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Risk assessment of insect-resistant and herbicide-tolerant genetically modified maize 1507 for cultivation, import, processing, food and feed uses under Directive 2001/18/EC and Regulation (EC) No 1829/2003

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

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2017: 21
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Assessed and approved

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The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed a working group consisting of VKM members to answer the requests from the Norwegian Environment Agency and the Norwegian Food Safety Authority. Project leaders from the VKM secretariat have been Nana Asare and Merethe Aasmo Finne.

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Competence of VKM experts

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

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Summary

The Norwegian Environment Agency (NEA) and the Norwegian Food Safety Authority (NFSA) requested the Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) for an opinion of potential risks to biodiversity and agriculture in Norway associated with import of seeds for sowing, and cultivation of insect-resistant and herbicide-tolerant genetically modified maize 1507 under Directive 2001/18/EC (Notification C/ES/01/01). The notification is still pending for authorisation in the European Union. VKM is also requested to assess the applicant's post-market environmental monitoring plan, and the management measures suggested in the draft implementing decision of the European Commission.

As VKM delivered a scientific opinion on this application including cultivation in 2014 (VKM, 2014), VKM is asked to assess whether the previous risk assessment is still valid concerning cultivation, and to update the opinion after current knowledge. The assessment shall specifically consider Norwegian conditions. Furthermore, as the notification does not cover food and feed uses of maize 1507, VKM was not asked for a health risk assessment of maize 1507. However, VKM has decided to update the previous safety evaluation of the food and feed uses of maize 1507 and derived products from 2014.

VKM appointed a working group consisting of members from the Panel on Genetically Modified Organisms, the Panel on Alien Organisms and trade in Endangered Species (CITES) and the VKM staff to answer the requests. The Panel on Genetically Modified Organisms has assessed and approved the final report.

The genetically modified maize 1507 was developed to provide protection against certain lepidopteran target pests, such as the European corn borer (ECB, *Ostrinia nubilalis*), and some species belonging to the genus *Sesamia*. The insect resistance is achieved by the expression of a synthetic version of the truncated *cry1F* gene derived from *Bacillus thuringiensis* subsp. *aizawai*, a common soil bacterium.

Maize 1507 also expresses the *phosphinothricin-N-acetyltransferase* (*pat*) gene, derived from the soil bacterium *Streptomyces viridochromogenes*. PAT protein confers tolerance to the herbicidal active substance glufosinate-ammonium. The PAT protein expressed in maize 1507 was used as a selectable marker to facilitate the selection process of transformed plant cells and is not intended for weed management purposes. Since the scope of the notification C/ES/01/01 does not cover the use of glufosinate-ammonium-containing herbicides on maize 1507, potential effects due to the use of such herbicides on maize 1507 are not considered by VKM.

In delivering its scientific opinion, VKM considered relevant peer-reviewed scientific publications and information provided by the applicant in the notifications C/ES/01/01,

C/NL/00/10, the applications EFSA/GMO/NL/2004/02 and EFSA/GMO/RX/1507, and scientific opinions and comments from EFSA and other EU member states.

VKM has evaluated maize 1507 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.

VKM has also decided to take into account, the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA, 2011a), the environmental risk assessment of GM plants (EFSA, 2010a), selection of comparators for the risk assessment of GM plants (EFSA, 2011b) and for the post-market environmental monitoring of GM plants (EFSA, 2011c).

The scientific opinion of maize 1507 include molecular characterisation of the inserted DNA and expression of novel proteins, comparative assessment of agronomic and phenotypic characteristics, nutritional assessments, toxicology and allergenicity. An evaluation of unintended effects on plant fitness, potential for gene transfer, interactions between the GM plant and target and non-target organisms, effects on biogeochemical processes, the post-market environmental monitoring plan and coexistence measures at the farm level has also been undertaken.

It is emphasised that VKM's 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 VKM.

Molecular characterisation

Appropriate analyses of the transgenic DNA insert, its integration site, number of inserts and flanking sequences in the maize genome, have been performed. The results show that one copy only of the insert is present in maize 1507. Homology searches with databases of known toxins and allergens have not indicated any potential production of harmful proteins or polypeptides caused by the genetic modification in maize 1507. Southern blot analyses and segregation studies show that the introduced genes *cry1F* and *pat* are stably inherited and expressed over several generations along with the phenotypic characteristics of maize 1507. VKM considers the molecular characterisation of maize 1507 satisfactory.

Comparative assessment

Comparative analyses of maize 1507 to its non-GM conventional counterpart have been performed during multiple field trials located at representative sites and environments in Chile (1998/99), USA (1999) and in Europe (1999, 2000 and 2002). With the exception of

small intermittent variations, no biologically significant differences were found between maize 1507 and the conventional maize.

Based on the assessment of available data, VKM concludes that maize 1507 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, except for the introduced characteristics, and that its composition fell within the normal ranges of variation observed among non-GM varieties. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of 1507 compared to conventional maize.

Food and feed safety assessment

Whole food feeding studies on rats, broilers, pullets, pigs and cattle have not indicated any adverse health effects of maize 1507. These studies further indicate that maize 1507 is nutritionally equivalent to conventional maize. The PAT and Cry1F proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE mediated allergic reactions. Some studies have however, indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, VKM concludes that maize 1507 is nutritionally equivalent to conventional maize varieties. It is unlikely that the PAT and Cry1F proteins will introduce a toxic or allergenic potential in food or feed based on maize 1507 compared to conventional maize.

Environmental risk assessment

Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivated maize 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. Since maize 1507 has no altered agronomic and phenotypic characteristics, except for the specific target insect resistance and herbicide tolerance, the likelihood of unintended environmental effects as a consequence of spread of genes from maize 1507 is considered to be extremely low.

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.

There are a limited number of published scientific studies on environmental effects of Cry1F protein. Published scientific studies show that the likelihood of negative effects of Cry1F protein on non-target arthropods that live on or in the vicinity of maize plants is low.

In Norway, the maize cultivation is marginal. The total crop area of forage maize is estimated to 2000-2800 decares, equivalent to less than 0.1 % of the areas with cereal crops. The area of individual fields is limited by the topography such that the quantity of maize pollen produced under flowering is also limited. The potential exposure of Cry1F-containing maize pollen on non-target lepidopteran species in Norway is therefore negligible. Cultivation of maize 1507 is not considered to represent a threat to the prevalence of red-listed species in Norway.

Exposure of non-target organisms to Cry proteins in aquatic ecosystems is likely to be very low, and potential exposure of Cry proteins to non-target organisms in aquatic ecosystems in Norway is considered to be negligible.

VKM concludes that, although the data on the fate of the Cry1F protein and its potential interactions in soil are limited, the relevant scientific publications analysing the Cry1F protein, together with the relatively broad knowledge about the environmental fate of other Cry1 proteins, do not indicate significant direct effects on the soil environment. Despite limited number of studies, 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.

Coexistence

VKM concludes that separation distances of 200 meters most likely will ensure coexistence between genetically modified maize and conventional and organic maize varieties in Norway.

Overall conclusion

Based on current knowledge, VKM concludes that maize 1507 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1F and PAT proteins will introduce a toxic or allergenic potential in food or feed derived from maize 1507. VKM likewise concludes that cultivation of maize 1507 is unlikely to have any adverse effect on the environment or agriculture in Norway.

Keywords: Maize, *Zea mays* L., genetically modified maize 1507, EFSA/GMO/NL/2004/02, C/NL/00/10, C/ES/01/01, EFSA/GMO/RX/1507, insect-resistance, herbicide-tolerance, Cry1F, PAT, glufosinate ammonium, cultivation, food/feed risk assessment, environmental risk assessment, Directive 2001/18, Regulation (EC) No 1829/2003

Sammendrag på norsk

Miljødirektoratet og Mattilsynet har bedt Vitenskapskomiteen for mattrygghet (VKM) om en vitenskapelig vurdering av risiko for negative konsekvenser for biologisk mangfold og norsk landbruk ved import og omsetning av såvare og dyrking av den insektresistente og herbicidtolerante maislinje 1507. Maislinjen er søkt godkjent til dyrking under direktiv 2001/18/EF (Notifisering C/ES/01/01), men søknaden er fortsatt under vurdering for godkjenning i EU. VKM er også bedt om å vurdere søkeres miljøovervåkingsplan, og om tiltakene som er beskrevet i vedlegget til EU-kommisjonens utkast til implementeringsbeslutning ivaretar hensynet til norsk natur og landbruk.

VKM leverte en risikovurdering av maislinje 1507 for alle bruksområder i 2014 (VKM, 2014). Miljødirektoratet og Mattilsynet har derfor bedt VKM om en oppdatering av denne risikovurderingen for bruksområdet dyrking. Det skal legges særlig vekt på særnorske forhold. Notifiseringen C/ES/01/01 omfatter ikke bruk av mais 1507 som mat og fôr, og VKM er ikke bedt om å vurdere disse bruksområdene. VKM har imidlertid besluttet å oppdatere den tidligere helserisikovurderingen av mais fra 2014.

VKM nedsatte en prosjektgruppe bestående av medlemmer fra faggruppen for genmodifiserte organismer, faggruppen for fremmede organismer og handel med truede arter (CITES), og ansatte i VKMs sekretariat for å besvare oppdragene. Faggruppen for genmodifiserte organismer har gjennomgått utkastet fra prosjektgruppen og godkjent den endelige rapporten.

Maislinjen 1507 har fått innsatt et *cry1F*-gen fra jordbakterien *Bacillus thuringiensis* var. *aizawai* og et *pat*-gen, som er isolert fra jordbakterien *Streptomyces viridochromogenes*. *Cry1F*-genet koder for et δ -endotoksin som gir resistens mot enkelte arter i sommerfuglordenen Lepidoptera, eksempelvis maispyralide (*Ostrinia nubilalis*) og enkelte arter i slekten *Sesamia*. *Pat*-genet koder for enzymet fosfinotricin acetyltransferase (PAT), som acetylerer og inaktiverer glufosinat-ammonium (fosfinotricin), virkestoffet i fosfinotricin-herbicerider av typen Finale. PAT-proteinet er benyttet som markør for seleksjon av transformerte planteceller under utviklingen av maislinjen. Bruksområdet for søknaden omfatter ikke sprøyting med dette herbicidet. Potensielle helse- og miljøeffekter ved bruk av glufosinat-ammonium er derfor ikke vurdert av VKM.

Risikovurdering av mais 1507 er basert på fagfellevurderte, vitenskapelige publikasjoner, informasjon fra søker i notifikasjonene og søknadene C/EC/01/01, C/NL/00/10, EFSA/GMO/NL/2004/02 og EFSA/GMO/RX/1507, samt vitenskapelige vurderinger og kommentarer fra EFSA og andre EU-medlemsland.

Vurderingen er gjort i henhold til tiltenkt bruk i EU/EØS-området, og i overensstemmelse med miljøkravene i genteknologiloven med forskrifter, først og fremst forskrift om konsekvensutredning etter genteknologiloven. Videre er kravene i EU-forordning 1829/2003/EF, utsetningsdirektiv 2001/18/EF (vedlegg 2,3 og 3B) og veiledende notat til

Annex II (2002/623/EF), samt prinsippene i EFSA's retningslinjer for risikovurdering av genmodifiserte planter og avledete næringsmidler (EFSA, 2010a; 2011a,b,c) lagt til grunn for vurderingen.

Den vitenskapelige vurderingen omfatter transformeringsprosessen og vektorkonstruksjonen, karakterisering og nedarving av genkonstruksjonen, komparativ analyse av ernæringsmessig kvalitet, mineraler, kritiske toksiner, metabolitter, antinæringsstoffer, allergener og nye proteiner. Videre er agronomiske egenskaper, potensiale for utilsiktede effekter på fitness, genoverføring, effekter på målorganismer og ikke-målorganismer og biogeokjemiske prosesser vurdert. VKM har også vurdert søkers miljøovervåkingsplan og tiltak for å sikre sameksistens fram til og med høsting av avlingen.

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

Molekylær karakterisering

Adekvate analyser av det transgene DNA-innskuddet, dets integreringssete, antall integreringer og flankerende DNA-sekvenser i mais-genomet, har blitt utført. Resultatene viser at ett transgent innskudd, altså en genkopi, er til stede i mais 1507. Homologisøk i databaser over kjente toksiner og allergener indikerer at genmodifiseringen ikke har ført til potensiell produksjon av skadelige proteiner eller polypeptider i mais 1507. Southern hybridiserings- og segresjonsanalyser viser at de introduserte genene *cry1F* og *pat* er stabilt uttrykt og nedarvet over flere generasjoner, og i samsvar med de fenotypiske egenskapene til mais 1507. VKMs faggruppe for genmodifiserte organismer vurderer den molekylære karakteriseringen av mais 1507 som tilfredsstillende.

Komparative analyser

Komparative analyser av mais 1507 og tilhørende umodifisert kontroll («konvensjonell motpart») er basert på feltforsøk i representative områder for maisdyrking i Chile (1998/99), USA (1999) og Europa (1990, 200, 2002). Med unntak av enkelte små variasjoner viste studiene ingen biologisk relevante forskjeller mellom mais 1507 og dens konvensjonelle motpart. Basert på vurdering av tilgjengelig data, konkluderer VKMs faggruppe for GMO at mais 1507 er ernæringsmessig, morfologisk og agronomiske vesentlig lik dens konvensjonelle motpart, med unntak av de introduserte egenskapene. Variasjonsområdene for de undersøkte parameterne ligger innenfor det normale variasjonsområdet til konvensjonelle maissorter.

Helserisiko

Føringsstudier utført på rotter, broiler, høns, gris og storfe har ikke indikert helseskadelige effekter av mais 1507. Disse studiene indikerer også at mais 1507 er ernæringsmessig vesentlig lik konvensjonell mais. Proteinene PAT og Cry1F viser ingen likhetstrekk til andre kjente toksiner eller allergener, og er heller ikke rapporterte å ha forårsaket IgE-medierte allergiske reaksjoner. Enkelte studier har derimot indikert at noen typer Cry-proteiner kan

forsterke andre allergiske reaksjoner, dvs. fungere som adjuvans. Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at mais 1507 er ernæringsmessig vesentlig lik konvensjonell mais, og at det er lite trolig at proteinene PAT og Cry1F vil introdusere et toksisk eller allergent potensiale i mat eller fôr basert på mais 1507 sammenliknet med konvensjonelle maissorter.

Miljørisiko

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 sammenliknet 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.

Målorganismene for den genmodifiserte maislinjen 1507 er ikke rapportert som skadegjørere i mais 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 at sannsynligheten for negative effekter av Cry1F-proteinet på ikke-målartropoder som lever på eller i nærheten av maisplanter er lav.

Maisdyrkingen i Norge er marginal. Det totale dyrkingsarealet av fôrmais er estimert til 2000-2800 dekar, tilsvarende under 0,1% av det totale kornarealet. Arealet av enkeltfelt er dessuten begrenset av topografiske forhold og mengden av maispollen som produseres under blomstring er begrenset. Den potensielle eksponeringen av maispollen med Cry1F-protein på ikke-målorganismer av lepidoptera i Norge er derfor ubetydelig.

Det vurderes ikke å være risiko for rødlistede arter ved dyrking av maislinjen 1507 i Norge

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

Det er publisert få studier som har undersøkt mulige effekter av Cry1F-protein på økosystemer i jord, mineralisering og næringsstoffomsetning eller effekter på jordsamfunn som bidrar til dette. De fleste studiene konkluderer med at effektene av Cry1F-proteinet er små og forbigående sammenliknet med effekter av dyrkingsmessige og miljømessige forhold. Tilgjengelige data er imidlertid basert på kortvarige studier, og mulige langsiktige effekter er derfor vanskelig å predikere.

Selv om datagrunnlaget er begrenset, indikerer relevante vitenskapelige studier av Cry1F-proteinet og kunnskapen om andre Cry-proteiners skjebne i jord, at Cry1F ikke har direkte effekter på jordmiljøet.

Sameksistens

VKM konkluderer med at dyrkingsavstander på 200 meter mest sannsynlig vil sikre sameksistens mellom genmodifisert mais og konvensjonelle og økologiske maissorter i Norge

Samlet konklusjon

Ut i fra dagens kunnskap konkluderer VKM med at maislinje 1507 er ernæringsmessig ekvivalent med konvensjonell mais. Det er videre lite trolig at Cry1F og PAT vil introdusere et toksisk eller allergent potensiale i mat eller fôr basert på mais 1507.

VKM finner det lite trolig at dyrking av maislinje 1507 vil medføre negative effekter på miljø eller landbruk i Norge.

Abbreviations and glossary

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.
Body condition scoring	Body condition scoring (BCS) serves as a useful, easy-to-use management tool to determine the nutritional needs of a cow herd.
bp	Basepair
Bt	<i>Bacillus thuringiensis</i>
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).
Concentrate	Feeds that contain a high density of nutrients, usually low in crude fibre content (less than 18% of dry matter (DM)) and high in total digestible nutrients.
Cry	Any of several proteins that comprise the crystal found in spores of <i>Bacillus thuringiensis</i> . Activated by enzymes in the insect’s midgut, these proteins attack the cells lining the gut, and subsequently kill the insect.
Cry1F	Cry1 class crystal protein from <i>Bacillus thuringiensis</i> var. <i>aizawai</i> . Provide protection against certain lepidopteran target pests.
CTP	Chloroplast transit peptide
DAP	Days after planting
DNA	Deoxyribonucleic acid
DT50	Time to 50% dissipation of a protein in soil
DT90	Time to 90% dissipation of a protein in soil
dw	Dry weight
dwt	Dry weight tissue
EC	European Commission

ECB	European corn borer, <i>Ostrinia nubilalis</i>
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
ERA	Environmental risk assessment
<i>E-score</i>	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
GLP	Good Laboratory Practice
Glufosinate-ammonium	Broad-spectrum systemic herbicide
GM	Genetically Modified
GMO	Genetically Modified Organism
GMP	Genetically Modified Plant
H	Hybrid
ha	Hectare
Heifer	A young cow over one year old that has not produces a calf
ILSI	International Life Sciences Institute
IPM	Integrated Pest Management
IRM	Insect Resistance Management
Locus	The position/area that a given gene occupies on a chromosome
LOD	Limit of detection
LOQ	Limit of quantification
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization-Time Of Flight. A mass spectrometry method used for detection and characterisation of biomolecules, such as proteins, peptides, oligosaccharides and oligonucleotides, with molecular masses between 400 and 350,000 Da.
MCB	Mediterranean corn borer, <i>Sesamia nonagrioides</i>
mRNA	Messenger RNA
MT	Norwegian Food Safety Authority (Mattilsynet)
NDF	Neutral detergent fibre, measure of fibre used for animal feed analysis. NDF measures most of the structural components in plant cells (i.e. lignin, hemicellulose and cellulose), but not pectin.

Northern blot hybridisation	Northern blot is a technique used to study gene expression by detection of RNA or mRNA separated in a gel according to size.
NTO	Non-target organism
Nicosulfuron	Herbicide for maize that inhibits the activity of acetolactate synthase
Near-isogenic lines	Term used in genetics/plant breeding, and defined genetic lines that are identical except for differences at a few specific locations or genetic loci.
OECD	Organisation for Economic Co-operation and Development
ORF	Open Reading Frame, in molecular genetics defined as a reading frame that can code for amino acids between two stop codons (without stop codons).
OSL	Over season leaf
OSR	Over season root
OSWP	Over season whole plant
<i>pat</i>	<i>Phosphinothricin-Acetyl-Transferase</i> gene
PAT	Phosphinothricin-Acetyl-Transferase protein
PCR	Polymerase chain reaction, a technique to amplify DNA by copying it
PMI	Phosphomannose Isomerase enzyme. Metabolizes mannose and allows positive selection for recovery of transformed plants.
R0	First transformed generation, parent
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 hybridisation	Method used for transfer of electrophoresis-separated DNA fragments to a filter membrane and possible subsequent fragment detection by probe hybridisation
T-DNA	Transfer DNA, the transferred DNA of the tumour-inducing (Ti) plasmid of some species of bacteria such as <i>Agrobacterium tumefaciens</i> and <i>A. rhizogenes</i> , into plant's nuclear genome. The T-DNA is bordered by 25-base-pair repeats on each end. Transfer is initiated at the left border and terminated at the right border and requires the <i>vir</i> genes of the Ti plasmid.
TI	Trait integrated
TMDI	Theoretical Maximum Daily Intake
U.S. EPA	United States Environmental Protection Agency.
Maize growth stages	<i>Vegetative</i>
	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
	<i>Reproductive</i>
	R0: Anthesis or male flowering. Pollen shed begins
	R1: Silks are visible
	R2: Blister stage. The grains are filled with a clear nourishing endosperm fluid and the embryo can be seen
	R3: Milk stage. The grain endosperm is milky white.
	R4: Dough stage. The grain endosperm has developed to a white paste
	R5: Dent stage. If the genotype is a dent type, the grains are dented
	R6: Physiological maturity
Western blot	Technique used to transfer proteins separated by gel electrophoresis by 3- D structure or denatured proteins by the length of the polypeptide to a membrane, where they might be identified by antibody labelling.
WHO	World Health Organisation
ZM	<i>Zea mays</i> L.
ZM-HRA	A modified version of the native acetolactate synthase protein from maize. Confers tolerance to the ALS-inhibiting class of herbicides

Background

2001-2005

In 2001, the notification C/ES/01/01 covering the placing on the market of seeds of varieties derived from maize 1507 for cultivation was submitted by Pioneer Hi-Bred International/Mycogen Seeds to the competent authority of Spain. The scope of the notification did not cover the commercial use of the product as a plant tolerant to glufosinate-ammonium herbicides in the EU. The PAT protein produced by maize 1507 was used as a selectable marker to facilitate the selection process of transformed plant cells and is not intended for weed management purposes.

On 13 February 2003, the European Commission received the full notification and an assessment report from Spain. On 27 May 2004, the notification was transmitted to the competent authorities of the other Member States for a 60-days public hearing. EFSA issued a scientific opinion on the notification for the placing on the market of maize 1507 for feed uses, import, processing and cultivation under Part C of Directive 2001/18/EC 19 January 2005 (EFSA 2005). In its 2005 opinion, the EFSA GMO Panel recommended that management measures be put in place to delay the possible evolution of resistance to the Cry1F protein in target Lepidoptera. The EFSA GMO Panel was also of the opinion that such measures would reduce the exposure of non-target Lepidoptera to maize 1507 pollen. Based on the evaluation of the environmental risk assessment, EFSA concluded that the cultivation of maize 1507 would not pose a significant risk to the environment.

2006-2008

In both 2006 and 2008, the European Commission successively requested the EFSA GMO Panel to consider whether new scientific evidence published in the scientific literature required a revision of the conclusions of its 2005 scientific opinion on maize 1507 (EFSA 2005). Following these requests, the EFSA GMO Panel evaluated the available new scientific information, and found no new evidence for adverse effects caused by cultivation of maize 1507 (EFSA 2006b, 2008).

2010-2011

In the course of the evaluation of three applications for renewal of authorisation of a similar insect resistant maize (event MON810), the EFSA GMO Panel used a new risk assessment methodology (Perry et al. 2010), in order to simulate and assess potential adverse effects on non-target Lepidoptera after ingestion of Cry1Ab-containing maize pollen deposited on their host-plants.

On 14 June 2010, the European Commission therefore requested the EFSA GMO Panel to consider whether new scientific elements might require a revision of the conclusions of its previous scientific opinion on maize 1507. EFSA confirmed that, considering recent studies and advances in methodology, there was a need to further analyse the potential adverse effects of maize 1507 pollen on non-target Lepidoptera, as well as to clarify its

recommendations to risk managers. On 16 December 2010, EFSA endorsed a self-task mandate of the EFSA GMO Panel to review its previous safety assessment of maize 1507 in the light of recent advances in methodology and knowledge.

In the scientific opinion published 18 November 2011 (EFSA 2011d), the EFSA GMO Panel recalibrated its mathematical model in order to simulate and assess potential adverse effects resulting from the exposure of non-target Lepidoptera (butterflies and moths) to pollen from maize 1507 under representative EU cultivation conditions, and extended it to estimate the efficacy of certain mitigation measures. The EFSA GMO Panel concludes that the cultivation of maize 1507 could have the following adverse effects on the environment in the context of its intended uses (1) the adoption of altered pest control practices with higher environmental load due to potential evolution of resistance to the Cry1F protein in populations of exposed lepidopteran target pests, and (2) reductions in populations of certain highly sensitive non-target lepidopteran species where high proportions of their populations are exposed over successive years to high levels of maize 1507 pollen deposited on their host-plants. In situations where highly sensitive non-target Lepidoptera populations might be at risk, the EFSA GMO Panel recommends that mitigation measures are adopted to reduce exposure.

