VKM Report 2014: 08

Final health and environmental risk assessment of genetically modified maize MON 89034

Food/feed and environmental risk assessment of insect resistant genetically modified maize MON 89034 for import, processing, food and feed uses under Regulation (EC) No 1829/2003 (EFSA/GMO/NL/2007/37)

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

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2014: 08

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Summary

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

The insect-resistant genetically modified maize MON 89034 (Unique Identifier MON-89Ø34-3) from Monsanto Company is approved under Regulation (EC) No 1829/2003 for food and feed uses, import and processing since 30 October 2009 (Application EFSA/GMO/NL/2007/37, Commission Decision 2009/813/EC).

Genetically modified maize MON 89034 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 application EFSA/GMO/NL/2007/37 in 2007 (VKM 2008a). VKM has also been requested to issue a preliminary scientific opinion on the safety of the genetically modified maize MON 89034 for cultivation, and submit relevant scientific comments or questions to EFSA on the application EFSA/GMO/BE/2011/90 (VKM 2012a). At the request from the Norwegian Environment Agency the VKM GMO Panel also submitted a final environmental risk assessment of MON 89034 for food and feed uses in 2013 (VKM 2013). Finally, maize MON 89034 has been evaluated by the VKM GMO Panel as a component of several stacked GM maize events (VKM 2008b, 2009a,b, 2010a,b).

The food/feed and environmental risk assessment of maize MON 89034 is based on information provided by the applicant in the applications EFSA/GMO/NL/2007/37 and EFSA/GMO/BE/2011/90, 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 MON 89034 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 MON 89034 include molecular characterisation of the inserted DNA and expression of novel proteins, comparative assessment of agronomic and phenotypic characteristics, nutritional assessments, toxicology and allergenicity, unintended effects on plant fitness, potential for gene transfer, interactions between the GM plant and target and non-target organisms and effects on biogeochemical processes.

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

The genetically modified maize MON 89034 was developed to provide protection against certain lepidopteran target pest, including European corn borer (*Ostrinia nubilalis*) and Mediterranean corn borer (*Sesamia nonagrioides*). Protection is achieved through expression in the plant of two insecticidal Cry proteins, Cry1A.105 and Cry2Ab2, derived from *Bacillus thuringiensis*, a common soil bacterium. Cry1A.105, encoded by the *cry1A.105* gene, is a chimeric protein made up of different functional domains derived from three wild-type Cry proteins from *B. thuringiensis* subspecies *kurstaki* and *aizawai*. The Cry2Ab2 protein is encoded by the *cry2Ab2* gene derived from *B. thuringiensis* subspecies *kurstaki*.

Molecular characterisation
Appropriate analyses of the transgenic DNA insert, its integration site, number of inserts and flanking sequences in the maize genome, have been performed. The results show that only one copy of the insert is present in maize MON 89034. Homology searches with databases of known toxins and allergens have not indicated any potential production of harmful proteins or polypeptides caused by the genetic modification in maize MON 89034. Southern blot analyses and segregation studies show that the introduced genes *cry1A.105* and *cry2Ab2* are stably inherited and expressed over several generations along with the phenotypic characteristics of maize MON 89034. The VKM GMO Panel concludes that the molecular characterisation of maize MON 89034 does not indicate a safety concern.

Comparative assessment
Comparative analyses of maize MON 89034 to its non-GM conventional counterpart have been performed during multiple field trials in representative areas for maize cultivation in USA, Argentina and Europe (2004, 2005 and 2007). With the exception of small intermittent variations, no biologically significant differences were found between maize MON 89034 and the conventional non-GM control. Based on the assessment of available data, the VKM GMO Panel concludes that maize MON 89034 is compositionally, agronomical and phenotypically equivalent to its conventional counterpart, except for the introduced characteristics.

Food and feed risk assessment
A 90-day subchronic feeding study on rats, as well as whole food feeding studies on broilers and feedlot steers have not indicated any adverse effects of maize MON 89034, and shows that maize MON 89034 is nutritionally equivalent to conventional maize. The Cry1A.105 and Cry2Ab2 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 MON 89034 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1A.105 or Cry2Ab2 proteins will introduce a toxic or allergenic potential in food or feed based on maize MON 89034 compared to conventional maize.

Environmental risk assessment
Considering the intended uses of maize MON 89034, 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 MON 89034.

Maize MON 89034 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 MON 89034. 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 MON 89034 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1A.105 and Cry2Ab2 proteins will introduce a toxic or allergenic potential in food or feed derived from maize MON 89034 compared to conventional maize.

The VKM GMO Panel likewise concludes that maize MON 89034, 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 MON 89034, EFSA/GMO/UK/2007/37, insect resistance, Cry proteins, Cry1A.105, Cry2Ab2, food and feed risk assessment, environmental risk assessment, Regulation (EC) No. 1829/2003, Directive 2001/18/EC

Norsk sammendrag

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

Den insektsresistente maislinjen MON 89034 (unik kode MON-89Ø34-3) fra Monsanto Company ble godkjent til import, videreforedling og til bruk som mat og fôr under EU-forordning 1829/2003 30. oktober 2009 (Kommisjonsbeslutning 2009/813/EU).

VKM risikovurderte maislinje MON 89034 på oppdrag fra Mattilsynet og Miljødirektoratet første gang i forbindelse med EFSAs offentlige høring av søknad EFSA/GMO/NL/2007/37 i 2007 (VKM 2008a). En utvidet søknad om godkjenning av MON 89034 til dyrking (EFSA/GMO/BE/2011/90) ble vurdert av VKM i tilknytting til EFSAs høring i 2012 (VKM 2012a). På oppdrag fra Miljødirektoratet har også VKM utarbeidet en endelig miljørisikovurdering av maislinjen for bruksområdene import, prosessering, mat og fôr (VKM 2013). Risikovurderingen ble utført på oppdrag fra Miljødirektoratet i forbindelse med nasjonal sluttføring av søknad EFSA/GMO/NL/2007/37). VKMs faggruppe for GMO har også risikovurdert en rekke hybrider der MON 89034 inngår som en av foreldrelinjene (VKM 2008b, VKM 2009a,b, 2010a,b).

Risikovurderingen av den genmodifiserte maislinjen er basert på uavhengige vitenskapelige publikasjoner og dokumentasjon som er gjort tilgjengelig på EFSAs nettside EFSA GMO Extranet. Vurderingen er gjort i henhold til tiltenkt bruk i EU/EØS-området, og i overensstemmelse med miljøkravene i genteknologiloven med forskrifter, først og fremst forskrift om konsekvensutredning etter genteknologiloven. Videre er kravene i EU-forordning 1829/2003/EF, utsettingsdirektiv 2001/18/EF (vedlegg 2,3 og 3B) og veiledende notat til Annex II (2002/623/EF), samt prinsippene i EFSAs retningslinjer for risikovurdering av genmodifiserte planter og avledete næringsmidler (EFSA 2010a, 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, samt effekter på målorganismer, ikke-målorganismer og biogeokjemiske prosesser 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 MON 89034 er utviklet for å gi plantene resistens mot enkelte skadegjørerere i ordenen Lepidoptera (sommerfugler), eksempelvis maispyralide (*Ostrinia nubilatis*) og *Sesamia nonagrioides*. Grunnlaget for insektresistensen er to *cry*-gener som er satt inn i maislinjen og som koder for toksinene Cry1A.105 og Cry2Ab2. *Cry1A.105* er et syntetisk gen, sammensatt av sekvenser fra genene *cry1Ac*, *cry1Ab* og *cry1F* fra jordbakterien *Bacillus thuringiensis* (underartene

kurstaki og *aizawai)*. I tillegg har MON 89034 fått innsatt *cry2Ab*-genet fra *B. thuringiensis* subsp. *kurstaki*.

Molekylær karakterisering
Adekvate analyser av det transgene DNA-innskuddet, dets integreringssete, antall integreringer og flankerende DNA-sekvenser i mais-genomet, har blitt utført. Resultatene viser at kun ett transgent innskudd er til stede i mais MON 89034. Homologisøk i databaser over kjente toksiner og allergener indikerer at genmodifiseringen ikke har ført til potensiell produksjon av skadelige proteiner eller polypeptider i mais MON 89034. Southern blot og segresjonsanalyser viser at de introduserte genene *cry1A.105* og *cry2Ab2* er stabilt uttrykt og nedarvet over flere generasjoner, og i samsvar med de fenotypiske karakterene til mais MON 89034. VKMs faggruppe for genmodifiserte organismer har ikke identifisert noen risiko knyttet til det som framkommer av den molekylærbiologiske karakteriseringen av de rekombinante innskuddene i maislinjen.

Komparative analyser
Komparative analyser av mais MON 89034 og tilhørende umodifisert kontroll («konvensjonell motpart») er basert på feltforsøk i representative områder for maisdyrking i USA, Argentina og Europa 2004, 2005 og 2007. Med unntak av enkelte små variasjoner viste studiene ingen biologisk relevante forskjeller mellom mais MON 89034 og dens konvensjonelle motpart. Basert på vurdering av tilgjengelig data, konkluderer VKMs faggruppe for GMO at mais MON 89034 er ernæringsmessig, morfologisk og agronomisk vesentlig lik dens konvensjonelle motpart, med unntak av de introduserte egenskapene.

Helserisiko
Fôringsstudier utført på rotter, broiler, og storfe har ikke indikert helseskadelige effekter av mais MON 89034. Disse studiene indikerer også at mais MON 89034 er ernæringsmessig vesentlig lik konvensjonell mais. Proteinene Cry1A.105 og Cry2Ab2 viser ingen likhetstrekk til andre 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 MON 89034 er ernæringsmessig vesentlig lik konvensjonell mais, og at det er lite trolig at proteinene Cry1A.105 og Cry2Ab2 vil introdusere et toksisk eller allergent potensiale i mat eller fôr basert på mais MON 89034 sammenliknet med konvensjonelle maissorter.

Miljørisiko
Med bakgrunn i tiltenkt bruksområde for søknaden 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 MON 89034 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 MON 89034 er ernæringsmessig, fenotypisk og agronomisk ekvivalent med konvensjonell mais. Det er lite trolig at Cry1A.105- og Cry2Ab2-proteinene vil introdusere et toksisk eller allergent potensiale i mat eller fôr basert på mais MON 89034 sammenliknet med konvensjonelle maissorter.

Faggruppen finner likeledes at mais MON 89034, ut fra dagens kunnskap og omsøkt bruk, er sammenlignbar med konvensjonell mais når det gjelder mulig miljørisiko i Norge.

Abbreviations and explanations

ALS	Acetolactate synthase, an enzyme that catalyses the first step in the synthesis of the branched-chain amino acids, valine, leucine, and isoleucine
ARMG	Antibiotic resistance marker gene
BC	Backcross. Backcross breeding in maize is extensively used to move a single trait of interest (e.g. disease resistance gene) from a donor line into the genome of a preferred or "elite" line without losing any part of the preferred lines existing genome. The plant with the gene of interest is the donor parent, while the elite line is the recurrent parent. BC_1, BC_2 etc. designates the backcross generation number.
BLAST	Basic Local Alignment Search Tool. Software that is used to compare nucleotide (BLASTn) or protein (BLASTp) sequences to sequence databases and calculate the statistical significance of matches, or to find potential translations of an unknown nucleotide sequence (BLASTx). BLAST can be used to understand functional and evolutionary relationships between sequences and help identify members of gene families.
bp	Basepair
Bt	*Bacillus thuringiensis*
CaMV	Cauliflower mosaic virus
Codex	Set by The Codex Alimentarius Commission (CAC), an intergovernmental body to implement the Joint FAO/WHO Food Standards Programme. Its principle objective is to protect the health of consumers and to facilitate the trade of food by setting international standards on foods (i.e. Codex Standards).
Cry	Any of several proteins that comprise the crystal found in spores of *Bacillus thuringiensis*. Activated by enzymes in the insects midgut, these proteins attack the cells lining the gut, and subsequently kill the insect.
Cry1A.105	Chimeric protein comprised of domains from the naturally occurring Cry1Ab, Cry1F, and Cry1Ac proteins of *Bacillus thuringiensis*
Cry2Ab2	A Cry2 class crystal protein from *Bacillus thuringiensis* subsp. *kurstaki*
CTP	Chloroplast transit peptide
DAP	Days after planting
DNA	Deoxyribonucleic acid
DT50	Time to 50% dissipation of a protein in soil
DT90	Time to 90% dissipation of a protein in soil
dw	Dry weight
dwt	Dry weight tissue
EC	European Commission
ECB	European corn borer, *Ostrinia nubilalis*

EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	Environmental risk assessment
E-score	Expectation score
EU	European Union
fa	Fatty acid
FAO	Food and Agriculture Organisation
FIFRA	US EPA Federal Insecticide, Fungicide and Rodenticide Act
Fitness	Describes an individual's ability to reproduce successfully relative to that of other members of its population.
fw	Fresh weight
fwt	Fresh weight tissue
GAT	Glyphosate N-acetyltransferase
GLP	Good Laboratory Practice
Glufosinate-ammonium	Broad-spectrum systemic herbicide
Glyphosate	Broad-spectrum systemic herbicide
GM	Genetically Modified
GMO	Genetically Modified Organism
GMP	Genetically Modified Plant
H	Hybrid
ha	Hectare
ILSI	International Life Sciences Institute
IPM	Integrated Pest Management
IRM	Insect Resistance Management
Locus	The position/area that a given gene occupies on a chromosome
LOD	Limit of detection
LOQ	Limit of quantification
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization-Time Of Flight. A mass spectrometry method used for detection and characterisation of biomolecules, such as proteins, peptides, oligosaccharides and oligonucleotides, with molecular masses between 400 and 350,000 Da.
MCB	Mediterranean corn borer, *Sesamia nonagrioides*
mRNA	Messenger RNA
MT	Norwegian Food Safety Authority (Mattilsynet)
NDF	Neutral detergent fibre, measure of fibre used for animal feed analysis. NDF measures most of the structural components in plant cells (i.e. lignin, hemicellulose and cellulose), but not pectin.

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

Maize growth stages	*Vegetative*
	VE: emergence from soil surface
	V1: collar of the first leaf is visible
	V2: collar of the second leaf is visible
	Vn: collar of the leaf number 'n' is visible

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

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Background

On 31 January 2007, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands an application (Reference EFSA-GMO-NL-2007-37) for authorisation of the insect-resistant genetically modified (GM) maize MON 89034 (Unique Identifier MON-89Ø34-3), submitted by Monsanto Company 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/2007/37 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 24 August 2007, 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 May 2008 (VKM 2008a). EFSA published its scientific opinion 3 December 2008 (EFSA 2008), and maize MON 89034 was approved for food and feed uses, import and processing 30 October 2009 (Commission Decision 2009/813/EC).

An application for authorisation of maize MON 89034 for cultivation in the EU was submitted by Monsanto Company in December 2010 (EFSA/GMO/BE/2011/90). VKM participated in the 90 days public consultation of the application in 2012, and issued a preliminary scientific opinion and submitted relevant scientific comments or questions to EFSA (VKM 2012a). In August 2013 the application was withdrawn by Monsanto.

MON 89034 has also been evaluated by the VKM GMO Panel as a component of several stacked GM events (VKM 2008b, 2009a,b, 2010a,b). At the request from the Norwegian Environment Agency the VKM GMO Panel also submitted a final environmental risk assessment of MON 89034 for food and feed uses in 2013 (VKM 2013).

Terms of reference

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

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

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

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

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

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

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

Assessment

1 Introduction

The genetically modified maize MON 89034 was developed to provide protection against certain lepidopteran insect larvae, including European corn borer (*Ostrinia nubilalis*) and Mediterranean corn borer (*Sesamia nonagrioides*).
Insect protection is achieved through expression in the plant of two insecticidal Cry proteins, Cry1A.105 and Cry2Ab2, derived from *Bacillus thuringiensis*, a common soil bacterium. Cry1A.105, encoded by the *cry1A.105* gene, is a chimeric protein made up of different functional domains derived from three wild-type Cry proteins from *B. thuringiensis* subspecies *kurstaki* and *aizawai*. The Cry2Ab2 protein is encoded by the *cry2Ab2* gene derived from *B. thuringiensis* subspecies *kurstaki*. The Cry proteins exert their effect on the host insect by causing lysis of midgut epithelial cells, which leads to gut paralysis, cessation of feeding and eventual death of the insect. The lysis of the midgut epithelial cells is mediated by the binding of the activated Cry protein to specialised receptors on these cells.

MON 89034 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 2006, 2011a), the environmental risk assessment of GM plants (EFSA 2010a), the selection of comparators for the risk assessment of GM plants (EFSA 2011b), and for the post-market environmental monitoring of GM plants (EFSA 2006, 2011c).

The environmental risk assessment of the GM maize MON 89034 is based on information provided by the applicant in the applications EFSA/GMO/NL/2007/37 and EFSA/GMO/BE/2011/90, and scientific 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.

In line with its mandate, VKM emphasised that assessments of 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, shall not be carried out by the Panel on Genetically Modified Organisms.

2 Molecular characterisation

2.1 Information related to the genetic modification

Maize event MON 89034 was developed through *Agrobacterium*-mediated transformation of the proprietary inbred maize line LH172 (Eggerling 1994) using the transformation vector PV-ZMIR245. MON 89034 expresses the Cry1A.105 and Cry2Ab2 insecticidal proteins that confer tolerance to certain lepidopteran insect pests.

2.1.1 Description of the methods used for the genetic modification

MON 89034 was developed through *Agrobacterium*-mediated transformation of maize to produce the *Bt* insecticidal proteins Cry1A.105 and Cry2Ab2 using the binary plasmid vector PV-ZMIR245 (Figure 1 (2), Appendix 1). PV-ZMIR245 contains two separate transfer DNAs (T-DNAs). The first T-DNA, designated as T-DNA I, contains the *cry1A.105* and the *cry2Ab2* expression cassettes. The second T-DNA, designated as T-DNA II, contains the *nptII* expression cassette that encodes the neomycin phosphotransferase enzyme that confers tolerance to certain antibiotics such as neomycin, kanamycin and paromomycin. The use of two separate T-DNAs enables the generation of marker free plants by allowing insertion of the T-DNA with the traits of interest (T-DNA I) and the T-DNA with the selectable marker (T-DNA II) into two independent loci within the maize genome. Following selection of the transformants, the inserted T-DNA encoding the selectable marker can be segregated from progeny through subsequent breeding and genetic selection, while the inserted T-DNA containing the traits of interest is maintained.

Freshly isolated immature maize embryos were used in the transformation and production of MON 89034. *Agrobacterium tumefaciens* strain ABI, containing plasmid PVZ-MIR245 was made virulent by the use of acetosyringone. Strain ABI also contains a helper plasmid that does not contain any T-DNA but allows for the transfer of T-DNA I and T-DNA II to the plant cells. Each T-DNA was integrated into the plant genome at separate loci. Following an incubation period on a co-culture medium, the immature embryos were transferred to a selection medium containing carbenicillin to eliminate *Agrobacterium*, and paromomycin to eliminate cells that were not transformed, so that only cells containing T-DNA II and/or T-DNA I + TDNA II survived. The resulting transformed cells were then subcultured several times on a selection medium and regenerated into the R0 plants. During subsequent breeding at the F1 generation, the unlinked insertions T-DNA I and T-DNA II were segregated. The plants that had only the insert containing the *cry1A.105* and *cry2Ab2* gene cassettes (T-DNA I) were selected using molecular analysis, while the plants containing the *nptII* cassette (T-DNA II) were eliminated from subsequent breeding. The absence of the *nptII* gene and the NPTII protein was further confirmed by both Southern blot and ELISA analyses.

2.1.2 Nature and source of vector used

Plasmid vector PV-ZMIR245 is a binary *Agrobacterium tumefaciens* transformation vector that contains sequences that are necessary for transfer of T-DNA into the plant cell. These sequences are contained in the Right and Left Border regions which flank both T-DNA I and T-DNA II allowing an independent integration of each T-DNA into the plant genome during transformation. The T-DNA I region containing the *cry1A.105* and *cry2Ab2* gene expression cassettes is the portion of plasmid PV-ZMIR245 maintained in MON 89034.

2.1.3 Source of donor DNA, size and intended function of each constituent fragment of the region intended for insertion

T-DNA I

The cry1A.105 gene and Cry1A.105 protein

The *cry1A.105* coding sequence encodes the 133 kDa Cry1A.105 insecticidal protein that provides protection against feeding damage by lepidopteran insect pests. The Cry1A.105 is a modified Bt Cry1A protein with amino acid sequence identity to Cry1Ab, Cry1Ac and Cry1F proteins of 90.0%, 93.6% and 76.7%, respectively. The Cry1A.105 protein consists primarily of domains I and II from Cry1Ab or Cry1Ac (these proteins share 100% amino acid sequence identity in domains I and II), domain III from Cry1F, and substantially the entire C-terminal domain of Cry1Ac. Figure 2 provides a schematic representation of Cry1A.105.

