Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Preliminary environmental risk assessment of insect resistant genetically modified maize MON 89034 for cultivation (EFSA/GMO/BE/2011/90)

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

Scientific comments submitted to the EFSA GMO Extranet

Date: November 5th, 2012

Doc. no.: 12/309 – final

ISBN: 978-82-8259-068-6

VKM Report 2012: 31

Contributors

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

Assessed by

Panel on Genetically Modified Organisms
Audun H. Nerland (Chair), Åshild Andreassen, Per Brandtzæg, Hilde-Gunn Hoen-Sorteberg, Askild Holck, Olavi Junttila, Heidi Sjursen Konestabo, Richard Meadow, Kåre M. Nielsen, Rose Vikse

Scientific coordinators from the secretariat
Merethe Aasmo Finne, Ville Erling Sipinen

Summary

The environmental risk assessment of the insect resistant genetically modified maize MON 89034 (Reference EFSA/GMO/BE/2011/90) has been performed by the Panel on Genetically Modified Organisms (GMO) of the Norwegian Scientific Committee for Food Safety (VKM). VKM has been requested by the Norwegian Directorate for Nature Management and the Norwegian Food Safety Authority to issue a preliminary scientific opinion on the safety of the genetically modified maize MON 89034 (Unique identifier MON-89Ø34-3) for cultivation, and submit relevant scientific comments or questions to EFSA on the application EFSA/GMOBE/2011/90. The current submission is intended to complement application EFSA-GMO-NL-2007-37, which was approved by Commission Decision 2009/813/EC of 30 October 2009, authorising the placing on the market of products containing, consisting of, or produced from genetically modified maize MON 89034 (scope import, processing, food and feed). Maize MON89034 has previously been assessed by the VKM GMO Panel in connection with EFSA´s public hearing of the application EFSA/GMO/NL/2007/37 (VKM 2008a). Preliminary health- and environmental risk assessments of several stacked events, with MON 89034 as one of the parental lines, have also been performed by the VKM GMO Panel (VKM 2009a, b, c; VKM 2010a,b).

The environmental risk assessment of the maize MON 89034 is based on information provided by the applicant in the application 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 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 2006, 2011a), the environmental risk assessment of GM plants (EFSA 2010), the selection of comparators for the risk assessment of GM plants (EFSA 2011b), and for the post-market environmental monitoring of GM plants (EFSA 2006, 2011c).

The scientific risk assessment of maize MON 89034 include molecular characterisation of the inserted DNA and expression of target proteins, comparative assessment of agronomic and phenotypic characteristics, unintended effects on plant fitness, potential for gene transfer, interactions between the GM plant and target and non-target organisms, effects on biogeochemical processes and evaluations of the post-market environmental plan.

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.

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 analysis of the integration site, including flanking sequence and bioinformatics analysis, has been performed to characterise the transformation event MON 89034. The results of the segregation analysis are consistent with a single site of insertion for the *cry1A.105* and *cry2Ab2* gene expression cassettes and confirm the results of the molecular characterisation. Molecular analysis of both self-pollinated and cross-fertilised lines, representing a total of seven different generations, indicates that the inserted DNA is stably transformed and inherited from one generation to the next. No genes that encode resistance to antibiotics are present in the genome of MON 89034 maize. The molecular characterisation confirmed the absence of both the *aad* and *nptII* genes, which were used in the cloning and transformation process.

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).

Comparative assessment

The field trials for comparative assessment of agronomic and phenotypic characteristics of maize MON 89034 in the USA (2004-2005) and Europe (2007), have been performed in accordance with the EFSAs guidelines for risk assessment of genetically modified plants and derived food and feed (EFSA 2010, 2011a). Based on results from the comparative analyses, it is concluded that maize MON 89034 is agronomically and phenotypically equivalent to the conventional counterpart and commercial available reference varieties, with the exception of the lepidopteran-protection trait. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of MON 89034 compared to conventional maize. Evaluations of ecological interactions between maize MON 89034 and the biotic and abiotic environment indicate no unintended effects of the introduced trait on agronomic and phenotypic characteristics.

Environmental risk

There are no reports of the target Lepidopteran species attaining pest status on maize in Norway. Since there are no Bt-based insecticides approved for use in Norway, and lepidopteran pests have not been registered in maize, issues related to resistance evolution in target pests are not relevant at present for Norwegian agriculture.

Published scientific studies show no or negligible adverse effects of Cry1A.105 and Cry2Ab2 proteins on non-target arthropods that live on or in the vicinity of maize plants. Cultivation of maize MON 89034 is not considered to represent a threat to the prevalence of red-listed species in Norway.

Few studies have been published examining potential effects of Cry1A.105 and Cry2Ab toxin on ecosystems in soil, mineralization, nutrient turnover and soil communities. Some field studies have indicated that root exudates and decaying plant material containing Cry proteins may affect population size and activity of rhizosphere organisms (soil protozoa and microorganisms). However, data are only available from short term experiments and predictions of potential long term effects are difficult to deduce. Most studies conclude that effects on soil microorganisms and microbial communities are transient and minor compared to effects caused by agronomic and environmental factors.

Few studies have assessed the impact of Cry proteins on non-target aquatic arthropods and the fate of these proteins in senescent and decaying maize detritus in aquatic environments. Further studies with better experimental design are needed for the assessment of the potential effects of Bt crops on aquatic organisms. However, exposure of non-target organisms to Cry proteins in aquatic ecosystems is likely to be very low, and potential exposure of Bt toxins to non-target organisms in stream ecosystems in Norway is considered to be negligible.

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. Vertical gene transfer in maize therefore depends on cross-pollination with other conventional or organic maize varieties. In addition, unintended admixture of genetically modified material in seeds represents a possible way for gene flow between different crop cultivations. The risk of pollen flow from maize volunteers is negligible under Norwegian growing conditions.

In addition to the data presented by the applicant, the VKM GMO Panel is not aware of any scientific report of increased establishment and spread of maize MON 89034 and any change in survival (including over-wintering), persistence and invasiveness capacity. Because the general characteristics of maize MON 89034 are unchanged, insect resistance are not likely to provide a selective advantage outside cultivation in Norway.

Since MON 89034 has no altered agronomic and phenotypic characteristics, except for the specific target pest resistance, the VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects due to the establishment and survival of maize MON 89034 will be no different to that of conventional maize varieties in Norway

The environmental risk assessment will be completed and finalized by the VKM Panel on Genetically Modified Organisms when requested additional information from the applicant is available.

Keywords

Maize, *Zea mays* L., genetically modified maize MON 89034, EFSA/GMO/BE/2011/90, insect resistance, Cry proteins, Cry1A.105, Cry2Ab2, cultivation, environmental risk assessment, Regulation (EC) No. 1829/2003, Directive 2001/18/EC

Norsk sammendrag

Miljørisikovurderingen av den genmodifiserte, insektresistente maislinjen MON 89034 (søknad EFSA/GMO/BE/2011/90) fra Monsanto Company er utført av Faggruppen for genmodifiserte organismer i Vitenskapskomiteen for mattrygghet (VKM). VKM er bedt av Direktoratet for naturforvalting og Mattilsynet om å vurdere miljørisiko og landbruksrelatert miljørisiko ved en eventuell godkjenning av maislinjen MON 89034 til dyrking, samt gi kommenterer og innspill til EFSA på søknaden.

MON 89034 ble godkjent til import, prosessering og til bruk som mat og fôr i EU/EØS-området i 2009 (søknad EFSA/GMO/NL/2007/37; Kommisjonsbeslutning 2009/813/EC). I forbindelse med EFSAs offentlige høring av søknaden i 2007, ble maislinjen vurdert av Faggruppe for genmodifiserte organismer (VKM 2008a). MON 89034 er også tidligere vurdert av VKMs faggruppe for genmodifiserte organismer i forbindelse med risikovurderinger av hybrider der MON 89034 inngår som en av foreldrelinjene (VKM 2009a,b,c; VKM 2010a,b).

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

Den vitenskapelige vurderingen omfatter transformasjonsprosess, vektorkonstruksjon, samt karakterisering, uttrykk og nedarving av genkonstruksjonen. Videre er agronomiske og fenotypiske egenskaper, potensialet for ikke tilsiktede effekter på fitness, genoverføring, effekter på målorganismer og ikke-målorganismer, biogeokjemiske prosesser, samt søkers overvåkingsplan 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 fremkommet ved *Agrobacterium*-mediert transformasjon av umodne maisceller fra en av Monsantos innavlede maislinjer. MON 89034-plantene har fått satt inn et rekombinant DNA-fragment med to genekspresjonskassetter, inneholdende genene *cry1A.105* og *cry2Ab2*. *Cry1A.105* er et syntetisk gen, som er sammensatt av sekvenser fra genene *cry1Ac*, *cry1Ab* og *cry1F* fra *Bacillus thuringiensis* subsp. *aizawai*. *Cry2Ab*-genet stammer fra *B. thuringiensis* subsp. *kurstaki*. *Cry1A.105*- og *cry2Ab2*-genene koder for δ-endotoksiner, som gir plantene resistens mot enkelte arter i ordenen Lepidoptera, eksempelvis europeisk maispyralide (*Ostrinia nubilalis*) og *Sesamia nonagrioides*.

Molekylær karakterisering
Faggruppen vurderer karakteriseringen av det rekombinante innskuddet i maislinjen MON 89034, og de fysiske, kjemiske og funksjonelle karakteriseringene av proteinene til å være tilfredsstillende. Faggruppen har ikke identifisert noen risiko knyttet til det som framkommer av den molekylærbiologiske karakteriseringen av de rekombinante innskuddene i maislinjen.

Komparative analyser
Feltforsøkene som ligger til grunn for søkers komparative analyser er i tråd med EFSAs retningslinjer for risikovurdering av genmodifiserte planter og avledete mat- og fôrvarer (EFSA 2010, 2011a).

Feltforsøk i USA (2004-2005) og Europa (2007) indikerer agronomisk og fenotypisk ekvivalens mellom den transgene maislinjen MON 89034 og umodifisert, nær-isogen kontroll og konvensjonelle referansesorter. Det konkluderes med at de innsatte genene i MON 89034 ikke har medført endringer i egenskaper knyttet til vekst og utvikling hos maisplantene.

Miljørisiko
I Norge er det kun registrert enkeltfunn av målorganismen *Ostrinia nubilialis*, men arten er ikke rapportert som skadegjører. Det er ikke gjort observasjoner av andre målorganismer av Lepidoptera i Norge. Siden det ikke er godkjente Bt-produkter til bruk i mais i Norge, og det ikke er registrert Lepidoptera-arter som skadegjørere i mais, er problematikken knyttet til resistens i målorganismene ikke relevant i norsk sammenheng.

Publiserte vitenskapelig studier viser ingen eller neglisjerbare effekter av Cry1A.105- og Cry2Ab2-proteinene på ikke-målartropoder som lever på eller i nærheten av maisplanter. Det vurderes ikke å være risiko for rødlistede arter i Norge.

Det er publisert få studier som har undersøkt effekter av Cry1A.105- og Cry2Ab-toksin på økosystemer i jord, mineralisering og næringstoffomsetning eller effekter på jordsamfunn som bidrar til dette. Det finnes enkeltstudier som viser små, men signifikante effekter av andre Bt-toksiner på jordlevende organismer og mikrobiell samfunnsstruktur i jord. De fleste studiene konkluderer imidlertid med at disse effektene er små og forbigående sammenlignet med effekter av dyrkingsmessige og miljømessige forhold.

Det er kunnskapsmangler med hensyn på effekter av Bt-toksiner på vannlevende organismer. Konsentrasjonene av Bt-endotoksiner er imidlertid vist å være svært lave i akvatiske systemer og eventuell eksponering av toksinene på disse organismene vil være marginal i Norge.

Det vurderes ikke å være økt risiko knyttet til spredning, etablering og invasjon av maislinjen i naturlige habitater, eller utvikling av ugraspopulasjoner av mais i dyrkingsmiljø sammenlignet med konvensjonelle sorter.

Det er ingen stedegne eller introduserte viltvoksende arter i den europeiske flora som mais kan hybridisere med, og vertikal genoverføring vil være knyttet til krysspollinering med konvensjonelle og eventuelle økologiske sorter. I tillegg vil utilsiktet innblanding av genmodifisert materiale i såvare representere en mulig spredningsvei for transgener mellom ulike dyrkingssystemer. En slik spredning vurderes som ubetydelig.

Miljørisikovurderingen av den genmodifiserte maislinjen MON 89034 vil ferdigstilles og sluttføres av VKMs faggruppe for genmodifiserte organismer når endelig dokumentasjon fra søker foreligger.

Abbreviations and explanations

ALS	Acetolactate synthase, an enzyme that catalyses the first step in the synthesis of the branched-chain amino acids, valine, leucine, and isoleucine
AMPA	Aminomethylphosphonic acid, one of the primary degradation products of glyphosate
ARMG	Antibiotic resistance marker gene
BC	Backcross. Backcross breeding in maize is extensively used to move a single trait of interest (e.g. disease resistance gene) from a donor line into the genome of a preferred or "elite" line without losing any part of the preferred lines existing genome. The plant with the gene of interest is the donor parent, while the elite line is the recurrent parent. BC_1, BC_2 etc. designates the backcross generation number.
BLAST	Basic Local Alignment Search Tool. Software that is used to compare nucleotide (BLASTn) or protein (BLASTp) sequences to sequence databases and calculate the statistical significance of matches, or to find potential translations of an unknown nucleotide sequence (BLASTx). BLAST can be used to understand functional and evolutionary relationships between sequences and help identify members of gene families.
bp	Basepair
Bt	*Bacillus thuringiensis*
CEW	Corn earworm, *Helicoverpa zea*
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
DN	Norwegian Directorate for Nature Management (Direktoratet for naturforvalting)
DNA	Deoxyribonucleic acid
DT50	Time to 50% dissipation of a protein in soil
DT90	Time to 90% dissipation of a protein in soil
dw	Dry weight
dwt	Dry weight tissue
EC	European Commission/Community
ECB	European corn borer, *Ostrinia nubilalis*
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	Environmental risk assessment
E-score	Expectation score
EU	European Union
fa	Fatty acid
FAO	Food and Agriculture Organisation
FIFRA	US EPA Federal Insecticide, Fungicide and Rodenticide Act

Fitness	Describes an individual's ability to reproduce successfully relative to that of other members of its population
fw	Fresh weight
fwt	Fresh weight tissue
GAT	Glyphosate N-acetyltransferase
GLP	Good Laboratory Practices
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 that a given gene occupies on a chromosome
LOD	Limit of detection
LOQ	Limit of quantitation
MALDITOF	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*
MON 89034	A Monsanto maize event which produces the *Bt*-proteins Cry1A.105 and Cry2Ab2
mRNA	Messenger RNA
MT	Norwegian Food Safety Authority (Mattilsynet)
NDF	Neutral detergent fibre, measure of fibre used for animal feed analysis. NDF measures most of the structural components in plant cells (i.e. lignin, hemicellulose and cellulose), but not pectin
Northern blot	Northern blot is a technique used in molecular biology research to study gene expression by detection of RNA or isolated mRNA in a sample
NTO	Non-target organism
Nicosulfuron	Herbicide for maize that inhibits the activity of acetolactate synthase
Near-isogenic lines	Term used in genetics, defined as lines of genetic codes that are identical except for differences at a few specific locations or genetic loci
OECD	Organisation for Economic Co-operation and Development
ORF	Open Reading Frame, in molecular genetics defined as the part of a reading frame that contains no stop codons
OSL	Overseason leaf
OSR	Overseason root
OSWP	Overseason whole plant
PCR	Polymerase chain reaction, a biochemical technology in molecular biology to amplify a single or a few copies of a piece of DNA
PV-ZMIR245	Plasmid vector used to develop MON 89034
R0	Transformed parent
Rimsulfuron	Herbicide, inhibits acetolactate synthase
RNA	Ribonucleic acid
RP	Recurrent parent
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis. Technique to separate proteins according to their approximate size
SAS	Statistical Analysis System
SD	Standard deviation

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

Table of contents

Background

On 21 December 2010, the European Food Safety Authority (EFSA) received from the Competent Authority of Belgium an application (Reference EFSA-GMO-BE-2011-90) for authorisation of the insect resistant genetically modified (GM) maize MON 89034 (Unique Identifier MON-89Ø34-3), submitted by Monsanto Company under Regulation (EC) No 1829/2003. The scope of the application is for cultivation in the European Union. The current submission is intended to complement application EFSA-GMO-NL-2007-37, which was approved by Commission Decision 2009/813/EC of 30 October 2009, authorising the placing on the market of products containing, consisting of, or produced from genetically modified maize MON 89034 (scope import, processing, food and feed).

After receiving the application EFSA-GMO-BE-2011-90 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 publicly 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 May 12 2012, 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 (MS) and the European Commission 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. All MS comments submitted during the consultation period will be considered by three working groups of the EFSA GMO Panel and responses to each individual comment will be provided.

The Norwegian Scientific Committee for Food Safety (VKM) has been requested by the Norwegian Directorate for Nature Management and the Norwegian Food Safety Authority to carry out a preliminary environmental risk assessment of maize MON 89034 for cultivation, and to submit relevant scientific comments or questions to EFSA on the application EFSA-GMO-BE-2011-90.

The environmental risk assessment will be completed and finalized by the VKM Panel on Genetically Modified Organisms when requested additional/final information from the applicant is available.

Terms of reference

The Norwegian Scientific Committee for Food Safety (VKM) carries out independent risk assessments for the Norwegian Food Safety Authority (Mattilsynet) across the Authority's field of responsibility as well as environmental risk assessments of genetically modified organisms for the Directorate for Nature Management (Direktoratet for naturforvalting (DN)).

The Norwegian Food Safety Authority

By way of letter from the Norwegian Food Safety Authority dated October 15 2010 (ref. 2010/195445) the Norwegian Scientific Committee for Food Safety (VKM), has been assigned to evaluate submissions sent to the European Commission under the Regulation (EC) 1829/2003. The Regulation concerns commercial approval of genetically modified organisms and their derivatives including processed non-germinating products, intended for use as or in food or feed. VKM is to evaluate any potential health risks of such products. In addition, VKM is requested to evaluate the potential risks of genetically modified plants (GMPs) to the Norwegian agriculture and/or environment, and whether they are relevant for cultivation in Norway. Depending on the intended use of the GMP(s), defined by the applicant, the environmental risk assessment will 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.

In the case of submissions regarding cultivation, VKM is further requested to assess risks concerning coexistence of cultivars. The assessment should cover the potential spread of plant materials from GMP-crops to areas of non-GMP crops as well as wild populations of endogenous plants of the same or similar species outside the cultivated areas, in addition to development and progression of weed populations. Evaluation of suggested measures for environmental monitoring provided by the applicants, in general or specific, are not covered by the assignment from the Norwegian Food Safety Authority.

The Norwegian Directorate for Nature Management

By way of letter from the Directorate for Nature Management (DN) dated June 15 2011 (ref. 2008/4367 ART-BI-BRH) the Norwegian Scientific Committee for Food Safety has been assigned to evaluate the potential environmental risks related to submissions of approval for the release of GMOs, i.e. cropping, sent to the EU Commission under the Directive (EC) 2001/18 and Regulation (EC) 1829/2003, and that are relevant to the Norwegian Gene Technology act. The task of VKM includes establishing scientific enquiries and comments as well as initial environmental risk assessments related to the submissions. VKM is also requested to deliver finalised reports on environmental risks in conjunction with national completion of the submissions.

The basis for evaluating the applicants environmental risk assessments is embodied in the act relating to the production and use of genetically modified organisms (Norwegian gene technology act), regulation on the assessment of potential impact based on the Norwegian gene technology act, the Directive 2001/18/EC on the deliberate release of genetically modified organisms into the environment, Guidance note in Annex II of the Directive 2001/18 (2002/623/EC) and the Regulation 1829/2003/EC. In addition, the EFSA guidance documents on risk assessment of genetically modified plants and food and feed from the GM plants (EFSA 2006, 2010, 2011a), and OECD guidelines will be useful tools in the preparation of the Norwegian risk assessments.

According to the assignment from the Directorate for Nature Management, VKM is to focus on environmental risk within the EEA and specific risks to Norway in particular.

Risk assessments provided by VKM on all submissions concerning approval of cultivation within the EEA are requested to include the potential environmental risks of the product related to any changes in agricultural practices. The assignment covers assessment of direct environmental impact of the intended use of pesticides with the GMO under Norwegian conditions, as well as changes to agronomy and possible long-term variations in the use of pesticides.

The preliminary reports on environmental risks provided by VKM should also consider the applicants recommended general and/or specific measures for monitoring. When recommended specific measures for monitoring are provided by the applicant, VKM must determine if these recommendations are sufficient to disclose potential direct short term effects, as well as delayed and indirect long term effects. If no specific measures are suggested in the application, VKM must also evaluate whether or not specific measures are required.

In accordance with the assignments given by the Norwegian Food Safety Authority, and the Directorate for nature management, VKM will provide input on said submissions without specific requirements, to the EFSA GMO EXTRAnet (initial input), with copies sent to both the Norwegian Food Safety Authority and the Directorate for nature management. Likewise, if no input or comments are made or submitted to EFSA on certain submissions, VKM will inform of this as well. The Norwegian Food Safety Authority also requests that it is made evident in the risk assessments provided by VKM whether or not the applicant has committed to the EFSA guidelines on risk evaluation of GMOs and their derived products for food and feed (EFSA 2006, 2010, 2011a).

VKM is further requested to follow up on EFSAs response and to consider whether the inputs by VKM to the EFSA GMO EXTRAnet are appropriately preserved in EFSAs own assessments.

The submission EFSA/GMO/BE/2011/101, genetically modified maize event MON 89034, was posted on the EFSA GMO Extranet May 12 2012. The VKM GMO Panel will in compliance with the letters of engagement prepare an environmental risk assessment with regards to cultivation of the maize event MON 89034. The evaluation will be implemented in light of the intended use and in accordance with the principles denoted in the EFSA guidelines on risk assessment of genetically modified plants and derived products for food and feed (EFSA 2006, 2010, 2011a).