In addition to the specific concern on non-target Lepidoptera, the EFSA GMO Panel considered the possible adverse effects of maize 1507 on other non-target organisms, in order to update, where appropriate, its previous evaluations in light of new relevant scientific literature. Having considered available relevant scientific literature, the EFSA GMO Panel concludes that no new scientific information has been made available that would invalidate the conclusions of its previous Scientific Opinions on maize 1507

2012

In 2012, the EFSA GMO Panel was asked by the European Commission to apply its mathematical model to simulate and assess potential adverse effects resulting from the exposure of non-target Lepidoptera to maize 1507 pollen under hypothetical agricultural conditions, and to provide information on the factors affecting the insect resistance management plan, additional to that in its 2011 Scientific Opinion updating the conclusions of the environmental risk assessment and risk management recommendations on maize 1507. Here, risk managers are provided with additional evidence and further clarifications to those previous conclusions and risk management recommendations. This Scientific Opinion provides background scientific information to inform the decision-making processes; the EFSA GMO Panel reiterates that risk managers should choose risk mitigation and management measures that are proportionate to the level of identified risk according to the protection goals pertaining to their regions.

The European Commission requested EFSA to provide additional evidence and to further clarify certain elements of the 2011 EFSA GMO Panel Scientific Opinion updating the evaluation of the environmental risk assessment and risk management recommendations on GM maize 1507 (EFSA 2011d). In particular, the EC requested EFSA to answer the following four questions by applying the mathematical model proposed by Perry et al. (2011, 2012) to

additional agricultural hypothetical conditions: (1) To calculate the local mortality of non-target Lepidoptera where there are no field margins; (2) To consider the influence of non-Bt-refugia spatial arrangements on the local mortality of nontarget Lepidoptera; (3) To calculate the local mortality of non-target Lepidoptera with increasing distances from the nearest maize 1507 field and where there are no field margins; (4) To consider the influence of local and regional conditions on insect resistance management plans (EFSA 2012a).

On June 2012, the EFSA GMO Panel was requested by the European Commission to deliver a new scientific opinion updating the risk assessment and/or management of maize 1507 in the light of new relevant scientific publications published from 2005 onwards. The EFSA GMO Panel performed a search of the scientific literature to identify new scientific publications specific to maize 1507 that may report new information relevant for the risk assessment and/or management of maize 1507. Subsequently, the EFSA GMO Panel evaluated whether the information reported in recent publications, identified by the literature search, would invalidate its previous risk assessment conclusions on maize 1507, as well as its previous recommendations on risk mitigation measures and monitoring.

Following a search of the scientific literature published between 2005 and September 2012, the EFSA GMO Panel identified 61 peer-reviewed publications containing evidence specific to the risk assessment and/or management of maize 1507, of which 25 publications were discussed and cited in previous EFSA GMO Panel scientific outputs. From the remaining 36 publications, two were relevant for the food and feed safety assessment of maize 1507, and 34 for the environmental risk assessment and/or risk management of maize 1507. EFSA did not identify new peer-reviewed scientific publications reporting new information that would invalidate its previous conclusions on the safety of maize 1507. Therefore, the EFSA GMO Panel considers that its previous risk assessment conclusions on maize 1507, as well as its previous recommendations for risk mitigation measures and monitoring, remain valid and applicable (EFSA 2012b).

2015-2017

Following new information reported by Hofmann et al. (2014) concerning maize pollen deposition in relation to distance from the pollen source, the EFSA GMO Panel assessed the consequences for its previous risk assessment conclusions and risk management for Bt-maize (EFSA, 2015). In particular, isolation distances to protected habitats were reviewed. For NT lepidopteran larvae of conservation concern potentially occurring in protected habitats, isolation distances of 30 m were recommended between protected habitats and the nearest fields of maize 1507.

Following a request from the EU-COM, EFSA assessed the relevance of two further scientific publications presenting new data on pollen deposition and potential exposure of butterflies in protected habitats by Bt maize cultivation (EFSA, 2016). EFSA concluded that neither Lang et al. (2015) nor Hofmann et al. (2016) provide data indicating the necessity to revise the previous ERA conclusions and risk management recommendations for Bt maize made in EFSA (2015). EFSA considers that the previous risk assessment conclusions and risk

management recommendations on maize MON810, Bt11 and 1507 for cultivation remain valid and applicable. In September 2016, EFSA issued a technical report that addresses the findings of theosinte and its impact on risk management recommendations in connection with the cultivation of MON810, Bt11 and 1507 (EFSA, 2016).

In 2016, EU-COM presented a proposal for approval of the application. The application was voted on at the committee meeting on 27 January 2017, but did not achieve qualified majority. On 27 March 2017, the Appeal Committee voted on the draft implementing acts concerning the authorisation for the cultivation of GM maize crops 1507 (and Bt11, and the re-authorisation of GM maize MON810). However, no qualified majority was achieved.

Terms of reference as provided by the Norwegian Environment Agency and the Norwegian Food safety Authority

The European Commission has proposed in their draft decision to grant consent to the application to market seeds of genetically modified maize 1507 for cultivation (notification C/ES/01/01 under Directive 2001/18/EC). The draft decision has been up for a vote in the Regulatory Committee of competent authorities under Directive 2001/18/EC, and subsequently an Appeal Committee. Both committees delivered no opinion. The Commission will therefore make the final decision. In preparation for the potential approval of the application, the Norwegian Environment Agency has initiated the process of final assessment of the application in Norway under the Gene Technology Act.

The Norwegian Environment Agency

With reference to the letter of assignment for 2017, The Norwegian Environment Agency requests the Norwegian Scientific Committee for Food Safety (VKM) to prepare a final environmental risk assessment of application C/ES/01/01 regarding approval of seeds of genetically modified maize 1507 for cultivation. Maize 1507 is genetically modified with resistance to certain insects of the order Lepidoptera and to herbicides containing the active ingredient glufosinate-ammonium.

As VKM delivered a scientific opinion on this application including cultivation in 2014, the Agency asks VKM to assess whether the previous risk assessment is still valid concerning cultivation, and to update it after current knowledge. The assessment shall specifically consider Norwegian conditions. The risk assessment shall be in line with the conditions set out in the Gene Technology Act, and shall identify eventual adverse effects on the Norwegian environment. EFSA's risk assessment of the application may be used as justification for the conclusions of the environmental risk assessment, however, conditions specific to Norway, must be addressed and evaluated in the assessment. VKM is also requested to assess the applicant's post-market environmental monitoring plan and if this is sufficient to capture identified and potentially un-identified adverse effects of the product. VKM shall evaluate if the control measures suggested in the draft decision of the Commission, amongst others but not limited to, the given isolation distances between cultivated area and protected habitats with potential populations of non-target Lepidoptera, is consistent with the protection level needed for species and nature in Norway.

The Norwegian Food Safety Authority

The Norwegian Food Safety Authority requests the Norwegian Scientific Committee for Food Safety to undertake environmental risk assessments for the cultivation applications C/F/96.05.10 and C/ES/01/01, regarding cultivation of GM maize, in accordance with our current order.

The EU Commission has made drafts for the implementing decisions concerning the placing on the market of genetically modified maize 1507 for cultivation. The EU-drafts have enclosed annexes, which include conditions or restrictions on the placing on the market, use or handling of the products, among other management measures. These management measures, in addition to the measures for securing co-existence, may consequently effect the cultivation system of these GM plants in Norway, as well as a potential regulation of such cultivation. The Norwegian Food Safety Authority requests the Norwegian Scientific Committee for Food Safety to assess whether the management measures described in the annexes of the EU-drafts will require adjustments to your previous recommended co-existence measures, and in that case, which adjustments that should be.

Assessment

1 Introduction

The genetically modified maize 1507 was developed to provide protection against certain lepidopteran target pests, such as the European corn borer (ECB, *Ostrinia nubilalis*), and some species belonging to the genus *Sesamia*. The insect resistance is achieved through expression of a synthetic version of the truncated *cry1F* gene derived from *Bacillus thuringiensis* subsp. *aizawai*, a common soil bacterium.

The general mode of action of Cry proteins is to bind selectively to specific receptors on the epithelial surface of the midgut of susceptible lepidopteran species, lethal to larvae through pore formation, cell burst and subsequently septicemia (OECD 2007; Raymond et al. 2009).

Maize 1507 also expresses the *phosphinothricin-N-acetyltransferase (pat)* gene, from the soil bacterium *Streptomyces viridochromogenes*. The encoded PAT protein confers tolerance to the herbicidal active substance glufosinate-ammonium. PAT produced by maize 1507 was used as a selectable marker to facilitate the selection process of transformed plant cells and is not intended for weed management purposes. Since the scope of the notification C/ES/01/01 does not cover the use of glufosinate-ammonium-containing herbicides on maize 1507, potential effects due to the use of such herbicides on maize 1507 are not considered by VKM.

Maize 1507 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.

VKM also considers the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2006a, 2011a), the environmental risk assessment of GM plants (EFSA, 2010a), the selection of comparators for the risk assessment of GM plants (EFSA, 2011b) and for the post-market environmental monitoring of GM plants (EFSA, 2011c).

The risk assessment of the GM maize 1507 is based on information provided by the applicant in the following notifications and applications:

- Application EFSA/GMO/NL/2004/02 for placing on the market of insect-tolerant genetically modified maize 1507 for food use under Regulation (EC) 1829/2003 Food

and food ingredients containing, consisting of or produced from maize 1507 approved since 3 March 2006 (Commission Decision 2006/197/EC)

- Notification C/NL/00/10 for import and processing use under Part C of Directive 2001/18/EC. Approved for importation, processing and feed use since 3 November 2005 (Commission Decision 2005/772/EC)
- Application EFSA/GMO/RX/1507 for renewal of authorisation of existing products of maize 1507 under Regulation (EC) no 1829/2003 Renewing of the authorisation of existing feed products from maize 1507 granted since 17 June 2011 (Commission Decision 2011/365/EC).
- Notification C/ES/01/01 for cultivation, import, processing and use as any other maize (excluding food uses) under Directive 2001/18/EC on the deliberate release of GMOs into the environment.

The risk assessment is also based on relevant peer-reviewed scientific publications, scientific opinions and comments from EFSA and other EU-member states and a risk analysis report of 1507 from the [Food Standards Australia New Zealand](#) (FSANZ, 2002).

It is emphasised 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 Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act and Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms. These considerations are therefore not part of the risk assessment provided by VKM.

2 Literature search

2.1 Search strategy

Literature searches were performed to retrieve publications addressing putative environmental and health effects of genetically modified maize 1507. Even though no systematic review of the literature is carried out in this scientific opinion, VKM adhered to some general principles for performing systematic review, in order to ensure methodological rigour and coherence in the retrieval and selection of publications, transparency, and reproducibility of the performed literature search (EFSA, 2010 c).

Literature searches were performed from January 2013 to April 2017 using the scientific databases ISI Web of Science, Medline, EMBASE, Scopus, Agris and CAB Abstracts. Each database was searched individually. Searches in ISI Web of Science, Medline, Embase and Scopus were conducted by a librarian at the Norwegian Institute of Public Health (NIPH). Searches in CAB Abstracts and Agris were conducted by librarians at the Norwegian University of Life Sciences. Additionally, PubMed searches were conducted by the VKM staff.

The literature was searched and screened in a stepwise manner. As a first step, a combination of generic keywords being both trait- and event-specific was used to retrieve all references for further consideration (TOPIC FIELD: maize AND 1507 OR TC1507 OR Cry*1F) The search by keywords using the topic field, enabled to retrieve publications that contain these keywords, either in the publications title, list of keywords, or abstract.

The following search terms were employed:

1. Gene flow and coexistence

(Organisms, Genetically Modified/ or Plants, Genetically Modified/) and Zea Mays/
((gmo or (Genetically adj1 (Modified or engineered)) or Transgenic or bt) adj2 (zea or corn or maize or teosinte)).tw.
(("Bt crop?" or "Bt GM crop?") and (zea or corn or maize or teosinte)).tw.
or/1-3
Gene Flow/
("gene flow" or "pollen dispersal" or "cross pollination?" or crosspollination? or "out crossing" or outcrossing or "co existence" or coexistence).tw.
5 or 6
4 and 7

limit 8 to yr="2013 -Current"

limit 9 to english language

2. Potential effects of non-target organisms

(Organisms, Genetically Modified/ or Plants, Genetically Modified/) and Zea Mays/

((gmo or (Genetically adj1 (Modified or engineered)) or Transgenic or "1507") adj2 (zea or corn or maize or teosinte)).tw.

("TC1507" or "cry1f").tw.

((("Bt crops" or "Bt GM crops") and (zea or corn or maize or teosinte)).tw.

or/1-4

Soil Microbiology/

(predator? or parasitoid? or lepidoptera or "pollinating insect?" or pollinator? or "pollen feeder?" or "natural enemy"? or "beneficial insect" or "soil arthropod?" or "aquatic arthropod?" or earthworm? or "enchytraeid worm?" or nematod* or isopod? or collembolan? or diplopod? or "non target organism?" or "nontarget organism?" or biodiversity or "soil microorganism?" or "soil microbiology" or "soil microbial" or rhizosphere).tw.

6 or 7

5 and 8

limit 9 to english language

3. Potential effects of target organisms

(Organisms, Genetically Modified/ or Plants, Genetically Modified/) and Zea Mays/

((gmo or (Genetically adj1 (Modified or engineered)) or Transgenic or "1507" or bt) adj2 (zea or corn or maize or teosinte)).tw.

("TC1507" or "cry1f") tw.

((("Bt crops" or "Bt GM crops") and (zea or corn or maize or teosinte)).tw.

or/1-4

(resistance or susceptibility or sensitivity or crossresistance or tolerance).tw.

Lepidoptera/

(sesamia? or ostrinia? or "european corn borer?" or lepidoptera?).tw.

7 or 8

5 and 6 and 9

limit 10 to english language

4. General search – Food and Feed

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("zea"[All Fields] AND "mays"[All Fields]) OR "zea mays"[All Fields] OR "maize"[All Fields] AND 1507[All Fields]

GM[All Fields] AND ("zea mays"[MeSH Terms] OR ("zea"[All Fields] AND "mays"[All Fields]) OR "zea mays"[All Fields] OR "maize"[All Fields]) AND 1507[All Fields]

GM[All Fields] AND ("zea mays"[MeSH Terms] OR ("zea"[All Fields] AND "mays"[All Fields]) OR "zea mays"[All Fields] OR "maize"[All Fields]) AND 1507[All Fields] AND cry1F[All Fields] AND ("Polym Adv Technol"[Journal] OR "pat"[All Fields])

2.2 Relevance screening

The titles of all hits were scanned, and for those that were of potential relevance, the abstracts were also inspected. The relevance screening was performed by the members of the project group.

Citations were excluded if they did not relate to the terms of reference. Publications related to detection, quantification, labelling, traceability and socio-economics were excluded, as these topics are not in the remit of VKM. Only full-text, peer-reviewed articles published in English were included in this assessment.

3 Molecular characterisation

3.1 Information related to the genetic modification

Maize 1507 was genetically modified to express the genes *cry1F* and *pat*. The resulting Cry1F protein produced by maize 1507 provides season-long resistance against certain lepidopteran pests, such as the European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp. The produced PAT protein confers tolerance to application of glufosinate-ammonium herbicides, only serving as a selectable marker in the transformation process.

3.1.1 Description of the methods used for the genetic modification

A particle acceleration method was used to introduce a purified linear DNA fragment (PHI8999A, 6235 bp; Figure 1) containing the *cry1F* and *pat* coding sequences, and the chosen regulatory components, into maize cells.

Immature embryos isolated from maize ears harvested soon after pollination were cultured on callus initiation medium for some days. On the day of transformation, microscopic tungsten particles were coated with the purified PHI8999A DNA, and next introduced to the cultured embryos by acceleration forces to reach the cell nuclei. This leads to insertion of DNA into the maize cell genome. The *nptII* gene was not part of the DNA fragment (PHI8999A) that was used in the transformation, and therefore not transferred to maize.

After transformation, the embryos were placed on callus initiation medium containing the herbicide glufosinate-ammonium as the selection agent for the production of GM maize. The embryos that survived and produced glufosinate-ammonium tolerant calli tissues were subsequently transferred to fresh selection medium. Maize plants were regenerated from tissues derived from each unique event and moved to a greenhouse. Leaf samples were taken for molecular analysis to confer the presence of the inserted genes by PCR and to measure protein levels of Cry1F and PAT by ELISA. Plants were further subjected to a whole plant bioassay and exposed to European corn borer.

3.1.2 Breeding pedigree

The notifier has provided details on the selective breeding program undertaken with the transformed line to demonstrate the production of elite maize cultivars with various commercial applications. The *cry1F* and *pat* genes were transformed into the original parental line known as Hi-II, which was subsequently known as maize line 1507. The genetic makeup of this transformed line was 100% Hi-II. Maize line 1507 was crossed to an elite inbred line, so the resulting progeny contained 50% Hi-II germplasm and 50% elite inbred germplasm and later self pollinated to get homozygote and hemizygote transgenics after selecting against the homozygote non-transgenic. This confirmed Mendelian inheritance according to a single transgenic construct segregating 1:2:1 homozygote GM, hemizygote

and homozygote non-transgenic, or 3:1 transgenic to non-transgenic. Backcrosses to the elite inbred, while simultaneously selecting for the transgenes, resulted in elite germplasm close to 100% while still containing the transferred *cry1F* and *pat* genes (Figure 1 and 2, Appendix 1).

High yielding hybrid maize seeds sold to farmers is produced by crossing two distinct inbred maize lines, explaining why these hybrid vigour plants would lose their yield if their seeds were sown due to inbreeding depression. This is one reason for farmers to buy hybrid seeds each season, to keep the yield and quality of these combined inbred lines respective effects. Each inbred maize line further has a different genetic background that allows the hybrid seed to be optimised for a specific geographical region where maize is grown. A new gene, such as *cry1F* in maize line 1507, is introduced into the many different inbred lines through conventional backcrossing.

3.1.3 Nature and source of vector used

No vector was used in the transformation of 1507 maize. A linear DNA fragment containing the *cry1F* and *pat* coding sequences and added regulatory DNA sequences was transferred to immature maize embryos through particle acceleration. No additional DNA was used for the transformation.

The insert was obtained from plasmid PHP8999 (Figure 2) following digestion of the plasmid DNA with the restriction enzyme PmeI. As a result, two linear fragments of DNA were obtained: a 6235 bp fragment, i.e. the intended insert containing the *cry1F* and *pat* genes; and a 3269 bp fragment not used in the transformation. The 6235 bp (PHI8999A) fragment was subsequently purified by agarose gel electrophoresis and used in the transformation of 1507 maize. A detailed description of the organisation, size and function of the genetic material present in the 6235 bp fragment and the 3269 bp fragment is provided in Table 1 and 2, respectively.

3.1.3.1 Size, source of donor organism(s) and intended function of each constituent fragment of the region intended for insertion

The insert PHI8999A consisted of a linear DNA fragment of 6235 bp containing a synthetic and truncated version of the *cry1F* gene from *Bacillus thuringiensis* subsp. *aizawai*, optimised for plant expression. Its transcription is directed by the ubiquitin promoter *ubiZM1(2)* from *Zea mays* and a termination sequence derived from ORF25PolyA from *Agrobacterium tumefaciens* extrachromosomal plasmid pTi15995. The insert also contained a synthetic version and plant optimised *phosphinothricin acetyltransferase* gene sequence, *pat*, from *Streptomyces viridochromogenes*. The transcription of *pat* is directed by the *CaMV 35S* promoter and *CaMV 35S terminator*, from cauliflower mosaic virus.

A restriction map of insert PHI8999A is shown on Figure 1, and a complete description of the size, position, source of donor organism and intended function of the DNA sequences contained in the insert, together with appropriate references, is presented in Table 1.

The size of the truncated *cry1F* gene in the intended insert was 1818 bp. It coded for amino acids 1-605 of the Cry1F protein from *Bacillus thuringiensis* subsp. *aizawai* and included the active core of the native Cry1F protein. A change in the coding sequence was made to introduce an XhoI restriction site at the 3' end of the truncated *cry1F* gene. According to the applicant, this change was designed so that it resulted in a single and conservative amino acid substitution in maize expressed Cry1F protein, leucine at position 604 instead of phenylalanine.

The *pat* gene was only introduced for selection of transgenic plants, not for agricultural use. The sequence of the *pat* gene in insert PHI8999A was 552 bp, and the CaMV 35S promoter and terminator sequences from cauliflower mosaic virus were 554 bp and 204 bp, respectively.

