Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Food/feed and environmental risk assessment of insect-resistant genetically modified maize MIR604 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (EFSA/GMO/UK/2005/11)

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

Date: 21 January 2014

Doc. no.: 13/335- final

ISBN: 978-82-8259-121-8

VKM Report 2014: 30

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Acknowledgements

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Summary

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

The insect-resistant genetically modified maize MIR604 from Syngenta Seeds S.A.S. (Unique Identifier SYN-IR604-5) is approved under Regulation (EC) No 1829/2003 for food and feed uses, import and processing since 30 November 2009 (Commission Decision 2009/866/EC).

Genetically modified maize MIR604 has previously been risk assessed by the VKM Panel on Genetically Modified Organisms (GMO), commissioned by the Norwegian Food Safety Authority and the Norwegian Environmental Agency related to the EFSAs public hearing of the applications EFSA/GMO/UK/2005/11 and EFSA/GMO/UK/2010/83 in 2005 (VKM 2005) and 2011 (VKM, unpublished. In addition MIR604 has been evaluated by the VKM GMO Panel as a component of several stacked GM maize events (VKM 2008, VKM 2009a,b,c VKM 2012, VKM 2013a,b,c).

The food/feed and environmental risk assessment of maize MIR604 is based on information provided by the applicant in the applications EFSA/GMO/UK/2005/11 and EFSA/GMO/UK/2010/83, 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 MIR604 with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed. The Norwegian Scientific Committee for Food Safety has also decided to take account of the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2011a), the environmental risk assessment of GM plants (EFSA 2010), selection of comparators for the risk assessment of GM plants (EFSA 2011b) and for the post-market environmental monitoring of GM plants (EFSA 2011c).

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

Genetically modified maize MIR604 was developed to provide protection against certain coleopteran target pests belonging to the genus *Diabrotica* such as the larvae of western corn rootworm (WCRW; *D. virgifera virgifera*), the northern corn rootworm (NCRW; *D. longicornis barberi*) by the introduction of a modified *cry3A* gene (*mcry3A*) derived from *Bacillus thuringiensis* subsp. *tenebrionis.* Maize MIR604 also contains the *pmi (manA*) gene from *Escherichia coli* which encodes the phosphomannose isomerise (PMI) protein as a selectable marker. PMI allows transformed maize cells to utilize mannose as a sole carbon source, while maize cells lacking the *pmi* gene fail to grow with mannose as single carbon source.

Molecular characterisation

The molecular characterisation data indicate that only one copy of the transgenic insert with the *mcry3A* and *pmi* genes is integrated in the genome of maize MIR604, and that it is stably inherited over generations. 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 MIR604 as adequate.

Comparative assessment

The applicant has performed comparative analyses of data from field trials located at representative sites and environments in North America during the 2002 and 2003 growing seasons. With the exception of small intermittent variations and the insect resistance conferred by mCry3A, the results showed no biologically significant differences between maize MIR604 and control maize. Based on the assessment of available data, the VKM GMO Panel concludes that maize MIR604 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, except for the newly expressed proteins.

Food and feed risk assessment

Whole food feeding studies on rats, rainbow trout and broilers have not indicated any adverse health effects of maize MIR604. These studies also indicate that maize MIR604 is nutritionally equivalent to conventional maize. The mCry3A and PMI proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

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

Environmental risk assessment

The scope of the application EFSA/GMO/UK/2005/11 includes import and processing of maize MIR604 for food and feed uses. Considering the intended uses of maize MIR604, excluding cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize MIR604.

Maize MIR604 has no altered survival, multiplication or dissemination characteristics, and there are no indications of an increased likelihood of spread and establishment of feral maize plants in the case of accidental release into the environment of seeds from maize MIR604. Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation. The VKM GMO Panel considers the risk of gene flow from occasional feral GM maize plants to conventional maize varieties to be negligible in Norway. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered by the GMO Panel to be an issue.

Overall conclusion

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

The VKM GMO Panel likewise concludes that maize MIR604, based on current knowledge, is comparable to conventional maize varieties concerning environmental risk in Norway with the intended usage.

Keywords

Maize, *Zea mays* L., genetically modified maize MIR604, EFSA/GMO/UK/2005/11, insect-resistance, Cry proteins, mCry3A, PMI, 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 maishybriden MIR604 (unik kode SYN-IR604-5) fra Syngenta Seeds S.A.S. ble godkjent til import, videreforedling og til bruk som mat og fôr under EU-forordning 1829/2003 i 2009 (søknad EFSA/GMO/UK/2005/11, Kommisjonsbeslutning 2009/866/EU).

Maishybriden har tidligere vært vurdert av VKMs faggruppe for genmodifiserte organismer med hensyn på mulig helserisiko i forbindelse med EFSAs offentlige høring av søknad EFSA/GMO/UK/2005/11 i 2005 (VKM 2005). En søknad om godkjenning av MIR604 til dyrking (EFSA/GMO/UK/2010/83) ble vurdert av VKM i 2011. VKM s faggruppe for GMO har også risikovurdert en rekke hybrider der MIR604 inngår som en av foreldrelinjene (VKM 2008, VKM 2009a,b,c VKM 2012, VKM 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.

Den genmodifiserte maislinjen MIR604 har fått satt inn et modifisert *cry3A*-gen (mcry3A) fra *Bacillius thuringiensis* subsp. *tenebrionis.* mCry3A er fremkommet ved endringer i basesekvensen til *cry3A*-genet, endringer som medfører et optimalt uttrykk i mais. mCry3A-proteinet gir plantene resistens mot angrep fra bladbiller i slekten *Diabrotica*, eksempelvis *D. virgifera virgifera* (’Western

Corn Rootworm), *D. barberi* ('Northern Corn Rootworm') og *D. virgifera zeae* ('Mexican Corn Rootworm'). Proteinet uttrykkes primært i røttene hos maisplantene.

MIR604 inneholder også *pmi (manA)*-genet fra *Escherichia coli,* som koder for enzymet PMI (fosfomannose isomerase). PMI gjør at transformerte celler kan benytte sukkerarten mannose som karbonkilde, noe som medfører at celler/vev som ikke har fått overført og inkorporert genkonstruktet kan selekteres. Genet er kun introdusert som markør for seleksjon av transformanter under regenerasjonen, og har ingen funksjon i det endelige produktet

Molekylær karakterisering
Data fra den molekylære karakteriseringen indikerer at de introduserte genene og egenskapene er intakt integrert i maisens genom og at disse er stabilt nedarvet over generasjoner. Passende bioinformatikk og sekvens -analyser er utført av integreringssete i plantens genom, og innsatt og flankerende DNA. Bioinformatikk- analysene har ikke avdekket potensielle nye åpne leserammer med sekvenslikhet til kjente toksiner eller allergener. Segresjonsanalyser for insektsresistens er i overenstemmelse med at det kun er integrert ett eksemplar av ekspresjonskassettene med de to genene i mais MIR604. VKMs faggruppe for genmodifiserte organismer vurderer den molekylære karakteriseringen av mais MIR604 som tilfredsstillende.

Komparative analyser
Feltforsøk over to vekstsesonger i USA i 2002 og 2003 viser små eller ingen signifikante forskjeller mellom den transgene maislinjen MIR604 og korresponderende, nær-isogene kontrollhybrider med hensyn på ernæringsmessige, morfologiske og agronomiske egenskaper, med unntak av insektsresistens. Resultatene viser ingen indikasjon på at de innsatte genene i MIR604 har medført utilsiktede endringer i egenskaper knyttet til vekst og utvikling hos maisplantene.

Helserisiko
Fôringsstudier utført på rotter, broiler og ørret har ikke indikert helseskadelige effekter av mais MIR604. Disse studiene indikerer også at mais MIR604 er ernæringsmessig vesentlig lik konvensjonell mais. Proteinene mCry3A og PMI viser ingen likhetstrekk til kjente toksiner eller allergener, og er heller ikke rapporterte å ha forårsaket IgE-medierte allergiske reaksjoner. Enkelte studier har derimot indikert at noen typer Cry-proteiner kan forsterke andre allergiske reaksjoner, dvs. fungere som adjuvans.

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at mais MIR604 er næringsmessig vesentlig lik konvensjonell mais, og at det er lite trolig at mCry3A - eller PMI proteinet vil introdusere et toksisk eller allergent potensiale i mat basert på mais MIR604 sammenliknet med konvensjonelle maissorter.

Miljørisiko
Søknad EFSA/GMO/UK/2005/11 gjelder godkjenning av mais MIR604 for import, prosessering og til bruk i næringsmidler og fôrvarer, og omfatter ikke dyrking. Med bakgrunn i tiltenkt bruksområde er miljørisikovurderingen avgrenset til mulige effekter av utilsiktet frøspredning i forbindelse med transport og prosessering, samt indirekte eksponering gjennom gjødsel fra husdyr fôret med genmodifisert mais.