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*). None of these insects are present in the Norwegian agriculture. 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 2010), the selection of comparators for the risk assessment of GM plants (EFSA 2011b), and for the post-market environmental monitoring of GM plants (EFSA 2006, 2011c).

The environmental risk assessment of the GM maize MON 89034 is based on information provided by the applicant in the application 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 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 PVZMIR245 was induced to be 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 1 provides a schematic representation of Cry1A. 105.

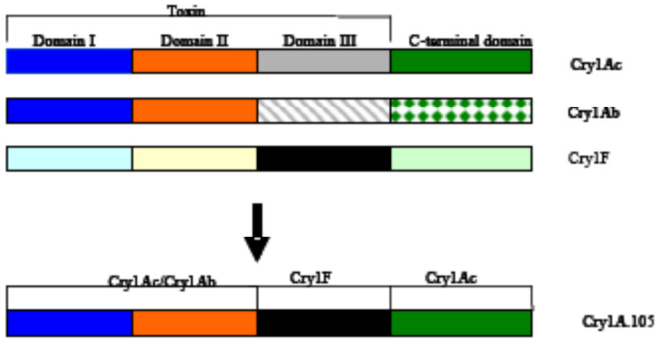

Figure 1. Schematic representation of the Cry1A.105 protein domain similarity to Cry1Ac, Cry1Ab and Cry1F

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, that the introduction of MON 89034 will provide superior maize hybrids with higher yields, better quality grain, reduced potential for the development of insect resistance to *Bt* proteins, as well as enhanced breeding efficiencies.

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 2. 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 2). 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.

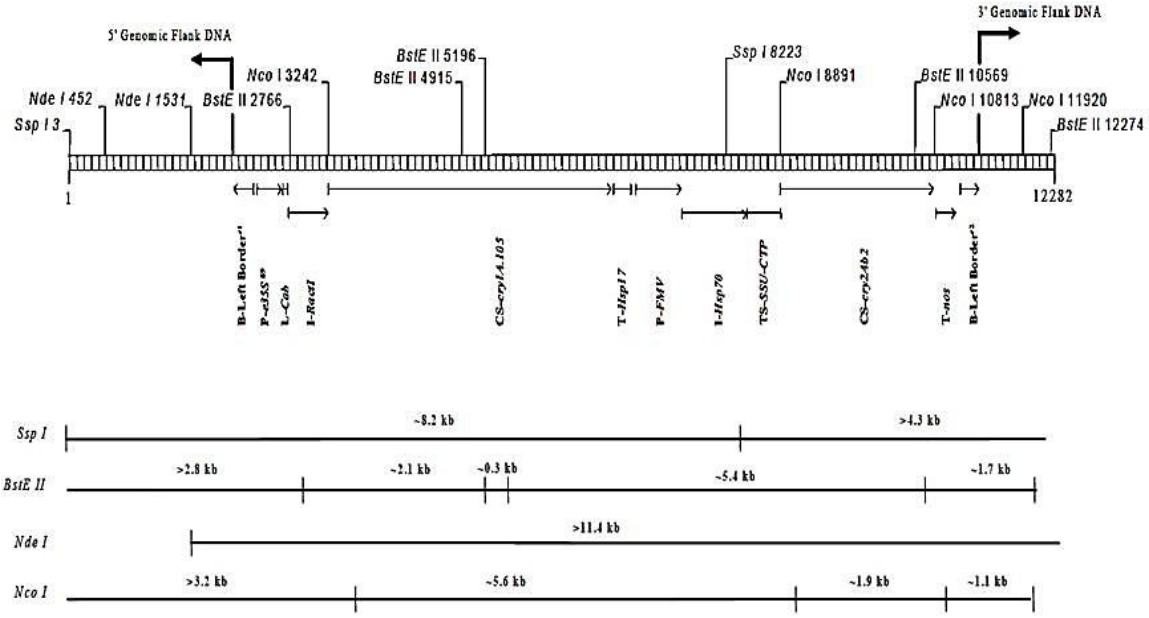

Figure 2. Schematic representation of the insert and genomic flanking sequences in MON 89034.
The linear DNA derived from T-DNA I of vector PV-ZMIR245 which was incorporated into MON 89034 is shown. Arrows in black indicate the end of the insert and the beginning of maize genomic flanking sequence. Identified 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 2.

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 3). 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.

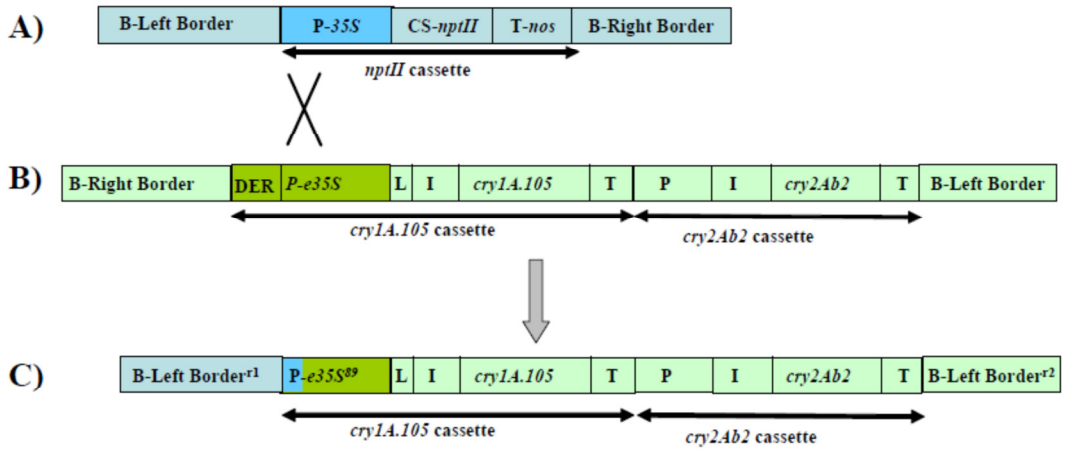

Figure 3. Description of the recombination process that explains the modified 5'end of the insert
A) Illustration of the T-DNA II of plasmid PV-ZMIR245
B) Illustration of the T-DNA I of plasmid PV-ZMIR245
C) Illustration of the modified T-DNA in MON 89034
Abbreviations and symbols: DER = duplicated enhancer 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 applicant concludes that only the two proteins, Cry1A.105 and Cry2Ab2, are encoded by the DNA insert present in MON 89034, that no endogenous genes were found in the analysed sequences that flank the MON 89034 T-DNA insertion site, and that 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. 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.

2.2.3 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). Data from these protein expression studies have previously been assessed by the VKM GMO Panel (VKM 2008). I 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).

2.2.3.1. 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; Niemeyer & Silvanovich 2008a).

Field sites were selected that represent the major maize growing region of the U.S., 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 2) 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

[1] stalk and leaf material remaining after harvest

grain was harvested at maturity. Stover was collected following harvest at approximately 130 – 160 days after planting.

2.2.3.2. 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 7 and table 4 & 6 in Appendix 1). Limits of detection and quantification are presented in Table 4 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 1, 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. 2006c). The Cry1A.105 and Cry2Ab2 protein levels obtained from these sites are presented in Table 2, 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 2008a). 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 3.

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 2). 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, 7.4 µg/g dwt in forage root, 40 µg/g dwt in forage, 24 µg/g dwt in pollen and 1.8 µg/g dwt in grain (Table 2). 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.

2.2.3.3 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

2.2.3.4 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.

Table 3. 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 4. 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.

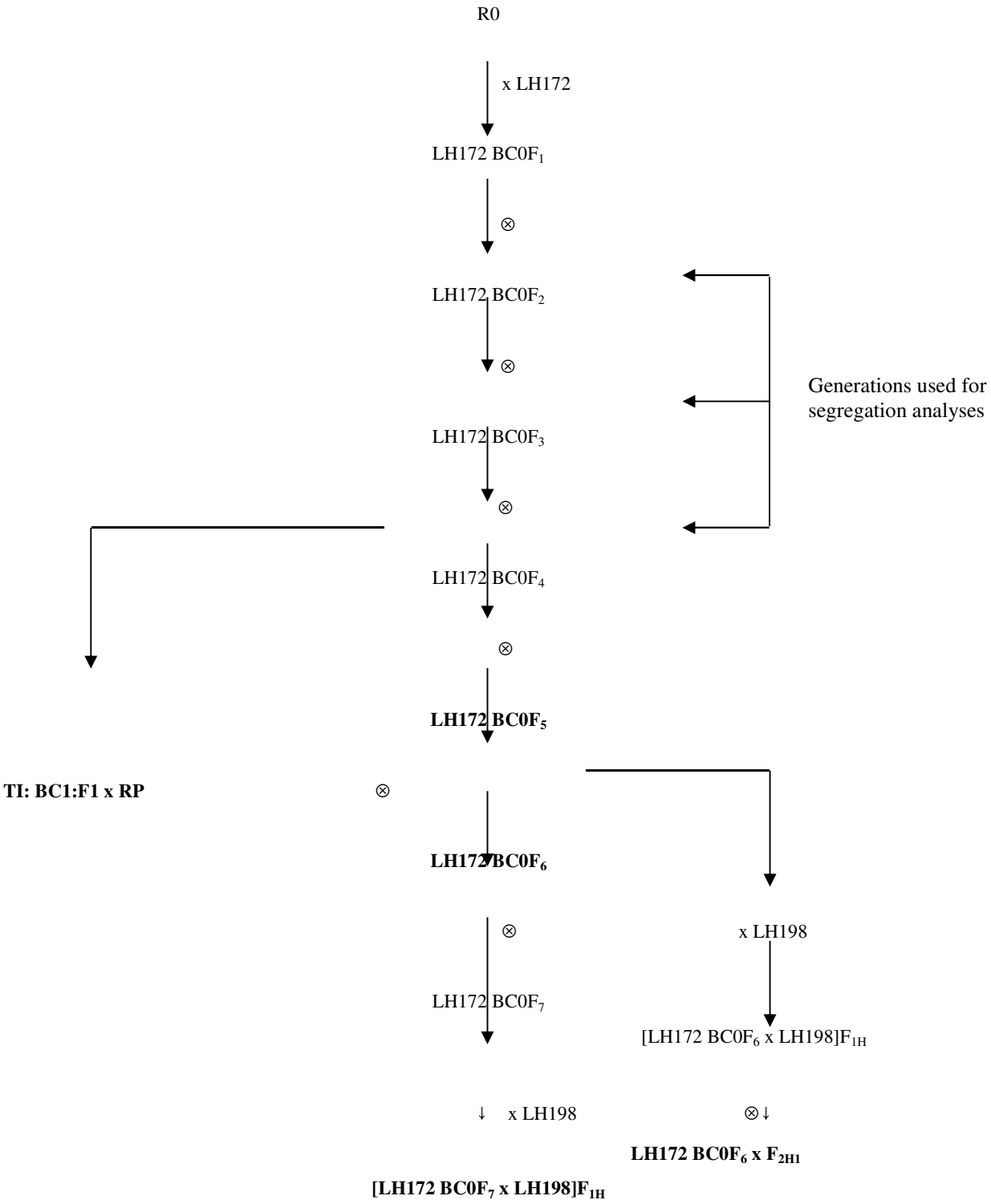

Figure 4. **Breeding history of maize MON 89034**
The LH172 $BC0F_6$ x F_{2H1} generation was used for all the molecular analyses. Generations in bold were used for molecular stability analyses.
Key: R_0 = primary transformant; F(#) = filial generation; ⊗ = self-pollination; BC(#) = backcross generation; RP = recurrent parent; H = hybrid; TI = trait integration.

2.3.4 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.

2.3.4.1 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 4; 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 5). 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 6.

Table 5. 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).

The results of the Chi-square test[2] are summarised in Table 6. 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.

Table 6. 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

2.5 Assessment based on available data

Appropriate analysis of the integration site, including flanking sequence and bioinformatics analysis, has been performed to characterise the transformation event MON 89034. The results of the segregation analysis are consistent with a single site of insertion for the *cry1A.105* and *cry2Ab2* gene expression cassettes and confirm the results of the molecular characterisation. Molecular analysis of both self-pollinated and cross-fertilised lines, representing a total of seven different generations, indicates that the inserted DNA is stably transformed and inherited from one generation to the next. No genes that encode resistance to antibiotics are present in the genome of MON 89034 maize. The molecular characterisation confirmed the absence of both the *aad* and *nptII* genes, which were used in the cloning and transformation process.

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).

[2] Computed as $\chi^2 = \Sigma [(\left| o - e \right| - 0.5)^2/e]$ 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 Maize crop production in Norway

There is no official agricultural statistics of the total crop area of maize in Norway. Most of the maize in Norway is grown for feed, where the whole plant is harvested for silage before grain ripening. Information from various seed companies indicates cropping areas of forage maize of about 2000-2800 decares the latest five years period. This is equivalent to less than 0.1% of the areas with cereal crops (Netland et al. 2012). In the period 2005-2010, the area of sweet corn for human consumption varied between 286 and 1183 decares (Statistics Norway 2011). According to Debio, the Norwegian control body for organic crop production, there are no cropland under organic management certified for maize production in Norway (www.debio.no). So far, no maize areas are in the process of conversion to organic farming.

The maize crop production is mainly located in the southeastern Norway, with the largest areas located in the counties of Østfold and Vestfold. There is also some cultivation of fodder maize in Agder and Rogaland.

There is a growing interest in commercial cultivation of forage maize in Norway (Netland et al. 2012). Silage of maize is especially suitable for cattle, and yields of 800-1000 kg dry matter per decare provide a profitable production and an energy-rich and palatable feed supplement which can replace traditional forage and concentrates for livestock. Maize is not labor intensive production, and when the growth season is long enough, maize provides a digestible and nutritious feed that can increase the forage intake. However, if the growing season is too short, and the maize cobs do not get time to evolve, the feed unit concentration becomes very low (0.75 FEm/kg TS; http://www.grovfôrnett.no).

Results from Norwegian field trials demonstrate large differences with respect to yields and qualities of forage maize, both between experimental years and field sites. In a field study from Nord-Trøndelag, Nesheim (2008) reported high dry matter yields of forage maize when growing maize under a plastic film cover (1100 kg t.s. per decare). Other studies have, however, denoted maize crop production in Trøndelag and Rogaland with the current varieties as risky, also if intensive farming methods as establishing maize under plastic cover are adopted (Bakken et al. 2005). In this experiment, Bakken et al. tested a selection of early maturing varieties at different locations in the South and Middle- Norway. The authors concluded that even in the best agricultural areas in the Oslofjord region, maize production will imply risk of crop failure and yields of varying quality. These results are consistent with recent, unpublished studies (T. Lunnan pers. com)

It is not expected a strong increase in the maize cultivation in Norway without a further improvement of adapted cultivars and technology that enables earlier sowing and/or that a larger proportion of the cattle production occurs in the southeastern Norway (Bakken et al. 2005; T. Lunnan pers. com). In the traditional livestock districts the growing season is too short that forage maize can be a real alternative to other forage productions (Netland et al. 2012). Climate change, which entails a longer growing season and higher average temperatures, however, can in the long term expand the maize cultivation area in Norway.

4 Comparative assessment

Agronomic and phenotypic characteristics 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 (Application EFSA-GMO-NL-2007-37), and have previously been assessed by the VKM GMO Panel (VKM 2008a). Since the data of the current application is intended to complement the data presented in application EFSA-GMO-NL-2007-37, the same information that was provided within the context of this application (2004-2005 US field trials) is presented in the current application. In addition, a summary of the results from field trials conducted in Germany and Spain in 2007 is also included in this application (CBI Technical Dossier: De Billot 2010).

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

4.1.1 Experimental design

Comparative assessments of phenotypic, agronomic and ecological characteristics of MON 89034 and conventional maize have been conducted in 2004-2005 at nine field locations within major US maize producing geographies, and in 2007 at eight field locations within two major European maize producing regions (Tech. Doss: De Billot 2010).

Field trials US (2004-2005)
Phenotypic and agronomic data were collected from 18 field locations over two consecutive years: nine locations in 2004 and nine locations in the 2005 growth season. 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 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 4). As a consequence, the 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. (EFSA Regulation (EC) No 1829/2003 defines a conventional counterpart as "a similar food or feed produced without the help of genetic modification and for which there is a well-established "history of safe use" (Art. 2.12). In line with this legal requirement the EFSA GMO Panel provides details on the criteria for the selection of appropriate comparators, under different scenarios, in the EFSA Guidance for the Selection of comparators for the risk assessment of GM plants (EFSA 2011b)).

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[3]. 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.

[3] H8751 and H9231 (Golden Harvest); N60-N2 (Northrup King); 590 (Burrus), 2784, 2E685, 2P682 and 2A791 (Mycogen); DKC62-15, DKC61-42 and DKC60-15 (Dekalb); 2730 (Pfister); SC1124A (Seed Consultants); 4908 (Crow's); RX708 (Asgrow).

Field trials Europe (2007)
Phenotypic and agronomic data were collected from eight field locations in Europe, five in Spain and three in Germany over one 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 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 to the study area at any site.

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). In the European study, separate combined site analyses were conducted for Germany and Spain. For each site and substance, various plant phenotypic characteristics (Table 5) and stressor damage types (Table 6) were measured.

Difference and equivalence tests were conducted using statistical models consistent with EFSA guidelines (EFSA 2010, 2011b). Difference testing was performed at the 10% level of significance ($\alpha = 0.10$), and equivalence testing performed at the 5% level ($\alpha = 0.05$).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. Due to lack of variability in the responses in the European study, phenotypic data from five parameters in the German trial and two of the parameters in Spain were excluded from the combined site analysis. Further, only five of the variables from the study of environmental interactions were included for a statistical combined site analysis.

Table 7. 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

4.2 Agronomic traits and GM phenotype

During field trials conducted over two 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 7. 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 9).

4.2.1 Agronomic and phenotypic results

Field trials USA (2004-2005)
VKM (2008a):
«I henhold til søkers dokumentasjon er det foretatt registreringer av karakterer knyttet til reproduksjon, spredning, vegetativ vekst, sjukdoms- og insektresistens, samt toleranse mot ulike abiotiske stressfaktorer (tørke, vind, næringsmangel etc.). Resultatene viser signifikante forskjeller ($p \leq 0,05$) mellom MON 89034 og umodifisert kontroll for karakterene plantehøyde og lengde i 2004-forsøkene. Gjennomsnittsverdiene for disse karakterene ligger imidlertid innenfor variasjonsområdene for referansesortene som er presentert i søknaden. For de øvrige agronomiske og morfologiske egenskapene ble det ikke funnet signifikante forskjeller. Med bakgrunn i manglende registreringer over flere vekstsesonger er alle statistiske analyser foretatt innen år. Det er derfor ikke mulig å vurdere effekt av år eller stabilitet over år for de fenotypiske egenskapene.

I tillegg til feltforsøkene er det er foretatt spiretester i vekstkamre under ulike temperaturregimer. Det ble ikke funnet signifikante forskjeller mellom den transgene linjen og kontrollsorter med hensyn på de undersøkte parameterne knyttet til frøkvile og spiring. Undersøkelser av pollendiameter og -vitalitet viste at uttrykk av Cry1A.105- og Cry2Ab2- proteiner ikke har effekt på morfologi og vitalitet av pollen fra MON 89034.»

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 8. 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 8). In the combined-site analysis for Spain, significant differences were detected between MON 89034 and the control for the parameter "stalk logded plants" ($p \leq 0.05$). There were fewer stalk logded plants in 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.

Table 8. 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

4.2.2 Ecological interactions

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 9). 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 3). 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.

Table 9. 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

4.3 Assessment based on available data

Based on results from comparative analyses of data from field trials located at representative sites and environments in the USA (2004-500) and Europe (2007), it is concluded that maize MON 89034 is agronomically and phenotypically equivalent to the conventional counterpart and commercially available reference varieties, with the exception of the lepidopteran-protection trait, conferred by the expression of the Cry1A.105 and Cry2Ab2 proteins. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of MON 89034 compared to conventional maize. Evaluations of ecological interactions between maize MON 89034 and the biotic and abiotic environment indicate no unintended effects of the introduced trait on agronomic and phenotypic characteristics.

5 Environmental risk assessment

5.1 Unintended effects on plant fitness due to the genetic modification

Maize is a highly domesticated annual plant and generally unable to survive in the environment without management intervention (Eastham & Sweet 2002). Maize propagates entirely by seed produced predominantly by cross-pollination (OECD 2003). In contrast to weedy plants, maize has a pistillate inflorescence (ear) with a cob enclosed with husks. Due to the structure of the cob, the seeds remain on the cob after ripening and natural dissemination of the kernels rarely occurs. In Norway, practically all maize is grown for feed, where the whole plant is harvested for silage before grain ripening. There is only a very limited production of sweet corn for human consumption (see section 3.0).

During harvest and post-harvest activities, some cobs, cob fragments and/or isolated kernels may remain in the field or accidentally be spilled outside agricultural areas. Survival of maize in Europe is, however, limited by a combination of absence of a dormancy phase, 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 cannot survive temperatures below 0ºC for more than 6 to 8 hours after the growing point is above ground (5 to 7 leaf stage) (OECD 2003), and in Norway and most of Europe, maize kernels and seedlings do not survive the winter cold (Gruber et al. 2008). In regions with mild winters, however, maize volunteers frequently occur (BEETLE Report 2009). Crop management and climatic conditions during the post-harvest and sowing periods are the main factors that determine the presence of volunteers. If the following autumn is wet, the kernels will germinate and plantlets will die without flowering. In dry conditions, the kernels remain in the field until the next sowing season, when they will germinate and reach the flowering stage (Devos et al. 2009). In Spain, volunteer densities from residuals of up to 7000-8000 plants/ha have been reported, which corresponds to approximately 10 % of the maize planting densities (Melé et al. 2007; Palaudelmás et al. 2009). Field observations performed on maize volunteers after cultivation of GM maize in Spain revealed that maize volunteers had low vigour, tended to flower asynchronously with the cultivated maize crops in which they occur and rarely had cobs (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.