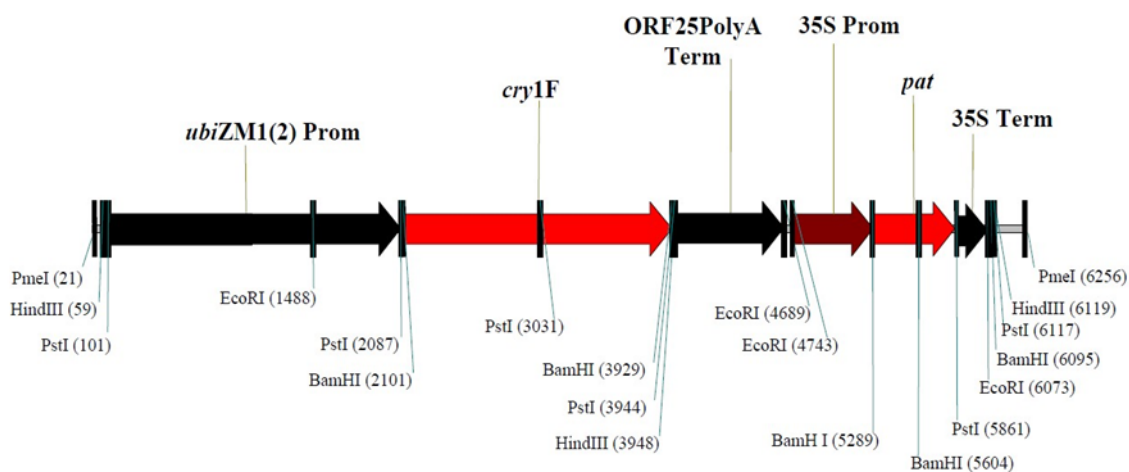


Figure 1. Restriction map of the 6235 bp insert PHI8999A used in the transformation of 1507 maize

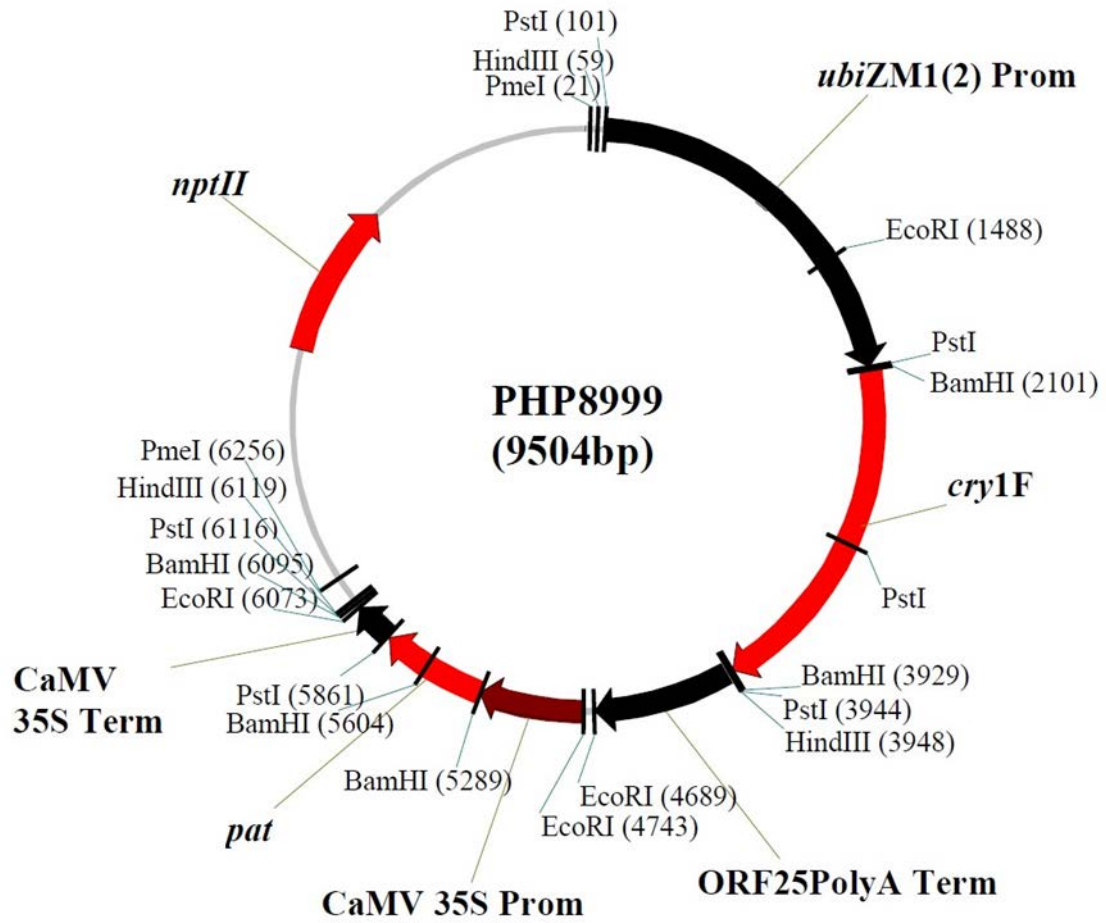


Figure 2. Plasmid map of PHP8999 used in the construction of insert PHI8999A

Table 1. Genetic elements in insert PHI8999A used in the transformation of 1507 maize

Location on insert PHI8999A (bp to bp incl.)	Location on plasmid PHP8999 (bp to bp incl.)	Genetic element	Size (bp incl.)	Function
1 – 80	21 – 100	Polylinker region	80	Contains restriction sites required for cloning of the genetic elements
81 – 2066	101 – 2086	<i>ubiZM1(2)</i>	1986	The ubiquitin promoter (plus 5' untranslated region) from <i>Zea mays</i> (Christensen <i>et al.</i> , 1992)
2067 – 2089	2087 – 2109	Polylinker region	23	Contains restriction sites required for cloning of the genetic elements
2090 – 3907	2110 – 3927	<i>cry1F</i>	1818	A synthetic version of truncated <i>cry1F</i> from <i>Bacillus thuringiensis</i> sbsp. <i>aizawai</i> (plant optimized)
3908 – 3952	3928 – 3972	Polylinker region	45	Contains restriction sites required for cloning of the genetic elements
3953 – 4666	3973 – 4686	ORF25PolyA	714	A terminator from <i>Agrobacterium tumefaciens</i> pTi15955
4667 – 4722	4687 – 4742	Polylinker region	56	Contains restriction sites required for cloning of the genetic elements
4723 – 5276	4743 – 5296	CaMV 35S promoter	554	35S promoter from Cauliflower Mosaic Virus (Odell <i>et al.</i> , 1985)
5277 – 5828	5297 – 5848	<i>pat</i>	552	The synthetic glufosinate-ammonium tolerance gene (plant optimized), based on a phosphinothricin acetyltransferase gene sequence from <i>Streptomyces viridochromogenes</i> (Wohlleben <i>et al.</i> , 1988; Eckes <i>et al.</i> , 1989)
5829 – 5846	5849 – 5866	Polylinker region	18	Contains restriction sites required for cloning of the genetic elements
5847 – 6050	5867 – 6070	CaMV 35S terminator	204	35S terminator from Cauliflower Mosaic Virus (Pietrzak <i>et al.</i> , 1986)
6051 – 6235	6071 – 6255	Polylinker region	185	Contains restriction sites required for cloning of the genetic elements

Table 2. Description of the genetic elements present in the 3269 bp fragment obtained from plasmid PHP8999 that were not intended for transformation of 1507 maize

Location on plasmid PHP8999 (bp to bp incl.)	Genetic element	Size (bp incl.)	Function
6256 – 6307	Polylinker region	65	Contains restriction sites required for cloning of the genetic elements
6308 – 6538 (Compl.)	<i>lac</i> promoter	218	The promoter for the <i>lac</i> operon in <i>E.coli</i> ; used to express cloned genes in bacteria, but non-functional in the context of PHP8999 (Blattner <i>et al.</i> , 1997). It also contains the M13 reverse primer binding site (6331 – 6354 bp, complementary)
6539 – 7493	Polylinker region	955	Contains restriction sites required for cloning of the genetic elements
7494 – 8309	<i>nptII</i>	816	Marker gene conferring kanamycin resistance (Beck <i>et al.</i> , 1982)
8310 – 8868	Polylinker region	559	Contains restriction sites required for cloning of the genetic elements
8869 – 9324 (Compl.)	Phage F1	456	The origin of replication from the bacteriophage F1; functional only in the presence of active phage F1; used to render the plasmid single-stranded DNA, rather than double-stranded DNA (Hill and Petersen, 1982).
9325 – 9504 and 1 – 20	Polylinker region	200	Contains restriction sites required for cloning of the genetic elements. It also contains the M13 forward primer binding site (9438 – 9461 bp)

3.2 Information relating to the GM plant

3.2.1 General description of the trait(s) and characteristics which have been introduced or modified

Maize 1507 was developed for protection against certain lepidopteran pests such as the European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp. and for tolerance to glufosinate-ammonium herbicides. Insect resistance is achieved by production of a truncated Cry1F protein from *Bacillus thuringiensis* ssp. *aizawai*, and tolerance to glufosinate-ammonium is conferred by the enzyme PAT from *Streptomyces viridochromogenes*, introduced only for use during development of the GM plant.

3.2.2 Information on the sequences actually inserted/deleted or altered

The size and structure of the insert present in 1507 maize was characterised by Southern hybridisation and DNA sequence analyses. These analyses indicate that the genetic material inserted in 1507 maize consists of an almost full-length copy of the linear fragment used in the transformation (i.e. 6186 bp from the 6235 bp of insert PHI8999A, containing the *cry1F* and *pat* genes together with regulatory sequences).

According to the applicant, the 1507 maize does not contain the *nptII* gene or any other detectable fragments from the portion of plasmid PHP8999 that was not intended for transformation of 1507 maize. Maize genomic DNA flanking regions at both the 5' and 3' borders of the 1507 maize insert were sequenced and characterised. In addition, analysis by PCR amplification indicate the presence of both maize genomic flanking regions in non-GM Hi-II maize used in the transformation of 1507 maize.

The following base pairs were missing from the almost full length insert: base pairs 1-10 at the 5' end of the PHI8999A linear DNA fragment, and base pairs 6197 to 6235 at the 3' end of the PHI8999A linear DNA fragment. These base pairs were probably lost during the integration of the insert into the maize genome. According to the applicant, the location of the missing base pairs indicate that they are not relevant, and that their absence will not give rise to any adverse effects on the expression or stability of the *cry1F* and *pat* genes introduced in 1507 maize.

3.2.2.1 *Size and copy number of all detectable inserts, both complete and partial*

The Southern hybridisation and sequence analyses indicate that the genetic material inserted in 1507 maize consists of an almost full-length copy of the linear fragment used in the transformation.

Plasmid PHP8999 DNA, genomic DNA from Hi-II maize, and genomic DNA from 1507 maize T1S1 and BC4 generations were digested with the restriction enzymes *PmeI*, *HindIII*, *PstI*, *BamHI*, *EcoRI*, and *BamHI* combined with *EcoRI*.

According to the applicant, the *PmeI* restriction site is lost during transformation because the specific sequence required for *PmeI* digestion (GTTT/AAAC) is not likely to be present at the point of integration into the maize genome. Therefore, the observed hybridisation fragments are larger than 6235 bp.

The *cry1F* gene

The digestions with *HindIII*, *PstI*, and *BamHI* were conducted for the purpose of characterising the *cry1F* gene and its *ubiZM1(2)* promoter in 1507 maize. The *HindIII* restriction enzyme cuts at the 5' end of the *ubiZM1(2)* promoter and the 3' end of the *cry1F* coding sequence. The purpose of the *HindIII* digestion was to determine whether the full-length *cry1F* gene is present with its promoter intact. The *PstI* digestion was intended to provide further information on whether the *ubiZM1(2)* promoter was intact as the enzyme cuts essentially at both ends of this promoter. *BamHI* digestion was intended to provide information on whether the *cry1F* coding sequence was intact as the enzyme cuts at both the 5' and 3' end of this coding sequence.

HindIII digestion was expected to produce a 3890 bp fragment containing the *ubiZM1(2)* promoter and *cry1F* gene. This fragment was observed after hybridisation with probes specific for the *ubiZM1(2)* promoter and *cry1F*. The *PstI* digestion supports the conclusion that *ubiZM1(2)* promoter is intact resulting in a 1986 bp fragment when genomic DNA was hybridised with the *ubi* probe. Finally, a 1828 bp fragment was present when genomic DNA was digested with *BamHI* and hybridised with the *cry1F* probe, which indicated that an intact *cry1F* coding sequence is present.

HindIII digestion and hybridisation with the *cry1F* probe resulted in two bands: one of 3890 bp size and a second, representing an additional copy that is larger and estimated at ~ 4000 bp in size. Hybridisation of the *HindIII* digest with the *ubi* probe resulted in one band of 3890 bp size and failed to reveal the ~4000 bp fragment. According to the applicant, this indicates that the promoter region is either absent in this additional copy or it is not intact.

According to the applicant, a small portion of the *ubiZM1(2)* promoter cannot be detected by the *ubi* DNA probe used in this study because the *ubi* probe was prepared with a fragment of the *ubiZM1(2)* promoter extending from 120 bp to 1707 bp. Therefore, an approximately 300 bp region of the *ubiZM1(2)* promoter that is 5' to the *cry1F* gene cannot be detected with this probe. None of the other digestions were designed to provide evidence for the presence or absence of the *ubiZM1(2)* promoter on the additional *cry1F* gene. Interpretation of hybridisation results with the *ubi* probe is made difficult by the fact that the *ubiZM1(2)* promoter was isolated from maize and, therefore, is also endogenous (present) in the non-GM control maize plants. Nevertheless, the results of the *HindIII* digestion support the conclusion that the *ubiZM1(2)* promoter on the additional copy of the *cry1F* coding sequence is either absent or not intact.

The *pat* gene

The digestions with *EcoRI*, *BamHI*, and the combination *BamHI/EcoRI*, were conducted for the purpose of characterising the *pat* gene and its CaMV promoter in 1507 maize. The *EcoRI* enzyme cuts at the 5' end of the CaMV 35S promoter and at the 3' end of the CaMV 35S terminator for the *pat* gene and was expected to result in a 1329 bp fragment if an intact copy of the *pat* gene and its CaMV 35S promoter and terminator was present in 1507 maize. The *BamHI* enzyme cuts at the 5' end of the *pat* gene and within approximately 150 bp of the 3' end of this gene. An additional digestion with the combination *BamHI/EcoRI* was conducted to determine whether a 546 bp fragment corresponding to the CaMV 35S promoter could be detected after hybridisation with the CaMV 35S promoter DNA probe.

A 1329 bp *EcoRI* fragment was observed after hybridisation with the CaMV 35S and *pat* DNA probes. The presence of an intact CaMV 35S promoter was shown because a 546 bp fragment was observed with the combined *BamHI/EcoRI* digestion. The presence of an intact *pat* gene was shown because the fragments were observed after *BamHI* digestion followed by hybridisation with the *pat* DNA probe. Finally, *HindIII* digestion was expected to produce 2170 bp fragment containing the CaMV 35S promoter, *pat* gene, and CaMV 35S terminator if the sequences were present as full-length copies. This fragment was observed after hybridisation with the CaMV 35S and *pat* DNA probes.

The *nptII* gene and sequences of plasmid PHP8999 not intended for transformation:

According to the applicant, the portion of the plasmid that was used as the insert for transformation did not contain the kanamycin resistance gene, *nptII*. To further verify that 1507 maize does not contain the *nptII* gene, genomic DNA was hybridised with an *nptII* probe. No bands hybridising to the *nptII* DNA probe were detected, which indicate that the *nptII* gene for kanamycin resistance is not present in 1507 maize. Additional Southern blot analyses of 1507 maize was been carried out to provide further evidence on the absence of all sequences of plasmid PHP8999 that were not intended for transformation of 1507 maize.

Genomic DNA samples of eight 1507 maize plants from two different generations (T1 and BC4, early and late generations, respectively) were digested with *HindIII* restriction enzyme and probed with *i*) whole-length plasmid PHP8999 (9054 bp); *ii*) 3.9 kb *HindIII* fragment corresponding to part of insert PHI8999A; and, *iii*) 2.2 kb *HindIII* fragment corresponding to another part of insert PHI8999A. Only bands relating to the *HindIII* fragments corresponding to parts of insert PHI8999A were obtained.

Assuming that unintended integration of plasmid PHP8999 had taken place in 1507 maize, a ~3.4 kb band would be expected in the Southern hybridisation probed with the whole length PHP8999 probe. However, there is no evidence for the presence of the ~3.4 kb *HindIII* fragment corresponding to the part of plasmid PHP8999 that was not used or intended for transformation of 1507 maize.

No unexpected bands of a smaller size (<3.4 kb) were observed. A ~2.2 kb and ~1 kb bands observed in the Southern blots were recognised by the 2.2 kb *HindIII* probe corresponding to

part of insert PHI8999A. The ~3.9 kb band observed corresponded to the 3.9 kb *HindIII* fragment expected from the insert PHI8999A. In addition, the largest fragment of ~6.7 kb observed was recognised by the ~3.9 kb probe corresponding to part of insert PHI8999A, and may correspond to the additional *cry1F* copy plus flanking maize genomic sequence.

Open reading frame analysis

Analysis of the 1507 maize insert sequence for the presence of potential open reading frames (ORFs) has been carried out. In searching for potential ORFs in the 5' and 3' border sequences and in the rest of the sequence of 1507 maize insert, no significant concern was associated with ORFs of less than 100 amino acids (300 bp) since these are commonly found unfunctional in maize.

Three potential ORFs longer than 300 bp (100 amino acids) were identified on the 1507 maize insert 5' border sequence: ORF1 spanning bases 362-691 (330 bp total); ORF2 spanning bases 433- 780 (348 bp total); and, ORF3 spanning bases 1896-2648 (753 bp total) extending from near the end of region 4 to region 7b.

Both ORF1 and ORF2 are contained within regions 1 and 2 of the 1507 maize insert which correspond to maize genomic DNA. This was shown by PCR analysis indicating that regions 1 and 2 are present in non- GM Hi-II maize used in the transformation of 1507 maize.

A potential ORF of 630 bp was identified in the full-length insert of 1507 maize partially spanning the ORF25PolyA terminator and the CaMV35S promoter sequences. According to the applicant, there are no regulatory sequences next to this potential ORF, named ORF4, which could direct its expression in 1507 maize. In particular, the sequences directly upstream of this potential ORF4 have not been identified as promoter-like sequences that might drive transcription and translation of the potential ORF4.

3.2.2.2 The organization and sequence of the inserted genetic material at each insertion site

The structural organisation of the insert in 1507 was analysed by Southern hybridisation analysis. Verification of the insert by DNA sequencing was conducted. The results of the molecular characterisation established that 1507 maize contains a single DNA insert, containing one intact copy of the restriction fragment PHI8999A that was used for transformation. According to the applicant, the 1507 maize does not contain the *nptII* gene nor any other detectable fragments from the portion of plasmid PHP8999 that was not used for transformation of 1507 maize. According to the applicant, the following base pairs were missing from the almost full length insert: base pairs 1-10 at the 5' end of the PHI8999A linear DNA fragment, and base pairs 6197 to 6235 at the 3' end of the PHI8999A linear DNA fragment.

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

Maize 1507 contains 6186 bp from the 6235 bp insert sequence containing the *cry1F* and *pat* genes.

3.2.2.4 *Sub-cellular location(s) of insert(s) and methods for its / their insertion*

Statistical analyses of segregation data show that the DNA insert is stably inherited by maize 1507 progeny, and that maize event 1507 segregates according to standard Mendelian patterns, as expected for a single site of insertion in the maize nuclear genome.

3.2.3 Information on the expression of the inserted/modified sequence

Protein productions of Cry1F and PAT were characterised in a range of tissues from 1507 maize, representing key developmental stages of a typical maize plant with specific Enzyme Linked Immunosorbent Assay (ELISA) developed for each protein.

Samples of leaf tissue were tested at the V9 (nine leaf) stage. Samples of pollen, silk and stalk were tested at approximately R1 (50% pollen shed), and whole plants at R4 (approximately four weeks after pollination). Additional measurements were also done on mature grain, senescent whole plants, and samples of whole plant tissues from selected sites at V9 and R1 stages of development.

Chile 1998/1999

A field study was conducted at four field sites in Chile in 1998/1999, located in the major maize growing regions of Chile. The results are summarised in Table 3. All tissue samples were collected from a single replication of 1507 maize and control at each site. For the leaf sample, the youngest whorl leaf was collected from five plants at approximately the V9 stage. Pollen, silk and stalk samples were collected from five discrete plants at approximately the R1 stage. Grain from five self-pollinated plants was collected after physiological maturity. The whole plant samples (entire plant except roots) consisted of three self-pollinated plants harvested at the R4 stage, that were pooled at each site. The senescent whole plant samples, including ears, were harvested when the plant tissues had turned brown and dried. None of the plots were sprayed with glufosinate-ammonium.

France and Italy 1999

A second study was conducted at three locations in France and three locations in Italy in 1999, all situated in the major maize growing regions of the EU. The results are summarised in Table 4. At each location in Italy, the trial consisted of 1507 maize sprayed with glufosinate-ammonium, 1507 maize not sprayed with glufosinate-ammonium, and a non-GM control maize with genetics representative of 1507 maize. At each location in France, the trial consisted of 1507 maize not sprayed with glufosinate- ammonium, and a non-GM control maize with genetics representative of 1507 maize. All tissue samples were collected from a single replication of 1507 maize and control maize. Leaf samples were collected at V9, whole

plant at V9, pollen, silk, stalk, and whole plant at R1, and whole plant at R4. Grain and senescent whole plant were also collected. In addition, samples of whole plant forage (R4) and grain were collected from plots sprayed with glufosinate-ammonium.

France, Italy and Bulgaria 2000

A third field study was conducted at three locations in France, two locations in Italy and one location in Bulgaria in 2000. At each location, the trial consisted of 1507 maize sprayed with glufosinate- ammonium, 1507 maize not sprayed with glufosinate-ammonium, and a non-GM control with genetics representative of 1507 maize. Leaf, grain, pollen, stalk, and whole plant were collected at V9 and R from all locations. The results from the study are presented in Table 5-8.

The PAT protein in 1507 maize does not occur at measurable levels throughout the developmental stages. It was only measured in samples collected at the V9 stage (leaf and whole plant tissues), and found to be below the limit of detection in R1 or R4 tissues, senescent plants and mature grain.

Table 3. Summary of Cry1F protein levels (in pg/μg total extractable protein) in tissue collected from 1507 hybrid maize from field trials in Chile in 1998/99

Tissue (growth stage) ^a	Mean ^b CRY1F (pg/μg TEP ^c)	Standard deviation	Min/max range (pg/μg TEP)
Leaf (V9 stage)	110.9	27.2	56.6 – 148.9
Pollen (R1 stage)	135.5	13.5	113.4 – 168.2
Silk (R1 stage)	50.3	16.5	26.8 – 79.8
Stalk (R1 stage)	550.0	104.0	355.9 – 737.4
Whole plant (R4 stage)	1063.8	361.7	803.2 – 1572.7
Grain (Physiol. maturity)	89.8	23.3	71.2 – 114.8
Senescent whole plant (Brown and dry)	714.3	95.5	622.2 – 845.3

a: Iowa State University (1997)

b: Values are means across all four sites from mean values calculated from the analysis of five individual samples per site for leaf, pollen, silk, stalk, grain and one pooled sample per site for both whole plant samples

c: TEP = total extractable protein

Table 4. Summary of CryF protein levels (in pg/μg total extractable protein) in tissue collected from 1507 maize from field trials in France and Italy in 1999

Tissue (growth stage) ^a	Mean ^b CRYIF (pg/μg TEP ^c)	Standard deviation	Min/max range (pg/μg TEP)
Leaf (V9 stage)	348.0	160.9	193.2 – 651.4
Whole plant (V9 stage)	743.7	394.2	409.6 – 1526.6
Pollen (R1 stage)	190.5	84.4	141.9 – 630.8
Silk (R1 stage)	133.0	58.1	61.1 – 265.3
Stalk (R1 stage)	630.8	141.6	417.9 – 917.7
Whole plant (R1 stage)	671.9	348.2	323.4 – 1206.4
Whole plant (R4 stage)	1073.1 ^d 569.4 ^e	338.2 11.0	874.4 – 1576.1 556.7 – 575.8
Grain (Physiol. maturity)	96.4 ^d 90.3 ^e	25.9 21.8	44.8 – 135.3 57.4 – 131.8
Senescent whole plant (Brown and dry)	198.9	21.4	171.2 – 219.5

a: Iowa State University (1997)

b: Values are means across all sites. Samples were taken from plants not sprayed with glufosinate-ammonium unless stated otherwise

c: TEP = total extractable protein

d: Unsprayed plants

e: Sprayed with glufosinate-ammonium

Table 5. Summary of levels of Cry1F protein (pg/μg TEP) measured in tissues collected from maize hybrid line 1507 (sprayed and unsprayed) and controls from field trials in France, Italy and Bulgaria in 2000

Tissue	Mean (pg/μg TEP ¹)	Standard Deviation	Min/Max Range (pg/μg TEP)	Number of Samples ²
Leaf				
Hybrid 1507	190	64	0 - 317	30/2<LLOQ(25pg/μg)
Hybrid 1507S	214	42	148 - 297	30/0<LLOQ(25pg/μg)
Control	0 ³	0	0	6/6<LLOQ(25pg/μg)
Grain				
Hybrid 1507	69	26	0 - 128	29/1<LLOQ(25pg/μg)
Hybrid 1507S	74	22	35 - 118	29/0<LLOQ(25pg/μg)
Control	0	0	0	6/6<LLOQ(25pg/μg)
Pollen				
Hybrid 1507	162	18	132 - 208	30/0<LLOQ(25pg/μg)
Hybrid 1507S	167	23	132 - 241	30/0<LLOQ(25pg/μg)
Control	0	0	0	6/6<LLOQ(25pg/μg)
Stalk				
Hybrid 1507	448	114	338 - 744	30/0<LLOQ(25pg/μg)
Hybrid 1507S	395	90	211 - 710	30/0<LLOQ(25pg/μg)
Control	0	0	0	6/6<LLOQ(25pg/μg)
Whole Plant (V9) WPV9				
Hybrid 1507	965	382	478 - 1450	5/0<LLOQ(250pg/μg)
Hybrid 1507S	883	372	495 - 1230	5/0<LLOQ(250pg/μg)
Control	0	0	0	5/5<LLOQ(250pg/μg)
Whole Plant (R1) WPR1				
Hybrid 1507	1310	630	739 - 2190	4/0<LLOQ(250pg/μg)
Hybrid 1507S	1230	498	767 - 1910	4/0<LLOQ(250pg/μg)
Control	0	0	0	5/5<LLOQ(250pg/μg)
Whole Plant (forage) WPF				
Hybrid 1507	515	295	0 - 752	5/1<LLOQ(250pg/μg)
Hybrid 1507S	396	273	0 - 708	5/1<LLOQ(250pg/μg)
Control	0	0	0	5/5<LLOQ(250pg/μg)
Whole Plant (Senescence)				
Hybrid 1507	410	184	255 - 695	5/0<LLOQ(50pg/μg)
Hybrid 1507S	446	231	204 - 803	5/0<LLOQ(50pg/μg)
Control	0	0	0	5/5<LLOQ(50pg/μg)

¹ TEP: Total Extractable Protein

² Number of Samples: number of samples analyzed/number of samples < sample LLOQ (pg/μg TEP). Sample LLOQ varies based on the amount (μg TEP/well) of tissue type analyzed, with the assay LLOQ remaining constant.