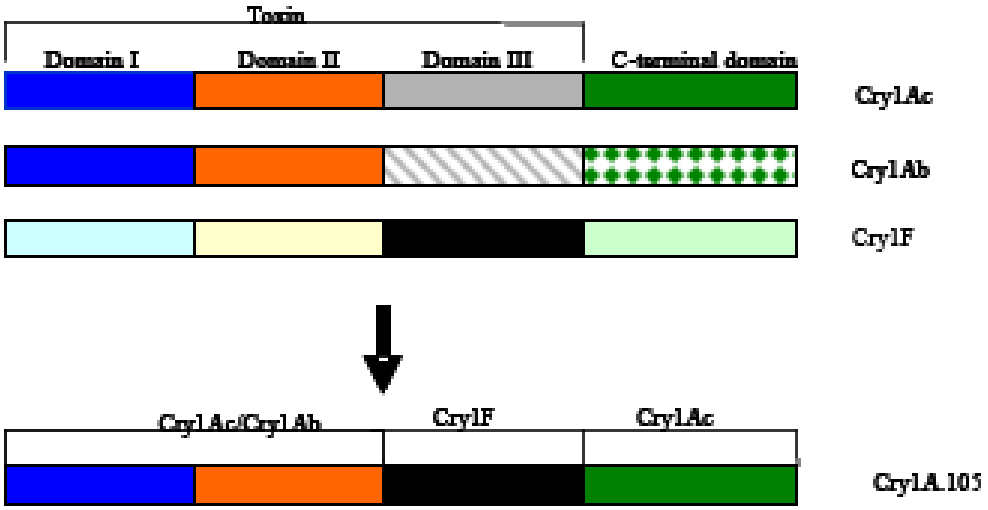

Figure 2. Schematic representation of the Cry1A.105 protein domain similarity to Cry1Ac, Cry1Ab and Cry1F

The cry1A.105 regulatory sequences

The expression cassette for the coding sequence of the Cry1A.105 protein consists of the promoter (P-e35S) and leader for the cauliflower mosaic virus (CaMV) 35S RNA containing a duplicated enhancer region. It contains the 5' untranslated leader of the wheat chlorophyll a/b/ binding protein (L-*Cab*), the intron from the rice actin gene (*I-Ract1*), the *cry1A.105* coding sequence that was optimised for expression in monocots, and the 3' nontranslated region of the coding sequence for wheat heat shock protein 17.3 (T-*Hsp17*), which terminates transcription and provides the signal for mRNA polyadenylation (Table 1).

The cry2Ab2 gene and Cry2Ab2 protein

The Cry2Ab2 protein present in MON 89034 is a member of the Cry2Ab class of proteins that share more than 95% amino acid sequence homology. It is a variant of the wild type Cry2Ab2 protein isolated from *Bacillus thuringiensis* subsp. *kurstaki*.

The cry2Ab2 regulatory sequences
The *cry2Ab2* gene expression cassette that produces the Cry2Ab2 protein consists of the 35S promoter from figwort mosaic virus (P-*FMV*) and the first intron from the maize heat shock protein 70 gene (I-*Hsp* 70). It also contains a *cry2Ab2* coding sequence with a modified codon usage (CS-*cry2Ab2*) fused to a chloroplast transit peptide region of maize ribulose 1,5-biphosphate carboxylase small subunit including the first intron (TS-*SSUCTP*). The 3' nontranslated region of the nopaline synthase (T-*nos*) coding region from *Agrobacterium tumefaciens* T-DNA terminates transcription and directs polyadenylation (Table 1).

Table 1. Description of the gene inserts in MON 89034

***cry1A.105* expression cassette**	
P-e35S	promoter and 9 bp leader for the cauliflower mosaic virus (CaMV) 35S RNA
L-Cab	5' untranslated leader of the wheat chlorophyll a/b/ binding protein. Not expressed in the plant
ract1 intron	intron from the rice actin gene
CS-cry1A.105	modified Bt Cry1A protein with amino acid sequence identity to Cry1Ab, Cry1Ac and Cry1F proteins
T-Hsp17	3' nontranslated region of the coding sequence for wheat heat shock protein 17.3. Terminates transcription and provides the signal for mRNA polyadenylation. Not expressed in the plant.
***cry2Ab2* expression cassette**	
P-FMV	promoter from figwort mosaic virus
I-Hsp 70	first intron from the maize heat shock protein 70 gene
TS-SSU-CTP	chloroplast transit peptide region of maize ribulose 1,5-biphosphate carboxylase small subunit including the first intron
cry2Ab2	coding sequence with a modified codon usage (CS-cry2Ab2) from *Bacillus thuringiensis subsp. kurstaki*
T-nos	3'nontranslated region of the nopaline synthase (T-nos) coding region from *Agrobacterium tumefaciens*. Terminates transcription and directs polyadenylation. Not expressed in the plant.

T-DNA II

nptII gene and NPTII protein
The *nptII* gene encodes the neomycin phosphotransferase II enzyme (NPTII) that inactivates certain aminoglycoside antibiotics such as kanamycin, neomycin and paromomycin.

nptII regulatory sequences
The *nptII* gene cassette that produces the NPTII protein consists of the promoter (P-*35S*) from the cauliflower mosaic virus (CaMV) 35S RNA. The sequence coding for the NPTII protein is followed by the 3' nontranslated region of the nopaline synthase (T*nos*) coding region from *Agrobacterium tumefaciens* T-DNA that ends transcription and directs polyadenylation.

T-DNA borders

The Right and Left Border regions each contain a border sequence that is a 24-26 bp sequence that defines the extent of the DNA that should be transferred into the plant genome. They flank both

TDNA I and T-DNA II, allowing for independent transfer and integration of each T-DNA into the plant genome during transformation. The Right Borders present in PV-ZMIR245 are made of a 24 bp nucleotide sequence originally derived from plasmid pTiT37 isolated from *A. tumefaciens*. The Left Borders present are made of a 25 bp nucleotide sequence from the *A. tumefaciens* plasmid pTi5955, a derivative of plasmid pTiA6.

Genetic elements outside the T-DNA borders

The backbone region outside of the inserted DNA contains two origins of replication necessary for replication and maintenance of the plasmid in bacteria. In addition, it contains a bacterial selectable marker gene, *aad*, which encodes an aminoglycoside-modifying enzyme that confers resistance to the action of the antibiotics spectinomycin and streptomycin.

2.2 Information relating to the GM plant

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

MON 89034 produces the Cry1A.105 and Cry2Ab2 insecticidal proteins that protects the plant from feeding damage caused by certain lepidopteran insect pests, e.g. the European corn borer (ECB, *Ostrinia nubilalis*) and the Mediterranean Corn borer (MCB, *Sesamia nonagrioides*). According to the applicant the Cry1A.105 protein provides increased activity against fall armyworm (FAW, *Spodoptera* sp.) and black cutworm (BCW, *Agrotis ipsilon*) compared to Cry1Ab. In addition it is also stated that the Cry2Ab2 protein provides improved control over Cry1Ab products from damage caused by corn earworm.

2.2.2 Information on the sequences actually inserted or deleted

Molecular analyses have been performed by the applicant to characterise the DNA inserted in MON 89034. Genomic DNA was digested using restriction enzymes and subjected to Southern blot analyses to determine: the insert number (number of insertions of the integrated DNA within the maize genome), the copy number (the number of copies of the integrated DNA within one locus), the intactness of T-DNA I, the presence or absence of the elements of T-DNA II, the presence or absence of *nptII* coding sequence and the presence or absence of plasmid backbone sequences. Schematic representation of the linear DNA derived from T-DNA I of vector PV-ZMIR245 inserted in MON 89034, including restriction enzyme sites and expected restriction fragments, is shown in Figure 3. A description of the genetic elements inserted, including the approximate size and function is provided in Table 2.

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

Insert and copy number analysis
Southern blot analyses were performed to assess insert sites and copy number of the DNA inserted in MON 89034. The insert site was evaluated by digesting the test and control DNA with *Nde* I, a restriction enzyme that does not cleave within T-DNA I. This enzyme generates a restriction fragment containing T-DNA I and adjacent plant genomic DNA. The number of restriction fragments detected indicates the number of insert sites present in MON 89034. The number of copies of the T-DNA present in MON 89034 was determined by digesting test and control genomic DNA samples with *Ssp* I, which cleaves once within the insert. If MON 89034 contains one copy of the insert, probing with overlapping T-DNA I should result in two bands, each representing a portion of the insert along with adjacent, plant genomic DNA.

According to documentation from the applicant the results confirm that MON 89034 contains one insert located on ~13 kb *Nde* I restriction fragment. The MON 89034 DNA digested with *Ssp* I produced two bands in addition to the endogenous background hybridisation observed in conventional maize control DNA. The ~8.2 kb band is the expected size for the border fragment containing the 5' end of the inserted DNA (corresponding to T-DNA I) along with the adjacent genomic DNA flanking the 5' end of the insert (Figure 3). The ~7.4 kb band, which was expected to be >4.3 kb, represents the 3' border fragment containing the 3' end of the inserted DNA along with the adjacent genomic DNA flanking the 3' end of the insert. According to the documentation provided MON 89034 contains only one copy of T-DNA I that resides at a single locus of integration on ~13 kb *Nde* I restriction fragment.

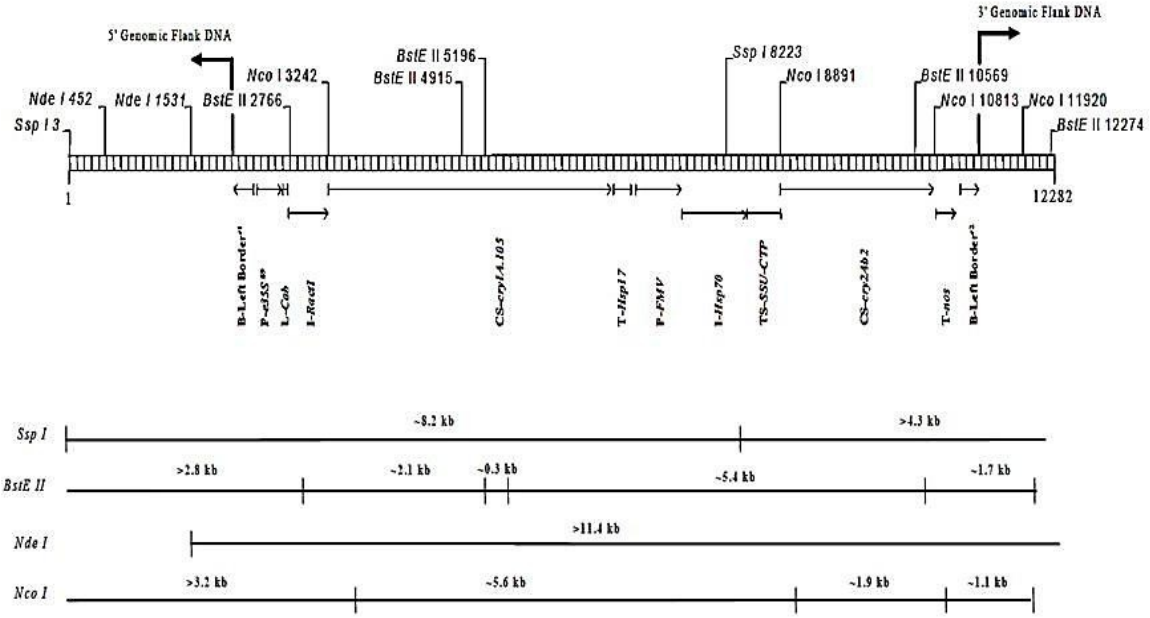

Figure 3. Schematic representation of the insert and genomic flanking sequences in MON 89034.
The linear DNA derived from T-DNA I of vector PV-ZMIR245 which was incorporated into MON 89034 is shown. Arrows in black indicate the end of the insert and the beginning of maize genomic flanking sequence. Identified on the map are genetic elements within the insert, as well as restriction sites with positions relative to the size of the linear map for enzymes used in the Southern blot analyses. Shown on the lower portion of the map are the estimated locations of the T-DNA probes and the expected sizes of the DNA fragments after digestion with the respective restriction enzymes. A portion of Left Border sequence and a *e35S*^{89} promoter sequence is present at the 5' insert-to-flank junction in MON 89034

Table 2. Summary of genetic elements inserted in MON 89034

Genetic Element	Size (~kb)	Function (Reference)
B-Left Border r1	2061-2299	239bp DNA region from the B-left Border region remaining after integration.
P-*e35S*^{89}	2350-2651	Modified *e35s* promoter and 9 bp leader resulting from a recombination between the P-*e35s* and P-*35s* promoters. Differing from *e35S* in that it does not contain the duplicated enhancer element
L-*Cab*	2678-2738	5' untranslated leader of the wheat chlorophyll a/b-binding protein (Lamppa *et al.*, 1985)
I-*Ract1*	2755-3234	Intron from the rice actin gene (McElroy *et al.*, 1991)
CS-*cry1A.105*	3244-6777	Coding sequence for the *Bacillus thuringiensis* Cry1A.105 protein (Monsanto unpublished data)
T-*Hsp17*	6809-7018	3' nontranslated region of the coding sequence for wheat heat shock protein 17.3, which ends transcription and directs polyadenylation (McElwain and Spiker, 1989)
P-*FMV*	7086-7649	Figwort Mosaic Virus 35S promoter (Rogers, 2000)
I-*Hsp70*	7672-8475	The first intron from the maize heat shock protein 70 gene (Brown and Santino, 1995)
TS-*SSU-CTP*	8492-8892	DNA region containing the targeting sequence for the transit peptide region of maize ribulose 1,5-bisphosphate carboxylase small subunit and the first intron (Matsuoka *et al.*, 1987)
CS-*cry2Ab2*	8893-10800	Coding sequence for a Cry2Ab2 protein from *Bacillus thuringiensis* (Donovan, 1991; Widner and Whiteley, 1989). This coding sequence uses a modified codon usage
T-*nos*	10827-11146	3' termination sequence of the nopaline synthase (*nos*) coding sequence from *Agrobacterium tumefaciens* which terminates transcription and directs polyadenylation (Bevan *et al.*, 1983)
B-Left Border r2	11147-11377	230 bp DNA region from the B-Left Border region remaining after integration

B - border region, P – promoter, L – leader, I – intron, CS - coding sequence,T - transcript termination sequence
TS - targeting sequence

According to documentation provided by the applicant, the molecular characterisation of MON 89034 by Southern blot analyses show that the DNA is inserted into the maize genome at a single locus and that the insert contains single functional copies of the *cry1A.105* and *cry2Ab2* expression cassettes. The data further demonstrates that no additional elements were detected other than those present in T-DNA I.

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

PCR and sequence analysis were performed on genomic DNA extracted from MON 89034 and conventional control to confirm the integrity of the inserted DNA in MON 89034, the organisation of the elements within the MON 89034 DNA insert; to demonstrate that the DNA sequences flanking the 5' and 3' ends of the insert in MON 89034 are native to the maize genome; and to characterise the insertion site in conventional maize. The DNA sequencing analyses complement the Southern blot analyses. A bioinformatics evaluation was also performed to determine if any endogenous genes were disrupted by the insertion of the *cry1A.105* and *cry2Ab2* expression cassettes present in MON 89034 or whether genes from the maize genome are present in the flanking genomic DNA and adjacent to the T-DNA after transformation.

Analysis of the insert structure by PCR

The organisation of the elements within the insert in MON 89034 was established using PCR analysis by amplifying seven overlapping regions of DNA that span the entire length of the insert. The generation of the predicted size PCR products from MON 89034 show that the arrangement or linkage of elements in the insert are the same as those in plasmid PV-ZMIR245 and that the elements within each gene cassette are arranged as depicted in the schematic of the insert in Figure 3.

Sequence analysis of the insert

According to the applicant, MON 89034 insert sequence - analyses confirm that both the *cry1A.105* and *cry2Ab2* coding sequences are identical to those of the corresponding genes in PV-ZMIR245. It is also shown that the e35S promoter that regulates expression of the cry1A.105 gene has been modified into a shorter promoter version, e35S^{89} (differing from e35S in that it does not contain the duplicated enhancer element) and that the Right Border region present in PV-ZMIR245 was replaced by a Left Border region. This molecular rearrangement is explained by a recombination event having taken place either before or during the process of T-DNA transfer to the plant cell genome (Figure 4). According to the applicant this modification did not affect any of the coding regions of the insert and enabled sufficient expression of the Cry1A.105 protein.

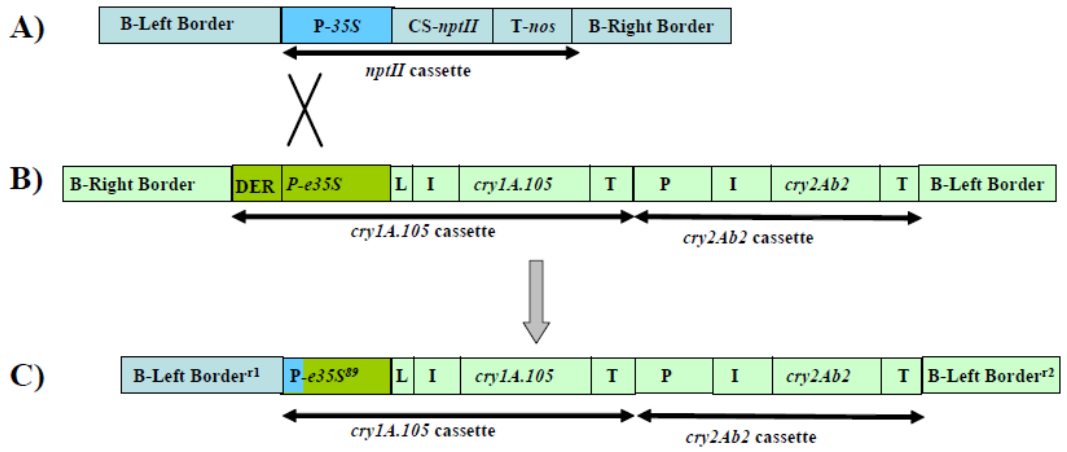

Figure 4. Description of the recombination process that explains the modified 5'end of the insert

A) Illustration of the T-DNA II of plasmid PV-ZMIR245

B) Illustration of the T-DNA I of plasmid PV-ZMIR245

C) Illustration of the modified T-DNA in MON 89034

Abbreviations and symbols: DER = duplicated enhancer region; L = leader sequence; I = intron sequence; P = promoter; T = termination sequence.

Molecular structure at the insertion site

PCR analysis was performed on genomic DNA extracted from MON 89034 and conventional maize to demonstrate that the DNA sequences flanking the 5' and 3' ends of the insert in MON 89034 are native to the maize genome. A sequence comparison between the product generated in conventional maize and the 5' and 3' flanking sequence generated previously from MON 89034 indicated that a 57 bp deletion occurred in MON 89034 upon insertion of the DNA. Additionally, MON 89034 was shown to contain a 10 bp sequence that is not present in the conventional maize genome at the insertion site of MON 89034, immediately adjacent to the 5' insert-to-plant junction. From this analysis, it was concluded that the DNA sequences flanking the 5' and 3' ends of the insert in MON 89034 are native to the maize genome.

The results show that only the two proteins, Cry1A.105 and Cry2Ab2, are encoded by the DNA insert present in MON 89034. No endogenous genes were found in the analysed sequences that flank the MON 89034 T-DNA insertion site. It is unlikely that any endogenous genes were disrupted at the insertion site.

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

The analysis of the molecular structure at the insertion site identified a 57 bp deletion in MON 89034 upon insertion of the DNA. MON 89034 was also shown to contain a 10 bp sequence that is not present in the conventional maize genome at the insertion site of MON 89034, immediately adjacent to the 5' insert-to-plant junction.

2.3 Information on the expression of the inserted sequences

Information on the protein expression of MON 89034 was previously described in application for authorisation of maize MON 89034 for import, processing, and food and feed use in the EU, according to Regulation (EC) No 1829/2003 (EFSA-GMO-NL-2007-37). The data of the current application is intended to complement the data presented in application EFSA-GMO-NL-2007-37, and the same information that was provided within the context of this application, is presented in the current application (2005 US field season and 2004 Argentina field season (Hartmann et al. 2006a,b)). Data from these protein expression studies have previously been assessed by the VKM GMO Panel (VKM 2008). In addition, protein expression analyses performed on tissue samples of MON 89034 collected from seven field trials conducted in Germany and Spain in 2007 are enclosed with the current application EFSA-GMO-BE-2011-90 (Niemeyer & Silvanovich 2008).

2.3.1 Methods used and reference to raw data of Cry1A.105 and Cry2Ab2 protein analysis

Cry1A.105 and Cry2Ab2 protein expression levels were determined by a validated enzyme-linked immunosorbent assay (ELISA) in tissues collected from MON 89034. The ELISA method used was optimised to minimise method bias. Protein extracts from the test substance were analysed by ELISA with the appropriate protein standard and inter-assay negative and positive controls (Hartmann et al. 2006a,b; Niemeyer & Silvanovich 2008).

Field sites were selected that represent the major maize growing region of the US, Argentina and Europe, and which provide a range of environmental conditions that would be encountered during commercial production. At each site, three replicated plots of MON 89034 maize (the [LH172 $BC0F_7$ x LH198]F_{1H} and LH172 $BC0F_6$ x F_{2H1}generations, see Figure 5) were grown along with a conventional hybrid maize variety with a similar genetic background to the test plants. In the European field trials, two different germplasms were included in the study, adaptet to northern (Germany) and southern (Spain) European growing regions, respectively.

Over season leaf (OSL 1-4), over season root (OSR 1-4), over season whole plant (OSWP 1-4), forage, stover[1], forage-root, senescent root, pollen, silk and grain were collected from each replicated plot at all field sites. The over season leaf and whole plant samples were collected four times at four different growth stages: (1) V2 – V4 stage (2-4 leaf stage); (2) V6 – V8 stage (6-8 leaf); (3) V10 – V12 (10-12 leaf) stage; and (4) pre-VT stage (pre-tasseling). The over season root samples were collected at: (1) V2 – V4 stage; (2) V6 – V8 stage; (3) V10 – V12 stage; (4) pre-VT stage; (5) early dent stage; and (6) after harvest. Pollen and silks were collected at approximately 100-120 days after planting and grain was harvested at maturity. Stover was collected following harvest at approximately 130 – 160 days after planting.