During the long process of domestication maize has lost the ability to survive outside cultivation. In spite of extensive 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 (ref. Eastham & Sweet 2002; Devos et al. 2009). There are no native or introduced sexually cross-compatible species in the European flora with which maize can hybridise and form backcross progeny (Eastham & Sweet 2002; OECD 2003). The only recipient plants that can be cross-fertilised by maize are other cultivated maize cultivars and types (e.g. Sanvido et al. 2008). The BEETLE report (2009) assessed the likelihood for increased fitness for Bt maize in Europe to be negligible.

It is considered very unlikely that the establishment, spread and survival of maize MON 89034 would be increased due to the insect resistance trait. The insect protection against Lepidoptera is not regarded as providing a significant selective advantage to maize plants in Europe, except under high infestation conditions in cultivated fields. In Norway, there have been only a few reports of the target pests (section 5.3), and this trait cannot be regarded as a potential selective advantage to maize MON 89034. Moreover, it is considered very unlikely that maize MON 89034 plants and their progeny will differ from conventional maize varieties 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 4.2).

The European agronomic and phenotypic 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 2003). Vertical gene transfer in maize therefore depends on cross-pollination with other conventional or organic maize varieties. All maize varieties which are cultivated in Europe can interbreed. In addition, unintended admixture/adventitious presences of genetically modified material/transgenes in seeds represent a possible way for gene flow between different 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 2004b, 2009b; 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 in 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 feces 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.

The origin and properties of the inserted genes does not suggest a novel directional positive selection of the plant transgenes in MON 89034 in bacterial recipients.

In conclusion, the VKM GMO Panel consider it is unlikely that genes from MON 89034 will transfer and established in the genome of microorganisms in the environment or in the intestinal tract of humans or animals

5.2.2 Plant to plant gene flow

Reproduction biology
Cultivated maize (*Zea mays* L.) is a member of the grass family *Poaceae*. Maize is presumed to have derived from teosinte (*Z, mexicana),* a plant native to Central America, and was introduced into Europe in the sixteenth century. Maize is a tall, monoecious, annual grass with separate male and female flowers on the same plant. The functional staminate flowers are borne in male tassels located terminally on the stems, and the female cobs are borne in the axils of the middle leaves.

Maize is predominantly a protandrous and out-crossing species, where the male inflorescence (tassel) appears around two to four days before silk emergence (Sleper & Poehlman 2006). There is however usually some overlap of pollen shedding and silk emergence on the same plant that can account for up to 5 % self-pollination (Eastham & Sweet 2002). Maize is predominantly wind-pollinated, although there is evidence to suggest that honey bees and other insects collect pollen from maize (Treu & Emberlin 2000). However, the female flowers of maize produce no nectars and pollinating insects usually do not contribute to fertilisation and cross-pollination of maize plants (Eastham & Sweet 2002; Malone & Burgess 2009; OGTR 2008; Tolstrup et al. 2003).

Pollen is released from the tassels in large quantities. It has been estimated that for each ovule developing into a kernel an individual plant delivers from 9000 to 50000 pollen grains. Assuming an average ear of maize grows approximately 500 kernels, a plant will yield between 4.5-25 million pollen grains (ref. Eastman & Sweet 2002). Compared to pollen of other wind-pollinated species, pollen grains of maize are relatively large (diameter 90-125 µm) and heavy (0.25 µg) (Aylor et al. 2003; Di-Giovanni et al. 1995; Raynor et al. 1972).

The longevity of maize pollen viability strongly differs according to air temperature and humidity, and published data on the length of time that maize pollen remains viable under natural conditions varies from about 24 hours to several days (Eastman & Sweet 2002). Dehydration is the main factor in maize pollen mortality and water loss in pollen grains during dispersal reduces their ability to germinate on

the stigma (Aylor 2004). In exceptionally hot, dry weather the viability could be reduced to a few hours, and extended up to nine days in cooler, humid conditions (Emberlin et al. 1999; Luna et al. 2001). It can therefore be expected that maize pollen on average has a longer viability under Norwegian growing conditions compared to most of the studies that have been published on outcrossing in maize (VKM 2005). The water content also affects the physical shape of the pollen grain and its flight dynamics (Aylor 2002; Aylor et al. 2003).

Pollen-mediated gene flow

Numerous studies have been conducted on pollen dispersal and outcrossing in maize (for a review, see BEETLE report 2009; Brookes & Barfoot 2004; Devos et al. 2005; Eastham & Sweet 2002; Feil & Schmid 2002; Sanvido et al. 2008). However, a general interpretation of the results is often difficult because of significant methodological differences and experimental conditions between studies and various environmental factors which are known to influence cross-fertilizations rates (Ingram 2000; Devos et al. 2005). In addition to direct measurements of pollen concentration at different distances from the pollen source, various qualitative and quantitative methods have been used to estimate the actual outcrossing in maize (phenotypic markers, protein analysis, molecular markers, quantitative DNA analysis) (Devos et al. 2005). More recent studies are based on different mathematical models for simulation of the potential for outcrossing under different growing conditions.

A number of abiotic and biotic parameters are known to influence outcrossing rates in maize (Hüsken et al. 2007; Sanvido et al. 2008; Palaudelmás et al. 2009). These factors include size, shape and orientation of both pollen source and recipient field, as well as distance, topography and vegetation between pollen source and recipient field. The size of the experimental donor and receptor fields determines the amount of competing pollen (Ingram 2000; Devos et al. 2005). E.g. a high donor to receptor ratio (large donor field, small receptor field) leads to a higher amount of pollen from the donor field resulting in high cross-fertilisation rates in the receptor field due to low competition with incoming pollen. The shape of the fields is another factor that may influence cross-pollination. The amount of cross-fertilisation is clearly higher in elongated recipient fields than in rectangular ones of the same surface area when the long side of the field faces the source (Messeguer et al. 2006). Using SSR analysis to identify the origin of pollen showed that while changes in the size of the donor field clearly influences the percentage of GMO detected, this effect is moderate (Palaudelmás et al. 2012). This study demonstrated that doubling the donor field size resulted in an approximate increase of GM content in the receptor field by 7 %. This indicates that variations in the size of the donor field have a smaller influence on GM content than variations in the size of the receptor field. Similarly, a buffer zone with the same competitive agricultural crop will produce pollen, as well as being a physical obstacle to wind-dispersed pollen between fields, and reduce the outcrossing effectively.

The rate of cross-fertilisation between fields also depends on pollen viability and longevity, male fertility and/or sterility, synchrony in flowering between anthesis of the pollen donor and silking of the recipient field, wind direction and velocity and weather conditions. However, distance between the fields, flowering coincidence and orientation to prevailing horizontal wind speed have been identified within the EU-project SIGMEA as the major factors affecting cross pollination in maize (Hüsken et al. 2007; SIGMEA 2009).

When assessing the frequencies of outcrossing, it is also important to take the intended use of the maize plant into consideration (Tolstrup et al. 2007). In forage maize, harvested as whole plants for ensilage or direct feed, the vegetative tissue that is not affected by cross-pollination will constitute a major part of the yield/final product (depending on cultivar and maturity level).

The basic pattern of outcrossing in maize is described by the leptokurtic pollen dispersal curve. The highest pollen concentrations and most of the crossing and fertilisation occur close to the pollen source with a strong exponential decrease near the source field followed by a very slow decline with increasing distance (e.g. Eastham & Sweet 2002). Due to its pollen characteristics, maize pollen has a high settling speed and usually has a short flight range, and pollen concentrations decline rapidly with the distance from the source (Jarosz et al. 2005). Most of the pollen falls within 5 m of the fields'

edge and approximately 95-99 % of the released pollen is deposited within about 30 m from the pollen source (Devos et al. 2005). At distances further than 30-50 m, the levels of pollen dispersion are very low but there is no clear cut-off distance beyond which these levels reach zero.

Under suitable meteorological conditions maize pollen can be lifted high up in the atmosphere and distributed over significant distances up to kilometers (Jarosz et al. 2005; Hofmann et al. 2010). However, vertical wind movements or gusts during pollen shedding only lead to very low levels of cross-fertilisation over longer distances (Palaudelmás et al. 2012). Most cross-pollination events occur within 40 m of the pollen source (reviewed by Eastham & Sweet 2002; Brookes at al. 2004; Devos et al. 2005; Hüsken et al. 2007; Sanvido et al. 2008; Riesgo et al. 2010; Palaudelmás et al. 2012).

Sanvido et al. (2008) have reviewed existing cross-fertilisation studies in maize and established relevant criteria for the evaluation of these studies and applied criteria to define science-based isolation distances. The results of their analysis showed that an isolation distance of 20 m for silage maize, and 50 m for grain maize, respectively, is sufficient to keep GM-inputs from cross-fertilisation below the arbitrary level of 0.5 % at the border of a conventional/non-GM maize field. The proposed isolation distances represent a rather conservative approach leaving an additional safety margin up to the current legal threshold of 0.9 % in the final product.

Occasionally, however, and particularly in the case of small fields less than 0.5 ha and/or of long, narrow fields that are located downwind from a larger GM maize field, the isolation distance may need to be extended to 50 m or more (Devos et al. 2005; Hüsken et al. 2007). Based on a statistical analysis of different datasets on cross-fertilisation rates, Riesigo et al. (2010) concluded that a separation distance of 40 m is sufficient to reduce admixture in maize cultivation to below the legal threshold of 0.9 % in the EU.

Cross-pollination in maize has been examined in great detail in several European countries in the EU Program 'Sustainable Introduction of GM Crops into European Agriculture' (SIGMEA 2007, 2009). These studies indicate that a separation distance of 20-50 m is enough to maintain the labelling threshold below 0.9 %. In certain cases, where there are particular spatial conditions and agricultural practices (e.g. small scale production systems, average field size smaller than one hectare and/or long and narrow fields), the separation distances may have to be extended.

Like separation distances, pollen barriers of maize plants effectively reduce out-crossing between neighbouring maize fields. Barrier plants located adjacent to the recipient field act on the one hand as a pollen trap and on the other as an additional source of pollen that dilutes the transgenic airborne pollen. Studies in Germany and Switzerland confirmed the high interception of pollen by the first few maize rows when open ground or low growing intervening crops separate maize fields. The removal of the first 10-20 m of a non-transgenic field facing a GM crop might therefore be more efficient for reducing the total level of cross-fertilisation in a recipient population than to recommend separation distances (Hüsken et al. 2007).

Seed mediated gene flow

In spite of extensive cultivation in many countries and accidental seed spillage, seed mediated establishment of maize and its survival outside cropped area in Europe is rare (see section 5.1). Maize is incapable of sustained reproduction outside cultivation and is non-invasive of natural habitats (ref. Eastham & Sweet 2002), but maize plants occasionally grow in uncultivated fields and by roadsides. The probability of a volunteer maize crop appearing in subsequent (maize) crops and then contributing to gene flow via cross pollination from the volunteer to a maize crop in Europe is very low due to the inability of the maize plant to shed seed naturally, a limited dormancy period, low competitiveness, the susceptibility to plant pathogens and herbivores, the common use of mechanical pre-planting soil preparation practices and the inability of maize seed to survive low winter temperatures (Hüsken et al. 2007). In addition, maize is mainly harvested as whole plants for silage. Since these characteristics are not altered in maize MON 89034, it is considered very unlikely that the transgenic maize line or its progeny will differ from conventional maize varieties in their ability to establish feral populations in

Europe. Although seeds from the previous crop year can overwinter and germinate the following year, the plant cannot persist as a weed. Based on the observations in central Europe (Grüber et al. 2008), volunteers may only occur after a warm winter period. Monitoring of maize volunteers after maize cultivation in Spain has shown that the vigour of the volunteer plants is low; they are much shorter than normal plants and rarely have cobs (if produced normally without grains). Tassels were frequently produced, but cross-pollination was estimated to be low, most probably due to loss of hybrid vigour and uniformity in plant size, asynchronous flowering with the cultivated maize crops in which they occur, and amount of fertile pollen etc. (Palaudelmás et al. 2009). The contribution of pollen flow from occasional feral maize plants to agricultural fields with conventional maize varieties is therefore considered to be insignificant.

Field trials in Europe and the USA do not indicate altered agronomic or phenotypic characteristics of maize MON 89034, except for the specific target pest resistance. Pollen production and pollen viability is not expected to be affected by the genetic modification, and it is therefore not likely that out-crossing frequencies to other maize fields will be different from conventional varieties. The VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of gene flow from maize MON 89034 is negligible.

National proposals for maize co-existence
An overview of mandatory separation distances adopted by EU member states shows a considerable range of variation (25-600 m), with respect to separation distances between GM and non-GM maize fields (EC 2009). The Norwegian Scientific Committee for Food Safety concluded that separation distances of 200 m most likely will ensure an upper limit of 1 % of adventitious presence as a result of introgression via pollination in maize (VKM 2006). These recommendations are based on the maize used being heterozygote for the inserted gene and that the maize grains constitute a maximum 50 % of the silage/yield.

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.

Adverse effects due to resistance evolution
Development of resistance to Cry proteins following exposure to Bt plants is an important aspect, with both agronomic and environmental implications (e.g. BEETLE Report 2009; Tabashnik et al. 2009). Resistance evolution to the Cry1A.105 and Cry2Ab2 proteins is not considered a direct environmental harm, but the consequences of the establishment of resistant Lepidoptera pests populations may lead to the use of other pest control tools with greater environmental harm. The first documented case of field resistance to Bt as a sprayed insecticide was observed in Hawaii, where populations of the diamondback moth (*Plutella xylostella*) showed a reduced susceptibility to Bt-sprays (Tabashnik et al. 1990). The main target for MON810 *O. nubilalis* has also developed resistance to Dipel® insecticide containing *B. thuringiensis* subsp. *kurstaki* (Li et al. 2005). When larvae from Dipel®-resistant populations were fed diet containing Cry1Ab, they were also resistant to the diet (Li et al. 2005). With Bt maize, the herbivores ingest the toxin whenever they feed on the plant. This has obvious implications for the development of resistance to the toxin.

When Bt is used as a sprayed insecticide, it is active on the plant for a relatively short time (days) and coverage is never so complete that all of the targets in the treated field will be affected. Development of resistance is expected to go faster in insect-resistant crops, where the Cry proteins are expressed constitutively throughout the growing season. In addition to resistance development in the target pest, polyphagous herbivores feeding on *Bt* maize can develop resistance to the Cry proteins. This in turn will render Bt sprays useless in controlling these herbivores in other crops.

According to the applicant, maize MON 89034 provides an effective approach for insect resistance management by producing two structurally very different insecticidal proteins. The Cry1A.105 and Cry2Ab2 proteins expressed in MON 89034 are different in their mode of action, particularly in the way in which they bind to the lepidopteran midgut. The probability of cross-resistance between the Cry1A.105 and the Cry2Ab2 proteins is therefore expected to be very low (ref. Monsanto reports MacRae et al. 2005, 2006). According to the applicant this strategy should delay the evolution of resistance if the target insects are not able to develop a single mechanism of resistance that overcomes both proteins simultaneously. The interaction study was carried out with target organisms (*O. nubilalis* and *Helicoverpa zea*), and showed that it is plausible that the combined effect of the Cry1A.105 and Cry2Ab2 proteins consist of dose-additive activity on these target organisms.

Since there are no Bt insecticides approved for use in Norway, and lepidopteran pests have not been registered in maize, issues related to resistance evolution in target pests are not relevant at present for Norwegian agriculture.

Internationally, much attention has been given to proactively avoiding and delaying the potential development of resistance in Bt crops. Resistance management strategies, relying on a "high dose/refuge strategy" have been endorsed in several countries (Andow 2008). Current practice is to set off a refuge of non-Bt maize adjacent to the Bt maize crop. This is to provide a habitat where the herbivores are not exposed to the Bt toxin and can develop populations that do not inherit the resistance genes. The strategies recommended are either to have 5% of the crop as non-Bt and unsprayed and adjacent to the Bt crop, or to incorporate (embedded) the 5% that are non-Bt into the area with the *Bt*-plants, or else to have 80% of the crop as Bt plants and 20% adjacent non-Bt plants that are sprayed with a non-Bt insecticide (Shelton et al. 2002). The methods using conventional cultivars in adjacent refuges are considered to be more effective than the embedded non-Bt plant method.

Monitoring data from five continents reported in 41 studies that evaluate responses of field populations of 11 Lepidopteran pests to four Bt toxins produced by Bt maize and cotton, have been

analysed (Tabasnik et al. 2008, 2009). After more than a decade since initial commercialisation of Bt crops, most target pest populations remain susceptible, whereas field-evolved resistance has been documented in some populations of three noctuid species; *Spodoptera frugiperda* to Cry1F in Bt maize, *H. zea* to Cry1Ac and Cry2Ab2 in Bt cotton and *Busseola fusca* to Cry1An in Bt maize. However, analyses of the monitoring data indicate that neither in the EU, nor in the USA, have populations of resistant *O. nubilalis* or *Sesamia nonagrioides* been found. Recent studies indicate increased frequency of field-evolved resistance to Cry1Ac in *H. armigera* in China (Zhang et al. 2011; Wan et al. 2012). The field outcomes documented with monitoring data are consistent with the theory underlying the refuge strategy, suggesting that refuges will not prevent the development of resistance but have helped to delay resistance (Tabasnik et al. 2008, 2009; Wan et al. 2012). In addition, other factors like recessive inheritance of resistance and two-toxin Bt crops deployed separately from one-toxin Bt crops will potentially delay resistance development.

A strain of *O. nubilalis*, obtained from field collections throughout the central USA Corn Belt was selected in the laboratory for resistance to Cry1F by exposure to the toxin incorporated into artificial diet (Pereira et al. 2008). The selected strain developed more than 3000-fold resistance to Cry1F after 35 generations of selection and readily consumed Cry1F expressing maize tissue; yet, it was as susceptible to Cry1Ab and Cry9C as the unselected control strain. Only a low level of cross-resistance (seven-fold) to Cry1Ac was observed. This lack of cross-resistance between Cry1F and Cry1Ab suggests that maize hybrids expressing these two toxins are likely to be compatible for resistance management of *O. nubilalis*.

According to Monsanto's insect resistance management (IRM) plan for cultivation of maize MON 89034 in the EU, a conservative 5 % refuge for ECB and MCB-protected maize will be implemented for planting areas larger than 5 hectares. The applicant claims that a 5 % refuge is adequately protective for the level of control provided by MON 89034 for ECB and MCB, and refers to experiments in the EU with the implementation of a refuge size of 20% associated with the cultivation of maize MON810.

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

The potential of maize MON 89034 to have direct or indirect adverse effects on non-target organisms and ecological functions they provide in agro-ecosystems was previously evaluated by the VKM GMO Panel in connection with EFSA official hearing of application EFSA/GMO/BE/2009/72 (MON 89034 x MON 88017) and EFSA/GMO/NL/2009/72 (MON 89034 x NK603) in 2010 (VKM 2010a,b). The outcome of these evaluations has been updated in light of new relevant scientific literature.

In agro-ecosystems, NTOs provide key ecological functions (including ecosystem services), such as plant pollination, biological control and decomposition, and form important components of farming systems (Arpaia 2010). Considering that every species cannot be tested, it is important that the main functional groups mediating the ecological functions as well as their response to GM plants are considered in the ERA of GM plants (EFSA 2010). Thus, toxicity of Cry proteins is generally tested on a representative subset of NTO species ("focal species") using a tier approach. Lower-tier studies represent a first step to reach reliable risk assessment conclusions, as they give indications of possible hazards associated with the cultivation of GM plants. In case a hazard has been identified in lower-tier studies, a detailed exposure characterisation is required to fully characterise the possible risk (EFSA 2010).

5.4.1 Effects on pollinating insects

Honeybees and other pollinators can be exposed to any genetically modified products expressed in pollen or nectar. Adult bees consume pollen during their first week after emergence and thus might be exposed to Bt proteins. Bee larvae also ingest pollen but in lesser amounts (e.g. BEETLE report 2009).

Because of their ecological and economic importance, the Western honey bees (*Apis melifera* L.) are often used as test-species in pre-market risk assessment studies to assess direct toxicity on non-target organisms, and are probably the most studied non-target arthropod with respect to potential effects of conventional pesticides. While numerous studies have been conducted on Cry1 Bt toxins, hardly any studies are published on potential risks of Cry2 Bt toxins on pollinating insects (Malone & Burgess 2009; Hendriksma et al. 2012). In addition, relatively few large scale field studies have been conducted to assess the possible ecological impact of transgenic crops on honey bee colonies under realistic agricultural conditions (Rose et al. 2007).

The applicant assessed possible adverse effects of the Cry1A.105 and Cry2Ab2 proteins on pollinators. In lower-tier dietary bioassays, honey bee larvae and adults were exposed to purified Cry1A.105 and Cry2Ab2 protein, respectively (Technical dossier: Richards 2006a, b; Maggi 2000a,b). No adverse effects were observed for survival and adult emergence of honey bee larvae exposed to Cry1A.105 (1200 µg protein/ml) and Cry2Ab2 (1 and 100 µg/ml of diet), or on survival or behavior of adult honey bees exposed to Cry1A.105 (550 µg/g) and Cry2Ab2 (1.7 and 170 µg/ml of diet).

A peer-reviewed paper assessing the impact of Cry2Ab2 protein, Hedriksma et al. (2012) came to similar conclusions as those reported by the applicant. In this study Hendriksma analysed combined effects of the three Bt proteins Cry1A.105, Cry2Ab2 and Cry3Bb1, simultaneously expressed in a transgenic maize hybrid variety (MON 89034 x MON 88017). Under a worst-case exposure scenario, using controlled *in vitro* larvae rearing, neither single Bt proteins nor a combination of the proteins showed adverse effects on developing honey bee larvae (larval survival and prepupal weight).

According to the BEETLE Report (2009), no adverse effects of Bt crops on honeybees have been reported so far, and no reports are available regarding harmful effects on other non-target organisms involved in pollination.