Det er ingen indikasjoner på økt sannsynlighet for spredning, etablering og invasjon av maislinjen i naturlige habitater eller andre arealer utenfor jordbruksområder som resultat av frøspill i forbindelse med transport og prosessering. Risiko for utkryssing med dyrkede sorter vurderes av GMO panelet til

å være ubetydelig. Ved foreskreven bruk av maislinjen MIR604 antas det ikke å være risiko for utilsiktede effekter på målorganismer, ikke-målorganismer eller på abiotisk miljø i Norge.

Samlet vurdering
Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at mais MIR604 er ernæringsmessig, fenotypisk og agronomisk ekvivalent med konvensjonell mais. Det er lite trolig at mCry3A- eller PMI- proteinet vil introdusere et toksisk eller allergent potensiale i mat eller fôr basert på mais MIR604 sammenliknet med konvensjonelle maissorter.

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

Abbreviations and explanations

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
Cry	Crystal protein.
Cry3A	Cry protein from *Bacillus thuringiensis*. Sp..
mCry3A	Modified Cry3A protein optimized for maize
CTP	Chloroplast transit peptide
DAP	Days after planting
DN	Norwegian Directorate for Nature Management (Direktoratet for naturforvalting)
DNA	Deoxyribonucleic acid
DT50	Time to 50% dissipation of a protein in soil
DT90	Time to 90% dissipation of a protein in soil
dw	Dry weight
dwt	Dry weight tissue
EC	European Commission/Community
ECB	European corn borer, *Ostrinia nubilalis*
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	Environmental risk assessment
E-score	Expectation score
EU	European Union
fa	Fatty acid
FAO	Food and Agriculture Organisation
FIFRA	US EPA Federal Insecticide, Fungicide and Rodenticide Act

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

SAS	Statistical Analysis System
SD	Standard deviation
Southern blot	Method used for detection of DNA sequences in DNA samples. Combines transfer of electrophoresis-separated DNA fragments to a filter membrane and subsequent fragment detection by probe hybridisation
T-DNA	Transfer DNA, the transferred DNA of the tumour-inducing (Ti) plasmid of some species of bacteria such as *Agrobacterium tumefaciens* and *A. rhizogenes*. The bacterium transfers this DNA fragment into the host plant's nuclear genome. The T-DNA is bordered by 25-base-pair repeats on each end. Transfer is initiated at the left border and terminated at the right border and requires the *vir* genes of the Ti plasmid.
TI	Trait integration
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, Kernels are filled with clear fluid and the embryo can be seen R3: Milk stage. Kernels are filled with a white, milky fluid. R4: Dough stage. Kernels are filled with a white paste R5: Dent stage. If the genotype is a dent type, the grains are dented R6: Physiological maturity Seedling growth (stages VE and V1); Vegetative growth (stages V2, V3... Vn); Flowering and fertilization (stages VT, R0, and R1); Grain filling and maturity (stages R2 to R6)
Western blot	Analytical technique used to detect specific proteins in the given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native proteins by 3-D structure or denatured proteins by the length of the polypeptide. The proteins are then transferred to a membrane where they are stained with antibodies specific to the target protein.
WHO	World Health Organisation.
ZM	*Zea maize* L.
ZM-HRA	A modified version of the native acetolactate synthase protein from maize. Confers tolerance to the ALS-inhibiting class of herbicides

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Background

On 12 January 2005, the European Food Safety Authority (EFSA) received from the Competent Authority of United Kingdom an application (Reference EFSA/GMO/UK/2005/11) for authorisation of the insect-resistant genetically modified (GM) maize MIR604 (Unique Identifier SYN-IR6Ø4-5), submitted by Syngenta Seeds S.A.S. within the framework of Regulation (EC) No 1829/2003.

The scope of the application covers:

- Food
  - ✓ GM plants for food use
  - ✓ Food containing or consisting of GM plants
  - ✓ Food produced from GM plants or containing ingredients produced from GM plants
- Feed
  - ✓ GM plants for feed use
  - ✓ Feed containing or consisting of GM plants
  - ✓ Feed produced from GM plants

- GM plants for environmental release
  - ✓ Import and processing (Part C of Directive 2001/18/EC)

After receiving the application EFSA/GMO/NL/2005/20 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the EU- and EFTA Member States (MS) and the European Commission and made the summary of the dossier publicity available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of regulation (EC) No 1829/2003. On 16 September 2005, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the EC and consulted nominated risk assessment bodies of the MS, including the Competent Authorities within the meaning of Directive 2001/18/EC (EC 2001), following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1929/2003, to request their scientific opinion. Within three months following the date of validity, all MS could submit via the EFSA GMO Extranet to EFSA comments or questions on the valid application under assessment. The VKM GMO Panel assessed the application in connection with the EFSA official hearing, and submitted a preliminary opinion in December 2005 (VKM 2005). EFSA published its scientific opinion 2 July 2009 (EFSA 2009a), and maize MIR604 was approved for food and feed uses, import and processing in 30 November 2009 (Commission Decision 2009/866/EC).

An application for authorisation of maize MIR604 for cultivation in the EU was submitted by Syngenta Seeds in July 2010 (EFSA/GMO/UK/2010/83). VKM participated in the 90 days public consultation of the application in 2011. MIR604 has also been evaluated by the VKM GMO Panel as a component of several stacked GM events (VKM 2008, VKM 2009a,b,c VKM 2012, VKM 2013a,b,c).

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 MIR604 was developed to provide protection against certain coleopteran target pests belonging to the genus *Diabrotica* such as the larvae of western corn rootworm (WCRW; *D. virgifera virgifera*), the northern corn rootworm (NCRW; *D. longicornis barberi*) by the introduction of a modified *cry3A* gene (*mcry3A*) derived from *Bacillus thuringiensis* subsp. *tenebrionis.* Maize MIR604 also contains the *pmi (manA*) gene from *Escherichia coli* which encodes the phosphomannose isomerise (PMI) protein as a selectable marker. PMI allows transformed maize cells to utilize mannose as a sole carbon source, while maize cells lacking the *pmi* gene fail to grow with mannose as single carbon source.

The genetic modification in maize MIR604 is intended to improve agronomic performance only, and is not intended to influence the nutritional properties, the processing characteristics and the overall use of maize as a crop.

Maize MIR604 has been evaluated with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed.

The Norwegian Scientific Committee for Food Safety has also decided to take 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 MIR604 is based on information provided by the applicant in the applications EFSA/GMO/UK/2005/11 and EFSA/GMO/UK/2010/83, and scientific opinions and comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment is also based on a review and assessment of relevant peer-reviewed scientific literature.

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

2 Molecular characterisation

Breeding pedigree indicating the generations that were tested in the molecular analysis of MIR604 are depicted in Figure 1 in Appendix.

2.1 Information related to the genetic modification

2.1.1 Description of the methods used for the genetic modification

Maize MIR604 was produced by transforming immature maize embryos derived from a proprietary *Zea mays* line (A188) *via Agrobacterium*-mediated transformation, with the transformation vector pZM26. By this method, genetic elements within the left and right border regions of the transformation vector are transferred and integrated into the genome of the plant cell, while genetic elements outside these border regions are generally not.

2.1.2 Nature and source of vector used

Plasmid pZM26 is a binary vector that contains the *mcry3A* and *pmi* genes and regulatory elements transferred to the maize embryos to produce MIR604. Plasmid map of pZM26, components of the vector backbone and T-DNA genetic elements are shown in Figure 1, Table 1, Table 2 and Figure 2, respectively.