[1] stalk and leaf material remaining after harvest

2.3.2 The range and mean values for the levels of Cry1A.105 and Cry2Ab2 protein

Tissue samples for analysis were collected from US, Argentinean and European field trials conducted in 2005, 2004 and 2007, respectively. Data from the different studies are presented in table 3 and table 4 & 5 in Appendix 1). Limits of detection and quantification are presented in Table 6 for all studies.

USA 2005
Tissue samples for analysis were collected from five field trials conducted in the USA in 2005 (Hartmann et al. 2006a). Field sites were selected to represent geographical regions where maize is grown for commercial purposes. A randomised complete block design with three replicated plots of MON 89034, as well as the conventional control, was planted at each test site. The following tissues were collected and analysed: over season leaf (OSL-1-4), over season root (OSR-1-4), over season whole plant (OSWP-1-4), forage, stover (leaves and stalks of maize), forage root, senescent root, silk, pollen, and grain. The over season samples (leaf, root, and whole plant) were collected four times at different growth stages: (1) V2 – V4, (2) V6 – V8, (3) V10 – V12, and (4) pre-VT. All protein levels for all tissue types were calculated on a microgram (µg) per gram (g) fresh weight (fw) basis. Moisture content was then measured for all tissue types and all protein levels were converted and reported on a dry weight (dw) basis. Results are presented in Table 3 (Appendix 1).

Cry1A.105 protein levels
In tissues harvested throughout the growing season, Cry1A.105 levels across all sites ranged from 27 to 850 µg/g dwt in leaf, 20 to 570 µg/g dwt in whole plant and 6.2 to 110 µg/g dwt in root. In forage, pollen and grain, Cry1A.105 levels ranged from 20 to 56, 8.5 to 16 and 4.7 to 7.0, respectively.

Cry2Ab2 protein levels
In tissues harvested throughout the growing season, Cry2Ab2 levels across all sites ranged from 48-270 µg/g dwt in leaf, 5-230 µg/g dwt in whole plant, and 13-100 µg/g dwt in root. In forage, pollen and grain, Cry2Ab2 levels ranged from 15 to 55, 0.49 to 0.79 and 0.77 to 2.1, respectively. In general, levels of the Cry1A.105 and Cry2Ab2 proteins declined over the growing season

Argentina 2004
Data was collected from studies performed at five field sites in Argentina during the 2004 season (Hartmann et al. 2006b). The Cry1A.105 and Cry2Ab2 protein levels obtained from these sites are presented in Table 4 (appendix 1). The means for Cry1A.105 protein levels across all sites were 2.6 µg/g dwt in grain, 30 µg/g dwt in forage, 7.7 µg/g dwt in pollen, 260 µg/g dwt in OSL-1, 200 µg/g dwt in OSL-4, 28 µg/g dwt in forage root, and 19 µg/g dwt in stover. In tissues harvested throughout the growing season, mean Cry1A.105 protein levels across all sites ranged from 160 – 260 µg/g dwt in leaf, 22 – 71 µg/g dwt in root, and 48 – 170 µg/g dwt in whole plant. The means for Cry2Ab2 protein levels across all sites were 0.95 µg/g dwt in grain, 45 µg/g dwt in forage, 0.56 µg/g dwt in pollen, 120 µg/g dwt in OSL-1, 270 µg/g dwt in OSL-4, 31 µg/g dwt in forage root, and 44 µg/g dwt in stover. In tissues harvested throughout the growing season, mean Cry2Ab2 protein levels across all sites ranged from 120 – 270 µg/g dwt in leaf, 23 – 48 µg/g dwt in root, and 61 – 98 µg/g dwt in whole plant.

Europe 2007
Tissue samples for analysis were collected from seven field trials conducted in Europe in 2007 (three in Germany and four in Spain) (Niemeyer and Silvanovich 2008). Field sites were selected to represent geographical regions where maize is grown commercially. There were two germplasms for this study, the first was adapted to northern European growing regions (Germany), and the second was adapted to the southern European growing regions (Spain). At each site, MON 89034 as well as the conventional control, were planted using a randomised complete block field design, with three replications. Over season leaf (OSL 1-4), over season root (OSR 1-4), over season whole plant (OSWP 1-4), forage, stover, forage root, senescent root, pollen, silk, and grain tissues were collected from each replicated plot at all field sites. The over season samples (leaf, root, and whole plant) were collected

four times at different growth stages: (1) V2 – V4, (2) V6 – V8, (3) V10 – V12, and (4) pre-VT. ELISA methods were developed and validated for each protein. Protein levels for all ten tissue types were calculated on a microgram (µg) per gram (g) fresh weight (fwt) basis. Moisture content was then measured for all tissue types and all protein levels were converted and reported on a dry weight (dwt) basis. Levels of Cry1A.105 and Cry2Ab2 proteins are summarised in Table 5 (Appendix 1).

Cry1A.105 protein levels
The mean concentration of Cry1A.105 in MON 89034 maize was highest in tissue samples from whole plant early in the growth season (V2-V4 stage; 240 µg/g dwt), with the mean level in pollen and grain being 24 µg/g dwt and 3.4 µg/g dwt (Table 5, Appendix 1). The mean Cry1A.105 protein levels across all sites were 130 µg/g dwt in OSL-1, 44 µg/g dwt in OSR-1, 7.4 µg/g dwt in forage-root, 60 µg/g dwt in OSWP-3, 31 µg/g dwt in forage, 24 µg/g dwt in pollen, and 3.4 µg/g dwt in grain.

Cry2Ab2 protein levels
The mean Cry2Ab2 protein levels in MON 89034 across all field sites were 250 µg/g dwt in leaf samples from growth stages V6-V8, 30 µg/g dwt in forage root, 49 µg/g dwt in forage, 0.59 µg/g dwt in pollen and 1.8 µg/g dwt in grain (Table 5, Appendix 1). In tissues harvested throughout the growing season, mean Cry2Ab2 protein levels at all sites ranged from 71-250 µg/g dwt in leaf, 23-33 µg/g dwt in root and 48-150 µg/g dwt in whole plant. Data on over season protein levels is not presented in this report but in general shows that the levels of both proteins declined over the growing season.

According to the applicant the overall range of the observed protein levels for Cry1A.105 and Cry2Ab2 were all spanning the range of the relative control in the USA, Argentinean and European field trials.

2.3.3 Part of the plant where the insert is expressed

Cry1A.105 and Cry2Ab2 proteins were found to be expressed in leaf, root, pollen, silk, forage, forage root, grain, stover and senescent root at appropriate times of plant development. Grain and forage are the most relevant tissues for the food and feed safety assessment of MON 89034, while leaf, root, pollen, silk and stover are relevant tissues in terms of environmental risk assessment

2.3.4 Expression of potential fusion proteins

Bioinformatic analyses of open reading frames found within the DNA spanning the 5´ and 3´ junctions between the MON 89034 inserted DNA and the maize genomic DNA were performed. The purpose of the assessment was to evaluate the potential for novel open reading frames (ORFs) that may produce proteins with similarity to known allergens and toxins. DNA sequences spanning the 5' and 3' inserted DNA-maize genomic DNA junctions in MON 89034 were translated from stop codon to stop codon in all six reading frames. The putative flank polypeptides encoded by the 5´ and 3´ junctions of the MON 89034 insertion site were subjected to similarity searches using:
1) FASTA and eight amino acid sliding window search of the 2011 allergen, gliadin and glutenin sequence database (AD_2011)
2) FASTA search of the 2011 protein database (PRT_2011)
3) FASTA search of the 2011 toxin database (TOX_2011) that was selected using a keyword search and filtered to remove likely non-toxin proteins.

According to the applicant, the FASTA and eight amino acid sliding window search analyses showed that no biologically relevant sequence similarities were observed between allergens, toxins or other biologically active proteins and the 10 putative sequences translated from junctions which were used in the queries against the respective databases. Furthermore that the results of the studies indicate that if any of the hypothetical fusion proteins were to be expressed in MON 89034, none would share

significant similarity or identity to known allergens, toxins, or other biologically active proteins that could affect human or animal health.

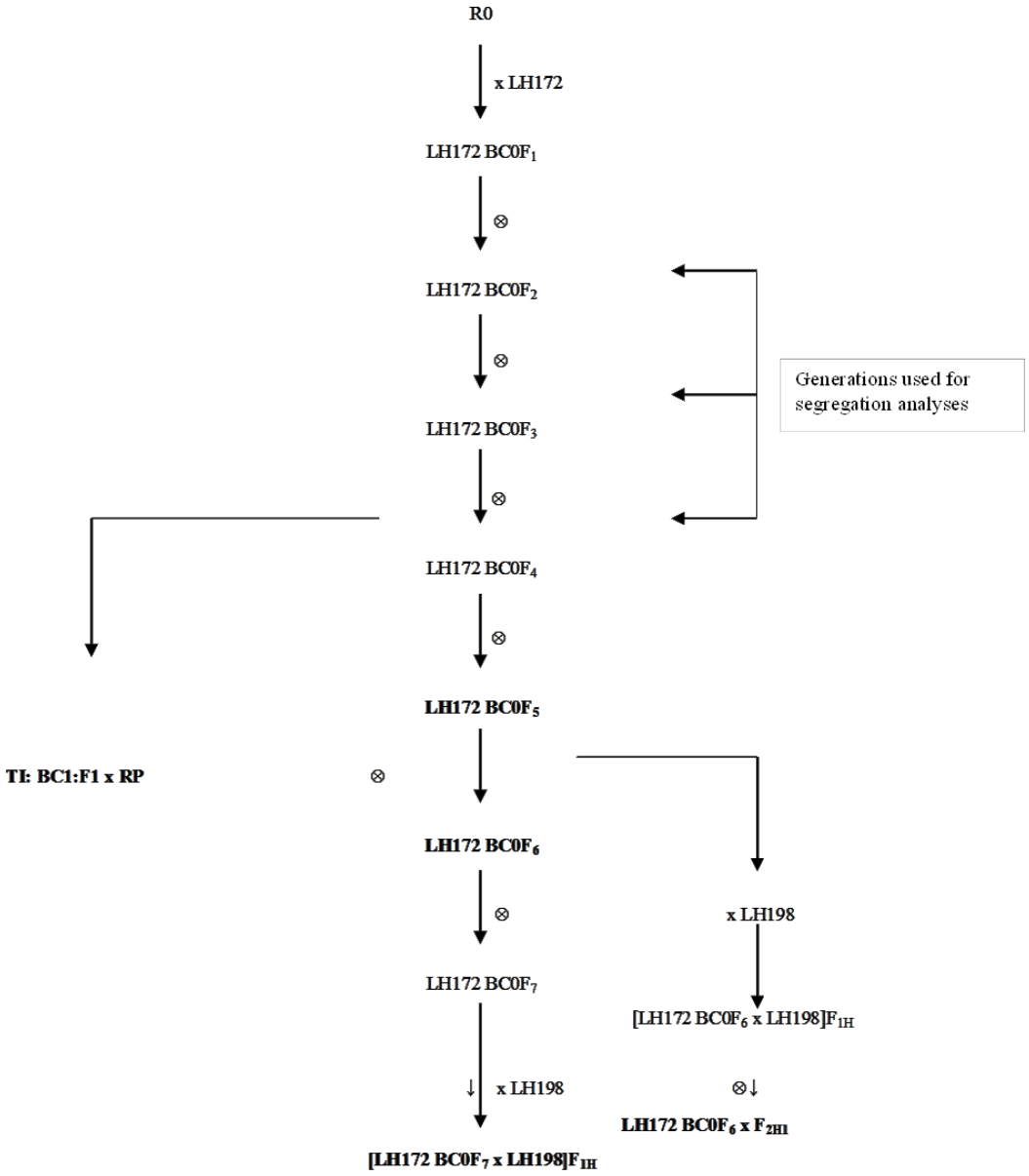

Figure 5. **Breeding history of maize MON 89034**
The LH172 $BC0F_6$ x F_{2H1} generation was used for all the molecular analyses. Generations in bold were used for molecular stability analyses.
Key: R_0 = primary transformant; F(#) = filial generation; ⊗ = self-pollination; BC(#) = backcross generation; RP = recurrent parent; H = hybrid; TI = trait integration.

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

A number of analyses were done to demonstrate the stability of the genetic changes in MON 89034. Segregation analysis over multiple generations was done to determine the heritability and stability of the new trait (the *cry1A.105* and *cry2Ab2* genes, and Cry1A.105 and Cry2Ab2 proteins). Southern blot analysis over multiple generations was done to determine the stability of the inserted DNA.

2.4.1 Genetic stability of the insert in MON 89034

Genetic stability of the inserted DNA was investigated by Southern blot analyses of genomic DNA isolated from multiple generations of MON 89034 maize (see Figure 5; the generations used are in bold). For these analyses, DNA samples were digested with *Ssp* I which cleaves once within the inserted DNA and in both the 5' and 3' genomic flanking regions. This produces two DNA fragments of ~8.2 and >4.3 kb. The stability of the inserted DNA was confirmed using overlapping T-DNA I probes spanning the entire inserted DNA sequence. Genomic DNA isolated from maize with the same genetic background as MON 89034 was used as a negative control, and was also spiked with DNA from PV-ZMIR245 to serve as a positive hybridisation control.The results showed that the single copy of T-DNA I in MON 89034 was stable in all selected generations. In addition, none of the generations tested contained any T-DNA II elements or backbone sequences from PV-ZMIR245

2.4.2 Phenotypic stability of the insect-resistance trait in MON 89034

Significance of the segregation pattern was assessed by a Chi-square test of inheritance data over four generations of MON 89034 maize to determine the heritability and stability of the new traits (Table 7, Appendix 1). The confirmation of the presence of the gene and stability of the trait was based on: (i) ELISA to detect the Cry2Ab2 and Cry1A.105 proteins; (ii) GeneCheck immunoassays to detect Cry2Ab2 protein (Cry2A QuickStix Lateral Flow test strips, Envirologix Inc., Portland, MN) and PCR assay to detect the presence of the *cry1A.105* and *cry2Ab2* genes. The Chi-square test is based on testing the observed segregation ratio of the Cry proteins to the ratio that is expected according to Mendelian principles as shown in Table 8 (Appendix 1).

The results of the Chi-square test[2] are summarised in Table 8 (Appendix 1). All Chi-square values indicate no significant differences between observed and expected genetic ratios across all tested generations of MON 89034 maize. These results are consistent with the molecular characterisation data indicating a single site of insertion for the *cry1A.105* and *cry2Ab2* gene expression cassettes.

2.5 Conclusion

Appropriate analyses of the transgenic DNA insert, its integration site, number of inserts and flanking sequences in the maize genome, have been performed. The results show that only one copy of the insert is present in maize MON 89034. Homology searches with databases of known toxins and allergens have not indicated any potential production of harmful proteins or polypeptides caused by the genetic modification in maize MON 89034. Southern blot analyses and segregation studies show that the introduced genes *cry1A.105* and *cry2Ab2* are stably inherited and expressed over several generations along with the phenotypic characteristics of maize MON 89034. The VKM GMO Panel concludes that the molecular characterisation of maize MON 89034 does not indicate a safety concern. Event MON 89034 and the physical, chemical and functional characteristics of the proteins have previously been evaluated by The VKM Panel on Genetically Modified Organisms, and considered satisfactory (VKM 2008a, 2013).

[2] Computed as $\chi^2 = \Sigma [(| o - e | - 0.5)^2/e]$ where o = observed frequency of the genotype, e = expected frequency of the genotype, and 0.5 = Yates correction factor for analysis with one degree of freedom (Little & Hills 1978).

3 Comparative assessment

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

The applications EFSA/GMO/NL/2007/37 and EFSA/GMO/BE/2011/90, covering food and feed uses, cultivation, import and processing of maize MON 89034 within the EU, presented compositional, agronomic and ecological data on seed and forage material collected in field trials performed in major maize producing regions in the USA, Argentina and Europe.

3.1.1 Experimental design

Compositional studies
In the compositional studies, field trials were carried out at five different geographical sites in the USA in the 2004 growing season and five sites in Argentina in 2004/2005. In these trials, maize MON 89034 was compared to the non-transgenic maize hybrid LH198 x LH172, which is a conventional maize hybrid with background genetics similar to maize MON 89034. In addition to the comparator, 15 commercially available maize hybrids were included in the field trials (3 varieties at each site). These maize varieties were used as reference material to estimate the naturally occurring variation in composition expected for the various analytes in conventional maize. Materials for the compositional analyses were collected from each field trial site and constituted grains and forage. At each field site, the test, control and reference seed were planted in a randomised complete block design with three replicates per block. All plants were grown under normal agronomic field conditions for their respective geographic regions.

Phenotypic and agronomic studies
USA
Phenotypic and agronomic data were collected from 18 field locations over two consecutive years in the USA: nine locations in 2004 and nine locations in 2005. According to the applicant, these locations provided a range of environmental and agronomic conditions representative of major US maize-growing regions where commercial production of MON 89034 would be expected. The number of field trial sites varied however from 1 to 18 depending on the specific character investigated.

The MON 89034 maize lines used for this study were hybrids between the F_6 and F_7 generations and a conventional inbred maize line, LH198 (see Figure 5). The maize line used as the comparator for this study is a conventional LH198 x LH172 hybrid, which has a genetic background representative of the MON 89034 hybrid lines, but without the transferred genes.

Commercially available maize hybrids were also included in the study as reference lines to provide data for the development of a 99% tolerance interval for each component analysed. The commercial hybrids used were all conventional lines. In the 2004 field trials 23 reference varieties were included in the study, while 12 and 14 commercially maize hybrids were included in the 2005 field trials (Study -1 & -2).

Plots were established at each of the field sites in a randomised complete block design with three replications. Each plot consisted of two to six rows of maize spaced approximately 75 cm apart and approximately 5-3-6.1 m in length. All the maize lines at each of the field sites were grown under normal agronomic field conditions for their respective geographic regions.

Europe

Phenotypic and agronomic data were collected from eight field locations in Europe, five in Spain and three in Germany in the 2007 growing season. According to the applicant, these locations provided a range of environmental and agronomic conditions representative of the northern and southern European maize growing regions where commercial production of MON 89034 is expected. In these field trials genetically modified maize MON 89034 was compared with a conventional counterpart having a comparable genetic background. Event MON 89034 was introgressed into two different genetic backgrounds; DKC3945 adapted to northern (Germany) and DKC5143 adapted to southern (Spain) European growing regions. The control substances included in the field trials were conventional maize DKC3945 (Germany) and DKC5143 (Spain). DKC3945 and DKC5143 have genetic backgrounds similar to the test plants grown in Germany and Spain, respectively, except for the insect-protection trait. 15 conventional, commercial available maize hybrids with similar relative maturities as the test and control substances were included in the comparative assessments to verify whether any differences observed between the GMO and its comparator fall within the range of natural variation. Six locally adapted conventional hybrids were used in Germany, and nine different locally adapted hybrids were grown in Spain.

Plots were established at each site in a randomised complete block design with three replications. Each plot consisted of six rows spaced approximately 70 cm apart and approximately 6-10 m in length. Rows 4 and 5 were designated for phenotypic and ecological interaction data, while row 3 and 6 were used as buffer rows. Agronomic practices used to prepare and maintain each study were characteristic of the respective region. Maintenance pesticides were applied as needed at the field sites. Pesticides containing *Bt* were not applied at any of the sites.

3.1.2 Statistical analysis

Analysis of variance for each site was conducted according to a randomised complete block design with three replications (mixed model analysis). SAS® (Version 9.2) was used to compare the test substance MON 89034 to its conventional counterpart within each site (by-site analysis) and for the combination of all five sites (i.e., the combined-site analysis). In the European study, separate combined site analyses were conducted for Germany and Spain. The level of statistical significance was predetermined to be 5% ($p \leq 0.05$). No statistical comparisons were made between the test and reference substances. For the phenotypic data, the reference range (minimum and maximum mean values) was determined from the reference varieties across the sites, for each country.

3.2 Compositional analysis

From the field trials performed in the United States in 2004 and Argentina in 2004/2005, data on chemical composition of maize MON 89034, conventional control and reference varieties were provided for plant material from each individual location. Analyses of forage material included proximates (protein, fat, ash, and moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), minerals (calcium, phosphorus), and carbohydrates. The composition of grains was analysed with respect to proximates (protein, fat, ash, and moisture), ADF, NDF, total dietary fibre (TDF), amino acids, fatty acids (C8-C22), vitamins (B_1, B_2, B_6, E, niacin, and folic acid), anti-nutrients (phytic acid and raffinose), secondary metabolites (furfural, ferulic acid, and p-coumaric acid), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and carbohydrates.

Altogether, 77 different analytical components were analysed. In total 9 components were analysed in forage and 68 in grain. Several of the compounds analysed occurred at levels below the limit of quantification. When more than 50% of the analytical data points were below the limit of quantification, which was the case for 16 of the 77 compounds analysed, no statistical analyses were performed. Statistical analyses of the remaining 61 components were conducted for comparison of

MON 89034 with conventional control maize. Table 9 and Table 10 (Appendix 1) summarises results of the compositional analyses of MON 89034 for all sites combined.