Malone & Burgess (2009) have reviewed available scientific data on potential adverse effects on honeybees of Cry proteins or Cry-containing maize pollen gathered either under lower- or higher-tier studies. The authors concluded that none of the Bt-maize events commercially available have significant impacts on the health of honeybees. A meta-analysis of 25 studies that assessed potential effects of Bt proteins on honeybee survival has been published by Duan et al. (2008). No adverse effects on honeybee larvae or adults, in laboratory settings, were reported when looking at studies performed with lepidopteran and coleopteran specific Bt proteins. However, Duan et al. (2008) considered that in field settings, honeybees might face additional stresses, which theoretically could affect their susceptibility to Cry proteins and generate indirect effects.

Feeding studies performed under controlled conditions with honeybees being fed either with Bt pollen or mixtures of honey and sugar syrup containing purified Cry1Ab protein have indicated no direct adverse effects on foraging activity, learning performance or survival of honeybees (Ramriez-Romero et al. 2005, 2008). Further studies with bees fed purified Bt-proteins, pollen from Bt crops, or bees allowed to forage on Bt crops in the field have confirmed the lack of effects on the mortality of honey bees (Malone & Pham-Delegue 2001; Babendreier et al. 2005; Bailey et al. 2005).

In order to assess the risk that insecticidal transgenic plants may pose for bumblebees, Babendreier et al. (2008) tested whether *Bombus terrestris* (L.) workers are able to detect insecticidal proteins dissolved in sucrose solution and whether consumption of these proteins will affect survival and offspring production. Feeders containing either Cry1Ab, soybean trypsin inhibitor (SBTI) or *Galanthus nivalis* agglutinin (GNA) were offered to bumblebee colonies at different concentrations.

No difference was found in the number of visits or the duration of visits among the different concentrations for each of the insecticidal proteins, indicating that bumblebees do not discriminate among the compounds. According to Babendreier et al. Cry1Ab protein did not affect microcolony performance, while the consumption of SBTI and especially GNA affected survival of *B. terrestris* workers and drones and caused a significant reduction in the number of offspring.

In a field study functional colonies of honeybees were exposed to Bt maize pollen (foraging in sweet maize plots, supplied with pollen cakes from Bt maize pollen) expressing Cry1Ab toxin for 28 days (Rose et al. 2007). No significant adverse effects on foraging behavior, bee body weight or colony performance were detected. Offspring development was not affected by exposure to Bt pollen, but significantly reduced by the positive insecticide control.

5.4.2 Effects on natural enemies (predators and parasitoids)

The exposure of natural enemies (predators and parasitoids) to Cry proteins expressed in Bt-plants can occur in different ways: natural enemies can be exposed to Cry proteins by feeding on plant material (including pollen) or honeydew excreted from sap-sucking species, and indirectly through feeding on prey/host organisms which have previously been feeding on Bt plants (ref. EFSA 2009b).

Potential effects of the Bt maize MON 89034 x MON 88017 on ground beetles and spiders were investigated in field and laboratory experiments in Germany in 2008-2011 (Priesnitz et al. 2011). The study compared the GM variety with its isogenic parent and two conventional maize varieties. More than 70 000 predatory arthropods were counted in soil traps and assessed over the three year investigation period. The density of ground beetles and spiders did not differ significantly between the Bt maize plots and the conventional maize plots. By contrast, on a few sampling dates there were clear differences between the maize MON 89034 x MON 88017 and the plots with the isogenic variety treated with insecticides. The composition of the ground beetle community varied over the course of the three years, but no differences were found between the different plots. Preliminary results from feeding trials, 600 beetle larva (*Poecilus cupreus*) were tested and fed on CryBb1 protein and a protein mix containing Cry1A.105, Cry2Ab2 and Cry3Bb1, respectively. No negative impacts were found on the pupation rate, hatching rate, development, weight at emergence or fertility of the beetles.

Bourguet et al. (2002) studied the effect of Bt maize on the field abundance of nontarget insects. In their experiments with MON810 they looked at the effect on aphids and their predators/parasitoids. There were no significant differences in the abundance of aphids or predators/parasitoids. The predators found were: *Orius insidiosus, Syrphus corollae, Coccinella septempunctata, Chrysoperla carnea* and thrips. The parasitoids were hymenopterans.

In a laboratory study, no effect was found of pollen from Bt maize expressing the Cry1Ab protein on *O. insidiosus, C. carnea* or *Coleomegilla maculata* (Pilcher et al. 1997). This study was followed by a 2 year field study where predators of *O. nubilalis* were monitored before pollen shed, at pollen shed and after pollen shed. The authors concluded that Bt maize pollen did not effect movement of these predators (Pilcher et al. 1997).

A different *Orius* species, *O. majusculus*, was investigated for non-target effects of Bt maize in a laboratory study (Zwahlen et al. 2000). The predator was fed thrips (*Anaphothrips obscurus*) that were either reared on Bt maize or non-Bt maize. Although the thrips is not sensitive to the Bt toxin, it was assumed that the toxin would be in the thrips' body when it was consumed by the predator. The study revealed that there were no differences in mortality or developmental time for the predator.

Torres & Ruberson (2008) studied the effect of Cry1Ac toxin on four species of predatory bugs; *Podisus maculiventris, Geocoris punctipes, Nabis roseipennis* and *O.insidiosus*. The bugs were fed with prey from Bt cotton. The authors concluded that the predatory bugs were not adversely affected by eating Cry1Ac-contaminated prey.

The effects of Cry toxins (Cry1Ac, Cry1Ab and Cry2Ab) on the anthocorid *O. albidipennis* were studied under laboratory conditions (González-Zamora et al. 2007). Tritrophic experiments were performed, in which the nymphs were fed *Helicoverpa armigera* larvae reared on a diet with Cry1Ac, Cry1Ab, or Cry2Ab toxins at different concentrations (0, 1, and 10 microg/ml), when supplemented with *Ephestia kuehniella* eggs. In complementary experiments, the Bt Cry1Ac toxin was directly fed to Orius nymphs at a very high concentration (1 mg/ml). No effects on prey consumption, developmental time, nymph survival, fecundity, and egg hatching of *O. albidipennis* were found in either experiment. It can be concluded that the toxins tested do not seem to pose a risk for the anthocorid *O. albidipennis*, especially when it is exposed through the prey.

Alvarez-Alfageme et al. (2008) investigated prey-mediated effects of two maize varieties expressing a truncated Cry1Ab toxin (Event Bt176, MON810) on the biology of the ladybird *Stethorus punctillum*. Although immuno-assays demonstrated the presence of Cry1Ab in both prey and predator collected from commercial maize-growing fields, neither transgenic variety had any negative effects on survival of the predator, nor on the developmental time through to adulthood. Furthermore, no subsequent effects on ladybird fecundity were observed. Corresponding results were shown by Alvarez-Alfageme et al. (2009). There were no significant effects on mortality, development time or growth of larvae and pupae of the ground-dwelling predator *Poecilus cupreus* L. fed with *Spodoptera littoralis* larvae reared on Bt176 maize leaves. To elucidate potential detrimental effects due to a reduction in the quality of the prey, the authors assessed the digestive proteolytic activities of *P. cupreus* adults from a laboratory culture and insects collected in commercial Bt and non-Bt maize fields. Field-collected *P. cupreus* adults had higher proteolytic activities than those reared in the laboratory, whereas no significant differences were found between *P. cupreus* adults reared on Bt and non-Bt maize fed *S. littoralis* or between *P. cupreus* adults collected in commercial Bt and non-Bt maize fields.

A comprehensive study using a tritrophic bioassay was conducted to evaluate the potential impact of Cry2Ab- and Cry1Ac-expressing cotton on fitness parameters of the lady beetle *Coleomegilla maculata,* a common and abundant predator found in many cropping systems worldwide (Li et al. 2011). Both larvae and adults of *C. maculata* are predaceous, feeding on aphids, thrips and lepidopteran eggs and young larvae. In addition to prey, *C. maculata* also feeds on plant tissues, such as pollen. Therefore the species can be directly and indirectly exposed to Cry proteins in several ways when feeding Bt crops. Li et al. (2011) used *Bt*-susceptible and –resistant larvae of *Tichoplusia ni* as prey. *C. maculata* survival, development time, adult weight and fecundity were not different when they were fed with resistant *T. ni* larvae reared on either Bt or control cotton. To ensure that *C. maculata* were not sensitive to the tested Cry toxins independent from the plant background and to add certainty to the hazard assessment, *C. maculata* larvae were fed artificial diet incorporated with Cry2Ab, Cry1Ac or both at >10 times higher concentrations than in cotton tissue. No differences were detected in any life-table parameters between Cry protein-containing diet treatments and the control diet.

Conflicting results regarding potential adverse effects of the Cry1Ab toxin to larvae of the ladybird *Adalia bipunctata* have been reported in the literature (Romeis et al. 2012). Hilbeck et al. (2012) reported lethal effects of the toxin on larvae of *A. bipunctata* when fed directly to the predator. Corresponding results were found in an earlier feeding study, where *A. bipunctata* suffered increased mortality during the first larval stage when ingesting the Cry1Ab protein (Schmidt et al. 2009). Such toxic effects were not observed in direct feeding bioassays conducted by Porcar et al. (2010) and Alvarez-Alfageme et al. (2011). In the higher tier, tri-trophic study using Bt maize-fed spider mites as prey did not revealed any adverse effects on lethal and sublethal parameters of the predator (Alvarez-Alfageme et al. 2011). This was despite the fact that the larvae had ingested high amounts of biologically-active Cry1Ab protein. Many ladybird species, including *A. bipunctata,* mainly feed on aphis that are known to contain, at best trace amounts of Cry protein when feeding on Bt maize, and Romeis et al. (2012) concluded that Bt maize expressing Cry1Ab poses a negligible risk to the predator under realistic worst case exposure conditions.

Adults of common green lacewing (*Chrysoperla carnea*) are prevalent pollen-consumers in maize fields. They are therefore exposed to insecticidal proteins expressed in the pollen of insect-resistant maize varieties expressing Cry proteins. Li et al. (2008) conducted two laboratory experiments to evaluate the impact of Cry1Ab and Cry3Bb1-expressing transgenic maize pollen (Event Bt176, MON 88017) on fitness parameters of adult *C. carnea.* Adults were fed pollen from Bt maize varieties or their corresponding near isolines together with sucrose solution for 28 days. Survival, pre-oviposition period, fecundity, fertility and dry weight were not different between Bt or non-Bt maize pollen treatments. In order to ensure that adults of *C. carnea* are not sensitive to the tested toxins independent from the plant background and to add certainty to the hazard assessment, adult *C. carnea* were fed with artificial diet containing purified Cry1Ab or Cry3Bb1 at an approximately 10 times higher concentration than in maize pollen. No differences were found in any life-table parameters between Cry protein-containing diet treatments and control diet.

A preference study was conducted in Switzerland using all three larval stages of the lacewing *C. carnea* and two prey species, the aphid *Rhopalosiphum padi* and the lepidopteran *Spodoptera littoralis*. The Bt maize used expressed Cry1Ab. It was not lethal to either of the prey species. In choice tests involving only one prey species, the predator showed a preference for the *S. littoralis* larvae feeding on non-Bt maize, but no preference for aphids based on food plant type (Meier & Hillbeck 2001). When given a choice of *S. littoralis* or *R. padi,* the lacewing preferred the aphids. The authors speculate that the aphids did not contain the toxin, as it is not present in the plant phloem on which they feed. If this is the case, then Bt maize should not pose a problem for *C. carnea*. Laboratory studies that showed that the aphids do not take up the Bt toxin from the phloem were done by Dutton et al. (2002). These studies also showed that when *C. carnea* are fed *S. littoralis* from Bt maize, they have an increase in mortality and a delay in development. However, this may be of little importance if the non-preference that *C. carnea* showed for *S. littoralis* in the lab also holds true for the field.

Similar studies were conducted to examine the effect on the Ichneumonid parasitoid *Campoletis sonorensis* when its host *O. nubilalis* was fed on Bt maize or non-Bt maize (Sanders et al. 2007). This study found that when the parasitoid developed in hosts feeding on Bt maize, the emerging adults were significantly smaller. The size of the adults was directly related to the size of the host at oviposition by the parasitoid, and the host's subsequent growth rate. When the new generation of adult parasitoids were analyzed, no Cry1Ab was found. This indicated that the smaller size was entirely host-mediated and not a direct effect of the toxin on the parasitoid. This study included a choice test where the parasitoid could choose hosts from Bt maize or non-Bt maize. No obvious preference were observed.

In a Chinese study *Helicoverpa armigera* was fed with a diet containing Cry1Ac-toxin (Ding et al. 2009). The effect on the Braconid parasitoide *Microplitis mediator* was a result of the host's growth rate and size. No adverse effects of the Bt toxin itself were found.

Romeis et al. (2004) fed Cry1Ab toxin directly to *C. carnea* larvae at concentrations that were approximately 10,000 times greater than the concentration in lepidopteran prey fed on Bt maize. This resulted in no direct toxic effect of the toxin on the lacewing. The authors concluded that the previously reported negative effects of Bt maize could be attributed to prey-mediated effects and not the Bt toxin. In a subsequent study of Lawo & Romeis (2008) no adverse effects were observed of Cry1Ac and Cry1Ab on larvae of *C. carnea.*

A field study was conducted comparing maize MON810 expressing Cry1Ab with near isogenic maize (Daly & Buntin 2005). They found a reduction in sap beetles (*Carpophilius* spp.) and an otitid fly (*Euxesta stigmatis*), which they attributed to less ear damage from the target species, the corn earworm (*H. zea*), as the damaged ear is what attracts these insects to the maize. They also found a reduction in predatory damsel bugs (*Nabis* spp.). They comment that the numbers of damsel bugs in both Bt maize and non-Bt maize were so low that no conclusions could be drawn. There are 8 reported species of damsel bugs in Norway (Coulianos & Ossiannilsson 1976).

In Spain, where Bt maize has been grown since 1998, a study was conducted to compare the abundance of predatory arthropods in Bt maize and non-Bt maize (de la Poza et al. 2005). The Bt maize contained the Cry1Ab gene. The predators were monitored visually on the plants or in pitfall traps. This study found no differences in the abundance of *Anthocoridae, Coccinellidae, Aranea* or *Carabidae* in the Bt maize compared to the non-Bt maize. All of these taxa are common in Norwegian maize fields.

Ludy and Lang (2006) also investigated spiders in their 3-year study in Germany of the effect of Bt maize expressing Cry1Ab. MON810. They found no significant differences in the numbers of spiders in Bt maize fields or their margins compared to non-Bt maize fields.

Perhaps the most thorough and detailed investigation of the impact of Bt maize on non-target arthropods to date is that of Dively (2005). This field study was over a 3 year period in Maryland, USA. Over 500,000 arthropods were counted, from 13 orders, with 112 families and 203 taxonomic groups. The maize lines had both the VIP3A and the Cry1Ab genes. The effects of Bt maize were compared to non-Bt maize with and without insecticide treatment. Arthropods were registered by visual inspection, sticky traps, pitfall traps and emergence traps. Registration was also carried out the following growing seasons to document carry-over effects. All of the arthropod families that are likely to occur in Norwegian maize fields are represented in the list of herbivores, saprovores, predators and parasitoids recorded in the isogenic non-Bt maize fields in this study. There were significant differences between the insecticide-treated maize and the other treatments (Bt and non-Bt maize).The author concludes that there were no significant differences in biodiversity and community-level responses caused by the Bt maize. The differences in abundance of certain species between the Bt maize and non-Bt maize that were recorded are regarded by the author to be the result of factors such as lack of prey or lack of plant injury. This is similar to the conclusion of several other studies mentioned above.

Mann et al. (2010) studied relative abundance of non-target insects on Bollgard cotton cultivars expressing Cry1Ac and Cry2A2 toxins over two cropping seasons. Densities of sucking insects (*Amrasca biguttula biguttula, Bemisia tabaci, Aphis gossopy, Trips tabaci*), the foliage feeder *Myllocerus undecimpustulatus* and of the predators *Chrysoperla* spp, *Brumus* spp*., Vespa* spp.*Lycosa* spp. and *Aranews* spp. were similar on the transgenic and conventional cultivars.

5.4.3 Effects on non-target Lepidoptera

Maize plants are not an important resource of food for indigenous Lepidoptera with the exception of a few pest species. Therefore, the main potential risk to non-target Lepidoptera is expected to be the exposure to potentially harmful amounts of pollen deposited on host-plants in or near maize MON 89034 fields.

A field study in Germany evaluated the impact of MON810 on nontarget lepidopteran larvae (Gathmann et al. 2006). Weed belts were established in plots containing MON810 and non-Bt maize both with and without insecticide treatment. The naturally occurring lepidopteran larvae on the weeds were recorded. The only species that were numerous enough to compare statistically were specialist species on Brassicaceae, *Plutella xylostella* and *Pieris rapae*, both of which were found on *Sinapis alba*. There were no differences detected between the MON810 plots and the untreated non-Bt maize plots.

The above-mentioned study in Germany was likely initiated in the wake of the controversy over the effect of pollen from Bt maize on larvae of the monarch butterfly (*Danaus plexippus*) in a laboratory experiment reported in Nature (Losey et al. 1999). This was followed by a paper that considered ecological factors in the field and their influence on the monarch's exposure to natural quantities of Bt maize pollen (Jesse and Obrycki 2000), where it was concluded that when the monarch fed on its host plant milkweed (*Asclepias syriaca*) with natural dusting of Bt maize pollen it suffered higher mortality

than on plants with non-Bt maize pollen. In a later paper, the same authors conclude that MON810 Bt maize pollen and anthers had no measurable effect on the oviposition or survival of the monarch (Jesse and Obrycki 2003).

The studies on the monarch butterfly were performed in the USA. Similar studies were later done in European laboratories using the common swallowtail butterfly (*Papilio machaon*) and its host *Pastinaca sativa*. When exposed to different densities of pollen from Cry1Ab maize, the larvae had lower weights, longer development time and lower survival, and smaller wing size as adults (Lang and Vojtech 2006). This result was more pronounced with higher pollen densities. This study used the Bt Maize Bt176. The paper mentions that MON810 expresses much lower levels of toxin in the pollen.

Schuppener et al. (2012) have assessed the risk posed by event MON89034 × MON88017 to the small tortoiseshell *Aglais urticae*, a butterfly species common in central Europe. The authors assessed the toxicity of Bt maize pollen on butterfly larvae, measured pollen deposition on leaves of the host plant *Urtica dioica* and mapped the occurrence and distribution of host plants and larvae in two arable landscapes in Germany during maize anthesis. The results showed that larvae-fed 200 Bt-maize pollen grains/cm^2 had a reduced feeding activity. Significant differences in developmental time were also detected at pollen densities of 300 Bt-maize pollen grains/cm^2 and in survival at 400 grains/cm^2. The highest pollen amount recorded was 212 grains/cm^2 at the field margin, and the mean densities were much lower. Schuppener et al. concluded that the amount of pollen from maize MON89034 × MON88017 found on host plants is unlikely to adversely affect a significant proportion of larvae of *A. urticae*, and that the risk of event MON89034 × MON88017 to populations of this species is negligible.

5.4.4 Effects on non-target soil arthropods

Springtails (Collembola) and mites (Acari) are key indicator organisms of soil fertility and health, as they are important in the breakdown and recycling of crop residues, and abundant populations of these microarthropods are generally in well-managed agricultural soils. Springtails and mites can be exposed to Cry proteins in crop residues, root exudates, live roots and associated fungi in the rhizosphere.

In general, no negative effects of the Cry1Ab, Cry1Ac and Cry2A toxins on springtails have been observed (reviewed by Icoz & Stotzky 2008). Microbially produced purified Bt insecticidal proteins (Cry1Ab, Cry1Ac, Cry2A and Cry3A) were added at concentrations of 200 µg/g fresh weight to the diet of the species *Folsomia candida* and *Xenylla grisea* for 21 days (Sims & Martin 1997, ref. Icoz & Stotzky 2008). In soils in the field, concentrations of Cry proteins in plant material exposed to soil organisms are usually lower and are estimated to be less than 30µg/g. The results showed no effects on adult survival or reproduction compared with the unamended diet, and are consistent with the findings of Yu et al. (1997).

In a laboratory toxicity study of the subacute effects of maize expressing Cry1Ab on springtails, Clark & Coats (2006) fed *F. candida* with ground up meal of leaves of Bt maize and corresponding non-Bt isolines. No deleterious effects on survival and reproduction of *F. candida* were observed. However, springtails receiving isoline material had significantly more offspring compared with those in the corresponding Bt line, but no other pairs were different. Time to reproduction of *F. candida* was only affected by the reference control treatment. The authors concluded that differences in growth of springtails were due to nutritional differences in the two varietal lines of maize, and not due to the Bt toxin.

Bakonyi et al. (2006) showed that Bt maize was less preferred as food by *F. candida* than near-isogenic maize. However, this was not the case for other species of Collembola, i.e. *Heteromurus*

nitidus and *Sinella coeca.* In a laboratory experiment, Heckmann et al. (2006) reported differences in springtail performance when they were reared on baker yeast versus maize, but no significant differences between Bt maize (Cry1Ab) and non-Bt maize. No significant differences in the population density of springtails were found in soils cultivated with Bt and non-Bt maize and between the application of an insecticide and no insecticide (Lang et al. 2006).

Griffiths and his partners in the EU-project ECOGEN investigated the effects of different soils collected from field sites in Denmark and France in which MON810 maize and non-Bt maize were grown. These studies, carried out in a glasshouse included an insecticide treatment, the pyrethroid deltamethrin, which increased the concentration of the Bt toxin in MON810 (Griffiths et al. 2006). The reasons for this are unclear. The experiments evaluated effects on the two microarthropod groups collembola and mites by soil extraction at different plant growth stages. To investigate the effect on macroarthropods, swedes (*Brassica napus*) were grown in the soils from the maize and were inoculated with eggs of the cabbage root fly (*Delia radicum*). Neither the micro- nor macroarthropods were affected by the soil from MON810. Corresponding results on soil microarthropods have been published by Cortet et al. (2007). This study was carried out at four European locations (2 in France and 2 in Denmark). The Danish sites are comparable climatically to regions in Norway where maize is grown. Cortet et al. reported some significant negative effects of Bt maize on microarthropods in soils with a high clay content. The authors concluded however, that the slight differences in abundance of some soil microarthropods were most likely due to maize variety and not the Bt toxin, and within the normal variation expected in conventional agricultural systems.

