Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Food/feed and environmental risk assessment of insect resistant genetically modified maize MON810 for cultivation, seed production, import, processing and feed uses under Directive 2001/18/EC (Notification C/F/95/12/02)

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

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

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Summary

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

MON810 notification C/F/95/12-02 is approved under Directive 90/220/EEC for cultivation, seed production, import and processing into feeding stuffs and industrial purposes since 22 April 1998 (Commission Decision 98/294/EC). In December 1997, food and food ingredients derived from the progeny of maize line MON810 were notified under Article 5 of Regulation (EC) No 258/97 on novel foods and novel food ingredients. In addition, existing food and feed products containing, consisting of or produced from MON810 were notified according to Articles 8 and 20 of Regulation (EC) No 1829/2003 and were placed in the Community Register in 2005.

Three applications for renewal of the authorisation for continued marketing of (1) existing food and food ingredients produced from MON810; (2) feed consisting of and/or containing maize MON810, and MON810 for feed use (including cultivation); and (3) food and feed additives, and feed materials produced from maize MON810 within the framework of Regulation (EC) No 1829/2003 were submitted in 2007.

Maize MON810 has previously been assessed by the VKM GMO Panel commissioned by the Norwegian Environment Agency in connection with the national finalisation of the procedure of the notification C/F/95/12/02 (VKM 2007a,b). In addition, MON810 has been evaluated by the VKM GMO Panel as a component of several stacked GM maize events (VKM 2005a,b,c, VKM 2007c, VKM 2008, VKM 2009, VKM 2012a). Due to the publication of new scientific literature and updated guidance for food/feed and environmental risk assessment of genetically modified plants, the VKM GMO Panel has decided to deliver an updated risk assessment of MON810.

The updated risk assessment of the maize MON810 is based on information provided by the applicant in the notification C/F/95/12/02 and application EFSA/GMO/RX/MON810, and scientific comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment also considered other peer-reviewed scientific literature as relevant.

The VKM GMO Panel has evaluated MON810 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 VKM GMO panel 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), and the selection of comparators for the risk assessment of GM plants (EFSA 2011b).

The scientific risk assessment of maize MON810 includes molecular characterisation of the transformation process, vector construction, expression, inheritance and stability of the transgene

construct, comparative assessment of agronomic and phenotypic characteristics, nutritional assessments, toxicology and allergenicity, unintended effects on plant fitness, potential for gene transfer, interactions between the GM plant and target and non-target organisms and effects on biogeochemical processes.

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

The genetically modified maize MON810 was developed to provide protection against certain lepidopteran target pests, including European corn borer (*Ostrinia nubilalis*) and species belonging to the genus *Sesamia.* Protection is achieved through expression in the plant of the insecticidal Cry protein, Cry1Ab, derived from *Bacillus thuringiensis* ssp. *kurstaki*, a common soil bacterium.

Molecular characterisation

Appropriate analysis of the integration site including flanking sequences and bioinformatics analyses have been performed to analyse the construct integrated in the GM plant. Updated bioinformatics analyses revealed that one ORF shared sequence similarity to a putative HECT-ubiquitin ligase protein.

The VKM GMO Panel found no safety implications from the interruption of this gene sequence. Analyses of leaf, grains, whole plant tissue and pollen from the maize MON810 demonstrated that the Cry1Ab protein is expressed at very low levels in all tissues tested and constitute less than 0.001% of the fresh weight in each tissue. The *cry1*Ab gene is the only transgene expressed in line MON810 and is expressed the highest in leaves. The stability of the genetic modification has been demonstrated over several generations.

Event MON810 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 2007a,b).

Comparative assessment

Compositional assessments were performed using the principles and analytes outlined in the OECD consensus document for maize composition (OECD 2002). For maize MON810 grain and forage, VKM previously concluded, based on data from risk assessments and field trials as presented in notification MON810 (C/F/95/12/02) and application NK603 x MON810 (EFA/GMO/UK/2004/1), MON 863 x MON810 (EFSA/GMO/DE/2004/03), MON863xMON810xNK603 (EFSA/GMO/BE/2004/07) and MON 88017 x MON810 (EFSA/GMO/ CZ/2006/33), that maize MON810 is compositionally similar to the non-GM counterparts and conventional maize varieties, except for the new trait (VKM 2005a,b,c, 2007a,b,c).

Comparative analyses of data from field trials located at representative sites and environments in the USA and Europe indicate that maize MON810 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 Cry1Ab protein. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of MON810 compared to conventional maize. Evaluations of ecological interactions between maize MON810 and the biotic and abiotic environment indicate no unintended effects of the introduced trait on agronomic and phenotypic characteristics.

Food and feed safety assessment

Based on current knowledge, there is no reason to assume that the characteristics of processed products derived from maize MON810 would be different from processed products derived from non-GM maize. The compositional and nutritional equivalence of MON810 to conventional non-GM maize varieties is supported by several animal studies.

Acute oral toxicity tests have not indicated any toxicity related to the Cry1Ab protein from *Bacillus thuringiensis.* Cry1Ab is readily degraded in simulated gastric fluids and no adverse health effects have been reported related to maize MON810 from whole food feeding studies performed on rats, broilers, pigs or dairy cows. Some studies on Atlantic salmon have however indicated possible immunological reactions related to MON810 in fish feed. Bioinformatics analyses show no resemblance of the Cry1Ab protein to known toxins or allergens. Cry1Ab has not been shown to cause IgE mediated allergic reactions and is considered a non-allergenic by EFSA. Some studies have however indicated a potential role of Cryproteins as adjuvants in allergic reactions (VKM 2012b).

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 Cry1Ab protein on non-target arthropods that live on or in the vicinity of maize plants. Cultivation of maize MON810 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 Cry1Ab 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). Most studies conclude that effects on soil microorganisms and microbial communities are transient and minor compared to effects caused by agronomic and environmental factors. However, data are only available from short term experiments and predictions of potential long term effects are difficult to deduce.

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. 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 aquatic 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 MON810 and any change in survival (including over-wintering), persistence and invasiveness capacity. Because the general characteristics of maize MON810 are unchanged, insect resistance are not likely to provide a selective advantage outside cultivation in Norway.

Since MON810 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 MON810 will be no different to that of conventional maize varieties in Norway.

Overall conclusion

The VKM GMO Panel has not identified toxic or altered nutritional properties of maize MON810 or its processed products compared to conventional maize. Based on current knowledge, it is also unlikely that the Cry1Ab protein will increase the allergenic potential of food and feed derived from maize MON810 compared to conventional maize varieties. The VKM GMO Panel likewise concludes that cultivation of maize MON810 is unlikely to have any adverse effect on the environment and agriculture in Norway.

Keywords

Maize, *Zea mays* L., genetically modified maize MON810, C/F/9512/02, insect resistance, *Bacillus thuringiensis,* Cry proteins, Cry1Ab, cultivation, feed safety, human health, environmental risk assessment, Directive 2001/18/EC

Norsk sammendrag

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

Mais MON810 (EU-notifisering C/F/95/12-02), har vært godkjent til dyrkning, frøproduksjon, import, og til fôr og industrielle formål under direktiv 90/220/EEC siden 22. april 1998 (Kommisjonsbeslutning 98/294/EC). I desember 1997, ble næringsmidler og næringsmiddelingredienser avledet fra maislinje MON810 notifisert i henhold til artikkel 5 under EU-forordning No 258/97 («Novel Foods-forordningen»). I tillegg ble mat og fôrprodukter som inneholder, består av, eller er avledet fra MON810, notifisert som eksisterende produktert i henhold til artikkel 8 og 20 under forordning 1829/2003.

I 2007 ble det innsendt tre fornyelsessøknader for MON810 under forordning 1829/2003/EF. Søknadene gjaldt autorisasjon for videre markedsføring av (1) eksisterende næringsmidler og næringsmiddelingredisenser avledet fra MON810; (2) fôr som består av og/eller som inneholder mais MON810 og MON810 til bruk som fôr (inkludert dyrking); og (3) MON810 til bruk som næringsmiddel- og fôrtilsetting, samt fôrvarer produsert fra MON810.

Mais MON810 har tidligere vært vurdert av VKM med hensyn på helse- og miljøeffekter i forbindelse med vurdering av markedsadgang i Norge (VKM 2007a,b). I tillegg har VKMs faggruppe for GMO også risikovurdert en rekke maishybrider der MON810 inngår som en av foreldrelinjene (VKM 2005a,b,c, VKM 2007c, VKM 2008, VKM 2009, VKM 2012a). Etablering av nye, reviderte retningslinjer for helse- og miljørisikovurderinger av genmodifiserte planter og publisering av ny vitenskapelig litteratur har medført at VKM har valgt å utarbeide en ny, oppdatert helse- og miljørisikovurdering av maislinje MON810.

Denne oppdaterte risikovurdering av mais MON810 er basert på dokumentasjon fra søker i notifikasjon C/F/95/12/02 og søknaden EFSA/GMO/RX/MON810, vitenskapelige kommentarer fra EFSA og andre medlemsland gjort tilgjengelig på EFSAs GMO Extranet, men også uavhengige vitenskapelige publikasjoner.

Risikovurderingen av den genmodifiserte maislinjen er gjort i henhold til tiltenkt bruk i EU/EØS-området, og i overensstemmelse med den norske matloven, genteknologiloven med forskrifter, først og fremst forskrift om konsekvensutredning etter genteknologilovens utsettingsdirektiv 2001/18/EF og EU-forordning 1829/2003/EF om genmodifisert mat og fôr. VKMs faggruppen for GMO har også valgt å ta i legge til grunn prinsippene i EFSAs retningslinjer for risikovurdering av genmodifiserte planter og avledete næringsmidler (EFSA 2006, 2011a), miljørisikovurdering av genmodifiserte planter (EFSA 2010), og valg av komparator til risikovurderinger av genmodifiserte planter (EFSA 2011b).

Den vitenskapelige risikovurderingen av MON810 omfatter molekylær karakterisering av transformasjonsprosessen, vektorkonstruksjonen, genuttrykk, nedarving og stabilitet av transgenet, komparative analyser av agronomiske og fenotypiske egenskaper, næringsmessige vurderinger, toksikologi og allergenisitet, utilsiktede effekter, potensialet for genoverføring, fitness, effekter på målorganismer og ikke-målorganismer og biogeokjemiske prosesser.

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.

Maislinjen MON810 inneholder genet *cry1Ab* fra *B. thuringiensis* ssp. *kurstaki* HD-1. Genet koder for et δ-endotoksin som gir resistens mot enkelte skadeinsekter i ordenen Lepidoptera, eksempelvis maispyralide (*Ostrinia nubilalis*), og enkelte arter i slekten *Sesamia*.

