, or produced from genetically modified maize Bt11, authorising foods and food ingredients containing or consisting of field maize Bt11 pursuant to Regulation (EC) No 1829/2003 (Commission Decision 2010/419/EU).

The authorisation covers:

- Foods and food ingredients containing, consisting of or produced from SYN-BTØ11-1 maize
- Feed containing, consisting of, or produced from SYN- BTØ11-1 maize
- Products other than food and feed containing or consisting of SYN-BTØ11-1 maize for the same uses as any other maize with the exception of cultivation.

Notification C/F/96/05.10
On 16 June 2003, the European Commission received from the Competent Authority of France a notification (reference C/F/96/02.10) under Part C of Directive 2001/18/EC, for authorisation of the insect-resistant and herbicide tolerant genetically modified maize Bt11 for cultivation, feed use and industrial processing.

On 20 April 2005, the EFSA GMO Panel issued a scientific opinion on the notification (EFSA 2005), and concluded that there was no evidence indicating that placing maize Bt11 on the market is likely to cause adverse effects on human or animal health or the environment in the context of its proposed uses. At the time, the EFSA GMO Panel also recommended that maize Bt11 cultivation should be accompanied by appropriate risk management strategies to delay the potential evolution of resistance to the Cry1Ab protein in target insects and to minimise exposure of non-target Lepidoptera (EFSA 2005).

In both 2006 and 2008, the European Commission requested the EFSA GMO Panel to consider whether new evidence published in the scientific literature required a revision of the conclusions of its

2005 Scientific Opinion on maize Bt11 (EFSA 2005). Following these requests, the EFSA GMO Panel evaluated the available new scientific information, and found no new evidence for adverse effects caused by the cultivation of maize Bt11 (EFSA 2006, 2008). Therefore, the EFSA GMO Panel concluded that no new scientific information had been made available that would invalidate its previous risk assessment conclusions.

On 8 December 2010, the European Commission requested the EFSA GMO Panel to consider whether new scientific elements might require a revision of the conclusions of its previous Scientific Opinion on maize Bt11 in particular in the light of the mathematical model applied to the risk assessment of the insect resistant maize 1507. On 30 November 2011, the EFSA GMO Panel adopted a Statement supplementing the environmental risk assessment conclusions and risk management recommendations on maize Bt11 cultivation. In its Statement, the EFSA GMO Panel concluded that: "subject to appropriate management measures, maize Bt11 cultivation is unlikely to raise additional safety concerns for the environment compared to conventional maize" (EFSA 2011a).

The EFSA GMO Panel further supplemented its previous risk management recommendations on maize Bt11 for cultivation by reapplying the mathematical model developed by Perry et al. (2010, 2011, 2012), in order to consider additional hypothetical agricultural conditions, and to provide additional information on the factors affecting the insect resistance management plan (EFSA 2012a). Following a request from the European Commission, the EFSA GMO Panel compiled its previous risk assessment conclusions and risk management recommendations on maize Bt11, and considered their validity in the light of new relevant scientific publications published from 2005 onwards. A scientific opinion updating the risk assessment conclusions and risk management recommendations on Bt11 was published 11 December 2012 (EFSA 2012b).

Norway

Genetically modified maize Bt11 has previously been risk assessed by the VKM Panel on Genetically Modified Organisms (GMO) as sweet maize in 2005 and field maize in 2007 (Notification C/F/96/05.10) (VKM 2005, 2007). 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 Bt11. Bt11 has also been evaluated by the VKM GMO Panel as a component of several stacked GM maize events (VKM 2008, VKM 2009a,b,c,d,e VKM 2012a,b, 2013a,b,c).

Through the Agreement of the European Economic Area (EEA), Norway is obliged to implement the EU regulations on GM food and feed (regulations 1829/2003, 1830/2003 et al). Until implementation of these regulations, Norway has a national legislation concerning processed GM food and feed products that are harmonised with the EU legislation. These national regulations entered into force 15 September 2005. For genetically modified feed and some categories of genetically modified food, no requirements of authorisation were required before this date. Such products that were lawfully placed on the Norwegian marked before the GM regulations entered into force, the so-called existing products, could be sold in a transitional period of three years when specific notifications were sent to the Norwegian Food Safety Authority. Within three years after 15. September 2005, applications for authorisation should be sent to the Authority before further marketing. Four fish feed producing companies have once a year since 2008, applied for an exemption of the authorisation requirements of 19 existing products, including maize Bt11. These 19 GM events are all authorised in the EU, and the Norwegian Food Safety Authority has granted exemption for a period of one year each time.
http://www.mattilsynet.no/planter_og_dyrking/genmodifisering/fire_virksomheter_har_faatt_dispensasjon_fra_kravet_om_godkjenning_av_genmodifisert_fiskefor.10951

Terms of reference

The Norwegian Environment Agency (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 Bt11 has been developed to provide protection against certain lepidopteran target pests, such as the European maize borer (*Ostrinia nubilalis*), and species belonging to the genus *Sesamia* (in particular the Mediterranean maize borer (*Sesamia nonagrioides*)). Protection from feeding damage by pest larvae is provided by expression in the tissues of Bt11 maize of a truncated form of a Cry1Ab protein encoded by a modified cry1Ab gene derived from the soil microorganism *Bacillus thuringiensis* subsp *kurstaki* HD-1. The mode of action of the Cry1Ab protein and other Cry proteins is to bind selectively to specific receptors on the epithelial surface of the midgut of larvae of susceptible insect species, leading to death of larvae through pore formation, cell burst and subsequently septicaemia. None of the target pests for maize Bt11 are present in the Norwegian agriculture.

Bt11 was also genetically modified to express the enzyme phosphinothricin acetyl-transferase (PAT), encoded by the *pat* gene from the soil actinomycete *Streptomyces viridochromogenes*. Expression of PAT confers tolerance to the herbicide glufosinate-ammonium, the active ingredient in the herbicides Basta, Rely, Finale, and Liberty). Glufosinate-ammonium acts by inhibiting the plant enzyme glutamine synthetase, the only enzyme in plants that detoxifies ammonia by incorporating it into glutamine. Inhibition of this enzyme leads to an accumulation of ammonia in the plant tissues, which kills the plant within hours of application. PAT catalyses the acetylation of the herbicide phosphinothricin and thus detoxifies glufosinate-ammonium into an inactive compound. The PAT protein expressed in maize Bt11 has been used as selectable marker to facilitate the selection process of transformed plant cells, and is not intended for weed management purposes.

The genetic modification in maize Bt11 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. Event Bt11 has been crossed info both field maize and sweet maize varieties.

Maize Bt11 (Unique Identifier SYN-BT Ø11-1) 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 Bt11 is based on information provided by the applicant in the application EFSA/GMO/RX/Bt11 and notification C/F/96/05.10), 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 Bt11 from the Australia New Zealand Food Authority (ANFZA 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

Bt11 maize was generated by transformation of a proprietary inbred maize line, H8540, with the vector pZ01502 to transfer two new genes, a truncated *cry1ab* gene (referred to as the *cry1Ab* gene) and the *pat* gene. The line was transformed using protoplast transformation. The protoplasts were transformed with a larger DNA fragment obtained by a restriction digest of the plasmid pZO1502 with the enzyme *Not* I. According to the applicant, the *Not* I fragment was not expected to contain plasmid backbone DNA sequences. Regenerated plants were backcrossed to a selected line resulting in a plant called Bt11 maize.

2.1.2 Nature and source of vector used

The vector used for transformation was pZO1502. The pZO1507 vector is a derivative of pUC18. *E. coli* is the common host of the pUC18 plasmid. A vector map of pZO1502 is presented in Figure 1. The *Not*I restriction fragment containing the expression cassette was used for the transformation. The components are presented in Table 1 and 2 below. Their origin is also described. The *Not*I restriction fragment intended for insertion contained the *cry1ab* and the *pat* genes, but did not contain the ampicillin resistance gene.

The elements intended for insertion are contained within the *Not*1 restriction fragment of plasmid pZO1502 shown in Figure 1. The source, size and intended function of each constituent are shown in Table 3.

Table 1. Vector backbone components of pZO1502

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 pZO1502

Vector Component	Description
35S promoter	Promoter from CaMV which triggers constitutive gene expression
IVS6	Intron sequence derived from maize enhancing protein expression
cry1Ab gene	encodes a truncated version of the full-length *cry1Ab* gene from *Bacillus thuringiensis* subsp. kurstaki strain HD-1 which confers tolerance to certain lepidopteran species
nos terminator	3' nontranslated region from the nopaline synthase gene from *Agrobacterium tumefaciens* (Bevan, 1983)
35S promoter	Promoter from CaMV which triggers constitutive gene expression
IVS2	Intron sequence derived from maize enhancing protein expression
pat gene	encodes for a phosphinothricine acetyl-transferase (PAT) enzyme from *Streptomyces viridochromogenes* which confers tolerance to glyfosinate herbicides
Nos 3' end	3' nontranslated region from the nopaline synthase gene from *Agrobacterium tumefaciens* (Bevan, 1983)

Table 3. Source, size and intended function of constituents intended for insertion in Bt11 maize

Vector component	Approx Size (kb)	Source	Intended function
35S promoter	**0.509**	*cauliflower mosaic virus 35S* gene	Promoter of high level constitutive gene expression in plant tissues (Gardner *et al.*, 1981)
IVS6 enhancer	0.471	intron from maize *alcoholdehydrogenase 1S* gene	regulatory sequence that enhances *crylAb* gene expression in the plant (Freeling and Bennet, 1985)
cry1ab gene	1.848	*Bacillus thuringiensis* subsp. *kurstaki* strain HD-1	encodes a truncated version of the full-length *crylAb* gene which confers tolerance to certain lepidopteran species (Perlak *et al.*,1991).
Nos'3 terminator	0.253	*A. tumefaciens* nopaline synthase gene	Contains the signal for the termination of transcription and directs polyadenylation (Bevan *et al.*,1983)
35S promoter	**0.418**	*cauliflower mosaic virus 35S* gene	Promoter of high level constitutive gene expression in plant tissues (Gardner *et al.*, 1981)
IVS2 enhancer	0.180	intron from maize *alcoholdehydrogenase 1S* gene	regulatory sequence that enhances *pat* gene expression in the plant (Freeling and Bennet, 1985)
pat gene	0.552	Phosphinothricin acetyl transferase from *Streptomyces viridochromogenes*	encodes for a phosphinothricine acetyl-transferase enzyme which confers tolerance to glufosinate ammonium herbicides (Strauch *et al.*,1988)
Nos'3 terminator	0.253	*A. tumefaciens* nopaline synthase gene	Contains the signal for the termination of transcription and directs polyadenylation (Bevan *et al.*,1983)

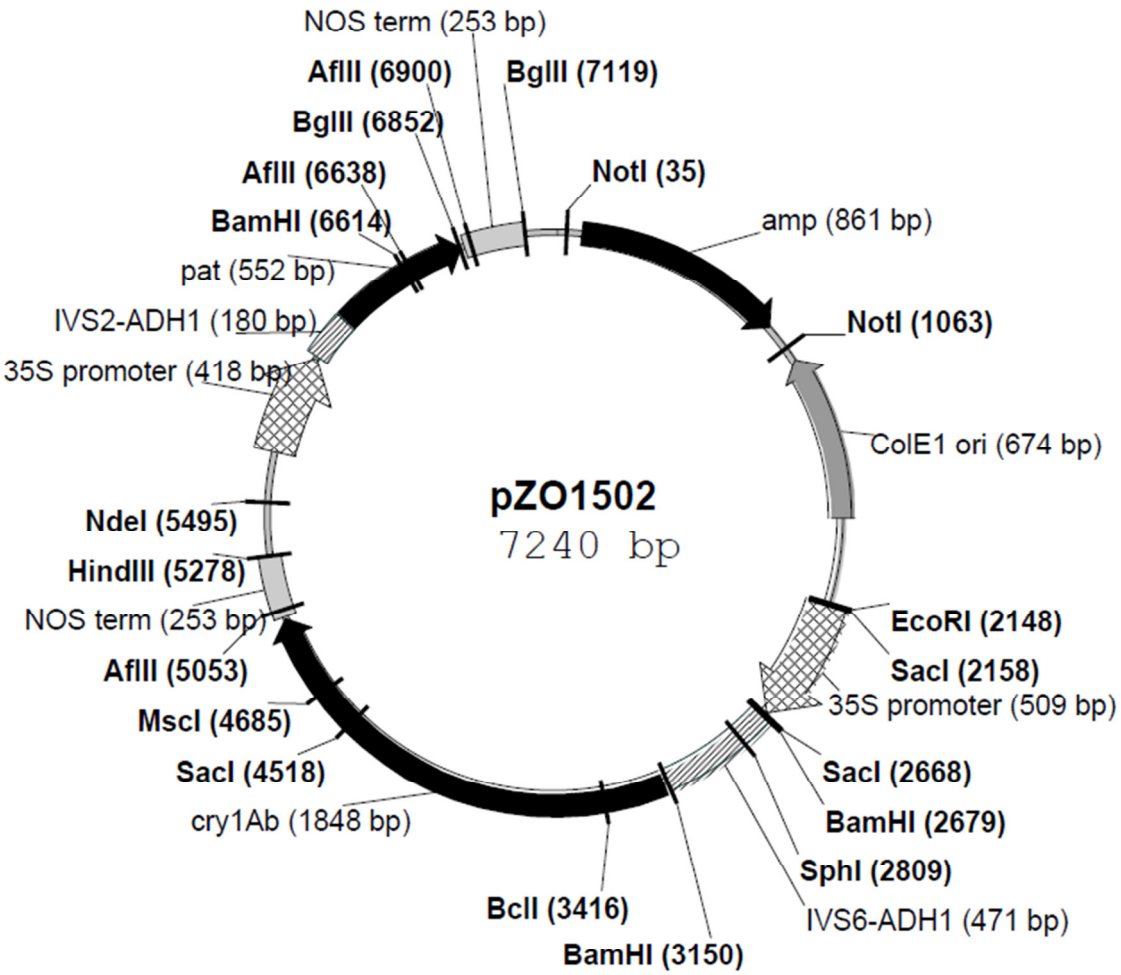

Figure 1. Plasmid map of transformation vector pZO1502

2.2 Information relating to the GM plant

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

Bt11 maize plants are protected from certain Lepidopteran insect pests (including *Ostrinia nubilalis* (European Maize Borer) and *Sesamia spp*.) and show tolerance to glufosinate-ammonium herbicides. Protection from feeding damage by pest larvae is provided by a truncated form of a Cry1Ab protein encoded by a modified *cry1Ab* gene derived from the soil microorganism *Bacillus thuringiensis* subsp *kurstaki* HD-1. The DNA sequence of the gene has been truncated at the 3' end and modified to increase the level of expression in maize, but the amino acid sequence of the protein has not been altered (Perlak et al. 1991). The *cry1Ab* gene in Bt11 maize codes for the Cry1Ab protein, a truncated version of the δ-endotoxin produced by *B.thuringiensis*.

The tolerance to glufosinate ammonium herbicides is accomplished by expression of a *pat* gene, derived from the soil microorganism *Streptomyces viridochromogenes* strain Tu494, which encodes an enzyme: phosphinothricin acetyl transferase (PAT), capable of detoxifying the herbicide.

According to the applicant, the truncated Cry1Ab protein and the PAT protein are produced within maize tissues and protect the plants from feeding damage by first and second brood ECB larvae.

2.2.2 Information on the sequences actually inserted or deleted

Bt11 maize was subjected to molecular analysis in order to determine the number of integration sites within the maize genome and the copy number (the number of copies of the DNA fragment used for transformation that were inserted in the GM plant), the integrity of the inserted cassettes and the absence of backbone sequences. Southern blot analyses were undertaken using a variety of DNA probes including the *pat* and *cry1Ab* genes, *amp* sequence and the entire plasmid to search for unintended insertions in the maize genome.

Sequence analysis of the entire insert present in Bt11 maize indicate overall integrity of the insert and that the contiguousness of the functional elements has been maintained. PCR analysis and DNA sequencing were used to establish a detailed transgene locus structure and to verify the 5' and 3' junction sequences of the insert with the plant genome. These analyses indicate intactness of the 5' and 3' ends of the inserted cassettes. The sequence of the inserted fragment was obtained by sequencing PCR fragments amplified directly from the Bt11 maize genomic DNA. According to the applicant, the resulting sequence was identical to the sequence of the corresponding fragment in the plasmid. Therefore, the applicant concludes that no rearrangements occurred within the *Not* I fragment during gene transfer and integration into the plant genome. Sequence information indicates that no vector backbone fragments, including *amp* sequences, are inserted and fused to the inserted *Not* I fragment.

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

Data from Southern analysis indicate that single copies of the *cry1Ab* gene, *pat* gene and ColE1 origin of replication derived from the transformation plasmid pZO1502 are present in the Bt11 maize. According to the applicant, the Bt11 maize insert contains two copies of the 35S promoter, corresponding to the two copies of the promoter present in the transformation plasmid pZO1502. The applicant also concludes that the Bt11 maize insert does not contain any unintended backbone sequences from the transformation plasmid pZO1502.