Potential effects of Bt maize expressing Cry1Ab on soil microarthropods (Collembola, Actinedida, Arcaridida, Gamasida and Oribatida) were assessed in a 4-month microcosm study in the ECOGEN project (de Vaufleury et al. 2007). Total soil microarthropod abundance and diversity were similar between the conventional control and the Bt maize microcosms.

Bakonyi et al. (2011) conducted a multiple generation laboratory study to investigate the potential effects of long-term feeding of the springtail *F. candida* on Bt maize MON810 (0,6, 16 and 22 months). Significant differences were found in food consumption, egg production and food preference between the populations in some cases, but no time-response effect was observed. The authors concluded that long-term feeding on maize containing Cry1Ab seems not to have adverse effects on this species.

In a laboratory study of Bt rice expressing Cry1Ab protein, growth, development, reproduction, and superoxide dismutase activity (indicator of environmental stress) of *F. candida* were investigated (Bai et al. 2011). The springtail populations were reared on leaf tissue or leaf-soil mixtures of two CrylAb rice lines and a non-Bt rice isoline in two independent tests. No significant differences between the populations reared on Bt and non-Bt rice leaf tissue were detected in all measured parameters, suggesting no significant effects of the CrylAb protein in Bt rice on *F. candida*.

No negative effects of Cry proteins on mites have been observed (Icoz & Stotzky 2008). Yu et al. (2007) fed the soil mite, *Oppia nitens,* fresh and old Bt cotton and Bt potato leaves expressing the Cry1Ab/Ac and Cry3A protein, respectively, as well as leaves of isogenic controls. After 7 weeks, no significant effects on oviposition, the number of eggs produced per female or final body length were observed.

The woodlouse *Porcellio scaber* is considered a model decomposer organism and has been a subject of a few studies on the effects of Cry proteins on isopods (Sims 1997; Escher et al. 2000; Pont & Nentwig 2005). Sims (1997) observed no effect of purified Cry2A protein on mortality and growth of *P. scaber.* In a laboratory feeding experiment with *P. scaber,* no adverse effects of Bt maize expressing Cry1Ab were found (Escher et al. 2000). *P. scaber* did not differ between Bt and the non-transgenic control in its food preference, and the number of offspring did not differ between the two maize varieties. In the study of Pont & Nentwig (2005) *P. scaber* was fed for 15 days on two different

transgenic maize varieties expressing Cry1Ab. The Cry protein was detected in the body and faeces of *P. scaber,* showing that the woodlouse ingested and excreted the protein. No adverse effects of the protein on survival and growth of *P. scaber* were detected.

5.4.5 Effects on non-target aquatic arthropods

Byproducts from genetically modified plants (e.g. pollen, detritus) can be transported in water courses to downstream water bodies where non-target aquatic arthropods can be exposed to transgene products through consumption.

In the present application for cultivation of MON 89034, the applicant has included a study on the effects of MON 89034 pollen on *Daphnia magna,* a freshwater cladoceran (Palmer & Kreuger 2006). *Dapnia magna* is the model system for ecotoxicological studies, but is not routinely used in the risk assessment of GM plants. Relevant aquatic test species compared to e.g. caddisflies?

In the current literature, the environmental risk assessment of aquatic environments concerning the cultivation of GM crops is under discussion (BEETLE report 2009; Carstens et al. 2012). So far, few studies have addressed the potential exposure of aquatic ecosystems to GM plant material and transgene products, and the potential impacts of Bt proteins on aquatic organisms (e.g. Douville et al. 2005, 2007; Rosi-Marshall et al. 2007; Griffiths et al. 2009; Jensen et al. 2010; Tank et al. 2010).

Exposure of non-target organisms to Cry proteins in aquatic ecosystems in Canada has been studied by Douville et al. (2005, 2007). In an initial study Douville et al. (2005) aimed to quantify levels of Cry1Ab endotoxin and locate its source in the environment. Agricultural soils and surface waters were spiked with crystals (biopesticide-Dipel®) or with pure Bt-maize endotoxin. Additionally, surface water, soils and sediments were sampled in an area sprayed with *Bt kurstaki* and at a site where maize expressing Cry1Ab protein was grown. The results showed that Bt-endotoxin was degraded more rapidly in water than in soils (4 and 9 days, respectively), while crystals appeared to be more resilient, as expected. The levels of Cry1Ab protein were generally below the detection limit, although it was detected at concentrations ranging from 0.1 to 1 ng/g in sediment and surface water, respectively. In a follow-up study the group spiked surface water and sediment of a surface water body with genomic maize DNA containing the *cry1Ab* gene (Douville et al. 2007). Samples from surface water and sediments were collected and tested for *cry1Ab* residues at different times during the growth season. The gene was detected 40 days after introduction in clay and sand-rich sediment. Persistence of the gene was significantly higher in the sediments than in the open water. Tank et al. (2010) reported occurrence of maize detritus and detectable levels of Cry1Ab protein (0.56 ng/mL) in the water column located less than 500 m from maize fields up to six months after harvest in water streams in the Midwestern USA.

Direct input of pollen and other by products from Bt maize into headwater streams nearby to maize fields cultivated with Bt maize in the Midwest of USA was investigated by Rosie-Marshall et al. (2007). They found evidence for transport of Bt containing maize residues downstream in the water bodies, but with respect to degradation rates of Bt containing plant litter no differences were found between Bt and non Bt-containing litter. On the basis of experimental data under laboratory conditions, Rosie-Marshall et al. claimed that this would reduce growth and increase mortality in larvae of caddisflies (Trichopterans), species that are closely related to Lepidoptera. Concentrations of Cry1Ab protein in leaves and pollen were not measured, so no dose-response relationship with the Bt-protein can be estimated (EFSA 2009b). Measurement of growth rates of the caddisflies genera *Hydropsyche* and *Cheumatopsyche* in three streams draining fields planted with Bt maize did not show effects of Bt pollen on growth or mortality (Pokelsek et al. 2007).

In a study of exposure and effects of Bt maize on four non-target aquatic arthropods, Jensen et al. (2010) showed that input of maize detritus after harvest was extended over months in a stream adjacent to maize fields in USA. The study documented no bioactivity of Cry1Ab protein in senesced

maize tissue after 2 weeks of exposure to terrestrial or aquatic environments, indicating rapid degradation of the protein. No toxic effects were observed on the larvae of caddisflies (*Lepidostoma* ssp. and *Pycnopsyche scabripennis*) when fed senesced leaf tissues of maize expressing Cry1Ab. However, Jensen et al. proved that near-isolines modified growth and survivorship of crane fly (*Tipula abdominalis*) and the isopod *Caecidita communis* in the control groups. These effects were attributed to tissue-mediated differences among the isogenic line treatments.

Laboratory experiments performed by Bøhn et al. (2008, 2010) revealed that *Daphnia magna* fed a suspension of 100 % maize MON810 flour had a higher mortality and reduced fitness as compared to the control group. However, it is unclear whether the delays in development of the water fleas were caused by nutrient deficiencies related to the feeding regime or the presence of Cry1Ab protein (EFSA 2009b; Ricroch et al. 2010).

In a case study, Cartstens et al. (2012) identified exposure pathways and calculated early tier exposure estimates for Bt maize in aquatic ecosystems. (Established models and worst-case assumptions were applied, and the resulting EECs for aquatic organisms were low. The shredders were identified as the functional group most likely to be exposed to insecticidal proteins). However, even using worst-case assumptions, the exposure of shredders to Bt maize was low. The research group concluded that because the potential exposure of aquatic particle feeders, predators and shredders to insecticidal proteins in current Bt crops is very low, additional hazard testing would provide useful information for the environmental risk assessments.

5.4.6 Effects on non-target organisms that are not arthropods

Maize MON 89034 may have potential direct or indirect adverse effects on non-target organisms that are not arthropods, as well as the ecological functions they provide. Potential adverse effects on soil microorganisms are considered in section 5.6.2, while this section focuses on earthworms, enchytraeid worms, nematodes and molluscs.

Annelida (earthworms and enchytraeid worms)
Earthworms and enchytraeid worms play an important role in decomposing plant litter, and are responsible for numerous physical changes that affect the biological properties and processes in soil (e.g. structure, quality, functionality) (EFSA 2011d). These species are considered important organisms in the regulation of nutrient cycling processes. As Cry toxins can enter the soil by root exudates, plant material and by plant residues (Icoz & Stotzky 2008), earthworms and enchytraeid worms can be exposed to Cry proteins.

The applicant has conducted two studies to evaluate the potential effects of acute exposure of the Cry1A.105 and Cry2Ab2 protein administered to the earthworm species *Eisenia fetida* during a 14-day exposure period (178 mg Cry1A.105 protein/kg of dry soil, and 33.0 and 330 mg Cry2Ab2 protein/kg of dry soil) (Techical dossier: Sindermann et al. 2006a; Palmer & Kreuger 2006). These studies gave no indications of adverse effects of the Cry proteins on *E. fetida* when mixed in artificial soil substrate.

According to reviews of Icoz & Stotzky (2008) and the BEETLE Report (2009) studies to date have found no/few significant effects of Bt maize on survival, growth and reproduction on the earthworm species *L. terrestris, E.fetida* and *A.caliginosa*.

Impacts of Bt maize expressing Cry1Ab on the earthworm species *L. terrestris* have been studied in the laboratory and under semi-field conditions (e.g. Saxena & Strotzky 2001b; Zwahlen et al. 2003b; Lang et al, 2006; Zeilinger et al. (2010). None of the studies showed consistent effects on *L. terrestris.* On the whole, laboratory experiments with adult earthworms feeding on either Bt- or non-Bt maize litter showed no significant difference in weight change between the two treatments.

In a study by Saxena & Stotzky (2001b), no significant differences in percent mortality or weight of earthworms were detected after 40 days exposure to root exudates in soils planted with Bt maize (Cry1Ab). Corresponding results were found after 45 days in soil amended with residues from Bt maize. It was nonetheless evident that Bt toxins were taken up as they were detectable in the casts as well as the guts of earthworms. Within two to three days after placing earthworms in fresh soils, the toxins, however, were cleared from the gut.

Zwahlen et al. (2003b) showed that mortality and weight of adult and juvenile earthworms were not significantly different when fed Bt or non-Bt maize residues over 160 days, with the exception that after 200 days, adults fed Bt maize residues had a significant reduction in weight (18 %) compared to those fed non-Bt maize. Under semi-field conditions, no significant differences in growth patterns were observed in immature earthworms feeding on Bt or non-Bt litter (Zwahlen et al. 2003b).

Lang (2006) found no significant differences in population density or biomass of *Lumbricidae* earthworms in soils planted with Bt maize or non-Bt maize and between soils with maize either treated or not treated with insecticide. The field experiment, which was conducted at five sites during four growth seasons, showed that field site and sampling years had greater effect on population density and biomass of the earthworms than the presence of Cry protein.

Clark & Coats (2006) conducted laboratory toxicity studies to determine the sub-acute effects of Cry1Ab in maize litter on non-target soil organisms. No significant differences in survival and growth of compost worm (*Eisenia fetida*) were detected between transgenic and isogenic maize residue consumption. In a corresponding Danish study, leaf or root exudates from Bt maize had no deleterious effects on survival, growth, development or reproduction of the grey worm *Aporrectodea caliginosa* var. *tuberculata*, probably the most abundant species in agricultural soils in the temperate climate zone (Vercesi et al. 2006). However, a slight, but statistically significant negative effect of Bt maize residues on cocoon hatchability was observed. Field studies in Denmark and France on responses by earthworms to reduced tillage in herbicide tolerant maize and Bt maize cropping systems, did not show significant effects of Bt maize expressing Cry1Ab on biomass and abundance of different earthworm populations (Krog et al. 2007a).

In a field study conducted in USA over four years, Zeilinger et al. (2010) did not observe significant differences in numbers and biomass of juvenile and adult individuals of four earthworm species (*Aporrectodea caliginosa, A.trapezoides, A.tuberculata* (collectively the *A. caliginosa* complex), and *L. terrestris*) in the soil of Bt maize varieties expressing Cry1Ab and Cry3Bb1 proteins and non-Bt maize. However, Zeilinger et al. underline that only a small number of earthworm species that are likely to be exposed in the field have been investigated in this and previous studies. Considering the difficulty in extrapolating effects and the low species diversity of earthworm communities in maize agroecosystems in temperate climates, these data do not merit any general conclusion on the effects of Bt maize on earthworms.

The fate of insecticidal Cry1Ab protein from crop residues (leaves and roots) of the transgenic maize variety MON 810 expressing Cry1Ab, was studied by Schrader et al. (2008) in the presence and absence of two earthworm species (*L. terrestris* and *Aporrectodea caliginosa*) in soil microcosms (artificial ecosystem). All earthworms survived in the microcosms over a period of 5 weeks, irrespective of whether they received transgenic or non-transgenic plant material. Weight loss was observed for both earthworm species, independent of the plant material. A strong decline of immunoreactive Cry1Ab in plant residues of MON810 was observed in all treatments, but in microcosms with earthworms this decline was significantly higher with less than 10 % of the initial Cry1Ab concentration remaining after 5 weeks. No immunoreactive Cry1Ab protein was found in earthworm tissues.

In a study of Shu et al. (2010), *E. fetida* were bred in substances with stover of Bt maize expressing Cry1Ab protein (MON810, Bt11) and their corresponding near-isogenic varieties. More than 90% individuals of *E. fetida* survived over a period of 30 d, irrespective of whether they received Bt or non-

Bt maize. ELISA results indicated immunoreactive Cry1Ab in casts and guts of the earthworms from Bt maize treatments. However, no significant deleterious effects on survival rate or reproduction were reported.

Hönemann & Nentwig (2009) analysed survival and reproduction of the enchytraeid worm *Enchytraeus albidus*, fed with diets containing Bt maize litter (Cry1Ab, Cry3Bb1). For the Cry1Ab treatment, survival was significantly higher than for the treatment with the corresponding near-isoline. In contrast, reproduction was significantly lower for the Cry1Ab compared to the isoline. According to Hönemann & Nentwig the transgenic variety expressing Cry1Ab was less degradable compared to the control, and suggested a variety effect on life history traits of *E. albidus.* Naturally enchytraeids do not feed on a single food source, but take up all degradable organic matter of adequate size in the soil. It is therefore not expected that Cry1Ab-expressing maize will endanger the survival or reproduction of *E. albidus,* provided that organic matter of sufficient quality is available in the soil (Hönemann & Nentwig 2009). For the Cry3Bb1 treatment, no effect was shown on survival or reproduction.

Nematodes
Nematodes are considered particularly good bio-indicators for assessing soil quality, due to their great diversity and participation in many functions at different levels of food webs in soil and due to their presence in virtually all habitats with a high population density and a large number of species (ref. EFSA 2011d).

Studies on the effects of Cry proteins on soil nematodes have shown different results (reviewed by Icoz & Stotzky 2008). Impacts of Cry1Ab toxins on nematodes were examined in four studies using soil samples from fields planted with Bt maize and near-isogen control (Saxena & Stotzky 2001b; Manachini & Lozzia 2002; Griffiths et al. 2005; Höss et al. 2008). Results from the study of Saxena & Stotzky (2001b) indicated that there were no significant differences in the number of nematodes between rhizosphere soil of Bt and Bt maize grown in a plant-growth room. In a field experiment comparing Bt maize expressing the Cry1Ab protein with near-isogenic non-Bt maize, Manachini & Lozzia (2002, ref. Icoz & Stotzky 2008) reported no overall significant influence on communities and biodiversity of nematodes. However, in one of the eight study regions, fungi feeding nematodes were found to be more abundant in the field with transgenic maize, while bacteria-feeding nematodes were more abundant in the field cultivated with the isogenic hybrid.

In field studies over two years conducted in the ECOGEN project covering different soil types and distinct climatic zones (three European sites), MON810, the near-isogenic non-Bt cultivar, a conventional maize cultivar and plots of grass were evaluated (Griffiths et al. 2005). In all sites, nematode numbers, as well as of protozoa, associated with the transgenic variety were reduced. Nematode community structure was different at each site and the Bt effect was not confined to specific nematode taxa. It was concluded that the effect of the Bt maize was small and fall within the normal variation expected in these agricultural systems. In later studies, Griffiths et al. (2006, 2007 a,b) concluded that effects on soil nematode abundance by Cry1Ab-expressing maize was not related to the Bt trait, but more likely to the effects of agricultural practices, environmental stresses or differences between localities and maize varieties.

In a study of maize MON 810, significant effects were found on reproduction and growth of *Caennorhabditis elegans* in rhizosphere and bulk soil from fields with Bt maize expressing Cry1Ab compared with soils from fields with the near-isogenic variety (Höss et al. 2008). According to the authors, the observed effect of the soil samples on the nematodes could not be explained by a direct toxicity of the Cry1Ab, however, the toxicity of the pure Cry1Ab protein to the reproduction and growth of *C. elegans* was concentration-dependent

Unpublished results from a German study on the effects of Bt maize MON 89034 x MON 88017 (Cry1A.105, Cry2Ab2, Cry3Bb1) on nematodes showed that the incidence of nematodes fluctuated slightly on all plots over the course of the study (http://www.gmo-safety.eu). On most of the sampling dates no significant differences between the maize varieties were detected. A significant difference

was found between the number of nematodes on the Bt maize plots and on the conventional plots only on the last sampling date. The composition of the nematode communities in the field was assessed by classifying the nematodes according to food type (plants or bacteria) and according to reproductive strategy. The authors reported a change in the composition of the different food types in all plots during the growing season, with one exception, there were no significant differences between the different maize varieties. In terms of reproductive strategy, with one exception, no significant differences were observed between the different varieties. *C. elegans* exposed to aqueous Cry1A.105-Cry2Ab2- and Cry3Bb1-containing solutions and in equimolar (1:1) mixtures showed a dose-dependent inhibitory effect for all three proteins and protein mixtures on growth and reproduction. Cry3Bb1 displayed the highest toxicity, followed by Cry1A.105 and Cry2Ab2.

Molluscs
Slugs can be abundant and play an important role in the food web of maize ecosystems as prey of spiders, carabids, birds and hedgehogs. In a study of effects of Bt maize material (Cry1Ab) on the life cycle of the land snail *Cantareus aspersus,* snails exposed to Bt toxin in food and soil had a growth coefficient 25 % lower than unexposed snails after 47 weeks of exposure (Kramarz et al. 2009). After the first period of reproduction (68 weeks) a significant difference remained for body mass between the two groups. Differences in body mass were not significant at the end of exposure (88 weeks).

In a laboratory experiment with two transgenic maize varieties expressing Cry1Ab and Cry3Bb1, a potential impact of Bt maize was examined for the non-target slug *Arion vulgaris* (Hönemann & Nentwig 2010). Lifespan after field collection, weight change and oviposition was examined for slugs fed with Bt maize, conventional control or dandelion (*Taraxacum offiscinale*). Test parameters were neither significantly different between transgenic and comparator nor among the maize varieties overall over an exposure period of 16 weeks. These results are in compliance with previous studies on effects of Cry1Ab and Cry3Bb1 on *A. lusitanicus* and *Deroceras reticulatum* (Zurbrügg & Nentwig 2009). Cry proteins were detected in the gut and faeces, but no differences in biomass or leaf consumption were observed between the treated and untreated groups.

5.4.7 The Norwegian red list of threatened species

The 2010 Norwegian Red List for species (www.artsdatabanken.no) (Kålås et al. 2010) contains 462 Lepidoptera, an increase of 34 species from the Red List published in 2006. 191 of these taxons are categorised as critically endangered (CR) or endangered (EN), and thus have an extremely or very high risk of extinction. Most of the species are red listed due to a narrow host range, limited distribution range and a reduction in/disappearance of accessible habitats for their host plants. Most species on the Red List live in open habitats, which are either becoming overgrown or being affected by increasing use of monoculture.

Because the Cry-proteins expressed in maize MON89034 are toxic to a wide range of Lepidoptera, it is likely that most of the endangered species would be affected when feeding on MON 89034 maize plants. Among the red listed Lepidoptera categorized as endangered, only two species live on grasses in the vicinity of agricultural areas. *Euthrix potatoria* (caterpillar) prefer habitats with open woodlands and wetlands, where the larvae feed on various grass species and reeds. The species are threatened because of severe fragmentation and decline in accessible habitats. Threats to *Coenonympha hero* (the Scarce Heath) are primarily related to changes in farming methods and in land use practices. The species is favoured by lightly managed hay meadows, and are negatively affected by both agricultural intensification and overgrowth (Endrestøl & Bengtson 2012). The Scarce Heath is listed on the Bern Convention (“strictly protected fauna species-list”) and was also protected by law in Norway in 2001.

Cultivation of maize MON 89034 is not considered to represent a threat to the prevalence of these endangered species.

5.5 Impacts of the specific cultivation, management and harvesting techniques

Apart from changes in insecticide regimes, there are no anticipated changes in cultivation practices, management or harvesting techniques associated with the cultivation of maize MON 89034. *Bt* crops, such as maize 89034, may reduce the use of insecticides and may cause changes in crop rotations in response to reduced pest pressure (ref. EFSA 2011d). However, this reduction in pesticide use and narrow spectrum of activity of Cry proteins may provide an opportunity for secondary pests, previously controlled by insecticides used against key target pests, to reach damaging levels (reported for mirid bugs in *Bt*-cotton in China – Lu et al 2010). Natural enemies failing to fully control secondary pests, and reducing competition with target pests might also play a role in secondary pest outbreaks (ref. EFSA 2011d). Incidence of secondary pests and the environmental consequences of changes in management measures are highly dependent upon farming systems and regional environmental factors.

The implementation of insect resistance management strategies is desirable to delay or prevent the potential evolution of insect resistance to Cry1A.105 and Cry2Ab2 in Lepidopteran target pest populations.