³ For values below sample LLOQ (pg/μg TEP) a value of zero was assigned for calculation purposes

Table 6. Summary of levels of Cry1F protein (ng/mg Tissue Dry Weight) measured in tissues collected from maize hybrid line 1507 (sprayed and unsprayed) and control from field trials in France, Italy and Bulgaria in 2000

Tissue	Mean (ng/mg Tissue Dry Weight)	Standard Deviation	Min/Max Range (ng/mg Tissue Dry Weight)	Number of Samples ¹
Leaf				
Hybrid 1507	12.1	6.2	0 - 24	30/2<LLOQ
Hybrid 1507S	14.2	6.9	5.2 - 31.8	30/0<LLOQ
Control	0 ²	0	0	6/6<LLOQ
Grain				
Hybrid 1507	2.2	0.8	0 - 4	29/1<LLOQ
Hybrid 1507S	2.5	0.9	0.9 - 3.9	29/0<LLOQ
Control	0	0	0	6/6<LLOQ
Pollen				
Hybrid 1507	21.9	2.9	16.4 - 27.2	30/0<LLOQ
Hybrid 1507S	22.4	2.9	17.5 - 30.7	30/0<LLOQ
Control	0	0	0	6/6<LLOQ
Stalk				
Hybrid 1507	5.8	1.7	3.3 - 10.3	30/0<LLOQ
Hybrid 1507S	5.8	1.5	2.9 - 10.3	30/0<LLOQ
Control	0	0	0	6/6<LLOQ
Whole Plant (V9) WPV9				
Hybrid 1507	5.2	1.9	2.6 - 6.8	5/0<LLOQ
Hybrid 1507S	6.0	2.9	3.4 - 10.2	5/0<LLOQ
Control	0	0	0	5/5<LLOQ
Whole Plant (R1) WPR1				
Hybrid 1507	3.6	1.1	2.5 - 4.7	4/0<LLOQ
Hybrid 1507S	3.6	1.4	2.1 - 5.1	4/0<LLOQ
Control	0	0	0	5/5<LLOQ
Whole Plant (forage) WPF				
Hybrid 1507	1.7	1.1	0 - 3.2	5/1<LLOQ
Hybrid 1507S	1.6	1.1	0 - 2.9	5/1<LLOQ
Control	0	0	0	5/5<LLOQ
Whole Plant (Senescence)				
Hybrid 1507	1.6	0.6	0.9 - 2.4	5/0<LLOQ
Hybrid 1507S	1.7	0.6	1 - 2.3	5/0<LLOQ
Control	0	0	0	5/5<LLOQ

¹ Number of Samples: number of samples analyzed/number of samples < sample LLOQ (pg/μg TEP)

² For values below sample LLOQ (pg/μg TEP) a value of zero was assigned for calculation purposes

Table 7. Summary of levels of PAT protein (pg/μg TEP) measured in tissues collected from maize hybrid line 1507 (sprayed and unsprayed) and control in France, Italy and Bulgaria from field trials in 2000

Tissue	Mean (pg/μg TEP ¹)	Standard Deviation	Min/Max Range (pg/μg TEP)	Number of Samples ²
Leaf				
Hybrid 1507	55.3	33	0 - 164	30/2<LLOQ(12pg/μg)
Hybrid 1507S	81.9	21.9	50 - 135	30/0>LLOQ(12pg/μg)
Control	0 ⁴	0	0	6/6<LLOQ(12pg/μg)
Grain				
Hybrid 1507	0	0	0	29/29<LLOQ(12pg/μg)
Hybrid 1507S	0	0	0	29/29<LLOQ(12pg/μg)
Control	0	0	0	6/6<LLOQ(12pg/μg)
Pollen				
Hybrid 1507	0	0	0	30/30<LLOQ(6pg/μg)
Hybrid 1507S	0	0	0	30/30<LLOQ(6pg/μg)
Control	0	0	0	6/6<LLOQ(6pg/μg)
Stalk				
Hybrid 1507	NA ³	NA	0 - 48.9	30/21<LLOQ(12pg/μg)
Hybrid 1507S	NA	NA	0 - 25.9	30/18<LLOQ(12pg/μg)
Control	0	0	0	6/6<LLOQ(12pg/μg)
Whole Plant (V9) WPV9				
Hybrid 1507	NA	NA	0 - 6.2	5/4<LLOQ(6pg/μg)
Hybrid 1507S	8.6	12.3	0 - 25.9	5/4<LLOQ(6pg/μg)
Control	0	0	0	5/5<LLOQ(6pg/μg)
Whole Plant (R1) WPR1				
Hybrid 1507	NA	NA	0 - 8.9	4/3<LLOQ(6pg/μg)
Hybrid 1507S	9.9	19.9	0 - 39.8	4/3<LLOQ(6pg/μg)
Control	0	0	0	5/5<LLOQ(6pg/μg)
Whole Plant (forage) WPF				
Hybrid 1507	NA	NA	0 - 6.9	5/4<LLOQ(6pg/μg)
Hybrid 1507S	NA	NA	0 - 11.9	5/3<LLOQ(6pg/μg)
Control	0	0	0	5/5<LLOQ(6pg/μg)
Whole Plant (Senescence)				
Hybrid 1507	0	0	0	5/5<LLOQ(6pg/μg)
Hybrid 1507S	NA	NA	0 - 7.4	5/4<LLOQ(6pg/μg)
Control	0	0	0	5/5<LLOQ(6pg/μg)

¹ TEP: Total Extractable Protein

² Number of Samples: number of samples analyzed/number of samples < sample LLOQ (pg/μg TEP). Sample LLOQ varies based on the amount (μg TEP/well) of tissue type analyzed, with the assay LLOQ remaining constant.

³ NA: Not Applicable – The mean of the sample values was below the LLOQ and was not included in the table

⁴ For values below sample LLOQ (pg/μg TEP) a value of zero was assigned for calculation purposes

Table 8. Summary of levels of PAT protein (ng/mg Tissue Dry Weight) measured in tissues collected from maize hybrid line 1507 (sprayed and unsprayed) and control in France, Italy and Bulgaria from field trials in 2000

Tissue	Mean (ng/mg Tissue Dry Weight)	Standard Deviation	Min/Max Range (ng/mg Tissue Dry Weight)	Number of Samples ¹
Leaf				
Hybrid 1507	3.8	3.0	0 - 10.8	30/2<LLOQ
Hybrid 1507S	5.2	2.6	1.7 - 11.8	30/0>LLOQ
Control	0 ³	0	0 - 0	6/6<LLOQ
Grain				
Hybrid 1507	0	0	0	29/29<LLOQ
Hybrid 1507S	0	0	0	29/29<LLOQ
Control	0	0	0	6/6<LLOQ
Pollen				
Hybrid 1507	0	0	0	30/30<LLOQ
Hybrid 1507S	0	0	0	30/30<LLOQ
Control	0	0	0	6/6<LLOQ
Stalk				
Hybrid 1507	NA ²	NA	0 - 0.6	30/21<LLOQ
Hybrid 1507S	NA	NA	0 - 0.3	30/18<LLOQ
Control	0	0	0 - 0	6/6<LLOQ
Whole Plant (V9) WPV9				
Hybrid 1507	NA	NA	0 - 0.03	5/4<LLOQ
Hybrid 1507S	0.05	0.06	0 - 0.12	5/4<LLOQ
Control	0	0	0 - 0	5/5<LLOQ
Whole Plant (R1) WPR1				
Hybrid 1507	NA	NA	0 - 0.03	4/3<LLOQ
Hybrid 1507S	0.04	0.08	0 - 0.16	4/3<LLOQ
Control	0	0	0 - 0	5/5<LLOQ
Whole Plant (forage) WPF				
Hybrid 1507	NA	NA	0 - 0.03	5/4<LLOQ
Hybrid 1507S	NA	NA	0 - 0.07	5/3<LLOQ
Control	0	0	0 - 0	5/5<LLOQ
Whole Plant (Senescence)				
Hybrid 1507	0	0	0 - 0	5/5<LLOQ
Hybrid 1507S	0	0	0 - 0.01	5/4<LLOQ
Control	0	0	0 - 0	5/5<LLOQ

¹ Number of Samples: number of samples analyzed/number of samples < sample LLOQ (pg/μg TEP)

² NA: Not Applicable – The mean of the sample values was below the LLOQ and was not included in the table.

³ For values below sample LLOQ (pg/μg TEP) a value of zero was assigned for calculation purposes

3.2.3.1 *Analytics, Reference Protein*

Bioinformatics tools were applied to assess the potential toxicity, allergenicity or pharmacological activity of putative peptides encoded at the junctions of the 1507 insert with its flanking sequences. Sequences spanning these junctions were translated from stop codon to stop codon in all reading frames and then compared for homology with databases of known toxins and allergens. The results of these 3' and 5' end bioinformatics analyses indicate that in the highly unlikely event that any of the junction polypeptides were translated, they do not share a sufficient degree of sequence similarity or identity to indicate that they are potentially toxic, allergenic or have other health implications.

3.2.3.2 *Parts of the plant where the insert/modified sequences are expressed*

As described above, the levels of Cry1F and PAT proteins in 1507 maize was analysed in leaf, pollen, silk, stalk, mature grain and whole plant tissues during field trials conducted in Chile in 1998/99 and in France and Italy in 1999.

3.2.3.3 *Potential unintended expression of new ORFs identified under point 1.2.2.2(f) in Dossier which raise a safety concern*

Not applicable.

3.2.4 Genetic stability of the inserted/modified sequence and phenotypic stability of the GM plant

Southern hybridisation analyses were undertaken to investigate the genetic stability of the inserted DNA in maize 1507. Genomic DNA extracted from 1507 maize T1S1 and BC4 generations and controls were digested with the restriction enzymes *PmeI*, *HindIII*, *PstI*, *BamHI*, *EcoRI*, and *BamHI* combined with *EcoRI*, and probed with the *ubi* probe, the *cry1F* probe, the *pat* probe, the *nptII* probe and the *HindIII* probe.

No significant differences in banding patterns were observed between DNA extracted from the T1S1 generation and the BC4 generation of 1507, indicating stability of the inserted DNA over multiple generations. This is consistent with a single site of integration into the genomic DNA of 1507.

Observations of the phenotype indicated that the transgenes are inherited as dominant genes according to Mendelian segregation patterns. The segregation of 1507 maize was recorded and analysed at two stages.

The original transformed Hi-II germplasm containing event 1507 (transformant T0) was crossed to an elite inbred line to give an F1 hybrid. The F1 hybrid was backcrossed twice to the elite inbred to give BC2F1 seed (Figure 2, Appendix 1). Spraying each generation with glufosinate-ammonium eliminated null segregants (those plants not containing a copy of the

transgene) and resulted in hemizygous seed. The seed from the BC2F1 generation was planted, and the plants were sprayed with glufosinate- ammonium. The expected segregation ratio was 1:1 (tolerant: susceptible) for glufosinate-ammonium tolerance (Table 9).

Later segregation data were obtained from F1 seeds as follows: after three backcrosses, 1507 maize seeds (BC3F1) were sown and self-pollinated. Resulting seeds (BC3F2) were expected to segregate 3:1 (tolerant:susceptible) for glufosinate-ammonium tolerance. These seeds were sown and sprayed with the herbicide to remove the homozygous susceptible plants. The remaining plants (one part homozygous tolerant and two parts hemizygous tolerant) were crossed to a susceptible inbred line to make F1 hybrid seeds. These hybrid seeds were sown and sprayed with glufosinate-ammonium to check for the expected 2:1 (tolerant: susceptible) ratio (Table 9).

Later 200 neonate European corn borers were used to infest each F1 plant that survived the glufosinate-ammonium spraying. All of the plants determined to be tolerant to glufosinate-ammonium were also found to be resistant to European corn borer infestation.

The results show that the levels of both the Cry1F and PAT proteins in 1507 maize are phenotypically stable for at least six generations.

Table 9. Mendelian segregation of 1507 maize. Early segregation data obtained from the BC2F1 generation; later segregation data obtained from the F1 generation

Generation	Observed ratio ^a	Expected ratio	Chi Square	Alpha value	Significant difference? ^b
BC2F1	248 : 278	263 : 263	1.711	0.1909	No
F1	910 : 493	935.3 : 467.7	2.903	0.0884	No

a: Data expressed as number of observed tolerant to glufosinate-ammonium : number of observed plants susceptible to glufosinate-ammonium herbicide

b: Significant at alpha = 0.05

3.3 Conclusions

Appropriate analyses of the transgenic DNA insert, its integration site, number of inserts and flanking sequences in the maize genome, have been performed. The results show that one copy only of the insert is present in maize 1507. Homology searches with databases of known toxins and allergens have not indicated any potential production of harmful proteins or polypeptides caused by the genetic modification in maize 1507. Southern blot analyses

and segregation studies show that the introduced genes *cry1F* and *pat* are stably inherited and expressed over several generations along with the phenotypic characteristics of maize 1507. VKM considers the molecular characterisation of maize 1507 satisfactory.

4 Comparative assessments

4.1 Production of material for comparative assessment

The notifications C/ES/01/01 and C/NL/00/10, and the applications EFSA/GMO/NL/2004/02 and EFSA/GMO/RX/1507 covering food and feed uses, cultivation, import and processing of maize 1507 within the EU, presented compositional data on seed and forage material collected in field trials performed in Chile in 1998/99, France and Italy in 1999, and France, Italy and Bulgaria in 2000. In addition, agronomic characteristics and performance of maize 1507 have been evaluated in field trials in the USA in 1999, France, Italy and Bulgaria in 2000 and Spain in 2002.

Studies submitted by Pioneer HiBreed & Dow AgroSciences

- Stauffer C, Rivas J (1999) Quantitative ELISA Analysis of Cry1F and PAT Expression Levels in and Compositional Analysis of Maize Inbred and Hybrid Lines 1362 and 1507. Pioneer Hi-Bred International Inc., Johnston, Iowa. Study Number 98-09-RA-NGLP-012.
Field trials in Chile in 1998/1999(Annex 1NF)
- Stauffer C, Zeph L (2000) Compositional Analysis of Maize MPS Hybrid Line 1507. Pioneer Hi-Bred International Inc, Johnston, Iowa and Woodson-Tenet Laboratories Inc, Delaware, Iowa. Study Number 98-09-RA-NGLP-012.
Field trials in Chile in 1998/1999 – (Annex 2NF)
- Stauffer C (2000) Quantitative ELISA Analysis of poCry1F and PAT Protein Expression Levels, Composition and Efficacy of Hybrid Lines 1360 and 1507 – EU Field Sites. Pioneer Hi-Bred International Inc, Johnston, Iowa. Study Number PH199-005. Field trials in France and Italy 1999. (Annex 3NF)
- Pavely C (2002) Quantitative ELISA Analysis of Cry1F and PAT Protein Expression Levels, and Composition of the Hybrid Maize Line Containing Event TC1507. Study Number PHI-2000-016. *Field trials in France, Italy and Bulgaria 2000 (Annex 3A)*
- Agronomic characteristics of 1507 maize (C/NL/00/10)
Field trials in USA 1999
- Agronomic characteristics of 1507 maize (C/ES/01/01)
Field trials in France, Italy and Bulgaria 2000
- Agronomic characteristics of 1507 maize (C/ES/01/01))
Field trials in Spain 2002

4.1.1 Field trials: experimental design and statistical analysis

Field trials in Chile (1998/99)

Composition data was obtained from field trials carried out at 4 locations in the major maize growing regions of Chile in the growth season 1998/1999. According to the applicant, these field sites are comparable to regions in North America and Europe suitable for commercial maize production.

Plots were established at each of the field sites in a randomised complete block design with six replications. Plant tissue samples were collected from two test lines; a hybrid line derived from maize 1507 and an inbred line derived from 1507. Two corresponding near-isogenic, non-transgenic lines designated as Hybrid AM and Inbred AM were included as conventional controls in the trials. No commercial reference varieties were included in the Chilean field trials. All test lines were sprayed with glufosinate-ammonium herbicides at approximately the V5-V6 stage of development, while the control plants were sprayed with conventional herbicides. All the maize lines at each of the field sites were grown under normal agronomic field conditions for their respective geographical regions.

Field trials in France and Italy (1999)

Maize compositional data was obtained from 6 locations in France and Italy in 1999. The study was conducted at three field sites in France and three field sites in Italy, located in the major maize growing regions of the EU. In France, the trials involved six replicates of 1507 maize not sprayed with glufosinate-ammonium, and non-GM control maize lines with comparable genetics. At each location in Italy, the trials involved three replicates of the test line 1507 sprayed with glufosinate-ammonium, 1507 maize not sprayed with glufosinate-ammonium, and non-GM control maize with comparable genetics.

Field trials in France, Italy and Bulgaria (2000)

Additional field trials were carried out within commercial maize growing regions of Europe at a total of six locations of which three locations were in France, two in Italy and one in Bulgaria, during the 2000 growing season. Nutrient composition of 1507 maize sprayed with glufosinate-ammonium, and 1507 maize not sprayed with glufosinate-ammonium, was evaluated and compared with non-GM maize with a comparable genetic background. At each location there were three replicates of 1507 maize sprayed with glufosinate ammonium, 1507 maize not sprayed with glufosinate-ammonium, and non-GM maize with comparable genetic background treated with conventional herbicides commonly used when growing maize.

Statistical analysis

Forage samples from 1507 maize and non-GM control maize were collected and analysed for crude protein, crude fat, ash, crude fiber, acid detergent fiber (ADF), neutral detergent fiber (NDF), carbohydrates, and minerals (calcium and phosphorus).

Grain samples from 1507 maize and non-GM control maize were collected and analysed for nutrient composition, including: crude protein, crude fat, crude fiber, acid detergent fiber, neutral detergent fiber, ash, carbohydrates, fatty acids, amino acids, minerals, vitamins, secondary metabolites, and anti-nutrients. Statistical analysis of agronomic characteristics and nutrient composition data was conducted using SAS/STAT software, Version 8.2 to generate analysis of variance (ANOVA), means, and standard deviations.

Two separate statistical analyses were carried out on the composition data. For the first analysis, the combination of data from all replicates and all locations were analysed. Least-square means and standard deviations were calculated for the data across all locations and statistically significant differences were identified using a t-test at a 5% level of significance.

For the second statistical analysis, the results obtained were evaluated on a per location basis using data from the 3 replicates of each maize entry at each location. The least-square means and standard deviations for each location and maize entry were calculated and a t-test was used to identify statistically significant differences at a 5% level of significance.

The baseline used for evaluation of natural variations

Publicly available data on commercial maize varieties was compiled from literature by the applicant and used as a baseline representing natural variation, in the comparisons performed for the sprayed and unsprayed maize 1507, and the non-GM control.

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

4.2 Compositional analysis

For each growing season, the results of compositional analyses were provided both for the individual sites and for the sites combined.

4.2.1 Feed

Forage samples were dried to between 3-7 % moisture before processing. The proximate and mineral analyses (fat, protein, acid detergent fibre (ADF), neutral detergent fibre (NDF), ash, carbohydrate, phosphorus, and calcium) of forage from maize line 1507 (glufosinate-treated and untreated) were comparable to forage from the non-transformed version of the hybrid and no statistical significant differences were obtained. The levels of protein,

carbohydrates, fat, and ash in 1507 maize forage were all within the reported literature ranges for maize forage. The average values for forage ADF and NDF are 30 % and 51 %, respectively in published literature; however, no ranges of values are available. The ADF and NDF levels in 1507 maize are similar to these average literature values. In summary, the analysis showed that forage from 1507 maize was comparable to forage from commercial maize.

4.2.2 Food

The compositional analysis of grain from 1507 maize and its control included proximate analyses (as for forage above), fatty acid composition [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3)], amino acids (twelve essential and six non-essential amino acids), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), vitamins (vitamin B1, vitamin B2, folic acid, and total tocopherols), secondary metabolites (inositol, raffinose, furfural, p-coumaric acid, and ferulic acid), and anti-nutrients (phytic acid and trypsin inhibitor). Grains from the 2000 season were in addition analysed for crude fibre, arachidic acid, provitamin A, and vitamin E.

In summary, the analysis of nutrient composition of 1507 maize grain (glufosinate-ammonium treated and untreated) during the 1998/1999 season, occasionally revealed statistically significant differences for some compounds. For example, 1507 maize grain contained higher overall levels of potassium (Table 2, Appendix 2), linoleic acid, linolenic acid (Table 3, Appendix 2), and tocopherols (Table 5, Appendix 2) compared to control, while fat (Table 1, Appendix 2), manganese (Table 2, Appendix 2), stearic acid, oleic acid (Table 3, Appendix 2), cysteine, methionine (Table 4, Appendix 2), and vitamin B1 (Table 5, Appendix 2), were lower in maize 1507.

The levels of protein, amino acids (Ala, Asp, Glu, Gly, His, Leu, Phe, Pro, Ser, Thr, Tyr, and Val), and potassium were increased, while the level of vitamin B2 was decreased, in 1507 maize grain (with and without glufosinate ammonium - treatment) compared to control in the field trials in 1999 (Tables 7 - 10, Appendix 2).

In the 2000 season, the nutrient levels in 1507 maize were compared to levels in the control and levels reported in the literature. In maize forage, statistically significant differences were observed for the proximates: fat, protein and carbohydrates when data were summarised across locations; however no trend was seen for the individual locations. In forage the minerals phosphorus and calcium were analysed. The mineral levels were within the literature ranges, both when averaged across all locations and at each individual location. However, at one single location the phosphorus content of the non- GM-control was significantly higher than in 1507 maize (Tables 11 and 12, Appendix 2).

In grains (2000 season), levels of the proximates: fat, protein, crude fiber, ADF, NDF, ash, carbohydrate, and moisture, were all within the reported literature values (Table 13,

Appendix 2). Across locations the protein content in 1507 maize not sprayed with glufosinate-ammonium was statistically significantly higher compared to the non-GM-control (10.9% vs 10.1% of DW). The amount of ash was statistically significantly higher in grain from both sprayed and unsprayed 1507 maize compared to the non-GM-control across locations. Some sporadic differences were also observed between 1507 maize grain and the non-GM-control at the single locations, however no trends were observed for any of these analytes across locations.

Both essential and non-essential amino acids were analysed in grain from maize 1507 (sprayed and unsprayed). Some statistically significant differences (Table 14, Appendix 2) were observed for the analysis across locations, but the results were not consistent with the analyses for the individual locations. Levels of all essential amino acids were within the reported literature range.

Grains from 1507 maize and control was analysed for the six major fatty acids in maize: palmitic, stearic, oleic, linoleic, linolenic and arachidonic acid, results are shown in Table 15 (Appendix 2). Summarised across all locations, the levels of oleic and linoleic acid were statistically significantly different from control (oleic was higher, linoleic was lower), in grain from maize 1507 not sprayed with glufosinate-ammonium. These differences were not observed at the individual sites. At two individual sites the linolenic acid levels were statistically significantly higher in 1507 maize grain compared to the control, this was however not a general trend. All fatty acid concentrations were within the reported literature values.

Levels of nine minerals (phosphorus, calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc) were analysed in sprayed and unsprayed 1507 maize grain and in the non-GM- control. The amount of copper was below LOQ. When comparing the levels of phosphorous, calcium, manganese and potassium in maize 1507 and control, statistically significant differences were found across locations. These differences were however not consistent when viewed on the individual location basis. All mineral levels in maize 1507 were within the values found in the literature.

Compared to literature values no significant differences were found for vitamin levels in 1507 maize grain. The content of vitamin B2 in grain from unsprayed 1507 maize was statistically significantly different from the control when data was summarised across all locations (Table 16, Appendix 2), this was however not observed at the individual sites. Some other differences were also noticed at the individual sites, but without a consistent trend.

The following secondary metabolites were analysed in 1507 maize (sprayed and unsprayed) and control; inositol, raffinose, furfural, P-coumaric acid and ferulic acid. The two potential anti-nutrients phytic acid and trypsin inhibitor were also analysed. The levels of raffinose were within the reported literature values (for the other secondary metabolites no such value exists). Levels of furfural and P- coumaric acid in the hybrid (both sprayed and unsprayed), were statistically significantly different from the control when analysed across locations

(Table 17, Appendix 2). These differences were not observed consistently at the individual sites.