The structure of the Bt11 maize locus is represented in figure 2. As described previously the DNA inserted in the maize genome is the fragment obtained by *NotI* restriction of the plasmid pZO1502 derived from pUC18 (figure 1). This fragment contains two gene cassettes: the CaMV35S/intron/*Bt/nos* cassette of the *Bt* gene and the CaMV35S/intron/*pat/nos* cassette of the *pat* gene. Additionally, it contains vector backbone upstream from the *Bt* cassette, between the two cassettes and downstream from the *pat* cassette.

The size of the plasmid is 7,24 Kb, and the fragment integrated in the Bt11 maize is 6,2 Kb.

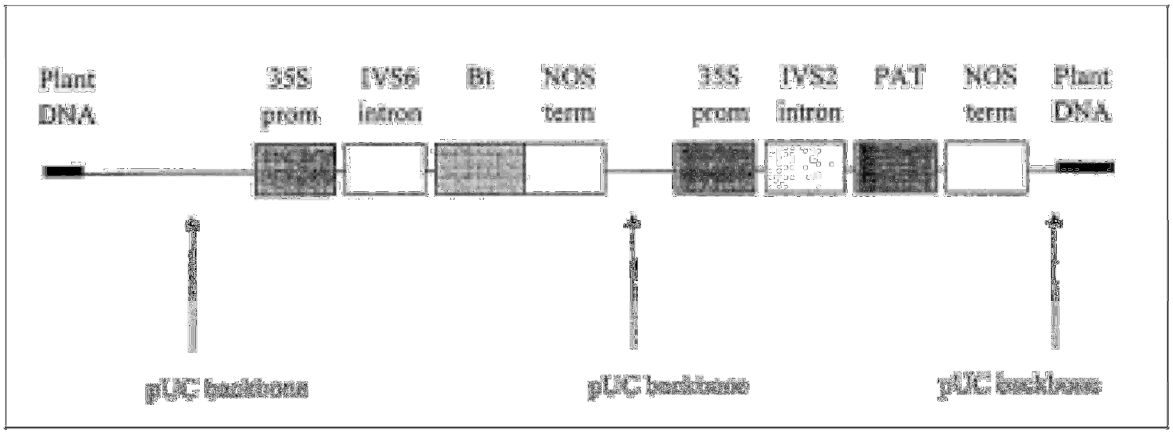

Figure 2. Structure of Bt11 maize locus.

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

According to the applicant, the entire Bt11 maize insert and flanking regions have been sequenced. The maize sequences flanking the Bt11 maize insert were also identified. A blast analysis of the sequences flanking the Bt11 maize insert was carried out against publicly available nucleotide databases (conf.). Bioinformatic analysis from 2008 confirmed the original analysis carried out by the applicant and supports the conclusion that the genomic sequences in both 5‟ and 3‟ regions flanking the insert of maize Bt11 show homology to highly repetitive, knob-associated sequences.

DNA sequences at the junctions between the insert and the parent genome were determined. At the 5' flank, approximately 350 bp of the plant DNA adjacent to the insert was sequenced. At the 3' flank, approximately 540 bp of the plant DNA adjacent to the insert was sequenced. The 5' and 3' flanking sequences were screened for homologies with sequences found in public databases. According to the applicant, BLAST analysis of both the 5' and 3' regions of the Bt11 maize insert revealed homology primarily to the *Zea mays* 180 bp knob-associated tandem repeat. Knobs are components of the maize heterochromatin, a class of chromatin known not to be expressed. The 180 bp tandem repeated sequences have been characterized. Therefore, the applicant concludes that the insertion of the *Not* I fragment in the maize genome does not disrupt any endogenous maize open reading frame.

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)

According to the applicant, the inserted DNA is located on the short arm of chromosome 8. The insert is stably integrated into the plant chromosome and is inherited as a single dominant gene in a Mendelian pattern.

2.3 Information on the expression of the insert

2.3.1 Part of the plant where the insert is expressed

To characterize the range of expression of Cry1Ab and PAT proteins in Bt11maize plants, the concentrations of Cry1Ab and PAT proteins were determined by ELISA in several plant tissues and whole plants at various growth stages (from whorl to senescence) from different hybrids of field and sweet maize.

According to the applicant, the Cry1Ab protein was found in all tissues examined, with a decrease in concentration at the time of plant maturation and senescence. To update the information, the concentrations of Cry1Ab protein (the active insecticidal principle) were determined in several plant tissues and whole plants at four developmental stages (whorl, anthesis, seed maturity and senescence) in two field maize hybrids. The quantity of Cry1Ab protein was also estimated on a per-acre and a per-hectare basis. Levels in pollen were below the lower limit of quantification, < 0.08 µg/g fresh wt. pollen and < 0.15 µg/g dry wt. pollen. Across all plant stages, mean Cry1Ab levels measured in leaves, roots and whole plants ranged from ca. 10 - 22 µg/g fresh wt. (12 – 154 µg/g dry wt.), 2 – 4 µg/g fresh wt. (9 – 22 µg/g dry wt.), and 4 – 9 µg/g fresh wt. (6 – 70 µg/g dry wt.), respectively. Mean Cry1Ab levels measured in grain at seed maturity and senescence were 1 – 2 µg/g fresh wt (2 µg/g dry wt.). The level of Cry1Ab was generally similar between hybrids for each tissue type at each time point.

The application also cover marketing and trading on the European market of Bt11 sweet maize for immediate consumption, for the consumption of tinned and frozen sweet maize and for the further processing into sweet maize powder.

The applicant has also performed a specific analysis to determine the level of the Cry1Ab protein in tissues from three Bt-11 sweet maize hybrid varieties and control lines with a similar genetic background from field tests in 1996. The Cry1Ab protein levels in grains tested at prime harvest stage was also assessed in these sweet maize hybrids that had been canned. The level of the Cry1Ab protein in Bt11 sweet maize hybrids grain at prime harvest was 1.97 ± 0.36 µg/g fresh weight, and at 21 days post prime harvest 2.98 ± 1.12 µg/g fresh weight. The range of Cry1Ab in grain for all three sweet maize hybrids was 0.51 to 3.80 µg Cry1Ab/g fresh weight. Cry1Ab protein was not detectable in any of the canned maize samples tested. The applicant concludes that since the Cry1Ab protein was not detected in canned maize and the levels of Cry1Ab in grain for all Bt11 maize varieties (field and sweet maize) were low, dietary exposure to the novel protein is expected to be very low.

According to the applicant, the level of the PAT protein was determined using Bt11 field maize plants; measurable levels (ng/g) were only found in leaves, silk and tassel. For grain, pollen, root and stalk concentrations were below the limits of detection. The PAT protein is present at less than 0.000008% fresh weight and 0.00016% of the total maize grain protein.

2.3.2 Expression of potential fusion proteins

Not applicable.

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

2.4.1 Genetic stability of the insert

The genetic stability of the inserted DNA in Bt11 maize was shown by both a classical approach and a molecular approach using Southern blot analysis on genomic DNA.

The Bt11 maize was subjected to a backcrossing program with the elite line H8540. The lines BC3 and BC6, developed as part of this program, were used to evaluate genetic stability. BC3 was developed from 3 backcrosses with H8540 and BC6 from 6 backcrosses. According to the applicant, no differences in banding pattern were observed between the DNA from these generations demonstrating the stability of the inserted DNA.

Segregation data for glufosinate ammonium tolerance and European maize borer resistance were collected at different points in the backcrossing experiment. BC3 and BC6 plants, identified as containing the *cry1Ab* and *pat* genes, were subjected to selfing experiments. According to the applicant, the results indicate heritability and stability of the two genes in the Bt11 maize. Data support the presence of a single insertion that segregates according to Mendelian inheritance patterns.

Restriction fragment length polymorphism (RFLP) mapping was used to determine the location of the novel genes in Bt11. The progeny of Bt11 plants crossed with the two inbred maize lines were screened with RFLP probes, corresponding to different regions of the corn genome. Comparison of the genotypes of the progeny with isogenic controls demonstrated that the site of integration for the genetic material in Bt11 maize is located on the long arm of chromosome 8.

2.4.2 Phenotypic stability of the GM plant

According to the applicant, no differences in the agronomic and phenotypic characteristics were found between the Bt11 maize and the non-transgenic counterpart during field trials at different locations (Spain, France, Italy and Portugal) conducted between 1994 and 2006 that would indicate unexpected pleiotropic effects of the genetic modification (Final reports at JRC web page).

The stability of the inserted DNA in Bt11 maize was demonstrated by a Mendelian inheritance pattern. The segregation of the *cry1Ab* and *pat* genes and their phenotypic traits was followed over multiple generations. F1 plants (first generation hybrids) were identified as containing the *cry1Ab* and *pat* genes. These plants were self-fertilised to produce the S1 population. This S1population was screened for protection against the European corn borer and for tolerance to glufosinate- ammonium. The S1 plants were again self-fertilised. The insect protection and herbicide tolerance traits were then backcrossed into two genetic backgrounds (H8540 and 977), and in some cases, followed by further self-fertilisation.

Seed was collected from maize plants exhibiting both new traits representing different backcross stages and planted in the field for analysis in 1994 and 1995. Plants were tested for protection against the European corn borer and tolerance to glufosinate ammonium. All plants were either both tolerant to the herbicide and protected against insect attack or susceptible to both with segregation patterns consistent with the expected ratio for a single dominant locus, for that particular generation.

2.5 Conclusion

The molecular characterisation data indicate that a single copy of the transgenic insert with the *cry1Ab* and *pat* genes is integrated in the nuclear genome of maize Bt11, and that it is 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 Bt11 as adequate.

3 Comparative assessment

3.1 Production of material for the comparative assessment

The application EFSA/GMO/RX/Bt11 for food and feed use, import and processing of maize Bt11 within the EU presented compositional data on seed and forage material collected in field trials performed in the USA in 1995, 1996 and 1998, and in France in 1998. These studies have been conducted on Bt11 seeds and major components in inbred and hybrid lines at different stages of maturity are assessed and the results are compared with their respective near-isogenic controls. In addition, the applicant has provided data from compositional analysis of Bt11 maize plants grown in greenhouses in Europe in 1999.

The applicant has provided data on the original transformant (H8540 and hybrids) and different maize hybrids widely used in food and feed production. This includes several field/field maize and sweet maize lines that have been developed from conventional breeding of the original transformed line. (See Table 1, Appendix 1 for a complete summary of the lines analysed).

Studies submitted by Syngenta Seeds Inc.

- Compositional analysis of Bt11 maize: determination of the substantial equivalence — chemical composition analysis done with Bt-11 maize with a European background.
  (Greenhouse study in Europe in 1999)

- Compositional analysis of Bt11 maize: determination of the substantial equivalence — chemical composition analysis done with Bt-11 maize with a US background. Part 1: Properties of grain produced from ECB protected maize hybrids; Part 2: Characterization of grain attributes of normal, wild-type maize hybrids and the Bt11 converted iso-hybrid counterparts; Part 3: Analyses of fatty acid and amino acid profiles of grain from Bt-11 maize. Report No. NSB-004-97.
  (Field trials conducted in the USA in 1995 (6 sites) with six field maize lines developed from conventional breeding with the original transformant)

- Comparison of vitamin and mineral composition of Bt11 maize and non-modified maize hybrids. Report No. NSB- 004-97. Novartis Seeds.
  (Field trials conducted in the USA in 1995 (3 sites) with six field maize lines developed from conventional breeding with the original transformant)

- Comparison of nutritional composition of fresh and canned grain prepared from Attribute insect protected and control sweet maize hybrids. Report No. NSV-002-98. Novartis Seeds Inc.
  (Field trials conducted in the USA in 1996 (1 site) with six sweet maize lines developed from conventional breeding with the original transformant)

- Goy PA (1999) Novartis Seed's genetically modified Bt11 maize: biochemical composition of grain from plants treated with a glufosinate ammonium herbicide.
  (Field trials conducted in France in 1998 (2 sites) with three field maize hybrids)

- Goy PA (2000) Novartis Seed's genetically modified Bt11 sweet maize: further determination of the biochemical composition of kernel- analysis of secondary metabolites.
  (Field trials conducted in the USA in 1998 (1 site) with three sweet maize hybrids)

3.2 Compositional Analysis

3.2.1 Study 1: Analysis of Bt11 maize grown in greenhouse in Europe (1999)

The components of several lines and hybrids of the Bt11 maize, derived through backcrossing from the original transformant, were analyzed and compared to isogenic non-modified lines and hybrids. The following greenhouse grown plants were analysed: an inbred line (H8540-Bt), a hybrid line (hybrid Bt+/Bt-) and their respective controls (isogenic non-modified H8540 and control hybrid). Between 45 and 56 ears were taken from each plant. Ears were harvested and dried four months after sowing and 500 g samples were analysed.

The following parameters were analysed: moisture, total nitrogen, ash, starch, cellulose, xanthophyll, fatty acid and amino acid composition. Statistical comparison with STATITCF software was made on the values of two replicate analyses, except in the case of xanthophyll, fatty acids and amino acids, where data points are the result of a single analysis.

Proximates

All values for proximates were within the range, except total nitrogen content (Table 1). The total nitrogen content of both Bt11 maize and non-transgenic maize were higher than the spectrum ranges found in the literature. As the protein content is influenced by the available soil nitrogen, the increase could be caused by the fertilizer used in culturing of the plants in the greenhouse.

Table 1. Proximate composition for Bt11 and control maize (ANZFA 2000).

	Inbred line H8540-Bt	***Isogenic control H8540***	**Hybrid Bt+/Bt-**	***Control hybrid***	**Normal range[2]**
Total nitrogen[3]	13.18 ± 0.07	12.35 ± 0.06	12.28 ± 0.03	12.30 ± 0.07	7.7–10[4]
Moisture	12.3	12.6	12.6	13.3	7–23
Ash	1.47 ± 0.04	1.79 ± 0.007	1.70 ± 0.02	1.6 ± 0.02	1.1–3.9
Starch	68.02 ± 0.4	67.57 ± 0.4	70.83 ± 0.81	70.25 ± 0.48	61–78
Cellulose	2.99 ± 0.007	2.9 ± 0.05	2.67 ± 0.28	2.92 ± 0.05	3.3–4.3 1.93–2.5[4]
Xanthophyll	24.2	21.0	21.6	19.1	19.2–33.1[4]

[1]Samples are 500g of kernels from: Bt^+/Bt^+ H8540 ears n=54, Control H8540 n=56, Bt^+/Bt^- hybrid n=50, Control hybrid ears n= 45. Each data point represents the mean of two replicate analyses made with the 500g sample. Data from AGPM. All data except moisture (% H_2O) and xanthophyll (mg/kg dry weight basis) are presented on a % dry weight basis.
[2]Wright, 1987 in Corn chemistry and technology, 1987, Watson SA and Ramstad PE (eds), American Association of Cereal Chemists, St. Paul, Minesota, USA.
[3]All values from control and genetically modified lines are significantly different to range.
[4]Data from AGPM

Fatty acids

A single analysis was done on 500g samples of grains from Bt_+/Bt_+ H8540 (number of ears n=54), isogenic control H8540 (number of ears n=56), Bt_+/Bt_- hybrid (number of ears n=50), control hybrid (number of ears n= 45). No range is available for arachidic acid, gadoleic acid or behenic acid. All other values regarding the fatty acids were within the range (Table 2). There were no differences in these values greater than 10% (which allows for experimental error) between the modified maize and controls. Literature ranges were available for most of the common fatty acids.

Table 2. Fatty acid composition for Bt11 and control maize (ANZFA 2000).

Fatty acid composition	Bt^+/Bt^+ line H8540	*Control H8540*	Bt^+/Bt^- hybrid line	*Control* hybrid line	Range
C16 palmitic acid	15.1	14.5	15.3	14.6	6–22[2]
C18 stearic acid	1.7	1.6	1.6	1.5	1–15[2]
C18:1 oleic acid	20.6	21.9	21.8	21.8	14–64[2]
C18:2 linoleic acid	58.9	58.2	58.1	60	19–71[2]; 56–65[3]
C18:3 linolenic acid	1.7	1.7	1.2	1.1	0.5–2[2]
C20 arachidic acid	0.5	0.4	0.4	0.4	
C20:1 gadoleic acid	0.2	0.2	0.2	0.2	
C22: behenic acid	0.2	0.2	0.1	0.1	

[1]Samples are 500g of kernels from: Bt^+/Bt^+ H8540 ears n=54, Control H8540 n=56, Bt^+/Bt^- hybrid n=50, Control hybrid ears n= 45. Values are expressed as % of the analysed fatty acid relative to the total amount of fatty acids.
[2]From Weber, "Lipids of the kernel", Chapter 10 in Corn chemistry and technology, 1987, Watson SA and Ramstad PE (eds), American Association of Cereal Chemists, St. Paul, Minesota, USA. data
[3]AGPM

Amino acids

A single analysis was done on 500g samples of grain from Bt+/Bt+ H8540 (number of ears n=54), isogenic control H8540 (number of ears n=56), Bt+/Bt- hybrid (number of ears n=50), control hybrid (number of ears n= 45). The experimental data regarding the amino acid composition are generally higher than the bibliographical ones (Table 3). According to the applicant, the reason is the high nitrogen content of the analysed material. The bibliographical values originated from maize grains have total nitrogen content of approximately 9.5%. According to the applicant these data, therefore, need to be corrected to give comparable values. Protein levels were higher than the normal range for all plants assessed. The data were multiplied by 1.33, the corrective factor shown in the total nitrogen analysis (Table 1). As protein content is affected by soil nitrogen, it is possible that the fertiliser used caused the high level of nitrogen for all plants in the study.