Field trials USA (2004)
A total of 90 samples were analysed from the US field trials. In summary, the comparison between forage and grains of MON 89034 and non GM-control showed statistical significant differences for 3 of the 61 compounds investigated when data from all locations were combined (Table 9 & 11, Appendix 1). In grains, increased levels of ***stearic*** and ***arachidic acid*** were observed in maize MON 89034 compared to the conventional control (1.89 vs. 1.82% and 0.39 vs. 0.38% of total fat, respectively) ($p < 0.01$ and $p < 0.001$). Likewise, the ***phosphorous*** level in forage from MON 89034 was increased compared to the concentional control (0.25 vs. 0.21% dw) ($p \leq 0.01$). All observed differences were small, and fell within the natural variation of conventional reference varieties included in the field trials, and within the ranges reported for maize in the literature (ILSI 2006).

When the compositional data were analysed for each separate site, 44 statistically significant differences were observed. Of these differences 33 were observed at one field site only. Remaining statistical significances (for arachidic acid, carbohydrates, copper and iron) were observed at two or three of the five sites (Table 11, Appendix 1). For the parameters carbohydrate and iron, the difference was small and not consistent – in one case increased, in the other decreased. The arachidic acid level was slightly higher in maize MON 89034 than in the control (0.38-0.41% vs 0.37-0.39%) at three of the five sites, but fell within the range of conventional reference varieties (0.32-0.47%). Copper levels were higher in MON 89034 than in its control at two sites. Also in this case mean levels of MON 89034 were well within the range of natural variation.

Field trials Argentina (2004/2005)
A total of 88 forage and grain samples were analysed from the field trials performed in Argentina in the growing season 2004/2005. Statistical evaluation of compositional data from these studies revealed five significant differences when the data was analysed across field sites (Table 10 & 11, Appendix 1). In grain, the statistically significant differences included increased levels of ***eicosenoic acid*** (0.29 vs 0.30%), ***manganese*** (6.81 vs 6.28 mg/kg dw) and ***vitamin B2*** (1.75 vs 1.94 mg/kg dw), as well as decreased levels of ***stearic acid*** (1.84 vs 1.79%), and ***ferulic acid*** (1894 vs 1759 µg/g dw) in maize MON 89034 as compared to its control.

For eicosenoic acid and ferulic acid the statistical difference was not noted at any of the single sites when the statistical analysis was performed per site (Table 12, Appendix 1). Stearic acid was found to be increased at one of the five sites, whereas manganese was increased at two of five sites, and vitamin B_2 was reduced at two of five sites. All the average values showing statistical significant differences were within the 99% tolerance interval of the reference substances and fell within the historical and literature ranges (ILSI 2006).

3.3 Agronomic and phenotypic characters

During field trials conducted over several seasons and different locations, phenotypic and agronomic data related to dormancy and germination, emergence and vegetative growth, reproductive growth and yield characteristics were collected. A description of evaluated phenotypic and agronomic characteristics and the designated developmental stages when evaluations occurred are listed in Table 13 (Appendix 1). In addition, the applicant has presented observational data from studies of plant environmental interactions several times during the growing seasons. The purpose of these evaluations was to assess whether plant response to abiotic and biotic stressors were altered compared to control maize. The evaluations of ecological interactions include plant response to abiotic stressors (e.g. drought, frost, wind, flood damage, nutrient deficiency, etc), disease damage and arthropod damage (Table 14, Appendix 1).

Field trials USA (2004, 2005)
The field studies in North America gave information on germination, dormancy, emergence, vegetative growth, reproductive growth (including pollen characteristics, seed retention on the plant, phenotypic and agronomic characteristics), and plant interactions with insects, diseases and abiotic stressors (e.g. drought, wind, nutrient deficiency). The following phenotypic characteristics were evaluated; seedling vigour, early stand count, days to 50% pollen shed, days to 50% silking, stay green, ear height, plant height, dropped ears, stalk lodging, root lodging, final stand count, grain moisture, test weight, and yield.

In the combined site analysis, statistically significant differences between maize MON89034 and its control plant were noted for plant height (2.14 vs 2.17 m) and stalk lodging (0.8 vs 2.4) ($p<0.05$) in 2004. No statistical differences were however observed for these parameters in the 2005 growing season. As the plant height of maize MON 89034 and the identified reduction in stalk lodging also fell within the range of conventional maize hybrids, the VKM GMO Panel is of the opinion that this statistical finding is of no biological relevance.

Of the 11 insect categories, 12 disease categories and eight abiotic stressors evaluated in the studies of ecological interactions in the 2004 growing season, only a few differences between maize MON 89034 and its comparator were noted at particular growth stages and trial sites. No consistent differences were observed. In 2005 more insect and disease categories and abiotic stressors were evaluated, but no indication of a different response between maize MON 89034 and its comparator was noted.

Field trials Europe (2007)
Results from the combined-site phenotypic comparisons of MON 89034 to the control for the field trials in Europe in 2007 are presented in Table 15 (Appendix 1). Minimum and maximum mean values (reference range) observed among 15 commercially available reference maize hybrids provide benchmark values common to maize for each characteristics. In the combined-site analysis for the German trial, no statistical differences were detected between MON 89034 and its conventional counterpart for the entire parameters measured (Table 15, Appendix 1). In the combined-site analysis for Spain, significant differences were detected between MON 89034 and the control for the parameter "stalk logded plants" ($p\leq0.05$). There were fewer stalk logded plants in the MON 89034 plots than in the control plots (0.0 vs. 0.5, respectively). However, mean values for MON 89034 for stalk lodging fall within the range of the reference varieties included in the study, and these differences are unlikely to have biological significance in terms of increased pest potential. Furthermore, less stalk lodging does not represent a change in the plant that would confer an increase in weediness potential. It is likely that the change in stalk lodging is a direct agronomic consequence of the presence of the lepidopteran protection trait in MON 89034. For the other parameters evaluated, no statistical differences were detected between the test line and the conventional counterpart in Spain.

Plots were rated for specific biotic (i.e. insect and disease) and abiotic (drought, wind nutrient deficiency etc.) stressors commonly occurring at each site (Table 14, Appendix 1). According to the applicant, no artificial infestation or inference was used. Therefore, the same stressors were not necessarily observed at each field site. Ecological interactions were assessed qualitatively by visual scoring (scale 0-9). Stressor variables which included sufficient variability were subjected to statistical analysis.

Results from evaluations of ecological stressors damage in the European field trials indicate no qualitative differences between MON 89034 and the conventional counterpart with respect to biotic and abiotic stressors (Appendix 1, Table 16. No overall differences were observed across sites between MON 89034 and the control in their susceptibility or tolerance to the ecological stressors assessed.

Based on 253 comparative observations recorded over two years in the USA, no consistent differences were observed across sites between MON 89034 and the control in their susceptibility or tolerance to the ecological stressors assessed during the 2004 and 2005 field trials. These results support the conclusion that compared to the conventional maize, the ecological interactions between MON 89034 and insects, diseases and abiotic stressors were not altered except for the introduced lepidopteran-protection trait.

3.4 Conclusion

Comparative analyses of maize MON 89034 to its non-GM conventional counterpart have been performed during multiple field trials in representative areas for maize cultivation in USA, Argentina and Europe in 2004, 2005 and 2007. With the exception of small intermittent variations, no biologically significant differences were found between maize MON 89034 and the conventional non-GM control. Based on the assessment of available data, the VKM GMO Panel concludes that maize MON 89034 is compositionally, agronomical and phenotypically equivalent to its conventional counterpart, except for the introduced characteristics.

4 Food and feed safety assessment

Maize MON 89034 and stacked events with MON 89034 have previously been evaluated by the VKM GMO Panel, and updated risk assessments were finalised in 2008 and 2009 (VKM 2008a,b; 2009 a,b,c).

4.1 Product description and intended uses

The genetic modification in maize MON 89034 will not impact the existing production processes used for maize. All MON 89034 maize products will be produced and processed for use in food, animal feed and industrial products in the same way as other commercial maize. Maize MON 89034 and all food, feed and processed products derived from maize MON 89034 are expected to replace a portion of similar products from commercial maize, with total consumption of maize products remaining unchanged. The total anticipated intake/extent of use of maize and all food, feed and processed products derived from maize will remain the same.

4.2 Effects of processing

Food manufacturing of MON 89034 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 Cry1Ab.105 and Cry2Ab2 proteins (Dien et al. 2002; Hammond & Jez 2011). These food processes will also lead to extensive degradation and substantial reduction of DNA-size, which will also apply to the *cry1Ab.105* and *cry2Ab2* genes (Gryson 2010 (review); Fernandes et al. 2013a). Concentrations of these proteins and genes will be below the limit of detection in wet-milled fractions, in maize chips and maize oil. Baking of the maize bread broa containing 11% of TC1507 maize flour and 20% MON810 maize flour, showed that the baking process sheared the DNA into small fragments, less than 1000 base pair (bp), the main part of sheared DNA was about 200 bp (Fernandes et al. 2013b). In the unprocessed grain and all dry-milled fractions the transgenic proteins and DNA will probably be found in quantifiable amounts.

4.3 Toxicological assessment

4.3.1 Toxicological assessment of the newly expressed protein

The VKM GMO Panel has previously evaluated the proteins Cry1Ab.105 and Cry2Ab2 in the risk assessments of MON 89034 (VKM 2008a,b, 2009a,b).

4.3.1.1 Acute oral toxicity studies

Information from VKM-secretariat: Following the OECD Council decision, the test guideline 401 'Acute Oral Toxicity' was deleted on 17th December 2002, and replaced by OECD test guideline No. 420.

Cry1A.105

The applicant has provided a single dose toxicity study on CD-1 mice with the Cry1A.105 protein (Bonnette 2005). The study was performed in general agreement with the US EPA, Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity (1998 and 2002); the OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects, Subsection 401, February 24, 1987(OECD 1987,

2001); and the EEC Part B: Methods for the Determination of Toxicity, B.1, No. L383 A/110, December 29, 1992.

Cry1A.105 produced by an *E. coli* culture was dosed by gavage as two doses separated by 4 hours (±20 minutes) to 10 females and 10 males (2072 mg/kg body weight). Two control groups were included in the study: a bovine serum albumin protein control, and a vehicle control. One male in the test protein group was moribund and sacrificed on day 1 due to a mechanical dosing error. All other mice survived the study. The mice were observed daily, weighed weekly, and food consumption was measured weekly. A gross necropsy examination was performed on all animals at the time of death or scheduled euthanasia (day 14).

There were no significant differences in body weight or body weight changes among the animals in the three groups during the study, and no treatment-related gross pathological findings were observed.
Since no indications of adverse effects were shown to be caused by Cry1A.105, even at this relatively high dose level, the applicant considers the Cry1A.105 protein not to be toxic. Also, the amino acid sequence comparisons showed no similarities between the Cry1A.105 and known toxic proteins in protein databases that could raise a safety concern.

Cry2Ab2
The applicant has provided two acute toxicity studies on CD-1 mice. In both studies the Cry2Ab2-protein was produced by *E. coli*.

Study I
Three groups of ten male and ten female mice were dosed by oral gavage with 30, 300, or 1000 mg/kg bodyweight of Cry2Ab2 protein (US EPA 2010). The study was performed according to the US EPA OPPTS 870.1100 Acute oral toxicity health effects test guidelines (US EPA 2002). Two negative control groups were included in the study: bovine serum albumin protein control, and a vehicle control (purified water). All mice survived the study. All animals were euthanised and necropsied on day 14. There were no significant differences between the test and control groups. The results indicate no adverse effects of Cry2Ab2 protein at an exposure level of up to 1000 mg/kg body weight.

Study II
The applicant has provided a single dose toxicity study on CD-1 mice with the Cry2Ab2 protein (Bonnette 2006). The study was performed according to US EPA, Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity, December 2002 (US EPA 2002); the OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects, Subsection 401, February 24, 1987 (OECD 1987); and the EEC Part B: Methods for the Determination of Toxicity, B.1, No. L383 A/110, December 29, 1992.

Cry2Ab2 protein produced by an *E. coli* culture was dosed by gavage as two separated doses to 10 females and 10 males (2198 mg/kg body weight). Two control groups were included in the study: 20 mice (10/sex) were given a dose of 2424 mg/kg bovine serum albumin as protein control, and 20 mice (10/sex) recieved the vehicle (2 mM carbonate-bicarbonate, 2mM reduced glutathione buffer).
Body weights were recorded prior to fasting, prior to dosing, and on days 7 and 14. The test animals were observed for clinical signs of toxicity two times post-dosing and daily for 14 days. A general health/mortality check was done twice daily.
All mice survived the study, and were euthanised and necropsied on day 14. There were no significant differences in body weights or body weight changes among the three groups during the study.
The results indicate no adverse effects of Cry2Ab2 protein at an exposure level of 2198 mg/kg body weight.

4.3.1.2 Comment by the VKM GMO Panel on the use of acute toxicity studies in risk assessments of GMOs

The acute dose toxicity tests described above have not indicated toxic effects of the Cry1Ab.105 and Cry2Ab2 in mice. However, these tests do not provide enough information to conclude on possible adverse health effects of maize MON 89034. In whole food and feed 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 toxicity studies in risk assessments of GMOs (EFSA 2011a).

4.3.2 Toxicological assessment of the whole GM food/feed

A 90-day subchronic feeding study in rats
The applicant has provided a sub-chronic 90-day feeding study performed on Sprague-Dawley rats (Kirkpatrick 2007, unpublished Monsanto technical report). The study, designated WIL-50297, was conducted in compliance with the United States Environmental Protection Agency (EPA) Good Laboratory Practice Standards (40 CFR Part 160), 16 October 1989, and with the OECD guidelines for Testing of Chemicals, Health Effects Test Guidelines, Section 408, 21 September 1998 (OECD 1998).

The study was performed at the animal facility of WIL Research Laboratories, LLC, and according to the standard operating procedures of WIL Research Laboratories. The diet preparation was performed by TestDiet, a unit of PMI which is not a GLP facility. TestDiet is an ISO 9002-certified facility that is periodically inspected by Monsanto Quality Assurance Unit. As the diet preparation was not conducted according to Good Laboratory Practice (GLP) standards, the diet preparation was conducted under the guidance of Monsanto toxicologists.

The study included three groups, each consisting of 20 animals/gender/group. One group was administered a diet containing approximately 11% (w/w) of MON 89034 maize grain, supplemented with approximately 22% (w/w) of the conventional parental control maize LH198 x LH172. The second group was fed a diet containing approximately 33% (w/w) of MON 89034 maize grain. The protein levels of Cry1A.105 and Cry2Ab2 in grain were 1.9 - 7.0 and 0.7 - 3.1µg/g dry weight, respectively. The diet containing 33% (w/w) of maize corresponded to a maize intake of 24.8 g/kg bw/day for males and 28.9 g/kg bw/day for females. The estimated intake of the Cry1A.105 and Cry2Ab proteins for male rats were 175 µg/kg bw/day and 77 µg/kg bw/day, respectively. For female rats the estimated intake was 202 µg/kg bw/day and 90 µg/kg bw/day, respectively.

A third group received the conventional parental control maize LH198 x LH172 formulated into the diet at approximately 33% (w/w). All diets were formulated according to the specifications for Purina Mills International (PMI) Certified Rodent LabDiet #5002. The composition of the diet and its quality, including herbicide residues and mycotoxin levels, were controlled by analyses. The diets were provided *ad libitum* for a minimum of 90 days. All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily and detailed physical examinations weekly. Individual body weights and food consumption were recorded weekly during the dosing period.

Clinical pathology evaluations (hematology, serum chemistry and urinalysis) were performed on 10 males and 10 females of each group at the scheduled necropsy (study week 13). Complete necropsies were conducted on all animals and selected organs were weighed at necropsy. Selected tissues were examined microscopically from all animals fed diets containing 33% MON 89034 or 33% parental control maize.

No clinically relevant effects related to treatment were observed. One female animal fed the 33% MON 89034 maize diet, died on study day 14. According to the study report, the microscopic examinations at necropsy revealed findings indicative of urinary tract obstruction, but the presence of 5 mL fluid in the thoracic cavity was considered to be the most clinically significant finding. The cause of death was undetermined, but it was considered most likely to be incidental and unrelated to consumption of the test diet.

There were no relevant differences in body weight, body weight gain or feed consumption between the groups. Of the 26 hematology, 18 serum chemistry and 14 urinalysis parameters determined for each gender and dose, only the mean platelet count in females given the lowest dose (11% MON 89034) was statistically significantly different from the controls. The value of this parameter was within the range recorded in the provided historical control data. There was no dose-response, no alterations in related parameters, and this finding did not occur in male animals.

Monsanto considered this finding not to be related to the exposure to maize MON 89034. Organ weight determinations at necropsy showed no statistically significant differences between groups in males. No differences in absolute organ weights were found in females, however, a statistically significant reduced mean thyroid/parathyroid weight relative to final body weight (0.006g/100g vs 0.007/100g) was observed in females given the highest amount of maize MON 89034 (33%) compared to controls. The value was within the range of the provided historical control data, and there was no statistically significant alteration in thyroid/parathyroid weight relative to brain weight.

Microscopic analyses did not reveal histopathological alterations. The lower relative thyroid/parathyroid weight in females was considered by Monsanto to be an incidental finding. Microscopic findings in organs and tissues were almost equally distributed and no statistically significant differences between males and females of the high dose group and the controls were found. Numerically higher incidences of kidney alterations were found in two females of the 33% dose group. These findings were seen in two rats, one died at day 14 of unknown cause, the other survived to the end of the study. Combined, the two rats represented two of five findings of nephropathy, minimal/moderate transitional cell hyperplasia, minimal sub-acute inflammation and moderate hydronephrosis. The animal that died on day 14 showed mild papillary necrosis and minimal tubular necrosis. Both rats had urinary bladder calculi and the study pathologist concluded that the lesions observed most likely were linked to these calculi.

According to WIL Research Laboratories it seems unlikely that the urinary bladder calculi and associated kidney alterations could have been induced by the tested maize in 14 days. Also, a low incidence of urinary bladder calculi is known to occur in this rat strain and therefore considered a spontaneous finding in sub-chronic studies. The historical control data supplied in the application also shows the occurrence of urinary bladder calculi in female control rats (CD rats) of previous studies conducted within the WIL Research Laboratories.

The VKM GMO Panel therefore considers the urinary bladder calculi as well as the associated kidney alterations as incidental findings unrelated to maize MON 89034. The same applies to the female animals of the control group, which were diagnosed with nephroblastomas, a very rare tumour of the kidney.

Administration of maize MON 89034 for at least 90 consecutive days at levels up to 33% (w/w) in the diet indicated no adverse effects on the growth or health of Sprague-Dawley rats.

Additional whole food feeding studies that also consider health effects of maize MON 89034
The applicant has performed a 42-day broiler feeding study with emphasis on nutritional properties of maize MON 89034, which also considers health effects. Another feeding study has been performed on feedlot cattle. Both studies are described under section 4.5.2.

4.4 Allergenicity assessment

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 2010b). Most food allergies are mediated by IgE (type-I reaction).

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 with, and structural similarity to, known human IgE-allergens with an array of bioinformatic 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.

4.4.1 Assessment of IgE mediated allergenicity of the newly expressed protein

A weighted risk analysis based on the decision tree approach has been performed by the applicant. The individual steps of this analysis starts by analysing the primary amino acid sequences of the novel proteins and looking for similarities with sequences of known IgE allergens, followed by serum screens for IgE cross-reactions to known allergens. Digestibility of the proteins was tested in simulated gastric and/or intestinal fluids, and by animal studies (FAO/WHO, 2001, Codex Alimentarius, 2003, König et al. 2004, Poulsen 2004). The proteins Cry1A.105 and Cry2Ab2 present in maize MON 89034 have previously been evaluated and found unlikely to be IgE allergenic (US EPA 2010).

These assessments were described by the applicant for the maize event MON 89034 (EFSA-GMO-NL-2007-37) and were based on the following aspects:

Cry1A.105:

i) The source of the transgene, *Bacillus thuringiensis*, has no known history of causing allergy.

ii) The Cry1A.105 protein is chimeric, with an overall amino acid sequence identity to the Cry1Ac, Cry1Ab and Cry1F proteins of 93.6, 90.0 and 76.7%, respectively. These proteins are not considered common food allergens (US EPA 2010).

iii) Immunoblot and glycosylation analyses of Cry1A.105 derived from recombinant *E.coli* and from extracts of leaf material from transgenic MON 89034 maize, indicate that post-translational glycosylation of Cry1A.105 protein has not occurred (US EPA 2010).

iv) A comparison of the amino acid sequence of Cry1A.105 with known IgE allergens indicated no homology at the level of 8 contiguous amino acids (US EPA 2010).

v) The Cry1A.105 protein is rapidly degraded by simulated gastric fluids *in vitro*. Digestability of the Cry1A.105 protein in simulated intestinal fluid assay showed that 99.5% of the full-length protein was digested within 5 minutes (Kapadia & Rice 2005, US EPA 2010).

Cry2Ab2:

vi) The Cry2Ab2 protein is isolated from *Bacillus thuringiensis* strain EG7699. This protein is not considered a common food allergen (US EPA 2010).

vii) The produced Cry2Ab2 protein in MON 89034 is a single polypeptide with similar sequence identity to the wild type and a peptide mass of 61 kDa. The plant-produced protein sample had an additional immunoreactive band migrating at approximately 50 kDa; N-terminal amino acid analysis of this protein indicated that it is a truncated Cry2Ab2 protein with its N-terminus starting at amino acid 145 (MON 89034 dossier).

viii) Immunoblot and glycosylation analyses of Cry2Ab2 derived from recombinant *E.coli* and from extracts of leaf material from MON 89034 maize, indicate that post-translational glycosylation of Cry2Ab2 protein has not occurred (US EPA 2010).

ix) A comparison of the amino acid sequence of Cry2Ab2 to known allergens indicated no homology at the level of 8 contiguous amino acids (US EPA 2010).

x) The Cry2Ab2 protein is rapidly degraded by simulated gastric and intestinal fluids in vitro (Kapadia and Rice 2006, US EPA 2010).

xi) At 4°C, 25°C, and 37° C there was little or no effect on Cry2Ab2 bioactivity, while at 65°C there was some reduction in the bioactivity. At 95°C Cry2Ab2 protein was completely inactivated (US EPA 2010).