The content of phytic acid in maize grain is typically low, and the concentration in 1507 maize grains was found to be within the values published in the literature (Table 17, Appendix 2). The level of trypsin inhibitor is also usually low, and was below LOQ for the assay in 1507 maize. The results show that the nutritional composition of 1507 maize (sprayed and unsprayed with glufosinate-ammonium) is comparable to commercial maize hybrids. Occasionally, statistically significant differences were observed for some of the components; however, these differences were not consistent over time and locations.

It has been suggested that lignin levels might be increased in transgenic maize lines expressing *B. thuringiensis* insecticidal proteins (Saxena & Stotzky, 2001a; Flores et al., 2005). However, a broader and more extensive study on lignin content in Bt-maize does not support this conclusion (Jung & Sheaffer, 2004). In addition, the levels of ADF and NDF, which includes lignin, in forage from 1507 maize were comparable with those in control maize and within the background range. Moreover, similar levels were observed for the lignin precursors p-coumaric acid and ferulic acid in grain from 1507 maize and control maize, except for a small but statistically significant difference in p-coumaric acid between glufosinate-ammonium treated 1507 maize and control maize in the 2000 season (Table 17, Appendix 2).

Aside from minor modifications, the selection of compounds analysed followed the recommendation of OECD (OECD 2002). During the Member State consultation under Article 6.4 of Regulation (EC) No. 1829/2003, it was suggested that additional compounds, including certain heavy metals, vitamins, and secondary metabolites, should be analysed. VKM is however of the opinion that such additional information would not add value to the data already provided, because of the high variability in content of some compounds (e.g. selenium and DIMBOA) due to either environmental conditions or the stage of plant development.

4.3 Agronomic and phenotypic characters

Notification C/ES/01/01

The notification C/ES/01/01, covering authorisation of maize 1507 for cultivation, import, feed and industrial processing, include results from field trials with maize 1507 from France, Italy and Bulgaria (2000), Spain (2002) and USA (1999).

Field trials in Europe 2000

Six field sites were employed in the study; all located within suitable maize-growing regions of Europe. The sites were located in France (3 sites), Italy (2 sites) and Bulgaria (1 site). Each site contained 3 blocks (replicates) with hybrid entries located randomly within each block. Each block contained 3 entries (TC1507, TC1507 treated with glufosinate-ammonium

and near-isogenic control). Glufosinate-ammonium was applied as a broadcast spray in sequential treatments. The first application (V4) was made at a rate of 0.50 kg ai/ha followed by a second application (V7) at a rate of 0.41 kg ai/ha. To prevent possible effects from herbicide drift, the unsprayed control was nested with the unsprayed TC1507 entry in each block. Agricultural practices (pesticide and fertiliser applications) were typical for commercial maize production in the regions chosen for this study. Besides the glufosinate-ammonium spray treatment, all maintenance and production practices were applied consistently across the entire trial site at each location.

According to the applicant, agronomic traits typically used when comparing maize lines in commercial maize breeding programs were evaluated in the field trials in France, Italy and Bulgaria in the 2000 growing season. The following traits were evaluated at each location: time to silking (accumulated heat units when approximately 50% of the plants in each plot are at silk stage), time to pollen shed (accumulated heat units when approximately 50% of the plants in each plot were shedding pollen), plant height (R6 stage), ear height (R6), stalk lodging, root lodging, final population (number of viable plants at the R6 growth stage), stay green (overall plant health –R6), disease incidence, insect damage and grain moisture.

Times to silking and pollen shed for the test line 1507 were 101% of the time required for the conventional control (Technical Dossier, Appendix 3A NF). Mean plant and ear height measurements for entry 1507 were 102% and 101%, respectively, of the heights observed in the control. Stay green, disease incidence and grain moisture of maize 1507 was 100 % of that observed in the control. There was no increase in stalk lodging, root lodging or differences in final populations for the test line when compared with the control. According to the applicant, the results indicate no unexpected differences in growth, development or performance between GM maize 1507 and the conventional control regardless of glufosinate-ammonium treatment.

4.4 Conclusion

Comparative analyses of maize 1507 to its non-GM conventional counterpart have been performed during multiple field trials located at representative sites and environments in Chile (1998/99), USA (1999) and in Europe (1999, 2000 and 2002). With the exception of small intermittent variations, no biologically significant differences were found between maize 1507 and the conventional maize.

Based on the assessment of available data, VKM concludes that maize 1507 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, except for the introduced characteristics, and that its composition fell within the normal ranges of variation observed among non-GM varieties. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of 1507 compared to conventional maize.

5 Food and feed safety assessment

5.1 Product description and intended uses

The genetic modification in 1507 field maize will not impact the existing production processes used for maize. All 1507 maize products will be produced and processed for use in food, animal feed and industrial products in the same way as other commercial maize. The 1507 field maize and all food, feed and processed products derived from 1507 field maize are expected to replace a portion of similar products from commercial maize, with total consumption of maize products remaining unchanged. The total anticipated intake/extent of use of maize and all food, feed and processed products derived from maize will remain the same.

5.2 Effect of processing

Food manufacturing of 1507 field maize includes many harsh processing steps, e.g. cooking, heating, high pressures, pH treatments, physical shearing, extrusion at high temperatures etc. under which the majority of DNA and proteins are denatured, which also applies to the Cry1F and PAT proteins and cry1F and pat genes (Dien et al. 2002; Hammond & Jez 2011, Fernandes et al., 2013). In the study performed by Fernandes et al (2013) it was shown that when baking the maize bread broa containing 11 % of 1507 maize and 20 % MON 810 maize flour, the baking process sheared DNA into small fragments less than 1000 bp, mostly the DNA was around 200 bp. In the unprocessed grain and all dry-milled fractions these proteins and DNA will probably be found in quantifiable amounts.

5.3 Toxicological assessment

The potential toxicity of 1507 maize expressing the cry1F and pat genes has been assessed in studies with rodents and broiler chicken.

5.3.1 Toxicological assessment of the newly expressed protein(s)

Phosphinothricin-N-acetyltransferase (PAT)

The PAT-protein originally obtained from *Streptomyces viridochromogenes* confers tolerance to the herbicidal active substance glufosinate-ammonium. The PAT protein has no known toxic potential. The PAT protein is enzymatically active but it has high substrate specificity to the active ingredient glufosinate. The PAT protein has already been found safe to human health during the assessment of glufosinate tolerant maize (VKM 2005a, 2005b, 2012a, 2012b; EFSA 2004a, 2012; US EPA 1995b, 2005, 2010).

Cry1F

Cry1F protein from *Bacillus thuringiensis* var. *aizawai* provide protection against certain lepidoptera target pests, such as European corn borer. The base sequence of the *cry1F* gene is modified to improve expression in maize, while the amino acid sequence of the translated Cry1F protein remains identical to the protein made by the bacteria. The expression of *cry1F* is regulated by the maize promoter *ubiZM1*. The Cry1F protein has already been found safe to human health during the assessment of insect resistant maize (VKM 2005a, 2005b, 2012a,b, 2013, EFSA 2004a, 2012, US EPA 2005, 2010).

Bacillus thuringiensis, from which Cry1F protein originates, has a history of safe use as a pesticide over several decades (US EPA 1995a, 1996, 2005, 2010). *B. thuringiensis* is a diverse group of Gram- positive, spore-forming bacteria, which were first discovered by Ishiwata in Japan on diseased silkworm, in 1901 (Federici et al 2006). It occurs naturally in the soil, and on plants including vegetables, cotton, tobacco, tree crops and forest crops (Cornell University 1996, Damgaard et al 1997). Several varieties of *B. thuringiensis* have been used as microbial insecticides since 1938 (Merritt, 1998). The subspecies *aizawai* (specific donor of the *cry1F* gene) is commercially used on crucifers such as broccoli to control wax moth larvae and various caterpillars, especially the diamondback moth caterpillar (Cornell University 1996; Schnepf et al. 1998).

Cry proteins originating from *Bacillus thuringiensis* have not been found to have harmful effects on the health of humans and animals (US EPA, 1995a, 1996, 2005, 2010; McClintock et al. 1995). In addition, there is no evidence for the presence of specific receptors in mammalian tissues for related Cry proteins such as Cry1A (Noteborn & Kuiper 1995; Kuiper et al. 2001).

5.3.2 Acute toxicity study

15 day intravenous acute exposure to PAT protein in rodents

Bayer Crop Sciences has performed an acute toxicity study of the PAT-protein in rats by a single intravenous administration. The study was performed in accordance with the principles of Good Laboratory practice (Organisation for Economic Cooperation and Development) Principles of Good Laboratory Practice, 1997, European Commission Directive 1999/1 I/EC, 1999, French decree n°98- 1312, regarding Good Laboratory Practice, December 31, 1998, E.P.A. (Environmental Protection Agency) 40 CFR part 160 Federal Insecticide, Fungicide and Rodenticide Act (FIFRA): Good Laboratory Practice Standards: Final Rule, August 17, 1989, and Good Laboratory Practice Standards for Toxicology studies on Agricultural Chemicals, Ministry of Agriculture, Forestry and Fisheries (M.A.F.F.), notification 12 NohSan n°8628, (December 06 2000).

The objective of this study was to assess the acute intravenous toxicity in OF1 mice of the PAT protein (>95% purity), a protein encoded by the *bar* gene. In addition, the acute intravenous toxicity of aprotinin (negative control) and melittin (positive control) were also

compared. Groups of 5 female OF1 mice were administered; PAT protein, aprotinin or melittin in physiological saline at dose levels of 1 and 10 mg/kg body weight.

All animals were observed for clinical signs daily for fifteen days whilst their body weights were measured weekly. No clinical signs were noted in PAT protein-treated animals or in control groups throughout the study period. The body weight evolution was unaffected by the treatment with either PAT protein at 1 and 10 mg/kg or control substances up to Day 15. At termination of the study period, animals were subjected to a necropsy including macroscopic examination. No treatment-related macroscopic abnormalities were detected in animals treated with either PAT protein at 1 and 10 mg/kg or control substances. The positive control (melittin), at 10 mg/kg, induced 100% mortality. Animals treated at 1 mg/kg of melittin and negative control animals treated with aprotinin at 1 and 10 mg/kg showed no visible signs of systemic toxicity (Hérouet et al. 2005).

Observations for mortality and/or clinical or behavioral signs of pathology as well as body weights were made during the course of the study, and gross necropsies were conducted at the end of the study. No mortality occurred during the course of the study. Additionally, no adverse clinical signs were observed during the study and no adverse findings were noted at necropsy. The range of doses tested in this study did not give rise to any toxicity and therefore the acute LD50 for Cry1F protein could not be determined.

14 day acute oral exposure to PAT protein in rodents

An acute oral toxicity study in mice was conducted using microbially-derived PAT protein (84% purity) prepared as a reference standard also for use in other analyses. Five male and five female CD-1 mice received 6000 mg/kg of the test material (containing approximately 5000 mg/kg PAT) as a 25% w/v suspension in aqueous 0.5% methylcellulose. Because the volume of the test material in methylcellulose exceeded 2 ml/100g body weight, the test material suspension was administered as two fractional gavage doses, given approximately one hour apart. Parameters evaluated during the two-week observation period included body weights and detailed clinical observation. All animals were examined for gross pathological changes. All mice survived to the end of the two-week observation period. All mice, except one female, gained weight over the duration of the study. There were no gross pathological lesions found in any of the animals. Under the condition of this study, the acute oral LD50 of PAT Microbial (FL) test material in male and female CD-1 mice was greater than 6000 mg/kg body weight (Brooks 2000). The results show that the acute oral LD50 of microbially- derived PAT protein in mice is greater than 5000 mg/kg.

14 day acute oral exposure to Cry1F protein in rodents

The potential toxicity of the Cry1F protein to humans and animals was examined in a study where Cry1F protein was evaluated for acute toxicity in CD1 mice (Kuhn 1998). The test substance, Cry1F *B.thuringiensis* subsp. *aizawai* Delta-toxin, was evaluated for its acute oral toxicity potential in mice when administered as a gavage dose at a level of 5050 mg/kg to males and females. The test substance was administered as a 15 % w/v concentration in

2% w/v aqueous carboxymethyl cellulose. The test substance consisted of *Bacillus thuringiensis* var. *aizawai* Cry1F protein at a net concentration of 11.4 %. The dose of Cry1F-protein was equivalent to 576 mg/kg body weight. No mortality occurred during the study. There were no clinical signs of toxicity exhibited at any time throughout the study. There was no significant effect on body weight gain. The gross necropsy conducted at termination of the study (day 14) revealed no observable abnormalities. The acute oral LD50, as indicated by the data, was determined to be greater than 5050 mg/kg of this microbially produced test substance. The actual dose administered contained 576 mg Cry1F protein/kg body weight, and at this dose, no LD50 was demonstrated as no toxicity was observed. The results show that the oral LD50 is greater than 576 mg/kg bodyweight in female and male mice.

5.3.2.1 Repeated-dose toxicity testing

Repeated dose 14-day oral toxicity study of PAT protein in rodents

PAT protein was administered by feed admixture in a powdered diet to Wistar rats at concentrations of 0 (group 1), 5000 (group 2), 50000 (group 3), and 0 ppm (group 4). Group 1 received a standard diet, where as groups 2 – 4 received a low-protein diet adjusted with a soya bean protein (Soyamin) to match the protein concentration of the group 1 diet (i.e. + 45000 ppm Soyamin in group 2, 0 ppm in group 3, and 50000 ppm in group 4). The average daily intakes of PAT protein in groups 2 and 3 were: 712, and 7619 mg/kg/day for male rats, and 703 and 7965 for female rats (Pfister et al. 1996; Hoechst Schering AgrEvo GmbH, RCC Project 616307, 1996, unpublished). The purity of the lyophilised PAT protein was assessed by SDS-PAGE analysis and estimated to contain 98% PAT protein, as described in the report by Pfister et al. (1996).

The results showed that food consumption and body weight were not influenced by the PAT treatment. There was no treatment-related mortality or behavior change observed in comparison to the control. Organ weights, gross pathology and histopathology findings did not indicate differences between treated and control animals. No changes were found in hematology or urine analysis. Immunological screening parameters indicated that PAT protein does not induce immunological effects.

Bayer Crop Sciences has also performed a sub-chronic oral toxicity study of the PAT-protein in rats (Pfister et al. 1999). The study was performed in accordance with the principles of OECDs Good Laboratory practice of OECD (Organisation for Economic Cooperation and Development) and Principles of Good Laboratory Practice, 1992. Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles, March 1986 and the Japanese Ministry of Agriculture, Forestry and Fisheries: On Good Laboratory Practice Standards for Toxicological Studies on Agricultural Chemicals, Agricultural Production Bureau, 59 NohSan Notification Number 3850, August 10, 1984. Test guidelines: The study procedures mostly conform to OECD Guidelines for Testing of Chemicals, number 407 "Repeated Dose 28-day Oral Toxicity Study in Rodents", adopted by the Council on July 27, 1995).

According to the OECD guidelines for testing of chemicals (OECD 1995) the duration of exposure should normally be 28 days although a 14-day study may be appropriate under certain circumstances; justification for use of a 14-day exposure period should be provided. The duration of this repeated dose oral toxicity study was 14-days, without providing any justification.

The study comprised four groups of five male and five female Wistar rats in each group. The rats in group 1 received a standard diet without PAT protein, whereas rats in group 2, 3 and 4 received diets with the inclusion of PAT and/or soybean protein: group 1 (standard diet), group 2 (0.5 % PAT + 4.5 % soybean), group 3 (5 % PAT), group 4 (5 % soybean), for a period of 14 days. The mean intake of PAT-protein in group 2 over the treatment period was 712 mg/kg body weight/day for males and 703 mg/kg body weight/day for females. In group 3 the mean intake of PAT-protein was 7965 mg/kg body weight/day for males and 7619 mg/kg body weight/day for females. The results showed no unscheduled deaths or clinical signs. Food consumption and body weights were unaffected by treatment. No treatment-related changes were seen in haematology or urinalysis parameters. Organ weight data, macroscopical and microscopical findings did not distinguish treated groups from controls.

The only changes which might be attributed to treatment were observed in clinical biochemistry parameters. They consisted of a slightly lower glucose level in males of group 4, slightly higher total cholesterol and phospholipid levels in males of groups 2, 3 and 4 and slightly higher triglyceride levels in females of group 4 when compared with rats of group 1. Animals of group 4 received no PAT- protein but - with respect to the protein content - a diet most similar to that of groups 2 and 3. The changes mentioned above were considered to reflect differences in the dietary composition and not related to the PAT protein itself. Further, when comparing the increased total cholesterol and phospholipid levels between group 3 (5 % PAT) and group 4 (5 % soybean) they were found to be within a similar range, which suggests a similar nutritional value of the proteins. The repeated dose toxicity study in rats gave no indications for adverse effects attributable to the PAT protein up to the highest dose tested. The results show that the LD50 of the PAT-protein is higher than 7965 mg/kg body weight.

The acute and repeated dose toxicity tests performed on rats and mice have not indicated toxic effects of the PAT or Cry1F proteins.

In whole food and feed the concentrations of these proteins are low, and acute toxic effects in humans and animals will most probably be negligible. Acute toxicity testing of the newly expressed proteins is of little additional value to the risk assessment of the repeated human and animal consumption of food and feed derived from GM plants. EFSA discourages the use of acute toxicity studies in risk assessments of GMOs (EFSA, 2011a).

5.3.3 Toxicological assessment of the whole plant

5.3.3.1 Design and performance of 90-day feeding study in rodents

90-day subchronic feeding study in rats

A 90-day feeding study was performed on Sprague-Dawley rats in accordance to OECD 408 guidelines with near isogenic control 33P66, non-GM commercial maize 33J56 and test diets (11% and 33% 1507 maize in feed) (MacKenzie et al., 2007). The concentration of Cry1F protein in the diet containing 33% grain from 1507 maize was at least 166 ng/g diet. The average daily intake of Cry1F protein by the rats was 0.0093 mg/kg body weight based on a consumption of 0.056 kg food/kg body weight/day.

The study showed no toxicologically significant differences between treatment groups. Observations included nutritional performance variables, clinical and neurobehavioral signs, ophthalmology, clinical pathology (haematology, clinical chemistry, coagulation, and urinalysis), organ weights, and gross and microscopic pathology. No diet-related differences were observed among the groups fed any of the different diets with respect to clinical signs of toxicity, ophthalmological observations, neurobehavioral assessments, clinical pathology, organ weights, and gross or microscopic pathology.

Additional whole food feeding studies that consider health effects of 1507 maize

The applicant has performed a 42-day broiler feeding study with emphasis on nutritional properties of 1507 maize, which also considers health effects. Additional feeding studies with maize 1507 include studies on pullets, Northern bobwhite quail, beef heifers, pigs and dairy cows. The studies are described in detail under section 5.5.2.

5.4 Allergenicity

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

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

35% identity over a segment of 80 or more amino acids, IgE cross-reactivity between the novel protein and the allergen should be considered a possibility.

5.4.1 Assessment of allergenicity of the newly expressed protein

The applicant has performed a weight-of-evidence approach (FAO/WHO 2001; Codex 2003) for an overall assessment of the IgE allergenic potential of the Cry1F and PAT proteins, which includes:

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

These assessments have previously been described by the applicant for Cry1F and PAT, and were based on the following aspects:

Cry1F and PAT

- The sources of the transgene genes are *Bacillus thuringiensis* subsp. *aizawai* (*cry1F* gene) and *Streptomyces viridochromogenes* (*pat* gene). These bacteria have no history of causing allergy.
- Cry proteins as microbial pesticides has a history of safe use (US EPA 2005, 2010), and there have been no indications of Cry proteins originating from *Bacillus thuringiensis* exhibiting harmful effects on human or animal health (US EPA 2005, 2010).
- The PAT protein has been subjected to previous safety assessments for genetically modified plants and found to have no allergenic potential (Herouet et al. 2005; US EPA 1997)
- The PAT protein has no homology to known toxins or IgE-allergenic proteins (Hérouet et al. 2005).
- The microbially produced Cry1F and PAT proteins were rapidly degraded in simulated gastric fluids *in vitro*.
- PAT and Cry1F do not resemble any characteristics of known IgE-allergens, and no significant homologies between the amino acid sequences of the PAT and Cry1F proteins and IgE-allergenic proteins have been found (Fard et al. 2013; Herouet et al. 2005; Kim et al. 2010; Randhawa et al. 2011; Meyer 1999; US EPA 2010).
- The PAT and Cry1F protein are not glycosylated (Herouet et al. 2005; Raybold et al. 2013; US EPA 2010)
- Cry1F and PAT are considered heat labile (Herouet et al. 2005; US EPA 2010)

The information listed above indicates that the newly expressed proteins in maize 1507 lack IgE allergenic potential with regard to human and animal health. However, it does not cover possible allergic reactions (e.g. enteropathies) that are not IgE mediated.

5.4.2 Assessment of allergenicity of the whole GM plant

Allergenicity of maize 1507 could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, e.g. through qualitative or quantitative modifications of the expression of endogenous proteins. However, given that no biologically relevant agronomic or compositional changes have been identified in field maize 1507 with the exception of the introduced traits, no increased allergenicity is anticipated for maize 1507. Moreover, maize is not considered a common allergenic food

5.4.3 Assessment of the allergenicity of proteins from the GM plant

It is the opinion of the VKM GMO Panel that a possible over-expression of any endogenous protein, which is not known to be allergenic, in maize 1507 would be unlikely to alter the overall allergenicity of the whole plant or the allergy risk for consumers.

5.4.4 Assessment of adjuvanticity

According to the EFSA Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed (EFSA 2010b) adjuvants are substances that, when co-administered with an antigen 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 potential role of these proteins as adjuvants should be considered. As for allergens, interactions with other constituents of the food matrix and/or processing may alter the structure and bioavailability of an adjuvant and thus modify its biological activity.

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

Studies with immunological mapping of the systemic and mucosal immune responses to Cry1Ac have shown that mice produce both systemic IgM and IgG and secretory IgA following intraperitoneal (i.p.), intragastric (i.g.) or intranasal (i.n.) immunisation, and that the adjuvant effects of Cry1Ac has been reported to be comparable to that of cholera toxin (CT) (Guerrero et al., 2004; Vazquez-Padron et al., 1999a, b; 2000; Moreno-Fierros et al., 2003). It is uncertain whether this applies to the same extent to other Cry proteins. A possible immunogenicity and adjuvanticity of Cry proteins has been considered by EFSA and VKM (EFSA, 2009a; EFSA, 2010b; VKM, 2012c).

“Bystander sensitisation”

“Bystander sensitisation” can occur when an adjuvant in food, or an immune response against a food antigen, results in increased permeability of the intestinal epithelium for other

components in food. Traditionally it was assumed that the epithelial cells of the intestine were permanently "glued together" by the so-called "tight junctions". Studies have however shown that these complex protein structures are dynamic and that they can be opened up by different stimuli.

Both *in vitro* and *in vivo* experiments have demonstrated that when an IgG response which can result in a complement activation (among other) is not balanced by an IgA response, the epithelial barrier may become leaky, allowing unwanted proteins to enter the body (bystander-penetration) and possibly lead to allergic sensitisation (Brandtzaeg & Tolo, 1977; Lim & Rowley, 1982).

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

5.5 Nutritional assessment of the GM food and feed

Compositional analyses of maize 1507 indicate nutritional equivalence to the non-GM control maize with a comparable genetic background and to the published range of values in the literature. The nutritional equivalence between maize 1507 and non-GM control maize has been further shown by the poultry feeding study, and other feeding studies described in section 5.5.2.

5.5.1 Intake information/exposure assessment

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

VKM has calculated a Theoretical Maximum Daily Intake (TMDI) for acute dietary consumption of Cry1F protein in maize and maize products. The mean level of Cry1F in grain was 69 pg/ μ g total extractable protein, or 6.9 μ g/g tissue dry weight. The TMDI is 30 μ g of Cry1F protein per adults per day, and 12 μ g per child per day.

These exposure estimates are based on the mean Cry1F protein expression levels reported for 1507 maize grain (6.9 μ g Cry1F per g grain dry weight) (Pavely, 2002).