After correction and taking in account experimental error (10%) the values are included in the range (Table 3). Comparison between Bt11 maize and control maize indicate no difference except for glutamic acid, which is in the limit of the allowed variation for the hybrid (experimental error of 10%).

The levels of glutamine, asparagine and tryptophan were not determined. Tryptophan is one of the essential amino acids and one of the limiting amino acids in maize. No spectrum or literature ranges were available for some of the amino acids, because some of these analyses are not routinely carried out by the laboratory assaying these samples.

Table 3. Amino acid composition for Bt11 and control maize (ANZFA 2000).

Amino acid composition	Bt^+/Bt^+ line H8540	*Control H8540*	Bt^+/Bt^- hybrid line	*Control hybrid line*	Range[2]
Aspartic acid	9.8	9	8.7	8.4	
Threonine	5.2	5	4.9	5	3.2–3.4
Serine	6.6	6.4	6.1	6.1	
Glutamic acid	28.1	25.7	26.2	25.1	
Proline	12	12.5	12	11.4	
Glycine	4.1	4.2	4	4	
Alanine	11.5	10.8	10.8	10.1	
Cysteine	2.4	2.4	2.5	2.7	
Valine	6.5	6.1	5.9	6.2	4.2–4.6
Methionine	2.5	2.5	2.7	2.9	1.8–1.9
Isoleucine	5.2	4.8	4.6	4.6	3.4–3.7
Leucine	19.4	17.5	17.7	17.3	10–11.3
Tyrosine	5.4	4.9	5	4.7	
Phenylalanine	7.2	6.5	6.4	6.3	4.4–4.5
Lysine	3.2	3.3	3.1	3	2.45–2.6
Histidine	3.5	3.4	3.4	3.5	
Arginine	4.4	4.8	4.9	4.8	4.1–5.2

[1]Values are expressed as g/Kg dry matter.
[2]Data from L'alimentation des animaux monogastriques: porc, lapin, volailles. INRA 1989, Feedstuffs ingredient analysis table, edition 1996, AEC Table and 1995 UCAAB data.

3.2.2 Study 2: Analysis of Bt11 field maize grown in USA (1995)

Grain property studies were conducted on Bt11 maize hybrids and non-transgenic maize hybrids grown in 3-6 field locations in the USA in 1995. The following traits were considered: grain size, density and the content of proximates: starch , protein , oil and fiber expressed as percentage of dry weight, and fatty acid,amino acid, and mineral and vitamin profiles.

Dataset 1:
An analysis of the major components and nutritional qualities of two elite Bt11 field maize lines (X6534CBR, X7634CBR) developed from conventional breeding with the original transformant, has been assessed. In this trial, the Bt11 hybrids and their near-isogenic controls (X6534 and X7634) were grown in three field locations in the USA (Table 1, Appendix 1).

Grains from the Bt11 hybrids and the conventional counterparts were analysed for percentage of starch, protein, oil and fibre. No significant differences were detected between the Bt hybrids and the control hybrids ($p>0.05$) (Table 4). The grains from insect-resistant maize hybrids were comparable to control hybrids regarding the content of starch, protein, oil and fibre and fell within the normal ranges expected for these components.

Table 4. Proximate composition for Bt11 and control maize (dataset 1) (ANZFA 2000).

	X6534CBR	Isogenic control X6514	X7634CBR	Isogenic control X7514	Normal range[2]
Protein	9.89 (9.40-10.60)	9.96 (9.10-11.40)	10.55 (10.24-11.00)	9.68 (8.90-10.94)	6-12
Oil	4.09 (4.00-4.16)	4.11 (4.10-4.13)	4.02 (4.00-4.02)	4.07 (3.80-4.31)	3.1-5.7
Starch	70.09 (68.80-71.07)	70.19 (67.80-71.50)	69.32 (68.60-70.36)	70.36 (69.07-71.40)	61-78
Fibre	2.95 (2.86-3.00)	2.97 (2.92-3.00)	2.93 (2.89-3.0)	2.91 (2.90-2.92))	2.5[3]

[1]Values presented as % dry weight. Values are means of 3 samples taken from 3 locations (ie 1 sample/location), ranges are given in brackets. Genetically modified corn lines are denoted CBR and are isogenic to their controls except for the presence of the novel genes.

Dataset 2:

A second nutritional study on Bt11 field maize that included additional hybrids was conducted. Three to five ears were picked from the center two rows of a four row strip plot for each hybrid per two sites within three geographical regions to give a total of six locations per hybrid. Two of the hybrids had a 'northern' (early-season) genetic background and two had a 'southern' (mid-late-season) genetic background. The hybrids were derived from separate backcross conversion processes using the same original transformation event.

Proximates

The compositional data for the Bt11 maize and control maize plants were analysed for significant differences by Analysis of Variance. The components measured were % protein, oil, starch and fibre (Table 5). Grain from the early season (northern hybrids) Bt11 maize hybrids (X4334CBR and X4734CBR) have a significantly lower protein content than grain from the control maize lines ($p<0.05$ and $p<0.01$), respectively). All other components were comparable between the Bt11 maize hybrids and their respective control maize lines.

Although the protein was lower in the northern hybrids, there is a lack of consistent differences between the non-modified hybrids and their genetically modified equivalents. These results may indicate that the effects observed, are not likely to be a result of the genetic modification itself but more likely from differences arising out of an incomplete backcross conversion in the normal breeding process. Values for all measured parameters fell within the literature ranges..

Table 5. Proximate composition for Bt11 and control maize (dataset 2) (ANZFA 2000).

Northern / Early	X4334CBR	Control N4242	X4734CBR	Control N4640	Normal range[2]
Protein	8.65[3] (8.03-9.11)	9.25 (8.63-9.63)	8.19[4] (7.74-9.16)	8.96 (8.28-9.53)	6-12
Oil	3.17 (2.81-3.73)	3.23 (3.04-3.50)	3.34 (3.36-3.48)	3.30 (3.12-3.68)	3.1-5.7
Starch	72.93 (71.8-73.2)	72.57 (71.7-73.4)	72.73 (71.5-73.7)	72.62 (71.3-73.2)	61-78
Fibre	2.69 (2.66-2.83)	2.75 (2.67-2.93)	2.77 (2.68-2.83)	2.77 (2.69-2.83)	2.5[5]
Southern / Mid-late	**X6534CBR**	**X6514**	**X7634CBR**	**X7514**	
Protein	9.52 (8.35-10.60)	9.93 (9.10-11.40)	9.85 (8.63-11.00)	9.87 (8.67-10.94)	6-12
Oil	3.80 (3.63-4.16)	3.93 (3.27-4.13)	3.37 (2.59-4.00)	3.48 (2.70-4.31)	3.1-5.7
Starch	70.77 (68.8-72.5)	71.07 (67.8-72.7)	71.33 (68.6-74.3)	71.12 (69.1-73.9)	61-78
Fibre	2.78 (2.55-3.00)	2.80 (2.61-3.0)	2.74 (2.53-3.00)	2.72 (2.46-2.92)	2.5[5]

[1]Values presented as % dry weight. Values are means of a total of 6 samples taken from 2 sites in 3 locations (ie 2 distinct samples from each of the 3 locations), ranges are given in brackets.
[2]From Corn chemistry and technology, 1987, Watson SA and Ramstad PE (eds), American Association of Cereal Chemists, St. Paul, Minesota, USA.
[3]Values are significantly different to that of control value at 5% level of probability.
[4]Values are significantly different to that of control value at 1% level of probability.
[5]Average value

Fatty acids and amino acids

Fatty acid and amino acid analyses were performed on twenty grain samples obtained from Bt11 and non-transgenic control maize. Two separate analyses were performed. In the first analysis, variation between Bt11 maize and non-transgenic control maize were analysed to determine whether there were significant differences between Bt11 maize and the control maize. In the second analysis, only the replicates of Bt11 maize versus the genetically similar near isogenic non-transgenic control maize were analysed.

Amino acids

Amino acid analysis was performed on twenty grain samples from Bt11 maize (6534CBR and 4734CBR) and non-transgenic control maize (N6800, N4640, N4242, N5220, N5666, N6223, N6822, N7070 and N7590). Significant difference between Bt11 maize and non-transgenic control maize were found for cysteine and arginine ($p < 0.05$). For cysteine Bt11 maize (4734CBR) was lowest in content at 0.170. The range among the maize plants was from 0.170 to 0.230. This difference is not consistent for all genetically modified maize hybrids and is consistent with the variability that is observed between lines. Some variability may arise as a result of incomplete backcrossing. This variation is not considered to be a result of the genetic modification nor is it biologically significant.

Table 6. Amino acid composition for Bt11 and control maize (ANZFA 2000).

	X6534-CBR	N6800	X4734-CBR	N4640	N4242[4]	N5220[5]
Tryptophan	0.05-0.06	0.05-0.06	0.05[2]	0.05-0.06	0.05[2]	0.07
Aspartic Acid	0.61-0.67	0.60-0.66	0.54[2]	0.55-0.57	0.55[2]	0.64
Threonine	0.35-0.38	0.35-0.38	0.29-0.30	0.30-0.31	0.30-0.32	0.36
Serine	0.50-0.55	0.50-0.55	0.42-0.43	0.43-0.44	0.43-0.44	0.52
Glutamic Acid	1.54-1.72	1.55-1.79	1.17-1.25	1.22-1.30	1.30-1.32	1.63
Proline	0.77-0.88	0.83-0.91	0.68-0.70	0.63-0.68	0.61-0.66	0.84
Glycine	0.34-0.37	0.35[2]	0.29-0.30	0.31-0.33	0.32-0.34	0.36
Alanine	0.75-0.82	0.75-0.87	0.60-0.62	0.58-0.63	0.61-0.63	0.74
Cysteine[3]	0.21-0.22	0.22-0.23	0.17[2]	0.20-0.21	0.18-0.21	0.22
Valine	0.41-0.43	0.40-0.45	0.32-0.33	0.32-0.34	0.32-0.36	0.43
Methionine	0.19-0.21	0.19-0.22	0.17-0.20	0.20-0.23	0.19-0.21	0.24
Isoleucine	0.28-0.32	0.28-0.33	0.23-0.25	0.24-0.26	0.23-0.27	0.32
Leucine	1.23-1.37	1.23-1.45	0.93-0.98	0.96-0.98	0.92-1.01	1.32
Tyrosine	0.14-0.18	0.14-0.16	0.13[2]	0.13-0.14	0.14[2]	0.17
Phenylalanine	0.44-0.49	0.44-0.51	0.37-0.39	0.36-0.40	0.35-0.38	0.50
Histidine	0.32-0.35	0.34-0.37	0.26-0.27	0.28-0.29	0.27-0.28	0.31
Lysine	0.25-0.26	0.24-0.26	0.23-0.24	0.24-0.25	0.23-0.25	0.27
Arginine[3]	0.36-0.37	0.37-0.38	0.31-0.32	0.32-0.34	0.33[2]	0.39

	N5866[5]	N6223[5]	N6822[5]	N7070[5]	N7590[5]	Range[6]
Tryptophan	0.06	0.07	0.06	0.06	0.08	
Aspartic Acid	0.58	0.68	0.59	0.71	0.67	
Threonine	0.34	0.38	0.34	0.40	0.39	0.32-0.34
Serine	0.47	0.55	0.45	0.53	0.56	
Glutamic Acid	1.54	1.83	1.53	1.61	1.83	
Proline	0.77	0.93	0.79	0.75	1.03	
Glycine	0.34	0.36	0.33	0.40	0.36	
Alanine	0.73	0.85	0.70	0.90	0.83	
Cysteine	0.22	0.22	0.20	0.21	0.23	
Valine	0.40	0.45	0.39	0.48	0.47	0.42-0.46
Methionine	0.23	0.26	0.24	0.27	0.34	0.18-0.19
Isoleucine	0.31	0.35	0.30	0.33	0.34	0.34-0.37
Leucine	1.24	1.46	1.20	1.28	1.47	0.10-0.11
Tyrosine	0.15	0.17	0.16	0.15	0.16	
Phenylalanine	0.47	0.54	0.46	0.46	0.54	0.44-0.45
Histidine	0.31	0.35	0.30	0.37	0.32	
Lysine	0.26	0.26	0.25	0.32	0.25	0.25-0.26
Arginine	0.36	0.40	0.36	0.36	0.38	0.41-0.52

[1]Values are ranges for three samples taken from 3 field sites (ie 1 sample/site) and are expressed as g/100g dry weight.
[2]The same value was obtained for all three samples.
[3]Values for genetically modified corn plants are significantly different to those of control corn plants.
[4]Range is obtained from two values
[5]Single value only.
[6]Data from L'alimentation des animaux monogastriques: porc, lapin, volailles. INRA 1989, Feedstuffs ingredient analysis table, edition 1996, AEC Table and 1995 UCAAB data.

Fatty acid

Fatty acid analyses were also performed on the grain sampled as described above. The grain was sampled from two locations, three samples per line from two Bt11 hybrid maize lines X6534CBR and X4734CBR and their genetically equivalent controls (N6800 and N4640 respectively). Additionally, grain from another seven non-modified reference hybrids was analysed (N4242, N5220, N5666, N6223, N6822, N7070 and N7590). As outlined above for the amino acid analysis, two separate statistical analyses were performed. The first analysed the variation between hybrids to determine whether there were significant differences between them. The second study analysed differences specifically between genetically modified hybrids and their isogenic controls. The results are shown in

Table 7. A statistical analysis to determine the variation between hybrids, as described above for the amino acid analysis, found no significant differences between the hybrids for fatty acid values ($p>0.05$).

A second statistical analysis of the fatty acid values investigated specifically differences between the genetically modified maize hybrid plants versus the non-modified control hybrids. Significant different between Bt11 maize and non-transgenic control maize were found for palmitic- and stearic acid. According to the applicant, Bt11 maize falls within the range of non-transgenic control maize for palmitic- and stearic acid content (Table 7).

Table 7. Fatty acid composition for Bt11 and control maize (ANZFA 2000).

	Palmitic	Stearic	Oleic	Linoleic	Linolenic
X6534CBR	10.99-11.14	1.99-2.16	27.15-27.36	56.88-57.31	1.16-1.25
N6800	10.78-11.11	2.11-2.24	26.85-26.90	56.81-57.07	1.29-1.43
X4734CBR	10.76-10.97	2.38-2.41	25.93-26.04	57.62-57.86	1.61-1.67
N4640	10.61-10.65	2.45-2.52	26.31-27.06	56.69-57.59	1.56-1.59
N4242[2]	10.76-11.27	2.15-2.31	25.51-25.89	57.32-57.85	1.59-1.66
N5220[3]	13.14	1.89	26.55	55.13	1.40
N5866[3]	9.17	2.18	21.05	64.53	1.28
N6223[3]	11.53	2.01	26.58	57.04	1.24
N6822[3]	12.05	2.27	18.79	64.30	1.18
N7070[3]	10.11	1.77	25.49	59.77	1.19
N7590[3]	9.86	2.17	20.59	64.68	1.18
Range[4]	6–22	1–15	14–64	19–71 56–65[5]	0.5–2

[1]Values are ranges for three samples taken from 3 field sites (ie 1 sample/site) unless otherwise indicated and are expressed as % of fatty acid as a proportion of total fatty acid.
[2]Values are the range for two samples.
[3]Single values given only.
[4]From Weber, "Lipids of the kernel", Chapter 10 in Corn chemistry and technology, 1987, Watson SA and Ramstad PE (eds), American Association of Cereal Chemists, St. Paul, Minesota, USA. data
[5]Data from AGPM.

Dataset 3:

An analysis of the major components and nutritional qualities of elite Bt11 field maize lines has also been assessed. These lines are derived from the original transformant. Two genetically modified Bt11 hybrid maize lines and their near-isogenic controls were grown in three field locations in the USA in 1995.

Vitamins and minerals

One-pound (2.24 kg) samples of grain were taken from each of three locations from the two Bt 11 maize hybrids N4242-Bt and N4640-Bt and their corresponding near-isogenic non-modified hybrids and analysed for their vitamin and mineral content.The data were analysed to determine if there were significant difference between the Bt11 maize and the non-transgenic control maize. For each analysed parameter, means and standard deviation were calculated. Transgenic maize was compared to non-transgenic control by *t-test* ($p = 0.05$).

The grain was analysed for the minerals copper, magnesium, manganese and zinc as well as the vitamins folic acid, niacin, vitamin B1 and vitamin B2 (Table 8). No significant differences ($p=0.05$) between Bt-11 maize hybrids and their corresponding control hybrids were observed for any of the selected components.

Table 8. Vitamin and mineral composition for Bt11 and control maize (ANZFA 2000).