5.6 Effects on biogeochemical processes

5.6.1 Fate of Bt-proteins in soil

Bt–toxin expressed in Bt crops can enter the soil system via root exudates released into the rhizosphere throughout the growth of the plant, and via senescent plant material remaining in the field after harvest and incorporated into the soil during tilling operations (Icoz & Stotzky 2008; BEETLE Report 2009). Beside root exudates and plant residues, pollen is another source of Bt proteins entering soils (e.g. Losey et al. 1999). Additionally, Bt proteins are found in the gastrointestinal tract of cows and their feces, as well as in the feces of decomposers (rew. Icoz & Stotzky 2008).

The stability, persistence and potential accumulation of the Bt proteins in soil are key factors for determining exposure and potential effects on soil biota related to the soil function. Persistence of Bt toxins in soil is primarily dependent on the protein quantity added and on the rate of inactivation and degradation by biotic and abiotic factors (Sanvido et al. 2006; Helassa et al. 2010). Degradation rates of Bt toxins are known to be influenced by varying environmental conditions (e.g. type of crop, soil characteristics, microbial activity, temperature, pH), protein source, method used for quantification of the protein as well as the particular Cry protein chosen (Sanvido et al. 2006; Icoz & Stotzky 2008). Cry proteins from e.g. *B. thuringiensis* subsp. *kurstaki* are rapidly absorbed and bound to clay minerals and humic substances which render the proteins resistant to biodegradation but with retention of larvicidal activity. Binding of Cry proteins to soil components indicates that there is a potential for long-term persistence and, thereby, prolonged exposure of the microbial and invertebrate communities in soils.

According to studies performed by the applicant, the Cry1A.105 and Cry2Ab2 proteins were subjected to rapid degradation in soil and were characterised by a short half-life (Cry1A.105 protein derived from MON 89034 tissues: DT50: ≤ 7 days and a DT90: ≤ 90 days; Cry2Ab2 protein derived from MON 89034: DT50: ≤ 6 days and a DT90: ≤ 14 days (Technical Dossier: Mueth et al. 2006). The applicant concludes that lack of persistence of these proteins strongly supports minimal exposure of Cry1A.105 and Cry2Ab2 to non-target organisms involved in decomposition and on soil-dwelling organisms in general. In a laboratory study, MON 89034 shoot and root tissues expressing Cry1A.105 and Cry2Ab2 proteins were shown not to pose any significant hazard to microorganisms and microbial mediated carbon and nitrogen mineralisation processes in soil (Technical Dossier: Huizinga et al. 2007)

Persistence, degradation and accumulation of Bt toxins in the soil has been assessed in a number of laboratory and field studies. However, reviews of the scientific literature reveal various results with regards to the persistence of Cry proteins. (The majority of the studies have been conducted with Bt maize expressing Cry1Ab). From studies dealing with potential impacts of Bt maize on soil processes and communities, some reveal a lower decomposition rate of residues of Bt crops compared to non-Bt crops (e.g. Flores et al. 2005; Saxena & Stotzky 2001a; Zwahlen et al. 2003a,b), while other laboratory and field studies show absence of negative effects of Bt toxins on decomposition processes and microbial community structure (e.g. Hopkins & Greogorich 2003, 2005; Devare 2004, 2007; Zwahlen et al. 2007; Hönemann et al. 2008; Zurbrügg et al. 2010; Gruber et al. 2012).

The Cry1Ab protein released in root exudates of Bt maize persisted in soil microcosms for at least 180 days and for at least three years from biomass of Bt maize (Saxena & Stotzky 2002; Stotzky 2002, 2004). Zwahlen et al. (2003a) has published the results from two Swiss field studies where the decomposition of the Cry1Ab toxin from leaf of Bt11 maize was recorded through autumn, winter and spring for a period of 200 days. At the end of the experimental period, 0.3% of the original proteins were still present in the soil.

Flores et al. (2005) investigated the deomposition of various species expressing Cry 1Ab toxin, and discussed the results in relation to the lignin content and potential environmental impacts. The authors concluded that Bt maize had higher ligning content than the conventional counterpart, and decomposed less in soil compared to non-Bt maize. Another study with different maize lines expressing Cry1Ab (MON 810, Bt11), showed no differences in lignin content of 12 Bt maize hybrids and isogenic non-Bt maize (Jung & Scheaffer 2004).

In the ECOGEN project, Cortet et al. (2006) investigated the effects of Cry1Ab protein on decomposition of wheat straw in three climatically different areas in Europe (Denmark, France). In the field-incubation trial, the Bt-maize and conventional, near-isogene lines were grown on 3 different soils and according to common cultivation practices. Results after 4 months showed that decomposition and mineralisation of organic matter were mainly driven by climatic parameters with no adverse effect of Bt proteins on these processes.

Devare (2004, 2007) reported no differences in N-mineralising potential, nitrification rates and soil respiration between fields planted with either Bt or non-Bt maize. Corresponding results have been reported by Hopkins & Gregorich (2003, 2005) and Dubelman et al. (2005). These studies showed that the Cry1Ab protein do not persist in biologically relevant concentrations in soil 3 months after harvest, and they found no evidence of accumulation of the Cry1Ab protein in soil from fields planted for at least 3 consecutive years with Bt maize, regardless of soil type, geographical region or climatic conditions.

In a field experiment, Zurbrügg et al. (2010) studied decomposition of leaf residues from three Bt maize cultivars expressing Cry1Ab and Cry3Bb1, corresponding near-isolines and three conventional hybrids using litterbags. The Cry protein concentrations in maize leaf residues were measured from harvest to the next growing season. The C:N ratios of Bt maize differed from their corresponding near-isolines, but more pronounced differences in C:N ratio, lignin, cellulose and hemicellulose content were present among conventional cultivars. Consequently, the decomposition dynamics of transgenic hybrids were similar to the non-transgenic near-isolines, but varied among conventional hybrids, demonstrating that Bt maize hybrids lie within the variation found in conventional maize agroecosystems. Expression levels and degradation patterns were different for Cry1Ab and Cry3Bb1, but leaf residues and Bt protein concentrations decreased rapidly in all Bt maize hybrids. Thus, non-target soil organism were exposed to relatively low *Bt* protein concentrations within a few months after harvest, and Zurbrügg et al. concluded that there is no indication of ecologically relevant, adverse effects on the activity of the decomposer community.

Helassa et al. (2010) investigated the adsorption properties, the mobility of the adsorbed protein and the decline of the Cry1Aa toxin as a function of time and microbial activity in contact with various

soils and soil minerals. No mobility of adsorbed toxin was observed at any pH and at different degrees of surface saturation.

In a recently published study, Gruber et al. (2012) investigated the fate of Cry1Ab protein in soil under long-term Bt maize cultivation in an experimental field trial performed over nine growing seasons on four field sites in Germany. The results from this study showed that on any of the four sites the climatic and field conditions led to complete degradation of the Bt-maize plant material containing the recombinant Cry1Ab protein by the following growth season. No persisting immunoreactive Cry1Ab protein was detected in any soil shortly before the next seeding over the experimental period of three years, which comprised the last third of nine years of Bt-maize planting. No experimental evidence for accumulation or persistence of Cry1Ab protein in different soils under long-term Bt-maize cultivation could be drawn from this field study.

5.6.2 Effects on soil microorganisms

Microorganisms are the dominant organisms both in terms of biomass and activity in the soil. The soil microbiota is involved in a number of important processes including decomposition of organic matter, nutrient mineralisation, regulation of plant pathogens, decomposition of agricultural chemicals and the improvement of soil structure (ref. Sanvido et al. 2006; BEETLE Report et al. 2009). Due to the close interaction between crop cultivation and soil processes, soil organisms in the rhizosphere are likely to be exposed to the Cry proteins released from root exudates and decaying plant material.

There have been numerous studies, with different methods (e.g. functional and structural composition of soil microbial communities) and different crops on the effects of Bt plants on soil microbial communities. Different effects, ranging from no effect to significant small transient negative effects on microbial communities/ soil protozoa and microorganisms have been reported (reviews by Sanvido et al. 2006; Icoz & Stotzky 2008; BEETLE Report 2009; Stefani & Hamelin 2010). (Data are however only available from short-term experiments and predictions of potential long-term effects are difficult to make). Based on available literature, The BEETLE Report (2009) concluded that the likelihood of adverse effects of Bt maize in EU is low. However, uncertainties remain regarding mycorrhizal fungi.

Root exudates of Bt maize (event Bt176) have been shown to reduce presymbiotic hyphal growth of the arbuscular mycorrhizal fungus *Glomus mosseae* compared with root exudates of another Bt maize hybrid (event Bt11) and conventional control (Turrini et al. 2004). A higher level of Cry1Ab toxin was measured in the event Bt176 (80.63 Cry1Ab/g protein) that negatively affected *G. mosseae* compared to Bt11 (<0.55 Cry1Ab/ g protein) and the authors stated that their findings could possibly be explained by the expression levels of Cry1A. Castaldini et al. (2005) have also reported consistent differences in rhizosphere heterotropic bacteria and mycorrhizal colonisation (including *G. mosseae*) between Bt-maize expressing Cry1Ab (Bt176, Bt11) and its conventional counterpart. In both transformed lines the intraradical colonisation of *G. mosseae* was significantly lower (about 50%) compared to wild type after 8 and 10 weeks of interaction under controlled conditions. The percentage of root length colonised by arbuscular mycorrhizal fungi was significantly lower in *Medigaco sativa* grown for four months in soil containing Bt11 residues. The reasons for which Bt maize were less susceptible to endomycorrhizal colonisation remain unknown (Stefani & Hamelin 2010).

By contrast, most studies, performed under laboratory, glasshouse or field conditions revealed only some minor changes in soil microbial community structure with Bt maize compared to non Bt maize (e.g. Blackwood & Buyer 2004; Griffiths et al. 2006; Mulder et al. 2006) or generally show no adverse effects of the Cry protein released by Bt maize in root exudates or from biomass incorporated into soil (e.g. Saxena & Stotzky 2001a; Hönemann et al. 2008; Icoz et al. 2008; Prischl et al. 2012).

Blackwood & Buyer (2004) has further investigated the effects of transgenic maize varieties expressing Cry1F and Cry1Ab protein on soil microbial community structure in three soils with different textures. The results of the growth chamber experiment showed significant effects of Bt-toxin

on microbial community structure in the loam samples. The authors assumed that Bt maize caused rapid growth in populations of special microorganisms due to increased protein content, and that soil types with a high content of clay increases retention of Cry-proteins.

Results from the ECOGEN project revealed that the small effects of Bt maize or a conventional insecticide on protozoa and microorganisms were less pronounced than effects due to soil and plant growth stage (Griffiths et al. 2006), and less than the variation seen between the eight maize cultivars (Griffiths et al. 2007b). No effects could be attributed to the Bt maize on mycorrhizal fungi in a separate mesocosm experiment (de Vaufleury et al. 2007). These field experiments, point to the conclusion that Bt maize (Cry1Ab) could have a significant, but small and transient, negative effect on soil protozoa and microorganisms (Griffiths et al. 2005, 2007a), but no effects on organic matter (wheat straw) decomposition (Cortet et al. 2006). EGOGEN developed a quantitative model to summarise the effects of the different cropping systems on soil quality (Bohanec et al. 2007). The authors concluded that Bt maize did not have deleterious effects on the soil biota, and that factors such as plant growth stage, season, soil type, tillage, crop type or variety produced larger effects on soil microbial community structures than the Bt maize (Griffiths et al. 2007b; Krog et al. 2007b).

Saxena & Stotzky (2001b) reported no significant differences in numbers of bacteria, fungi and protozoa between soils amended with biomass of Bt and non-Bt maize or in rhizosphere soil of Bt and non-Bt maize grown in a plant-growth room.

Prischl et al. (2012) compared the endophytic bacterial communities in plants of the transgenic Bt maize lines MON 89034, MON 88017 (*cry3Bb1*) and the stacked event MON 88017 x MON 89034, with those of the respective near-isogenic line and three additional conventional maize lines. The maize plants were grown in a containment system on two different soils that were commonly used for maize cultivation in Lower Austria. A 700 bacterial endophytes were obtained and characterised regarding their phylogenetic diversity and specific plant growth promoting functions. Both the soil environment and the plant cultivars had an effect on the phylogenetic diversity of the endophytic communities, but there were no specific effects of the transgenic varieties. Diversity measures of endophytic isolates were not different in Bt-versus non Bt-maize varieties.

5.7 Assessment based on available data

There are no reports of the target Lepidopteran species attaining pest status on maize in Norway. Since there are no Bt-based insecticides approved for use in Norway, and lepidopteran pests have not been registered in maize, issues related to resistance evolution in target pests are not relevant at present for Norwegian agriculture.

Published scientific studies show no or negligible adverse effects of Cry1A.105 and Cry2Ab2 proteins on non-target arthropods that live on or in the vicinity of maize plants. Cultivation of maize MON 89034 is not considered to represent a threat to the prevalence of red-listed species in Norway.

Few studies have been published examining potential effects of Cry1A.105 and Cry2Ab toxin on ecosystems in soil, mineralization, nutrient turnover and soil communities. Some field studies have indicated that root exudates and decaying plant material containing Cry proteins may affect population size and activity of rhizosphere organisms (soil protozoa and microorganisms). However, data are only available from short term experiments and predictions of potential long term effects are difficult to deduce. Most studies conclude that effects on soil microorganisms and microbial communities are transient and minor compared to effects caused by agronomic and environmental factors.

Few studies have assessed the impact of Cry proteins on non-target aquatic arthropods and the fate of these proteins in senescent and decaying maize detritus in aquatic environments. Further studies with better experimental design are needed for the assessment of the potential effects of Bt crops on aquatic organisms. However, exposure of non-target organisms to Cry proteins in aquatic ecosystems is likely

to be very low, and potential exposure of Bt toxins to non-target organisms in stream ecosystems in Norway is considered to be negligible.

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. Vertical gene transfer in maize therefore depends on cross-pollination with other conventional or organic maize varieties. In addition, unintended admixture of genetically modified material in seeds represents a possible way for gene flow between different crop cultivations. The risk of pollen flow from maize volunteers is negligible under Norwegian growing conditions.

In addition to the data presented by the applicant, the VKM GMO Panel is not aware of any scientific report of increased establishment and spread of maize MON 89034 and any change in survival (including over-wintering), persistence and invasiveness capacity. Because the general characteristics of maize MON 89034 are unchanged, insect resistance are not likely to provide a selective advantage outside cultivation in Norway.

Since MON 89034 has no altered agronomic and phenotypic characteristics, except for the specific target pest resistance, the VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects due to the establishment and survival of maize MON 89034 will be no different to that of conventional maize varieties in Norway

6 Post-Market Environmental Monitoring Plan

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

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

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

6.1 Case-specific GM plant monitoring

When potential adverse effects or important gaps in scientific information or significant levels of critical uncertainty linked to the GM plant and its management have been identified in the environmental risk assessment, then case-specific monitoring should be carried out after placing on the market, in order to confirm assumptions made in the ERA and to further inform the ERA (EFSA 2011c). Case-specific monitoring should be targeted at assessment endpoints and environmental protection goals identified in the ERA conclusion as being at risk or where levels of critical uncertainty were identified in relation to potential risks associated with the GM plant. Monitoring of potentially adverse cumulative long-term or large-scale effects and the resolution of areas of critical uncertainty, identified in the ERA are important objectives of monitoring (EC 2002).

The scope of the application EFSA/GMO/BE/2011/90 is the authorisation of MON 89034 in the EU under Regulation (EC) No. 1929/2003 for use as any other maize, including the cultivation of MON 89034 varieties. The environmental risk assessment, conducted by the applicant, support a conclusion that cultivation of MON 89034 in the EU, represent negligible risk to human and animal health and the environment. Because no immediate adverse risk effects are expected, the probability of long-term adverse effects is also negligible. The applicant has therefore considered that there is no need for case-specific monitoring. The VKM GMO Panel is of the opinion that case-specific monitoring is not appropriate.

Specific strategies for risk management are however required with regard to the interactions between the GM plant and target organisms. Insect resistance management measures will be put in place in

MON 89034 cultivation countries to proactively avoid and in any case delay insect resistance development.

6.2 General surveillance for unanticipated adverse effects

According to the principles and objectives outlined in Annex VII of Directive 2001/18/EC, the objectives of general surveillance is to detect any unanticipated adverse effects on protected and valued entities of the environment, including biodiversity and ecosystem services (EFSA 2011c).

The general surveillance proposed by the applicant is based on four pillars: (1) the use of annual farm questionnaires to feed a general surveillance database; (2) the review of scientific information provided by existing observation networks; (3) the implementation of company stewardship programs; and (4) the follow-up of various information sources such as scientific publications, expert reports etc to identify potential adverse effects associated with the intended uses of maize MON 89034. The applicant proposed to conduct general surveillance for maize MON 89034 throughout the period of validity of the authorisation.

The applicant will submit an annual monitoring report covering results of the general surveillance in accordance with the conditions of the authorisation. The report will contain information of any unanticipated adverse effects that have arisen from cultivation and/or use of MON 89034. According to the monitoring plan, the report will include a scientific evaluation of the confirmed adverse effect, a conclusion of the safety of MON 89034 and, as appropriate, measures that were taken to ensure the safety of human and animal health or the environment.

Comments:
The setting or population in which these effects might occur is either not, or hardly predictable. The central tool for general surveillance in the case of cultivation of MON 89034 is an annual farmers´ questionnaire which is addressed to a subset of farmers that cultivate maize MON 89034.

More detailed information about the management of data collected and the statistical analyses performed are however required, especially for those obtained from the questionnaires distributed to farmers. In addition, it is not clear if the questionnaire compilation is mandatory or not, for example, if it is foreseen in contract of sale between those who put the GM plant on the market and the farmers themselves.

General surveillance should be considered a period of time longer than 10 years (authorisation term) to assess any adverse effects not foreseen by ERA. The applicant is also invited to take into account the reporting format set out in Annex II of Decision 2009/770/EC as technical guidance notes in order to facilitate the implementation and explanation of Annex VII to Directive 2001/18/EC.

7 Data gaps

- Knowledge of possible target and non-target species present in Norway, in environments where GM maize could be grown.
- Effects of Cry proteins on the population size and activity of rhizosphere organisms present in Norwegian agricultural conditions.
- Effects of Bt toxins on aquatic organisms in Norway.
  Further studies with better experimental design are needed for the assessment of the potential effects of Bt crops on aquatic organisms.

8 Comments to the EFSA GMO Extranet - application EFSA/GMO/BE/2011/90

No comments from the VKM GMO Panel in connection with EFSAs official hearing of application EFSA/GMO/BE/2011/90.

Preliminary assessment based on available data

Molecular characterisation

Appropriate analysis of the integration site, including flanking sequence and bioinformatics analysis, has been performed to characterise the transformation event MON 89034. The results of the segregation analysis are consistent with a single site of insertion for the *cry1A.105* and *cry2Ab2* gene expression cassettes and confirm the results of the molecular characterisation. Molecular analysis of both self-pollinated and cross-fertilised lines, representing a total of seven different generations, indicates that the inserted DNA is stably transformed and inherited from one generation to the next. No genes that encode resistance to antibiotics are present in the genome of MON 89034 maize. The molecular characterisation confirmed the absence of both the *aad* and *nptII* genes, which were used in the cloning and transformation process.

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).

Comparative assessment

Based on results from comparative analyses of data from field trials in the USA (2004-500) and Europa (2007), it is concluded that maize MON 89034 is agronomically and phenotypically equivalent to the conventional counterpart and commercial available reference varieties, with the exception of the lepidopteran-protection trait. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of MON 89034 compared to conventional maize. Evaluations of ecological interactions between maize MON 89034 and the biotic and abiotic environment indicate no unintended effects of the introduced trait on agronomic and phenotypic characteristics.

Environmental risk

There are no reports of the target Lepidopteran species attaining pest status on maize in Norway. Since there are no Bt-based insecticides approved for use in Norway, and lepidopteran pests have not been registered in maize, issues related to resistance evolution in target pests are not relevant at present for Norwegian agriculture.

Published scientific studies show no or negligible adverse effects of Cry1A.105 and Cry2Ab2 proteins on non-target arthropods that live on or in the vicinity of maize plants. Cultivation of maize MON 89034 is not considered to represent a threat to the prevalence of red-listed species in Norway.

Few studies have been published examining potential effects of Cry1A.105 and Cry2Ab toxin on ecosystems in soil, mineralization, nutrient turnover and soil communities. Some field studies have indicated that root exudates and decaying plant material containing Cry proteins may affect population size and activity of rhizosphere organisms (soil protozoa and microorganisms). However, data are only available from short term experiments and predictions of potential long term effects are difficult to deduce. Most studies conclude that effects on soil microorganisms and microbial communities are transient and minor compared to effects caused by agronomic and environmental factors.

Few studies have assessed the impact of Cry proteins on non-target aquatic arthropods and the fate of these proteins in senescent and decaying maize detritus in aquatic environments. Further studies with better experimental design are needed for the assessment of the potential effects of Bt crops on aquatic organisms. However, exposure of non-target organisms to Cry proteins in aquatic ecosystems is likely to be very low, and potential exposure of Bt toxins to non-target organisms in stream ecosystems in Norway is considered to be negligible.

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. Vertical gene transfer in maize therefore depends on cross-pollination with other conventional or organic maize varieties. In addition, unintended admixture of genetically modified material in seeds represents a possible way for gene flow between different crop cultivations. The risk of pollen flow from maize volunteers is negligible under Norwegian growing conditions.

In addition to the data presented by the applicant, the VKM GMO Panel is not aware of any scientific report of increased establishment and spread of maize MON 89034 and any change in survival (including over-wintering), persistence and invasiveness capacity. Because the general characteristics of maize MON 89034 are unchanged, insect resistance are not likely to provide a selective advantage outside cultivation in Norway.

Since MON 89034 has no altered agronomic and phenotypic characteristics, except for the specific target pest resistance, the VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects due to the establishment and survival of maize MON 89034 will be no different to that of conventional maize varieties in Norway

The environmental risk assessment will be completed and finalized by the VKM Panel on Genetically Modified Organisms when requested additional information from the applicant is available.