These levels are several orders of magnitude below the levels shown to have no effect in laboratory toxicology testing. Also, these levels are considerably below the proposed threshold of toxicological concern (TTC) level of 1800 μ g/person/day (Class 1, oral exposure) for chemicals considered to have a low potential for toxicity based on metabolism and mechanistic data (Vermeire et al., 2010). Transgenic proteins produced by genetically modified plants are

generally considered non-toxic to humans. This dietary exposure assessment is very conservative, as it assumes that all consumed maize consists of maize 1507 and that protein levels are not reduced by processing.

5.5.2 Interpretation of the relevance of the animal studies

A 42-day feeding study of broiler chickens

A 42-day poultry feeding study was conducted to confirm the nutritional equivalence of the 1507 and 1360 Bt-maize with its non-transgenic maize (Mycogen Seeds control line 7250) and four non-GM commercial maize (available lot of grain grown during the 1999 season) (Zeph 2000). The non-transgenic maize 7250 has a genetic background representative of 1507 maize, but is not genetically modified and does not express either the Cry1F or PAT proteins.

This study was completed on November 2000 (annex 4NF, applicant dossier). A total of 245 male broilers, commercial strain of Cobb x Cobb, were randomly distributed into 49 pens at one day of age. Each pen contained 5 broilers. Birds were identified by a wingband indicating animal number. According to the OECD guidelines of animal feedstuffs derived from genetically modified plants (OECD 2003) broiler chicks are useful for comparative growth studies. Because of their rapid weight gain, broiler chicks are particularly sensitive to any change in nutrient supply or the presence of toxic elements in their feed and are particularly useful for this purpose.

The test, control and reference substance diet mixtures were fed continuously for 42-days. Broilers were fed starter feed on trial days 0-20 (54.21 % maize), and grower feed on trial days 21-42 (57.03 %). Cry1F maize 1507, Cry1F maize 1360, control hybrid 7250 and four non-GM commercial maize (samples of starter and grower feed) were analysed for the concentration of Cry1F protein. The protein was not detected in control substances, but Cry1F was present in both test substances. Cry1F concentration in 1507 maize was measured to 2.8 µg/g and in maize 1360 to 3.2 µg/g dry weight basis. No Cry1F was found in control line 7250. Samples of maize grain lots were analysed for proximates, amino acids and minerals. No analysis of mycotoxins (aflatoxin, deoxynivalenol, fumonisin, T2 toxin and zearalenone) was performed.

Data generated during the study was subjected to following statistical tests: For all parameters, a multi-factorial procedure was used to compare the means of treatment groups, using Analysis of Variance (ANOVA). Treatment means were compared for Least Significant Difference. Significant differences were reported at the $p \leq 0.05$ level. Parameters that were measured were mortality, body weight, body weight gain, feed conversion (corrected for body weight). Body weight, daily weight gain (gram per bird per day) and survival data were analysed to determine statistical differences between maize grain diets.

No statistically significant decreases in clinical findings of health were observed during the studied period. Consistent with historical data and study type, a low incidence of mortality

occurred among all study groups. The results showed that at day 0, start of feeding, there was no overall statistically significant difference in the mean body weight of broiler chicks in the different treatment groups. On trial day 42 there were no statistically significant differences in mean body weight among any of the seven treatments. Further, there were no statistically significant differences in mean feed conversion corrected for body weight among any of the seven treatments, and for cumulative unadjusted and adjusted feed conversion ratios at any time. Mortality was recorded daily between trial days 0-42. There were no statistical differences in mean percent mortality among any of the seven treatments. All survival rates were consistently high.

The results of the broiler feeding study show that there were no differences in the parameters tested between broilers fed a diet containing 1507 or 1360 maize grain, and a diet containing grain from either a non-transgenic control line or a standard diet containing non-GM commercial maize grain. The results show that maize 1507 and 1360 are equivalent to the four non-GM commercial maize in the ability to provide adequate nutrition to rapidly growing broiler chickens.

A 16-week feeding trial of laying hens

A 16-week feeding study was performed on laying hens. Grain from maize 1507 (Pioneer Hi-Bred Inc. and Dow AgroSciences LLC) was compared with its isoline equivalent and two conventional maize lines (Scheideler et al. 2008). All procedures used in this experiment were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee.

Healthy pullets (young hens; Bovans White) were obtained from a pullet supplier at 16 wk of age and fed a common diet to 21 week of age. The hens were randomly placed in cages (7 hens/cage at 68 in.²/hen). The hens were 21 weeks of age at the start of the feeding trial. A randomised complete block design was used. The number of pens per treatment was selected to adequately detect, at $P < 0.05$, a 5% difference from the mean using a type I error rate of 0.05 and a type II error rate of 0.20.

Cages (12 cages per treatment, 336 hens total) were randomly assigned to 4 dietary treatments. The 4 dietary treatments were as follows: a near-isoline control (same genetic background excluding the 1507 event), the transgenic maize containing event 1507, conventional maize 1 and conventional maize 2. The isoline control treatment was included to evaluate the effects of the gene addition, whereas inclusion of the conventional maize sources allowed an additional comparison between hens fed the transgenic 1507 grain and those fed commercially available nontransgenic maize. Nutrient analysis for maize varieties and soybean meal protein, amino acids, fat, fiber, and gross energy were conducted on samples submitted by Pioneer Hi-Bred International to Eurofins Laboratories; gross energy analysis was performed by Pioneer Hi-Bred International.

Nutrient analysis for dietary protein, fat, dry matter, ash, fiber, calcium, phosphorus, xanthophylls and amino acids was performed on each diet also at Eurofins Laboratories. Diets were fed for a 16-wk period divided into four 4-wk periods called phases.

Live hen weights were taken at the start of the study and every 4 week during the trial. Hens were fed *ad libitum*, and weekly feed intakes (g/hen per day) were calculated as follows: each week on the same day, excess feed in the feeders was weighed back to measure unconsumed feed, and that amount was subtracted from the amount added to the feeders.

Egg production (number and percentage per hen) was measured daily. On storage day-2 (2-d) egg weight was measured of the total egg production every 4 wk. Albumen, yolk and shell weights, Haugh units, and Roche color scores were recorded on eggs/pen every 4 week according to procedures as reported by Novak et al 2004. In the event of hen mortality, an evaluation was performed at the University of Nebraska Veterinary Diagnostic Laboratory to determine if mortality was treatment- related.

Three mortalities occurred during the experimental period. Deaths were not diet-related. Feed intake was not different between hens fed 1507 maize diet and those fed the diet formulated with its near- isoline control maize.

Egg production parameters for pullets fed 1507 maize grain were comparable to the respective values for hens fed diets formulated with non-transgenic grain. Egg quality measures were equivalent for the transgenic maize and near-isoline control maize, but differed among the conventional maize tested for egg albumen, Haugh units and egg yolk color scores. Overall, hens fed the diet formulated with maize 1507 performed as well as hens fed diets formulated with isoline or non-transgenic conventional maize.

A 5-day feeding study of juvenile Northern bobwhite quail

A 5-day dietary toxicity study was conducted on Northern bobwhite quail with 1507 maize (10% inclusion in diet) expressing Cry1F (Mycogen c/o Dow AgroScience project no.: 354-116, 1999). The level of Cry1F in maize 1507 feed ranged from 0.2-1.1 µg/g. Limit of detection by ELISA was 0.04

µg/g feed. The control maize was from a maize line that is genetically similar to maize 1507. All birds were 10 days old at the start of the feeding study. Each tested maize included six replicates of five birds each. The feed consumption was 5-13 g/quail/day. Following the five day exposure period all groups were given untreated basal diet for three days.

The results showed no acute treatment-related mortality or behavioral changes among birds fed the maize 1507 diet in comparison to birds fed the control diet.

This study is regarded as supplemental since the amount of maize tested is too low and the duration of the study too short to assess potential hazards to non-target birds from continuous exposure to higher levels of Cry1F protein through their diet (e.g. populations of birds residing near fields of 1507 maize).

A 118- day growth performance study of beef heifers

Sindt et al. (2007) reported the growth performance and carcass quality of 19 beef heifers fed finishing diets that were based on steam-flaked grain from maize 1507, 20 beef heifers fed its conventional counterpart (maize 33P66), 20 heifers fed the non-GM maize commercial variety 33J56 and 19 heifers fed the non-GM maize commercial variety 33R77. Each diet was fed to heifers that were randomly assigned to the four groups. The average body weight of the heifers was 360 kg. The study ran for 118 days.

The four maize lines were grown in the same field but in physically isolated plots. Maize 1507 received two sequential applications of glufosinate-ammonium-based herbicides. Nutrient composition was determined for the whole and flaked maize grain. Diets were formulated to meet nutrient requirements (NRC 2000) and the nutrient composition of each diet was determined. Most nutrient values were found to be similar for these four types of maize. The starch content of the test diets differed statistically, ranging from approximately 52% to 56%. It is not clear whether the presence of the Cry1F protein in the maize 1507 diet was verified. With regard to growth performance, there were no statistically significant differences in body weight, body weight gains or gain/feed ratio between treatment groups. Carcass traits, yield and quality grades were not different between treatment groups.

The results showed that beef heifers fed diets containing 1507 maize grain had similar growth performance and carcass traits compared with heifers fed diets containing grain from both the conventional counterpart and the two commercial non-GM maize varieties.

A 13- week growth performance study of pigs

Stein et al. (2009) reported the growth performance and carcass composition of growing and finishing pigs. Ninety-six pigs were allotted to 4 dietary treatments with 3 pigs/pen and 8 pen replicates/treatment. The 4 dietary treatments consisted of maize-soybean meal diets formulated with a commercial source of maize (bin run corn), standard maize (33J36), near isoline 33P66 maize, or 1507 maize.

A three-phase feeding program was used to meet the nutritional needs of pigs during the growing period (~ 3 mth). Diets were formulated by mixing maize, soybean meal, soybean oil, vitamins and minerals. The inclusion rate of maize was approximately 65%, 73% and 81% in the grower (up to 60 kg), early finisher (60 - 90 kg) and late finisher (90 – 120 kg) diets, respectively. Biochemical analysis and gross energy determination of the diets confirmed that they met the nutrient requirements (NRC 2000). Animals were fed *ad libitum* until their body weight reached approximately 120 kg. Average daily gain, average daily feed intake, and gain/feed ratios were calculated throughout the entire study to measure growth performance. Live weights at slaughter were determined, standard carcass measurements (hot carcass weight, 10th-rib backfat thickness, loin eye area, and loin eye depth) were performed, and dressing percentage and lean meat percentage calculated.

The results showed that pig performance was not affected by dietary treatments in any of the three phases or in the overall experimental period. No effects of dietary treatment on any carcass parameter were observed.

Feeding study in dairy cows

Twenty lactating dairy cows were used in a single cross-over design in which there was 2 feeding periods lasting for 28 days each (Faust et al., 2007). The aim was to compare the effect of using maize silage and maize grain derived from 1507 maize on feed intake and milk production when compared with maize silage and maize grain derived from a non-GM control hybrid, isogenic to maize 1507, without the *cry1F* or *pat* genes.

Diets contained on average 43.0% dry matter (DM) maize silage and 22.1% concentrate of which 70.2% was in the form of ground maize. Other feed ingredients included alfalfa hay, soybean meal, and cotton seeds. The diet composition was analysed for proximates, minerals (Ca, P, Mg, K), mycotoxins (nivalenol, deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, fusarenon X, diacetoxyscirpenol, scirpentriol, 15-acetoxyscirpentriol, T-2 toxin, iso-T-2 toxin, acetyl-T-2 toxin, T-2 triol, t-2 tetraol, HT-2 toxin, neosolaniol, zearalenol, and zearalenone) and silage fermentation products and found to be similar for both treatment groups.

Cry1F was detected in 1507 maize grain and silage. PAT was not detectable in grain, and ranged from not detectable to slightly above the detection threshold in forage, of 1507 maize. The following measurements were made: (1) Physical (weekly): body weight, body condition score, temperature, pulse, feed intake; (2) Milk production (daily); (3) Milk composition (weekly): protein, fat, dry matter, lactose, urea N, somatic cell count, Cry1F; (4) Chemical and haematological blood analysis (prior to and at the end of both trials).

One cow was positive for the presence of Cry1F in milk prior to and during both treatments, which therefore can be considered a false positive ELISA-reaction. No differences were detected in milk production, milk composition, or cow health as indicated by physical measures, blood chemistry, and hematological analyses between dairy cows fed diets containing maize grain plus whole-plant maize silage from maize 1507 and dairy cows fed grain plus silage from its near-isoline counterpart. Health and productivity of lactating dairy cows were not different between cows fed maize silage plus grain feed containing Cry1F and cows fed a control maize silage plus grain.

The results of this study showed no significant differences in health parameters or milk quality and no significant differences between dietary treatments, indicating nutritional equivalence between the 1507 maize and the non-GM control maize.

Notably, the body condition scoring technique (BCS), used in the feeding study in dairy cows serves as a useful, easy-to-use management tool to determine the nutritional needs of a cow herd. Using a numeric scoring system from 1 to 9, cattlemen can evaluate cows in the field to estimate body energy reserves. This information can be used to adjust feeding strategies

to reach optimal BCS. Thus, monitoring body condition greatly affects reproductive performance as well as feeding efficiency.

New studies 2013-2017

To the best of our knowledge, PubMed search as well as literature searches in the EFSA Journal and Google Scholar did not identify newly published information deemed relevant for human and animal health effects of maize 1507, other than previously described in VKMs report published in 2014, presented herein.

5.6 Conclusion

Whole food feeding studies on rats, broilers, pullets, pigs and cattle have not indicated any adverse health effects of maize 1507. These studies further indicate that maize 1507 is nutritionally equivalent to conventional maize. The PAT and Cry1F proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE mediated allergic reactions. Some studies have however, indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, VKM concludes that maize 1507 is nutritionally equivalent to conventional maize varieties. It is unlikely that the PAT and Cry1F proteins will introduce a toxic or allergenic potential in food or feed based on maize 1507 compared to conventional maize.

6 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 annual cropping areas of forage maize of about 2000-2800 decares (Netland et al., 2013). This is equivalent to less than 0.1% of the areas with cereal crops.

In the period 2010-2016, the area of sweet corn for human consumption varied between 84 and 709 decares (Statistics Norway, 2017). According to Debio, the Norwegian control body for organic crop production, there are no cropland under organic management certified for maize production in Norway (Lene Nilssen pers. com. 2017). 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 FEu/kg TS; <http://www.grovførnett.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 recently, unpublished studies (T. Lunnan pers. com., 2012).

In the traditional livestock districts in Norway, the growing season is too short, such that forage maize can be a real alternative to other forage productions (Netland et al., 2013). The major prerequisites for a significant increase in the maize cultivation are therefore a higher proportion of livestock production in the southeastern Norway, improved varieties and technology that enables earlier sowing (Bakken et al., 2005; T. Lunnan pers. com., 2012).

Climate change, which entails a longer growing season and higher average temperatures, however, may expand the maize cultivation area in Norway in the long term.

7 Environmental risk assessment

7.1 Unintended effects on plant fitness due to the genetic modification

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 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 maize for human consumption (see section 6.0).

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 0°C 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; Palauelmás 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 (Palauelmás 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 very unlikely that the establishment, spread and survival of maize 1507 would be increased due to the insect resistance and herbicide tolerance traits. The insect protection against Lepidoptera is not regarded to provide 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 7.3), and this trait cannot be regarded as a potential selective advantage to maize 1507. Moreover, we consider it very unlikely that maize 1507 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 present European environmental conditions. The herbicide tolerant trait can only be regarded as providing a selective advantage for the GM maize plant where and when glufosinate ammonium-based herbicides are applied. Glufosinate ammonium-containing herbicides have been withdrawn from the Norwegian market since 2008, and the substance will be phased out in the EU in 2017 due to reproductive toxicity.

Field trials carried out by the applicant do not indicate altered fitness, persistence and invasiveness of maize 1507 relative to its conventional counterpart. A series of field trials with maize 1507 were carried out in Chile, USA, France, Italy and Bulgaria from 1998 to 2002. Information on phenotypic (e.g. crop physiology, morphology, development) and agronomic (e.g. grain yield) characteristics was provided to assess the agronomic performance of maize 1507 in comparison with its conventional counterpart (see section 4.3). No differences in the general appearance of the plants or other phenotypic differences that would indicate unexpected pleiotropic effects of the genetic modification were found in these studies.

VKM is not aware of any scientific reports indicative of increased establishment or spread of maize 1507, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of maize 1507 are unchanged, insect resistance and herbicide tolerance is not likely to provide a selective advantage outside of cultivation in Europe, VKM is of the opinion that the likelihood of unintended environmental effects based

on the establishment and survival of maize 1507 will not differ from that of conventional maize varieties.

7.2 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 (Ryffel, 2014; Trtikova et al., 2015). Transgenic DNA is also a component of a variety of food and feed products derived from maize 1507. 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 that 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.

7.2.1 Plant to micro-organisms gene transfer

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

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 1507 to unrelated species such as bacteria.

It is, however, pointed out that there are limitations in the methodology used in field sample based studies (Nielsen & Townsend, 2004) and that experimental studies of limited size and duration should be interpreted with caution given the scale and exposure differences between 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). Oral intake of genetically modified soybean has 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, VKM considers it unlikely for the introduced genes in maize 1507 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 *cry1F* and *pat* genes from 1507 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.

7.2.2 Plant to plant gene flow

7.2.2.1 *Reproduction biology*

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 evidence suggests that honeybees 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 (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 and 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).

7.2.2.2 *Pollen-mediated gene flow*

Numerous studies have been conducted on pollen dispersal and outcrossing in maize (for a review, see BEETLE report, 2009; Brookes et al., 2004; Devos et al., 2005; Eastham & Sweet, 2002; Feil & Schmid, 2002; Sanvido et al., 2008; Nieh et al., 2014; Nadal et al., 2016; Ricci et al., 2016; Stachow et al., 2013). However, a general interpretation of the results is often difficult because of significant methodological differences and experimental conditions between studies and various environmental factors that are known to influence cross-fertilization rates (Ingram, 2000; Devos et al., 2005; Trtikova et al., 2015). 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.

The field trial data are often restricted to small-scale releases and the range of studied conditions (landscape patterns, climate and crop management) is limited. Gene flow modelling at the landscape level makes it possible to take into consideration additional factors affecting gene flow. It also allows the prediction of adventitious presence under different environmental and agronomic conditions, including large-scale adoption (Schenkelaars & Wesseler, 2016).

A number of abiotic and biotic parameters are known to influence outcrossing rates in maize (Hüsken et al., 2007; Sanvido et al., 2008; Palauelmás 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 influence the percentage of GMO detected, this effect is moderate (Palauelmás 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 (Devos et al. 2009, 2014).

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 (Eastham & Sweet, 2002; Messeguer et al., 2006). 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 (size/weight), 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. There is, however, 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 et al., 2004; Devos et al., 2005, 2014; Hüsken et al., 2007; Sanvido et al., 2008; Riesgo et al., 2010; Palaudelmás et al., 2012)

In a recent study, Hofmann et al. (2014) aimed to analyse data on maize pollen deposition in relation to the distance from the nearest maize field. The authors employed a standardised method to record maize pollen grains at 216 sites in Germany, Switzerland and Belgium from 2001 to 2010, using a pollen mass filter (PMF) sampler. The study confirms that the highest pollen deposition is within the nearest maize field and decreases with increasing distance from this field. Maize pollen was sampled up to 4.45 km from the nearest maize field and this made it possible to gather an extended dataset on pollen dispersal. The 95 % confidence interval for a predicted value of pollen deposition spans almost two orders of magnitude. Hofmann et al. (2014) also discussed the implications of their study on previous risk assessments of Bt-maize and the associated recommendations for mitigation measures. The pollen dose–distance distribution curve used by Perry et al. (2010, 2011, 2012) differs

significantly from that used by Hofmann et al. (2014) for long distances. According to Hofmann et al. (2014), this difference leads to the underestimation of maize pollen deposition over long distances.

Sanvido et al. (2008) reviewed existing cross-fertilisation studies in maize and established relevant criteria for the evaluation of these studies and applied criteria to define science-based separation distances. The results of their analysis showed that a separation 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 separation 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 separation 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 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).

7.2.2.3 *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 areas in Europe is rare (see section 7.1). 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 1507, 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. (Palauelmás 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, Chile and the USA do not indicate altered agronomic or phenotypic characteristics of maize 1507, except for the specific target pest resistance (Pioneer, unpublished data).

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. VKM is of the opinion that the likelihood of unintended environmental effects as a consequence of gene flow from maize 1507 is negligible.

7.2.2.4 National proposals for coexistence

Coexistence refers to the choice of farmers and consumers between conventional, organic or GM based crop production, in compliance with the legal obligations for labelling and/or purity standards. Coexistence always refers to GM plants that have passed the authorisation process. Therefore, environmental risks or risks to human or animal health do not concern the formulation of coexistence rules. EU regulations have introduced a 0.9 per cent labelling threshold for the adventitious presence of approved GM material in non-GM products.

A number of the EU member states (MS) have implemented coexistence regulations to ensure that GM and non-GM crops can be cultivated side by side without excluding any agricultural option. As cross-fertilization due to pollen flow between neighbouring fields represents the major potential biological source of on-farm mixing in maize (e.g. Sanvido et

al., 2008), most member states have proposed separation distances as the main coexistence measure to comply with legal tolerance threshold requirements (EC, 2009). Some MS differentiate additionally between distances to conventional non- GM crops, organic crops and crops for seed production. Separation distance can be used as the unique requirement, as in Germany and Denmark, or in conjunction with buffer zones that often allow a reduction of the distance requirement (Schenkelaars & Wesseler, 2016).

An overview of mandatory separation distances adopted by EU member states shows a considerable range of variation, with respect to separation distances between GM and non-GM maize fields (Schenkelaars & Wesseler, 2016). Spain, the country with the highest share of GM maize cultivation in the EU requires the lowest separation distance for maize (20 m), while Bulgaria has the largest distances for maize (600- 30 000 m). Hungary imposes a minimum distance of 400 m that can be extended on a case- by- case approval process.

In 2007, the Norwegian Food Safety Authority requested VKM to assess coexistence measures at farm level in different GM crop production systems (VKM, 2007). In this report, VKM concluded that separation distances of 200 m most likely would ensure an upper limit of 1 % of adventitious presence as a result of introgression via pollination in maize. In general, VKM assumed that the adventitious presence, most likely, would be less than 0.3% than in the range of 0.3 to 1.0%.

These assessments were based on the maize used being heterozygote for the inserted genes and that the maize plants are harvested as whole plants for ensilage or direct feed (maize grains constitute maximum 50 % of the silage/yield).

7.3 Interactions of the GM plant with target organisms

Genetically modified maize 1507 has been developed to provide protection against certain lepidopteran target pests, such as the European corn borer (ECB, *Ostrinia nubilalis*), and some species belonging to the genus *Sesamia*. The insect resistance is achieved through expression of a synthetic version of the truncated *cry1F* gene derived from *Bacillus thuringiensis* subsp. *aizawai*, a common soil bacterium.

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 NIBIO has never received samples of this pest or plant material damaged by this pest (K. Ørstad, pers. com.). Consequently, there are no insecticides authorised or previous applications for registrations of insecticides against this herbivore in Norway.

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

7.3.1 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 Cry1F 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.

Field resistance is defined as a genetically based decrease in susceptibility of a population to a toxin caused by exposure of the population to the toxin in the field (Andow, 2008). 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 Cry protein 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.

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 maize 1507 *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.

Monitoring data from five continents reported in 41 studies that evaluate responses of field populations of 11 lepidopteran pests to four Cry proteins 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 the noctuid species; *Spodoptera frugiperda*, *H. zea* and *Busseola fusca*. 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 first instance of field resistance to Bt-maize has been reported in a population of the African stem borer (*B. fusca*) in South Africa, where some larvae were able to survive on Cry1Ab-expressing maize (EFSA, 2011d). It appeared that the field resistance in stem borer in this area has resulted from a combination of a late general planting date with consequent increased levels of infestation and variance in time of planting providing a continuous supply of moths (Kruger et al., 2009). A survey by Kruger et al. (2011) revealed that compliance with refugia requirements in the region was low especially during the initial 5-7 years after release and high number of farmers applied insecticides as preventative sprays on Bt-maize and refugia irrespective of stem borer infestation levels.