	N4242Bt	Control N4242	N4640Bt	Control N4640
Copper	0.17 ± 0.06	0.17 ± 0.06	0.20 ± 0.0	0.20 ± 0.0
Magnesium	95.7 ± 1.15	91.7 ± 5.51	90.0 ± 1.73	86.3 ± 4.73
Manganese	0.47 ± 0.06	0.43 ± 0.06	0.40 ± 0.0	0.33 ± 0.06
Zinc	1.93 ± 0.06	2.03 ± 0.29	1.77 ± 0.12	1.70 ± 0.10
Folic acid	0.051 ± 0.010	0.045 ± 0.002	0.57 ± 0.03	0.57 ± 0.03
Niacin	8.62 ± 1.32	8.03 ± 0.14	8.96 ± 0.21	9.49 ± 0.41
B_1	1.44 ± 0.10	1.37 ± 0.21	1.26 ± 0.23	1.48 ± 0.15
B_2	0.71 ± 0.04	0.70 ± 0.09	0.72 ± 0.04	0.71 ± 0.02

[1]Values are means of 3 samples, one from each of 3 locations. Minerals are expressed as % and vitamins are expressed in mg/lb.

3.2.3 Study 3: Comparison of nutritional composition of fresh and canned Bt-11 sweet maize (North America 1996)

A fifth analysis of Bt-11 maize lines was done, specifically to assess the nutritional value of three Bt-11 sweet maize varieties.

Maize was harvested from the Bt-11 sweet maize hybrids, Bt 98- 0943, Bt 95-0937 and Bt 95-0941, and from their corresponding near-isogenic non-modified hybrids, grown in 1996 at one location in the North America. The field trials were designed following a random block design with three replicate plots of each genotype. Data for each genotype were subjected to analysis of variance. For each analyte the statistical significance of the genotype effect was determined using a standard F-test at the 5% probability. The significance of the location x genotype interaction was also assessed using an F-test. The results were compared to compositional analysis data for grain and forage published in the literature and in compositional analysis databases.

Ten ears of each of the hybrids were harvested at prime harvest and analysed as fresh maize on the cob. Maize from each hybrid was canned and also analysed (processed maize analysis). Fresh and canned sweet maize was analysed for moisture, protein, fat, ash, carbohydrates, fibre, vitamins and minerals (Table 9). Given that there was only duplicate analysis of one sample taken for each line, no statistical analysis was performed. Comparable nutritional composition was observed between Bt11 sweet maize (Bt 95-0943, Bt 95-0937 and Bt 95-0941) and corresponding non-transgenic hybrids (Jubilee, Bonus and Empire) for the selected compounds (Table 9). According to the applicant, the values obtained in the study were consistent with reported literature values.

Table 9. Compositional analysis for fresh and canned sweet Bt11 maize and control maize (ANZFA 2000).

Fresh	Bt 95-0943	Jubilee	Bt 95-0937	Bonus	Bt 95-0941	Empire
Moisture (g)	69.88 – 69.78	69.67-69.70	73.65	72.20-72.24	71.15-71.28	70.34-70.56
Protein (g)	3.7-4.09	3.20-4.35	3.75-3.37	3.89-4.06	3.75-3.83	4.17-4.26
Fat (g)	0.76-1.34	1.10-0.97	0.75-0.91	0.81-0.88	0.85-1.18	0.91-1.13
Ash (g)	0.90-0.93	0.91	0.99-1.05	1.00-1.03	1.01-1.02	0.91-0.95
Carbohydrates - total[2] (g)	24.28	24.63	20.94	22.06	22.89	23.36
Calories[2]	111	112	93	100	105	110
Calories[2] from fat	10	9	7	7	10	9
Sugars[2] (g)	6.8	6.31	4.14	4.38	5.21	4.86
Other Carbohydrates[2] (g)	14.71	15.59	13.77	15.01	14.81	16.04
Total Dietary Fibre (g)	2.83-2.71	2.93-2.54	2.61-3.44	2.64-2.70	2.36-3.38	2.38-2.54
Vitamin A[2] (IU)	230	137	280	211	95.8	160
Vitamin C[2] (mg)	0.869	1.63	7.35	6.53	7.25	7.69
Sodium (mg)	9.9-14.2	5.9-7.2	10.0-13.0	3.9-5.3	5.8-7.2	4.9-8.6
Potassium (mg)	293.5-286.2	326.0-322.6	287.6-307.4	292.6-306.7	372.7-391.8	255.6-322.9
Calcium (mg)	3.4-8.6	1.6	0.7-7.1	0.0-0.4	7.1-8.0	0.7-7.1
Iron (mg)	0.49-0.85	0.49-0.56	0.57-0.61	0.6-0.90	0.54-0.63	0.71-0.74
Canned	**Bt 95-0943**	**Jubilee**	**Bt 95-0937**	**Bonus**	**Bt 95-0941**	**Empire**
Moisture (g)	77.81 – 77.83	76.81-76.85	77.66-77.76	77.77-77.80	76.44-76.52	77.80-77.96
Protein (g)	2.95-2.99	2.62-2.97	2.95-3.00	3.09-3.18	2.85-2.94	2.93-3.02
Fat (g)	0.85-1.77	1.02-1.90	1.01-1.09	0.68-0.75	0.83-0.96	0.62-0.85
Ash (g)	0.97-1.01	1.01	0.84-0.85	0.85-0.87	0.85-0.87	0.83-0.83
Carbohydrates - total[2] (g)	16.91	17.92	17.42	17.5	18.87	17.59
Calories[2]	83	87	81	79	86	79
Calories[2] from fat	12	13	9	6	8	7
Sugars[2] (g)	1.8	1.92	1.54	1.3	1.89	1.53
Other Carbohydrates[2] (g)	12.99	13.85	13.38	13.72	14.65	13.56
Total Dietary Fibre (g)	1.99-2.23	2.01-2.29	2.47-2.55	2.41-2.54	2.19-2.48	2.18-2.82
Vitamin A[2] (IU)	175	209	192	185	175	206
Vitamin C[2] (mg)	2.07	2.32	2.25	2.31	2.15	1.99
Sodium (mg)	262.8-285.0	266.1-304.1	245.9-248.0	212.5-230.2	225.7-239.6	191.9-235.6
Potassium (mg)	199.9-202.8	212.2-262.4	210.3-228.4	191.4-202.6	181.1-205.3	176.3-200.2
Calcium (mg)	3.1-8.8	2.4-4.2	0.0-1.8	5.1-8.2	3.7-10.2	5.2-8.2
Iron (mg)	0.29-0.55	0.289-0.614	0.31-0.25	0.23-0.34	0.348-0.387	0.31-0.37

[1]Values are expressed per 100 g serving basis.
[2]Only one sample determined.

3.2.4 Study 4: Analysis of Bt11 field maize and sweet maize grown in North America (1998)

Compositional analyses of three Bt11 maize hybrids and non-transgenic control maize, grown in one location, were analysed. An *F test* was conducted to see whether the values obtained for the Bt11 sweet maize were statistically significantly different from the values obtained for the isogenic hybrids, taking the various hybrids as "repetitions" (P = 5%). The values obtained were compared to those listed for maize grain in the *NOTIS plus* database, when available (Table 11). The following traits were considered: furfural, *p*-coumaric acid, ferulic acid, *myo*-inositol and raffinose.

Secondary metabolites

There are no generally recognised anti-nutrients in maize at levels considered to be harmful, but for the purposes of assessment of substantial equivalence, the OECD has asked for analytical data for the following secondary metabolites in maize: ferulic acid, p-coumaric acid, furfural, inositol, phytic acid, raffinose and trypsin inhibitor.The study indicate that grains from Bt11 sweet maize are substantially equivalent to grain from non-transgenic maize, by showing that the levels of furfural, *p*-coumaric acid, ferulic acid, *myo*-inositol and raffinose are similar in both types of maize (Table 10). The values were not significantly different at P = 5%, for any of the parameters measured. One striking difference observed are the higher amount of myo-inositol in one hybrid pair (GS0975 2330 and the corresponding isogenic GSS9377 322), compared to the other hybrids. Phytic acid and trypsin inhibitor were not analysed.

Table 10. Secondary metabolites composition for Bt11 sweet maize and control maize

Hybrid	furfural (ppm*)	*p*-coumaric acid (ppm*)	ferulic acid (ppm*)	*myo*-inositol (ppm*)	raffinose (ppm*)
GH0937 2132	11	11800	940	21	280
GS0975 2330	12	10900	860	79	260
GH0913 2117	12	7900	650	23	230
mean ± sd	**11.7 ± 0.5**	**10200 ± 1667**	**817 ± 122**	**41 ± 27**	**257 ± 21**
Bonus 2133	22	10800	840	31	150
GSS9377 322	8	11900	720	72	390
Sprint 2118	3	11100	730	22	480
mean ± sd	**11.0 ± 8.0**	**11267 ± 464**	**763 ± 54**	**42 ± 22**	**340 ± 139**

*: mean of the three replicate analyses

The values obtained in Table 10 were compared to those listed for maize grain in the *NOTIS plus* database, when available. The comparison is shown in Table 11. Significant differences were observed. According to the applicant, these differences are most likely due to the fact that the values reported in the *NOTIS plus* database are for "field maize", whereas the analyses reported here were conducted on sweet maize. Sweet maize has a different biochemical composition than field maize, the major difference being that energy is stored in the endosperm of the sweet maize grain in the form of various "sugars", whereas field maize accumulates starch. According to the applicant, differences are not unexpected. No literature values for these compounds of sweet maize were found..

Table 11. Comparison of the values measured in maize samples compared to the values in the NOTIS database

Compound	NOTIS database (ppm)			Novartis Seeds' results (mean, ppm)	
	mean	minimum	maximum	Bt-maize	isogenic maize
p-coumaric acid	167.3	18.9	222.9	10200	11267
ferulic acid	294.1	241.4	380.5	817	763
raffinose	11733.3	2100.0	30000.0	257	340

3.2.5 Study 5: Analysis of Bt11 field maize lines treated with herbicide in Europe (1998)

An additional study was done to assess the potential effects of herbicide treatment on the major components of the maize grain. Three Bt-11 hybrids representing different maturity types (Madera, Manuel and Magister) and their isogenic controls were grown in open fields at two locations in France in 1998.

An *F test* was conducted to see whether the values obtained for the "treated Bt11 hybrids", the "untreated Bt11 hybrids" and the "non-modified control hybrids" were statistically significantly different at $p<0.05$, taking the various hybrids as "repetitions". The evaluation of the data was done by comparing the values obtained for the "treated Bt11 hybrids" to those obtained for the "untreated Bt11 hybrids" and for the "control hybrids".

Proximate analysis (carbohydrate, protein, fat and fibre), fatty acids and amino acid composition were compared between transgenic crops treated with a glufosinate ammonium herbicide (Liberty®) at a rate of 2.25 L/ha active ingredient at the 3 and 6–7 leaf stages and untreated transgenic and isogenic

controls (Table 12). Values presented in this experiment are not directly comparable to values for other experiments because they have been performed by a different laboratory using slightly different methods.

Proximates

No significant differences in the content of proximates were observed between the different groups of hybrids, for none of the parameters measured (Table 12).

Table 12. Proximate composition for Bt11 and1 maize and control (treated or untreated with glufosinat ammonium) (ANZFA 2000).

	Treated	Untreated	Control
Energy	1441 ± 37	1430 ± 35	1433 ± 29
Carbohydrate	70.0 ± 2.0	69.5 ± 1.5	68.8 ± 1.5
Protein	7.6 ± 0.9	8.2 ± 0.8	8.4 ± 0.8
Fat	3.3 ± 0.6	3.0 ± 0.6	3.3 ± 0.8
Fibre	8.0 ± 1.0	8.0 ± 0.8	7.7 ± 0.2

[1]Values are means of 3 samples, one from each of the hybrids Madera, Manuel and Magister. Values are all expressed as a % except for energy (KJ/100g). *(ii)*

Amino acids

Bt11 hybrids, untreated or treated with glufosinate ammonium, as well as control hybrids gave comparable results, with the exception of proline and alanine (Table 13). When comparing the three "treatments" using an *F test*, the values obtained for glutamic acid, proline, alanine, isoleucine, leucine and phenylalanine were statistically different at P = 5%. However, when comparing only the "treated Bt11 hybrids" to the "control hybrids", the values for glutamic acid, isoleucine, leucine and phenylalanine were not statistically significantly different. According to the applicant, concerning proline and alanine, the differences observed are unlikely to be due to the genetic modification itself in interaction with the glufosinate ammonium treatment, as not all three hybrids showed this. The difference between the treated modified and non-modified line was not consistent for all lines and may be a result of variability between the lines. This difference is not considered to raise safety or nutritional concerns.

The values for proline and alanine for each of the three hybrids is shown in detail in Table 14. The difference between the treated modified and non-modified line was not consistent for all lines and may be a result of variability between the lines. This difference is not considered to raise safety or nutritional concerns. The levels of glutamine and tryptophan were not determined. Tryptophan is one of the essential amino acids and one of the limiting amino acids in maize.

Table 13. Amino acid composition for Bt11 and control maize (treated or untreated with glufosinate-ammonium) (ANZFA 2000).

	Treated	Untreated	Control
Aspartic Acid	4690 ± 406	5033 ± 439	4703 ± 142
Threonine	2690 ± 423	2850 ± 165	2690 ± 423
Serine	3537 ± 353	3750 ± 260	3537 ± 353
Glutamic Acid	14533 ± 1595	16233 ± 1626	15700 ± 625
Proline	6967 ± 1154	8367 ± 234	8590 ± 769
Glycine	3047 ± 238	3187 ± 111	2920 ± 26
Alanine	5057 ± 415	5760 ± 606	5500 ± 207
Valine	2963 ± 552	3327 ± 654	3210 ± 183
Methionine	1030 ± 183	1270 ± 122	1170 ± 30
Isoleucine	1717 ± 315	2320 ± 368	2013 ± 42
Leucine	8153 ± 918	9320 ± 1105	8787 ± 420
Tyrosine	3800 ± 573	4240 ± 455	3957 ± 172
Phenylalanine	3163 ± 440	3540 ± 243	3363 ± 280
Histidine	1867 ± 376	2147 ± 170	1853 ± 169
Lysine	1967 ± 228	2223 ± 228	1967 ± 163
Arginine	3257 ± 319	3443 ± 119	3160 ± 236

[1]Values are means of 3 samples, one from each of a different maturity type. Values are all expressed as mg/kg.
[2]Data from L'alimentation des animaux monogastriques: porc, lapin, volailles. INRA 1989, Feedstuffs ingredient analysis table, edition 1996, AEC Table and 1995 UCAAB data.

Table 14. Significant differences in amino acid composition between treated Bt11 and control maize (ANZFA 2000).

Hybrid	Proline (mg/kg)		Alanine (mg/kg)	
	Bt11 hybrid[2]	Control hybrid	Bt11 hybrid[2]	Control hybrid
Madera	5640	7730	4720	5330
Manuel	7520	9210	5520	5730
Magister	7740	8830	4930	5440

[1]Values are all expressed as mg/kg.

Fatty acids

The results of the determination of palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid are presented in Table 15. The values obtained for the "treated Bt11 hybrids", the "untreated Bt11 maize" and the "non-modified control maize" was comparable. An *F test* confirmed the lack of significant differences at P = 5%.

Table 15. Fatty acid composition for Bt11 and control maize (treated or untreated with glufosinate-ammonium) (ANZFA 2000).

	Treated	Untreated	Control
Palmitic	12.4 ± 1.9	12.3 ± 1.2	11.2 ± 1.2
Stearic	2.3 ± 0.2	2.4 ± 0.3	2.2 ± 0.2
Oleic	28.0 ± 1.9	27.4 ± 2.0	27.2 ± 1.3
Linoleic	55.1 ± 2.7	55.8 ± 3.0	57.0 ± 2.3
Linolenic	1.1 ± 0.2	1.1 ± 0.2	1.2 ± 0.2

[1]Values expressed as a % of total fatty acids. Values are means of 3 samples, one from each of the hybrids Madera, Manuel and Magister.

3.2.6. Determination of DIMBOA levels in Bt11 maize (field maize and sweet maize)

DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) is a naturally occurring hydroxamic acid, a benzoxazinoid. DIMBOA is a powerful antibiotic. In maize, DIMBOA functions as natural defense against European corn borer larvae and other damaging pests. MBOA (on 6-methoxybenzoxazolin-3-one (MBOA) is a degradation products of DIMBOA and was analysed in Bt11 maize. Bt11 maize has been back-crossed into several field maize and sweet maize varieties through traditional breeding methods. Therefore, "Bt11 field maize" and "Bt11 sweet maize" represents the same transformation event. The report presents data on the level of MBOA in seedlings of 4 different Bt11 maize hybrids (N6800Bt, N4640Bt, N4242Bt and Attribute), compared to their (near) isogenic hybrids (N6800, N4640, N4242, Attribute non-modified control). One of the hybrid pair tested was a sweet maize variety. No statistically significant differences were found.

Considerable plant-to-plant variability was observed, as reflected by the large standard deviation. A t-test was conducted and indicates that the DIMBOA levels (as determined after conversion to MBOA) were not significantly different between the Bt11 maize plants and the isogenic controls, for any of the hybrid pairs analyzed. An F-test was also conducted, taking all data together and considering the "Bt11-trait" as a "treatment". No statistically significant difference was identified this way either.