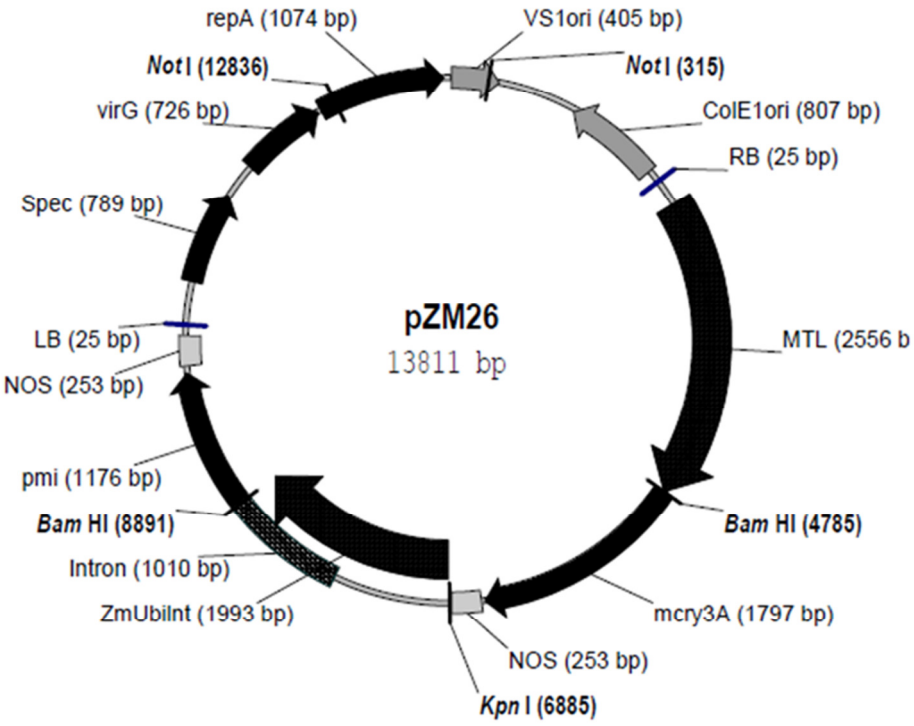

Figure 1. Plasmid map of pZM26

Table 1. Vector backbone components

Component	Size (bp)	Function and origin of the sequence
Spec	789	Streptomycin adenylyltransferase, *aadA* gene from *E. coli* Tn7. Confers resistance to erythromycin, streptomycin, and spectinomycin; used as a bacterial selectable marker.
VS1ori	405	Consensus sequence for the origin of replication and partitioning region from plasmid pVS1 of Pseudomonas. Serves as origin of replication in *Agrobacterium tumefaciens* host.
ColE1ori	807	Origin of replication that permits replication of plasmid in *E. coli*.
LB	25	Left border region of T-DNA from *Agrobacterium tumefaciens* nopaline ti-plasmid. Short direct repeat that flanks the T-DNA and is required for the transfer of the T-DNA into the plant cell.
RB	25	Right border region of T-DNA from *Agrobacterium tumefaciens* nopaline ti-plasmid. Short direct repeat that flanks the T-DNA and is required for the transfer of the T-DNA into the plant cell.
virG	726	VirGN54D from pAD1289. The N54D substitution results in a constitutive *virG* phenotype. VirG is part of the two-component regulatory system for the vir regulon in Agrobacterium.
repA	1074	pVS1 replication protein from Pseudomonas, which is a part of the minimal pVS1 replicon that is functional in gram-negative plant associated.

Table 2. T-DNA genetic elements

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

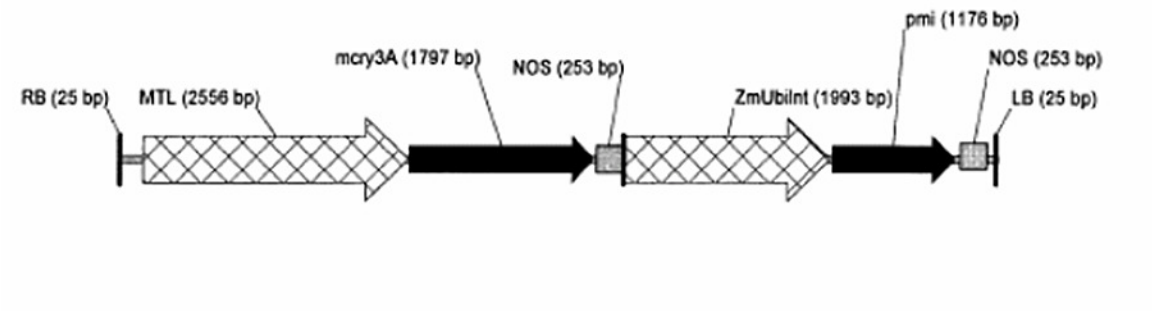

Figure 2. Genes and regulatory elements inserted in MIR604

2.2 Information relating to the GM plant

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

Maize MIR604 expresses the *mcry3A* gene, which is a modified version of the *cry3A* gene from *Bacillus thuringiensis*. The *mcry3A* gene encodes the mCry3A protein that confers resistance to the Western Corn rootworm (*Diabrotica virgifera virgifera*) and other related coleopteran pests of maize like the Northern Corn rootworm (*Diabrotica longicornis barberi*). The native *cry3A* gene was modified to incorporate a cathepsin-G serine protease recognition site within the expressed protein. The original N-terminal region of this protein has been removed and the mCry3A protein commences at a methionine residue in position 48 of the native protein. The entire coding region of the *mcry3A* gene was synthesised with codons that are preferred in maize. The amino acid sequence of the synthetic version of Cry3A is the same as the native protein, except for the modified serine-protease recognition site. The *mcry3A* gene is regulated by the promoter from the metallothionein-like gene from Zea mays, which is preferentially expressed in root tissue, and the nopaline synthase (NOS) terminator from *A. tumefaciens.*

MIR604 also expresses the *pmi* (*manA*) gene from *Escherichia coli*, that and encodes the enzyme phosphomannose isomerase (PMI). The gene was introduced as a selectable marker for the development of maize Mir604. Mannose is taken up by plants and converted to mannose-6-phosphate by hexokinase. Usually this product cannot be further utilised in plants as they lack the PMI enzyme. The accumulation of mannose-6-phosphate inhibits phosphoglucose isomerase, causing a block in glycolysis. It also depletes cells of orthophosphate required for the production of ATP. Therefore, while mannose has no direct toxicity on plant cells, it causes growth inhibition. This does not occur in plants transformed with the *pmi* gene as they can utilise mannose as a source of carbon. The *pmi* gene is regulated by the polyubiquitin promoter (ZmUbilnt) from Zea mays and the NOS terminator from *A. tumefaciens*.

2.2.2 Information on the sequences actually inserted or deleted

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

Southern blot analysis was used to determine the insert and copy number of the *mcry3A* and *pmi* genes and to verify absence of DNA sequence from outside the T-DNA borders of the transformation vector pZM26. Southern blot analyses of leaf tissue from plants in MIR604 backcross generation six (BC6) and negative segregants from BC4 indicate that maize MIR604 occurred as an integration of a single intact T-DNA from plasmid pZM26. Plasmid backbone DNA is not present in MIR604.

To further investigate the integrity of the inserted T-DNA, the entire insert was sequenced and compared to the DNA sequence of the plasmid pZM26. The results showed that a total of 8416 bp of T-DNA had become inserted into the maize genome. A 44bp segment was found to be missing from the Right border region, as well as 43bp at the Left border region. Three base pair changes were also observed within the MIR604 insert: one within the MTL promoter, and two within the *pmi* gene. These modifications have resulted in two amino acid changes: valine at position 61 has been substituted by alanine (V61A) and glutamine at position 210 has been substituted by histidine (Q210H). The first of these changes is a conservative one (both aliphatic amino acids) and the second

change results in the substitution of an acidic residue for a basic residue. These changes have not affected the function of the enzyme.

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

The entire T-DNA insert and the 5' and 3' flanking regions have been sequenced and analysed. According to the applicant the sequence analyses have shown that the overall integrity of the insert and the contiguousness of the functional elements in pZM26 are maintained.
The applicant has performed a BLAST analysis of the *Zea mays* genomic sequences flanking the T-DNA insert in MIR604 with publicly available nucleotide databases to determine if the T-DNA insertion occurred in a known functional gene of *Zea mays*. The genomic sequences flanking the T-DNA insert were also screened for novel open reading frames (ORF's) that may have occurred at the junction of the T-DNA insert and the genome of *Zea mays*. According to the applicant, the results obtained indicated that the insertion of the T-DNA had occurred in a region of the *Zea mays* genome that was not well annotated and that the MIR604 T-DNA insert did not appear to disrupt any identified endogenous *Zea mays* genes. ORF analyses of six potential reading frames at both the 5' and 3' T-DNA to genome junctions did not show the presence of any novel ORF's (application Appendix CBI.2, Hart 2004).

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

Only the 44bp and 43bp segments deleted at the Right and Left border region mentioned in 2.2.2.1.

2.2.2.4 Chromosomal location(s) of insert(s)

According to the applicant, the inheritance pattern of the T-DNA insert in maize MIR604 show that the insertion has taken place in the nucleus, and that the insert is stably integrated in the maize genome. Segregation analysis was performed on MIR604 plants from generation T5 (original transformant was out-crossed and progeny were selfed twice, out-crossed once and selfed again to produce the T5 generation). Individual plants from generation T5 were analysed for the presence of the mCry3A protein by enzyme-linked immuno-sorbent assay (ELISA) and both the *mcry3A* and *pmi* genes by PCR analysis. The expected and observed ratios of positive vs. negative plants were analysed by Chi square analysis to determine if the traits were segregating in a Mendelian fashion. The expected ratio was 3:1 positive to negative for the introduced traits. No significant difference was observed for either the ELISA assay or the PCR assay.

2.3 Information on the expression of the inserts

2.3.1 Parts of the plant where the insert is expressed

Levels of mCry3A and PMI proteins in maize plants derived from maize MIR604, were determined by ELISA at four growth stages (whorl, anthesis, seed maturity and senescence).

Levels of mCry3A protein were detected in all MIR604-derived plant tissues analysed except pollen. Across all growth stages, mean mCry3A levels measured in leaves, roots and whole plants ranged from 3 - 23 µg/g fresh wt. (4 - 94 µg/g dry wt.), 2 - 14 µg/g fresh wt. (7 - 62 µg/g dry wt.), and 0.9 - 11 µg/g fresh wt. (3 - 28 µg/g dry wt.), respectively. Mean mCry3A levels measured in grain at seed

maturity and senescence ranged from 0.6 – 1.4 µg/g fresh wt. (0.8 – 2.0 µg/g dry wt.). Mean mCry3A levels measured in silk tissue at anthesis were below the lower limit of quantification (LOQ), <0.1 µg/g fresh wt. (<1.0 µg/g dry wt.). Mean mCry3A levels measured in silk tissue at seed maturity ranged from 0.6 – 1.9 µg/g fresh wt. (1 – 3 µg/g dry wt.). No mCry3A protein was detectable in pollen.