Molekylær karakterisering
Adekvate analyser har blitt utført av både integreringssted og flankesekvenser til det innsatte genkonstruktet, inkludert bioinformatikkmetoder brukt til å beskrive konstruktet i mais MON810. Oppdaterte bioinformatikkanalyser har avdekket en åpen leseramme (ORF) med sekvenslikhet til et gen man antar koder for et HECT-ubiquitin ligase protein. VKMs faggruppe for GMO anser ikke avbrudd av dette genet å medføre noen økt risiko.

Analyser av blad, korn, hel plante og pollen fra MON810 viser at Cry1Ab er uttrykt ved veldig lave nivåer i alle deler av planten og utgjør mindre enn 0,001 % av råvekten av de ulike plantedelene. *Cry1Ab*-genet er det eneste uttrykte transgenet i MON810, og er høyest uttrykt i blad. Genmodifiseringen har blitt vist å være stabil over flere generasjoner.

VKMs faggruppe for GMO har tidligere vurdert MON810 og de fysiske, kjemiske og funksjonelle egenskapene til det uttrykte proteinet, Cry1Ab og funnet den molekylære karakteriseringen tilfredsstillende (VKM 2007a,b).

Komparative analyser
Den ernæringsmessige sammensetningen av MON810 er vurdert i henhold til OECDs konsensusdokument for mais (OECD 2002). Når det gjelder korn og fôr fra mais MON810 har VKM tidligere konkludert på grunnlag av data fra risikovurderinger og feltforsøk presentert i notifikasjon MON810 (C/F/95/12/02) og søknadene NK603 x MON810 (EFA/GMO/UK/2004/1), MON 863 x MON810 (EFSA/GMO/DE/2004/03), MON863xMON810xNK603 (EFSA/GMO/BE/2004/07) og MON 88017 x MON810 (EFSA/GMO/ CZ/2006/33), og konkluderer med at mais MON810, med unntak av de tilsiktede endringene, er lik umodifisert, og konvensjonelle referansesorter (VKM 2005a,b,c, 2007a,b,c).

Feltforsøk i USA og Europa indikerer agronomisk og fenotypisk ekvivalens mellom den transgene maislinjen MON810 og umodifisert, nær-isogen kontroll og konvensjonelle referansesorter. Det konkluderes med at det innsatte genet i MON810 ikke har medført endringer i egenskaper knyttet til vekst og utvikling hos maisplantene

Helserisiko
Det er ikke grunnlag for å anta at egenskapene til prosesserte produkter fra mais MON810 vil være forskjellige fra proseserte produkter basert på konvensjonelle maissorter. At MON810 er komposisjonelt og næringsmessig lik konvensjonell mais støttes også av flere dyrestudier, og EFSA. Akutte oral-eksponeringsstudier indikerer ingen toksisitet relatert til proteinet Cry1Ab fra *Bacillus thuringiensis.* Cry1Ab degraderes lett i simulert fordøyelsesvæske. Ingen negative helseeffekter relatert til MON810 har blitt rapportert fra fôringsstudier med hel mat utført på rotter, broilere, gris eller melkekyr. Derimot har enkelte fôringsstudier på Atlantisk laks indikert mulige immunologiske reaksjoner knyttet til MON810 i fiskefôr. Bioinformatikk-analyser viser ingen likheter mellom Cry1Ab og kjente toksiner eller allergener. Cry1Ab har ikke blitt vist å kunne utløse IgE-medierte allergiske reaksjoner, og er ikke ansett som et allergen av EFSA. Enkelte studier har derimot indikert at Cry-proteiner muligens kan fungere som adjuvans i allergiske reaksjoner (VKM 2012b).

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 Cry1Ab-proteinet 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 Cry1Ab-toksin på økosystemer i jord, mineralisering og næringsstoffomsetning eller effekter på jordsamfunn som bidrar til dette. Det finnes enkeltstudier som viser små, men signifikante effekter av 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.

Samlet konklusjon
VKMs faggruppe for GMO har ikke identifisert toksiske eller endrede ernæringsmessige egenskaper til mais MON810 eller prosesserte produkter sammenliknet med konvensjonell mais. Basert på dagens kunnskap er det også lite trolig at Cry1Ab-proteinet vil øke det allergene potensialet til mat og fôr produsert fra mais MON810 sammenliknet med konvensjonelle maissorter. Faggruppen finner det lite trolig at dyrking av maislinje MON810 vil medføre negative effekter på miljø eller landbruk i Norge.

Abbreviations and explanations

ADF	Acid detergent fibre, the fibrous component represents the least digestible fiber portion of forage or other roughage. During laboratory analysis, ADF is the residue remaining after boiling a forage sample in acid detergent solution. ADF is often used to calculate digestibility, total digestible nutrients and/or net energy.
Allele	An allele is an alternative form of a gene (one member of a pair) that is located at a specific position on a specific chromosome. These DNA codings determine distinct traits that can be passed on from parents to offspring.
ALS	Acetolactate synthase, an enzyme that catalyses the first step in the synthesis of the branched-chain amino acids, valine, leucine, and isoleucine
AMPA	Aminomethylphosphonic acid, one of the primary degradation products of glyphosate
ARMG	Antibiotic resistance marker gene
BC	Backcross. Backcross breeding in maize is extensively used to move a single trait of interest (e.g. disease resistance gene) from a donor line into the genome of a preferred or "elite" line without losing any part of the preferred lines existing genome. The plant with the gene of interest is the donor parent, while the elite line is the recurrent parent. BC_1, BC_2 etc. designates the backcross generation number.
BLAST	Basic Local Alignment Search Tool. Software that is used to compare nucleotide (BLASTn) or protein (BLASTp) sequences to sequence databases and calculate the statistical significance of matches, or to find potential translations of an unknown nucleotide sequence (BLASTx). BLAST can be used to understand functional and evolutionary relationships between sequences and help identify members of gene families.
bp	Basepair
Bt	*Bacillus thuringiensis*
CaMV	Cauliflower mosaic virus
Codex	Set by The Codex Alimentarius Commission (CAC), an intergovernmental body to implement the Joint FAO/WHO Food Standards Programme. Its principle objective is to protect the health of consumers and to facilitate the trade of food by setting international standards on foods (i.e. Codex Standards)
Cry	Any of several proteins that comprise the crystal found in spores of *Bacillus thuringiensis*. Activated by enzymes in the insect's midgut, these proteins attack the cells lining the gut, and subsequently kill the insect
Cry1Ab	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*
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
NOAEL	No observed adverse effect level
NOEL	No observed effect level
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
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* L.
ZM-HRA	A modified version of the native acetolactate synthase protein from maize. Confers tolerance to the ALS-inhibiting class of herbicides

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Background

Based on a safety assessment of the Scientific Committee on Plants (SCP 1998), MON810 notification C/F/95/12-02 (Unique Identifier MON-ØØ81Ø-6) was approved for cultivation, seed production, import and processing into animal feeding stuffs and industrial purposes under Directive 90/220/EEC April 22 1998 (Commission Decision 98/294/EC). The authorising decision under the Directive covers the seeds from inbred lines and hybrids derived from maize line MON810. In December 1997, food and food ingredients derived from the progeny of maize line MON810 were notified under Article 5 of Regulation (EC) No 258/97 on novel foods and novel food ingredients. In addition, existing food and feed products[1] containing, consisting of or produced from MON810 were notified according to Articles 8 and 20 of Regulation (EC) No 1829/2003 and were placed in the Community Register in 2005.

The authorizations expired in April 2007 and three applications for renewal of the authorisation for continued marketing of (1) existing food and food ingredients produced from MON810; (2) feed consisting of and/or containing maize MON810, and MON810 for feed use (including cultivation); and (3) food and feed additives, and feed materials produced from maize MON810 were submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on GM food and feed.

The scopes of the renewal applications cover the continued marketing of:

- Existing food and food ingredients produced from maize MON810 (Reference EFSA-GMO-RX-MON810$_{[8\text{-}1a]}$ that have been placed on the market in accordance with Article 5 of Regulation (EC) No 258/97
- Feed consisting of and/or containing maize MON810 that were authorized under Directive 90/220/EEC, including the use of seed for cultivation (Reference EFSA-GMO-RX-MON810$_{[20\text{-}1a]}$
- Food additive produced from maize MON810 that were authorized under Directive 89/107/EEC, and feed produced from maize MON810, i.e., feed additives placed on the market under Directive 70/524/EEC and feed materials (Reference EFSA-GMO-RX-MON810$_{[8\text{-}1b/20\text{-}1b]}$

The EFSA GMO Panel assessed the three renewal applications together, and published its scientific opinion in July 2009 (EFSA 2009b).

By 2012, seven Member States (MS) had submitted safeguard clauses according to Article 23 of Directive 2001/18/EC to temporarily restrict or prohibit the use and/or sale of maize MON810 within their territory. On requests from the European Commission related to the safeguard clauses invoked by France, Hungary, Austria, Germany, Italy and Greece on concerns related to health- and environmental risk assessments and post market environmental monitoring, EFSA has provided scientific replies to the questions raised by the MS (e.g. EFSA 2012a). In the scientific opinion related to the safeguard clause notified by Greece, the EFSA GMO Panel could not identify any new data subjected to scientific scrutiny or scientific information that would invalidate its previous risk assessment of maize MON810. With regard to issues related to management and monitoring of maize MON810, the EFSA GMO Panel refers to its recent recommendations for management and monitoring measures of maize MON810. In conclusion, based on the scientific evidence currently available, the EFSA GMO panel finds that cultivation of maize MON810 is unlikely to have an adverse effect on human and animal health and the environment (EFSA 2012a).

[1] Approval granted based on pre-2003 regulations. "Existing products" are GMOs that were lawfully placed on the EU market before the entry into force of Regulation 1829/2003 on GM food and feed on 18 April 2004.

National bans on cultivation of maize MON810 varieties have been implemented in several European countries including France, Germany, Hungary, Austria, Poland, Luxembourg, Greece and Italy.

Norway
Through the Agreement of the European Economic Area (EEA), Norway is obliged to implement the EU regulations on GM food and feed (regulations 1829/2003, 1830/2003 et al). Until implementation of these regulations, Norway has a national legislation concerning processed GM food and feed products that are harmonised with the EU legislation. These national regulations entered into force 15 September 2005. For genetically modified feed and some categories of genetically modified food, no requirements of authorisation were required before this date. Such products that were lawfully placed on the Norwegian marked before the GM regulations entered into force, the so-called existing products, could be sold in a transitional period of three years when specific notifications were sent to the Norwegian Food Safety Authority. Within three years after 15. September 2005, applications for authorisation should be sent to the Authority before further marketing.

Four fish feed producing companies have once a year since 2008, applied for an exemption of the authorisation requirements of 19 existing products, including maize MON810. These 19 GM events are all authorised in the EU, and the Norwegian Food Safety Authority has granted exemption for a period of one year each time.
http://mattilsynet.no/genmodifisering/dispensasjon_fra_godkjenningskrav_i_f_ocirc_rvareforskriften_73820

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

Maize MON810 has previously been assessed by the VKM GMO Panel commissioned by the Norwegian Environment Agency in connection with the national finalisation of the proceedings of the notification C/F/95/12/02 (VKM 2007a,b). In addition, maize MON810 has been evaluated by the VKM GMO Panel as a component of several stacked GM maize events under Directive 2001/18/EC and Regulation (EC) 1829/2003 (VKM 2005a,b,c, VKM 2007c, VKM 2008, VKM 2009, VKM 2012a). Due to the publication of new scientific literature and updated guidance for food/feed and environmental risk assessment of genetically modified plants, the VKM GMO Panel has decided to deliver an updated risk assessment of MON810.