The second instance concerns *S. frugiperda*. Larvae surviving on Cry1F-expressing maize in some fields on an isolated tropical island in the USA (Puerto Rico) were collected and exposed to high concentrations of the Cry1F protein in laboratory bioassays, where no mortality was observed (Matten et al., 2008; Moar et al., 2008; Tabashnik, 2008; Tabashnik et al., 2008). Storer et al. (2010) and Velez et al. (2013), confirmed via laboratory bioassays that *S. frugiperda* collected from the affected area exhibited lower sensitivity to the Cry1F protein compared with typical colonies from other regions, and that the resistance was shown to be autosomally inherited and highly recessive. The unusual combination of biological, geographic, and operational factors (such as high selection pressure for resistance by continuous silage maize production with sequential year-round plantings, high level of overall *S. frugiperda* pest pressure during the year of observing its damage on Cry1F expressing hybrids, drought conditions reducing availability of alternative host plants that encouraged movement of the adult and larval populations into irrigated agricultural maize fields) led to *S. frugiperda* evolving resistance to the Cry1F protein in Puerto Rico. Moreover, no insect resistance management (IRM) measures were put in place at that time in Puerto Rico.

Storer et al. (2012a) provided an update on the status of the previously reported instance of field-evolved resistance to Cry1F-expressing maize in Puerto Rico. Resistant populations in Puerto Rico and susceptible ones in the southern USA were further monitored, showing high

levels of Cry1F resistance and full susceptibility, respectively. The authors concluded that the resistant populations have not spread to any measurable extent from Puerto Rico to mainland USA, and that local selection from Cry1F-expressing maize in the southern USA has caused no measurable change in population susceptibility. However, these data indicate that resistance may persist in a population, and that slowing the spread of resistance genes is more practical than eradicating resistance. Therefore, the authors advocated the deployment IRM measures to delay the evolution of resistance, and to manage the sustainable use of Bt-crops.

Monitoring data indicate that neither in the EU, nor in the USA, have populations of resistant *O. nubilalis* or *Sesamia nonagrioides* been found. 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 deployment of pyramided *Bt*-crops will potentially delay resistance development. According to Storer et al. (2012b), pyramiding in the same plant of two or multiple *Bt*-toxins, acting independently on target insect pest midgut receptors, is expected to delay the evolution of resistance to either Cry protein effectively when most individuals that are resistant to one Cry protein are killed by the other, and when selection for resistance to one of the Cry protein does not cause cross-resistance to the other.

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*.

Xu et al. (2010) and Crespo et al. (2011) investigated the potential for cross-resistance between Cry1Ab and Cry1F. Laboratory-selected Cry1Ab-resistant *O. furnacalis* and *O. nubilalis* colonies were shown to exhibit low levels of cross-resistance to Cry1F, ranging between < 4- and 6-fold, respectively.

Several recent laboratory studies have demonstrated the development of resistance and cross-resistance to Cry-proteins in various species including *Ostrinia furnacalis* (Zhang et al., 2014, Wang et al., 2016), *Helicoverpa zea* (Welch et al., 2015) and *Diatraea saccharalis* (Girón-Pérez et al., 2014, Huang et al., 2015).

Omoto et al. (2015) have registered field resistance (decreased susceptibility) to Cry1Ab in Fall armyworm (*S. frugiperda*) in Brazil. The authors conclude that the Cry1Ab resistance can be the result of exposure to this protein, but that it is indistinguishable from cross-resistance developed from exposure to Cry1F. Jakka et al. (2014) had very similar results in a *S. frugiperda* population from Puerto Rico, USA.

7.4 Interactions of the GM plant with 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, 2010a). 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 characterization is required to fully characterize the possible risk (EFSA, 2010a).

7.4.1 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 honeybees (*Apis mellifera* 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).

The applicant assessed possible adverse effects of the Cry1F protein on pollinators. In lower-tier dietary bioassays with *Apis mellifera* using either purified Cry1F protein or Cry1F-containing maize pollen incorporated into diet, no adverse effects on larval survival or adult behaviour were reported (EFSA, 2011d). In a peer-reviewed paper assessing the impact of the Cry1F protein on honeybee, Hanley et al. (2003) came to similar conclusions as those reported by the applicant. Feeding honeybee larvae with the Cry1Ab- or Cry1F-containing maize pollen did not affect larval mortality, pupal mortality, pupal weight or haemolymph protein concentration, compared with larvae fed regular bee-collected pollen or non-transgenic maize pollen.

A number of studies on effects of purified or pollen-enclosed single Cry proteins demonstrate that there is to date no indication of acute or chronic toxicity either for larvae or adult bees (ref. BEETLE Report, 2009, Duan et al., 2008, Malone & Burgess, 2009, Hendriksma et al.,

2011; 2013; Lima et al. ,2011; 2013; Grabowski & Dabrowski, 2012; Dai et al., 2013; Geng et al., 2013; Niu et al., 2013).

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.

Hendriksma et al. (2013) examined the response of nurse bees and their gut bacteria to pollen from Bt maize expressing three different insecticidal Cry proteins (Cry1A.105, Cry2Ab2, and Cry3Bb1). Colonies of *Apis mellifera carnica* were kept during anthesis in flight cages on field plots with the Bt maize, two different conventionally bred maize varieties, and without cages, 1 km outside of the experimental maize field to allow *ad libitum* foraging to mixed pollen sources. Honey bee nurses which were forced to cover their full protein demand by pollen from a stacked Bt maize showed no apparent effects on survival rates, body weight and pollen digestibility. The community structure of the gut bacteria significantly responded to the different pollen diets, but differences found with the Bt maize pollen were in the range of those occurring between pollen from conventionally bred varieties or mixed pollen sources. The relatively low Cry protein concentration measurements compared to the high exposure of nurse bees indicate that the recombinant proteins were actively digested. The natural occurrence of Cry proteins in the gut of nurse bees with no exposure to Bt maize and the lack of detectable effects on nurse bees and their gut bacteria give no indication for harmful effects of this Bt maize on honey nurse bees.

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 honeybees (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. (2008), 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 protein 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.

7.4.2 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 (EFSA, 2009b).

The applicant reported on a number of higher-tier studies. These studies did not reveal adverse effects on the number and abundance of beneficial and non-target arthropods associated with the cultivation of maize 1507, though in some cases fewer parasitic hymenoptera were observed.

Higgins et al. (2009) conducted a 3-year field study with maize 1507 at four locations in the USA, and surveyed a large group of non-target arthropods, including predators, parasitoids, herbivores and detritivores. The range of sampled taxa (including ladybird beetles, lacewings, rove beetles, ground beetles, aphids, thrips, springtails, parasitic wasps, spiders) can be considered sufficiently representative (in functional terms) of maize ecosystems in Europe. Visual counts on maize plants, sticky traps, pitfall traps and litterbags were used to sample specific groups of NTOs. Field data were analysed with a multivariate method to account for general community level responses, whereas an analysis of variance on individual taxa was performed when species abundance was sufficiently high to detect statistically 50% differences. No significant differences in abundance were observed between arthropod assemblages in maize 1507 and its near-isogenic control in any of the field experiments. The first component of the multivariate analysis explained on average the high percentage of 60.3 of the overall variability. All taxa contributed similarly to indices of community abundance and analysis of single taxa always produced differences falling between confidence limits of taxon abundance, thus indicating no significant treatment effects.

Analysis of soil dwelling rove beetles (Coleoptera: Staphylinidae) in experiments with cultivated maize fields containing the Cry proteins, Cry34/35Ab1 and Cry1F during one

growing season indicated that GM maize varieties expressing Cry34Ab1, Cry35Ab1 or Cry1F proteins have no impact on rove beetles through their diet (Balog et al., 2011).

In a large scale experiment in Poland in the 2008-2010 growing seasons, Twardowski et al. (2014) monitored the population density of surface-active invertebrates of the Staphylinidae family. The average number of rove beetle populations in Bt maize expressing Cry1Ab protein were shown not to differ significantly from the number of beetles in conventional maize fields.

A four year on farm study was conducted in the Philippines between 2006 and 2009 to investigate if Bt maize (Cry1Ab) has long term effects on arthropod communities in commercial farms and in adjacent riparian areas (Alcantara, 2012). The arthropod composition was monitored through visual inspection in commercial farms and through sweep sampling in nearby riparian areas. Results of the sampling revealed that the abundance and diversity of arthropods were similar in Bt and conventional farms and in adjacent areas.

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.

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).

Mason et al. (2008) studied whether pollen from Cry1F-expressing maize event 1507 and Cry1Ab- expressing maize events MON 810 and Bt176 causes adverse effects to adult *Chrysoperla plorabunda*. Adult lacewings are not predacious, but are prevalent pollen consumers in maize fields (Meissle et al., 2012), so they could be exposed to the Cry1F protein contained in the pollen when feeding on pollen. Males fed pollen from maize 1507 showed a trend for living longer than males fed non-Bt-maize pollen. Such a trend was not observed for females fed pollen from maize 1507 or non-Bt-maize. The mean number of eggs produced per female per day was similar for those fed maize 1507 pollen compared with females fed pollen from non-Bt-maize. No difference in total egg production was

observed between females fed pollen from maize 1507 or non-*Bt*-maize. These results confirm that Cry1F and pollen of maize 1507 are not toxic to *C. plorabunda*

Tian et al. (2012) evaluated the potential impact of Cry1F-expressing maize on some life-history parameters (development time, weight) and reproductive parameters (fecundity, fertility) of the predatory ladybird beetle *Coleomegilla maculata* in a tri-trophic study. *C. maculata* larvae were fed Cry1F-resistant FAW larvae reared on leaves of maize 1507 or its near-isogenic line. Cry1F-resistant *S. frugiperda* were used to overcome prey-mediated effects. The authors found no difference in life-history and reproductive parameters of *C. maculata*. ELISA analyses confirmed the uptake of Cry1F by the ladybird beetle larvae, as larvae contained 20-32 ng Cry1F/g by fresh weight. The authors concluded that Cry1F protein did not accumulate but was strongly diluted when transferred through trophic interactions. These results confirm that Cry1F is not toxic to *C. maculata*.

In laboratory experiments using aphids (*Myzus persicae*) that were reared on an artificial diet containing Cry1F as prey for adult ladybird beetle (*Harmonia axyridis*), Paula et al. (2015) found that the beetles sequestered the Cry1F and passed the Cry1F to the offspring (eggs and neonate larvae). No detrimental effects were found on age of first reproduction, total number of eggs laid per female, age-specific fecundity, egg development time, hatching rate or fertility rate.

Zhang et al. (2014) developed a rape seed pollen-based diet suitable for use in a dietary exposure assay for *Propylea japonica*. Using the diet, the authors established and validated a dietary exposure assay by using the protease inhibitor E-64 as positive control. Dose-dependent responses were documented for all observed life-table parameters of *P. japonica* including survival, pupation and eclosion rates, development time and adult weight. Results suggested that the dietary assay can detect the effects of insecticidal compounds on the survival and development of *P. japonica*. Using the established dietary assay, Zhang et al. subsequently tested the toxicity of Cry1Ab, Cry1Ac and Cry1F proteins that are expressed by transgenic maize, cotton or rice plants to *P. japonica* larvae. The diet containing E-64 was included as a positive control. Survival and development of *P. japonica* larvae were not adversely affected when the diet contained purified Cry1Ab, Cry1Ac, or Cry1F at 500 µg/g diet representing a worst-case exposure scenario. In contrast, *P. japonica* larvae were adversely affected when the diet contained E-64. The bioactivity and stability of the Cry proteins in the diet and Cry protein uptake by the ladybird larvae were confirmed by bioassay with a Cry-sensitive insect species and by ELISA. The current study describes a suitable experimental system for assessing the potential effects of gut-active insecticidal compounds on ladybird beetle larvae. The experiments with the Cry proteins demonstrate that *P. japonica* larvae are not sensitive to Cry1Ab, Cry1Ac and Cry1F.

The predator *Orius 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 Cry protein, 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.

In a study under laboratory conditions, Lumbierres et al. (2012) examined the effects on the reproduction and nymphal development of *O. majusculus* caused by feeding on Bt plant materials (Cry1Ab) and on herbivore prey fed on Bt plants in three experiments. To measure the effects of Bt maize plants on *O. majusculus* fecundity and fertility, nymphs were provided with combinations of Bt or non-Bt pollen and leaf in addition to prey, and egg laying and egg hatching in the resulting adults were measured. Second, the effect of Bt vs. non-Bt pollen and leaf with no additional prey on nymphal development, survival, sex ratio and teneral adult weight and size were measured. Third, prey-mediated effects of Bt protein on nymphal developmental time, survival, sex ratio and teneral adult weight were evaluated using *Tetranychus urticae* Koch fed on Bt and non-Bt maize plants as prey. The study confirmed that ingestion of Bt protein by *O. majusculus* via plant leaves or pollen or via the food web has no negative effects on predator survival, development, fecundity and fertility. On the contrary, in such circumstances a positive effect on fecundity and developmental time of the predator was observed. Fecundity was increased when they feed on Bt plant material, and nymphal development was shortened when nymphs were fed on Bt-containing spider mites and when they were fed on Bt plant material in the absence of lepidopteran eggs prey.

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 protein 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 reveal 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 aphids 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.

Li et al. (2014) used an artificial diet system to study the toxicity of Cry1Ab, Cry1Ac and Cry2Aa proteins, produced by transgenic maize, cotton or rice plants on larvae of the green lacewing *C. sinica*. The concentrations of the proteins were 200 µg Cry protein/g FW of diet to the larvae, which can be regarded as a worst-case exposure scenario. The Cry protein in the diet was >>10-times higher than the Cry protein concentration likely encountered by green lacewing larvae in the field; in Bt crop fields, lacewing larvae feed mainly on aphids that contain only trace amounts or no amount of Cry protein. The experiments revealed no detrimental impact of the Cry proteins on any of the life-table parameters measured.

In a study of Tian et al. (2013), they expand on the previous work on lacewings by using different hosts, multiple toxins and several Bt plant species. Two different Bt-resistant Lepidoptera species, *Trichoplusia ni* and *S. frugiperda*, were used to assess the direct toxic effects of Cry1Ac produced in Bt broccoli, Cry1Ac/Cry2Ab produced in Bt cotton, and Cry1F produced in Bt maize on larvae of the green lacewing *Chrysoperla rufilabris*. Larvae of these species were fed Bt plants or non-Bt plants and then exposed to redaceous larvae of *C. rufilabris*. Fitness parameters (larval survival, development time, fecundity and egg hatch) of *C. rufilabris* were assessed over two generations. There were no differences in any of the fitness parameters regardless if *C. rufilabris* consumed prey (*T. ni* or *S. frugiperda*) that had consumed Bt or non-Bt plants. Additional studies confirmed that the prey contained bioactive Cry proteins when they were consumed by the predator. According to the authors, this study confirm that Cry1F, Cry1Ac and Cry2Ab do not pose a hazard to the important predator *C. rufilabris*, and demonstrates the power of using resistant hosts when assessing the risk of genetically modified plants on non-target organisms.

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, which 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 assume that the aphids did not contain the toxin, as it is not present in the plant phloem on which they feed. This indicates that Bt maize should not pose a problem for *C. carnea*. Laboratory studies that showed that the

aphids do not take up the Cry protein 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 would 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 analysed, 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 parasitoid *Microplitis mediator* was a result of the host's growth rate and size. No adverse effects of the Cry protein 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 Cry protein. 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 (*Carpophilus* 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 thorough and detailed investigation of the impact of Bt maize on non-target arthropods to date is that of Dively (2005). This field study was over a 3-year period in Maryland, USA. Over 500,000 arthropods were counted, from 13 orders, with 112 families and 203 taxonomic groups. The maize lines had both the *vip3a* and the *cry1Ab* genes. The effects of Bt maize were compared to non-Bt maize with and without insecticide treatment. Arthropods were registered by visual inspection, sticky traps, pitfall traps and emergence traps. Registration was also carried out the following growing seasons to document carry-over effects. All of the 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.

In a field study from Brazil, insects were collected directly from plants, over two months in large fields of conventional and Bt maize over a wide geographic area (Chaves et al., 2016). The study included *Spodoptera frugiperda* (the target of Bt treatment) and insect community (non-target pests, natural enemies of pests, other insects) of maize agroecosystems. Though there were large differences among maize fields, there was no general negative effect of Bt proteins on insect species richness (including on natural enemy richness). Species richness was generally low.

In a laboratory study with herbivorous two-spotted spider mite, *Tetranychus urticae*, and its predator, *Amblyseius andersoni*, Guo et al. (2016) found no effects of Bt on the biology of spider mites, or on their predator. *T. urticae* reared on Cry1Ac/Cry2Ab and non-Bt cotton or Cry1F and non-Bt maize did not significantly differ in any developmental or reproductive life history parameters even though they had ingested relevant levels of Bt proteins from maize and cotton.

In a three year field study in Hungary, Palinkas et al. (2017) investigated effects of Bt maize expressing Cry1F, Cry34Ab1/Cry35Ab1 on rove beetle abundance and competition between rove beetles and two other predatory groups (spiders, ground beetles). Nearly 6000 rove beetles, 76000 ground beetles, and 11000 spiders were collected. No variation in dominant rove beetle species between years or between treatments (Bt or non-Bt). Authors claim there is a negative relationship between rove beetle and carabid or spider abundance, implying 'interspecific competition' (rove beetles vs. spiders or vs. ground beetles), but this can't be seen in the figure they refer readers to or in other data in the paper. There were large (unexplained) decreases in abundances per sample over the three years.

Tian et al. (2014) studied the effects of three Cry proteins (Cry1Ac or Cry2Ab cotton, Cry1F maize) on the life history of two generalist predators, exposed to Cry proteins through their prey, through two generations. The group used cry resistant prey, to control for potentially poorer quality of susceptible prey. Insect survival, development, adult mass, fecundity, and fertility were measured. The predators acquired Cry proteins through their prey, and protein concentrations were diluted up the food chain. There were no effects of Cry proteins on either predator.

In a laboratory study, Su et al. (2015) studied the effects of Cry proteins (Cry1Ac or Cry2Ab cotton, Cry1F maize) on the life history of a generalist predator assassin bug *Zelus renardi*, exposed to Cry proteins through prey. The predator acquired Cry proteins through their herbivore prey, and Cry protein concentrations were diluted up the food chain. There were no effects of Cry proteins on fitness parameters of this predator.

Han et al. (2016) review published research on the effects of insect-resistant GM crops on the behaviors of phytophagous, predaceous, and parasitic insects and mites. Almost all such studies have been conducted on Bt crops, some of which were Cry1Ab or Cry1F maize. Altered behaviors could have indirect effects on insect or mite fitness and hence could impact non-target the non-target native arthropod fauna. However, the behavioral studies were usually carried out in the laboratory or were otherwise quite artificial (as acknowledged by the authors), so caution must be used in drawing conclusions for the effects of Bt crops on natural populations. Overall, while there are documented effects on some behaviors, most of these effects are unlikely to impact natural populations negatively.

Of 12 reviewed, 10 studies found significant effects on the behaviors of non-target herbivores (phytophages); 22/47 studies on target herbivores found significant effects. With respect to Cry1Ab and Cry1F Bt maize, all studies were on target species and none involved non-target species. The significant results for these maize studies primarily involved studies on spatial distribution (e.g. aphids clustering in sites with lower Cry protein concentrations, on Bt cotton) or movement (e.g. nematodes abandoned Bt maize more frequently). There were also experiments on host preference for feeding or oviposition (Bt vs non-Bt), which gave mixed results.

Overall, 11/50 reviewed studies found significant effects. For Cry1Ab or Cry1F maize, only 2 of 11 reviewed papers on arthropod natural enemies found a significant behavioral effect. In one study, a wasp parasitoid of a target lepidopteran foraged less efficiently in the presence of frass from Cry1Ab maize-fed host; a different study of the same host-parasite system found no significant effects on foraging behavior, though. The majority of studies on parasitoids found no effects on foraging behavior (including two others on Cry1Ab maize), but a few found minor effects. In the other study with significant results, a mite predator of herbivorous spider mites showed that the predator species spent more time near non-Bt maize fed prey than Bt maize fed prey. Experiments using ladybird beetles (Coccinellidae, 2 species), the garden spider *Araneus diadematus*, a ground beetle, a minute pirate bug and a lacewing, found no effects on predatory behavior for target species lepidopteran larval prey;

these are all generalist predators found in maize fields. A three-year field study of spiders and ground beetles in Cry1Ab maize found no phenological effects of Bt maize.

7.4.3 Effects on non-target Lepidoptera

Maize plants are not an important resource of food for indigenous Lepidoptera in Norway. 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 1507 fields.

A number of laboratory, field and theoretical exposure studies have assessed the potential risks of Bt-maize pollen to non-target Lepidoptera. Most of these studies have been performed with Bt maize expressing Cry1Ab protein.

In a laboratory experiment reported in Nature (Losey et al., 1999), the effect of pollen from Bt maize on larvae of the monarch butterfly (*Danaus plexippus*) was studied. 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 & Obrycki, 2003).

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 & Vojtech, 2006). This result was more pronounced with higher pollen densities. This study used event Bt176, and the paper mentions that MON810 expresses much lower levels of Cry protein in the pollen.

A field study in Germany evaluated the impact of MON810 on non-target 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.

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/cm² had a reduced

feeding activity. Significant differences in developmental time were also detected at pollen densities of 300 Bt-maize pollen grains/cm² and in survival at 400 grains/cm². The highest pollen amount recorded was 212 grains/cm² 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.

Perry et al. (2010, 2011) developed an 11-parameter mathematical model of exposure of larvae of non-target Lepidoptera to Bt-maize MON810 pollen in Europe. This model integrated a relationship between mortality and pollen dose based on laboratory bioassays with a relationship between dose and distance from a maize crop based on field measurements. Hence, Perry et al. (2010) derived predictions of mortality within a Bt-maize crop and at various distances from it into the field margins. The model structure distinguished between parameters relating to worst-case local exposure at small spatial and temporal scales (within-field and within the duration of anthesis) to large-scale effects (within-region; within-season; utilization rate of GM technology; allowance for physical effects and larval behaviour).

It provides a novel structure by which exposure may be quantified for other GM crops, a variety of traits and a range of non-target lepidopteran species. The model generated realistic data for three widespread European species, the butterflies *Inachis io* (L.) and *Vanessa atalanta* (L.) and the moth *Plutella xylostella* (L.) in 11 representative maize ecosystems in four European countries and demonstrated that the likely impact of maize MON810 pollen on non-target lepidopteran populations is low.

Maize 1507 could however pose a greater risk for non-target Lepidoptera than MON810, because: (i) while Lepidoptera are on average five times less sensitive (Wolt et al., 2005) to Cry1F than to Cry1Ab, the Bt-protein content expressed in the pollen of maize 1507 is more than 350 times that expressed in the pollen of maize MON810 (Mendelsohn et al. 2003; US EPA 2005); (ii) reported species sensitivities of laboratory populations, quoted as the average lethal concentration (units: grains of pollen per cm² leaf) that kills half of the susceptible larvae (LC₅₀), range widely from 0.065 to 410 lg Cry1F per gram diet (Wolt et al., 2005), leaving open the possibility that some species of conservation concern might be highly sensitive (Lang & Otto, 2010); (iii) more recent studies have shown considerable additional variability in LC₅₀ values, because of differences in toxin batches, methodologies (Saeglitz et al., 2006) and origin of test populations (Gaspers et al., 2010).

Perry et al. (2012) extended the model to assess potential adverse effects resulting from exposure of non-target lepidopteran species to Cry1F-containing maize pollen deposited on their host-plants under representative cultivation conditions and to provide recommendations for management to mitigate this risk. The 14-parameter mathematical model integrating small- and large-scale exposure was used to estimate the larval mortality of hypothetical species with a range of sensitivities, and under a range of simulated mitigation measures consisting of non-Bt maize strips of different widths placed around the field edge.

The greatest source of variability in estimated mortality was species sensitivity. Before allowance for effects of large-scale exposure, with moderate within-crop host-plant density and with no mitigation, estimated mortality locally was <10% for species of average sensitivity. For the worst-case extreme sensitivity considered, estimated mortality locally was 99.6% with no mitigation, although this estimate was reduced to below 40% with mitigation of 24-m-wide strips of non-Bt maize. For highly sensitive species, a 12-m-wide strip reduced estimated local mortality under 1.5%, when within-crop host-plant density was zero. Allowance for large-scale exposure effects would reduce these estimates of local mortality by a highly variable amount, but typically of the order of 50-fold. According to Perry et al. (2012), mitigation measures of risks of Bt-maize to sensitive larvae of non-target lepidopteran species can be effective, but depend on host-plant densities which are in turn affected by weed-management regimes.