PMI protein was detected in most of the maize MIR604 plant tissues, although at low levels. Across all plant stages, mean PMI levels measured in leaves, roots and whole plants ranged from not detectable (ND) to 0.4 µg/g fresh wt. (ND – 2.1 µg/g dry wt.), below the LOQ (<0.03 µg/g fresh wt.) to 0.2 µg/g fresh wt. (<0.1 – 1.0 µg/g dry wt.), and below the LOQ (<0.02 µg/g fresh wt.) to 0.3 µg/g fresh wt. (<0.04 – 2 µg/g dry wt.), respectively. Mean PMI levels measured in grain at seed maturity and senescence ranged from below the LOQ (<0.06 µg/g fresh wt.) to *ca.* 0.4 µg/g fresh wt. (<0.07 – 0.5 µg/g dry wt.). Mean PMI levels measured in silk tissue at anthesis and seed maturity ranged from below the LOQ (<0.1 µg/g fresh wt.) to 0.8 µg/g fresh wt. (<0.2 – 6.8 µg/g dry wt.). PMI in pollen ranged from 1.9 – 2.6 µg/g fresh wt. (3.9 – 5.2 µg/g dry wt.). (Details in application Appendix III (Joseph and Hill 2003)).

2.3.2 Expression of potential fusion proteins

No novel ORF's were identified that spanned either the 5' or 3' junctions between the MIR604 T-DNA and *Zea mays* genomic sequence. No fusion proteins are therefore expected.

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

2.4.1 Genetic stability of the insert

Genomic DNA was isolated from pooled leaf tissue from ten plants of backcross four (BC4), five (BC5), and six (BC6) of maize MIR604, and assayed by Southern blot analysis. In addition all plants used for DNA isolation were individually analysed with TaqMan® PCR to verify the presence of a single copy of the *mcry3A* and *pmi* gene. For the negative segregant controls, DNA was isolated from pooled leaf tissue of ten plants representing the BC6 generation of maize MIR604. These plants were also individually analysed with TaqMan® PCR, and were negative for the *mcry3A* and *pmi* gene, but positive for an assay internal control (endogenous maize gene).
According to the applicant, the results confirmed the presence of only single copies of the *mcry3A and pmi* genes in MIR604 plants, and that the T-DNA insert is stably incorporated into maize MIR604 over several generations (applicant Appendix CBI.1. Hart & Rabe 2005).

2.4.2 Phenotypic stability of the GM plant

The applicant has measured the levels of mCry3A and PMI protein over multiple generations. Seeds from four successive backcross generations (representing genotypes that were hemizygous for the maize MIR604 transgenes) were grown under greenhouse conditions, and leaf material was collected at anthesis for the analysis of mCry3A and PMI protein levels.

Mean mCry3A protein levels across the four generations were 2.3 – 3.1 µg/g fresh wt. (11.8 – 15.5 µg/g dry wt.). Overall, levels were similar across the four generations analysed and there was no

evidence of any significant trend either up or down, indicating that the expression of mCry3A protein was stable.

A similar result was obtained for the PMI protein. Mean PMI protein levels across the four generations were 0.2 – 0.3 µg/g fresh wt. (1.1 – 1.3 µg/g dry wt.). Overall, levels were similar across the four generations analysed and there was no evidence of any significant trend either up or down, indicating that the expression of PMI protein was stable. Based on these results both proteins appear to be stably expressed in maize MIR604 across multiple generations. (applicant Appendix III, Joseph & Hill 2003).

2.5 Conclusion

The genetically modified maize MIR604 was produced by *Agrobacterium*-mediated transformation of a proprietary *Zea maize* line to provide protection against certain coleopteran pests of maize, e.g. the Northern Corn rootworm. The molecular characterisation data indicate that only one copy of the transgenic insert with the *mcry3A* and *pmi* genes is integrated in the genome of maize MIR604, and that it is stably inherited over generations. 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 MIR604 as adequate.

3 Comparative assessment

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

Key nutritional components in grain and forage derived from maize MIR604 and near-isogenic non-transgenic control plants were analysed in samples from hybrid pairs (a hybrid pair consisting of transgenic maize MIR604 and near-isogenic control plants) grown at 10-12 locations in the USA over two growing seasons (2002 and 2003). A complete breeding pedigree is shown in Figure 2 in Appendix, including control hybrids.

2002 growing season
All plant materials harvested in 2002 were from the two hybrid pairs:

1. **C** (Control) and **D** (MIR604)
2. **E** (Control) and **F** (MIR604)

Each hybrid pair was grown at the following locations:

Bloomington IL (BMI)	both hybrid pairs
Hawaii (HWI)	both hybrid pairs
Stanton MN (SMN)	C and D only
Shirley IL (SIL)	E and F only

2003 growing season
All plant materials harvested in 2003 were from the two hybrid pairs:

1. **E1** (Control) and **E3** (MIR604)

2. **E2** (Control) and **E4** (MIR604)

Hybrids E2 and E4 are more inbred varieties of the 2002 hybrids E and F.
Hybrid pairs E2 and E4 were grown at seven locations whereas hybrid pairs E1 and E3 were grown at all nine of the following locations:

Bondville IL	both hybrid pairs
Bloomington, IL	both hybrid pairs
Shirley IL	both hybrid pairs
Stanton MN	E1 and E3 only
Fairbault MN	E1 and E3 only
Glidden IA	both hybrid pairs
Granger IA	both hybrid pairs
Hawaii	both hybrid pairs
Puerto Rico	both hybrid pairs

Data for each genotype pair were subjected to analysis of variance. For each analyte the statistical significance of the genotype effect was determined with a standard F-test at the 5% probability. The significance of the location x genotype interaction was also assessed with a F-test. The results were compared to compositional analysis data for grain and forage published in the literature.

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

3.1.1 Experimental design and statistical analysis

The field trials were designed following a random block design with three replicate plots of each genotype. Plants were grown according to local agronomic practices. Prior to anthesis silks were bagged to ensure self-pollination.

For each analyte, data for each season were considered separately, and were subjected to analysis of variance across locations with the model

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

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

For each analyte, the statistical significance of the genotype effect was determined with a standard test. An F-test probability of <5% indicates that the difference between the genotypes was statistically significant at the customary 5% level. An F-test was also used to assess the significance of the location x genotype interaction; a significant outcome (F-test probability <5%) indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotypes averaged across locations is questionable. In the study, over 11000 individual data points were assembled into 274 pair-wise comparisons of transgenic and control values for each analyte, across locations, and these datapoints were subjected to analysis of variance for each growing season. The statistical analysis summaries (presented in tables 1-15, Appendix) also show the standard deviation and coefficient of variation for each analyte. Both are measures of random variation, the former expressed on the same scale as the data, the latter expressed as a percentage relative to the overall mean of the analyte in question. Their derivation takes into account all data from both genotypes and all locations. While both are informative in showing the level of variation present in the data, neither is used directly in the comparison of genotypes.

Details of this study can be found in the technical dossier from the applicant (Appendix 4), considered confidential by Syngenta.

Forage sampling and processing
At each location the entire above-ground portion of five plants per replicate plot of the hybrids described above were harvested at dough stage (*ca*. the stage at which silage would be prepared), pooled, and ground with a chipper-shredder. These were sub-sampled and the 3 replicate samples/genotype/location were shipped overnight on wet ice to the Syngenta Biotechnology, Inc. Regulatory Science Laboratory. The ground plant samples were stored at -20ºC or -80°C until further processing. Prior to analysis, each sample was further homogenised in the presence of dry ice and shipped overnight frozen on dry ice to contract analytical laboratories for compositional analysis.

Grain sampling and processing
Grain samples were from pooled ears harvested from 10-15 plants from each genotype from each replicate plot at each location. After harvesting and dry down, the grain samples were shipped to Syngenta Biotechnology Inc. Regulatory Science Laboratory, RTP NC, where they were stored at room temperature. Prior to analysis grain was ground to a fine powder and shipped overnight frozen on dry ice to contract analytical laboratories for compositional analysis.

Analyses performed
All 2002 analyses were conducted by Woodson-Tenent Laboratories Inc., Goldston NC. All 2003 analyses were conducted by Covance Laboratories Inc., Madison WI. Methods for measurement of phytosterols in maize grain were developed and validated by Covance, for the 2003 grain analysis. Based on the moisture content, all other units of measure were converted to a dry weight basis.

3.2 Compositional Analysis

As recommended by OECD (2002) grain from maize MIR604 plants and isogenic non-transgenic control plants were analysed for proximates (including starch), minerals, amino acids and selected fatty acids, vitamins, anti-nutrients and secondary metabolites. Forage from maize MIR604 and isogenic non transgenic control plants were analysed for proximates and minerals.