Terms of reference

The Norwegian Environment Agency has the overall responsibility for processing applications for the deliberate release of genetically modified organisms (GMOs). This entails inter alia coordinating the approval process, and to make a holistic assessment and recommendation to the Ministry of the Environment regarding the final authorization process in Norway. The Directorate is responsible for assessing environmental risks on the deliberate release of GMOs, and to assess the product's impact on sustainability, benefit to society and ethics under the Gene Technology Act.

The Norwegian Food Safety Authority (NFSA) is responsible for assessing risks to human and animal health on deliberate release of GMOs pursuant to the Gene Technology Act and the Food Safety Act. In addition, the NFSA administers the legislation for processed products derived from GMO and the impact assessment on Norwegian agriculture according to sector legislation.

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

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

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

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

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

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

Assessment

1 Introduction

The genetically modified maize MON810 was developed to provide protection against certain lepidopteran insect larvae, including European corn borer (*Ostrinia nubilalis*) and species belonging to the genus *Sesamia*. None of these target pests are present in the Norwegian agriculture.

Insect protection is achieved through expression in the plant of the insecticidal Cry protein Cry1Ab, derived from *Bacillus thuringiensis* ssp. *kurstaki*, a common soil bacterium. During sporulation, *B. thuringiensis* produces cytoplasmic inclusions containing one or more of the insecticidal crystal protein or deltaendotoxin. Most crystal proteins are synthesised intracellularly as inactive protoxins that spontaneously form small crystals, approximately 1 µm in size. Upon ingestion by susceptible insects, the highly alkaline pH of the midgut promotes solubilisation of the protoxincontaining crystals. The protoxin is then activated by trypsinlike gut proteases that cleave off domains from the carboxyand aminotermini leaving a proteaseresistant core that is the active toxin. The active toxin binds to a highly specific glycoprotein receptor on the surface of midgut epithelial cells in the insect. When about eight of these core proteins aggregate together, they form a pore through the cell membrane. These cells eventually swell and burst, causing loss of gut integrity and resulting in larval death within 1 to 2 days (Cooper 1991).

MON810 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)., EFSA principles of risk assessment of GM plants and derived food and feed are described in Appendix 1.

The risk assessment of the GM maize MON810 is based on information provided by the applicant in the notification C/F/95/12/02 and application for renewal of authorisation for continued marketing of MON810 (EFSA/GMO/RX/MON810), previous risk assessments performed by the VKM GMO Panel (VKM 2005a,b,c, VKM 2007 a,b,c, VKM 2013) and scientific opinions and comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment is also based on a review and assessment of relevant peer-reviewed scientific literature.

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

2 Molecular characterisation

2.1 Transformation process and vector constructs

Maize event MON810 was generated by particle acceleration technology using plasmids PV-ZMBK07 and PV-ZMGT10. Plasmid PV-ZMBK07 contained the *CaMV35S* promoter with duplicated enhancer region (*e35S*); an intron from the maize *Hsp70* (heat-shock protein) gene; the *cry1Ab* gene encoding the nature identical Cry1Ab protein; *nos 3'* - a 3' non-translated region of the nopaline synthase gene (transcriptional termination; polyadenylation); a *lac* operon fragment (a partial *Escherichia coli lacI* coding sequence, the promoter *lac* and a partial coding sequence for β-D-galactosidase or *lacZ* protein from pUC119); *ori-pUC* (replication origin for pUC plasmids, originally derived from plasmid ColE1); and the *npt*II gene as a selectable marker.

Plasmid PV-ZMGT10 contained the e35S promoter; the *Hsp70* intron; transit peptides *CPT1* and *CPT2* (from *Arabidopsis thaliana*); the *CP4 epsps* gene (from *Agrobacterium* sp.) which allows for selection on glyphosate; and the *gox* gene (from *Ochrobactrum anthropi* sp.) which encodes a glyphosate metabolising enzyme, the *nos 3'* terminator, the *lacZ* region, *ori-pUC* and the *npt*II gene.

Table 1. Components of the inserted DNA fragment of maize MON810

Sequence	Size (Kb)	Source	Function
P-e35S	0.32	Cauliflower mosaic virus	DNA sequences derived from cauliflower mosaic virus (CaMV) containing a portion of the CaMV promoter with the duplicated enhancer region and 5´untranslated region.
Hsp70	0.81	Maize (*Zea mays* L.)	DNA sequence derived from maize containing the intron sequence from the maize *hsp* 70 gene (heat-shock) protein present to stabilize the level of transcription.
CS-Cry1Ab	2.45	*Bacillus thuringiensis* subsp. *kurstaki*	DNA sequence containing synthetic linker and a portion of the synthetic coding sequence for a variant of Cry1Ab1 protein from *Bacillus thuringiensis* subsp. *kurstaki*

2.2 Transgenic construct in the genetically modified plant

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

MON810 produces the Cry1b insecticidal protein 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*).

2.2.2 Information on the sequences actually inserted or deleted

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

The molecular characterisation of maize MON810 shows that MON810 contains a single insertion event which consists of elements derived from plasmid PV-ZMBK07, including the enhanced *35S* promoter, the maize *Hsp70* intron, and a *cry1Ab* coding sequence sufficient to encode an active insecticidal Cry1Ab protein (Table 2). Additional experiments confirmed that the MON810 insert contains a portion of the 3' end of the *e35S* promoter as well as a portion of the 5' end of the *cry1Ab* coding sequence. Data indicated that no other portion of plasmid PV-ZMBK07 DNA and no portion of plasmid PV-ZMGT10 were present in maize MON810. This included the absence of the *nptII* gene. Probes that were derived from sequences spanning the *cry1Ab* expression unit in PV-ZMBK07, the plasmid backbone sequence that encompasses both PV-ZMBK07 and PV-ZMGT10 backbone, and elements from plasmid PV-ZMGT10, show that MON810 contains part of the *e35S* promoter, the *Hsp70* intron, and part of the *cry1Ab* coding sequence, but does not contain the *nos* transcriptional sequence.

Schematic representation of the linear DNA derived from T-DNA of vector PV-ZMBK07 inserted in MON810, including restriction enzyme sites and expected restriction fragments, is shown in Figure 1. A description of the genetic elements inserted, including the approximate size and function is provided in Table 1.

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

The organisation of the elements within the insert in maize MON810 was confirmed by PCR. The insert was sequenced to further confirm the organisation of the elements within the insert. Sequence data indicate that the *e35S* promoter that regulates expression for the *cry1Ab* gene has been modified into a shorter promoter version $e35S^{MON810}$ (307 bp at the 3' end of the 620 bp promoter), that the *Hsp*70 is intact and that 2448 bp of the *cry1Ab* coding sequence (corresponding to the 5' end of the 3470 bp gene) encompassing the insecticidal active tryptic core is present. A portion from the 3' end of the *cry1Ab* gene as well the *nos* terminator have been deleted as the result of the integration process.

The PCR was performed with a forward primer specific to the genomic DNA sequence flanking the 5' end of the insert paired with a reverse primer specific to the genomic DNA sequence flanking the 3' end of the insert (Figure 1). The amplified PCR product from the conventional counterpart was subjected to DNA sequence analysis. DNA sequence analyses performed on MON810 determined the DNA sequence of the insert in MON810, confirmed the predicted organisation of the genetic elements within the insert, determined the sequences flanking the insert, and examined the MON810 insertion site.

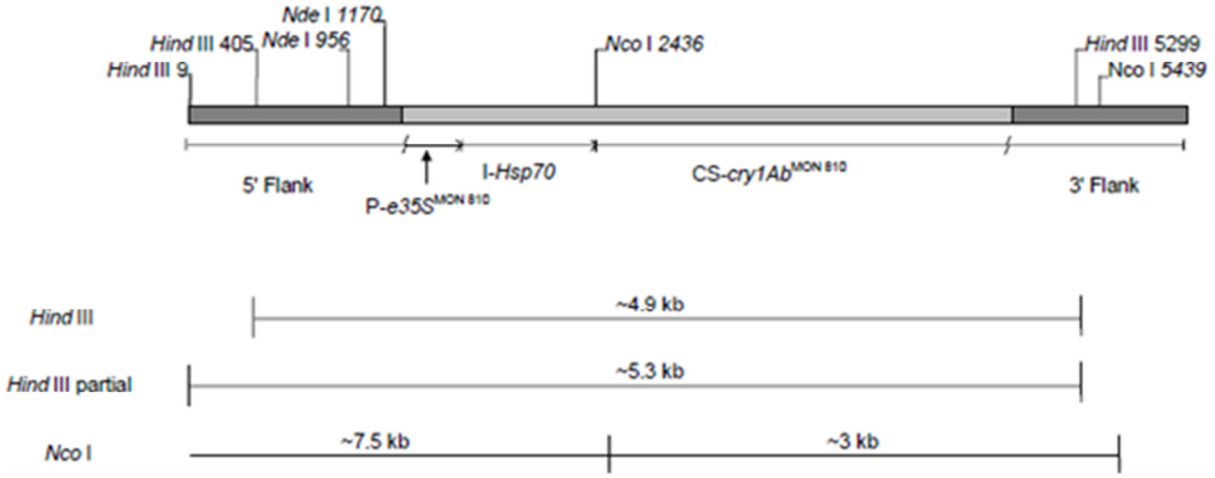

Figure 1. Schematic representation of the insert and flanking DNA in MON810.

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

Additional information submitted by the applicant confirmed the DNA sequences of the 5' and 3' DNA flanking regions originally provided. The applicant has also supplied additional sequence information. This revealed an additional 400 bp of maize DNA at the 3' flank and an additional 1000 bp of maize DNA at the 5' flank

2.2.2.4 Sub-cellular location(s) of inserts(s)

The presence of MON810 insert in the nuclear genome is best shown by the Chi square analysis of the segregation results. The Chi square analysis of the segregation pattern, according to Mendelian genetics, was consistent with a single site of insertion into maize nuclear DNA.

2.2.2.5 Sequence information for both 5´ and 3´flanking regions and bioinformatics analyses on flanking regions and ORFs

Analysis of open reading frames (ORFs) indicated no new potential chimeric proteins showing homologies with potential toxins or allergens, confirming the original bioinformatic assessment. *In silico* analysis did reveal that the 3' genomic region corresponded to a gene putatively coding for the HECT-ubiquitin ligase protein.