The information listed above indicates that the newly expressed proteins in maize MON 89034 lack IgE allergenic potential with regard to human and animal health. However, it does not cover possible allergic reactions (e.g. enteropathies) that are not IgE mediated.

4.4.2 Assessment of the allergenicity of the whole GM plant

Allergenicity of maize MON 89034 could be increased as an unintended effect of the random insertion of the transgenes in the genome of the recipient, e.g. through qualitative or quantitative modifications of the expression of endogenous proteins. However, given that no biologically relevant agronomic or compositional changes have been identified in maize MON 89034 with the exception of the introduced traits, no increased allergenicity is anticipated for maize MON 89034. Moreover, maize is not considered a common allergenic food.

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

It is the opinion of the VKM GMO Panel that a possible over-expression of any endogenous protein, which is not known to be allergenic, in maize MON 89034 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 2010b) adjuvants are substances that, when co-administered with an antigen increases 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 potential 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). 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 2009b, EFSA 2010b, VKM 2012d).

"Bystander sensitisation"

"Bystander sensitisation" can occur when an adjuvant in food, or an immune response against a food antigen, results in 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 may become leaky, allowing unwanted proteins to enter the body (bystander-penetration) and possibly 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 2012d).

4.5 Nutritional assessment of GM food/feed

Compositional analyses of maize MON 89034 indicate nutritional equivalence to the non-GM control maize with comparable genetic backgrounds, as well as several commercially available reference maize hybrids. The nutritional equivalence is further supported by the broiler and steer feeding studies described in section 4.6.2.

4.5.1 Intake information/exposure assessment

Net import of maize staple, e.g. flour, starch and mixed products, in Norway in 2007 was 7600 tons, corresponding to 4.4 g dry weight/person/day or an estimated daily energy intake for adults of 0.6%. The estimated median daily intake of sweet maize was 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 of 0.6% for a 6 month child (Vikse 2009, unpublished).

Since most foods and foodstuffs from maize are derived from field maize grains, an estimated maximum daily intake for a Norwegian adult of Cry1A.105 and Cry2Ab2 proteins from maize MON 89034 is calculated to be 26 µg and 13 µg, respectively, based on intake of maize staple (4.4 g/person/day) and the maximum protein levels in grain (kernel) at physiological maturity in Table 5 (Appendix 1). The estimated maximum daily intake of Cry1A.105 and cry2Ab2 proteins from sweet maize is calculated to be 103 µg and 53 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 toxicological tests.. Also, these levels are considerably below the proposed threshold of toxicological concern (TTC) level of 1800 µg/person/day (Class 1, oral exposure) for chemicals considered to have a low potential for toxicity based on metabolism and mechanistic data (Vermeire et al. 2010). Transgenic proteins produced by genetically modified plants are generally considered non-toxic to humans.

The VKM GMO Panel notes that farm (production) animals e.g. pigs and poultry often are fed diets with a substantial inclusion of unprocessed maize grain, and that the exposure to transgenic proteins from maize MON 89034 may be higher for these animals. This dietary exposure assessment is very conservative as it defines all maize as MON 89034, and assumes that the transgenic protein levels are not reduced by processing.

4.5.2 Nutritional studies

A 42-day feeding study on broiler chickens

A 42 day poultry (Ross x Ross 308) feeding study was carried out by the applicant to compare the nutritional properties of maize MON 89034 to its non-GM control: LH198 x LH172, and the four commercial maize varieties: ASGROW RX690, ASGROW RX772, DKC60-15, and DKC57-01 (Monsanto Study No. 05-01-50-13, unpublished, Davis 2006 **CI**). The main part of the study meets the FDA's Good Laboratory Practice Regulations for Nonclincal Laboratory Studies as specified in 21 CFR Part 58. Portions of the study meet the US EPA's Good Laboratory Practice Requirements as specified in 40 CFR Part 160.

The following aspects were not conducted under GLP: Semi-annual water analysis (total coliforms) by Stewart Environmental Consultants, Northern Colorado Water Association water testing, starter and grower/finisher diet formulations by Global Poultry Consulting, Inc., diet and meat sample analyses at the University of Missouri Experiment Station Chemical Laboratories, yearly scale licensing by the State of Colorado and test, control and reference maize grain containers were not retained for the duration of the study. According to the applicant these exceptions had no effect on the integrity or quality of the study.

Poultry studies are considered useful because chickens are fast growing animals that can consume large quantities of maize in the diet and are thus sensitive to potentially toxic effects of maize dietary components (OECD 2003b).

Three different diets were prepared for each of the maize lines: 1) Starter (approximately 55% maize grain), 2) Grower (approximately 59% maize grain), and 3) Finisher (approximately 59% maize grain). Maize grain was mixed with soybean oil cake (33.5% to 37.8%) and other nutrients with an increasing inclusion of maize from starter to finisher diets. Diets were also formulated to be isocaloric based on nutrient analyses performed before diet formulation. Feed and water was given *ad libitum*.

Prior to the study, maize samples were analysed for proximates, amino acids, pesticides and mycotoxins. The mycotoxin determinations showed only low levels of contaminations in the different maize grains: aflatoxins (not detected (ND)), ochratoxin (ND), fumonisins (ND in MON 89034, about 0.3-4 ppm in controls), zearalenone (ND in MON 89034, 271ppb in one control), T-2 and HT-2 toxin (ND) and deoxynivalenol (vomitoxin, 0.1 ppm in MON 89034, 1.6 ppm in two controls), citrinin (336 ppb in MON 89034).

The study followed a randomised complete block design with 5 blocks of 12 pens each. Birds were randomly assigned to the pens, with two extra birds in each pen during the first seven days to compensate for losses due to starv-outs, dehydration etc. Surplus birds were removed on day seven. There were six different treatments corresponding to the six maize lines tested. 100 birds were assigned to each treatment, 50 males and 50 females, a total of 600 birds. The birds were housed 10/pen/sex with a total of 5 pens/sex in each treatment group (10 birds x 2 (sex) x 5 pens x 6 treatments = 600 birds).

Birds were observed daily and were weighed by pen on study Day 0, at study end (Day 42), and individually immediately prior to slaughter for processing. Performance was determined by calculating the average weight gain per bird on Day 42. The average feed: gain ratio was calculated for Days 0 – 42 by dividing the total feed consumption by the total weight gain of surviving birds for each pen. The

adjusted feed:gain ratio was calculated by dividing the total feed consumption by the weight gain of surviving birds plus weight gain of birds that died or were removed from the pen. All surviving birds in each pen were slaughtered then processed for determination of carcass and meat characteristics. Statistical analysis was conducted on performance, carcass yield and meat quality parameters.

All treatments resulted in comparable and somewhat low mortality rates. Chickens fed diets containing maize MON 89034 showed no effect on total feed intake, feed conversion and growth across treatments. However, a small but statistically significant difference in adjusted feed conversion (a calculated parameter) in males was observed between broilers fed maize MON 89034 and control broilers (1.59 vs 1.64 kg/kg). The adjusted feed conversion was 1.52, 1.53, 1.54 and 1.61 kg/kg for the four conventional maize varieties. Compared to three of the four conventional maize varieties, the adjusted feed conversion was not significantly different in birds fed maize MON 89034. There were no difference in chilled carcass, fat pad, breast, thigh, drum and wing weight, and no difference in percentage of moisture, protein, and fat in thigh and breast meat between broilers fed maize MON 89034 and broilers fed the control maize.

No biologically relevant differences were observed in the parameters measured between broilers fed the MON 89034 diet and the control diet. For the individual treatment comparisons, broilers in general had similar performance values and carcass yield and meat composition, regardless of whether the diets contained maize grain from MON 89034, the conventional counterpart or commercial maize hybrids. The 42-day broiler feeding study showed that maize MON 89034 is nutritionally equivalent to the non-GM comparator and conventional maize varieties.

Study on feedlot cattle performance, feed conversion and carcass characteristics

Weber et al. (2011) have reported growth performance and carcass quality of finishing steers fed maize and mazie silage from either MON 89034, the parental non-GM maize hybrid DKC 63-78 (PAR), or the two reference maize hybrids DKC 61-42 (REF1) and DKC 62-30 (REF2). British x Continental crossbred steers (n = 240, initial body weight (BW) = 290 ± 14 kg) were used in a randomised complete block design experiment. Steers were adjusted to the final diet over 21 days, with ground alfalfa hay replacing maize. Prior to initiation of the study, samples of maize and maize silage were tested for the presence of mycotoxins. Small amounts of zearalenone and deoxynivalenol (vomitoxin) were found in maize silage from MON 89034 and the parental non-GM maize. Small amounts of deoxynivalenol were also detected in all samples of whole maize, as well as zearalenone and fumonisin B1 and B2 in DKC 62-30 (REF2). In all samples, the levels of mycotoxins detected were well below the level of concern for feeding studies. Ingredient and diet samples were collected weekly and sent to a commercial laboratory for nutrient analyses.

All steers were slaughtered on day 175. Hot carcass weight (HCW) and liver abscess data were recorded on the day of slaughter. After a 48-hour chill, USDA marbling score (so named because the streaks of fat in the meat resemble a marble pattern), 12th rib fat thickness, and lean meat area (LM) were collected. Hot carcass weights were used to calculate adjusted final BW by dividing HCW by a common dressing percentage of 63%. Average daily gain (ADG) and gain: feed (G:F) were determined from adjusted final BW. Data were analysed as G:F and reported as feed:gain (F:G).

One steer from the MON 89034 treatment group and one from the REF1 treatment group died from non-treatment related illnesses during the trial. One steer from REF1 was also removed from the study for a reason not related to the treatment. Data were analysed using the MIXED procedures of SAS (SAS Institute, Cary, N.C.). Pens were the experimental unit (6 pens/treatment). Block was treated as a fixed effect in the model, with 5 replications in each block (except for “heavy block” which only had one replication). Least square means were not presented due to adjustment for unequal replication of blocks. Arithmetic means were presented by treatment.

The study was blind to personell to avoid bias. Each hybrid was assigned a letter before beginning the trial. All treatments, silage bags, commodity bays, pen assignments, feed sheets, and observation documents were designated by letter to limit possible partiality to treatment.

No significant differences in average daily gain, feed:gain or carcass characteristics were observed among treatments. Due to a statistical difference in initial BW, initial BW was used as a covariate of analysis and unadjusted treatment means were reported. The average across all treatments for ADG and F:G were 1.69 kg and 2.72 kg, respectively. According to the authors, steers fed MON 89034 and REF1 had numerically better F:G ratios, with a tendency (P = 0.13) for more efficient feed conversion in steers fed REF1.

The results of the study indicate that there were no significant effects of maize MON 89034 compared to controls on the performance or carcass characteristics of the cattle, and that MON 89034 is nutritionally comparable to the non-GM maize.

4.6 Conclusion

A 90-day subchronic feeding study on rats, as well as whole food feeding studies on broilers and feedlot steers have not indicated any adverse effects of maize MON 89034, and shows that maize MON 89034 is nutritionally equivalent to conventional maize. The Cry1A.105 and Cry2Ab2 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 MON 89034 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1A.105 or Cry2Ab2 proteins will introduce a toxic or allergenic potential in food or feed based on maize MON 89034 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 2003a). 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 2003a), 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 2003a). 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 MON 89034 would be increased due to the insect resistance. Insect resistance against certain lepidopteran target pests provides a potential advantage in cultivation of MON 89034 under infestation conditions. It is considered very unlikely that maize MON 89034 plants or their progeny will differ from conventional maize cultivars in their ability to survive as volunteers until subsequent seasons, or to establish feral populations under European environmental conditions.

A series of field trials with maize MON 89034 have been conducted by the applicant at nine replicated field locations within major maize-growing areas of the USA over two years (9 locations in 2004 and 9 locations in 2005), and across eight representative EU maize growing locations in 2007 (5 locations in Spain and 3 locations in Germany). Information on phenotypic and agronomic characteristics of maize MON 89034 and its comparators was generated to compare their growth habit, vegetative vigour and reproductive characters. Several endpoints related to growth habit, vegetative growth, reproduction, yield and grain characteristics were measured (section 3.3).

The field trials did not show major changes in plant characteristics that indicate altered fitness, persistence and invasiveness of maize MON 89034 plants. No visually observable response to naturally occurring insects, diseases and/or abiotic stressors recorded during the growing season

provided any indication of altered stress responses of maize MON 89034 as compared with its conventional counterpart. Laboratory experiments, analysing seed dormancy and pollen morphology and viability, revealed no relevant differences in seed germination, pollen morphology or pollen viability characteristics between MON 89034 and its conventional counterpart.

The VKM GMO Panel is not aware of any scientific reports indicative of increased establishment or spread of maize MON 89034, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of maize MON 89034 are unchanged, insect resistance is 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 MON 89034 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 MON 89034. 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 2003a). 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 cultivation/production systems. The risk of pollen flow from maize volunteers is negligible under Norwegian growing conditions.

5.2.1 Plant to micro-organisms gene transfer

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

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 MON 89034 to unrelated species such as bacteria.

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

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

In conclusion, the VKM GMO Panel consider it is unlikely that the introduced genes from maize MON 89034 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 *cry1A.105* and *cry2Ab2* genes from MON 89034 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 MON 89034 (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 2003a).

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 MON 89034 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

MON 89034 is a second generation genetically modified insect resistant maize, and was developed to provide protection against a variety of target pests of the order Lepidoptera. Protection is achieved

through expression in the plant of two insecticidal Cry proteins, Cry1A.105 and Cry2Ab2, derived from *Bacillus thuringiensis*, a common soil bacterium. Cry1A.105, encoded by the *cry1A.105* gene, is a chimeric protein made up of different functional domains derived from three wild-type Cry proteins from *B. thuringiensis* subspecies *kurstaki* and *aizawai*. The Cry2Ab2 protein is encoded by the *cry2Ab2* gene derived from *B. thuringiensis* subspecies *kurstaki.*

Two Lepidoptera pests are primarily targeted by MON 89034; *Ostrinia nubilalis* (European corn borer, ECB) and *Sesamia nonagrioides* (Mediterranean corn borer, MCB). According to the applicant, the Cry1A.105 protein also provides increased activity against fall armyworm (*Spodoptera* spp.) and black cutworm (*Agrotis ipsilon*) compared to Cry1Ab. Further, the Cry2Ab2 toxin provides improved control over Cry1Ab products from damage caused by corn earworm (*Helicoverpa zea*).

The European corn borer is widely distributed in Europe covering the Iberian Peninsula, Czech Republic and Slovakia, southwest of France, northern Italy and the southern regions of Germany and Poland. The Mediterranean corn borer is present in the Mediterranean region (Andreadis 2011). There are ten reports of *O. nubilalis* in Norway, restricted to the counties of Vestfold, Telemark, Aust-Agder and Vest Agder. *Sesamia* spp., *Spodoptera frugiperda* or *H. zea* have not been reported in Norway. There are no reports of *O. nubilalis* attaining pest status in Norway, and the Plant Clinic (Planteklinikken) at Bioforsk has never received samples of this pest or plant material damaged by this pest (K. Ørstad pers. com.). Consequently, there are no insecticides authorised or previous applications for registrations of insecticides against this herbivore in Norway.

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

Considering the intended uses of maize MON 89034, 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 Cry1A.105 and Cry2Ab2 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 MON 89034, 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). 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 2008).

Data supplied by the applicant indicate that a limited amount of the Cry1A.105 and Cry2Ab2 protein enters the environment due to expression in the grains (range 1.97-7 µg/g d.w. and 0.77-3.2 µg/g d.w,

respectively). In addition, the data show that at least 99% of microbially produced Cry1A.105 and Cry2Ab2 protein was rapidly degraded in simulated gastric fluid (US EPA 2010).

In conclusion, the VKM GMO Panel considers that the exposure of potentially non-target organisms to the Cry1A.105 and Cry2Ab2 proteins is likely to be very low and of no ecological relevance.

5.5 Potential interactions with the abiotic environment and biochemical cycles

Considering the intended uses of maize MON 89034, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with the abiotic environment and biogeochemical cycles were not considered an issue by the VKM GMO Panel.

5.6 Conclusion

Considering the intended uses of maize MON 89034, 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 MON 89034.

Maize MON 89034 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 MON 89034. 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

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
Appropriate analyses of the transgenic DNA insert, its integration site, number of inserts and flanking sequences in the maize genome, have been performed. The results show that only one copy of the insert is present in maize MON 89034. Homology searches with databases of known toxins and allergens have not indicated any potential production of harmful proteins or polypeptides caused by the genetic modification in maize MON 89034. Southern blot analyses and segregation studies show that the introduced genes *cry1A.105* and *cry2Ab2* are stably inherited and expressed over several generations along with the phenotypic characteristics of maize MON 89034. The VKM GMO Panel concludes that the molecular characterisation of maize MON 89034 does not indicate a safety concern.

Comparative assessment
Comparative analyses of maize MON 89034 to its non-GM conventional counterpart have been performed during multiple field trials in representative areas for maize cultivation in USA, Argentina and Europe (2004, 2005 and 2007). With the exception of small intermittent variations, no biologically significant differences were found between maize MON 89034 and the conventional non-GM control. Based on the assessment of available data, the VKM GMO Panel concludes that maize MON 89034 is compositionally, agronomical and phenotypically equivalent to its conventional counterpart, except for the introduced characteristics.

Food and feed risk assessment
A 90-day subchronic feeding study on rats, as well as whole food feeding studies on broilers and feedlot steers have not indicated any adverse effects of maize MON 89034, and shows that maize MON 89034 is nutritionally equivalent to conventional maize. The Cry1A.105 and Cry2Ab2 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 MON 89034 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1A.105 or Cry2Ab2 proteins will introduce a toxic or allergenic potential in food or feed based on maize MON 89034 compared to conventional maize.

Environmental risk assessment
The scope of the application EFSA/GMO/NL/2007/37 includes import and processing of maize MON 89034 for food and feed uses. Considering the intended uses of maize MON 89034, 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 MON 89034.

Maize MON 89034 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 MON 89034. 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 MON 89034 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1A.105 and Cry2Ab2 proteins will introduce a toxic or allergenic potential in food or feed derived from maize MON 89034 compared to conventional maize.

The VKM GMO Panel likewise concludes that maize MON 89034, based on current knowledge, is comparable to conventional maize varieties concerning environmental risk in Norway with the intended usage.

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VKM (2010b) Foreløpig miljørisikovurdering av genmodifisert mais MON89034 x NK603 (EFSA/GMO/NL/2009/72). Uttalelse fra Faggruppe for genmodifiserte organismer 7.04.2010. Vitenskapskomiteen for mattrygghet, Oslo, Norge.
http://www.vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&6563=6566:3&Content_6504=6187:1770406::0:6566:15:::0:0

VKM (2012a) Foreløpig miljørisikovurdering av genmodifisert mais MON 89034 (EFSA/GMO/BE/2011/90). Uttalelse fra Faggruppe for genmodifiserte organismer 5.11.2012. Vitenskapskomiteen for mattrygghet, Oslo, Norge.
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VKM (2013) Endelig miljørisikovurdering av genmodifisert mais MON 89034 (EFSA/GMO/NL/2007/37). Uttalelse fra Faggruppe for genmodifiserte organismer 19.3.2013. Vitenskapskomiteen for mattrygghet, Oslo, Norge.
http://vkm.no/eway/default.aspx?pid=277&trg=Content_6504&Main_6177=6504:0:31,2365&Content_6504=6187:2013469::0:6569:1:::0:0

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Appendix 1

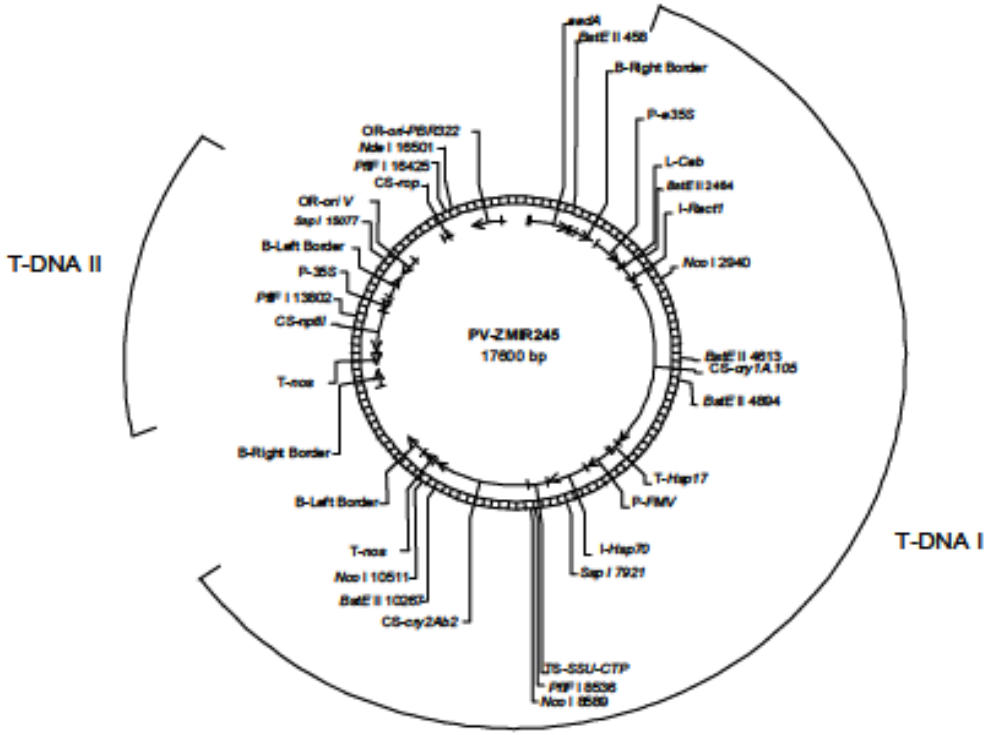

Figure 2. Plasmid map of vector PV-ZMIR245

A circular map of the plasmid vector PV-ZMIR245 used in *Agrobacterium*-mediated transformation to develop MON 89034 is shown. PV-ZMIR245 contains two T-DNA regions designated as T-DNA I and T-DNA II. In this procedure, only the DNA present between the left and right border regions was transferred into the host maize cells.