Proximates
The major constituents of maize grain and forage are carbohydrates, protein, fat and ash. Fibre is the predominant form of carbohydrate present in forage, and starch is the major carbohydrate in corn grain. Fibre is measured by the neutral detergent fibre method (NDF), which measures the insoluble fibre: lignin, cellulose and hemicellulose. This method has replaced the crude fibre method, which underestimates the cell wall content due to hydrolysis of hemicellulose and cellulose. Total dietary fibre (TDF) consists of the insoluble and soluble fibre (pectin). The soluble fibre fraction in maize is negligible, so the NDF value in maize grain is comparable to that of TDF. The acid detergent fibre (ADF) method solubilises hemicellulose, measuring only cellulose and lignin (Watson 1987).

An F-Test Probability of <5% was observed for carbohydrates in grain of both 2003 hybrid pairs, but the actual average carbohydrate levels in the transgenic and control values differed by only 1.0-1.5%. A statistically significant difference was observed in protein levels in the 2003 grain, with average % dry weight of protein in the transgenic grain only 4-7% higher than in the non-transgenic control. In 2002 grain samples the differences in protein were not significant. Other scattered statistically significant differences were noted but none were consistent across hybrid pairs and growing seasons and all values were within ranges reported in the literature for these analytes (data not shown).

Moisture
Levels in the 2003 transgenic samples were *ca*. 3% higher than in the control samples, but in 2002 they were not significantly different. Other scattered statistically significant differences observed were not consistent across hybrid pairs and growing seasons and all were within literature ranges.

Minerals
Several mineral ions are recognised as essential plant nutrients and are required by the plant in significant quantities. These macronutrients include calcium, phosphorous, potassium, and sodium. The micronutrient minerals, iron, copper and zinc are incorporated in plant tissues in only trace amounts. Macro- and micro-nutrient minerals were analysed in grain grown in 2002 and 2003 and in forage grown in 2003. Maize is an important source of selenium in animal feed (Watson 1987), and was also included in the 2003 analysis of grain and forage.

Statistically significant differences were observed between MIR604 and control maize for calcium and zinc in grain samples from 2003, but not 2002. Other random statistically significant differences were not consistent across hybrid pairs and growing seasons and were within published ranges.

In forage, the level of potassium in the MIR604 samples was approximately. 9 -10% higher than in the control samples (1196 vs. 1074 ± 102 for E3 and E1, respectively, and 1271 vs. 1162 ± 154 for E4 and E2). According to the applicant there is insufficient literature data to interpret the relevance of the observed differences, including whether the measured values are within normal variation in maize populations. Selenium and sodium levels were at or below the limit of quantitation in most of the forage samples. Other differences noted between transgenic and control samples were isolated and inconsistent. Results are presented in.

Vitamins
The analyses were performed by different contract labs each of the years, so the units used for quantification of some vitamins differ. A more extensive analysis of vitamin composition was

performed for the 2003 samples. Scattered significant differences were observed, but most were not consistent across growing seasons or consistently associated with the transgene, and the direction of the difference was inconsistent. A significant difference for gamma-tocopherol was observed for both hybrid pairs grown in 2003. Average gamma-tocopherol levels were 9-18% lower in the transgenic grain than in the control grain, however, values for both transgenic and control were within the ranges reported in the literature (data not shown).

Amino acids
Grain from both the 2002 and 2003 locations were analysed for eighteen amino acids. Several statistically significant differences between the 2003 transgenic and control grain were observed, but these differences were not detected in 2002 grain samples. The degree of difference between the average values for the transgenic and control samples ranges from 1-10%. All values of amino acids were within the ranges reported in the literature.

Fatty acids
The five most abundant fatty acids were measured in grain grown in 2002 and 2003 (data not shown). No difference was observed between the transgenic and control grain for palmitic acid. Some significant differences were observed for stearic, linoleic and linolenic acids, however these were within the range of literature values. A significant difference was also observed for oleic acid for three of the four hybrid pairs. The levels of oleic acid in MIR604 grain were closer to the literature range than those of the control and therefore attributed to normal variability rather than the genetic modification. Other sporadic significant differences, especially for hybrid pair E4 and E2 were not consistent across the other hybrid pairs, across growing seasons, or in the direction of the difference. All fatty acid levels for both transgenic and control samples were within the values reported in the literature.

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

Statistically significant differences were observed for both ferulic acid and p-coumaric acid, with lower levels of both in the transgenic samples as compared to the control samples, but all values were within ranges reported in the literature.

Phytosterols
Grain from the 2003 growing season was analysed for the phytosterols campesterol, β-sitosterol and stigmasterol. Small but statistically significant differences were observed for campesterol and stigmasterol for both hybrid pairs, with the transgenic samples having higher amounts of both phytosterols, compared to the control samples. The levels in both the transgenic and control grain were below the levels published in the literature, but because there is very limited historical data available, it is not possible to determine if these levels are within conventional ranges (data not shown).

3.3 Agronomic and phenotypic characters

According to the applicant up to 18 separate agronomic traits were assessed during field trials at 22 locations in 8 states in 2002 and 2003, in addition to greenhouse trials. The tests included corn

rootworm damage, pathogen infestation, yield and other physiological characteristics (Techical Dossier, Appendix CBI.4 (Steiner 2004)). According to the applicant, most of the agronomic traits evaluated showed no statistically significant differences between MIR604-derived hybrids and the non-transformed isogenic counterparts. While some differences between transgenic and control plants were found to be significant, there were no consistent trends in the data across locations or across years that would indicate that any of these differences were due to the presence of the transgene. According to the applicant, these differences were within the normal range of variation experienced in agronomic field trials conducted with transgenic events by Syngenta over the past decade.

3.4 Conclusion

The applicant has performed comparative analyses of data from field trials located at representative sites and environments in North America during the 2002 and 2003 growing seasons. With the exception of small intermittent variations and the insect resistance conferred by mCry3A, the results showed no biologically significant differences between maize MIR604 and control maize. Based on the assessment of available data, the VKM GMO Panel concludes that maize MIR604 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, except for the newly expressed proteins.

4 Food and feed risk assessment

The genetic modification in maize MIR604 will not impact the existing production processes used for maize. All maize MIR604 products will be produced and processed for use in food, animal feed and industrial products in the same way as other commercial maize. MIR604 maize and all food, feed and processed products derived from maize MIR604 are expected to replace a portion of similar products from commercial maize, with total consumption of maize products remaining unchanged. The total anticipated intake/extent of use of maize and all food, feed and processed products derived from maize will remain the same.

4.1 Effects of processing

Food manufacturing of 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 the mCry3A and PMI-proteins (Hammond & Jez 2011). E.g. analyses of maize chips prepared from flour containing 0.32 µg mCry3A/g showed no presence of the protein in the chips (U.S. EPA 2010).

4.3 Toxicological assessment

Potential toxicity of MIR604 maize expressing the mCry3A and PMI proteins has been assessed in toxicity studies performed on rodents, broilers, bobwhite quail and rainbow trout.

4.3.1 Toxicological assessment of the newly expressed proteins

mCry3A and PMI

For toxicological testing both the PMI – and the mCry3A protein was produced in a recombinant *E. coli* system for the purposes of human health risk assessments. Protein characterisation, i.e. several biochemical and functional parameters, established that the *E. coli*-produced proteins showed sufficiently similar biological activity to that of plant produced proteins.
When using microbial proteins in a study for risk assessment, it is essential that their suitability as surrogates for the plant-produced transgenic proteins is established, i.e. the proteins are equivalent for the purposes of the study (Raybould et al. 2013).

4.3.1.1 Acute oral toxicity testing

MCry3A-0102-protein.
Two modified Cry3A protein variants were produced in a recombinant *E. coli* system (designated as test material MCRY3A-0102) for the purposes of human health and ecological effect risk assessments. SDS-PAGE and western blot analyses indicated single intense bands consistent with the predicted molecular weights of approx. 69,500 Da for the mCry3A-LF (long form) and approx. 67,700 Da for the mCry3A-SF (short form). A FIFRA Scientific Advisory Panel (SAP) meeting addressed the scientific issues that arose during the risk assessment of mCry3A (US EPA 2006). US EPA asked the SAP to comment on the equivalence of the mCry3A proteins from maize MIR604 and recombinant *E. coli* - specifically the presence of two forms in the bacterially produced mCry3A protein and the differences in bioactivity in the WCRW bioassay. The majority of the Panel concluded that the two forms of the mCry3A are of relatively comparable biological activity for the purposes of the human health assessments based on the amino acid sequence identity, lack of glycosylation, and general stability. According to SAP, protein characterisation data demonstrate that the plant-produced protein is of sufficiently similar biological activity to that of the two modified Cry3A protein variants. The MCRY3A-0102 test material was not as active towards target pests as the plant-produced modified Cry3A protein, however, the doses in submitted studies were much higher than would occur via the modified maize (Table 4 and US EPA 2006).

15–day single dose oral exposure of MCRY3A-0102 protein in mice
Syngenta has performed a single dose oral toxicity study in mice. The acute oral study was conducted in compliance with UK Principles of good laboratory practice (The United Kingdom GLP Regulations 1999, Statutory Instruments No. 3106, except for the deviation listed in the dossier), US-EPA Guidelines (Health effects test guidelines and Microbial pesticide test guidelines), US-FDA (Redbook 2000, Toxicological principles for the safety of food ingredients), EU (Guidelines for the assessment of additives in feeding stuffs).