No significant differences in the levels of DIMBOA were observed between the genetically modified Bt11 hybrids and their isogenic counterparts, which could be linked to the genetic modification itself.

3.3 Agronomic and phenotypic characters

According to the applicant, agronomic and phenotypic data on maize Bt11 and its non-transgenic counterpart were collected from field trials conducted in Spain, France, Italy and Portugal between 1994 and 2006, and in the USA and Canada in 1994. The technical dossier (Appendix 4), however, contains only a draft summary of the results obtained in the USA and a visual comparison of Bt11 to its non-modified isogenic hybrid in France. Data on the mentioned field trials in Spain, France, Italy and Portugal are not presented in the application EFSA-GMO-RX-Bt11.

North America 1994
Four generations of backcrossing (to BC_4) supplemented by selection, were used to transfer the chromosome fragment containing Btk coding sequence into the genetic background of elite maize inbred lines. A series of three indepenfield conversions of the inbred 2043 were derived through parallel backcrossing schemes. Likewise, two indepenfield conversions of the inbred 2044, were used to obtain a Btk version of the original 2044.These BC_4 derivates were then subjected to extensive experimental analysis to assure that they represented adequate recoveries of the essential features of the original elite lines.

The two 2044 conversions, along with the original 2044, were crossed to a series of five, unrelated, elite inbred lines to produce F_1 hybrid seed for evaluation in replicated trials in 1994. Eighteen hybrids were produced for evaluation. Evaluation consisted of planting each hybrid at each of 17 locations which were distributed across eight US States and one Canadian providence. The trial design at each site was a randomized complete block design with two replicates. Each plot was represented by two rows (paired) of 20 feet in length, including three foot alleys. The plant population varied within the range of 25.000 to 27.000 plants per acre at each location. Notes and measures were taken on the plots during the growing season, as is usual for commercial variety trials. At harvest, a grain combine, which is adapted to harvest of maize plot trials, was used to determine plot yield and grain moisture at harvest.

Data were collected for the following agronomic and phenotypic characteristics: yield, grain moisture, stalk lodging rating, root lodging rating, ear height, plant height, heat units to silking, heat units to pollen shed and intactness rating (data not shown). The parameter “intactness rating” was however not specified, and the method for its evaluation not indicated.Data was subjected to factorial analysis of variance using the SAS GLM Procedure. Contrast was used to test the significance of the difference between the original, non-transformed, 2044 line effects and those of the transformed derivates. An ifieldical process was used in testing the Btk conversions of the elite inbred 2043. Btk-converted lines were tested in combination with 6 elite inbreds and resulting hybrids were evaluated in 17 locations.

The VKM GMO Panel considered the data presented for the USA/Canada trial inadequate for a complete evaluation, as information on the varieties assessed and their relationship (Bt hybrids and conventional control), use of the herbicides, pesticides and the applied fertilizer regime is missing. Furthermore information is missing on environmental conditions during trials. A complete breeding history of the maize varieties analysed in the trials should be provided as well as a complete description of the agricultural management applied in each location. In particular, as this GMO is tolerant to glufosinate-ammonium, a herbicide-treated and a herbicide untreated variant must be included and potential differences assessed, which are due to the different treatments.

Apart from the incomplete presentation of material and methods used in the USA/Canadian field trials in 1994, only a summary of results is contained in the submitted study. No raw data are presented or data on a per location basis.

According to the applicant, statistically significant differences were observed between the elite line 2044 and the Bt11 conversions for the parameters stalk lodging ($p<0.001$), heat units to silking and pollen shed ($p<0.05$) and intactness rating ($p<0.01$) (data not shown). Similarly for 2043 and its

derivates, differences were observed for moisture ($p<0.05$), stalk lodging ($p<0.001$) and intactness rating ($p<0.001$). The parameters stalk lodging rate and intactness rating (general performance) are expected to be affected by maize borer damage, and thus the results are as expected. According to the applicant, the significant differences between the test line and control for heat units to silking and pollen shed and moisture at harvest are related to different genetic backgrounds and different maturities of the elite lines.

France 1995
During the field trial in one growing season in France, visual observations were taken on maize Bt11 (H8540 Bt11) and the non-transgenic counterpart (near-isogenic line H8540). The following agronomic and phenotypic parameters were assessed: anthocyanin coloration at the level of the ear, tassel, leaf, internodes and glumes; plant and tassel length, grain type, resistance to pests and diseases, number of primary lateral branches, height of insertion of ears, length of peduncle, shape/length of ears and number of rows of grain.

According to the applicant, the data from the field in France confirmed the equivalence of Bt11 maize phenotype to its non-transgenic counterpart. Furthermore, no differences in the agronomic and phenotypic characteristics were found between the Bt11 maize and the non-transgenic counterpart during field trials at different locations in Spain, France, Italy and Portugal conducted between 1994 and 2006 that would indicate unexpected pleiotropic effects of the genetic modification.

The data presented for the 1995 France trial are however insufficient for an assessment of agronomic behavior of maize Bt11. Appendix 4 contains only a overview over the parameters assessed and , visual comparison of Bt11 to its near-isogenic hybrid. No data from the analyses of phenotypic characters from these field trials is provided in the application. Further, no information is contained on the location of the sites, the methods applied to assess the indicated parameters, the agronomic management conditions (herbicide, pesticide, fertilizer regime), the environmental conditions, the field trial design (replication, plot size etc) and the breeding history of the GM and non-GM varieties used.

Data on the mentioned field trials in Spain, France, Italy and Portugal are not presented in the dossier. Appendix 4 contains only a draft summary on results obtained in the USA and a visual comparison of Bt11 to its non-modified isogenic hybrid in France.

Due to the insufficient data provided by the notifier, no conclusions can be made on the agronomic behavior and characteristics of the GM maize Bt11 as well as the related phenotypic characteristics such as reproduction, dissemination and survivability.

3.4 Conclusion

Comparative analyses of data from field trials located at representative sites and environments in North America and Europe indicate that maize Bt11 is compositionally equivalent to its conventional counterpart, with the exception of the herbicide tolerance and insect resistance traits, conferred by the expression of the Cry1Ab and PAT proteins. However, data on the amino acid tryptophan, is only given in one out of six studies. Based on current knowledge, the VKM GMO panel concludes that maize Bt11 is compositionally equivalent to conventional maize.

The data provided by the applicant are not sufficient to show that Bt11 maize is phenotypically and agronomically equivalent to conventional near-isogenic maize lines. The agronomic assessment data are provided from one growing season in the North America and one growing season in France. This is not considered to be sufficient for representative testing of agricultural environments.

4 Food /feed risk assessment

According to ISAAA (The International Service for the Acquisition of Agri-biotech Applications) the trade name of Bt11 is Agrisure™ CB/LL, or Attribute (Bt Maize registrations-2010).

4.1 Product description and intended uses

The genetic modification in Bt11 field maize will not impact the existing production processes used for maize. All Bt11 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 Bt11 field maize and all food, feed and processed products derived from Bt11 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.

The Bt11 sweet maize is grown exclusive (100 %) for human consumption. By-products from processing are used as animal feeds. Sweet maize to be used as fresh vegetable is harvested at an immature stage, around 24 % dry matter. According to Syngenta about 12 million hectares Bt11 sweet maize were planted in USA in 2012 (information on Syngenta web-page).

4.2 Processing of maize

Food manufacturing of Bt11 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, which also applies to CRY1Ab1 and PAT proteins (Hammond et al. 2011).

Bt11 sweet maize is able to consumers as fresh vegetable (maize on the cob) to be eaten as steamed cooked, or roasted on a barbecue. Sweet maize is also processed for freezing, canning and dehydrated powder. The canning process includes a cooking/sterilisation process at or above 110 °C, and duration of approximately 10 minutes. The freezing process includes a short bleaching process. Generally, the grain are exposed to steam (100 °C) from a few second to one minute.

4.3 Toxicological assessment

The potential toxicity of Bt11 maize expressing the Cry1Ab and PAT protein has been assessed in toxicity study in rofields, broiler chicken, pigs and cattle.

4.3.1 Toxicological assessment of the newly expressed protein

The *cry1Ab* gene was originally obtained from *Bacillus thuringiensis* strain. There is no evidence of Cry1Ab having harmful effects on the health of humans or animals (EPA 1995a; McClintock et al. 1995; EPA 1996). The Cry1Ab protein has a very specific mode of action and selective toxicity against certain lepidopteran and coleopteran insect pests (target organisms).

Test for possible binding of Cry1Ab to proteins of bovine intestinal cells
Shimada et al. (2006b) performed immunohistochemical and ligand blot analysis to investigate the interaction of Cry1Ab toxin with bovine intestinal epithelium. Shimada et al. (2006) found that Cry1Ab binds to actin, but not to aminopeptidase N, cadherin, or alkaline phosphatase, all which are cell membrane receptors or candidate proteins for development of toxicity in susceptible insects cells.

The results indicate that Cry1Ab toxin is able to bind to cytoskeletal actin, but that the bovine intestinal epithelial cells lack membrane receptors, which are necessary for the toxin to exert its toxicity on cells.

Test for possible binding Cry1Ab to mammalian cellular brush border membrane vesicle (BBMV)
Shimada et al. (2006a) investigated the affinity of Cry1Ab toxin, a lepidopteran-specific Cry1-type toxin, to the cellular BBMV of two mammalian intestinal cells as well as the effect of the toxin on the membrane potential of three mammalian intestinal cells compared to its effects on the silkworm midgut cell. Shimada et al. (2006) found that Cry1Ab toxin did bind to the bovine and porcine BBMV, but far more weakly than it did to the silkworm midgut BBMV. Although the silkworm midgut cells developed severe membrane potential changes within 1 h following the toxin treatment at a final concentration of 2 μg/ml, no such membraneous changes were observed on the bovine, porcine, and human intestinal cells. The present *in vitro* results suggest that, although Cry1Ab toxin may bind weakly or nonspecifically to certain BBMV components in the mammalian intestinal cell, it does not damage the cell's membrane integrity, thus exerting no subsequent adverse effects on the cell.

Test for possible toxic potential of Cry1Ab on porcine culture intestinal cells
Bondzio et al. (2013) performed *in vitro* testing on porcine culture intestinal cells (IPEC-J_2) with Cry1Ab protein. For comprehensive risk assessment they used WST-1 conversion and ATP content as metabolic markers for proliferation, lactate dehydrogenase release as indicator for cells with compromised membrane and transepithelial electrical resistance as parameter indicating membrane barrier function. The results were compared to the effects of valinomycin, a potassium ionophore, known to induce cytotoxic effects in most mammalian cell types. Whereas no toxicity was observed after Cry1Ab treatment, valinomycin induced a decrease in IPEC-J2 viability. Two dimensional differential in-gel electrophoresis was performed on the cell proteins. Only three proteins were differentially expressed. The functions of these proteins were associated with responses to stress. The up-regulation of heat shock protein Hsp70 was verified by Western blotting as well as by enzyme-linked immunosorbent assay and may be related to a protective function.

The present study shows that cultured porcine gastrointestinal cells can tolerate Cry1Ab even in a dose range that greatly exceeds any amount that may accumulate in the gastrointestinal tract of pigs. No influence on viability of IPEC-J2 cells was found using a screening with different assays including real-time monitoring of cell viability (Bondzio et al 2013). Consistent with a previously published studies in fish fed maize MON810 [Sagstad et al; 2007], the proteomic data at 24 h were indicative of a mild stress response to Cry1Ab.

According to the authors long-term investigations are needed to determine whether increased Hsp70 expression is only a transient short-term adaptive response to Cry1Ab or may be the cause of further unintended side effects of this protein.

Test for possible toxic potential of Cry1Ab on human culture kidney cells
Mesnage et al. (2012) performed *in vitro* testing on human culture kidney cells (HEK293) with Cry1Ab- and Cry1Ac protein. Time- and dose depenfield effects of relatively high concentrations of Cry1Ab on viability of HEK293 cells, respiration inhibition and plasma membrane alterations, were detected.When treating the cells with 100 ppm of Cry1Ab the mitochondrial succinate dehydrogenase activity was decreased by 11%. Lower doses were tested from 10 ppb to 10 ppm, but significant effects were not observed. The adenylate kinase (AK) activity, when released in the medium, reveals possible membrane alterations. A Cry1Ab concentration of 100 ppm increased AK leakage in the medium 2-fold, revealing plasma membrane alterations. Lower doses were tested from 10 ppb to 10 ppm, but significant effects were not observed. Mesnage et al. argue that modified Bt toxins are not inert on non-target human cells, and that they can present combined side effects with other residues of pesticides specific to GM plants.

PAT
The *pat* gene was originally obtained from *Streptomyces viridochromogenes* strain Tü494 which has no known toxic or pathogenic potential. The PAT protein is enzymatically active but it has high substrate specificity to the active ingredient L-phosphinothricin (L-PPT) of glufosinate-ammonium. The PAT protein has already been found safe to human health during the assessment of glufosinate-ammonium tolerant maize (OECD 1999).

4.3.2 Acute toxicity testing

*Acute oral exposure of **PAT** protein in rodents*
Syngenta has rights for citing the acute oral toxicity PAT study on mice conducted by Monsanto. The acute oral study was conducted in compliance with EPA Guidelines no. 81-1, and with the following good laboratory standards: US Environ. Protection agency FIFRA: Good laboratory standards, 40 CFR 160 and 40 CFR 158, Japan Minisry of Agric., Forestry and Fisheries, Notf. No. 59 Noshan 3850, Director –General of Agricultural Production Bureau, August 1984 and OECD Principles of GLP, Annex 2, C(81)30.

PAT Microbial Protein (FL), which was 51% pure microbial protein, was evaluated for acute oral toxicity. Group I animals (five male and five female Harlan Sprague Dawley (HSD:ICR) mice received 5050 mg/kg (51 % pure) of the test material as a 26% w/v suspension in aqueous 2% carboxymethylcellulose (CMC), group II (5 males and 5 females) received heath inactivated PAT (52 % pure) and group III received 19.42 ml/kg bw doses of 2 % CMC only. Parameters evaluated during the two-week observation period included body weights and detailed clinical observation. All animals were examined for gross pathological changes. One male in Groupe I died during the study. Necropsy finding in the animal that died on test pertained to material logged in the esophagus. The ifieldity of the material blocking the esophagus was not determined. There were no treatment-related clinical observations in the rest of the mice. There were no gross pathological lesions for any other animal in the study. Under the condition of this study, the acute oral LD_{50} of PAT Microbial protein in male and female HSD:ICR mice was greater than 2575 mg/kg bw.

*Acute oral exposure of **Cry1Ab** protein in rodents*
An acute oral toxicity study was performed with the Cry1Ab protein in mice. An acute oral study was considered appropriate since toxic proteins are only known to exert acute effects. The Cry1Ab protein was administered orally by gavage to three groups of ten male and female mice; additionally, one group of mice was dosed with a vehicle control lacking the Cry1Ab the protein. The targeted doses of Cry1Ab protein administered to mice were 0, 400, 1000, and 4000 mg/kg. A mice control group was dosed with Bovine serum albumin (BSA) at 4000 mg/kg. At the time of sacrifice, 7 days after dosing, there were no statistically significant differences in mortality, body weights, cumulative body weight or total food consumption between the BSA control groups and Cry1Ab protein-treated groups. Results from this study demonstrated that the Cry1Ab protein is not acutely toxic to mammals.

The acute oral toxicity test performed on mice did not indicate toxic effects of *E. coli* produced CryAb1 and PAT protein. However, acute tests do not provide enough information to conclude on possible adverse health effects of maize Bt11. 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

14-day oral toxicity study of PAT protein in rats
Bayer Crop Sciences has performed a sub-chronic oral toxicity study of the PAT-protein in rats (Pfister et al. 1996). The study was performed in accordance with the principles of Good Laboratory of O.E.C.D. (Organization for Economic Cooperation and Development) and Principles of Good Laboratory Practice, 1992. Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles, March 1986 and the Japanese Ministry of Agriculture, Forestry and Fisheries: On Good Laboratory Practice Standards for Toxicological Studies on Agricultural Chemicals, Agricultural Production Bureau, 59 NohSan Notification Number 3850, August 10, 1984. Test guidelines: The study procedures mostly conform to OECD Guidelines for Testing of Chemicals, number 407 "Repeated Dose 28-day Oral Toxicity Study in Rofields", adopted by the Council on July 27, 1995.

The study comprised four groups of five male and five female Wistar rats in each group. The rats in group 1 received a standard diet without PAT protein, whereas rats in group 2, 3 and 4 received diets with the inclusion of PAT and/or soybean protein: group 1 (standard diet), group 2 (0.5 % PAT + 4.5 % soybean), group 3 (5 % PAT), group 4 (5 % soybean), for a period of 14 days. According to the OECD guidelines the duration of exposure should normally be 28 days although a 14-day study may be appropriate under certain circumstances; justification for use of a 14-day exposure period should be provided. The duration of this repeated dose oral toxicity study was 14-days. No justification for using 14-days has been found in the dossier from the applicant.