A publication by Rosati et al. (2008) confirmed that the 3' genomic region corresponded to a gene putatively coding for the HECT E3 ubiquitin ligase. In addition, using RT-PCR they showed that this 3' region produced cDNA variants of different length. *In silico* translation of these transcripts identified 2 and 18 putative additional amino acids in different variants, all derived from the adjacent host genomic sequences, added to the truncated Cry1Ab protein. These putative recombinant proteins did not show homology with any known protein. Results of this analysis confirm that it is unlikely that endogenous ORFs that encode protein sequences have been disrupted by the insertion of T-DNA in MON810.

2.2.3 Information on the expression of the insert

Tissue samples for analysis were collected from American and European field trials conducted in 1994 and 1995, respectively. Data from the different studies are presented in Table 1-4.

USA 1994
Tissue samples for analysis were collected from six field trials conducted in the USA in 1994. 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 MON810, as well as the conventional control MON818, was planted at each test site. The whole plant samples were collected two weeks following pollination. Young leaves were collected three times at two week intervals for estimating foliar expression levels during the growing season (overseason leaf expression).

Tissues of MON810 plants were analysed for the three proteins, Cry1Ab, CP4 EPSPS, and GOX using ELISA. The CP4 EPSPS and GOX proteins were not detected in any of the plant tissues of maize MON810. This was expected since the molecular analysis of maize MON810 established that the *cp4 epsps* and *gox* genes were not present in the nuclear genomic DNA.

The level of Cry1Ab protein ranged from 7.93-10.34 µg/g fresh weight (fw) in young leaf tissue; 3.65-4.65 µg/g fw in whole plant tissue; and 0.19-0.39 µg/g fw in harvested grain (Table 2). The foliar expression of Cry1Ab protein remained high during the vegetative growth stages of the maize plant as measured in overseason leaf samples.

Table 2. Summary of protein levels in tissues of MON810 from the field trials in USA in 1994

		Protein (µg/g fwt)		
		Cry1Ab	**CP4 EPSPS**	**GOX**
Leaf	Mean[1]	9.35	N.D.[4]	N.D.
	Range	7.93-10.34	N.A.[5]	N.A.
Whole plant[2]	Mean	4.15	N.D.	N.D.
	Range	3.65-4.65	N.A.	N.A.
Grain	Mean[1]	0.31	N.D.	N.D.
	Range	0.19-0.39	N.A	N.A
Overseason Leaf[3]	Mean 1st	9.78		
	Mean 2nd	8.43		
	Mean 3rd	4.91		

[1] The means were calculated from the analysis of one plant sample of pooled tissue from several plants per site unless noted otherwise.
[2] The mean and range were calculated from the analysis of two plants collected from a single site.
[3] The youngest leafs were collected at two week intervals during growing season from one site
[4] Not detected
[5] Not applicable

Europe 1995
Tissue samples from MON810 for analysis of protein expression were collected from five field trials conducted within the major maize growing regions of France and Italy in 1995. According to the applicant, the locations (4 in France, 1 in Italy) encompass a range of environmental conditions and insect pressure from agronomic important pests. Young leaf samples from MON810 and conventional control MON820 were collected from all sites. Forage and grain samples were collected from all sites, except in Italy, which was destroyed prematurely.

The level of Cry1Ab protein ranged from 7.59-9.39 µg/g fw in young leaf tissue; 4.21-9.23 µg/g fw in forage tissue; and 0.42-0.69 µg/g fw in harvested grain (Table 3). The 1995 analysis confirmed that CP4 EPSPS and GOX proteins were not present in plant tissues of maize MON810. With regard to Cry1Ab, the protein levels were similar for plants grown in the USA and European field trials over two consecutive generations.

Field trials were also conducted at two field sites in Italy and France in 1995 to produce leaf, forage and grain samples for expression analysis of MON810 hybrids. The five MON810 hybrids were developed through crossing of maize MON into commercial maize inbred lines. Near isogenic hybrids were used as conventional controls. Leaf samples were collected at the Italy site only, while forage and grain samples were collected at both sites. The Cry1Ab protein levels were assessed in the maize samples using a validated ELISA. The ELISAs for CP4 EPSPS and GOX protein were not performed in this study.

The level of Cry1Ab protein in progeny of MON810 ranges from 8.20-10.51 µg/g fwt in young leaf tissue, 4.00-5.11 µg/g fwt in forage tissue, and 0.35-0.60 µg/g fwt in harvested grain (Table 4). The Cry1Ab protein levels were similar for MON810 plants derived from backcrosses to B73/Mo17 and commercial hybrids.

Table 3. Summary of protein levels in tissues of MON810 from the field trials in Europe in 1995 (five field sites)

		Protein (µg/g fwt)		
		Cry1Ab	**CP4 EPSPS**	**GOX**
Leaf	Mean[1]	8.60	N.D.[4]	N.D.
Forage[2]	Range	7.59-9.39	N.A.[5]	N.A.
	Mean	4.80	N.D.	N.D.
Grain[3]	Range	4.11-5.56	N.A.	N.A.
	Mean[1]	0.53	N.D.	N.D.
	Range	0.42-0.69	N.A	N.A

[1] The means were calculated from the analysis of a single pooled sample from each site.
[2] The mean and range were calculated from the analysis of two pooled plants collected from four sites.
[3] The mean and range were calculated from the analysis of pooled ears collected from four sites.
[4] Not detected, [5] Not applicable

Table 4. Summary of Cry1Ab protein levels in tissues of progeny from MON810 grown in the 1995 European field trials (five hybrids planted at two field sites)

		Protein (µg/g fwt)
		Cry1Ab
Leaf	Mean[1]	9.26
	Range	8.20-10.51
Forage[2]	Mean	4.52
	Range	4.00-5.11
Grain[3]	Mean[1]	0.46
	Range	0.35-0.60

[1] The means were calculated from the analysis of an aliquot of pooled sample from Italy site.
[2] The mean and range were calculated from the analysis of one or two plants collected from both sites.
[3] The mean and range were calculated from the analysis of pooled grain samples collected from both sites.

Germany (2001-2003)

Nguyen & Jehle (2007) conducted a quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in maize MON810 plants (cultivar "Novelis") from two field trials in Germany. The Cry1Ab contents of various plant tissues (root, stalk, leaf, anther, pollen and kernel) were determined at four different growth stages (BBCH19, BBCH30, BBCH61 and BBCH83) collected in the growth seasons 2001, 2002 and 2003. The highest Cry1Ab levels were detected in the leaves (5.5-6.4 µg/g fw) at BBCH83, whereas the lowest Cry1Ab contents were detected in the pollen (1-97 ng/g fw) (Table 5). Cry1Ab content of residual root stocks collected in the field nine months after harvest was 15-17 ng/g fw, equivalent to about one-hundredth of the fresh root. This large-scale monitoring of Cry1Ab expression in maize MON810 showed a considerable variation in the expression levels of Cry1Ab between genotypes, plant tissues and growth stages

The expression levels of Cry1Ab in maize MON810 and several stacked GM maize events containing MON810 (MON863xMON810xNK603; MON863xMON810 and NK603xMON810), MON88017xMON810; 1507x59122xMON810xNK603) have also been reported and reviewed by the VKM GMO Panel, who concluded that the levels of expression of Cry1Ab protein do not raise safety concerns (VKM 2005 a,b,c, 2007a,b,c, 2012a,b).

Table 5. Mean Cry1Ab levels in various tissues of event maize MON810 (Cv. Novelis) sampled during two growing seasons (2001-2003) at two field sites in Germany (Nguyen & Jehle 2007)

		Cry1Ab (µg/g fresh weight) Growth stage			
Tissues	**Field site**	**BBCH19**	**BBCH30**	**BBCH61**	**BBCH83**
Root	Bonn	1.449[1] (0.098)[2] 0.471-2.389[3]	1.386 (0.112) 0.266-2.402	1,421 (10.108) 0.589-2.429	1.419 (0.087) 0.748-2.139
	Halle	1.594 (0.229) 0.279-3.947	1.683 (0.200) 0.466-4.174	1.606 (0.105) 0.608-2.690	1.583 (0.137) 0.336-0.789
Stalk	Bonn	0.404 (0.038) 0.133-1.096	0.333 (0.028) 0.078-0.621	0.988 (0.100) 0.511-2.402	1.127 (0.073) 0.354-1.910
	Halle	0.463 (0.047) 0.174-1.034	0.433 (0.046) 0.180-0.850	1.017 (0.108) 0.356-1.982	1.238 (0.127) 0.467-2.605
Lower leaf	Bonn	nd	4.373 (0.267) 1.138-7.759	2.541 (0.188) 1.265-4.750	3.946 (0.204) 2.227-5.868
	Halle	nd	4.618 (0.298) 2.553-6.976	4.205 (0.295) 2.026-7.043	5.779 (0.504) 1.359-9.603
Upper leaf	Bonn	2.451 (0.149) 0.316-4.620	3.236 (0.244) 0.699-6.591	2.718 (0.212) 1.241-4.518	5.521 (0.242) 3.589-8.597
	Halle	3.333 (0.188) 1.964-4.707	2.911 (3.11)	5.060 (0.365) 1.960-8.580	6.367 (0.436) 1.878-11.072
Anther	Bonn	-	-	2.050 (0.268) 0.485-4.658	-
	Halle	-	-	2.808 (0.294) 0.301-6.650	-
Kernel	Bonn	-	-	-	0.268 (0.023) 0.057-0.509
	Halle	-	-		0.235 (0.026) 0.008-0.461

[1] Mean, [2] SE= standard error, [3] Range (minimum and maximum value during the survey of three years)

2.2.4 Inheritance and stability of inserted DNA

The integrity of the insert originally described in 1995 and 2001 has been confirmed by a study performed by the applicant in 2007, indicating stability of the insert. According to the dossier from the applicant, stability of the insert over generations was established by Southern analyses. MON810 has been planted for two years in field trials in the USA, representing various stages in the breeding program. Leaf tissue samples from two sets of MON810 plants, representing three generations, were used to assess the stability of the inserted DNA by Southern blot analyses. This analysis demonstrates that the insertion event has been stable during the maize breeding. The continued efficiency of this line in controlling the target pests during breeding also supports the molecular stability of the inserted DNA.