Table 3. Summary of Cry1A.105 and Cry2Ab2 protein levels in tissues from MON 89034 maize. From field trials in US in 2005

Tissue Type	Growth Stage	Cry1A.105 Mean (SD) [Range], n=15		Cry2Ab2 Mean (SD) [Range], n=15	
		µg/g fwt	µg/g dwt	µg/g fwt	µg/g dwt
Young leaf	V2 – V4	85 (21) [56 – 130]	520 (130) [380 – 850]	29 (6.8) [19 – 43]	180 (59) [94 – 270]
Pollen	R1 (silking)	6.4 (1.5) [3.8 – 8.8]	12 (1.7) [8.5 – 16]	0.34 (0.084) [0.21 – 0.47]	0.64 (0.091) [0.49 – 0.79]
Silk	R1 (silking)	3.0 (0.57) [2.0 – 3.8]	26 (3.9) [20 – 31]	8.2 (3.6) [3.3 – 16]	71 (35) [33 – 160]
Forage	R4 – R6 (early dent)	14 (3.6) [8.3 – 24]	42 (9.4) [20 – 56]	12 (4.0) [6.5 – 18]	38 (14) [15 – 55]
Forage root	R4 – R6 (early dent)	2.2 (0.35) [1.3 – 2.7]	12 (3.1) [6.2 – 16]	4.1 (1.4) [2.2 – 6.5]	21 (5.9) [14 – 33]
Grain	R6 (maturity)	5.1 (0.67) [4.1 – 6.0]	5.9 (0.77) [4.7 – 7.0]	1.1 (0.31) [0.67 – 1.8]	1.3 (0.36) [0.77 – 2.1]
Stover	R6 (after harvest)	17 (4.4) [9.5 – 26]	50 (17) [26 – 85]	22 (3.6) [17 – 29]	62 (15) [46 – 97]
Senescent root	R6 (after harvest)	2.2 (0.36) [1.7 – 3.1]	11 (1.4) [9.4 – 15]	5.3 (2.0) [2.4 – 9.1]	26 (8.8) [13 – 43]

Table 4. Cry1A.105 and Cry2Ab2 protein levels in maize tissues collected from MON 89034 produced in the 2004 Argentinean growing season.

Tissue Type[1]	Cry1A.105		Cry2Ab2	
	Mean (SD)[2] Range[3] (µg/g fwt)	Mean (SD) Range (µg/g dwt)	Mean (SD) Range (µg/g fwt)	Mean (SD) Range (µg/g dwt)
Over Season Leaf, OSL-1	38 (7.1) 26 – 53	260 (61) 150 – 350	17 (3.5) 11-22	120 (30) 64 – 160
OSL-2	32 (8.2) 21 – 50	160 (32) 110 – 210	26 (5.4) 17 – 38	130 (23) 83 – 160
OSL-3	34 (14) 14 – 63	160 (56) 60 – 270	46 (8.0) 35 – 59	210 (42) 150 – 280
OSL-4	47 (11) 30 – 69	200 (45) 130 – 300	65 (23) 41– 130	270 (76) 150 – 470
Over Season Root, OSR-1	8.6 (1.2) 5.7 – 10	71 (9.5) 53 – 89	5.7 (1.4) 2.7 – 8.1	48 (11) 25 – 63
OSR-2	4.8 (0.90) 3.6 – 6.7	32 (6.7) 22 – 44	6.0 (2.9) 2.6 – 11	38 (17) 19 – 74
OSR-3	4.1 (0.83) 3.0 – 5.7	28 (4.1) 23 – 35	3.4 (0.93) 2.0 – 5.2	23 (5.9) 13 – 32
OSR-4	3.3 (0.83) 2.0 – 4.4	22 (3.5) 17 – 28	3.9 (1.1) 2.5 – 5.8	26 (4.9) 19 – 34
Forage Root	5.0 (1.2) 3.8 – 7.6	28 (3.9) 20 – 36	5.6 (1.8) 4.0 – 9.3	31 (5.3) 25 – 40
Senescent Root	5.7 (1.6) 3.5 – 10	30 (8.5) 18 – 48	1.9 (0.72) 0.78 – 3.3	10 (4.4) 4.1 – 21
Over Season Whole Pl OSWP-1	17 (4.7) 10 – 26	170 (59) 71 – 270	8.1 (1.8) 4.7 – 11	80 (22) 43 – 130
OSWP-2	5.0 (0.73) 3.9 – 6.3	48 (8.6) 32 – 63	10 (1.7) 7.7 – 14	98 (12) 77 – 120
OSWP-3	6.8 (3.1)	60 (27)	7.2 (1.7)	61 (15)

	3.9 – 13	32 – 120	4.5 – 10	45 – 92
OSWP-4	8.8 (2.9) 2.8 – 13	72 (24) 23 – 110	8.9 (1.6) 5.4 – 12	73 (13) 45 – 98
Forage	8.6 (2.1) 5.4 – 13	30 (7.3) 19 – 41	13 (2.5) 9.2 – 17	45 (7.7) 33 – 61
Stover	6.1 (1.6) 3.9 – 8.9	19 (4.4) 11 – 26	13 (3.8) 5.4 – 19	44 (15) 13 – 64
Silk	3.5 (2.2) 2.5 – 11	41 (25) 25 – 130	5.2 (1.2) 2.8 – 6.6	61 (16) 31 – 79
Pollen	5.6 (0.71) 4.4 – 6.6	7.7 (0.90) 6.1 – 9.1	0.40 (0.077) 0.27 – 0.57	0.56 (0.089) 0.41 – 0.73
Grain 4	2.3 (0.30) 1.7 – 2.7	2.6 (0.36) 1.9 – 3.2	0.82 (0.13) 0.59 – 1.1	0.95 (0.16) 0.67 – 1.3

1 Tissues were collected at the following growth stages (Ritchie *et al.*, 1997):
OSL-1: V2 – V4; OSL-2: V6 – V8; OSL-3: V10 – V12;
OSL-4: pre-VT; OSR-1: V2 – V4; OSR-2: V6 – V8;
OSR-3: V10 – V12; OSR-4: pre-VT; Forage-root: early dent;
Senescent root: after harvest; OSWP-1: V2 – V4; OSWP-2: V6 – V8;
OSWP-3: V10 – V12; OSWP-4: pre-VT; Forage: early dent;
Stover: after harvest; Silk: at pollination; Grain: at physiological maturity;
Pollen: at pollination;
2 The mean and standard deviation were calculated across sites (n=19, except OSWP-3, n=15; forage, n=16; silk, n=25 and grain, n=18).
3 Minimum and maximum values were determined for each tissue type across sites.
4 Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis.
5 Protein levels are expressed as µg/g on a dry weight tissue (dwt) basis. The dry weight values were calculated by dividing the fwt by the dry weight conversion factors obtained from moisture analysis data.
6 The mean and standard deviation were calculated across sites (n=19, except OSWP-3, n=15; forage, n=16; pollen n=29 and grain n=18).

Table 5. Cry1A.105 and Cry2Ab2 protein levels in maize tissues collected from MON 89034 produced in the European field trials collected in 2007 (Germany and Spain)

Tissue type[1]		Growth stages	Cry1A.105 (µg/g dwt)[2]	Cry2Ab2 (µg/g dwt)
			Mean (SD) Range	Mean (SD) Range
Leaf	OSL-1	V2-V4	130(50) 85-240	180 (57) 110-280
	OSL-2	V6-V8	190 (44) 130-280	250 (100) 110-400
	OSL-3	V10-V12	59 (8.4) 45-73	91 (25) 42-130
	OSL-4	Pre-VT	120 (30) 55-150	71 (21) 35-110
Root	OSR-1	V2-V4	44 (12) 27-66	31 (9.6) 19-58
	OSR-2	V6-V8	36 (11) 16-56	33 (15) 4.4-65
	OSR-3	V10-V12	22 (3.9) 15-32	28 (11) 16-55
	OSR-4	Pre-VT	25 (3.8) 17-32	23 (5.7) 16-35
Forage root		Early dent	7.4 (1.9) 5.0-12	30 (9.0) 17-48
Senescent root		After harvest	20 (9.7) 5.8-32	39 (12) 18-62
Whole plant	OSWP-1	V2-V4	240 (54) 160-320	110 (23) 77-150
	OSWP-2	V6-V8	95 (37) 45-160	150 (89) 29-270
	OSWP-3	V10-V12	60 (17) 34-85	74 (18) 37-120
	OSWP-4	Pre-VT	31 (9.0) 17-55	48 (15) 31-90
Forage		Early dent	40 (6.3) 31-53	49 (15) 25-89
Stover		Harvest	29 (12) 12-52	44 (13) 25-72
Silk		Pollination	13 (5.4) 4.9-22	31 (13)
Pollen		Pollination	24 (4.5) 15-30	0.59 (0.32) 0.21-1.5

Grain	Physiological maturity	3.4 (1.2) 1.7-5.9	1.8 (0.70) 0.58-3.0

Table 6. ELISA limits of detection[1] and quantitation[2] for Cry1A.105 and Cry2Ab2 proteins – US, Argentina and European Field trials conducted in 2005, 2004 and 2007, respectively

Tissue Type	Cry1A.105		Cry2Ab2	
	LOD[1] (µg/g fwt)	LOQ[2] (µg/g fwt)	LOD (µg/g fwt)	LOQ (µg/g fwt)
Forage	0.372	0.44	0.191	0.44
Leaf	0.568	0.66	0.081	0.44
Pollen	0.412	1.1	0.055	0.11
Root	0.254	0.33	0.056	0.22
Silk	0.275	0.44	0.04	0.22
Grain	0.262	1.1	0.123	0.22

1 The limit of detection (LOD) was calculated as the mean value plus three SD using the data generated with conventional sample extracts for each tissue type. The LOD value in "ng/ml" was converted to "µg/g fwt" using the respective dilution factor and tissue-to-buffer ratio.

2 The limit of quantitation (LOQ) was calculated based on the lowest standard concentration. The "ng/ml" value was converted to "µg/g fwt" using the respective dilution factor and tissue-to-buffer ratio.

Table 7. Expected segregation ratios for MON 89034 maize generations

Generation[a]	Expected ratio[b]	Comment
LH172BC0F_1	n.a	Screened for copy number and absence of *nptII* (segregation data not shown)
LH172BC0F_2	3:1	Positive:negative (product of self-pollination)
LH172BC0F_3	1:0	Positive:negative (homozygous plant selection)
LH172BC0F_4	1:0	Positive:negative (homozygous plant selection)
LH172BC1F_1[c]	1:1	Positive:negative (product of backcrossing)
LH172BC1F_2[d]	3:1	Positive:negative (product of self-pollination)
LH172BC1F_2[d]	3:1	Positive:negative (product of self-pollination)

[a]See breeding tree in Figure 2., [b]n.a. = not applicable.
[c]To confirm segregation, LH172BC0F1 plants were backcrossed to the recurrent parent (LH172) to produce this generation (not shown in the breeding tree, Figure 2).
[d]To confirm segregation, the LH172BC1F1 plants were self-pollinated to produce two different plant populations of this generation (not shown in the breeding tree, Figure 2).

Table 8. Segregation analyses of maize MON 89034.

Generation	No. of Plants	Observed positives	Expected positives	Observed negatives	Expected negatives	Chi-square	Probability
LH172BC0F_2	11	7	8.25	4	2.75	0.2727	>0.05
LH172BC0F_3	24	24	24	0	0	Fixed +	n.a
LH172BC0F_4	30	30	30	0	0	Fixed +	n.a
LH172BC1F_1	28	13	14	15	14	0.0357	>0.05
LH172BC1F_2	24	20	18	4	6	0.5	>0.05
LH172BC1F_2	24	17	18	7	6	0.0556	>0.05

Table 9. Compositional analysis of MON 89034 compared to control and commercial varieties – 2004 US field trials – all sites combined

	MON 89034		Control		Commercials[1]		
Tissue/Component (Units)[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Forage							
Fibre (% dw)							
ADF	28.95	22.60-35.85	27.26	19.93-35.59	[16.76,43.76]	18.3-41.0[b] ; 17.5-38.3[a]	16.13-47.39
NDF	39.69	33.99-46.82	37.60	31.44-43.96	[25.94,55.67]	26.4-54.5[b]; 27.9-54.8[a]	20.29-63.71
Proximates							
Ash (% dw)	3.70	2.51-4.67	3.90	2.59-5.10	[1.93,6.31]	2.43-9.64[a]; 2-6.6[b]	1.527-9.638
Carbohydrates (% dw)	86.90	84.93-89.13	86.69	84.36-89.57	[83.05,90.74]	83.2-91.6[b]; 76.5-87.3[a]	76.4-92.1
Total fat (% dw)	1.57	0.63-3.17	1.71	0.77-2.91	[0.4,54]	0.35-3.62[b] ; 1.42-4.57[a]	0.296-4.570
Moisture (% fw)	72.20	68.50-75.40	71.53	65.90-76.80	[57.62,86.45]	56.5-80.4[a] ; 55.3-75.3[b]	49.1-81.3
Protein (% dw)	7.82	6.34-8.98	7.70	6.06-8.87	[4.78,10.38]	4.98-11.56[a]	3.14-11.57
Minerals (% dw)							
Calcium	0.20	0.16-0.24	0.19	0.13-0.28	[0.016,0.38]	0.0969-0.3184[b]	0.0714-0.5768
Phosphorus	0.25*	0.22-0.32	0.21	0.15-0.25	[0.071,0.32]	0.1367-0.2914[b]	0.0936-0.3704

* significant difference at 5% level when compared with the control

1 15 commercial maize hybrids.

2 dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre, TDF = total dietary fibre, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

3 The mean of 15 replicate values

4 Tolerance Interval : with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

5 Literature range references: [a] Ridley *et al.* (2002); [b] Sidhu *et al.* (2000); [c] Jugenheimer (1976); [d] Watson (1987); [e] Watson (1982); [f] Classen *et al.* (1990); [g] Dowd and Vega (1996); [h] Choi *et al.* (1999).

6 ILSI range is from ILSI Crop Composition Database, 2006.

Table 9. Compositional analysis of MON 89034 compared to control and commercial varieties – 2004 US field trials – all sites combined. Continued

	MON 89034		Control		Commercials[1]		
Tissue/Component[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain							
Amino acids (% of total aa)							(% dw)
Alanine	0.77	0.64-0.89	0.78	0.67-0.89	[0.48,1.08]	N/A	0.439-1.393
Arginine	0.48	0.38-0.52	0.47	0.41-0.51	[0.33,0.56]	N/A	0.119-0.639
Aspartic acid	0.68	0.56-0.78	0.67	0.60-0.76	[0.43-0.90]	N/A	0.335-1.208
Cystine	0.23	0.20-0.26	0.23	0.21-0.25	[0.18,0.27]	N/A	0.125-0.514
Glutamic acid	1.97	1.63-2.29	1.99	1.70-2.26	[1.25,2.75]	N/A	0.965-3.536
Glycine	0.38	0.32-0.41	0.38	0.36-0.41	[0.28,0.46]	N/A	0.184-0.539
Histidine	0.31	0.25-0.35	0.31	0.28-0.34	[0.22,0.38]	N/A	0.137-0.434
Isoleucine	0.36	0.30-0.43	0.36	0.30-0.42	[0.23,0.51]	N/A	0.179-0.692
Leucine	1.31	1.09-1.57	1.32	1.08-1.55	[0.77,1.92]	N/A	0.642-2.492
Lysine	0.33	0.26-0.36	0.32	0.29-0.36	[0.20,0.40]	N/A	0.172-0.668
Methionine	0.23	0.20-0.27	0.22	0.20-0.24	[0.14,0.25]	N/A	0.124-0.468
Phenylalanine	0.51	0.43-0.61	0.52	0.43-0.60	[0.32,0.73]	N/A	0.244-0.930
Proline	0.93	0.79-1.05	0.93	0.83-1.01	[0.68,1.21]	N/A	0.462-1.632
Serine	0.52	0.44-0.61	0.52	0.46-0.60	[0.34,0.71]	N/A	0.235-0.769
Threonine	0.33	0.27-0.37	0.33	0.29-0.36	[0.24,0.41]	N/A	0.224-0.666
Tryptophan	0.056	0.048-0.064	0.056	0.045-0.063	[0.032,0.072]	N/A	0.0271-0.215
Tyrosine	0.37	0.22-0.43	0.36	0.24-0.42	[0.17,0.52]	N/A	0.103-0.642
Valine	0.49	0.40-0.55	0.49	0.43-0.55	[0.35,0.62]	N/A	0.266-0.855

1 15 commercial maize hybrids.

2 dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre, TDF = total dietary fibre, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

3 The mean of 15 replicate values

4 Tolerance Interval : with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

5 Literature range references: [a] Ridley *et al.* (2002); [b] Sidhu *et al.* (2000); [c] Jugenheimer (1976); [d] Watson (1987); [e] Watson (1982); [f] Classen *et al.* (1990); [g] Dowd and Vega (1996); [h] Choi *et al.* (1999).

6 ILSI range is from ILSI Crop Composition Database, 2006.

Table 9. Compositional analysis of MON 89034 compared to control and commercial varieties – 2004 US field trials – all sites combined. Continued

	MON 89034		Control		Commercials[1]		
Tissue/Component[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain – continued							
Fatty acids (% of total fa)						(% total fat)	
16:0 palmitic acid	9.19	8.98-9.46	9.12	8.91-9.34	[6.12,15.67]	7-19[e]	7.94-20.71
16:1 palmitoleic acid	0.13	0.11-0.14	0.12	0.048-0.14	[0.0.28]	1[e]	0.095-0.447
18:0 stearic acid	1.89*	1.79-2.03	1.82	1.76-1.87	[0.86,2.98]	1-3[e]	1.02-3.40
18:1 oleic acid	24.96	23.38-25.75	24.84	23.62-26.66	[7.51,46.46]	20-46[e]	17.4-40.2
18:2 linoleic acid	61.82	60.85-63.61	62.07	60.51-63.41	[39.41,76.74]	35-70[e]	36.2-66.5
18:3 linolenic acid	1.19	1.12-1.23	1.22	1.15-1.43	[0.63,1.77]	0.8-2[e]	0.57-2.25
20:0 arachidic acid	0.39*	0.36-0.42	0.38	0.36-0.40	[0.23,0.54]	0.1-2[e]	0.279-0.965
20:1 eicosenoic acid	0.28	0.26-0.29	0.28	0.25-0.29	[0.15,0.39]	N/A	0.170-1.917
22:0 behenic acid	0.16	0.13-0.20	0.15	0.13-0.18	[0.081,0.23]	N/A	0.110-0.349
Fibre (% dw)							
ADF	5.48	3.82-7.24	5.27	4.17-7.00	[2.77,7.56]	3.3-4.3[d]; 2.46-11.34[a,b]	1.82-11.34
NDF	10.06	8.59-12.08	9.75	8.48-11.75	[5.93,13.63]	8.3-11.9[d]; 7.58-15.91[b]	5.59-22.64
TDF	15:17	13.39-17.02	14.67	12.82-17.62	[9.20,20.27]	10.99-11.41[h]	8.82-35.31

* significant difference at 5% level when compared with the control

1 15 commercial maize hybrids.

2 dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre, TDF = total dietary fibre, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

3 The mean of 15 replicate values

4 Tolerance Interval : with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

5 Literature range references: [a] Ridley *et al.* (2002); [b] Sidhu *et al.* (2000); [c] Jugenheimer (1976); [d] Watson (1987); [e] Watson (1982); [f] Classen *et al.* (1990); [g] Dowd and Vega (1996); [h] Choi *et al.* (1999).