The mean intake of PAT-protein in group 2 over the treatment period was 712 mg/kg body weight/day for males and 703 mg/kg body weight/day for females. In group 3 the mean intake of PAT-protein was 7965 mg/kg body weight/day for males and 7619 mg/kg body weight/day for females.

The results showed no unscheduled deaths or clinical signs. Food consumption and body weights were unaffected by treatment. No treatment-related changes were seen in haematology or urinalysis parameters. Organ weight data, macroscopical and microscopical findings did not distinguish treated groups from controls.

The only changes which might be attributed to treatment were observed in clinical biochemistry parameters. They consisted of a slightly lower glucose level in males of group 4, slightly higher total cholesterol and phospholipid levels in males of groups 2, 3 and 4 and slightly higher triglyceride levels in females of group 4 when compared with rats of group 1. Animals of group 4 received no PAT-protein but - with respect to the protein content - a diet most similar to that of groups 2 and 3. The changes mentioned above were considered to reflect differences in the dietary composition and not related to the PAT protein itself. Further, comparing the increased total cholesterol and phospholipid levels between group 3 (5 % PAT) and group 4 (5 % soybean) they were found to be within similar range, which may suggest a similar nutritional value of the proteins.

28-day toxicity study in F344 male rats with impaired gastrointestinal function and gut damage, administered Cry1Ab protein
The study comprised four groups of eight male F344 rats in each group (Onose et al. 2008). In the control group rats were fed basal diet alone. The other rats were divided a Bt –group fed 10 ppm Cry1Ab-protein in feed, a GI-group with impaired gastrointestinal function and gut damage induced by treatment with famotidine and indomethacin and a GI + Bt-group. During the experimental period, general condition was checked daily, and body weights and food consumption were recorded every week.

The concentration for the Bt dosage was selected based on the content of the Cry1Ab-protein in Bt maize (Betz et al. 2000), assuming that all the food consumed by the animals was Bt maize.

At the end of the experiment, all animals were fasted overnight, anesthetized with ether and euthanized by exsanguination. Blood samples were collected from the abdominal aorta. The blood was applied for hematology and serum biochemistry. The red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT) and white blood cell count (WBC) were determined with an automatic hematology analyzer (K-4500, Sysmex, Hyogo, Japan). Differential leukocyte counts and the reticulocyte count were obtained with an automatic blood cell analyzer (Microx HEG-120A, Tateishi Electronic, Tokyo, Japan). For serum biochemistry, total protein (TP), albumin (Alb), albumin/globulin ratio (A/G), total bilirubine (T-Bil.), triglycerides (TG), total cholesterol (T-Cho), blood urea nitrogen (BUN), creatinine (CRE), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), cglutamyl transpeptidase (c-GTP), calcium (Ca), inorganic phosphorus (IP), sodium (Na), chloride (Cl) and potassium (K) were measured at the medical laboratory SRL, Inc. (Tokyo, Japan). The medical laboratory SRL, Inc. was certified by the College of American Pathologists.

All animals were subjected to a complete necropsy, and the brain, thymus, lungs, heart, spleen, liver, adrenals, kidneys and testes were resected and weighed. These organs and the following organs and tissues were fixed in 10% neutral buffered formalin and paraffin-embedded sections were cut and stained with hematoxylin and eosin (HE) for histopathological examination: skin, mammary gland, sternum with marrow, femur with marrow, submandibular and mesenteric lymph nodes, salivary glands, aorta, trachea, tongue, esophagus, stomach, small and large intestines, pancreas, urinary bladder, epididymides, seminal vesicles, prostate, bulbourethral gland, pituitary, thyroids, parathyroids, spinal cord, trigeminal nerve, sciatic nerve, nasal cavity, Harderian gland, eyes and thigh muscle.

The twenty-eight-day study in rats showed no adverse effects that can be attributed to diet containing Cry1Ab. The administration of diet containing Cry1Ab protein (10 ppm Cry1Ab) had no significant effect on any physiological or biochemical parameter, except a lower level of AST in the serum of animals fed such maize, when compared with control. However, no changes in organ weights or histo-pathological changes were observed in organs like heart, liver, and kidneys. Also, serum AST levels are usually elevated with tissue injury, but the interpretation of relatively small changes in AST in toxicology studies should be done carefully, since the range of variation of this parameter can be broad in healthy animals. The decrease of AST in this experiment, therefore, is not considered to be toxicologically significant.

4.4 Toxicological assessment of the whole GM food/feed

42-day feeding study on broiler chickens
The wholesomeness and safety of the Bt11 maize has been shown in a 42-day feeding study using broiler chickens. A poultry feeding study was conducted to confirm the nutritional equivalence of the Bt11 maize with its non-GM commercial maize equivalent (Brake et al. 2003). Broilers were fed over a 42-day period.

Four kinds of maize grain were used in this study:
(1) grain from the Bt-expressing field maize hybrid N7070Bt,
(2) grain from the N7070Bt hybrid that had been sprayed with Liberty brand herbicide (glufosinate) according to manufacturer's instructions (N7070Bt + Liberty),
(3) grain from standard N7070 (non-Bt isoline of N7070Bt) grain,
(4) grain from North Carolina grown the 2000 growing season (NC2000).

Poultry studies are considered to be very useful because they utilize a fast growing organism that can eat a high percentage of maize in the diet, thus, it is very sensitive to potentially toxic effects of dietary components (OECD 2003).
At 41 day of the experiment, there was a sudden and extreme increase in ambient temperature and humidity. The heat index exceeded 43°C during the afternoon, and birds began to die. All mortalities were weighed as quickly as possible, and on the morning of 42 day, the decision was made to terminate the growth performance portion of the experiment by weighing all live birds and total feed consumed. For completeness, BW of live and dead birds are presented; however, group differences were minor and unimportant. Access to feed was limited on 42 and 43 day of the experiment to allow broilers to lose body heat. After the ambient heat stress subsided, the broilers were returned to full feed for 44 day through 47 day of the experiment prior to processing.

The results of the study show that there were no significant differences in percentage survivors for birds that received the two transgenic maize diets and those that received the N7070 isoline and commercial NC2000 maize diets on an overall basis at any age.

However, there were significant differences due to sex, with males exhibiting higher mortality during the finisher phase (35 to 42 d) and cumulatively (0 to 42 day). This should be expected in extremely hot weather conditions as males are well known to be more susceptible to heat stress. There was a significant interaction of sex and maize source for the combined starter-grower periods (0 to 35 day) due to some erratic mortality during the grower period (21 to 35 day) that was not evifield on a cumulative basis.

According to the authors this interaction did not follow any logical or explainable pattern and probably represents chance occurrences.
The diets prepared with transgenic maize did not have any adverse effects on performance of broiler chickens when compared to diets prepared with nontransgenic (isogenic) control and commercial maize. Only minor differences were found due to maize source; performance was poorer for birds fed the commercial NC2000 maize diets.

According to the applicant this study shows that Bt11 maize is nutritionally equivalent to non-GM maize. These findings also provide further confirmation of the safety of the proteins Cry1Ab and PAT expressed in the Bt11 maize. The applicant concludes that the Bt11 maize is nutritionally equivalent to, and as safe as, non-GM commercial maize.

90-day feeding study in calves
Twelve healthy four-month-old cross-breed calves (Japanese Black × Holstein) were fed 43.3% Bt11 or 43.3% non-genetically modified isoline maize grain as dry matter for 90 days, according to the feeding experiment procedure for safety assessment of feeds recommended by the Ministry of Agriculture, Forestry and Fisheries of Japan (Shimada et al. 2006c).

At the end of the experiment, tissues from the liver, spleen, kidney, mesenteric lymph nodes, and musculus longissimus were sampled after slaughter. These tissue samples were examined histopathologically and measured for hematological and biochemical parameters, i.e. red blood cells, white blood cells, hematocrit, hemoglobin, aspartate aminotransferase, γ-glutamyltransferase, alkaline phosphatase, total-bilirubin, total protein, albumin, total cholesterol, triacylglycerol, blood urea nitrogen, creatinine, calcium, inorganic phosphorus, magnesium, glucose, sodium, potassium, and chlorine in peripheral blood and ruminal pH, volatile fatty acid, lactic acid, ammonia nitrogen, and free lipopolysaccharides in rumen juice.

No significant gross or histopathological lesions and no discernible clinical, hematological, biochemical, or ruminal abnormalities were found in calves fed Bt11 maize as compared with control calves fed non-Bt maize.

28-day feeding study in pigs
Ten castrated pigs weighing 42 kg (Large White/Duroc cross) were fed diets containing 60% Bt11 or non-Bt isoline maize grain for four weeks (Shimada et al. 2008). At the end of the experiment, the liver, spleen, kidney, heart, lung, lymph nodes, thymus, tonsils, stomach, duodenum, pancreas, jejunum, ileum, ileocecum, cecum, colon, rectum, and spinal cord were sampled after slaughter. These tissue samples were examined histopathologically. There were no significant differences in histopathological observation between Bt and control groups.

28-day feeding study in poultry
Ten one-week-old male White Leghorns were fed diets containing 61.22% of Bt11 or non-Bt isoline maize for four weeks (Shimada et al. 2008).

At the end of the experiment, the liver, spleen, kidney, heart, lung, thymus, thyroid, glandular stomach, gizzard, duodenum, jejunoileum, and spinal cord were sampled after slaughter. These tissue samples were examined histopathologically. No significant differences in histopathological observation were found between Bt and control groups.

Mice reproduction toxicity study over five generation.
A long term performance of multi-generations or life span was assessed using genetically modified (GM) insect-resistant Bt11 maize (Haryu et al. 2009). Diet containing 68% of GM Bt11 or non-Bt isoline with sufficient nutrient composition was fed to male and female ICR mice through 5 generations. The study was performed according to OECD test guideline408 Repeated dose 90-day oral toxicity study in rofields(OECD 1998), OECD test guideline 43 Guidance Document On Mammalian Reproductive Toxicity Testing and Assessment (OECD 2008) and OECD Environmental Health and Safety Publications: Considerations for the Safety Assessment of Animal Feedstuffs Derived from Genetically Modified Plants (OECD 2003).

Parent generation (F0) consisted of 31 females and 16 males. They were cross-bred at the age of 60-70 days. The rate of vaginal plug formation, delivery rate and period, number of fetuses, sex ratio of fetuses, weaning period and feed intake and individual body weight were recorded. F1 mice were bred in the same manner to generate the follow-up generations. One day before delivery of F4 fetuses, the pregnant mice were sacrificed to count the number of fetuses, determine possible abnormalities as well as size and weight of placenta and ovaries.

The results of growth, mating, gestation, milking periods, reproduction and life span were not different between the GM Bt11 and non-Bt fed groups. The percentage of embryonic death, litter size, newborn sex ratio and body weight (21-60 days after birth) were not different between these groups. The life span of the third-generation mice did not differ over 1,072 days of observation. In addition, there was a tendency for a weight decrease among each group as the generations progressed, but there was no significant difference in performance among each group in each generation of mice. There was no relevant difference in growth, reproduction performance and life span between the GM and control groups. (Haryu et al. (2009).

Short term and multigenerational mouse studies.
A short term and a multigenerational study were performed on mice (Brake et al. 2004). *Bt* maize (Bt11 (38PO6)) was studied with mouse testes as a sensitive biomonitor of potential toxic effects. Pregnant mice were fed a Bt maize (38PO6) or a non-transgenic (conventional maize 38PO5) diet during gestation and lactation.

Short term study.
Bt or conventional maize diets were fed to 20 randomly selected female mice (10 for each diet). Following breeding, gestation, and parturition, three male progeny of the same age (by day) were chosen at random for each of six time points, 8, 16, 26, 32, 63, and 87 days after birth. Time points were selected to show the various stages of cellular proliferation and differentiation that occur during the development of the testes and ongoing cycles of spermatogenesis. The relative percent of testicular cell populations reflects the kinetics of the developing testes.

After they were weaned, young male mice were maintained on the respective diets. At 8, 16, 26, 32, 63, and 87 days after birth, three male mice and an adult reference mouse were killed, the testes were surgically removed, and the percentage of germ cell populations was measured by flow cytometry. No differences between conventional and Bt maize-fed mice were seen at 87 days after birth as ongoing cycles of spermatogenesis ensued.

General health parameters of mice fed the Bt maize diet showed no differences in body weight through day 63 ($p < 0.1074$) as compared to those on the conventional maize diet suggesting that the Bt maize diet caused no negative impact on body growth. However, the 87 day old conventional diet mice were fatter. However, the 63 day old mice were the exact opposite (Bt maize diet weighed more), and this was attributed to individual eating behavior after adulthood is achieved. Average litter sizes in the two groups were similar (Bt = 7.2 ± 1.0, n = 9; conventional = 7.3 ± 1.0, n = 9).

Multigenerational studies.
Multigenerational studies were conducted in the same manner. Transgenic or conventional diets were fed to 16 randomly selected male and female mice (two of each sex and strain for each diet). Following breeding, gestation, parturition, and weaning, six female and three male 2nd generation progeny of each strain and diet were randomly selected for growth, development, and breeding to obtain the 3rd generation. Surplus mice from each generation were culled. When all 3rd generation mice were at least 6 weeks old, six C57Bl/6J females and three C3H/HeJ males were randomly chosen and bred to obtain 4th generation cross-bred progeny. Three male progeny of the same age (by day) were chosen at random for each of five time points. Time points and sampling followed the same procedure as the subchronic study, except that the extended adult time point at 87 days postpartum was deleted.

General Health Parameters. Mean body weights showed differences between conventional and transgenic maize-fed mice at 26 ($p < 0.0001$) and 63 ($p < 0.0100$) days (Figure 3B). At both time periods, the Bt maize-fed mice were heavier than the control mice. Mean body weight results suggest that ingestion of a Bt maize diet resulted in no multigenerational impacts on animal growth. Average litter sizes were comparable (Bt = 8.2 ± 1.9, n = 5; conventional = 6.6 ± 2.1, n = 5).

There were no apparent differences in percentages of testicular cell populations (haploid, diploid, and tetraploid) between the mice fed the Bt maize diet and those fed the conventional diet. Because of the high rate of cell proliferation and extensive differentiation that makes testicular germ cells highly susceptible to some toxic agents, it was concluded that the Bt maize diet had no measurable or observable effect on fetal, postnatal, pubertal, or adult testicular development. If data from this study were extrapolated to humans, Bt maize is not harmful to human reproductive development.

Nakajima et al. (2007) characterised the IgE antibodies of human sera by enzyme-linked immunosorbent assay (ELISA) and immunoblotting. For the ELISA assay, a soluble form of the Cry1Ab protein, purified from *E. coli* transformed with a DNA sequence from maize Bt11, was used as antigen. All tested sera from patients allergic to major food allergens did not contain IgE antibodies directed against Cry1Ab.

Nakasuji et al. (2008) monitored the presence of common enterobacterial genes, a ubiquitous plant chloroplast gene, maize intrinsic zein (*Ze1*) and recombinant *cry1Ab* gene in the gastrointestinal contents, peripheral blood mononuclear cells and visceral organs of mice and their progeny fed with maize Bt11. The enterobacterial and chloroplast genes were detected inconsistently in peripheral blood mononclear cells, visceral organs, milk or liver of suckling mice, while *Ze1* and *cry1Ab* were not detected. These results suggest that feed-derived enterobacterial and plant DNAs were incompletely degraded in the gastrointestinal tract, and part of them were absorbed into organs or milk as a source of transfer into suckling mice, but the *cry1Ab* gene was not transferred.

14-day feeding study in high producing dairy cows
Three groups of 4 dairy cows were fed fresh chopped whole plant maize (ca. 22.7 kg of dry matter per animal and per day) of either Bt11 maize, another insect tolerant transgenic maize (Bt 176) and the non-transgenic, near isogenic counterpart of Event 176. Both Bt11 maize and Bt 176 have been modified with the Cry1Ab and PAT proteins. Bt 176 derived from plants contained intermediate levels, and plants from the Bt11 maize variety contained relatively high levels of Cry1Ab protein. While the aim of this study was to determine whether transfer of Cry1Ab and PAT to milk from cows fed transgenic maize would occur, it also provides information on animal performance. Milk production, feed intake, milk composition, and udder health were similar for all study groups. Cry1Ab and PAT proteins could not be detected in milk of cows fed the genetically modified maize lines.

Cry1Ab protein and DNA fragments of the cry1Ab gene in the gastrointestinal content of animals fed Bt11 maize.
Chowdhury et al. (2003a) examined the presence of maize intrinsic and recombinant *cry1Ab* gene by PCR, and the Cry1Ab protein by immunological tests in the gastrointestinal contents of five genetically modified maize Bt11-fed and five non-genetically modified maize-fed pigs.

Fragments of maize zein (242 bp), invertase (226 bp) and of ribulose-1,5-bisphosphate carboxylase/ oxygenase genes (1,028 bp) were detected in the gastrointestinal contents of both Bt11 and nongenetically modified maize-fed pigs. Fragments of recombinant cry1Ab gene (110 bp and 437 bp) were detected in the gastrointestinal contents of the Bt11-fed pigs but not in the control pigs. Neither maize intrinsic nor cry1Ab gene fragments were detected in the peripheral blood by PCR. The gastrointestinal contents were positive for Cry1Ab protein by ELISA, immune chromatography, and immunoblot; however, these methods did not work for blood and precluded conclusions about any potential absorption of the protein. These results suggest that ingested maize DNA and Cry1Ab protein were not totally degraded in the gastrointestinal tract, as shown by their presence in a form detectable by PCR or immunological tests.