Groups of five male and five female Alpk:APfCD-1 mice (8-9 weeks old) were dosed orally by gavage with 0 (control) or 2632 mg MCRY3A-0102 protein (purity of 90.3 %)/kg bodyweight as a single dose on day 1 with 1 % w/v aqueous methylcellulose as the control substance and vehicle. The total dose of MCRY3A-0102 protein after adjustment was 2377 mg/kg bodyweight.
The animals were observed for signs of systemic toxicity frequently following dosing on day 1. Subsequent observations were made daily, up to day 15. The animals were weighted daily, except on day 13 (because of an error), for the reminder of the study.

With the exception of one female in the test group that was euthanised on day 2 (due to adverse in clinical signs consistent with a dosing injury), all other mice survived the study, gained weight, had no test material-related clinical signs, and had no test material-related findings at necropsy, i.e. external observation and a detailed examination of all cranial, thoracic and abdominal organs and structures, and brain, kidneys, liver and spleen were weighted.

According to the applicant the estimated LD_{50} value for pure MCRY3A-0102 protein in male and female mice was > 2,377 mg/kg body weight, the single dose used. According to the applicant this LD_{50} also applies to pure mCRY-protein expressed in maize.

14–day single dose oral exposure of MCRY3A-0102 protein in bobwhite quail
Young adult (25 week old) northern bobwhite quail (*Colinus virginianus*) were administered a single nominal oral dose of 722 mg MCRY3A-0102/kg body wt and observed for 14 days. There were no adverse treatment-related clinical signs or mortality. Body weight and feed consumption of the test birds were comparable to those of the negative control. The acute oral LD_{50} of MCRY3A-0102 was shown to be greater than a nominal concentration of 722 mg MCRY3A-0102/kg body wt (approximately 652 mg MCRY3A-102 protein/kg body wt).

The estimated LD_{50} value for pure MCRY3A-102 protein in young adult northern bobwhite quail was > 722 mg/kg body weight (approximately 652 mg pure mCry3A protein/kg body wt.), the single dose used. According to the applicant this LD_{50} also applies to pure mCry3A-protein expressed in maize.

15-day single dose oral exposure of PMI-0198 protein in mice
The PMI-0198 protein was produced in a recombinant *E. coli* system (designated as test material PMI-0198) for the purposes of human health risk assessments. Protein characterisation, i.e. several biochemical and functional parameters, established that the *E. coli*-produced protein is of sufficiently similar biological activity to that of plant produced PMI-enzyme.

Syngenta has performed a single dose oral toxicity study in mice. The acute oral study was conducted in compliance with US Environ. Protection Agency FIFRA: Good laboratory standards, 40 CFR 160, US Environ. Protection Agency TSCA 40 CFR 792, Good laboratory standards, 40 CFR 160, Japan Ministry 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(97)186.

Groups of seven male and six female Harlan Sprague Dawley (HSD:ICR) mice (males 22.2-28.2 g; females 18.6-24.7 g at the start of experiment) were dosed orally by gavage 5050 mg PMI-0198 protein (purity of 60 %)/kg bodyweight as a single dose on day 1 with 0,5 % w/v aqueous carboxymethyl cellulose (CMC) as the vehicle. The total dose of PMI-0198 protein after adjustment was 3030 mg/kg bodyweight. A vehicle control group (6 males/5 females) receiving 25.25 ml/kg bw of 0.5 % CMC was run concurrently.

The animals were observed for signs of systemic toxicity frequently following dosing on day 1. Subsequent observations were made daily, up to day 15. The animals were weighted prior the day of dosing and on day 14. Observations for mortality and clinical/behavioral signs of toxicity were made three times on the day of dosing, and at least once daily thereafter for 14 days.

One male in the control group and two males in the test group died, or were noted in distress shortly after dosing and subsequently died. One replacement animal was available for each group and each animal was dosed according to the described manner.

All other mice survived the study, gained weight, had no test material-related clinical signs, and had no test material-related findings at necropsy, i.e. external observation and a detailed examination of all cranial, thoracic and abdominal organs and structures, and brain, kidneys, liver and spleen were weighted.

The necropsy of the two males in the test group and the one male in the control group that died soon after dosing revealed perforated esophagus, evidence of gavage error, therefore their deaths were not considered test substance related.

According to the applicant the estimated LD_{50} value for pure maize PMI protein in male and female mice is > 3030 mg/kg body weight, the single dose used.

In the EFSA opinion from 2009 on maize MIR604, The EFSA GMO panel evaluated an acute toxicity study in mice performed by the applicant on the protein PMI-0105, - a bacterially produced analogue of PMI in maize MIR604. Additional information on the PMI-0105 protein was requested by EFSA. These data showed that both PMI-0105 and the PMI from MIR604 have similar specific enzymatic activities and that both have the same molecular size based on identical electrophoretic mobilities as detected by immunoblotting. The EFSA GMO panel concluded that PMI-0105 did not induce adverse effects in the mice after administration of a single dose of 2072 mg PMI/ kg body weight. The data on the testing with PMI-0198 protein (considered identical to the native protein encoded by the bacterial *manA* gene) were considered supplementary by the EFSA GMO Panel.

The acute oral toxicity tests performed on mice and bobwhite quail did not indicate toxic effects of *E. coli* produced mCry3A or PMI proteins. However, acute tests do not provide enough information to conclude on possible adverse health effects of maize MIR604. In whole food the concentrations of these proteins are low, and acute toxic effects in humans and animals will most probably be negligible. Acute toxicity testing of the newly expressed proteins 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).

4.3.1.2 Repeated dose toxicity testing

No repeated dose 14-day oral toxicity study of MCRY3A-0102 and PMI-0198 proteins in rodents has been performed by the applicant.

4.3.2 Toxicological assessment of the whole GM food/feed

28-day feeding study, juvenile rainbow trout (*Onchorhynchus mykiss*)
In this 28-day toxicity study, juvenile rainbow trout (*Onchorhynchus mykiss*) were fed fish feed containing 50% w/w maize MIR604 (0.09 µg mCry3A/g test diet) or non-transgenic (negative control) maize grain (US EPA 2010). To minimise degradation of mCry3A protein in the feed, a 'cold' pelleting method was used in the preparation of the feed. The study was carried out according to OECD Guideline 215 and US EPA OPPTS Guideline 885.4200. Prior to test initiation, 40 fish were placed in each of two test vessels, the exposure tank and the control tank. Mortality and symptoms of toxicity were assessed on a daily basis and detailed observations of symptoms and feeding responses were made on days 4, 7, 10, 15, and 22. No significant differences were detected in the weight of the control or test fish at 0, 14, or 28 days. No significant difference in length was seen at 14 or 28 days. In the MIR604 test group, transient discoloration and surfacing were seen in one to three fish after day 15, and one fish (2.5% of test group) was found dead on day 21. No mortality was seen in the control group (US-EPA 2010). The results indicate that exposure to mCry3A in fish feed prepared from maize MIR604 for 28 days had no significant effects on the growth and mortality of juvenile rainbow trout.

The GMO panel questions the statistical design and diet composition of the 28-day feeding study with juvenile rainbow trout. The following remarks have been raised 1) Feeding studies with fish use the tank as the experimental unit, not the fish. This is because fish confined in the same tank is not independent 2) Rainbow trout is a carnivorous fish and requires a certain amount of proteins and lipids. Unprocessed maize as a feed ingredient would provide mostly carbohydrates. It is questioned whether the diets in this trial were compositionally sufficient.

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

A broiler feeding study was conducted to compare the nutritional properties of maize MIR604 with its isogenic control (derived from the non-transgenic version of the same inbred parent), and a conventional maize reference (Appendix X, applicant dossier).

Diets were prepared from maize MIR604, isogenic control, and the conventional maize NC2003. One day old male (commercial strain Ross344) and female (commercial strain Ross 308) birds were distributed into 36 pens assigned in a randomised complete block design. Male and female birds were housed separately, and for each test group there were six replicated pens of 25 birds/gender. A total of 900 birds.

The broilers were fed over a 49-day period. Starter, grower (days 16-31), and finisher diets (days 31-49) contained 57.5, 63.0 and 67.5% maize, respectively. The levels of plant produced mCry3A in the transgenic diets were reported to be 0.04, 0.06, and 0.08 μg/g dry weight of the starter, grower, and finisher diets, respectively. The amount of PMI protein was not stated. Samples of maize grain lots were analysed for proximates, amino acids and mycotoxins (aflatoxin, deoxynivalenol, fumonisin, T2 toxin and zearalenone, were all below regulatory limits).