References

Alvarez-Alfageme F, Ferry N, Castanera P, Ortego F, Gatehouse AMR (2008) Prey mediated effects of *Bt* maize on fitness and digestive physiology of the red spider mite predator *Stethorus punctillum* Weise (Coleoptera: Coccinellidae). Transgenic Research 17: 943-954

Alvarez-Alfageme F, Ortego F, Castanera P (2009) *Bt* maize fed-prey mediated effect on fitness and digestive physiology of the ground predator *Poecilus cupreus* L. (Coleoptera: Carabidae). Journal of Insect Physiology 55: 143-149

Alvarez-Alfageme F, Bigler F, Romeis J (2011) Laboratory toxicity studies demonstrate no adverse effects of Cry1Ab and Cry3Bb1 to larvare of *Adalia bipunctata* (Coleoptera: Coccinellidae): the importance of study design. Transgenic Research 20: 467.479

Andow DA (2008) the risk of resistance evolution in insects to transgenic insecticidal crops. Collection of Biosafety Reviews 4: 124-199

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

Arpaia S (2010) Genetically modified plants and "non-target" organisms: analysing the function of the agro-ecosystem. Collection of Biosafety Reviews 5: 12-80

Aylor DE (2002) Settling speed of corn (*Zea mays*) pollen. Journal of Aerosol Science 33: 1601.1607

Aylor DE, Schultes NP, Shields EJ (2003) An aerobiological framework for assessing crosspollination in maize. Agriculture and Forest Meteorology 119: 111-129

Aylor DE (2004) Survival of maize (*Zea mays*) pollen exposed to the atmosphere. Agricultural and Forest Metereology 123: 125-133

Babendreier D, Kalberer NM, Romeis J, Fluri P, Mulligan E, Bigler F (2005) Influence of Bt-transgenic pollen, Bt-toxin and protease inhibitor (SBTI) ingestion on development of the hypopharyngeal glands in honeybees. Apidologie 36: 585-594

Babendreier D, Reichhart B, Romeis J, Bigler F (2008) Impact of insecticidal proteins expressed in transgenic plants on bumblebee microcolonies. Entomologia Experimentalis Et Applicata 126(2): 148-157

Bai Y, Yan R, Ke X, Ye G, Huang F, Luo Y, Cheng J (2011) Effects of transgenic Bt rice on growth, reproduction, and superoxide dismutase activity of Folsomia candida (Collembola: Isotomidae) in laboratory studies. J Econ Entomology 104: 1892-8199

Bailey J, Scott-Dupree C, Harris R, Tolman J, Harris B (2005) Contact and oral toxicity to honey bees (*Apis mellifera*) of agents registered for use for sweet corn insect control in Ontario, Canada. Apidologi 36: 623-633

Bakken AK, Nesheim L, Harbo O, Johansen A, Wikmark T (2005) Potensial for dyrking av fôrmais i Norge. Grønn kunnskap 9: 1-6

Bakonyi G, Szira F, Kiss I, Villanyi I, Seres A, Szekacs A (2006) Preference tests with collembolas on isogenic and Bt maize. European Journal of Soil Biology 42: S132-S135

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

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

Blackwood CB, Buyer JS (2004) Soil microbial communities associated with Bt and non-Bt corn in three soils. Journal of Environmental Quality 33: 832–836

Bohanec M, Cortet J, Griffiths b, Znidarsic M, Debeljak M, Caul S et al. (2007) A qualitative multi-attribute model for assessing the impact of cropping systems on soil quality. Pedobiologia 51: 239-250

Bourguet D, Chaufaux J, Micoud A, Delos M, Nabio B, Bombarde F et al. (2002). *Ostrinia nubilalis* parasitism and the field abundance of non-target insects in transgenic *Bacillus thuringiensis* corn (*Zea mays*). Environmental Biosafety Research 1: 49-60

Brookes G, Barfoot P, Mele E, Messeguer J, Benetrix D, Foueillassar X, Fabie A, Poeydomenge C (2004) Genetically Modified Maize: Pollen movement and crop coexistence. Report from PG Economics. 20 s

Brookes G, Barfoot P (2005) GM crops: the global economic and environmental impact- the first nine years 1996-2004. AgBioForum 8: 187-196

Bøhn T, Primiceria R, Hessen D, Traavik T et al (2008) Reduced fitness of *Dapnia magna* fed a Bt-transgenic maize variety. Archives of Environmental Contamination and Toxicology 55: 585-592

Bøhn T, Traavik T, Primicero R (2010) Demographic responses of *Dapnia magna* fed transgenic Bt-maize. Ecotoxicology 19: 419-430

Carstens K, Anderson J, Bachman P, De Schrijver A, Dively G, Frederici B et al. (2012) Genetically modified crops and aquatic ecosystems: considerations for environmental risk assessment and non-target organism testing. Transgenic Res. 21: 813-842

Castaldini M, Turrini A, Sbrana C, Benedetti A, Marchionni M, Mocali S et al. (2005) Impact of Bt corn on rhizospheric and soil eubacterial communities and on beneficial mycorrhizial symbiosis in experimental microcosms. Applied and Environmental Microbiology 71: 6719-6729

CERA (2012) Center for Environmental Risk Assessment. GM Database for safety information. http://cera-gmc.org/index.php?action=gm_crop_database

Chambers P, Whiles MR, Rosi-Marshall MJ, Tank J, Royer TJ, Griffiths NJ et al. (2010). Responses of stream macroinvertebrates to Bt maize leaf detritus. Ecol Appl 20:1949-1960

Clark BW, Coats JR (2006) Subacute effects of CrylAb Bt corn litter on the earthworm *Eisenia fetida* and the springtail *Folsomia candida*. Environmental Entomology 35: 1121-1129

Cortet J, Andersen MN, Caul S, Griffiths BS, Joffre R, Lacroix B et al. (2006) Decomposition processes under Bt (*Bacillus thuringiensis*) maize: Results of a multi-site experiment. Soil

Biology & Biochemistry 38: 195-199

Cortet J, Griffiths BS, Bohanec M, Demsard D, Andersen MN, Caulc A et al. (2007) Evaluation of effects of transgenic Bt maize on microarthropods in a European multi-site experiment. Pedobiologia 51: 207-218

Coulianos CC, Ossiannilsson F (1976) Catalogus Insectorum Sueciae VII. Hemiptera-Heteroptera. 2nd ed. 97:135-173

Daly T, Buntin GD (2005) Effect of *Bacillus thuringiensis* transgenic corn for lepidopteran control on nontarget arthropods. Environmental Entomology 34:1292-1301

de la Poza M, Pons X, Farinos GP, Lopez C, Ortego F, Eizaguirre M et al. (2005) Impact of farm-scale *Bt* maize on abundance of predatory arthropods in Spain. Crop Protection 24: 677-684

de Vaufleury, Kramarz, P.E., Binet, P., Cortet, J., Caul, S., Andersen, M.N., Plumey, E., Coeurdassier, M. & Krogh, P.H. (2007). Exposure and effects assessments of *Bt*-maize on non-target organisms (gastropods, microarthropods, mycorrhizal fungi) in microcosms. Pedobiologi, 51: 185-194

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

Devare M, Jones CM, Thies JE (2004) Effect of Cry3Bb transgenic corn and tefluthrin on the soil microbial community. Journal of Environmental Quality 33: 837-843

Devare M, Londono RLM, Thies JE (2007) Neither transgenic Bt maize (MON863) nor tefluthrin insecticide adversely affected soil microbial activity or biomass: A 3 –year field analysis. Soil Biology & Biochemistry 39: 2038-2047

Devos Y, Reheul D, De Schrijver A (2005) The co-existence between transgenic and non-transgenic maize in the European Union: a focus on pollen flow and cross-fertilization. Environmental Biosafety Research 4: 71-87

Devos Y, Cougnon M, Thas O, Reheul D (2008) A method to search for optimal field allocations of transgenic maize in the context of co-existence. Environmental Biosafety Research 7: 97-104

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

Dhillon MK, Sharma HC (2009) Effects of Bacillus thuringiensis -endotoxins Cry1Ab and Cry1Ac on the coccinellid beetle, *Cheilomenes sexmaculatus* (Coleoptera, Coccinellidae) under direct and indirect exposure conditions. Biocontrol Science and Technology 19: 407-420

Di-Giovanni F, Kavan P, Nasr M (1995) The variability in settling velociyies of some pollen and spores. Grana 34 : 39-44

Ding J, Li JC, Liu XX, Zhang QW (2009) The life parameters of a parasitoid *Microplitis mediator* (Hymenoptera: Braconidae), reared on cotton bollworm *Helicoverpa armigera* (Hubner) with Cry1Ac diet. Biocontrol Science and Technology 19: 931-941

Dively GP (2005) Impact of transgenic VIP3A x Cry1A(b) lepidopteran-resistant field corn on the nontarget arthropod community. Environmental Entomology 34: 1267-1291

Douville M, Gagne F, Masson L, McKay J, Blaise C (2005) Tracking the source of *Bacillus thuringiensis* Cry1Ab endotoksin in the environment. Biochemical Systematics and Ecology: 33: 219-232

Douville M, Gagne F, Blaise C, André C (2007) Occurence and persistence of *Bacillus thuringiensis* Bt corn *cry1Ab* gene from an aquatic environment. Ecotoxicology and Environmental Safety 66: 195-203

Duan JJ, Marvier M, Huesing J, Dively G, Huang ZY (2008) A meta-analysis of effects of Bt crops on honey bees (Hymenoptera: Apidae). PLos ONE 3:1-6 (e1415)

Dubelman S, Ayden BR, Bader BM, Brown CR, Jiang C, Vlachos D et al. (2005) Cry1Ab protein does not persist in soil after 3 years of sustained Bt corn use. Environmental Entomology 34: 915-921

Dutton A, Klein H, Romeis J, Bigler F (2002) Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator Chrysoperla carnea. Ecological Entomology 27: 441-447

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

EC (2009) Report from the Commission to the Council and the European Parliament on the coexistence of genetically modified crops with conventional and organic farming. http://ec.europa.eu/agriculture/coexistence/com2009_153_en.pdf

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

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

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

EFSA (2009b) Application (EFSA-GMO-RX-MON810) for renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON810; (2) feed consisting of and/or containing maize MON810, including the use of seed for cultivation; and of (3) food and feed additives, and feed materials produced from maize MON810, all under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 1149: 1-85

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

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

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

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

EFSA (2011d) Scientific Opinion on application (EFSA-GMO-CZ-2008-54) for placing on the market of genetically modified insect resistant and herbicide tolerant maize MON 88017 for cultivation under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 9 (11):2428

Emberlin J Adams-Groom B, Tidmarsh J (1999) The dispersal of maize (Zea mais) pollen. A report commissiones by the Soil Assosiation: A National Pollen Research Unit, University College Workcester, UK.

Endrestøl A & Bengtson R (2012) Faglig grunnlag for handlingsplan for heroringvinge *Coenonympha hero.* NINA Rapport 860.

Escher, N., Käch, B. & Nentwig, W. (2000). Decomposition of transgenic *Bacillus thuringiensis* maize by microorganisms and woodlice *Porcellio scaber* (Crustacea: Isopoda). Basic and Applied Ecology 1: 161–169

Feil B, Schmid JE (2002) Dispersal of maize, wheat and rye pollen: A contribution to determining the the necessary isolation distances for the cultivation of transgenic crops. Shaker Verlag GMBH Aachen, Germany.

Flores S, Saxena D, Stotzky G (2005) Transgenic *Bt*-plants decompose less in soil than non-*Bt* plants. Soil Biology & Biochemistry 37: 1073-1082

Gathmann A, Wirooks L, Hothorn LA, Bartsch D, Schuphan I. (2006). Impact of *Bt* maize pollen (MON 810) on lepidopteran larvae living on accompanying weeds. Molecular Ecology, 15: 2677-2685

González-Zamora JE, Camúnez S, Avilla C (2007) Effects of *Bacillus thuringiensis* Cry toxins on developmental and reproductive characteristics of the predator Orius albidipennis (Hemiptera: Anthocoridae) under laboratory conditions. Environmental Entomology 36: 1246-1253

Griffiths BS, Caul S, Thompson J, Birch ANE, Scrimgeour C, Andersen MN (2005) A comparisation of soil microbial community structure, protozoa and nematodes in field plots of conventional and genetically modified maize expressing the *Bacillus thuringiensis* Cry1Ab toxin. Plant and Soil 275: 135-146

Griffiths BS, Caul S, Thompson J, Birch ANE, Scrimgeour C, Cortet J et al. (2006) Soil microbial and faunal community responses to *Bt*-maize and insecticide in two soils. Journal of Environmental Quality 35: 734-741

Griffiths BS, Caul S, Thompson J, Birch ANE, Cortet J, Andersen MN, Krogh PH (2007a) Microbial and microfaunal community structure in cropping systems with genetically modified plants. Pedobiologia 51: 195-206

Griffiths BS, Heckmann LH, Caul S, Thompson J, Scrimgeour C, Krogh PH (2007b) Varietal effects of eight paired lines of transgenic *Bt* maize and near-isogenic non-*Bt* maize on soil microbial and nematode community structure. Plant Biotechnology Journal 5: 60-68

Griffiths BS, Caul S, Thompson J, Hackett CA, Cortet J, Pernin C, Krogh, PH (2008) Soil microbial and faunal responses to herbicide tolerant maize and herbicide in two soils. Plant Soil 308: 93-103

Griffiths NA, Tank JL, Royer TV, Rosi-Marshall EJ, Whiles MR, Chambers CP et al. (2009) Rapid decomposition of maize detritus in agricultural headwater streams. Ecol Appl 19: 133-142

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

Gruber H, Paul V, Meyer HH, Müller M (2012) Determination of insecticidal Cry1Ab protein in soil collected in the final growing seasons of a nine-year field trial of Bt-maize MON810. Transgenic Research 21:77-88

Hallauer AR (2000) Potential for outcrossing and weediness of genetically modified insect protected corn. APHIS-USDA.

Halsey ME, Remund KM, Davis CA, Qualls M, Eppard PJ, Berberich S (2005) Isolation of Maize from Pollen-Mediated gene Flow by Time and Distance. Crop Science, 45: 2172-2185

Hanley AV, Huang ZY & Pett WL (2003) Effects of dietary transgenic Bt corn pollen on larvae of *Apis mellifera* and *Galleria mellonella*. Journal of Apicultural Research 42: 77-81

Heckmann LH, Griffiths BS, Caul S, Thompson J, Pusztai-Carey M, Moar WJ et al. (2006) Consequences for *Protaphorura armata* (Collembola: Onychiuridae) following exposure to genetically modified *Bacillus thuringiensis (Bt)* maize and non-Bt maize. Environmental Pollution 142: 212-216

Helassa N, Daudin G, Noinville S, Janot JM, Dejardin P, Staunton S, Quiquampoix H (2010) Mobilty of adsorbed Cey1A insecticidal toxin from *Bacillus thuringiensis* (Bt) on montmorillonite measured by fluorescence recovery after photobleaching (FRAP). Philosophical Magazine 90: 17-18

Hendriksma HH, Härtel S, Babendreier D, von der Ohe W, Steffan-Dewenter I (2012) Effects of multiple BT proteins and GNA lectin on in vitro-reared honey bee larvae. Apidologie 43: 549-560

Hilbeck A, Schmidt JEU (2006) Another view on Bt proteins – How specific are they and what else might they do? Biopestic. Int. 2: 1-50

Hilbeck A, McMillan JM, Meier M, Humbel A, Schlaepfer-Miller J, Tritkova M (2012) A controversy re-visited: Is the coccinellid *Adalia bipunctata* adversely affected by Bt toxins? Environ Sci Europe 24:10

Hofmann F, Epp R, Kruse L, Kalchschmied A, Maisch B, Müller E et al. (2010) Monitoring of Bt-Maize pollen exposure in the vicinity of the nature reserve Ruhlsdorfer Bruch in northeastern Germany 2007-2008. Umweltwissenschaften und Schadstoff-Forschung 22: 229-251

Holland JM (2004) The environmental consequences of adopting conservation tillage in Europe: reviewing the evidence. Agriculture, Ecosystem & Environment 103: 1-25

Hopkins DW, Gregorich EG (2003) Detection and decay of the *Bt* endotoxin in soil from a field trial with genetically modified maize. European Journal of Soil Science 54: 793-800

Hopkins DW, Gregorich EG (2005) Decomposition of residues and loss of the delta-endotoxin from transgenic (*Bt*) (*Zea mays* L.) in soil. Canadian Journal of Soil Science 85: 19-26

Hönemann L, Nentwig W (2009) Are survival and reproduction of *Enchytraeus albidus* (Annelida: Enchytraeidae) at risk by feeding on *Bt*-maize litter? European Journal of Soil Biology 45: 351-355

Hönemann L, Nentwig W (2010) Does feeding on Bt maize affect the slug *Arion vulgaris* (Mollusca: Arionidae)? Biocontrol Sci Technol 20: 13-18

Hönemann LC, Zurbrugg C, Nentwig W (2008) Effects of Bt-corn decomposition on the composition of the soil meso- and macrofauna. Applied Soil Ecology 40: 203-209

HössS , Arndt M, Baumgarte S, Tebbe CC, Nguyen HT, Jehle JA (2008) Effects of transgenic corn and Cry1Ab protein on the nematode, *Caenorhabditis elegans*. Ecotoxicology and Environmental Safety 70: 334-340

Hüsken A, Amman K, Messeguer J, Papa R, Robson P, Scieman P et al. (2007) A major European synthesis of data on pollen and seed mediated gene flow in maize in the SIGMEA project. In: Stein . & Rodríguez-Cerezo E (Eds.), Books of abstracts of the third International Conference on Coexistence between Genetically Modified (GM) and non-GM-based Agricultural Supply Chains, European Commission, pp. 53-56

Icoz I, Stotzky G (2007) Cry3Bb1 protein from *Bacillus thuringiensis* in root exudates and biomass of transgenic corn does not persist in soil. Transgenic Research 17: 609-620

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

Icoz I, Saxena D, Andow D, Zwahlem C, Stotzky G (2008) Microbial populations and enzyme activities in soil in situ under transgenic corn expressing Cry proteins from *Bacillus thuringiensis*. Journal of Environmental Quality 37: 647-662

Ingram J (2000) Report on the separation distances required to ensure cross-pollination is below specific limits in non-seed crops of sugar beet, maize and oilseed rape. MAFF Project No RG0123.

Jarosz N, Loubet B, Durand B, Foueillasser X, Huber L (2005) Variations in maize pollen emission and deposition in relation to microclimate. Environmental Science and Technology 39: 4377-4384

Jensen PD, Dively GP, Swan CM, Lamp WO (2010) Exposure and nontarget effects of transgenic Bt Corn debris in streams. Environmental Entomology 39: 707-714

Jesse LCH, Obrycki JJ (2000) Field deposition of *Bt* transgenic corn pollen: lethal effects on the monarch butterfly. Oecologia 125: 241-248

Jesse LCH, Obrycki JJ (2003) Occurrence of *Danaus plexippus* L. (Lepidoptera : Danaidae) on milkweeds (*Asclepias syriaca*) in transgenic *Bt* corn agroecosystems. Agriculture Ecosystems and Environment 97: 225-233

Jung, H.G. & Sheaffer, C.C. (2004). Influence of Bt Transgenes on Cell Wall Lignification and Digestability of Maize Stover for Silage. Crop Science 44: 1781-1789

Koskella J, Stozky G (2002) Larvicidal toxins from *Bacillus thuringiensis* subspp, *kurstaki, morrisoni* (strain tenebrionis), and *israelensis* have no microbicidal or microbiostatic activity against selected bacteria, fungi, and algae in vitro. Canadian Journal of Microbiology 48: 262–267

Kramarz P, de Vaufleury, Gimbert F, Cortet J, Tabone E, Andersen MN, Krogh PH (2009) Effects of *Bt*-maize material on the life cycle of the land snail *Cantareus asperses.* Applied Soil Ecology 42: 236-242

Krogh PH, Griffiths B, Demsar D, Bohanec M, Debeljak M, Andersen MN et al. (2007a) Responses by earthworms to reduced tillage in herbicide tolerant maize and *Bt* maize cropping systems. Pedobiologia 51: 219-227

Krogh PH, Griffiths B (2007b) ECOGEN-Soil ecological and economic evaluation of genetically modified crops. Pedobiologia 51: 171-173

Krogh PH, Griffiths B, Cortet J, Andersen MN, Dzeroski S, Wessler J et al. (2008) ECOGEN Final Reportet. Introduction and Summary. www.ECOGEN.dk.

Kvaløy, K., Klemsdal, S.S., Eklo, O.M., Netland, J., Schanke, T. & Tømmerås, B.Å., (1998). *Konsekvenser ved bruk av herbicidresistente genmodifiserte jordbruksplanter.* NINA Oppdragsmelding 536.

Kålås JA, Viken Å, Henriksen S, Skjelseth S (eds.) (2010) The 2010 Norwegian Red List for Species. Norwegian Biodiversity Information Centre, Norway. 489 p

Lang A, Vojtech E (2006) The effects of pollen consumption of transgenic Bt maize on the common swallowtail, *Papilio machaon* L. (Lepidoptera, Papilionidae). Basic and Applied Ecology 7: 296-306

Lang A, Arndt M, Beck R, Bauchhenss J, Pommer G (2006) Monitoring of the environmental effects of the Bt gene. Bavarian State Research Center for Agriculture, No 2006/10.