Chowdhury et al (2003b) examined the fate of insecticidal Cry1Ab protein in the gastrointestinal (GI) contents and visceral organs of calves fed insect-resistant genetically modified maize Bt11. Twelve cross-breed (Japanese black x Holstein) calves were fed either Bt11 or non-genetically modified isoline maize for 90 days. Peripheral blood, rumen juice and feces were collected fortnightly, and GI contents and visceral organs were collected at slaughter at the end of the experiment. Samples were checked for Cry1Ab protein by immunological methods, and visceral organs were examined pathologically. Trace amounts of Cry1Ab protein were detected in the GI contents but not in the liver, spleen, kidney, muscle or mesenteric lymph nodes. No lesions were observed pathologically. Cry1Ab protein in the feces was degraded quickly at atmospheric temperature. These results suggested that only a trace amount of Cry1Ab protein survived passage through the GI tract but was not transferred to liver, spleen, kidney, lymph nodes or muscles.

Chowdhury et al (2004) examined the presence of maize intrinsic and recombinant cry1Ab genes in the gastrointestinal (GI) contents, peripheral blood mononuclear cells (PBMC), and visceral organs of

calves fed genetically modified Bt11 maize.during a subchronic 90-day performance study. Samples were collected from six Japanese Black/Holstein calves fed Bt11 maize and from six calves fed non-Bt maize. DNA-fragments of maize *zein (Ze1)*, *invertase*, *chloroplast*, and *cry1Ab* measured by PCR were detected inconsistently in the rumen fluid and rectal contents 5 and 18 h after feeding. The chloroplast DNA fragments of *ribulose-1,5-bisphosphate carboxylase/oxygenase* and tRNA were detected inconsistently in the PBMC, the visceral organs, and the longissimus muscle, while the *cry1Ab* gene was never detected in PBMC or in the visceral organs. These results suggest that feed-derived maize DNA was mostly degraded in the GI tract but that fragmented DNA was detectable in the GI contents as a possible source of transfer to calf tissues. These results also suggest that the recombinant *cry1Ab* genes were not transferred to the PBMC and tissues of calves fed Bt11 maize.

The applicant has provided several animal studies; some of these are taken into account in the evaluation. However, other studies are either performed with only dose or with too short exposure time.

4.5 Allergenicity assessment

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

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

4.5.1 Assessment of IgE mediated allergenicity of the newly expressed protein

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

These assessments have previously been described by the applicant for Cry1Ab and PAT, and were based on the following aspects:

Cry1Ab and PAT

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

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

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

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

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

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

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

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

4.5.2 Assessment of the IgE-mediated allergenicity of the whole GM plant

Allergenicity of the maize Bt11 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 Bt11 or sweet maize Bt11with the exception of the introduced traits, no increased allergenicity is anticipated for maizeBt11. Moreover, maize is not considered a common allergenic food.

4.5.3 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 Bt11 would be unlikely to alter the overall allergenicity of the whole plant or the allergy risk for consumers.

4.5.4 Adjuvanticity

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

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

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

"Bystander sensitisation"
"Bystander sensitisation" can occur when an adjuvant in food, or an immune response against a food antigen, results in an increased permeability of the intestinal epithelium for other components in food. Traditionally it was assumed that the epithelial cells of the intestine were permanently "glued together" by the so-called "tight junctions". Studies have however shown that these complex protein structures are dynamic and that they can be opened up by different stimuli.
Both *in vitro* and *in vivo* experiments have demonstrated that when an IgG response which can result in a complement activation (among other) is not balanced by an IgA response, the epithelial barrier can be opened and unwanted proteins are able to enter the body (bystander-penetration) and lead to allergic sensitisation (Brandtzaeg & Tolo 1977; Lim & Rowley 1982).

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

4.6 Nutritional assessment of GM food/feed

Compositional analyses of maize Bt11 and sweet maize Bt11 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 Bt11 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.

4.6.1 Intake information/exposure assessment

Net import of maize staple, e.g. flour, starch and mixed products, in Norway in 2007 was 7600 tons, corresponding to 4.4 g dry weight/person/day or an estimated daily energy intake for adults to be 0.6 % (Vikse 2009). The estimated median daily intake of sweet maize is 3.25 g/day, with a 97,5 % percentile of 17.5 g/day. The production of maize porridge for children in 2007 was about 37.5 tons, corresponding to a daily intake of 1.7 g/day or an estimated daily energy intake to be 0.6 % for a 6 month child (Vikse 2009).

The comparable composition and nutritional value of the maize, together with the results of the assessment of dietary intake and nutritional impact, indicate that food products derived from Bt11 maize are nutritionally equivalent to food products derived from commercial maize. Hence, anticipated dietary intake is not expected to change.

Since most foods and foodstuffs from maize are derived from field maize grains, an estimated maximum daily intake for a Norwegian adult of Cry1Ab protein from maize staple is calculated to be 8.8 µg, based on grain dry weight, and the estimated maximum daily intake of Cry1Ab from sweet maize is calculated to be 67 µg, based on a daily intake of 17.5 g fresh sweet maize/day (97.5 % percentile) These levels are several orders of magnitude below the levels shown to have no effect in laboratory toxicology testing. Also, these levels are considerably below the proposed threshold of toxicological concern (TTC) level of 1800 µg/person/day (Class 1, oral exposure) for chemicals considered to have a low potential for toxicity based on metabolism and mechanistic data (Vermeire et al., 2010). Transgenic proteins produced by genetically modified plants are generally 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 Bt11 may be higher for these animals.

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

4.6.2 Nutritional assessment of feed derived from the GM plant

Utilization of Bt maize residue and maize silage for growing beef steers and dairy cows.
a) Utilization of Bt maize residues by grazing beef steers and Bt maize silage and grain by growing beef cattle and lactating dairy cows has been reported by Folmer et al. (2002). Sixteen lactating dairy cows received diets containing silage of an early- and late-maturing variety of Bt11 maize or a control with the corresponding non-transgenic near isogenic maize line during 21-day feeding periods. No differences were observed between Bt11 maize and control maize for feed intake, body weight, milk production, and milk composition (lactose, protein, fat), as well as ruminal pH and volatile fatty acids. In addition no effects either were observed of the transgenic trait on in situ ruminal digestion of neutral detergent fibre of maize.

b) The same silages as those used for the dairy cow study were used in a beef cattle study which lasted for 101 days. Measurements included feed intake and body weight. Dry matter intake was significantly higher in steers fed early- and late-maturing Bt11 maize when compared with those fed diets containing non-GM silage. In addition, average daily weight gain in early maturing Bt11 maize-fed steers was higher than in control-fed steers, while final body weight and feed efficiency was decreased in steers fed late maturing Bt11 maize compared with steers fed control maize. In conclusion, the slightly higher dry matter intake was not associated with other effects on performance of beef cattle fed Bt11 maize that would be consistent for diets of both Bt11 maize lines. (see Appendix 8.4).

According to the applicant, the maize and derived feed products are substantially equivalent to, nutritionally equivalent to and as safe as commercial maize and derived feed products. This is based on the compositional analyses comprising proximates, minerals, fatty acids, amino acids, vitamins, secondary metabolites and anti-nutrients of forage and grain samples from; nutritional equivalence shown in a poultry feeding study; and, safety evaluation of the Cry1Ab and PAT proteins expressed in maize.

4.7 Conclusion

Whole food feeding studies have not indicated any adverse health effects of maize Bt11. These studies also indicate that maize Bt11 is nutritionally equivalent to conventional maize. The Cry1Ab and PAT proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they

been reported to cause IgE mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that maize Bt11 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1Ab and PAT proteins will introduce a toxic or allergenic potential in food or feed based on maize Bt11 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 grain 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 grain and seedlings do not survive the winter cold (Gruber et al. 2008). Observations made on cobs, cob fragments or isolated grains shed in the field during harvesting indicate that grains may survive and overwinter in some regions in Europe, resulting in volunteers in subsequent crops. The occurrence of maize volunteers has been reported in Spain and other European regions (e.g. Gruber et al. 2008). However, maize volunteers have been shown to grow weakly and flower synchronously with the maize crop (Palaudelmás et al. 2009). Cross-pollination values recorded were extremely variable among volunteers, most probably due to the loss of hybrid vigour and uniformity. Overall cross-pollination to adjacent plants was estimated as being low.

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

It is considered very unlikely that the establishment, spread and survival of maize Bt11 would be increased due to the insect resistance and herbicide tolerance traits. The herbicide tolerant trait can only be regarded as providing a selective advantage for the GM maize plant where and when glufosinate ammonium-based herbicides are applied. Glufosinate ammonium-containing herbicides have been withdrawn from the Norwegian market since 2008, and the substance will be phased out in the EU in 2017 for reasons of reproductive toxicity. Similarly insect resistance against certain lepidopteran pests provides a potential advantage in cultivation of Bt11 under infestation conditions. It is considered very unlikely that maize Bt11 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.

Due to the insufficient data provided by the notifier, no conclusions can be made on the agronomic behavior and characteristics of the GM maize Bt11 as well as the related phenotypic characteristics such as reproduction, dissemination and survivability. There are, however, no indications of altered fitness of maize Bt11 relative to its conventional counterpart and the VKM GMO Panel is not aware of any scientific reports indicative of increased establishment or spread of maize Bt11, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general

characteristics of maize Bt11 are unchanged, insect resistance, glufosinate tolerance are not likely to provide a selective advantage outside of cultivation in Europe. The VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects based on establishment and survival of maize Bt11 will not differ from that of conventional maize varieties.

5.2 Potential for gene transfer

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

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

5.2.1 Plant to micro-organisms gene transfer

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

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

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

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

In conclusion, the VKM GMO Panel consider it is unlikely that the introduced gene from maize Bt11 will transfer and establish in the genome of microorganisms in the environment or in the intestinal

tract of humans or animals. In the rare, but theoretically possible case of transfer of the *cry1Ab* and *pat* genes from Bt11 to soil bacteria, no novel property would be introduced into or expressed in the soil microbial communities; as these genes are already present in other bacteria in soil. Therefore, no positive selective advantage that would not have been conferred by natural gene transfer between bacteria is expected.

5.2.2 Plant to plant gene flow

Considering the intended uses of maize Bt11 (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 accifieldal grain spillage during transport and/or processing.

The extent of cross-pollination to other maize cultivars will mainly depend on the scale of accifieldal 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 accifieldal release during transportation and processing is however unlikely to disperse significant amounts of GM maize pollen to other maize plants. Field observations performed on maize volunteers after GM maize cultivation in Spain revealed that maize volunteers had a low vigour, rarely had cobs and produced pollen that cross-pollinated neighbour plants only at low levels (Palaudelmás et al. 2009).

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

5.3 Interactions between the GM plant and target organisms

Genetically modified maize Bt11 was developed to provide protection against a variety of target pests of the order Lepidoptera. Protection is achieved through expression in the plant of insecticidal Cry protein Cry1Ab, derived from *Bacillus thuringiensis*, a common soil bacterium. Cry1Ab, encoded by the *cry1Ab* gene, is derived from *B. thuringiensis* subspecies *kurstaki*. Two Lepidoptera pests are primarily targeted by Bt11; *Ostrinia nubilalis* (European corn borer, ECB) and *Sesamia nonagrioides* (Mediterranean corn borer, MCB).

The European corn borer is widely distributed in Europe covering the Iberian Peninsula, Czech Republic and Slovakia, southwest of France, northern Italy and the southern regions of Germany and Poland. The Mediterranean corn borer is present in the Mediterranean region (Andreadis 2011). There are ten reports of *O. nubilalis* in Norway, restricted to the counties of Vestfold, Telemark, Aust-Agder and Vest Agder. *Sesamia* spp. has not been reported in Norway. There are no reports of *O. nubilalis*

attaining pest status in Norway, and the Plant Clinic (Planteklinikken) at Bioforsk has never received samples of this pest or plant material damaged by this pest (K. Ørstad pers. com.). Consequently, there are no insecticides authorised or previous applications for registrations of insecticides against this herbivore in Norway.

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

Considering the intended uses of maize Bt11, excluding cultivation, the environmental exposure is limited to exposure through manure and faeces from the gastrointestinal tract mainly of animals fed on the GM maize as well as to the accifieldal release into the environment of GM seeds during transportation and processing and subsequently to potential occurrence of sporadic feral plants. Thus the level of exposure of target organisms to the Cry1Ab protein is likely to be extremely low and of no ecological relevance.

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

Considering the intended uses of maize stack Bt11, excluding cultivation, the environmental risk assessment is concerned with accifieldal release of GM maize viable grains into the environment during transportation and processing, and exposure through manure and faeces from the gastrointestinal tracts of animals fed the GM maize.

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

Data supplied by the applicant indicate that a limited amount of the Cry1Ab protein enters the environment due to expression in the grains (mean value of 2.0 µg/g d.w). In addition, the data show that at least 99% of microbially produced Cry1Ab1 protein was rapidly degraded in simulated gastric fluid.

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

5.5 Potential interactions with the abiotic environment and biochemical cycles

Considering the intended uses of maize Bt11, 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 ifieldify 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 ifieldify 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 Bt11 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/RX/Bt11 includes import and processing of maize Bt11 for food and feed uses. Considering the intended uses of maize Bt11, excluding cultivation, the environmental risk assessment is concerned with accidential release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize Bt11.

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

6 Data gaps

Adjuvanticity

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

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

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

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

Herbicide residue levels

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

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

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

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

7 Conclusions

Molecular characterisation

The molecular characterisation data indicate that a single copy of the transgenic insert with the *cry1Ab* and *pat* genes is integrated in the nuclear genome of maize Bt11, and that it is 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 Bt11 as adequate.

Comparative assessment

Comparative analyses of data from field trials located at representative sites and environments in North America and Europe indicate that maize Bt11 is compositionally equivalent to its conventional counterpart, with the exception of the herbicide tolerance and insect resistance traits, conferred by the expression of the Cry1Ab and PAT proteins. However, data on the amino acid tryptophan, is only given in one out of six studies. Based on current knowledge, the VKM GMO panel concludes that maize Bt11 is compositionally equivalent to conventional maize.

The data provided by the applicant are not sufficient to show that Bt11 maize is phenotypically and agronomically equivalent to conventional near-isogenic maize lines. The agronomic assessment data are provided from one growing season in the North America and one growing season in France. This is not considered to be sufficient for representative testing of agricultural environments.

Food and feed risk assessment

Whole food feeding studies have not indicated any adverse health effects of maize Bt11. These studies also indicate that maize Bt11 is nutritionally equivalent to conventional maize. The Cry1Ab and PAT proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

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

Environmental risk assessment

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

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

Overall conclusion

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

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

References

ANZFA (2000) Draft risk analysis report. Application A386. Food derived from insect-protected, herbicide-tolerant Bt-11 maize. Report from the Australia New Zealand Food Authority 4 October 2000. 07/01. 74 p.

Andreadis S (2011) Origin and taxonomic status of the Palearctic population of the stem borer *Sesamia nonagiodes* (Lefèbvre) Lepidoptera: Noctuidae. Biological Journal of the Linnean Society 103: 904–922

BEETLE report (2009). Long term effects of genetically modified (GM) crops on health and the environment (including biodiversity): prioritization of potential risks and delimitation of uncertainties. German Federal Office of Consumer Protection and Food Safety, BLaU-Umweltstudien and Genius GmbH. http://ec.europa.eu/environment/biotechnology/pdf/beetle_report.pdf

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

Betz FS, Hammond BG, Fuchs RL (2000) Safety and advantages of Bacillus thuringiensis-protected plants to control insect pests. Regul. Toxicol. Pharmacol. 32: 156–173

Bondzio A, Lodemann U, Weise C, Einspanier R (2013) Cry1Ab treatment has no effects on viability of cultured porcine intestinal cells, but triggers Hsp70 expression. PLoS ONE 8(7): e67079. doi:10.1371/journal.pone.0067079

Brake J, Faust MA, Stein J (2003a) Evaluation of Transgenic Event Bt11 Hybrid Maize in Broiler Chickens. North Carolina and Iowa State University. Poultry Science 82:551–559

Brake DG, Thaler R, Evenson, DP, (2004) Evaluation of Bt (*Bacillus thuringiensis*) maize on mouse testicular development by dual parameter flow cytometry. J. Agric. Food Chem. 52: 2097–2102

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

Chowdhury EH, Kuribara H, Hino A, Sultana P, Mikami O, Shimada N et al. (2003b) Detection of maize intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified maize Bt11. J. Anim. Sci. 81: 2546–2551

Chowdhury EH, Shimada N, Murata H, Mikami O, Sultana P, Miyazaki S et al. (2003c) Detection of Cry1Ab protein in gastrointestinal contents but not visceral organs of genetically modified Bt11-fed calves. Vet. Hum. Toxicol. 45: 72–75

Chowdhury EH, Mikami O, Murata H, Sultana, P., Shimada, N., Yoshioka, M et al. (2004) Fate of maize intrinsic and recombinant genes in calves fed genetically modified maize Bt11. J. Food Protect.

de Vries J, Wackernagel W (2002) Integration of foreign DNA during natural transformation of Acinetobacter sp. by homology-facilitated illegitimate recombination. The Proceedings of the National Academy of Sciences USA 99: 2094-2099

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

Devos Y, Demont M, Dillen K, Reheul D, Kaiser M, Sanvido O (2009) The coexistence of genetically modified (GM) and non-GM crops in the European Union. Agronomy for Sustainable Development 29: 11-30

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

Eastham K, Sweet J (2002) Genetically modified organisms (GMO): The significance of gene flow through pollen transfer. Environmental issue report. No 28. European Environment Agency (EEA), Copenhagen. http://reports.eea.eu.int/environmental_issue_report_2002_28/en

EFSA (2004) Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. The EFSA Journal, 48, 1-18. http://www.efsa.europa.eu/en/science/gmo/gmo_opinions/384.html.