Segregation data for the BC0F1 plants (derived from crossing R0 with an inbred line), BC1F1 plants (derived from crossing the BC0F1 plants to the same inbred used to cross with the R0 plant), and BC1F2 progeny (derived from crossing individual BC0F2 plants by a non-transgenic tester and analysing subsequent generation ear to row) are presented in Table 6. The results are consistent with a single, dominant gene segregating according to Mendelian genetics. The *cry1Ab* gene in maize MON810 has also been shown to be stable through seven generations of crosses to one recurrent parent (B73) and six generations of crosses to a second, unrelated inbred (Mo17) (Table 7). The Chi square tests for the backcross to B73 and to Mo17 did not deviate from expectations at $p<0.05$. The insect-protected phenotype and inheritance pattern have been consistent over multiple generations

Table 6. Segregation data and analysis of progeny of maize MON810

Generation	Actual	Expected ratio	Chi Square
BC0F1[1]	44:47	45.5:45.5	0.044^{ns}
BC1F1[2]	10:4	7:7	1.786^{ns}
BC1F2 progeny[3]	69:181:77	81.75:163.5:81.75	4.138^{ns}

[1] Data expressed as number of expressing plants: number of non-expressing plants based on European corn borer feeding assay
[2] Data expressed as number of expressing plants: number of non-expressing plants based on Cry1Ab ELISA
[3] Data expressed as number of ear rows with homozygous expressing plants: number of ear rows with segregating plants: number of ear rows with homozygous susceptible plant based on European corn borer feeding assay
[ns] Not significant at p=0.05 (chi square =3.84, 1 df; chi square = 5.99, 2 df)

Table 7. Stability of gene transfer based on segregation data for backcross derivates of MON810 in two untreated inbred lines (B73 and Mo17)

Generation	Actual	Expected	Chi Square
BC6F1 (B73)[1]	8:13	10.5:10.5	0.762^{ns}
BC5F1 (Mo17)[1]	11:11	11:11	0.045^{ns}

[1] Data expressed as number of expressing plants: number of non-expressing plants based on *B.t.k.* Cry1Ab ELISA
[ns] Not significant at p=0.05 (ch square= 3.84, 1 df)

2.3 Conclusion

Sufficient analyses of the integration site including flanking sequences and bioinformatics analyses have been performed to describe the construct integrated in the GM plant. Updated bioinformatics analyses revealed that one ORF shared sequence similarity to a putative HECT-ubiquitin ligase protein. The VKM GMO Panel found no safety implications from the interruption of this gene sequence.

Analyses of leaf, grains, whole plant tissue and pollen from the maize MON810 demonstrated that the Cry1Ab protein is expressed at very low levels in all tissues tested and constitute less than 0.001% of the fresh weight in each tissue. The *cry1*Ab gene is the only transgene expressed in event MON810 and was expressed highest in the leaves. The stability of the genetic modification has been demonstrated over several generations.

Event MON810 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 2007a,b, VKM 2013).

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. 2013). 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. 2013). 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. 2012).

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. 2013). 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

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

The original field trials with maize MON810 were performed in major maize-growing areas of the USA during the 1994 growth season (6 field sites). The non-GM maize control material was maize MON818 in all 1994 field trials and maize MON820 in the 1995 field trials. Both control materials were similar in pedigree to the tested maize MON810. Only grain material was analysed from the field trials in 1994, whereas both grain material and forage was analysed from the field trials performed in 1995. The set of compounds analysed in grain material were proximates, 18 amino acids, 9 fatty acids, carbohydrates (5 compounds or fractions), vitamins (3 tocopherols), minerals (calcium and phosphorous), and anti-nutrients (phytic acid). Forage was analysed for proximates, and neutral and acidic fibre. Leaf, forage and grain were also analysed for the expression of the Cry1Ab protein. In total 44 compounds were analysed. In addition, European field trials with MON810 and MON810 hybrids and conventional control maize were grown in France and Italy during the 1995 field season (5 locations) and France in 1995 (4 field sites).

To support the original compositional data, the applicant provided compositional data on forage and grain material collected from field trials with 3 different stacked GM maize events where maize MON810 was one of the parental GM maize lines. The studies were on MON810xMON863 grown at 4 replicated sites in Argentina in 1999, MON810xNK603 grown at 3 replicated sites in France in 2000, and MON810xMON863xNK603 (expresses Cry1Ab, Cry3Bb1, and CP4 EPSPS) grown at 4 replicated sites in Argentina during the season 2002-2003. The triple-stacked GM maize MON810xMON863xNK603 was produced from single maize events by first crossing maize MON863 with NK603, producing the double-stacked GM maize MON863xNK603, and then, after inbreeding, crossing this double-stacked GM maize with maize MON810. The field trials in Argentina 1999 compared the double-stacked GM maize MON810xMON863 with maize MON810, maize MON863 and a hybrid between non-GM maize varieties (MON846) having a comparable genetic background to that of the parental GM maize events. In the French field trials in 2000, the double-stacked GM maize MON810xNK603 was compared to maize MON810, maize NK603 and a hybrid between non-GM maize (name not specified) with comparable genetic background to that of the parental GM maize events. Finally, in the field trials in Argentina 2002-2003, the triple-stacked GM maize MON810xMON863xNK603 was compared to a non-GM maize control having a comparable genetic background (DKC46-26). Although these studies on stacked GM maize events did not statistically compare levels of key compounds in maize MON810 and in the maize hybrid created by crossing the appropriate non-GM maize controls, the raw data were available for the VKM GMO Panel to analyse and draw conclusions from. In total, there were data on 9 compositional parameters from forage and 54 from grain.

4.2 Compositional analysis

Grain materials from the field trials in the USA in 1994 were analysed for proximates (moisture, total protein, total fat, calories, carbohydrate, crude fibre and ash) and 44 specific maize constituents (amino acids, fatty acids, starch, sugars, calcium, phosphorous, tocopherols and phytic acid). For 11 of the studied compounds: 8 amino acids, crude fibre, calcium and α-tocopherol, levels were significantly higher in maize MON810 than in the control maize (MON818). However, for 8 of these compounds, concentrations in maize MON810 and its control line were within the ranges reported for maize in the literature.

Thirty-six compounds were analysed in the grains collected from the French field trials in 1995 (proximates, amino acids and fatty acids). In these trials also proximates in forage were analysed. In

this material, statistically significant differences in constituent levels between maize MON810 and its control (MON820) were observed for 5 compounds (increased grain moisture and palmitic acid content, and reduced levels of methionine and tryptophan, as well as increased crude protein in forage) which does not reflect the findings from the 1994 USA trials.

Several independent investigators have reported on the lignin levels in maize varieties expressing the Cry1Ab protein. Some claim that lignin levels are higher in maize MON810 than in an appropriate non-GM maize control (Saxena and Stotzky 2001b; Flores et al. 2005; Poerschmann et al. 2005), whereas other investigators claim that it is unchanged or reduced (Folmer et al. 2002; Jung and Sheaffer 2004; Mungai et al. 2005; Anonymous 2006). More recent literature identifies no compositional difference in lignin content between various GM maize events containing MON810 (Cry1Ab), MON863 (Cry3Bb1), and DKC60-14 (stacked Cry1Ab and Cry3Bb1), and their appropriate non-GM maize controls (Lehman et al. 2008). Furthermore, Tarkalson et al. (2008) found no difference in decomposition of lignin over time in maize genetically modified to express Cry1Ab and non-GM maize control.

According to the applicant grain of maize MON810 frequently exhibit lower mycotoxin levels than control maize grain. This observation has been supported by The EFSA GMO Panel, which based on reviewed literature concluded that the Cry1Ab expressing maize MON810 may contain lower levels of fumonisins, and possibly aflatoxin, than control maize (EFSA 2009b).

4.3 Agronomic and phenotypic characters

The VKM GMO Panel has considered available information submitted by the applicant in connection with several stacked GM maize events, as well as scientific publications and monitoring reports on MON810. No new data on agronomic and phenotypic characteristics of MON810 have been provided in the applications for renewal of authorisation for continued marketing of MON810 (EFSA-GMO-RX-MON810), apart from the monitoring reports on cultivation on MON810. In this application, the notifier refers to agronomic observations during field trials and to commercial experience since 1997.

The GMO Panel considered this set of information sufficient for the classification of the agronomic characteristics of the GM plant and conclude that the information available in the renewal applications gives no reason to change the opinion that maize MON810 is agronomically and phenotypically equivalent to currently grown non-GM varieties, with exception of the insect resistance conferred by the Cry1Ab protein. The VKM GMO Panel has already assessed the agronomic and phenotypic characteristics of maize MON810 in relation to an appropriate non-GM control (VKM 2007a,b). In addition, MON810 has been evaluated by the VKM GMO Panel as a component of several stacked GM maize events (VKM 2005a,b,c, VKM 2007c, VKM 2008, VKM 2009, VKM 2012a).

4.4 Conclusion

Part from the deliberate modifications in MON810, the VKM GMO Panel finds MON810 to be compositionally equivalent to conventionally grown maize varieties.

Comparative analyses of data from field trials located at representative sites and environments in the USA and Europe indicate that maize MON810 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 Cry1Ab protein. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of MON810 compared to conventional maize. Evaluations of ecological interactions between maize MON810 and the biotic and abiotic environment indicate no unintended effects of the introduced trait on agronomic and phenotypic characteristics.

5 Food and feed safety assessment

5.1 Product description and intended uses

The scope of the notification C/F/95/12/02 is for cultivation, seed production, import and processing and use of maize MON810 as animal feed.

Maize MON810 was developed to express a Cry1Ab protein from *Bacillus thuringiensis* subsp. kurstaki, rendering the maize protected against certain lepidopteran target pests such as the European corn border (*Ostrinia nubilalis*) and species belonging to the genus Sesamia. The genetic modification of maize MON810 is not intended to influence the nutritional properties, the processing characteristics and the overall use of maize MON810 as a crop.

5.2 Effect of processing

Food manufacturing includes many harsh processing steps, e.g. cooking, heating, high pressures, pH treatments, physical shearing, extrusion at high temperatures etc. under which the majority of both DNA and proteins are denatured, which also applies to Cry1Ab protein and the *cry1Ab gene* (Dien et al. 2002; Vijayakumar et al. 2009).

5.3 Toxicology

5.3.1 Toxicological assessment of expressed novel protein in maize MON810

Given the low expression level of Cry1Ab protein in maize MON810 and the very difficult task of isolating a sufficient quantity of purified protein from the maize plant for safety testing, Cry1Ab was produced in a recombinant *Escherichia coli* strain. As the Cry1Ab protein produced by MON810 is converted to the trypsin-resistant core protein by digestive proteases, the trypsin-resistant core protein (HD-1t), obtained through trypsinolysis of the *Escherichia coli*-produced Cry1Ab protein, was used for risk assessment. The identity of the *Escherichia coli*-expressed trypsin-resistant core protein to the trypsin-resistant core protein present in maize MON810 has been confirmed by amino acid sequencing, amino acid composition, immunoreactivity (ELISA and Western blot), molecular weight (SDS-PAGE and Coomassie blue staining), and insect bioassay. *In vivo* experience, backed by *in vitro* studies, have led to the conclusion that both the Cry1Ab protein expressed in *Bacillus thuringiensis* and the Cry1Ab protein expressed in plants are highly selective and do not target mammalian organisms (Wolfersberger, 1992; Wieczorek et al., 1999; Griffitts and Aroian, 2005; Shimada et al., 2006a,b; Stumpff et al., 2007; Bondzio et al., 2008).The VKM GMO Panel accepts the use of the trypsin-resistant core of Cry1Ab protein derived from *Escherichia coli* for the toxicity testing of the trypsin-resistant core of the Cry1Ab protein present in maize MON810.