6 ILSI range is from ILSI Crop Composition Database, 2006.

Table 9. Compositional analysis of MON 89034 compared to control and commercial varieties – 2004 US field trials – all sites combined. Continued

	MON 89034		Control		Commercials[1]		
Tissue/Component[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain – continued							
Minerals							
Calcium (% dw)	0.0050	0.0038-0.0066	0.0049	0.0040-0.0059	[0.0016,0.0059]	0.01-0.1[d]	0.00127-0.02084
Copper (mg/kg dw)	1.74	1.33-2.38	2.07	1.26-4.54	[0,4.20]	0.9-10[d]	0.73-18.50
Iron (mg/kg dw)	21.40	19.23-25.23	22.20	19.03-28.26	[8.88,34.51]	1-100[d]	10.42-49.07
Magnesium (% dw)	0.12	0.10-0.14	0.12	0.11-0.14	[0.075,0.17]	0.09-1[d]	0.0594-0.194
Manganese (mg/kg dw)	6.79	5.43-9.32	6.51	5.57-8.00	[3.17,9.99]	0.7-54[d]	1.69-14.30
Phosphorus (% dw)	0.33	0.27-0.36	0.33	0.29-0.36	[0.18,0.45]	0.26-0.75[d]	0.147-0.533
Potassium (% dw)	0.36	0.32-0.40	0.36	0.34-0.40	[0.26,0.46]	0.32-0.72[d]	0.181-0.603
Zinc (mg/kg dw)	22.05	18.91-26.89	21.91	18.81-26.04	[7.16,38.55]	12-30[d]	6.5-37.2
Proximates (% dw)							
Ash	1.41	1.25-1.56	1.39	1.28-1.51	[0.74,1.96]	1.1-3.9[d] ; 0.89-6.28[b]	0.616-6.282
Carbohydrates	84.85	83.29-86.52	84.96	83.58-86.22	[81.08,88.80]	77.4-87.2[b] ; 82.2-88.1[a]	77.4-89.5
Total fat	3.32	3.05-3.89	3.29	3.05-3.75	[2.20,4.55]	3.1-5.7[d] ; 2.48-4.81[b]	1.742-5.823
Moisture	9.52	7.89-12.80	9.50	7.86-13.10	[0.45,19.52]	7-23[d] ; 8.18-26.2[b]	6.1-40.5
Protein	10;43	8.54-11.98	10.36	9.22-11.52	[7.54,13.13]	6-12[d]; 9.7-16.1[c]	6.15-17.26

1 15 commercial maize hybrids.

2 dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre, TDF = total dietary fibre, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

3 The mean of 15 replicate values

4 Tolerance Interval : with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

5 Literature range references: [a] Ridley *et al.* (2002); [b] Sidhu *et al.* (2000); [c] Jugenheimer (1976); [d] Watson (1987); [e] Watson (1982); [f] Classen *et al.* (1990); [g] Dowd and Vega (1996); [h] Choi *et al.* (1999).

6 ILSI range is from ILSI Crop Composition Database, 2006.

Table 9. Compositional analysis of MON 89034 compared to control and commercial varieties – 2004 US field trials – all sites combined. Continued

	MON 89034		Control		Commercials[1]		
Tissue/Component[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain – continued							
Vitamin (mg/kg dw)							
Folic acid	0.35	0.26-0.48	0.36	0.23-0.53	[0.012.0.69]	0.3[d]	0.147-1.464
Niacin	30.08	25.72-34.84	29.59	24.93-35.75	[6.97.37.83]	9.3-70[d]	10.37-46.94
Vitamin B1	3.07	2.39-3.44	2.94	2.39-3.36	[0.37.6.35]	3-8.6[e]	1.26-40.00
Vitamin B2	1.42	1.24-1.65	1.42	1.16-1.61	[0.91.2.30]	0.25-5.6[e]	0.50-2.36
Vitamin B6	6.22	5.28-6.99	6.26	5.37-6.80	[3.12.9.30]	5.3[d]; 9.6[e]	3.68-11.32
Vitamin E	6.77	5.55-8.62	6.63	2.72-9.02	[0.20.49]	3-12.1[e]; 17-47[d]	1.5-68.7
Antinutrient (% dw)							
Phytic acid	0.75	0.53-0.87	0.73	0.56-0.88	[0.21.1.22]	0.48-1.12[a]	0.111-1.570
Secondary metabolite (µg/g dw)							
Ferulic acid	2131.38	1790.25-2525.31	2148.05	1878.66-2669.85	[1136.69.2806.24]	113-1194[f]; 3000[g]	291.9-38885.8
p-coumaric acid	194.25	166.11-253.04	183.96	167.76-210.13	[0.378.57]	22-75[f]	53.4-576.2

1 15 commercial maize hybrids.

2 dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre, TDF = total dietary fibre, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

3 The mean of 15 replicate values

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5 Literature range references: [a] Ridley *et al.* (2002); [b] Sidhu *et al.* (2000); [c] Jugenheimer (1976); [d] Watson (1987); [e] Watson (1982); [f] Classen *et al.* (1990); [g] Dowd and Vega (1996); [h] Choi *et al.* (1999).

6 ILSI range is from ILSI Crop Composition Database, 2006.

Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw.

Table 10. Compositional analysis of MON 89034 compared to control and commercial varieties – 2004-2005 Argentinian field trials – all sites combined

	MON 89034		Control		Commercials[1]		
Tissue/Component (Units)[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Forage							
Fibre (% dw)							
ADF	25.70	19.22-32.80	25.72	20.27-30.52	[17.39,38.71]	18.3-41.0[b] ; 17.5-38.3[a]	16.13-47.39
NDF	35.50	29.38-42.68	36.33	29.19-46.82	[23.84,55.56]	26.4-54.5[b]; 27.9-54.8[a]	20.29-63.71
Proximates							
Ash (% dw)	5.22	4.27-7.22	4.98	3.76-5.87	[2.22,8.69]	2.43-9.64[a]; 2-6.6[b]	1.527-9.638
Carbohydrates (% dw)	84.44	82.56-86.11	84.87	82.83-88.30	[79.06,89.42]	83.2-91.6[b]; 76.5-87.3[a]	76.4-92.1
Total fat (% dw)	2.33	1.16-3.49	2.45	1.46-3.13	[0,5.13]	0.35-3.62[b] ; 1.42-4.57[a]	0.296-4.570
Moisture (% fw)	70.26	64.20-75.40	70.13	65.90-74.10	[56.88,84.19]	56.5-80.4[a] ; 55.3-75.3[b]	49.1-81.3
Protein (% dw)	8.01	7.04-9.05	7.70	4.32-8.70	[3.90,12.06]	4.98-11.56[a]	3.14-11.57
Minerals (% dw)							
Calcium	0.14	0.11-0.18	0.14	0.11-0.16	[0,0.32]	0.0969-0.3184[b]	0.0714-0.5768
Phosphorus	0.26	0.15-0.39	0.23	0.14-0.29	[0,0.56]	0.1367-0.2914[b]	0.0936-0.3704

1 15 commercial maize hybrids.

2 dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre, TDF = total dietary fibre, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

3 The mean of 15 replicate values

4 Tolerance Interval : with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

5 Literature range references: [a] Ridley *et al.* (2002); [b] Sidhu *et al.* (2000); [c] Jugenheimer (1976); [d] Watson (1987); [e] Watson (1982); [f] Classen *et al.* (1990); [g] Dowd and Vega (1996); [h] Choi *et al.* (1999).

6 ILSI range is from ILSI Crop Composition Database, 2006.

Table 10. Compositional analysis of MON 89034 compared to control and commercial varieties – 2004-2005 Argentinian field trials – all sites combined. Continued

	MON 89034		Control		Commercials[1]		
Tissue/Component (Units)[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain							
Amino acids (% of total aa)							
Alanine	0.78	0.65-0.88	0.75	0.69-0.85	[0.54,1.06]	N/A	0.439-1.393
Arginine	0.46	0.39-0.53	0.46	0.39-0.49	[0.33,0.58]	N/A	0.119-0.639
Aspartic acid	0.68	0.61-0.76	0.67	0.61-0.71	[0.50,0.89]	N/A	0.335-1.208
Cystine	0.23	0.20-0.25	0.23	0.21-0.25	[0.18,0.28]	N/A	0.125-0.514
Glutamic acid	1.96	1.64-2.23	1.89	1.75-2.14	[1.34,2.73]	N/A	0.965-3.536
Glycine	0.39	0.36-0.42	0.38	0.35-0.40	[0.30,0.47]	N/A	0.184-0.539
Histidine	0.29	0.27-0.32	0.29	0.27-0.30	[0.20,0.40]	N/A	0.137-0.434
Isoleucine	0.35	0.29-0.39	0.35	0.32-0.38	[0.26,0.47]	N/A	0.179-0.692
Leucine	1.31	1.05-1.49	1.27	1.14-1.47	[0.85,1.89]	N/A	0.642-2.492
Lysine	0.31	0.28-0.36	0.31	0.29-0.34	[0.23,0.37]	N/A	0.172-0.668
Methionine	0.22	0.19-0.24	0.22	0.20-0.25	[0.13,0.28]	N/A	0.124-0.468
Phenylalanine	0.53	0.44-0.60	0.52	0.48-0.59	[0.38,0.73]	N/A	0.244-0.930
Proline	0.91	0.77-1.00	0.89	0.82-0.98	[0.66,1.26]	N/A	0.462-1.632
Serine	0.52	0.47-0.60	0.51	0.46-0.56	[0.36,0.72]	N/A	0.235-0.769
Threonine	0.36	0.31-0.41	0.36	0.32-0.39	[0.28,0.47]	N/A	0.224-0.666
Tryptophan	0.065	0.060-0.071	0.065	0.061-0.070	[0.050,0.075]	N/A	0.0271-0.215
Tyrosine	0.34	0.22-0.40	0.35	0.21-0.41	[0.23,0.48]	N/A	0.103-0.642
Valine	0.48	0.43-0.53	0.47	0.45-0.51	[0.37,0.63]	N/A	0.266-0.855

1 15 commercial maize hybrids.

2 dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre, TDF = total dietary fibre, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

3 The mean of 15 replicate values

4 Tolerance Interval : with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

5 Literature range references: [a] Ridley *et al.* (2002); [b] Sidhu *et al.* (2000); [c] Jugenheimer (1976); [d] Watson (1987); [e] Watson (1982); [f] Classen *et al.* (1990); [g] Dowd and Vega (1996); [h] Choi *et al.* (1999).

6 ILSI range is from ILSI Crop Composition Database, 2006.

Table 10. Compositional analysis of MON 89034 compared to control and commercial varieties – 2004-2005 Argentinian field trials – all sites combined. Continued

	MON 89034		Control		Commercials[1]		
Tissue/Component[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain – continued							
Fatty acids (% of total fa)						(% total fat)	
16:0 palmitic acid	8.91	8.71-9.25	8.96	8.80-9.19	[7.54,13.55]	7-19[e]	7.94-20.71
16:1 palmitoleic acid	0.11	0.055-0.14	0.13	0.061-0.16	[0.0029,0.23]	1[e]	0.095-0.447
18:0 stearic acid	1.84*	1.76-1.98	1.79	1.73-1.87	[0.63,3.01]	1-3[e]	1.02-3.40
18:1 oleic acid	24.47	23.50-25.17	24.32	23.22-25.02	[8.77,43.80]	20-46[e]	17.4-40.2
18:2 linoleic acid	62.66	61.64-63.86	62.77	61.83-64.02	[41.30,77.09]	35-70[e]	36.2-66.5
18:3 linolenic acid	1.21	1.11-1.27	1.21	1.18-1.27	[0.63,1.66]	0.8-2[e]	0.57-2.25
20:0 arachidic acid	0.37	0.35-0.40	0.37	0.35-0.47	[0.15,0.66]	0.1-2[e]	0.279-0.965
20:1 eicosenoic acid	0.29*	0.27-0.31	0.30	0.28-0.37	[0.14,0.48]	N/A	0.170-1.917
22:0 behenic acid	0.14	0.12-0.17	0.15	0.13-0.31	[0.059,0.30]	N/A	0.110-0.349
Fibre (% dw)							
ADF	5.79	4.07-7.19	5.66	4.68-6.84	[2.74,9.13]	3.3-4.3[d]; 2.46-11.34[a,b]	1.82-11.34
NDF	10.84	8.20-14.45	10.85	9.22-12.34	[6.21,16.18]	8.3-11.9[d]; 7.58-15.91[b]	5.59-22.64
TDF	16.14	11.65-21.87	15.48	12.40-18.19	[7.95,25.13]	10.99-11.41[h]	8.82-35.31

* significant difference at 5% level when compared with the control

1 15 commercial maize hybrids.

2 dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre, TDF = total dietary fibre, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

3 The mean of 15 replicate values

4 Tolerance Interval : with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

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Table 10. Compositional analysis of MON 89034 compared to control and commercial varieties – 2004-2005 Argentinian field trials – all sites combined. Continued

	MON 89034		Control		Commercials[1]		
Tissue/Component[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain – continued							
Minerals							
Calcium (% dw)	0.0060	0.0045-0.0070	0.0061	0.0051-0.0070	[0.0019,0.0079]	0.01-0.1[d]	0.00127-0.02084
Copper (mg/kg dw)	2.37	1.09-4.82	4.16	1.44-18.36	[0.8,23]	0.9-10[d]	0.73-18.50
Iron (mg/kg dw)	18.91	16.73-21.00	19.39	16.63-27.75	[12.48,29.03]	1-100[d]	10.42-49.07
Magnesium (% dw)	0.13	0.12-0.14	0.12	0.11-0.14	[0.078,0.18]	0.09-1[d]	0.0594-0.194
Manganese (mg/kg dw)	6.81*	5.42-7.62	6.28	5.33-6.88	[2.33,11.13]	0.7-54[d]	1.69-14.30
Phosphorus (% dw)	0.34	0.29-0.39	0.34	0.28-0.40	[0.20,0.48]	0.26-0.75[d]	0.147-0.533
Potassium (% dw)	0.38	031-0.43	0.39	031-0.47	[0.21,0.58]	0.32-0.72[d]	0.181-0.603
Zinc (mg/kg dw)	20.74	17.53-23.60	20.80	16.86-23.64	[7.91,33.26]	12-30[d]	6.5-37.2
Proximates (% dw)							
Ash	1.43	1.16-1.70	1.41	1.20-1.61	[0.64,2.18]	1.1-3.9[d] ; 0.89-6.28[b]	0.616-6.282
Carbohydrates	85.32	84.17-86.73	85.42	84.53-86.22	[81.06,88.33]	77.4-87.2[b] ; 82.2-88.1[a]	77.4-89.5
Total fat	3.29	2.77-3.66	3.22	2.96-3.40	[1.94,5.07]	3.1-5.7[d] ; 2.48-4.81[b]	1.742-5.823
Moisture	13.44	12.30-14.50	13.51	12.90-14.10	[11.40,15.35]	7-23[d] ; 8.18-26.2[b]	6.1-40.5
Protein	9.96	8.91-11.17	9.94	9.29-10.92	[7.56,13.21]	6-12[d]; 9.7-16.1[c]	6.15-17.26

* significant difference at 5% level when compared with the control

1 15 commercial maize hybrids.

2 dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre, TDF = total dietary fibre, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

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6 ILSI range is from ILSI Crop Composition Database, 2006.

Table 10. Compositional analysis of MON 89034 compared to control and commercial varieties – 2004-2005 Argentinian field trials – all sites combined. Continued

	MON 89034		Control		Commercials[1]		
Tissue/Component[2]	Mean[3]	Range	Mean[3]	Range	99% T.I.[4]	Literature range[5]	ILSI range[6]
Grain – continued							
Vitamin (mg/kg dw)							
Folic acid	0.50	0.27-0.81	0.56	0.27-0.92	[0.1.74]	0.3[d]	0.147-1.464
Niacin	35.26	28.39-44.69	34.18	27.13-39.47	[0.56.72]	9.3-70[d]	10.37-46.94
Vitamin B1	3.34	2.97-3.70	3.28	2.76-3.94	[1.92.5.79]	3-8.6[e]	1.26-40.00
Vitamin B2	1.75*	1.54-1.95	1.94	1.65-2.16	[1.19.3.11]	0.25-5.6[e]	0.50-2.36
Vitamin B6	6.01	5.23-7.07	6.55	5.02-10.83	[2.34.10.88]	5.3[d]; 9.6[e]	3.68-11.32
Antinutrient (% dw)							
Phytic acid	0.81	0.61-0.98	0.78	0.53-1.03	[0.39.1.12]	0.48-1.12[a]	0.111-1.570
Raffinose	0.060	0.029-0.081	0.062	0.029-0.076	[0.0.32]	0.08-0.30[e]	0.020-0.320
Secondary metabolite (µg/g dw)							
Ferulic acid	1894.10*	1484.46-2165.31	1759.10	1471.61-2034.48	[552.46.3057.71]	113-1194[f]; 3000[g]	291.9-38885.8
p-coumaric acid	155.22	139.70-185.35	146.00	118.66-174.57	[0.326.22]	22-75[f]	53.4-576.2

* significant difference at 5% level when compared with the control

1 15 commercial maize hybrids.

2 dw = dry weight, ADF = acid detergent fibre, NDF = neutral detergent fibre, TDF = total dietary fibre, Vitamin B_1= Thiamine, Vitamine B_2 = Riboflavin, Vitamin B_6 = Pyridoxine

3 The mean of 15 replicate values

4 Tolerance Interval : with 95% confidence, interval contains 99% of the values expressed in the population of commercial hybrids. Negative limits were set to zero.

5 Literature range references: [a] Ridley *et al.* (2002); [b] Sidhu *et al.* (2000); [c] Jugenheimer (1976); [d] Watson (1987); [e] Watson (1982); [f] Classen *et al.* (1990); [g] Dowd and Vega (1996); [h] Choi *et al.* (1999).

6 ILSI range is from ILSI Crop Composition Database, 2006.

Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw.

Table 11. Summary of the statistical differences for the compositional comparison of MON 89034 to control maize – 2004 US field trials

Tissue/Site/ Component (Units)[1]	Mean MON 89034	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 89034 (Range)	99% Tolerance Interval[2]
Statistical differences observed in combined site analyses						
Mineral						
Forage Phosphorus	0.25	0.21	19.24	0.010	(0.22-0.32)	[0.071, 0.32]
Fatty acid						
Grain 18:0 Stearic (% total fa)	1.89	1.82	3.97	0.002	(1.79-2.03)	[0.86, 2.98]
Grain 20:0 Arachidic (% total fa)	0.39	0.38	3.43	<0.001	(0.36-0.42)	[0.23, 0.54]
Statistical differences observed in more than on site						
Proximate						
Site IA Grain Carbohydrates (% dw)	83.38	84.52	-1.34	0.008	(83.29-83.55)	[81.08,88.80]
Site OH Grain Carbohydrates (% dw)	84.26	83.80	0.55	0.009	(83.99-84.59)	[81.08, 88.80]
Mineral						
Site IL-1 Grain Copper (mg/kg dw)	1.76	1.36	29.35	0.023	(1.51-2.21)	[0, 4.20]
Site NE Grain Copper (mg/kg dw)	2.15	1.67	28.66	0.023	(1.92-2.38)	[0, 4.20]
Site IL-1 Grain Iron (mg/kg dw)	20.86	19.48	7.11	0.048	(19.23-21.79)	[8.88, 34.51]
Site OH Grain Iron (mg/kg dw)	21.37	25.74	-17.00	0.006	(20.59-21.76)	[8.88, 34.51]
Fatty acid						
Site IL-1 Grain 18:0 Stearic (% total fa)	1.96	1.82	7.94	<0.001	(1.89-2.02)	[0.86, 2.98]
Site IL-2 Grain 18:0 Stearic (% total fa)	1.98	1.82	9.05	<0.001	(1.93-2.03)	[0.86, 2.98]
Site IL-1 Grain 20:0 Arachidic (% total fa)	0.41	0.39	5.23	0.007	(0.40-0.42)	[0.23, 0.54]
Site IL-2 Grain 20:0 Arachidic (% total fa)	0.39	0.37	6.83	0.021	(0.38-0.40)	[0.23, 0.54]
Site OH Grain 20:0 Arachidic (% total fa)	0.38	0.37	3.12	0.035	(0.38-0.39)	[0.23, 0.54]

[1] dw=dry weight; fw=fresh weight; fa=fatty acids. Combined site = analyses of the combined data from each of the five replicated field trials

[2] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

States codes : IA: Iowa ; HO: Ohio; IL : Illinois ; NE : Nebraska

Table 11. Summary of the statistical differences for the compositional comparison of MON 89034 to control maize – 2004 US field trials. Continued

Tissue/Site/ Component (Units)[1]	Mean MON 89034	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 89034 (Range)	99% Tolerance Interval[2]
Statistical differences observed in one site only						
Amino acid						
Site IA Grain Alanine (% dw)	0.88	0.81	7.83	0.030	(0.87-0.88)	[0.48, 1.08]
Site IA Grain Arginine (% dw)	0.51	0.46	10.83	0.005	(0.50-0.52)	[0.33, 0.56]
Site IA Grain Aspartic acid (% dw)	0.77	0.71	8.66	0.003	(0.77-0.78)	[0.43, 0.90]
Site IA Grain Cystine (% dw)	0.25	0.23	7.54	0.014	(0.24-0.26)	[0.18, 0.27]
Site IA Grain Glutamic acid (% dw)	2.27	2.09	8.66	0.011	(2.26-2.28)	[1.25, 2.75]
Site IA Grain Glycine (% dw)	0.41	0.38	6.94	0.020	(0.40-0.41)	[0.28, 0.46]
Site IA Grain Histidine (% dw)	0.34	0.32	7.16	0.022	(0.339-0.343)	[0.22, 0.38]
Site IA Grain Leucine (% dw)	1.49	1.37	8.96	0.032	(1.48-1.51)	[0.77, 1.92]
Site IA Grain Lysine (% dw)	0.35	0.32	6.66	0.028	(0.33-0.36)	[0.20, 0.40]
Site IA Grain Methionine (% dw)	0.25	0.23	11.20	0.003	(0.25-0.27)	[0.14, 0.25]
Site IA Grain Phenylalanine (% dw)	0.58	0.53	9.45	0.028	(0.57-0.59)	[0.32, 0.73]
Site IA Grain Proline (% dw)	1.05	0.98	7.29	0.028	(1.04-1.05)	[0.68, 1.21]
Site IA Grain Serine (% dw)	0.60	0.56	8.28	0.004	(0.60-0.61)	[0.34, 0.71]
Site IA Grain Threonine (% dw)	0.37	0.34	8.45	0.004	(0.369-0.372)	[0.24, 0.41]
Site IA Grain Tyrosine (% dw)	0.43	0.36	17.50	0.006	(0.42-0.43)	[0.17, 0.52]
Proximate						
Site IA Grain Protein (% dw)	11.89	10.85	9.59	0.005	(11.73-11.98)	[7.54, 13.13]
Site IL-1 Forage Moisture (% fw)	69.03	66.53	3.76	0.031	(68.50-69.40)	[57.62, 86.45]
Site NE Forage Ash (% dw)	3.20	4.39	-27.12	0.021	(2.93-3.38)	[1.93, 6.31]
Site NE Forage Carohydrates (% dw)	88.16	84.98	3.74	0.004	(86.86-88.84)	[83.05, 90.74]