EFSA (2006) Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. ISBN: 92-9199-019-1. European Food Safety Authority, Parma, Italy. 100 p. http://www.efsa.europa.eu/en/science/gmo/gmo_guidance/660.html

EFSA (2008) Scientific Opinion of the Panel on Genetically Modified Organisms on application (Reference EFSA-GMO-UK-2005-20) for the placing on the market of the insect-resistant and herbicide-tolerant genetically modified maize Bt11, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred International. The EFSA Journal 874,1-34.

EFSA (2009a) Use of antibiotic resistance genes as marker genes in genetically modified plants. Scientific Opinion of the Panel on Genetically Modified Organisms (GMO) and the Panel on Biological Hazards (BIOHAZ). The EFSA Journal 1034: 1-82 http://www.efsa.europa.eu/cs/BlobServer/Statement/gmo_biohaz_st_ej1108_ConsolidatedARG_en.pdf?ssbinary=true

EFSA (2009b) Application (Reference EFSA-GMO-NL-2005-15) for the placing on the market of the insect-resistant and herbicide-tolerant genetically modified maize Bt11, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Mycogen Seeds, c/o Dow AgroSciences LLC and Pioneer Hi-Bred International, Inc. as represented by Pioneer Overseas Corporation. The EFSA Journal 1074: 1-28 http://www.efsa.europa.eu/en/efsajournal/pub/1074.htm

EFSA (2010) Guidance on the environmental risk assessment of genetically modified plants. Scientific option from the EFSA Panel on Genetically Modified Organisms (GMO). The EFSA Journal 8 (11):1-111 http://www.efsa.europa.eu/en/efsajournal/doc/1879.pdf

EFSA (2011a) Guidance for risk assessment of food and feed from genetically modified plants. The EFSA Journal 9(5): 2150. http://www.efsa.europa.eu/en/efsajournal/doc/2150.pdf

EFSA (2011b) EFSA Panel on Genetically Modified Organisms (GMO). Scientific Opinion on Guidance on selection of comparators for the risk assessment of genetically modified plants and derived food and feed. EFSA Journal 9(5):2149

EFSA (2011c) Guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. The EFSA Journal 9(8):2316

EFSA (2013) Scientific Opinion on an application from Pioneer Hi-Bred International and Dow AgroSciences LLC (EFSA-GMO-NL-2005-23) for placing on the market of genetically modified maize 59122 for food and feed uses, import, processing and cultivation under Regulation (EC) No 1829/20031. The EFSA Journal 11: 3135 http://www.efsa.europa.eu/en/search/doc/3135.pdf

Folmer JD, Grant RJ, Milton CT, Beck J (2002) Utilization of Bt maize residues by grazing beef steers and Bt maize silage and grain by growing beef cattle and lactating dairy cows. J. Anim. Sci. 80:1352–1361

Gruber S, Colbach N, Barbottin A, Pekrun C (2008) Post-harvest gene escape and approaches for minimizing it. Cab reviews: Perspective in Agriculture, Veterinary Science, Nutrition and Natural Resources, 3, No. 015, 17 pp.

Guertler P, Lutz B, Kuehn R, Meyer HHD, Einspanier R, Killermann B, Albrecht C (2008) Fate of recombinant DNA and Cry1Ab protein after ingestion and dispersal of genetically modified maize in comparison to rapeseed by fallow deer (*Dama dama*). European Journal of Wildlife Research 54: 36–43.

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

Hammond BG, Dudek R, Lemen JK, Nemeth MA (2006) Results of a 90-day safety assurance study with rats fed grain from maize borer-protected maize. Food and Chemical Toxicology 44(7): 1092-1099

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

Haryu Y, Taguchi Y, Itakura E, Mikami O, Miura K, Saeki T, Nakajima Y (2009) Longterm biosafety assessment of a genetically modified (GM) plant: the genetically modified (GM) insect-resistant Bt11 maize does not affect the performance of multi-generations or life span of mice. Open Plant Science Journal 3, 49 – 53.

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

Jung HG, Sheaffer CC (2004) Influence of *Bt* transgenes on cell wall lignification and digestibility of maize stover for silage. Crop Science 44:1781-1789.

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

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

Lutz B, Wiedermann S, Einspanier R, Mayer J, Albrecht C (2005) Degradation of Cry1Ab protein from genetically modified maize in the bovine gastrointestinal tract. Journal of Agricultural and Food Chemistry 53: 1453–1456

Mesnage R, Clair E, Gress S, Then C, Székács A, Séralini G-E (2012) Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. J. Appl. Toxicol. 33(7): 695-699

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

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

Netherwood T, Martín-Orúe SM, O'Donnell AG, Gockling S, Graham J, Mathers JC, Gilbert HJ (2004) Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. Nature Biotechnology 22: 204-209

Nielsen KM, van Elsas JD, Smalla K. (2000) Transformation of *Acinetobacter* sp. 13(pFG4delta*nptII*) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. Applied Environmental Microbiology 66: 1237-42

Nielsen KM (2003) An assessment of factors affecting the likelihood of horizontal transfer of recombinant plant DNA to bacterial recipients in the soil and rhizosphere. Collection of Biosafety Reviews 1: 96-149

Nielsen KM, Townsend J P (2004) Monitoring and modeling horizontal gene transfer. Nature Biotechnology 22(9):1110-1114

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

OECD (2003) Consensus Document on the biology of *Zea mays* subsp. *Mays* (Maize). Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO, No. 27, 1-9

Onose JI, Imai T, Hasumura M, Ueda M, Ozeki Y, Hirose M (2008) Evaluation of subchronic toxicity of dietary administrated Cry1Ab protein from *Bacillus thuringiensis* var *kurustaki* HD-1 in F344 male rats with chemically induced gastrointestinal impairment. Food and Chemical Toxicology 46:2184-2189

Palaudelmás M, Peñas G, Melé E, Serra J, Salvia J, Pla, M-, Nadal A, Messeguer J (2009) Effect of volunteers on maize gene flow. Transgenic Research 18: 583-594

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

Rizzi AN, Raddadi C, Sorlini L, Nordgård L, Nielsen KM, Daffonchio D (2012) The stability and degradation of dietary DNA in the gastrointestinal tract of mammals - implications for

horizontal gene transfer and the biosafety of GMOs. Crit. Rev. Food Science Nutr. 52:142-161

Schubbert GW, Lettmann C, Doerfler W (1994) Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. Molecular & general Genetics 242: 495-504

SCP (2002) Opinion of the Scientific Committee on Food on a request to place genetically modified sweet maize line Bt11 on the market. SCF/CS/NF/DOS/14 ADD2 Final. http://ec.europa.eu/food/fs/sc/scf/out129_en.pdf

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

Shimada N, Miyamoto K, Kanda K, Murata H (2006a) *Bacillus thuringiensis* insecticidal Cry1Ab toxin does not affect the membrane integrity of the mammalian intestinal epithelial cells: an in vitro study. In vitro Cell. Dev. Biol. - Animal, 42 (1 & 2): 45-49

Shimada N, Miyamoto K, Kanda K, Murata H (2006b) Binding of Cry1Ab toxin, a *Bacillus thuringiensis* insecticidal toxin, to proteins of the bovine intestinal epithelial cell: An in vitro study. Appl. Entomol. Zool. 41(2): 295-301

Shimada, N., Murata, H., Mikami O, Yoshioka M, Guruge KS, Yamanaka N, Nakajima Y, Miyazaki S (2006c) Effects of feeding calves genetically modified maize Bt11: A clinic-biochemical study. J. Vet. Med. Sci. 68(10): 1113-1115

Shimada N, Murata H, Miyazaki S. (2008) Safety evaluation of Bt plants. JARQ 42(4): 251-259

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

van de Wiel CCM, van den Brink L, Bus CB, Riemens MM, Lotz LAP (2011) Crop volunteers and climatic change. Effects of future climatic change on the occurrence of maize, sugar beet and potato in the Netherlands. COGEM report (CGM 2011-11).

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

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

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

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

VKM (2005a) Foreløpig helserisikovurdering av genmodifisert maislinje Bt11 fra Syngenta Seeds. Uttalelse fra Faggruppe for genmodifiserte organismer 10.12. 2005 (05/324-endelig). Vitenskapskomiteen for mattrygghet, Oslo, Norge.

http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&Content_6504=6187:2032251::0:6271:10:::0:0

VKM (2005b) Report from an *Ad Hoc* Group appointed by the Norwegian Scientific Panel on Genetically Modified Organisms and Panel on Biological Hazards – An assessment on potentially long-term health effects caused by antibiotic resistance marker genes in genetically modified organisms based on antibiotic usage and resistance patterns in Norway. Opinion 05/302-1-final. Norwegian Scientific Committee for Food Safety, Oslo, Norway. 62 p.
http://www.vkm.no/eway/default.aspx?pid=277&trg=Content_6500&Main_6177=6500:0:31,2296&Content_6500=6187:1685412::0:6295:14:::0:0

VKM (2007a) Helserisikovurdering av genmodifisert åkermais Bt11 (C/UK/94/M3/1) fra Syngenta Seeds. Uttalelse fra Faggruppe for genmodifiserte organismer 9.2. 2007 (05/308-endelig). Vitensk apskomiteen for mattrygghet, Oslo, Norge.
http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&Content_6504=6187:2031919::0:6271:10:::0:0

VKM (2008) Foreløpig helse- og miljørisikovurdering genmodifisert maishybrid Bt11 x MIR604 x GA21 (EFSA/GMO/UK/2008/56). Uttalelse fra Faggruppe for genmodifiserte organismer 19.11.2008 (08/332). Vitenskapskomiteen for mattrygghet, Oslo, Norge.
http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&Content_6504=6187:2031671::0:6271:10:::0:0

VKM (2009a) Foreløpig helse- og miljørisikovurdering genmodifisert maishybrid Bt11 x GA21 fra Syngenta Seeds Inc. (EFSA/GMO/UK/2007/49). Uttalelse fra Faggruppe for genmodifiserte organismer 3.4.2009 (08/316). Vitenskapskomiteen for mattrygghet, Oslo, Norge.
http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&Content_6504=6187:2031701::0:6271:10:::0:0

VKM (2009b) Foreløpig helse- og miljørisikovurdering av genmodifisert maishybrid Bt11 x MIR604 (EFSA/GMO/UK/2007/50). Uttalelse fra Faggruppe for genmodifiserte organismer 3.4.2009 (08/317). Vitenskapskomiteen for mattrygghet, Oslo, Norge.
http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&Content_6504=6187:2031696::0:6271:10:::0:0

VKM (2009c) Foreløpig helse- og miljørisikovurdering av genmodifisert maishybrid MIR604 x GA21 (EFSA/GMO/UK/2007/48). Uttalelse fra Faggruppe for genmodifiserte organismer 17.4.2009 (08/319). Vitenskapskomiteen for mattrygghet, Oslo, Norge.
http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&Content_6504=6187:2031691::0:6271:10:::0:0

VKM (2009d) Foreløpig helse- og miljørisikovurdering av genmodifisert mais Bt11 x MIR162 x MIR604 x GA21 (EFSA/GMO/DE/2009/66). Uttalelse fra Faggruppe for genmodifiserte organismer 22.10.2009 (09/309). Vitenskapskomiteen for mattrygghet, Oslo, Norge
http://vkm.no/eway/default.aspx?pid=277&trg=Main_6177&Main_6177=6187:1694583::0:6735:4:::0:0

VKM (2009e) Foreløpig helse- og miljørisikovurdering av genmodifisert mais Bt11 x MIR162 x GA21 (EFSA/GMO/DE/2009/67) til mat, fôr, import og prosessering under forordning (EF) nr. 1829/2003. Uttalelse fra Faggruppe for genmodifiserte organismer 22.10.2009 (09/310). Vitenskapskomiteen for mattrygghet, Oslo, Norge.

EFSA/GMO/RX/Bt11 – Genetically modified maize Bt11

http://vkm.no/eway/default.aspx?pid=277&trg=Content_6508&Main_6177=6504:0:31,2365&Content_6504=6508:0:31,2625&Content_6508=6187:1694582::0:6336:23:::0:0

VKM (2012a) Førebels helse- og miljørisikovurdering av genmodifisert mais Bt11 x MIR162 x 1507 x GA21 (EFSA/GMO/DE/2010/86). Uttalelse fra Faggruppe for genmodifiserte organismer 14.6.2012 (12/311). Vitenskapskomiteen for mattrygghet, Oslo, Norge. http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&Content_6504=6187:1969240::0:6271:7:::0:0

VKM (2012b) Førebels helse- og miljørisikovurdering av genmodifisert mais Bt11 x 59122 x MIR604 x 1507 x GA21 (EFSA/GMO/DE/2011/99). Uttalelse fra Faggruppe for genmodifiserte organismer 14.6.2012 (12/312). Vitenskapskomiteen for mattrygghet, Oslo, Norge. http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&Content_6504=6187:1969184::0:6271:6:::0:0

VKM (2012c) Helserisikovurdering av Cry-proteiner adjuvanseffekt. Uttalelse fra Faggruppe for genmodifiserte organismer 25.4.2012 -11/313/3-endelig. Vitenskapskomiteen for mattrygghet, Oslo, Norge. http://www.english.vkm.no/eway/default.aspx?pid=278&trg=Content_6444&Main_6359=6582:0:31,2564&Content_6444=6393:1986886::0:6596:1:::0:0

VKM (2013a) Endelig miljørisikovurdering av genmodifisert mais Bt11 x MIR604 x GA21 (EFSA/GMO/UK/2008/56). Uttalelse fra Faggruppe for genmodifiserte organismer 19.3.2013 (13/311). Vitenskapskomiteen for mattrygghet, Oslo, Norge. http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&Content_6504=6187:2015173::0:6566:6:::0:0

VKM (2013b) Endelig miljørisikovurdering av genmodifisert mais Bt11 x MIR604 (EFSA/GMO/UK/2007/50). Uttalelse fra Faggruppe for genmodifiserte organismer 19.3.2013 (13/310). Vitenskapskomiteen for mattrygghet, Oslo, Norge. http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&Content_6504=6187:2015171::0:6566:5:::0:0

VKM (2013c) Endelig miljørisikovurdering av genmodifisert mais Bt11 x GA21 (EFSA/GMO/UK/2007/50). Uttalelse fra Faggruppe for genmodifiserte organismer 19.3.2013 (13/310). Vitenskapskomiteen for mattrygghet, Oslo, Norge. http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&Content_6504=6187:2015163::0:6566:3:::0:0

Appendix 1

Table 1. Summary of lines evaluated (ANFZA 2000).

Lines	Protein Expression	Proximate[2]	Fatty Acids	Amino Acids[3]	Vitamins & Minerals
Initial transformant (greenhouse data)					
H8540 Bt$^+$/Bt$^+$		+	+	+	
Control H8540		+	+	+	
H8540 Bt$^+$/Bt$^-$ hybrid		+	+	+	
Control hybrid		+	+	+	
Dent Corn					
N4640-CBR					+
X4734-CBR	+	+	+	+	
X4334-CBR	+	+			
N4242-CBR					+
N4640		+	+	+	+
NK4242	+	+			+
X6534-CBR	+	+	+	+	
X6514		+			
N6800			+	+	
X7634-CBR	+	+			
X7514	+	+			
Sweet Corn Varieties					
0943	+	+			+
Jubilee	+	+			+
0937	+	+			+
Bonus	+	+			+
0941	+	+			+
Empire	+	+			+
Herbicide treated plants					
Madera-Bt		+			+
Madera		+			+
Manuel-Bt		+			+
Manuel		+			+
Magister-Bt		+			+
Magister		+			+

[1]A "+" indicates the data that was provided for that line. Control lines are in italics and genetically modified corn lines are in bold and are denoted as CBR – corn borer resistant or Bt. Control lines are either corresponding isogenic non-GM lines or are of a similar genetic background.

[2]Proximate components analysed were: *Initial transformants*: Total nitrogen, moisture, ash, starch, cellulose, xanthophyll; *Dent corn*: protein, oil, starch and fibre; *Sweetcorn*: moisture, protein, fat, ash, carbohydrates (total), calories, calories from fat, sugars, other carbohydrates, total dietary fibre; *Treated*: energy, carbohydrate, protein, fat, fibre.

[3]Some analyses did not assess all amino acids.