Lawo NC, Romeis J (2008) Assessing the utilization of a carbohydrate food source and the impact of insecticidal proteins on larvae of the green lacewing, *Chrysoperla carnea*. Biological Control 44(3): 389-398

Lei Z, Liu TX, Greenberg SM (2009) Feeding, oviposition and survival of *Liriomyza trifolii* (Diptera: Agromyzidae) on Bt and non-Bt cottons. Bulletin of Entomological Research 99: 253-261

Li HR, Oppert B, Higgins RA, Huang FN, Buschman LL. & Zhu KY (2005) Susceptibility of Dipel-resistant and -susceptible *Ostrinia nubilalis* (Lepidoptera : Crambidae) to individual *Bacillus thuringiensis* protoxins. Journal of Economic Entomology 98:1333-1340

Li YH, Meissle M, Romeis J (2008) Consumption of Bt Maize Pollen Expressing Cry1Ab or Cry3Bb1 Does Not Harm Adult Green Lacewings, Chrysoperla carnea (Neuroptera: Chrysopidae). Plos One 3(8)

Li Y, Romeis J, Wang P, Peng Y, Shelton AM (2011) A Comprehensive Assessment of the Effects of Bt Cotton on *Coleomegilla maculata* Demonstrates No Detrimental Effects by Cry1Ac and Cry2Ab. Plos One 6(7)

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

Losey JE, Rayor LS, Carter ME (1999) Transgenic pollen harms monarch larvae. Nature 399: 214-214

Lu Y, Wu K, Jiang Y, Xia B, Li P, Feng H et al. (2010) Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. Science 28: 5982

Ludy C, Lang A (2006) A 3-year field-scale monitoring of foliage-dwelling spiders (Araneae) in transgenic *Bt* maize fields and adjacent field margins. Biological Control 38: 314-324

Luna S, Figueroa J, Baltazar B, Gomez R, Townsend R, Schoper JB (2001) Maize pollen longevity and distance isolation requirements for effective pollen control. Crop Science 41: 1551-1557

Macintosh SC, Stone TB, Sims S.R, Hunst PL, Greenplate JT, Marrone PG, Perlak FJ, Fischhoff DA, Fuchs RL (1990) Specificity and Efficacy of Purified Bacillus-Thuringiensis Proteins against Agronomically Important Insects. Journal of Invertebrate Pathology 56: 258-266

Malone LA, Pham-Delegue MH (2001) Effects of transgene products on honey bees (*Apis mellifera*) and bumblebees (*Bombus* sp.). Apidologie 32: 287-304

Malone LA, Burgess EPJ (2009) Impact of genetically modified crops on pollinators. In: Ferry. N., Gatehouse, A.M.R. (EDS.). Environmental Impact of Genetically Modified Crops, CAB International, pp. 199-122.

Mann R S, Gill RS, Dhawan AK, Shera PS (2010) Relative abundance and damage by target and non-target insects on Bollgard and Bollgardll cotton cultivars. Crop Protection 29(8): 793-801

Meier MS, Hilbeck A (2001) Influence of transgenic *Bacillus thuringiensis* corn-fed prey on prey preference of immature *Chrysoperla carnea* (Neuroptera : Chrysopidae). Basic and Applied Ecology 2: 35-44

Melé E, Peñas G, Palaudelmás M, Serra J, Salvia J, Pla M, Nadal A, Messeguer J (2007) Effect of volunteers on maize gene flow. In: Stein, A. & Rodríguez-Cerezo, E. (Eds.), Books of abstracts of the third International Conference on Coexistence between Genetically Modified (GM) and non-GM-based Agricultural Supply Chains, European Commission, pp. 249-250.

Messeguer J, Penas G, Ballester J et al. (2006) Pollen-mediated gene flow in maize in real situations og coexistence. Plant Biotechnol J 4: 633-645

Mulder C, Wouterse M, Raubuch M, Roelofts W, Rutgers M (2006) Can transgenic maize affect soil microbial communities? PLoS Computation Biology 2: 1164-1172

Nesheim L (2008) Fôrmais I Stjørdal 2007: Tilfredstillende avling med plastdekke. Nyhetsmelding. www.bioforsk.no.

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

Netland J, Eklo OM, Aasmo Finne M (2012) Effekt på agronomi og miljø ved dirking av genmodifisert glyfosattolerant mais. Bioforsk Rapport -manuskript

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

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

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

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

OGTR (2008) The Biology of *Zea mays* L- ssp. *mays* (maize or corn). Australian Government Office of the Gene Technology Regulator. 80 pp.

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

Palaudelmás M, Melé E, Monfort A, Serra J, Salvia J, Messeguer J (2012) Assessment of the influence of field size on maize gene flow using SSR analysis. Transgenic Research 21: 471-483

Pereira EJG, Lang BA, Storer NP, Siegfried BD (2008) Selection for Cry1F resistance in the European corn borer and cross-resistance to other Cry toxins. Entomologia Experimentalis Et Applicata 126: 115-121

Perry JN, Devos Y, Arpaia S, Bartsch D, Gathmann A, Hails RS et al. (2010) A mathematical model of exposure of non-target Lepidoptera to Bt-maize pollen expressing Cry1Ab within Europe. Proceedings. Biological sciences / The Royal Society 277: 1417–1425

Pilcher CD, Obrycki JJ, Rice ME, Lewis LC (1997) Preimaginal development, survival, and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn. Environmental Entomology 26: 446-454

Pokelsek JD, Rosi-Marshall EJ, Chambers CP, Griffiths NA, Evans-White MA, Tank et al. (2007) Effects of Bt corn pollen on caddisfly growth rates in Midwestern agricultural streams. NABS Annual Meeting, Columbia, South Carolina 2007

Pont B, Nentwig W (2005) Quantification of Bt protein digestion and excretion by the primary decomposer *Porcellio scaber,* fed with two Bt corn varieties. Biocontrol Science and technology 15: 341-352

Priesnitz KU, Thieme T, Benker U (2011) Potential impact of a genetically engineered stacked Bt-maize on ground beetles. Ecological Impact of Genetically Modified Organisms. IOBC-WPRS 5th EIGMO MEETING June 22-25, 2011

Prischl M, Hackl E, Pastar M, Pfeiffer S, Sessitsch A (2012) Genetically modified Bt maize lines containing *cry3Bb1, cry1A105* or *cry1Ab2* do not affect the structure and functioning of root-associated endophyte communities. Applied Soil Ecology 54: 39-48

Ramirez-Romero R, Chaufaux J, Pham-Delegue MH (2005) Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee *Apis mellifera*, a comparative approach. Apidologie 36: 601-611

Ramirez-Romero R, Desneux N, Decourtye A, Chaffiol A, Pham-Delegue MH (2008) does Cry1Ab protein affect learning performance of the honey bee *Apis mellifera* L. (Hymenoptera, Apidae). Ecotoxicology and Environmental Safety 70: 327-333

Raynor G, Ogden E, Hayes J (1972) Dispersion and deposition of corn pollen from experimental sources. Agron J 64: 420-427

Ricroch A, Berge JB, Messéan A (2009) Literature review of the dispersal of transgenes from genetically modified maize. Comptes Rendus Biologies 332: 861-875

Ricroch A, Berge JB, Kuntz M (2010) Is the German suspension of MON810 maize cultivation scientifically justified? Transgenic Research 19: 1-12

Riesgo L, Areal FJ, Sanvido O, Rodriguez-Cerezo E (2010) Distances needed to limit cross-fertilization between GM and conventional maize in Europe. Nat Biotechnol 28:780-782

Rizzi AN, Raddadi C, Sorlini L, Nordgård L, Nielsen KM, Daffonchio D (2012) The stability and degradation of dietary DNA in the gastrointestinal tract of mammals - implications for horizontal gene transfer and the biosafety of GMOs. Crit. Rev. Food Science Nutr. 52:142-161

Rognli OA (1994) Økologisk risiko ved utsetting av genmodifiserte kulturplanter. Faginfo 2 (21): 81-197

Romeis J, Dutton A, Bigler F (2004). *Bacillus thuringiensis* toxin (Cry1Ab) has no direct effect on larvae of the green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). Journal of Insect Physiology 50: 175-183

Romeis J, Alvarez-Alfageme F, Bigler (2012) Putative effects of Cry1Ab to larvae of Adalia bipunctata – reply to Hilbeck et al. (2012) Environmental Sciences Europe 24: 18

Rose R, Dively GP, Pettis J (2007) Effects of Bt corn pollen on honey bees: emphasis on protocol development. Apidologie 38: 368-377

Rosie-Marshall EJ, Tank JL, Royer TV, Whiles MR, Evans-White M, Chambers C, Griffiths NA, Pokelsek J, Stephen ML (2007) Toxins in transgenic crop by-products may affect headwater stream ecosystems. PNAS 104: 16204-16208

Sanders CJ, Pell JK, Poppy GM, Raybould A, Garcia-Alonso M, Schuler TH (2007) Host-plant mediated effects of transgenic maize on the insect parasitoid *Campoletis sonorensis* (Hymenoptera : Ichneumonidae). Biological Control 40**:** 362-369

Sanvido O, Stark M, Bigler F (2006) Ecological impacts of genetically modified crops. Experiences from ten years of experimental field research and commercial cultivation. ART-Schriftenreihe 1: 1-84

Sanvido O, Widmer F, Winzeler M, Streit B, Szerencsits E, Biger F (2008) Definition and feasibility of isolation distances for transgenic maize cultivation. Transgenic Research 17: 317-355

Saxena D, Stotzky G (2001a) Bt corn has a higher lignin content than non-Bt corn. American Journal of Botany 88: 1704-1706

Saxena D, Stotzky G (2001b) *Bacillus thuringiensis* (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil. Soil Biology & Biochemistry 33:1225-1230

Schelton AM, Zhao JZ, Roush RT (2002) Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. Annual Review of Entomology 47: 845-881

Schmidt JEU, Braun CU, Whitehouse LP, Hilbeck A (2009) Effects of activated Bt transgene products (Cry1Ab, Cry3Bb) on immature stages of the ladybird *Adalia bipunctata* in laboratory ecotoxicity testing. Arch Environ Cont Toxicol 56: 221-228

Schrader S, Münchenberg T, Baumgarte S, Tebbe C (2008) Earthworms of different functional groups affect the fate of the *Bt*-toxin Cry1Ab from transgenic maize in soil. European Journal of Soil Biology 44: 283-289

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

Schuppener M, Mühlhause J, Müller A-K, Rauschen S (2012) Environmental risk assessment for the small tortoiseshell *Aglais urticae* and a stacked Bt-maize with combined resistances against Lepidoptera and Chrysomelidae in central European agrarian landscapes. Molecular ecology 21: 4646-4662

Shu Y, Ma H, Du Y, Li Z, Feng Y, Wang J (2011) The presence of *Bacillus thuringiensis* (Bt) protein in earthworms *Eisenia fetida* has no deleterious effects on their growth and reproduction. Chemosphere 85: 1648-1656

SIGMEA (2007) Sustainable Introduction of GM Crops into European Agriculture. Publishable Final Activity Report http://www.inra.fr/sigmea/final_report

SIGMEA (2009) SIGMEA WP2: conclusions relevant to GM coexistence from research on gene flow by pollen and seed. http://www.scri.ac.uk/scri/file/EPI/SIGMEA_WP2_species_summaries_May09.pdf

Sims SR, Martin JW (1997) Effect of *Bacillus thuringiensis* insecticidal proteins Cry1A(b), Cry1A(c), CryIIA, and CryIIIA on *Folsoma candida* and *Xenylla grisea* (Insecta: Collembola). Pedobiologia 41: 412-416

Skaland, N. (1993). Grønnfôrvekster. Forelesningshefte, Institutt for plantekultur, NLH.

Sleper DA, Poehlman JM (2006) Breeding filed crops. Fift Edition. Iowa State University Press Ames XV 424 pp

Statistics Norway (2011) Statistikkbanken. Avling av ymse hagebruksvekster. http://statbank.ssb.no/statistikkbanken/Default_FR.asp?PXSid=0&nvl=true&PLanguage=0&tilside=selectvarval/define.asp&Tabellid=06046

Stefani FOP, Hamelin RC (2010) Current state of genetically modified plant impact on target and non-target fungi. Environmental Reviews 18:441-475

Stotzky G (2002) Release, persistence and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis.* In: Letourneau DK, Burrows BE (Eds) Genetically Engineered Organisms: assessing Environmental and Human Health Effects. CRC Press, Boca Raton, FL, pp 187-222

Stotzky G (2004) Persistence and biological activity in soil of the insecticidal proteins from *Bacillus thuringiensis,* especially from transgenic plants. Plant and Soil 266: 77-89

Tabashnik BE, Cushing NL, Finson N, Johnson MW (1990) Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). J Econ Entomol 83: 1671-1676

Tabashnik BE, Gassmann A, Crowder DW, Carriére Y (2008) Insect resistance to *Bt* crops: evidence versus theory. Nature Biotechnology 26:199-208

Tabashnik BE, Rensburg JB, Carriére Y (2009) Field-evolved insect resistance to Bt crops: definition, theory and data. J Econ Ento 102:2011-2025

Tank JL, Rosi-Marshall EJ, Royer TV,Whiles MR, Griffiths NA, Fraukendorf TC, Treering DJ (2010) Occurrence of maize detritus and a transgenic insecticidal protein (Cry1Ab) within the stream network of an agricultural landscape. PNAS 107: 17645-17650

Tolstrup K, Andersen SB, Boelt B, Buus M, Gylling M, Holm PB, Kjellson G, Pedersen S, Østergård H, Mikkelsen SA (2003) Report from the Danish working group on the co-existence of genetically modified crops with conventional and organic crops. DIAS report Plant Production no. 94, Fredriksberg Boktryk, Denmark. 275 p.

Tolstrup K, Andersen SB, Boelt B, Gylling M, Holm PB, Kjellson G, Pedersen S, Østergård H, Mikkelsen SA (2007) Supplementary Report from the Danish Working Group on the Co-existence of Genetically Modified Crops with Conventional and Organic Crops. Update of the 2003 Report. DFJ Plant Science No 131

Torres JB, Ruberson JR (2008) Interactions of *Bacillus thuringiensis* Cry1Ac toxin in genetically engineered cotton with predatory heteropterans. Transgenic Research 17 345-354.

Treu R, Emberlin J (2000) Pollen dispersal in the crops maize (*Zea mays*), oil seed rape (*Brassica napus* ssp- *oleifera*), potatoes (*Solanum tuberosum*), sugar beet (*Beta vulgaris* ssp.*vulgaris*) and wheat (*Triticum aestivum*). Evidence from publications. A report from the Soil Assosiation, January 2000.

Turrini A, Sbrana C, Nuti MP, Pietrangeli B, Giovanetti M (2004) Development of a model system to assess the impact of genetically modified corn and aubergine plants on arbuscular

van de Wiel CCM, Lotz LAP (2006) Outcrossing and coexistence of genetically modified with (genetically) unmodified crops: a case study of the situation in the Netherlands. Netherlands Journal of Agricultural Science 54: 17-35

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

Vercesi ML, Krogh PH, Holmstrup M (2006) Can *Bacillus thuringiensis* (Bt) corn residues and Bt-corn plants affect life-history traits in the earthworm *Aporrectodea caliginosa*? Applied Soil Ecology 32: 180-187

Vettori C, Paffetti D, Saxena D, Stotzky G, Giannini R (2003) Persistence of toxins and cells of *Bacillus thuringiensis* subsp. *kurstaki* introduced in sprays to Sardinia soils. Soil Biology and Biochemistry 35: 1635-1642

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

VKM (2006) Vurdering av foreslåtte virkemidler for sameksistens mellom genmodifiserte vekster og konvensjonelt/økologisk landbruk, og rangering av spredningsrisiko av transgener fra relevante genmodifiserte planter som kan dyrkes i Norge. Uttalelse fra Faggruppe for genmodifiserte organismer 21.12.06. (06/305). Vitenskapskomiteen for mattrygghet, Oslo, Norge. http://www.vkm.no/eway/default.aspx?pid=277&trg=Main_6177&Main_6177=6187:1663908::0:6720:4:::0:0

VKM (2008a). Helse- og miljørisikovurdering av genmodifisert maislinje MON 89034 fra Monsanto (EFSA/GMO/NL/2007/37). Uttalelse fra Faggruppe for genmodifiserte organismer *9.05.08.* 07/318-endelig. Vitenskapskomiteen for mattrygghet, Oslo, Norge. http://www.vkm.no/eway/default.aspx?pid=277&trg=Content_6301&Main_6177=6301:0:31,2365:1:0:0:::0:0&Content_6301=6187:1670927::1:6318:17:::0:0&RiskList_6303=6319:0:31,2365:1:0:0:::0:0

VKM (2008b*).* Helse- og miljørisikovurdering av Monsantos genmodifiserte maishybrid MON 89034 x NK603 (EFSA/GMO/NL/2007/38). Uttalelse fra Faggruppe for genmodifiserte organismer 9.05.08. (07/319). Vitenskapskomiteen for mattrygghet, Oslo, Norge. http://www.vkm.no/dav/f25f42ecd7.pdf

VKM (2010a) Foreløpig miljørisikovurdering av genmodifisert mais MON89034 x MON88017 (EFSA/GMO/BE/2009/71). Uttalelse fra Faggruppe for genmodifiserte organismer 1.04.2010. 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=6187:1758627::0:6566:16:::0:0

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

Wan P, Huang Y, Wu H, Huang M, Cong S, Tabashnik BE, Wu K (2012) Increased frequency of pink bollworm resistance to Bt toxin Cry1Ac in China. PLOS ONE Vol 1, Issue 1

Weber WE, Bringezu T, Broer I, Eder J, Holz F (2007) Coexistence between GM and non-GM maize crops- Tested in 2004 at the field scale level. J Agron Crop Sci 193: 79-92

Yu L, Berry RE, Croft BA (1997) Effects of *Bacillus thuringiensis* toxins in transgenic cotton and potato on *Folsomia candida* (Collembola: Isotomidae) and *Oppia nitens* (Acari: Orbatidae). Journal of economic Entomology 90: 113-118

Yu H-L, Li Y-H, Wu K-M (2011) Risk assessment and ecological effects of transgenic *Bacillus thuringiensis* crops non-target organisms. Journal of Integrative Plant Biology 53: 520-538

Zeilinger A, Andow D, Zwahlen C & Stotzky G (2010) Earthworm populations in a northern U.S. Cornbelt soil are not affecyed by long-term cultivation of Bt maize expressing Cry1Ab and Cry3Bb proteins. Soil Biology and Biochemistry 42: 1284-1292

Zhang H, Yin W, Zhao J, Jin L, Yang Y, Wu S, Tabashnik BE, Wu Y (2011) Early warning of cotton bollworm resistance associated with intensive planting of Bt cotton in China. Vol 6, Issue 8

Zurbrügg C, Nentwig W (2009) Ingestion and excretion of two transgenic BT corn varieties by slugs. Transgenic Research 18: 215-225

Zurbrügg C, Hönemann L, Meissle M, Romeis J, Nentwig W (2010) Decomposition dynamics and structural plant components of genetically modified Bt maize leaves do not differ from leaves of conventional hybrids. Transgenic Research 19: 257-267

Zwahlen C, Nentwig W, Bigler F, Hilbeck A (2000) Tritrophic interactions of transgenic *Bacillus thuringiensis* corn, *Anaphothrips obscurus* (Thysanoptera : Thripidae), and the predator *Orius majusculus* (Heteroptera : Anthocoridae). Environmental Entomology 29: 846-850

Zwahlen C, Hilbeck A, Gugerli P, Nentwig W (2003a) Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field. Molecular Ecology 12: 765-775

Zwahlen C, Hilbeck A, Howald R, Nentwig W (2003b) Effects of transgenic Bt corn litter on the earthworm *Lumbricus terrestris.* Molecular Ecology 12: 1077-1086

Zwahlen C, Hilbeck A, Nentwig W (2007) Field decomposition of transgenic Bt maize residue and the impact on non-target soil invertebrates. Plant Soil 300: 245-257

Appendix 1

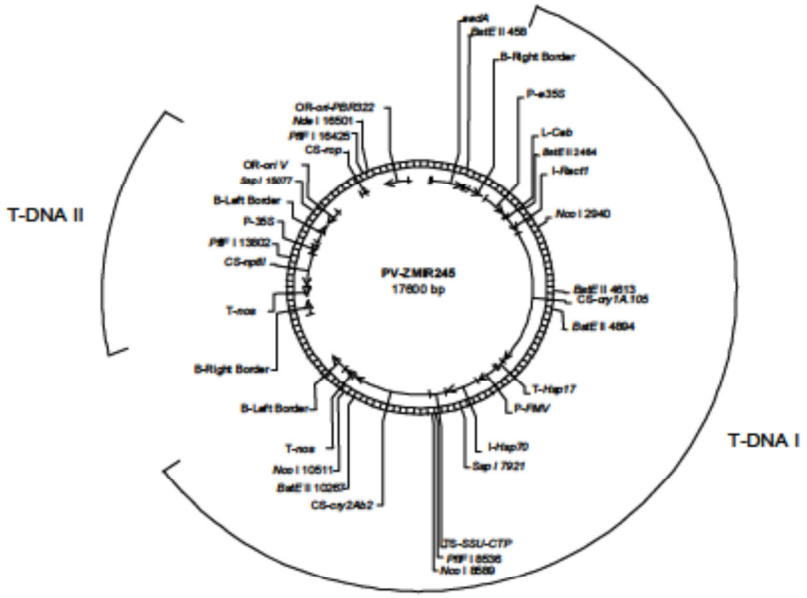

Figure 2. Plasmid map of vector PV-ZMIR245

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 1. 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 2. **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) 3.9 – 13	60 (27) 32 – 120	7.0 (1.7) 4.5 – 10	61 (15) 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 3. 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-V12)			Reproductive (VT-R2)			Harvest (R6)		
Stressors[1]	Sites[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	0.9
	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	3.3	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-V12)			Reproductive (VT-R2)			Harvest (R6)		
Stressors[1]	Sites[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-V12)			Reproductive (VT-R2)			Harvest (R6)		
Stressors[1]	Sites[2]	Test	Ctrl	Ref.	Test	Ctrl	Ref.	Test	Ctrl	Ref.	Test	Ctrl	Ref.
Abiotic													
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
	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						
	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
	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 (V12-V18)			Reproductive (VT-R2)			Harvest (R6)		
Stressors[1]	Site[2]	Test	Ctrl	Ref.	Test	Ctrl	Ref.	Test	Ctrl	Ref.	Test	Ctrl	Ref.
Diseases													
Smut (head & root)	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	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. (wireworms)	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
	5	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 (V12-V18)			Reproductive (VT-R2)			Harvest (R6)		
Stressors[1]	Site[2]	Test	Ctrl	Ref.	Test	Ctrl	Ref.	Test	Ctrl	Ref.	Test	Ctrl	Ref.
Agriotes 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	5	2.3	2.3	2.7									
Fruit fly	3	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