Pen weights (25 birds/pen) were recorded at days 1 (hatch), 16, 31, and 49. On the later three dates, feed conversion ratios were determined. Feeding was terminated approximately 16 hours before slaughter on day 51. Body weight, feed conversion, and survival data were recorded at 0, 16, 31, and 49 days. The carcass characteristics that were measured in six male and six female animals per group included body weight and weights of fat pads, drums, thighs, wings, pectoralis major and pectoralis minor. A statistically significant difference was observed in thigh weights of female animals, which were slightly higher in animals fed MIR604 than those fed the non-GM control maize, but not different from those fed the reference diet. No adverse clinical signs or statistically significant differences in carcass yield or mortality were noted among treatment groups, except for the expected differences in development related to gender.

According to the applicant poultry diets prepared with maize MIR604 grain supported rapid broiler growth at low mortality rates and good feed conversion ratios without any substantial differences in overall carcass yield. There were no obvious deleterious effects associated with consumption of maize MIR604 grain when compared to controls.

90-day subchronic feeding study in rats
The applicant has provided a subchronic (90-day) feeding study in rats with maize MIR604 grain as a component of the diet ((Appendix IX, applicant dossier)). The study (PR1282) was conducted in compliance with the UK Principles of Good Laboratory Practice (GLP) (The United Kingdom GLP

Regulations 1999, Statutory Instrument No. 3106, as amended 2004, Statutory Instrument No.994). These Principles are in accordance with the OECD Principles of Good Laboratory Practice (GLP), revised 1997 (ENV/MC/CHEM(98)17). The study was conducted according to a) OECD guideline reference 408 (1998): Repeated dose 90 day oral toxicity study in rodents. b) United States Environmental Protection Agency, Health Effects Test Guidelines, OPPTS. 870.3100 (August 1998): 90-Day Oral Toxicity in Rodents.

Groups of 12 male and 12 female Wistar-derived rats (Alpk:APfSD) were fed diets containing 10% or 41.5% (w/w) grain from maize MIR604 (MIR604 "positive") or 10% or 41.5% grain from a near isogenic non GM control maize (called MIR604 negative by the applicant). The applicant does not state the specific isogenic control, nor was any conventional reference maize used in the study. MIR604 breeding history can be found in Figure 2 (Appendix).

Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity.
Clinical observations, bodyweights and food consumption were measured throughout the study. The bodyweight of each rat was recorded immediately before feeding of the experimental diets commenced on day 1, on days 2 to 8 and weekly thereafter throughout the study and on the day of termination. A functional observation battery of tests and locomotor activity monitoring were performed during week 13. The observations were made by one observer who was 'blind' with respect to the animal's treatment.

An ophthalmoscopic examination was performed on all animals pre-study and in week 13, by an observer who was 'blind' with respect to the animal's treatment. At the end of the scheduled period, the animals were killed and examined post mortem. Cardiac blood samples were taken for clinical pathology evaluation. Selected organs were weighed and specified tissues were taken for subsequent histo-pathological examination.

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

Food consumption in males was slightly lower in the 10% and 41.5% MIR604 maize grain groups throughout the study compared to the respective control groups. These differences were statistically significant in weeks 3, 6, 7 and 13 in the 10% MIR604 group and in weeks 1 to 3 and 5 to 6 in the 41.5% MIR604 group. In females, food consumption was lower in weeks 1 and 2 in the 10% and 41.5% MIR604 groups compared to the corresponding control groups. However, all values fell within the historical control ranges. In the absence of indications of adverse effects, the applicant does not consider the reduction in bodyweight as toxicologically relevant.

Several statistically significant differences in haematology and clinical chemistry parameters compared with the controls were noted: in males fed 10 % MIR604, hemoglobin, hematocrit and red blood cell counts were higher, whereas mean cell volume, white blood cell, monocyte, neutrophil and large unstained cell counts were lower when compared to the corresponding control group. The values overlapped with the concurrent control group and the parameters were not significantly different between the group fed 41.5% MIR604 maize grain diet and the corresponding control group. In the group fed 41.5% MIR604 maize grain platelet count was slightly lower in males; this was not reflected

in any other red blood cell parameters and was considered not to be treatment related. The differences in haematology parameters were in one sex only, of relatively small magnitude and were considered to be incidental to treatment. The differences in clinical chemistry parameters were of relatively small magnitude, inconsistent between the sexes and treatment groups and were considered to be incidental to diets containing MIR604 maize grain.

In the 10% MIR604 maize group the spleen weights of females were significantly higher, and in the 41.5% group, testes weights in males, and heart weights in females were higher than the corresponding control groups. However, the differences were small, limited to one sex and in the absence of pathology findings are according to the applicant considered not to be treatment related.

Amongst the macroscopic and microscopic findings, a small number of common spontaneous lesions were observed; the applicant considers these changes not to be related to the administration of MIR604 maize grain in the diet.

According to the applicant, the results show that the inclusion of MIR604 maize grain at 10 or 41.5% in feed did not cause treatment related effects in rats fed for 90 consecutive days.

4.4 Allergenicity assessment

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

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

4.4.1 Assessment of IgE mediated allergenicity of the newly expressed proteins

The applicant has performed a weight-of-evidence approach (FAO/WHO, 2001; Codex, 2003) for an overall assessment of the IgE allergenic potential of the PMI and mCry3A proteins, which includes:

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

These assessments have previously been described by the applicant for PMI and mCry3A, and were based on the following aspects:

PMI:

i) The recipient of the transgenes is maize (*Zea mays*), which is not considered a common food allergen.
ii) PMI enzymes are found in various plants and microorganisms.
iii) The *pm*i (*manA*) gene came from *Escherichia coli*,
iv) The manA protein is a member of the superfamily of "cupins," which are proteins with a specific 3-D structure. Some members of this super family are known IgE allergens.
v) The gene coding for the PMI in the MIR604 was expressed in bacteria and the resulting enzyme compared to the MIR604 derived PMI by Western blot. The enzymes expressed from the two sources were shown not to be identical, two amino acids were changed, valine-61 was substituted by alanine, and glutamine-210 by histidine.
vi) Bioinformatic analysis did not reveal any relevant sequence homology between the PMI expressed in maize MIR604 and known IgE allergens of the cupin superfamily.
vii) No significant similarity was found between any of the PMI 80-amino acid peptides and any entries in the SBI Allergen Database.
viii) In the eight or more contiguous amino acids homology search, there was an alignment between the PMI protein and a recently identified allergen, α-parvalbumin from Rana species CH2001 (a frog of Indonesian origin).
ix) Serum screening with serum IgE obtained from an allergic individual who displayed food-induce anaphylaxis against α-parvalbumin showed no cross-reactivity with PMI.
x) The PMI protein is also in some but not all plants.
xi) The *E. coli* expressed PMI protein is also found in human intestinal microbiota, e.g. *E. coli*
xii) There has always been a background of human exposure and a low quantity of PMI found in the human diet.
xiii) The PMI-protein has previously been assessed for genetically modified plants and found to have no potential for IgE allergenicity (EFSA 2009; Delany et al. 2008,).

mCry3A

i) The expressed mCry3A protein is a single polypeptide with a 92.9 % sequence identity to the wild type.
ii) Immunoblot and glycosylation analysis of mCry3A derived from recombinant *E.coli* and from extracts of leaf material from MIR604 maize, indicate that both mCry3A proteins are not glycosylated.
iii) A comparison of amino acid sequences of known IgE allergens uncovered no evidence of any homology with mCry3A, even at the level of 8 contiguous amino acids residues.
iv) The mCry3A protein is rapidly degraded by gastric fluids *in vitro* (US EPA 2010).

The information listed above indicates that the newly expressed proteins in MIR604 maize lack IgE allergenic potential with regard to human and animal health. However, it does not cover allergic reactions that are not IgE mediated, e.g. some gluten-sensitive enteropathies or other enteropathies that are not IgE-mediated.

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

Food allergies to maize are of low frequency and mainly occur in populations of specific geographic areas. Rare cases of occupational allergy to maize dust have been reported. However, allergenicity of the maize MIR604 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. Given that no biologically relevant agronomic or compositional changes have been identified in field maize MIR604 with the exception of the introduced traits, no increased allergenicity is anticipated for maize MIR604.

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

It is the opinion of the VKM GMO Panel that a possible over-expression of any endogenous protein, which is not known to be allergenic, in maize MIR604 would be unlikely to alter the overall allergenicity of the whole plant or the allergy risk for consumers.

4.4.4 Adjuvanticity

According to the EFSA 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).

4.5 Nutritional assessment of GM food/feed

The compositional analyses indicate nutritional equivalence between maize MIR604, the non-GM control maize with comparable genetic background, and the published ranges of values in the literature. The data show no indications of unexpected alterations in the measured nutrients, anti-nutrients or other food components as a result of the genetic modification in MIR604. The nutritional equivalence between maize MIR604 and non-GM control maize is further supported by the poultry feeding study and the feeding study in fish.

4.5.1 Intake information/exposure assessment

According to the applicant, modified Cry3A protein (mCry3A) was analysed in wet- and dry milled fractions generated from standard food processing procedures carried out on maize grain derived from maize MIR604, together with a corresponding non-transgenic control. Quantifiable levels of mCry3A were detected in various wet- and dry-milled fractions ranging from 2.12 µg mCry3A/g in flaking grits to below detectable levels in coarse fiber, germ, and starch. Although the concentration of mCry3A measured in the flour used to prepare corn chips was 0.32 µg mCry3A/g, no mCry3A was detected in the chips. Similarly, mCry3A was not detectable in oil, whereas the starting material, flaking grits, contained the highest level of mCry3A (US EPA 2006).