5.3.1.1 Acute toxicity testing

The applicant has provided a single dose acute oral toxicity study in mice using the *Escherichia coli* produced protein. No signs of systemic toxicity were observed up to the highest Cry1Ab protein dose of 4000 mg/kg body weight.

5.3.1.2 Repeated dose toxicity testing

Potential toxicity after repeated dosage of the Cry1Ab protein purified from *Bacillus thuringiensis* var. *kurustaki* (strain HD-1), has been studied by Onose et al. (2008) in F344 rats with or without chemically induced gastrointestinal impairment. The protein was administered by gavage during the second and fourth week of feeding in the 28-days study. No significant changes indicative of toxicity

of the Cry1Ab protein from *Bacillus thuringiensis* were noted on any of the parameters tested, e.g. food consumption and general health, body and organ weights, histopathology, and blood chemistry.

5.3.1.3 Degradation in simulated digestive fluids

The applicant has tested the digestibility of the Cry1Ab protein produced in *Escherichia coli in vitro* with simulated gastric fluid containing pepsin. The degradation occurred rapidly (within 2 minutes) as demonstrated by Western analysis and Cry1Ab activity measurements in an insect bioassay. When simulated intestinal fluid containing a trypsin-like protease was used the Cry1Ab protein was not degraded within 19.5 hours (only time measured), also demonstrated by Western analysis.

The digestibility of Cry1Ab derived from *Bacillus thuringiensis* subsp. *kurstaki* (strain HD-1) has been studied in simulated gastric fluid and simulated intestinal fluid also by Okunuki et al. (2002). In simulated gastric fluid, the purified protein (85% pure) was degraded to undetectable levels within 60 seconds. Degradation of a heat-treated protein was quicker. The same protein extracted from maize MON810 was degraded within 120 seconds. Simulated intestinal fluid degraded Cry1Ab from the bacteria within 240 minutes, whereas only 20% of the protein extracted from maize was degraded during the same time. Preheating the proteins resulted in a dramatically increased degradability. No degradation fragments were identified.

The *in vitro* digestion experiments demonstrate that the Cry1Ab protein is rapidly degraded under simulated gastric conditions.

5.3.1.4 Degradation in the gastrointestinal tract

Several publications report on the degradability of the Cry1Ab protein, and transfer of the protein to mammalian tissue in animal feeding studies (or a feed-material in which the protein is expressed). There are even more studies on the fate of the transgenic DNA. Jennings et al. (2003) were unable to detect fragments of the *cry1Ab* gene and the Cry1Ab protein in the breast muscle of broiler chicken that had been fed a diet with 50-60% maize MON810 for 42 days. Rossi et al. (2005) were able to detect fragments of the *cry1Ab* gene in the contents of the crop and gizzard of chickens fed maize MON810 in a study on male broiler chickens, but not in any of the bird tissues. Nemeth et al. (2004) were not able to detect DNA fragments from the *cry1Ab*-gene, although they did find the high copy number maize endogenous chloroplast encoded gene *rubisco* in 5%, 15% and 53% of the muscle samples from steers, broilers, and pigs fed maize MON810, respectively. In milk samples taken from dairy cows 86% were positive for a DNA fragment of the *rubisco* gene, but contained no transgenic fragments. Using a sensitive analytical technique to quantify the Cry1Ab protein in blood of cows fed for 1 or 2 months on a diet containing 70% of its dry matter as maize MON810, Paul et al. (2008) were unable to detect the Cry1Ab protein in any of the plasma samples taken before or after end of feeding. Similarly, neither the intact *cry1Ab* gene nor its minimal functional unit was detected in blood, spleen, liver, kidney or muscle tissue from piglets fed a diet containing either 50% maize MON810 or conventional non-GM maize, for 35 days (Mazza et al. 2005). The authors did find small fragments of the *cry1Ab* transgene as well as fragments of certain maize genes in blood, liver, spleen and kidney of the piglets, but except for the transgene fragments there were no observed differences between the GM-test group and control animals. Walsh et al. (2012b) did not detect Cry1Ab protein or the *cry1Ab* gene in blood or organs of pigs fed MON810 for 110 days. The *cry1Ab* gene was detected in the stomach digests and at low frequency in the ileum, but not in the distal gastrointestinal tract, while protein fragments of Cry1Ab protein were detected at all sites in the gastrointestinal tract.

In a 20 day zebrafish feeding study, dietary DNA fragments from maize rubisco (a multi-copy plant gene) were detected in brain, muscle, liver and intestinal organs, and DNA fragments from the transgene in maize MON810 were detected in intestinal organs and liver, but not in the brain and muscle (Sissener et al. 2010). It was concluded from this study that there is no reason to suspect that transgenic sequences could be taken up more frequently than “regular” plant DNA and that the reason

why maize rubisco was detected in all investigated organs is related to the high copy number in the plant cells.

Taken together, transgenic DNA does not behave differently from non-transgenic DNA with respect to transfer to animal tissues.

5.3.2 Toxicological assessment of the whole GM food/feed

5.3.2.1 Repeated dose (90-day) oral toxicity study on rats

The applicant has provided a 90-day feeding study in Sprague-Dawley rats with grains of maize MON810 as a component in the diet. This study is available in the scientific literature (Hammond et al. 2006). Groups of 20 male and 20 female rats were fed diets containing 11% or 33% maize MON810 grain, the corresponding levels of the non-GM maize grain with a comparable genetic background, or 33 % (w/w) maize grain from 6 commercial non-GM maize reference varieties. When the dietary dose was 11% maize MON810 grain, a supplementation of the diet with 22 % of the commercial non-GM maize used by the feed-formulating company was required to bring all diets up to 33 % maize grain. No clinically relevant reactions were noted when observing the animals. The detailed examination of the animals revealed no biologically relevant differences between treatment groups regarding body weight gain, food consumption, clinical pathology parameters (haematology, blood chemistry, and urinalysis), organ weights, and gross and microscopic appearance of tissues. The only statistically significant differences observed in the haematology determinations were a slightly reduced mean corpuscular haemoglobin concentration and an increased number of platelets in female rats fed the lower dose of maize MON810. In the absence of effect at the higher dose level, and no effect in the males these effects were considered to be spurious as they were also within the literature reference and historical control ranges. In relation to serum chemistry, male rats given the diet with 33% maize MON810 showed a reduced albumin/globulin ratio, without any change in serum levels of albumin and globulin. The difference in albumin/globulin ratio in male rats given the high dose of maize MON810 was attributed to slightly lower albumin and slightly higher globulin levels.

5.3.2.2 Feeding studies on Atlantic salmon

Sagstad et al. (2007) fed post-smolt Atlantic salmon (*Salmo salar* L.) for 82 days diets containing 15% or 30% maize MON810, its near-isogenic non-GM maize variety, or a reference maize variety not genetically related to the other two maize varieties. They then evaluated what was termed stress- and immune-response biomarkers at the transcriptional and protein levels. Small changes in stress protein activity (Superoxide dismutase (SOD), and Catalase (CAT)), which were not correlated with gene expression, were noted in the liver and distal intestine. In addition, the study identified a change in white blood cell populations of fish fed high levels of the MON810 containing diet as compared to the near-isogenic non-GM maize variety. However, no difference was observed to the reference maize variety. Unfortunately the authors have not provided data on blood cell counts for the two doses separately. Although the fish performance in the study was good, the investigators interpreted their findings as a potential immune response.

The work of Sagestad et al. is included in investigations of maize MON810 as a feed ingredient in Atlantic salmon diets (Sanden et al. 2005, Sanden et al. 2006, Sagstad et al. 2007, Hemre et al. 2007, Bakke-McKellep et al. 2008, Sissener et al. 2010, Froystad-Saugen et al. 2009). Cry1Ab content in the maize MON810 fish feed was reported as 110-130 ng/g (Sanden et al 2005). A few differences have been observed between fish fed MON810 and the near-isogenic maize line, however these changes are not dose related (Sissener et al. 2011b). In Atlantic salmon fed 30% MON810 as reported by Sagestad and colleagues, significantly higher levels of blood granulocytes and lymphocytes were detected compared with salmon fed 30% of the near-isogenic maize line, but these differences were not

observed when compared to fish fed the fishmeal based reference diet. In the same study Atlantic salmon fed maize MON810 also had a reduced feed intake resulting in reduced growth compared to fish fed the isogenic maize line, but this difference was not observed when compared to fish fed the fishmeal based reference diet (Hemre et al. 2007).

In the study by Frøystad-Saugen et al. 2009, differences were observed in the glucose transport mechanism, and intestinal maltase enzyme activity in the gastrointestinal tract of Atlantic salmon fed MON810 compared to Atlantic salmon fed the near-isogenic control line. It was also found that the expression of a PCR clone showing high protein similarity to a proton-dependent high-affinity oligopeptide transporter was significantly upregulated in fish fed feed containing MON810 compared to fish fed feed with near isogenic maize.

Sissener et al. (Sissener et al. 2011a) have suggested that the effects observed in Atlantic salmon fed maize MON810 could be related to the content of the mycotoxin Deoxynivalenol (DON) in the MON810 ingredient (0.09 ppm).

A recent study on overall health and growth performance showed that Bt-maize (MON810) in diets (inclusion level 19,6%) for normal and soybean meal-sensitised Atlantic salmon resulted in lower protein and mineral digestibility as well as lipid and energy retention (Gu et al. 2013). This indicated that Atlantic salmon fed Bt-maize utilised and metabolised the feed less efficiently compared to those fed the non-GM maize diet. There was however no effects on growth performance between the Bt and non-GM maize fed salmon. It was also indicated that Bt-maize may potentiate intestinal oxidative cellular stress in fish afflicted with an intestinal hypersensitivity reaction.

A cross generational feeding study with zebrafish (*Danio rerio*) using the same Bt-maize event (MON810) and with similar inclusion levels (19%) found no adverse growth or reproductive effects on the parental or offspring generation (Sanden et al 2013). In this study the offspring generation fed the control-maize exhibited a better growth performance compared to fish fed the Bt-maize. Authors emphasised however, that parameters related to potential allergenic reactions from the Cry1Ab protein were not investigated in the study.

5.3.2.3 A short (32 days) and a longterm (100 days) feeding study on pigs

Walsh et al. (2012a,b) have performed a short- (32 days), and a long term feeding study (110 days), on pigs fed maize MON810. The kidneys of the pigs fed GM maize in the short term study, tended to be heavier than those of control pigs, however, no histopathological changes or alterations in blood biochemistry were evident (Walsh et al. 2012a). Small intestinal morphology was not different between treatments, although duodenal villi of GM maize-fed pigs tended to have fewer goblet cells/µm of villus compared with control pigs. The authors attribute this to a possible change of gut microbiota population caused by a small difference in fermentable carbohydrates.