[1] dw=dry weight; fw=fresh weight; fa=fatty acids. Combined site = analyses of the combined data from each of the five replicated field trials

[2] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

States codes : IA: Iowa ; HO: Ohio; IL : Illinois ; NE : Nebraska

Table 11. Summary of the statistical differences for the compositional comparison of MON 89034 to control maize – 2004 US field trials. Continued

Tissue/Site/ Component (Units)[1]	Mean MON 89034	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 89034 (Range)	99% Tolerance Interval[2]
Statistical differences observed in one site only - continued						
Fiber						
Site NE Grain Neutral Detergent Fiber (% dw)	10.52	9.05	16.27	0.028	(10.43-10.69)	[5.93, 13.63]
Site OH Forage Acid Detergent Fiber (% dw)	31.31	23.58	32.78	0.012	(26.92-34.93)	[16.76, 43.76]
Site OH Forage Neutral Detergent Fiber (% dw)	43.21	37.87	14.11	0.027	(40.07-46.82)	[25.94, 55.67]
Fatty acid						
Site IA Grain 18:3 Linolenic (% total fa)	1.21	1.34	-9.40	0.009	(1.20-1.23)	[0.63, 1.77]
Site IL-1 Grain 16:1 Palmitoleic (% total fa)	0.13	0.14	-6.87	0.012	(0.12-0.13)	[0, 0.28]
Site IL-2 Grain 18:1 Oleic (% total fa)	24.75	23.82	3.93	0.003	(24.14-25.25)	[7.51, 46.46]
Site IL-2 Grain 18:2 Linoleic (% total fa)	61.87	63.17	-2.07	0.001	(61.19-62.42)	[39.41, 76.74]
Site NE Grain 20:1 Eicosenoic (% total fa)	0.28	0.29	-1.50	0.030	(0.279-0.283)	[0.15, 0.39]
Mineral						
Site IA Grain Calcium (% dw)	0.0064	0.0058	10.96	0.012	(0.0062-0.0066)	[0.0016, 0.0059]
Site IA Grain Mangenese (mg/kg dw)	8.34	6.99	19.32	0.017	(7.62-9.32)	[3.17, 9.99]
Site IA Forage Calcium (% dw)	0.24	0.26	-8.77	0.033	(0.239-0.243)	[0.016, 0.38]
Site NE Forage Phosphorus (% dw)	0.25	0.17	46.95	0.036	(0.23-0.28)	[0.071, 0.32]
Vitamin						
Site IL-2 Grain Folic acid (mg/kg dw)	0.37	0.32	13.81	<0.001	(0.35-0.38)	[0.012, 0.69]
Secondary metabolite						
Site OH Grain p-Coumaric acid (µg/g dw)	218.38	185.63	17.64	0.032	(187.79-253.04)	[0, 378.57]

[1] dw=dry weight; fw=fresh weight; fa=fatty acids. Combined site = analyses of the combined data from each of the five replicated field trials

[2] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Site codes : IA: Iowa ; HO: Ohio; IL : Illinois ; NE : Nebraska

Table 12. Summary of the statistical differences for the compositional comparison of MON 89034 to control maize – 2004-2005 Argentinean field trials

Tissue/Site/ Component (Units)[1]	Mean MON 89034	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 89034 (Range)	99% Tolerance Interval[2]
Statistical differences observed in combined site analyses						
Fatty acid						
Grain 18:0 Stearic (% total fa)	1.84	1.79	2.61	0.017	(1.76-1.98)	[0.63.3.01]
Grain 20:1 Eicosenoic (% total fa)	0.29	0.30	-4.35	0.039	(0.27-0.31)	[0.14.0.48]
Mineral						
Grain Manganese (mg/kg dw)	6.81	6.28	8.56	0.017	(5.42-7.62)	[2.33.11.13]
Vitamin						
Grain Vitamin B2 (mg/kg dw)	1.75	1.94	-9.78	0.002	(1.54-1.95)	[1.19.3.11]
Secondary metabolite						
Grain Ferulic acid (µg/g dw)	1894.10	1759.10	7.67	0.036	(1484.46-2165.31)	[552.46.3057.71]
Statistical differences observed in more than on site						
Proximate						
Site B2 Grain Moisture (%fw)	12.93	13.63	-5.13	0.001	(12.80-13.10)	[11.40.15.35]
Site B3 Grain Moisture (%fw)	12.50	13.03	-4.09	0.041	(12.3-12.60)	[11.40.15.35]
Site SF Grain Moisture (%fw)	14.37	13.80	4.11	0.027	(14.10-14.50)	[11.40.15.35]
Site B2 Forage Moisture (%fw)	66.43	69.23	-4.04	0.035	(64.20-67.80)	[56.88.84.19]
Site B3 Forage Moisture (%fw)	68.03	69.23	-1.73	0.040	(67.40-68.90)	[56.88.84.19]
Vitamin						
Site B1 Grain Vitamin B2 (mg/kg dw)	1.69	2.02	-16.38	0.010	(1.54-1.81)	[1.19.3.11]
Site SF Grain Vitamin B2 (mg/kg dw)	1.82	2.08	-12.59	0.022	(1.79-1.89)	[1.19.3.11]

[1] dw=dry weight; fw=fresh weight; fa=fatty acids. Combined site = analyses of the combined data from each of the five replicated field trials

[2] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Site codes : Site B1- Pergamino, Buenos Aires; Site B2-Tacuari, Buenos Aires; Site B3-Gahan, Buenos Aires; Site CB-Marcos Juarez, Córdoba; Site SF-Uranga, Santa Fe

Table 12. Summary of the statistical differences for the compositional comparison of MON 89034 to control maize – 2004-2005 Argentinean field trials. Continued

Tissue/Site/ Component (Units)[1]	Mean MON 89034	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 89034 (Range)	99% Tolerance Interval[2]
Statistical differences observed in more than on site - continued						
Mineral						
Site B1 Grain Manganese (%dw)	7.20	5.95	20.94	0.009	(6.69-7.56)	[2.33,11.13]
Site B3 Grain Manganese (%dw)	6.99	6.39	9.28	0.045	(6.89-7.05)	[2.33,11.13]
Site CB Forage Calcium (%dw)	0.17	0.13	29.48	0.022	(0.16-0.18)	[0,0.32]
Site SF Forage Calcium (%dw)	0.13	0.15	-13.75	0.019	(0.128-0.131)	[0,0.32]
Statistical differences observed in one site only						
Proximate						
Site B1 Grain Total Fat (%dw)	3.59	3.38	6.15	0.027	(3.55-3.66)	[1.94,5.07]
Site B1 Forage Protein (% dw)	8.47	8.08	4.82	0.011	(8.33-8.66)	[3.90,12.06]
Mineral						
Site CB Grain Iron (mg/kg dw)	20.04	18.43	8.77	0.025	(19.56-21.00)	[12.48,29.03]
Site CB Grain Zinc (mg/kg dw)	20.20	18.73	7.83	0.013	(19.68-20.54)	[7.91,33.26]
Site SF Grain Calcium (mg/kg dw)	0.0049	0.0056	-12.08	0.016	(0.0045-0.0054)	[0.0019,0.0079]
Site SF Grain Potassium (mg/kg dw)	0.39	0.44	-10.41	0.024	(0.38-0.41)	[0.21,0.58]
Site B1 Forage Phosphorus (mg/kg dw)	0.36	0.26	35.53	0.031	(0.31-0.39)	[0,0.56]

[1] dw=dry weight; fw=fresh weight; fa=fatty acids. Combined site = analyses of the combined data from each of the five replicated field trials

[2] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Site codes : Site B1- Pergamino, Buenos Aires; Site B2-Tacuari, Buenos Aires; Site B3-Gahan, Buenos Aires; Site CB-Marcos Juarez, Córdoba; Site SF-Uranga, Santa Fe

Table 12. Summary of the statistical differences for the compositional comparison of MON 89034 to control maize – 2004-2005 Argentinean field trials. Continued

Tissue/Site/ Component (Units)[1]	Mean MON 89034	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 89034 (Range)	99% Tolerance Interval[2]
Statistical differences observed in one site only - continued						
Amino acid						
Site B1 Grain Glutamic acid (% dw)	2.02	1.85	9.34	0.048	(1.88-2.16)	[1.34,2.73]
Site B1 Grain Serine (% dw)	0.54	0.49	10.10	0.034	(0.49-0.58)	[0.36,0.72]
Fatty acid						
Site SF 18:0 Stearic (% total fa)	1.82	1.75	4.03	0.012	(1.76-1.85)	[0.63,3.01]
Site SF 18:3 Linolenic (% total fa)	1.23	1.20	2.83	0.026	(1.19-1.27)	[0.63,1.66]
Vitamin						
Site B2 Niacin (mg/kg dw)	37.06	34.00	8.98	0.002	(34.98-40.25)	[0,56.72]
Secondary metabolite						
Site B1 Grain p-Coumaric acid (µg/g dw)	150.88	132.04	14.27	0.046	(141.04-169.75)	[0,326.22]

[1] dw=dry weight; fw=fresh weight; fa=fatty acids. Combined site = analyses of the combined data from each of the five replicated field trials

[2] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Site codes : Site B1- Pergamino, Buenos Aires; Site B2-Tacuari, Buenos Aires; Site B3-Gahan, Buenos Aires; Site CB-Marcos Juarez, Córdoba; Site SF-Uranga, Santa Fe

Table 13. Phenotypic and agronomic characteristics of insect resistant maize MON 89034 evaluated during the 2007 European field trials

Characteristics	Evaluation Stage[1]	Evaluation Description
Seedling vigour	V2-V4	Rated on a 0-9 scale, where 0=dead and 9=above average vigor
Early stand count (#/plot)	V2-V4	Number of emerged plants
Days to 50% pollen shed	Pollen shed	Number of days after planting when 50% of the plants in a plot have begun to shed pollen
Days to 50% silking	Silking	Number of days after planting when 50% of the plants have multiple silks exposed
Stay green	Maturity	Rated on a 0-9 scale, where 0=entire plant is dried and 9=entire plant is green
Ear height (cm)	Maturity	Distance from the soil surface at the base of the plant to the ear attachment node
Ear/kernel root	Harvest	Rated on a 0-9 scale, where 0=no symptoms and 9= severe symptoms
Plant height (cm)	Maturity	Distance from the soil surface at the base of the plant to the flag leaf collar
Dropped ears (#/plot)	Pre-harvest	Number of mature ears dropped from plants
Stalk logded plants (#/plot)	Pre-harvest	Number of plants broken below the ear
Rot logded plants (#/plot)	Pre-harvest	Number of plants leaning at the soil surface greater than 30º from vertical
Final stand count (#/plot)	Pre-harvest	Number of plants
Stalk rot	Harvest	Rated on a 0-9 scale, where 0=no symptoms and 9= severe symptoms
Yield (t/ha)	Harvest	Harvested shelled grain, adjusted to relevant moisture

Table 14. Stressors damage types evaluated at each study site (European field trials 2007)

Stressor (type)	Type	Evaluation stage	Evaluation Description
Abiotic	Animal damage	V2-V4, V10-15	Rated on a 0-9 scale where 0=damage and 9= severe damage
	Drought stress	V10-15	Rated on a 0-9 scale where 0=no stress and 9=severe stress
	Flood damage	V10-15, VT-R3, R6	Rated on a 0-9 scale where 0=damage and 9= severe damage
	Frost damage	R6	Rated on a 0-9 scale where 0=damage and 9= severe damage
	Hail damage	V10-15	Rated on a 0-9 scale where 0=damage and 9= severe damage
	Heat damage	V2-V4, V10-15, VT-R3	Rated on a 0-9 scale where 0=damage and 9= severe damage
	Nutrient deficiency	V2-V4, V10-15, VT-R3, R6	Rated on a 0-9 scale where 0=symptoms and 9= severe symptoms
	Wind damage	V2-V4, V10-15, VT-R3, R6	Rated on a 0-9 scale where 0=damage
Disease	Ear rot	R6	Rated on a 0-9 scale where 0=damage and 9= severe damage
	Leaf blight	V2-V4, V10-15, VT-R3, R6	Rated on a 0-9 scale where 0=no symptoms and 9=severe symptoms
	Pythium	V2-V4, V10-15, VT-R3, R6	Rated on a 0-9 scale where 0=no symptoms and 9=severe symptoms
	Rust	V2-V4, V10-15, VT-R3, R6	Rated on a 0-9 scale where 0=no symptoms and 9=severe symptoms
	Smut (head+ear)	V2-V4, V10-15, VT-R3, R6	Rated on a 0-9 scale where 0=no symptoms and 9=severe symptoms
Insect	*Aphis* sp. (Aphids)	V2-V4, V10-15, VT-R3, R6	Rated on a 0-9 scale where 0=no symptoms and 9=severe symptoms
	Cutworm	V2-V4	Rated on a 0-9 scale where 0=damage and 9= severe damage
	Oscinella frit	V2-V4	Rated on a 0-9 scale where 0=damage and 9= severe damage
	Ostrinia nubalis	V2-V4, V10-15, VT-R3, R6	Rated on a 0-9 scale where 0=damage and 9= severe damage
	Thrip	V2-V4, V10-15, VT-R3, R6	Rated on a 0-9 scale where 0=damage and 9= severe damage

	Agriotes sp.	V2-V4, V10-15, VT-R3, R6	Rated on a 0-9 scale where 0=damage and 9= severe damage

Table 15. Combined field trials analysis: phenotypic characteristics of insect resistant maize MON 89034 compared to the control – European field trials conducted in 2007 (Germany and Spain)

Phenotypic Characteristics (units)	Northern EU field trials				Southern EU field trials			
	MON89034	Control	References Range[1]		MON89034	Control	References Range[1]	
	Mean	Mean	Min.	Max.	Mean	Mean	Min.	Max.
Seedling vigour	5.7	5.8	4.7	7.3	2.1	2.1	1.0	3.0
Early stand count (#/plot)	91.4	93.4	75.7	100.0	76.4	79.1	43.2	79.7
Days to 50% pollen shed	72.1	71.4	66.0	73.3	81.6	81.8	75.0	91.0
Days to 50% silking	71.1	70.3	65.0	73.3	77.0	77.0	69.0	88.0
Stay green	5.6	5.3	2.8	6.3	9.0	9.0	8.7	9.0
Ear height (cm)	81.2	84.7	63.1	118.3	93.7	97.6	83.0	126.2
Plant height (cm)	189.5	203.6	177.9	233.7	195.2	196.2	165.0	226.2
Dropped ears (#/plot)	0.0	0.0	0.0	0.0	1.9	1.9	0.0	13.3
Stalk logded plants (#/plot)	0.0	0.0	0.0	0.0	0.0	0.0*	0.0	0.3
Root logded plants (#/plot)	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.3
Final stand count (#/plot)	76.3	76.4	69.2	76.4	75.5	75.5	41.7	80.3
Ear/Kernel rot	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Stalk rot	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Yield (t/ha)	6.1	6.4	5.1	9.3	10.3	10.3	5.7	11.7

⋆ Indicates a significant difference between the test substances (MON 89034) and the control ($p \leq 0.05$).
[1] Data not analysed due to lack of variation

Table 16. Ecological stressor data for MON 89034 compared to its conventional counterpart and commercial reference varieties. European field trials conducted in Germany and Spain in 2007

		Seedling (V2-V4)			Vegetative (V10-V15)			Reproductive (VT-R3)			Harvest (R6)		
Stressors[1]	Site[2]	Test	Ctrl	Ref.	Test	Ctrl	Ref.	Test	Ctrl	Ref.	Test	Ctrl	Ref.
Abiotic													
Animal damage	6	0.0	0.0	0.0									
	8	0.0	0.0	0.0	0.0	0.0	0.0						
	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	1.7	0.0
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Flood damage	6				6.0	3.7	3.2	5.0	3.3	3.0	5.0	3.3	1.5
	8										0.0	0.0	0.0
	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0*	1.3	1.5
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Frost damage	4										3.0	3.0	3.0
	6										4.0	4.0	3.9
	8										5.0	5.0	5.0

Test = MON 89034; Ctrl = Control; Ref. = Reference

* Indicates a significant difference between test and control ($p \leq 0.05$).

[1] Data collected by evaluating each stressor at various stages of growth using a 0-9 scale where 0 = no symptoms and 9 = severe symptoms

[2] German sites: 4, 6, and 8; Spanish sites: 9, 10, 11, 12, and 13

		Seedling (V2-V4)			Vegetative (V10-V15)			Reproductive (VT-R3)			Harvest (R6)		
Stressors[1]	Site[2]	Test	Ctrl	Ref.	Test	Ctrl	Ref.	Test	Ctrl	Ref.	Test	Ctrl	Ref.
Abiotic													
Hail damage	6				0.0	0.0	0.0						
	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	11	0.0	0.0	0.0	0.0	0.0	0.0						
	12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	2.7	1.5
Heat damage	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
	6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
	8	0.0	0.0	0.0	0.0	0.0	0.0						
Drought stress	9				0.0	0.0	0.0	0.0	0.0	0.0			
	10				0.0	0.0	0.0	0.0	0.0	0.0			
	11				0.0	0.0	0.0	0.0	0.0	0.0			
	12				0.0	0.0	0.0	0.0	0.0	0.0			
	13				0.0	0.0	0.0	0.0	0.0	0.0			
Nutrient deficiency	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.0	0.0	0.0	0.0	0.0	0.0				0.0	0.0	0.0
Wind damage	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	6	0.0	0.0	0.0				0.0	0.0	0.0	0.0	0.0	0.0
	8	0.0	0.0	0.0	0.0	0.0	0.0						

Test = MON 89034; Ctrl = Control; Ref. = Reference
1 Data collected by evaluating each stressor at various stages of growth using a 0-9 scale where 0 = no symptoms and 9 = severe symptoms
2 German sites: 4, 6, and 8; Spanish sites: 9, 10, 11, 12, and 13.

		Seedling (V2-V4)			Vegetative (V10-V15)			Reproductive (VT-R3)			Harvest (R6)		
Stressors[1]	Site[2]	Test	Ctrl	Ref.	Test	Ctrl	Ref.	Test	Ctrl	Ref.	Test	Ctrl	Ref.
Disease													
Ear rot	6										0.0	0.0	0.0
	8										0.0	0.0	0.0
Leaf blight	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
	8	0.0	0.0	0.0	0.0	0.0	0.0						
	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pythium	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rust	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.0	0.0	0.0	0.0	0.0	0.0				0.0	0.0	0.0
	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	11	0.0	0.0	0.0	0.0	0.0	0.0				0.0	0.0	0.0
	12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Test = MON 89034; Ctrl = Control; Ref. = Reference

[1] Data collected by evaluating each stressor at various stages of growth using a 0-9 scale where 0 = no symptoms and 9 = severe symptoms

[2] German sites: 4, 6, and 8; Spanish sites: 9, 10, 11, 12, and 13

		Seedling (V2-V4)			Vegetative (V10-V15)			Reproductive (VT-R3)			Harvest (R6)		
Stressors[1]	Site[2]	Test	Ctrl	Ref.	Test	Ctrl	Ref.	Test	Ctrl	Ref.	Test	Ctrl	Ref.
Disease													
Smut (head & ear)	6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	11	0.0	0.0	0.0	0.0	0.0	0.0				0.0	0.0	0.0
	12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Insects													
Agriotes sp. (wireworm)	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.0	0.0	0.0	0.0	0.0	0.0						
	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Test = MON 89034; Ctrl = Control; Ref. = Reference

[1] Data collected by evaluating each stressor at various stages of growth using a 0-9 scale where 0 = no symptoms and 9 = severe symptoms

[2] German sites: 4, 6, and 8; Spanish sites: 9, 10, 11, 12, and 13

		Seedling (V2-V4)			Vegetative (V10-V15)			Reproductive (VT-R3)			Harvest (R6)		
Stressors[1]	Site[2]	Test	Ctrl	Ref.	Test	Ctrl	Ref.	Test	Ctrl	Ref.	Test	Ctrl	Ref.
Aphis sp. (Aphids)	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	2.7	3.0
	6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	3.0	3.0
	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cutworm	6	2.0	3.0	2.7									
Fruit fly	8	0.3	0.0	1.3									
Ostrinia nubilalis (European corn borer)	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0*	0.7	0.3

Test = MON 89034; Ctrl = Control; Ref. = Reference

* Indicates a significant difference between test and control ($p \leq 0.05$).

[1] Data collected by evaluating each stressor at various stages of growth using a 0-9 scale where 0 = no symptoms and 9 = severe symptoms

[2] German sites: 4, 6, and 8; Spanish sites: 9, 10, 11, 12, and 13