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

Since most foods and foodstuffs from maize are derived from field maize grains, an estimated maximum daily intake for a Norwegian adult of mCry3A and PMI proteins is calculated to be 13.8 µg and 2.64 µg, respectively, based on grain dry weight (4.4 g/person/day). The estimated maximum daily intake of mCry3A and PMI proteins from sweet maize is calculated to be 54.8 µg and 10.5 µg, respectively, based on a daily intake of 17.5 g fresh sweet maize/day (97.5 % percentile). These levels are several orders of magnitude below the levels shown to have no effect in laboratory toxicology testing. Also, these levels are considerably below the proposed threshold of toxicological concern (TTC) level of 1800 µg/person/day (Class 1, oral exposure) for chemicals considered to have a low potential for toxicity based on metabolism and mechanistic data (Vermeire et al., 2010). Transgenic proteins produced by genetically modified plants are generally 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 MIR604 may be higher for these animals.

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

4.5.2 Nutritional assessment of feed derived from the GM plant

According to the applicant, the maize and derived feed products are substantially and nutritionally equivalent to 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 maize MIR604, nutritional equivalence shown in a poultry feeding study, and safety evaluation of the mCry3A and PMI proteins expressed in maize MIR604.

4.6 Conclusion

Whole food feeding studies on rats, rainbow trout and broilers have not indicated any adverse health effects of maize MIR604. These studies also indicate that maize MIR604 is nutritionally equivalent to conventional maize. The mCry3A and PMI proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

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

5 Environmental risk assessment

5.1 Unintended effects on plant fitness due to the genetic modification

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

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

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

It is considered very unlikely that the establishment, spread and survival of maize MIR604 would be increased due to the insect resistance. Insect resistance against certain coleopteran target pests provides a potential advantage in cultivation of MIR604 under infestation conditions. It is considered very unlikely that maize MIR604 plants or their progeny will differ from conventional maize cultivars in their ability to survive as volunteers until subsequent seasons, or to establish feral populations under European environmental conditions.

Field trials were carried out by the applicant do not indicate altered fitness of maize MIR604 relative to its conventional counterpart. The agronomic performance of transgenic maize MIR604 and controls was analysed in field trials at 22 locations in USA during two growing seasons (2002 and 2003). The parameters tested included corn rootworm damage, pathogen infestation, yield and other physiological characteristics. The field data provided in the application showed enhanced biomass production in conditions of *Diabrotica* spp. infestation but do not show changes in plant characteristics that

indicate altered fitness and invasiveness of maize MIR604 plants (Corn rootworm damage was lower in MIR604 compared with the non-GM comparators, and yields of maize MIR604 were higher in locations where corn rootworm and drought were prevalent). No other consistent differences in agronomic performance and pathogen infestation were observed. The VKM GMO Panel therefore concludes that, with the exception of expected differences in agronomic performance linked with the introduced insect-resistance trait of maize MIR604, the phenotypic and agronomic performance of this maize is equivalent to that of the non-GM comparators.

In addition to the data presented by the applicant, the VKM GMO Panel is not aware of any scientific reports indicative of increased establishment or spread of maize MIR604, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of maize MIR604 are unchanged and insect resistance 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 MIR604 will not differ from that of conventional maize varieties.

5.2 Potential for gene transfer

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

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

5.2.1 Plant to micro-organisms gene transfer

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

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

It is however pointed out that there are limitations in the methodology used in these experimental studies (Nielsen & Townsend 2004). Experimental studies of limited scale should be interpreted with

caution given the scale differences between what can be experimental investigation and commercial plant cultivation.

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

In conclusion, the VKM GMO Panel consider it is unlikely that the introduced gene from maize MIR604 will transfer and establish in the genome of microorganisms in the environment or in the intestinal tract of humans or animals. In the rare, but theoretically possible case of transfer of the *mcry3A* and *pmi* genes from MIR604 to soil bacteria, no novel property would be introduced into or expressed in the soil microbial communities; as these genes are already present in other bacteria in soil. Therefore, no positive selective advantage that would not have been conferred by natural gene transfer between bacteria is expected.

5.2.2 Plant to plant gene flow

Considering the intended uses of maize MIR604 (excluding cultivation) and the physical characteristics of maize seeds, possible pathways of gene dispersal are grain spillage and dispersal of pollen from potential transgenic maize plants originating from accidental grain spillage during transport and/or processing.

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

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

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

As maize MIR604 has no altered survival, multiplication or dissemination characteristics, the VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this GM maize in Norway will not differ from that of conventional maize

varieties. The likelihood of cross-pollination between cultivated maize and the occasional feral maize plants resulting from grain spillage is considered extremely low.

5.3 Interactions between the GM plant and target organisms

Maize MIR604 was transformed to express a modified version of the Cry3A protein from *Bacillus thuringiensis* subsp. *tenebrionis*. The insecticidal toxin is active in the control of certain coleopteran insect pests belonging to the genus *Diabrotica,* such as larvae of western corn rootworm (WCR; *D. virgifera virgifera*) and the northern corn rootworm (NCR; *D. barberi*). WCR has been introduced to Europe from North America, where it is native and widespread (Miller et al. 2005, ref. EFSA 2013). *D. virgifera virgifera* was first detected in Serbia in 1992, but has since spread across the continent, resulting in well-established populations in approximately 19 European countries (EC 2012). There have been no reports of *D. virgifera virgifera* in Norway (http://www.faunaeur.org/distribution.php)

Considering the intended uses of maize MIR604, excluding cultivation, the environmental exposure is limited to exposure through manure and faeces from the gastrointestinal tract mainly of animals fed on the GM maize as well as to the accidental release into the environment of GM seeds during transportation and processing and subsequently to potential occurrence of sporadic feral plants. Thus the level of exposure of target organisms to mCry3A 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 MIR604, excluding cultivation, the environmental risk assessment is concerned with accidental release of GM maize viable grains into the environment during transportation and processing, and exposure through manure and faeces from the gastrointestinal tracts of animals fed the GM maize.

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

Data supplied by the applicant indicate that a limited amount of the mCry3A protein enters the environment due to expression in the grains (range 0.33-2.35 µg/g d.w.). In addition, the data show that at least 99% of microbially produced mCry3A 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 mCry3A 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 MIR604, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with the abiotic environment and biogeochemical cycles were not considered an issue by the VKM GMO Panel.

5.6 Post-market environmental monitoring

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

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

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

No specific environmental impact of genetically modified maize MIR604 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/NL/2005/11 includes import and processing of maize MIR604 for food and feed uses. Considering the intended uses of maize MIR604, excluding cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize MIR604.

Maize MIR604 has no altered survival, multiplication or dissemination characteristics, and there are no indications of an increased likelihood of spread and establishment of feral maize plants in the case of accidental release into the environment of seeds from maize MIR604. Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation. The 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.

7 Conclusions

Molecular characterisation
The molecular characterisation data indicate that only one copy of the transgenic insert with the *mcry3A* and *pmi* genes is integrated in the genome of maize MIR604, and that it is stably inherited over generations. 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 MIR604 as adequate.

Comparative assessment
The applicant has performed comparative analyses of data from field trials located at representative sites and environments in North America during the 2002 and 2003 growing seasons. With the exception of small intermittent variations and the insect resistance conferred by mCry3A, the results showed no biologically significant differences between maize MIR604 and control maize. Based on the assessment of available data, the VKM GMO Panel concludes that maize MIR604 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, except for the newly expressed proteins.

Food and feed risk assessment
Whole food feeding studies on rats, rainbow trout and broilers have not indicated any adverse health effects of maize MIR604. These studies also indicate that maize MIR604 is nutritionally equivalent to conventional maize. The mCry3A and PMI proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE mediated allergic reactions. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

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

Environmental risk assessment
The scope of the application EFSA/GMO/UK/2005/11 includes import and processing of maize MIR604 for food and feed uses. Considering the intended uses of maize MIR604, excluding cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure, mainly through manure and faeces from animals fed grains from maize MIR604.

Maize MIR604 has no altered survival, multiplication or dissemination characteristics, and there are no indications of an increased likelihood of spread and establishment of feral maize plants in the case of accidental release into the environment of seeds from maize MIR604. Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivation. The VKM GMO Panel considers the risk of gene flow from occasional feral GM maize plants to conventional maize varieties to be negligible in Norway. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered by the GMO Panel to be an issue.

Overall conclusion

Based on current knowledge, the VKM GMO Panel concludes that maize MIR604 is nutritionally equivalent to conventional maize varieties. It is unlikely that the mCry3A and PMI proteins will introduce a toxic or allergenic potential in food derived from maize MIR604 compared to conventional maize.

The VKM GMO Panel likewise concludes that maize MIR604, based on current knowledge, is comparable to conventional maize varieties concerning environmental risk in Norway with the intended usage.

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