On day 100 in the long term study, lymphocyte counts were higher ($P<0.05$) in pigs first fed MON810 followed by isogenic maize, than pigs fed either MON810 or isogenic maize, whereas erythrocyte counts were lower in pigs fed only MON810, or isogenic maize followed by MON810, than pigs fed MON810 followed by isogenic maize ($P<0.05$), (Walsh et al. 2012b). Neither the truncated Cry1Ab toxin nor the *cry1Ab* gene was detected in the organs or blood of pigs fed MON810 maize. The *cry1Ab* gene was however detected in stomach digesta and at low frequency in the ileum but not in the distal gastrointestinal tract (GIT), while the Cry1Ab toxin fragments were detected at all sites in the GIT. Results on autopsy and histopathology are unavailable.

5.4 Allergenicity

The strategies used when assessing the potential allergenic risk focuses on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation,

or to elicit allergic reactions in already sensitised individuals and whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (EFSA 2006, EFSA 2011a).

5.4.1 Assessment of allergenicity of Cry1Ab protein

The *cry1Ab* gene originates from *Bacillus thuringiensis* subsp. *kurstaki,* a soil microorganism that is not known to be allergenic. Cry1Ab protein has been subjected to bioinformatics analyses using relevant up to date databases to determine allergenic potential. The results of amino acid sequence homology searches for identical sequences of at least 8 continuous amino acids of the Cry1Ab protein expressed by maize MON810 with a sliding window of a similar size of known allergenic proteins have so far not identified any parts of the Cry1Ab protein to be identical to short stretches of known allergenic proteins. Neither have searches for overall similarity between the Cry1Ab protein and known allergens indicated any sequence identity above 35%. The Cry1Ab protein is readily degraded under acidic conditions as shown in tests simulating gastric conditions, and is therefore expected to be degraded when digested. The Cry1Ab protein is not considered to be allergenic by the EFSA GMO Panel (EFSA 2009b).

5.4.2 Assessment of allergenicity of the products derived from MON810

Allergenicity of the whole crop could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, e.g. through qualitative or quantitative modifications of the expression of endogenous proteins. However, given that no biologically relevant agronomic or compositional changes have been identified in maize MON810 or in stacked GM maize events containing MON810 (VKM 2005a,b,c, 2007a,c), with the exception of the introduced trait, no increased allergenicity is anticipated for maize MON810. Moreover, maize is not considered a common allergenic food.

There are independent reports indicating no, or very low risk, for allergenicity of maize MON810. Nakajima et al. (2007) monitored the occurrence of IgE antibodies specific to the Cry1Ab protein expressed in maize MON810 in food allergic patients of the Japanese population. IgE levels were within background levels in sera of all the 44 participating patients. When sera from maize allergic patients were tested against extracts of non-GM maize and maize MON810, similar staining patterns were found for both types of maize. Thus, no significant level of IgE antibodies specific to the Cry1Ab protein could be found in the studied food-allergic patients.

Batista et al. (2005) performed skin prick tests with extracts of maize MON810 on children with food and inhalant allergy and individuals with asthma-rhinitis. None of the individuals tested reacted differently to the MON810 and the non-transgenic maize samples. Similarly, when IgE of sera from food allergic patients were blotted to the transgenic Cry1Ab protein expressed in maize MON810, none of the tested samples contained detectable levels of IgE antibodies against the tested protein.

5.5 Adjuvanticity

According to the EFSA guidance document for risk assessment of food and feed from GM plants (EFSA 2011a), adjuvants are substances that, when co-administered with an antigen increase the immune response to the antigen and therefore might increase the allergic response. In cases when known functional aspects of the newly expressed protein or structural similarity to known strong adjuvants may indicate possible adjuvant activity, the possible role of these proteins as adjuvants should be considered. As for allergens, interactions with other constituents of the food matrix and/or

processing may alter the structure and bioavailability of an adjuvant and thus modify its biological activity.

The applicant has performed a theoretical assessment on the Cry1Ab protein and on the portion of the maize used for human food. According to the applicant Cry1Ab protein does not share any structural similarity to known strong adjuvants such as certain lectins and bacterial toxins (e.g. cholera toxin (CT) and *E. coli* heat-labile enterotoxin). The applicant has based his theoretical analysis on bioinformatics comparisons using toxin databases. According to these analyses Cry1Ab protein does not share functional aspects of the aforementioned bacterial toxins and plant lectin adjuvants.

Only two of the 10 Cry proteins that are currently used in genetically modified plants, Cry1Ab and Cry1Ac, have been studied experimentally regarding adjuvant effects. To the knowledge of the VKM GMO Panel, adjuvant effects have not been investigated for the other 8 Cry proteins used in GM plants, or for other groups of Cry proteins. Immunological mapping of the systemic and mucosal immune responses to Cry1Ac have shown that mice produce both systemic IgM and IgG and secretory IgA following intraperitonal and intragastric immunisation. In a mouse study by Vazquez et al., the adjuvant effect of Cry1Ac was found to be as strong as the effect of cholera toxin (CT) (Vazquez et al. 1999). The adjuvant effect of CT is thus a relevant basis for comparison in a risk assessment of Cry1Ac. It is uncertain whether this applies to the same extent to Cry1Ab.

Cry1Ab studies in animals have been examined in conjunction with allergic sensitisation against peanut extracts. In these experiments, IgE antibody was not induced by oral sensitisation, whereas CT provided effective stimulation of the IgE response. However, Cry1Ab had a significant effect on the production/release of leukotrienes C4 and E4, and influx of eosinophils, indicating that an immune response had been triggered (Guimaraes et al. 2008).

"Bystander sensitisation"

"Bystander sensitisation" can occur when an adjuvant in food, or an immune response against a food antigen, results in an increased permeability of the intestinal epithelium for other components in food. Previously it was assumed that the epithelial cells of the intestine were permanently "glued together" by the so-called "tight junctions". More recent knowledge shows that these complex protein structures are dynamic and can be opened up by different stimuli.

Both *in vitro* and *in vivo* experiments have demonstrated that when an IgG response which can result in a complement activation (among other) is not balanced by an IgA response, the epithelial barrier can be opened and unwanted proteins are able to enter the body (bystander-penetration) and lead to allergic sensitisation (Brandtzaeg P, Tolo K 1977; Lim PL, Rowley D1982).

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

5.6 Nutritional assessment

In the EFSA guidance document (EFSA 2011a) it is stated that:
The applicant should provide a nutritional evaluation to demonstrate that the introduction of food and feed derived from a GM plant into the market is not nutritionally disadvantageous to humans and animals, respectively. This evaluation should include an assessment of: (i) the nutritional relevance of newly expressed proteins and other new constituents; (ii) the changes in the levels of endogenous constituents in the GM plant and derived food and feed; (iii) the potential alterations in the total diet for the consumers/animals.

For maize MON810 grain and forage, the VKM GMO Panel concluded in 2007 (VKM 2007a), based on data from field trials as presented in application MON810, that maize MON810 is compositionally, phenotypically and agronomically not different from the non-GM counterparts and conventional maize varieties, except for the new trait (VKM 2007a).

Broiler chicken feeding studies
A 42-day broiler feeding study was provided by the applicant to evaluate the nutritional performance of maize MON810. The study included a group of broilers fed diets with maize MON810, broiler chickens fed diets with grains of a non-GM with a comparable genetic background to maize MON810, the stacked event MON810xGA21 (expressing the Cry1Ab and an EPSPS proteins) and its control, and 4 commercial maize varieties. Each treatment consisted of 100 broilers kept in 10 pens with 5 birds of each sex. The broilers were fed a starter diet (days 1-20) and grower/finisher diet (days 20-42) containing approximately 50% and 60% w/w maize, respectively, for all treatments (Taylor et al. 2003a). According to the data provided by the applicant, no biologically relevant differences were measured between broilers fed MON810 diets and the control diets. This conclusion is supported by EFSA who concluded that maize MON810 is as wholesome as conventional maize (EFSA 2009b).

Rossi et al. (2005) came to a similar conclusion in their 42-day feeding performance study mentioned in section 5.3.1.4., where male broiler chickens fed diets containing 55-60% GM or no-GM maize. MON810 showed no difference in influence on live weight, average daily weight gain, feed intake, or feed conversion compared to the non-GM maize control.

Performance of dairy cows
MON810 equivalency as feed to conventional maize on the performance of dairy cows have also been shown by independent research groups, as previously reported by EFSA (EFSA 2009b). None of these studies (Donkin et al. 2003; Sung et al. 2006), reported relevant differences related to GM-maize feed on feed intake, milk production, milk composition, ruminal fermentation and digestibility, or ruminal degradability of maize, compared to control maize.

Longterm effects on Atlantic salmon
Sanden et al. (2005, 2006) who investigated long-term effects of feeding plant products to Atlantic salmon, concluded that the inclusion of maize MON810 at a level of about 12% of the salmonid diets poses little, or no, health risks to Salmon parr and promotes normal growth. In a similar experiment, Bakke-McKellep et al. (2008) studied a large number of histological, digestive, metabolic, and immunological parameters, and concluded that the Salmon parr fed maize MON810 did not respond differently to the diet compared to fish fed non-GM maize of a comparable genetic background.

5.7 Conclusion

Food manufacturing includes many harsh processing steps, under which the majority of both DNA and proteins are denatured and there is no reason to assume that the characteristics of the processed products derived from maize MON810 would be different from that of processed non-GM maize products.

Neither a single dose (4000 mg/kg bw) acute oral toxicity study in mice or repeated dosage in rats have indicated toxicity of the Cry1Ab protein from *Bacillus thuringiensis.* Cry1Ab is readily degraded in simulated gastric fluids and no adverse health effects have been reported related to maize MON810 from whole food feeding studies performed on rats, broilers, pigs, and dairy cows. Some studies on Atlantic salmon have however indicated possible immunological reactions related to MON810 in fish feed. Bioinformatics analyses show no resemblance of the Cry1Ab-protein to known toxins or allergens. Cry1Ab has not been shown to cause IgE mediated allergic reactions and is considered a non-allergenic by EFSA. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergic reactions (VKM 2012b).

The VKM GMO Panel has not identified any concerns regarding nutritional aspects of maize MON810 or its processed products, or the properties of the Cry1Ab-protein with regard to toxicity. Based on current knowledge the VKM GMO Panel concludes that it is unlikely that Cry proteins will increase the allergenic potential of food/feed derived from MON810 compared to conventional maize varieties or by themselves cause adverse effects (VKM 2012b.

6 Environmental risk assessment

6.1 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 (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 MON810 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 MON810. Moreover, it is considered very unlikely that maize MON810 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 MON810 have been conducted by the applicant at nine replicated field locations within major maize-growing areas of the US 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 MON810 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 MON810 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 MON810 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 MON810 and its conventional counterpart (VKM 2007b).

The VKM GMO Panel is not aware of any scientific reports indicative of increased establishment or spread of maize MON810, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of maize MON810 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 MON810 will not differ from that of conventional maize varieties.

6.2 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 MON810. This means that micro-organisms in the digestive tract in humans and animals (both domesticated animals and other animals feeding on fresh or decaying plant material from the transgenic maize line) may be exposed to transgenic DNA.

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

different production systems. The risk of pollen flow from maize volunteers is negligible under Norwegian growing conditions.

6.2.1 Plant to micro-organisms gene transfer

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

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 MON810 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.
In conclusion, the VKM GMO Panel considers it unlikely for the introduced gene in maize MON810 to transfer and integrate with the genome of microorganisms in the environment or in the intestinal tract of humans or animals. In the rare, but theoretically possible event of transfer of the *cry1Ab* gene from MON810 to soil bacteria, no novel property would be introduced into, nor expressed by the soil microbial communities as sequence-similar genes are already present in other bacteria in soil. Therefore, no positive selective advantage that would not have been conferred by natural gene transfer between bacteria is expected.

6.2.2 Plant to plant gene flow

6.2.2.1 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 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 2007d). The water content also affects the physical shape of the pollen grain and its flight dynamics (Aylor 2002; Aylor et al. 2003).

6.2.2.2 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 (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).

6.2.2.3 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 MON810, 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 MON810, 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 MON810 is negligible.

6.2.2.4 National proposals for 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 2007d). 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.

6.3 Interactions between the GM plant and target organisms

MON810 was developed to provide protection against a variety of target pests of the order Lepidoptera. Protection is achieved through expression in the plant of insecticidal Cry protein Cry1Ab, derived from *Bacillus thuringiensis*, a common soil bacterium. Cry1Ab, encoded by the *cry1Ab* gene, is derived from *B. thuringiensis* subspecies *kurstaki.*Two Lepidoptera pests are primarily targeted by MON810; *Ostrinia nubilalis* (European corn borer, ECB) and *Sesamia nonagrioides* (Mediterranean corn borer, MCB).

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

Aphids are the only pests reported on maize in Norway. Studies have shown that aphids are not affected by the Cry1Ab protein (Bourguet et al. 2002). Under the development of Bt maize expressing Cry1Ab, the noctuid *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.

6.3.1 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 Cry1Ab protein 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.

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 MON810 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 MON810 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.

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

In agro-ecosystems, non-target organisms (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).

6.4.1 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. However, 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).

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.

6.4.2 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 MON810 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 MON810 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 non-target 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 affect the 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 was 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 and 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. 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 conducted 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.). The authors 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 (Cry1Ab) and non-Bt maize (de la Poza et al. 2005). 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 (event 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 throughout 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 families of arthropod predators and parasitoids that are likely to occur in Norwegian maize fields are represented 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.

6.4.3 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 MON810 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 & 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, and 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.

6.4.4 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 in well-managed agricultural soils populations of these microarthropods are generally abundant. 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.

6.4.5 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 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.

6.4.6 Effects on non-target organisms that are not arthropods

Maize MON810 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.

According to reviews of Icoz & Stotzky (2008) and the BEETLE Report (2009) studies to date have found no or 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 MON810 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% of the 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 MON810, 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 MON89034 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.

6.4.7 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 MON810 are toxic to a wide range of Lepidoptera, it is likely that most of the endangered species would be affected when feeding on MON810 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 MON810 is not considered to represent a threat to the prevalence of these endangered species in Norway.

6.4.8 Conclusion

Based on a review of available scientific literature the VKM GMO Panel concludes that the likelihood of adverse effects of Cry1Ab protein from cultivation of GM maize on non-target organisms in Norway and the EU/EEA area is low (The BEETLE Report 2009; Dively 2005; Krogh et al. 2007b; Yu et al. 2011).

6.5 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 MON810. Bt crops, such as maize MON810, 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. This is 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 Cry1Ab in lepidopteran target pest populations.

6.6 Effects on biogeochemical processes

6.6.1 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.

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 decomposition 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 lignin content than the conventional counterpart, and decomposed less in soil compared to non-Bt maize. Another study with different maize lines expressing Cry1Ab (MON810, 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-isogenic 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-isogenic lines 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.

6.6.2 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 rhizosphere organisms (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 deduce. Based on available literature, The BEETLE Report (2009) concluded that the likelihood of

adverse effects of Bt maize in the 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 MON810, MON 88017 (*cry3Bb1*) and the stacked event MON 88017 x MON810, 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. 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.

6.7 Conclusion

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 Cry1Ab protein on non-target arthropods that live on or in the vicinity of maize plants. Cultivation of maize MON810 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 Cry1Ab 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). Most studies conclude that effects on soil microorganisms and microbial communities are transient and minor compared to effects caused by agronomic and environmental factors. However, data are only available from short term experiments and predictions of potential long term effects are difficult to deduce.

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. 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 aquatic 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 MON810 and any change in survival (including over-wintering), persistence and invasiveness capacity. Because the general characteristics of maize MON810 are unchanged, insect resistance are not likely to provide a selective advantage outside cultivation in Norway.

Since MON810 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 MON810 will be no different to that of conventional maize varieties in Norway

7 Data gaps

- **Adjuvanticity**

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

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

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

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

- Insufficient knowledge of soil organisms present in Norway in environments where GM maize could be grown.
- Effects of Cry proteins on rhizosphere organisms.
- Effects of Bt toxins on aquatic organisms.

8 Conclusions

Molecular characterisation
Appropriate analysis of the integration site including flanking sequences and bioinformatics analyses have been performed to analyse the construct integrated in the GM plant. Updated bioinformatics analyses revealed that one ORF shared sequence similarity to a putative HECT-ubiquitin ligase protein. The VKM GMO Panel found no safety implications from the interruption of this gene sequence. Analyses of leaf, grains, whole plant tissue and pollen from the maize MON810 demonstrated that the Cry1Ab protein is expressed at very low levels in all tissues tested and constitutes less than 0.001% of the fresh weight in each tissue. The *cry1*Ab gene is the only gene expressed in event MON810 and was expressed highest in the leaves. The stability of the genetic modification has been demonstrated over several generations.

Event MON810 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 2007a,b).

Comparative assessment
Comparative analyses of data from field trials located at representative sites and environments in the USA and Europe indicate that maize MON810 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 Cry1Ab protein. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of MON810 compared to conventional maize. Evaluations of ecological interactions between maize MON810 and the biotic and abiotic environment indicate no unintended effects of the introduced trait on agronomic and phenotypic characteristics.

Food and feed safety assessment
Food manufacturing includes many harsh processing steps, under which the majority of both DNA and proteins are denatured; there is no reason to assume that the characteristics of the processed products derived from maize MON810 would be different from that of processed non-GM maize products.
Neither a single dose (4000 mg/kg bw) acute oral toxicity study in mice or repeated dosage in rats have indicated toxicity of the Cry1Ab protein from *Bacillus thuringiensis.*

The Cry1Ab protein is readily degraded under simulated digestive conditions and no adverse health effects have been reported related to maize MON810 from whole food feeding studies performed on rats, broilers, pigs, and dairy cows. Some studies on Atlantic salmon have however indicated possible immunological reactions related to MON810 in fish feed. The Cry1Ab-protein does not resemble known allergens or toxins, has not been shown to elicit IgE-mediated allergenic reactions, and is considered a non-allergenic by EFSA. Morover, maize is not considered a common allergenic food. Some studies have however indicated a potential role of Cry-proteins as adjuvants in allergenic reactions (VKM 2012b).

The VKM GMO Panel has not identified any concerns regarding nutritional aspects of maize MON810 or its processed products, or the properties of the Cry1Ab-protein with regard to toxicity. Based on current knowledge the VKM GMO Panel concludes that it is unlikely that Cry proteins will increase the allergenic potential of food/feed derived from MON810 compared to conventional maize varieties or by themselves cause adverse effects (VKM 2012b.

Environmental
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 Cry1Ab protein on non-target arthropods that live on or in the vicinity of maize plants. Cultivation of maize MON810 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 Cry1Ab 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). Most studies conclude that effects on soil microorganisms and microbial communities are transient and minor compared to effects caused by agronomic and environmental factors. However, data are only available from short term experiments and predictions of potential long term effects are difficult to deduce

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. 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 aquatic 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 MON810 and any change in survival (including over-wintering), persistence and invasiveness capacity. Because the general characteristics of maize MON810 are unchanged, insect resistance are not likely to provide a selective advantage outside cultivation in Norway.

Since MON810 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 MON810 will be no different to that of conventional maize varieties in Norway.

Overall conclusion
The VKM GMO Panel has not identified toxic or altered nutritional properties of maize MON810 or its processed products compared to conventional maize. Based on current knowledge, it is also unlikely that the Cry1Ab protein will increase the allergenic potential of food and feed derived from maize MON810 compared to conventional maize varieties. The VKM GMO Panel likewise concludes that cultivation of maize MON810 is unlikely to have any adverse effect on the environment and agriculture in Norway.

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Appendix 1

EFSA principles of risk assessment of GM plants and derived food and feed

The comparative approach

The risk assessment starts with the comprehensive molecular characterisation of the GM plant in question, followed by the comparative analysis of the relevant characteristics of the GM plant and its comparator(s). In particular, the comparative compositional, phenotypic and agronomic assessment requires the simultaneous application of two complementary tests: the test of difference and the test of equivalence (EFSA 2010b). The test of difference is used to verify whether the GM plant, apart from the introduced genetic modification(s), is different from its comparator(s) and has therefore the potential to cause adverse effects. The test of equivalence is used to verify whether the agronomic, phenotypic and compositional characteristics of the GM plant fall within the range of natural variation. The range of natural variation is estimated from a set of non-GM reference varieties with a history of safe use (EFSA 2011b). The outcome of this comparative analysis will further structure the risk assessment.

Objectives of the steps of the risk assessment

Hazard identification

Hazard identification is the identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food and feed or group of foods and feeds (Codex Alimentarius 2007). Hazard identification is the first step in the risk assessment and focuses on the identification of differences and/or lack of equivalences between the GM plant and its comparator, taking into account natural variation, through the comparative analyses of compositional, agronomic and phenotypic characteristics. Identification of differences and/or lack of equivalences will determine the additional studies required to assess the possible impact on human and animal health.

Exposure assessment

The aim of the exposure assessment is the quantitative estimation of the likely exposure of humans and animals to the food and feed derived from GM plants (e.g. exposure to food, feed, pollen, and new plant constituents). With regard to humans and animals, an exposure assessment characterises the nature and size of the populations exposed to the food and feed derived from GM plants, and the magnitude, frequency and duration of such exposure.

Risk characterisation

Risk characterisation is defined as the qualitative and/or quantitative estimation, including associated uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterisation and exposure assessment (Codex Alimentarius 2007). The risk characterisation should demonstrate whether the hazard identification and subsequent characterisation is complete or not. Integration and evaluation of data from hazard characterisation and exposure assessment allow evaluating whether an appropriate risk characterisation may be finalised, or whether further data is needed to complete the risk characterisation. For instance if an increased intake of food and feed derived from GM plants by humans or animals is expected, further data on toxicity at extended dose ranges may be needed.