Based on this mathematical model of exposure, the EFSA GMO Panel has assessed the potential impact of maize 1507 cultivation on a range of non-target lepidopteran species. According to the GMO Panel local and global estimated mortality increase monotonically with the five levels of species sensitivity studied, from „below-average“ to „extreme“ and with the level of exposure. Maize 1507 pollen grains found in and up to 30 m distance from maize 1507 fields could locally adversely affect differing proportions of non-target lepidopteran larvae, the proportion depending upon the sensitivity spectrum of the lepidopteran species under consideration, and other factors. However, global estimated mortality decreases monotonically with exposure level (measured by the parameter R) determined by factors such as the proportion of the land cropping maize 1507. For typical maize production conditions where maize represents 25% or less of arable land and as long as the proportion of maize 1507 is only moderate (uptake below 20%), the global mortality is likely to be less than 1%, even for extremely sensitive non-target lepidopteran species. The EFSA GMO Panel concludes that there is a risk to certain highly sensitive non-target lepidopteran species where high proportions of their populations are exposed over successive years to high levels of maize 1507 pollen deposited on their host-plants (EFSA, 2011d, EFSA, 2012b).

Following new information reported by Hofmann et al. (2014) (see section 7.2.2.2) concerning maize pollen deposition in relation to distance from the pollen source, EFSA assessed the consequences for its previous risk assessment conclusions and risk management for Bt-maize (EFSA, 2015). In particular, isolation distances to protected habitats were reviewed. For non-target lepidopteran larvae of conservation concern potentially occurring in protected habitats, isolation distances of 30 m were recommended between protected habitats and the nearest fields of maize 1507.

In 2016, the EFSA GMO Panel assessed the relevance of two further scientific publications (Lang et al., 2015; Hofmann et al., 2016) presenting new data on pollen deposition and potential exposure of butterflies in protected habitats by Bt maize cultivation (EFSA, 2016). EFSA concluded that neither Lang et al. (2015) nor Hofmann et al. (2016) provide data indicating the necessity to revise the previous ERA conclusions and risk management recommendations for Bt maize made in EFSA (2015).

In a recent commentary, Kruse-Plass et al. (2017) respond to the EFSA GMO Panel's criticism of the conclusions of the previous articles by their research group (Hofmann et al., 2014; 2016). In their commentary, they reiterate that measurements of pollen dispersal indicate that there is a need for specific environmental impact assessments for Bt-maize cultivation in relation to distance from protected habitats.

Perry et al. (2017) responded to this commentary stating that there are no new data that refute the GMO Panel's criticism. The main points that apply to the Norwegian situation are the proximity to protected habitats for the endangered lepidopteran species in Norway and the degree of exposure to maize pollen grains containing Cry proteins. The latter point is affected by (i) the distance from the Bt maize crop, (ii) the amount of pollen deposited on the host plant of the endangered species and (iii) coincidence of maize pollen spread with the feeding larval stage of the endangered species.

In Norway, the maize cultivation is marginal. The total crop area of forage maize is estimated to 2000-2800 decares, equivalent to less than 0.1 % of the areas with cereal crops. The area of individual fields is limited by the topography such that the quantity of maize pollen produced under flowering is also limited. The potential impact/exposure of Cry1F-containing maize pollen on non-target lepidopteran species in Norway is therefore negligible.

7.4.4 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. 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 Cry proteins on springtails and soil mites have been reported in the scientific literature (reviewed by Icoz & Stotzky, 2008). Furthermore, in a lower-tier study performed by the applicant the springtail *Folsomia candida* fed a diet containing the Cry1F protein was not adversely affected. In addition, Cry1F protein concentrations in decaying plant residues from maize 1507 decreased rapidly and did not accumulate in soil. Therefore, non-target soil organisms will be exposed to relatively low Cry1F protein concentrations within a few months after harvest.

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 *F. 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 is consistent with the findings of Yu et al. (1997).

In a study by Yuan et al. (2013), roots, stems, and leaves of different Bt rice varieties expressing Cry1Ab and Cry1Ac were exposed to *F. candida* under laboratory conditions, with survival, reproduction and growth of the collembolan as ecological fitness parameters. Significant differences in ecological fitness were found among the different treatments, including differences in the plant parts and varieties of non-Bt rice, presumably as the result of three factors: gene modification, plant parts and rice varieties. The fitness of *F. candida* was less affected by the different diets than by the exposure to the same materials mixed with soil. According to the authors, the results clearly showed that there was no negative effect of different Bt rice varieties on the fitness of *F. candida* through either diet or soil exposure.

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 isolate 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 Cry protein.

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 Cry protein 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 Cry protein, 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 Cry1Ab 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 Cry1Ab 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.

7.4.5 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 (Trichoptera), 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, 2009c). 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* spp. 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 2012c; Ricoch et al., 2010).

In a similar experiment, Holderbaum et al. (2015), fed a chronic high dose of ground leaf material from MON810 maize, compared to a chronic high dose of a near isogenic maize or the normal diet of algae to *D. magna*. Those that were fed on their normal diet of algae had a mortality rate of 20% at 21 days, while the mortality on both types of maize was higher, indicating that maize is deficient in some nutrients that *D. magna* needs or is unable to assimilate. Comparing only the MON810 maize and the near isoline, there were no significant differences in survival or survival time, although the median survival time of *D. magna* fed MON810 was slightly longer than those fed on the isoline. There was little difference in body size between the two maize diets until the *D. magna* were beyond their median lifespan. After that time, a significant number of *D. magna* fed on MON810 had a slight reduction in body size (<5%) compared to those fed on the near isoline. There were no significant differences in incidence of reproduction or age at first reproduction between the two populations fed on the two varieties. There were significant differences in the stage fecundity, with the *D. magna* that were fed MON810 reaching peak reproduction earlier. There were no significant differences between cumulative fecundity rates at any given time. A small number (ca. 10%) of the *D. magna* produced ephippia (resting stage eggs produced under adverse conditions), 14 of these were from the MON810 diet and 4 were from the near isoline diet. The authors suggest that the differences between the *D. magna* fed MON810 and those fed the near isoline can be due to the Cry1Ab proteins or an effect of nutritional differences in the MON810.

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.

7.4.6 Effects on non-target organisms that are not arthropods

Maize 1507 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 proteins 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 & Stotzky, 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 Cry proteins 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 et al. (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 (Krogh 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. (2011), *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; 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 *Caenorhabditis 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.

Höss et al. (2013) examined the potential toxic effects of the genetically modified maize MON 89034 x MON88017, expressing different the Cry proteins Cry1.105, Cry2Ab2 and Cry3Bb1 on the free-living bacterivorous soil nematode *Caenorhabditis elegans*. All three insecticidal Cry proteins showed dose-dependent inhibitory effects on *C. elegans* reproduction, however, at concentrations that were far above the expected soil concentrations. Moreover, a reduced toxicity was observed when Cry proteins were added jointly. A *C. elegans* mutant strain deficient for receptors for the nematicidal Cry5B was also resistant against the lepidopteran Cry proteins Cry1.105 and Cry2Ab2, suggesting that these Cry proteins bound to the same or similar receptors as nematicidal Cry proteins and thereby affect the reproduction of *C. elegans*.

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 Cry protein 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 officinale*). 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.

7.4.7 The Norwegian red list of threatened species

The 2015 Norwegian Red List for species (www.artsdatabanken.no) (Henriksen & Hilmo, 2015) contains 459 Lepidoptera, a decrease of 3 species from the Red List published in

2010. 172 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. As the CR and EN species' habitats and host plant range do not include agricultural crops, exposure to the Cry-protein in maize 1507 would be extremely low or none if this maize were to be cultivated in Norway.

Although the Cry-protein expressed in maize 1507 is toxic to a wide range of Lepidoptera, none of the endangered species in Norway feed on maize plants. Among the red listed Lepidoptera categorized as endangered, only two species live on grasses in the vicinity of agricultural areas. *Euthrix potatoria* 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 is negatively affected by both agricultural intensification and overgrowth (Endrestøl & Bengterson, 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.

As none of these endangered species occur in agricultural crops and they do not feed on maize, cultivation of maize 1507 is not considered to represent a threat to the prevalence of these endangered species in Norway.

7.4.8 Conclusion

Based on a review of available scientific literature VKM concludes that the likelihood of adverse effects of Cry1F protein from cultivation of GM maize on non-target organisms in Norway is negligible.

7.5 Impacts of the specific cultivation, management and harvesting techniques

The PAT protein expressed in maize 1507 has been used as a selectable marker during the transformation process. The scope of the application for maize 1507 cultivation does not cover the use of glufosinate-ammonium-containing herbicides on maize 1507. Therefore, potential environmental adverse effects due to the applications of glufosinate-ammonium-containing herbicides and possible changes in weed management are not considered by VKM in this Scientific Opinion.

Apart from changes in insecticide regimes, there are no anticipated changes in cultivation practices, management or harvesting techniques associated with the cultivation of maize 1507. Bt crops, such as maize 1507, may reduce the use of insecticides and may cause

changes in crop rotations in response to reduced pest pressure (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 (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 Cry1F in lepidopteran target pest populations.

As there are no pests which are controlled by Bt-based insecticides, cultivation of maize 1507 will not lead to changes in cultivation practices in Norway.

7.6 Effects on biogeochemical processes

7.6.2 Fate of Bt-proteins in soil

Cry protein 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, Cry 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 Cry proteins in soil are key factors for determining exposure and potential effects on soil biota related to the soil function. Persistence of Cry proteins 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 of Cry proteins are known to be influenced by different factors like type of crop, microbial communities, environmental conditions like the soil surface versus below the soil surface, temperature, pH, moisture, etc. (Sanvido et al., 2006; Icoz & Stotzky, 2008). Furthermore, various salt and hydroxides in soil may alter Cry proteins levels in the ecosystem. The soil accumulation of Cry proteins depends on their absorption onto soil components, and bioavailability. In particular the absorption of Cry to soil components are little understood (Singh & Dubey, 2016). For evaluation of Cry1F degradation in soil, these factors should be taken in consideration.

Persistence, degradation and accumulation of Cry proteins 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 Cry proteins 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).

Herman et al. (2002) assessed the stability of the Cry1F ICP in soil in a laboratory study designed to determine the persistence of the active protein residue in soil over time, using insect bioassay as the analytical quantification method. The GI50 (concentration estimated to inhibit growth by 50%) rose at each consecutive incubation interval, indicating a consistent decline in Cry1F activity over time. The residue data were poorly described by a first-order model when fit to either the full data or a truncated data set where the last interval (28 days) was excluded. Data were well described by a shift-log model, and this model predicted DT50 (time until 50% decay) and DT90 (time until 90% decay) values of 0.6 and 6.9 days, respectively. This rapid degradation rate was consistent with other Cry proteins evaluated.

Shan et al. (2008) evaluated the potential Cry1F protein accumulation in soil after three subsequent years of cultivation of maize 1507 at three different sites. The maize plants were incorporated into the soil through postseason tillage or no tillage each year. Soil samples were collected from the fields, and the level of Cry1F protein was determined using an enzyme-linked immunosorbent assay (ELISA). The ELISA was validated in soil matrices over the concentration range of 18-180 ng/g dry weight, with a limit of detection of 4.5 ng/g dry weight. No detectable Cry1F protein was found in any of the soil samples collected from the Cry1F maize fields, and no biological activity was observed against *Heliothis virescens neonates*. These results indicate that the level of Cry1F protein accumulated in soil after 3-yr continuous planting of transgenic Cry1F maize is negligible.

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 protein, 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 Cry 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 protein 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.

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.

7.6.3 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.

Cheeke et al. (2012) investigated the impact of several Bt-maize events (including Cry1F-expressing maize) on symbiotic arbuscular mycorrhizal fungi (AMF) under greenhouse potting conditions. The authors observed lower levels of AMF colonisation in the roots of Bt maize, as compared with the non-Bt-maize (parental) lines. The reduced mycorrhization was not related to the expression of a specific Bt-toxin, but may be the result of other factors such as unintended changes in Bt-maize due the genetic modification process. The authors themselves state that scientific uncertainty remains on how the reported observations translate to the field situation, as low levels of fertilisation had to be applied during the experiment to favour mycorrhization (Verbruggen et al., 2012). With the example of Cry1Ab-expressing maize event Bt11, the authors demonstrated in a previous study that differences between the Bt-maize and non-Bt-maize in fact disappeared when fertilisers were added to soil (Cheeke et al., 2011). For the cultivation of maize, in which normally larger amounts of organic or inorganic fertilisers are added to improve maize yield, the effects as observed by Cheeke et al. (2011) are therefore most likely insignificant (EFSA, 2012b). Furthermore, under common agricultural practices, the contribution of AMF to improve health or increase yield of maize appears to be negligible or not existent (e.g. as reviewed by Ryan & Kirkegaard, 2012).

In 2014, Cheeke et al. published a study of effects of *Bacillus thuringiensis* (Bt) maize cultivation history on arbuscular mycorrhizal fungal colonization, spore abundance and diversity, and plant growth. In this field experiment, the authors used split plots to evaluate the effect of Bt maize or non-Bt maize cultivation history on AMF spore abundance, diversity,

root colonization, and growth of seven different genotypes of Bt maize expressing Cry1F, Cry1Ab, Cry34/35Ab and Cry3Bb1 and five corresponding non-Bt parental isolines. Cheeke et al. found that Bt plants had higher leaf chlorophyll content when they were grown in plots that had been cultivated with Bt maize the previous year, and similarly, non-Bt plants had higher chlorophyll content when they were grown in plots with a non-Bt cultivation history, indicative of a positive feedback effect. There was a lower density of AMF spores in plots with a Bt maize cultivation history than in plots where conventional maize had been grown in the previous year, but no difference in spore diversity. There were no significant differences in AMF colonization or root or shoot biomass between plots with a cultivation history of Bt and conventional maize. The study was a field investigation and was performed in USA.

In a follow-up-study, Cheeke et al. (2015) evaluated the effects of Bt maize and their corresponding non-Bt parental isolines on AMF colonization and community diversity in plant roots. The research group used seven different genotypes of Bt maize that exhibited reduced AMF colonization in previous greenhouse studies. The Bt genotypes differed in the Cry protein expressed (Cry1Ab, Cry34/35Ab1, Cry1F + Cry34/35Ab1, Cry1F, Cry3Bb1). Plants were harvested 60 days after sowing, and data were collected on plant growth and percent AMF colonization of roots. AMF community composition in roots was assessed using 454 pyrosequencing of the 28S rRNA genes, and spatial variation in mycorrhizal communities within replicated experimental field plots was examined. Growth responses, per cent AMF colonization of roots and AMF community diversity in roots did not differ between Bt and non-Bt maize, but root and shoot biomass and per cent colonization by arbuscules varied by maize cultivar. The authors concluded that spatial soil heterogeneity in the field has a greater effect on the structure of AMF communities and plant growth than host plant cultivar or modification by Cry protein genes. The study was a field investigation and was performed in USA.

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 Cry1Ab. Castaldini et al. (2005) have also reported consistent differences in rhizosphere heterotrophic 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 *Medicago 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).

Blackwood & Buyer (2004) has 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 maize 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.

Cotta et al. (2013) evaluated temporal dynamics of microbial communities in the rhizosphere of two GM maize hybrids in tropical agrosystems using PCR-DGGE profiles. The study aimed to evaluate the possible effects of Bt maize expressing Cry1Ab and Cry1F protein, in comparison to the parental line, on the structure and abundance of microbial communities in the rhizosphere. Moreover, the effect of soil type was addressed. For this purpose, the bacterial and fungal communities associated with the rhizosphere of GM plants were compared by culture-independent methodologies to the near-isogenic parental line. Two different soils and three stages of plant development in two different periods of the year were included. As evidenced by principal components analysis (PCA) of the PCR-DGGE profiles of evaluated community, clear differences occurred in these rhizosphere communities between soils and the periods of the year that maize was cultivated. However, there were no discernible effects of the GM lines as compared to the parental line. For all microbial communities evaluated, soil type and the period of the year that the maize was cultivated were the main factors that influenced their structures. No differences were observed in the abundances of total bacteria between the rhizospheres of GM and parental plant lines. The investigation was a field experimental study and performed in Brazil.

Results from the EU-project ECOGEN 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). ECOGEN 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; Krogh 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.

Prischi 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.

Most published studies indicate that the host plant cultivar or modification has minor or no effect on soil microorganisms. Spatial variation and heterogeneity in the field has probably greater effects on soil microorganisms. There is need for evaluation of the methods regarding effects of Cry proteins on microorganisms in soil. There are lack of in vitro data regarding antibacterial/anti-fungal activity of Cry proteins. Furthermore, there is lack of data regarding the quantity of microbial species in soil, which may be performed using q or RT-PCR. The change of microbial species in non-microbial organisms, living in the soils, is not investigated.

7.7 Conclusion

Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivated maize 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. Since maize 1507 has no altered agronomic and phenotypic characteristics, except for the specific target insect resistance and herbicide tolerance, the likelihood of unintended environmental effects as a consequence of spread of genes from maize 1507 is considered to be extremely low.

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.

There are a limited number of published scientific studies on environmental effects of Cry1F protein. Published scientific studies showed that the likelihood of negative effects of Cry1F protein on non-target arthropods that live on or in the vicinity of maize plants is low. In Norway, the maize cultivation is marginal. The total crop area of forage maize is estimated to 2000-2800 decares, equivalent to less than 0.1 % of the areas with cereal crops. The area of individual fields is limited by the topography such that the quantity of maize pollen produced under flowering is also limited. The potential exposure of Cry1F-containing maize pollen on non-target lepidopteran species in Norway is therefore negligible.

Cultivation of maize 1507 is not considered to represent a threat to the prevalence of red-listed species in Norway

Cry1F protein does not negatively affect honeybee larvae and adults in laboratory settings. Considering that the proportion of maize pollen as a total of all pollen collected and fed to larvae during a summer will be low, VKM does not consider that maize 1507 will cause reductions to pollinating insects that are significantly greater from those caused by cultivation of conventional maize.

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, and no specific lower-tier studies, assessing the impact of the Cry1F protein on non-target aquatic arthropods have been reported in the scientific literature so far. However, exposure of non-target organisms to Cry proteins in aquatic ecosystems is likely to be very low, and potential exposure of Cry proteins to nontarget organisms in aquatic ecosystems in Norway is considered to be negligible.

Few studies have been published examining potential effects of Cry1F protein 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.

VKM concludes that, although the data on the fate of the Cry1F protein and its potential interactions in soil are limited, the relevant scientific publications analysing the Cry1F protein, together with the relatively broad knowledge about the environmental fate of other Cry1 proteins, do not indicate significant direct effects on the soil environment.

VKM concludes that separation distances of 200 m most likely will ensure an upper limit of 0.9 % of adventitious presence as a result of introgression via pollination in maize.

8 Post-market environmental monitoring

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

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

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

Summary and evaluation of the monitoring plans from the applicant

8.1 Case-specific GM plant monitoring

When potential adverse effects or important gaps in scientific information or significant levels of critical uncertainty linked to the GM plant and its management have been identified in the environmental risk assessment, then case-specific monitoring should be carried out after placing on the market, in order to confirm assumptions made in the ERA and to further

inform the ERA (EFSA 2011c). Case-specific monitoring (CSM) should be targeted at assessment endpoints and environmental protection goals identified in the ERA conclusion as being at risk or where levels of critical uncertainty were identified in relation to potential risks associated with the GM plant. Monitoring of potentially adverse cumulative long-term or large-scale effects and the resolution of areas of critical uncertainty, identified in the ERA are important objectives of monitoring (EC, 2002).

CSM should be put in place, in order (1) to confirm that any assumption in the ERA regarding the occurrence and impact of potential adverse effects is correct, and (2) to determine the efficacy of risk mitigation measures and/or ultimately to allow the modification of risk mitigation measures, so that their efficacy and proportionality can be improved (see EFSA, 2011a).

To assess the efficacy of risk mitigation measures put in place to reduce levels of risk and scientific uncertainty, the EFSA GMO Panel recommends CSM to address (1) resistance evolution to the Cry1F protein in lepidopteran target pests, and (2) the risk to sensitive non-target Lepidoptera to maize 1507 pollen.

Monitoring resistance evolution to the Cry1F protein in lepidopteran target pests

The applicant proposed to measure the baseline susceptibility of corn borer populations to the Cry1F protein and changes in that susceptibility in the EU. Resistance monitoring, through targeted field sampling in areas where maize 1507 adoption is the highest and selection pressure is greatest, should reveal changes in susceptibility of these populations. In this way, changes relative to the baseline susceptibility could be detected in time to enable proactive management before control failures occur (Tabashnik et al. 2008a,b, 2009). The EFSA GMO Panel agrees this approach and considers that susceptibility data can reveal potential changes in resistance levels in corn borer populations. Such data will also indicate the efficacy of the implemented „high dose/refuge“ strategy in delaying resistance evolution in the target pest species, and reduce the remaining scientific uncertainty related to the adequacy of the IRM plan proposed by the applicant.

The EFSA GMO Panel considers that the overall framework to monitor resistance evolution proposed by the applicant is consistent with those described in the scientific literature (reviewed by Tabashnik et al. 2009).

The EFSA GMO Panel makes the following additional recommendations to the applicant:

- to focus the sampling of lepidopteran target pests in „hotspot areas“ over time to increase the likelihood of detecting resistance evolution. Sampling in areas with lower adoption rate of maize 1507 is also required but at a lower frequency in order to establish susceptibility baselines;
- to include in the samplings surviving lepidopteran target pests within maize 1507 fields in order to detect potentially resistant individuals. The sampling should be mainly done as late as possible within the growing season in order to increase the likelihood of detecting surviving individuals;

- to consider regionally important lepidopteran pests (other than corn borers, see section 3.1.2.1) of maize 1507 in the context of CSM for IRM strategy and, where appropriate, adjust the design and implementation of the IRM plan accordingly;
- to revise the monitoring protocol aiming at a detecting resistance allele frequency below 5% (between 1% and 3%) in „hotspot areas“. The EFSA GMO Panel recommends to increase the number of larvae collected or to use a F2 screening (see EFSA, 2011b for further details).

Monitoring the risk to non-target Lepidoptera

The EFSA GMO Panel recommends to carry out further field studies on non-target Lepidoptera and considers that the purpose of these studies should be:

- to estimate whether non-target Lepidoptera larvae, with a high sensitivity to the Cry1F protein, are in reality feeding on plants in and adjacent to maize fields at the time of pollen deposition, and if so:
- to estimate the proportions of these populations likely to be affected and in specific cases
- to determine the overall effect on maintaining a favourable status of these populations

The EFSA GMO Panel considers that monitoring and additional studies are only required in situations where there is a potential risk to populations of sensitive non-target Lepidoptera due to high adoption of maize 1507 (i.e., above adoption rate of 20%).

There are no reports of the target lepidopteran species attaining pest status on maize in Norway. There have been ten reports of *O. nubilalis*, restricted to the counties of Vestfold, Telemark, Aust-Agder and Vest Agder. *Sesamia* spp. has not been reported 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 Cry 1F protein on non-target arthropods that live on or in the vicinity of maize plants. Likewise, cultivation of maize is not considered to represent a threat to the prevalence of red-listed species in Norway.

VKM therefore concludes that a case-specific monitoring plan is not necessary in Norway.

8.2 General surveillance (GS) for unanticipated effects

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

The applicant proposed to conduct GS for maize 1507 throughout the period of validity of the authorisation. The GS will take into consideration and be proportionate to the extent of

cultivation of maize 1507 in the EU Member States. The applicant proposed to build its GS on four approaches (1) the use of annual farmer questionnaires, (2) the review of scientific information provided by existing monitoring networks, (3) the monitoring and review of ongoing research and development, as well as scientific literature, and (4) the implementation of industry stewardship programs, in order to identify potential adverse effects associated with the intended uses of maize 1507.

Farmer questionnaires

The EFSA GMO Panel agrees with the approach of the applicant to establish farmer questionnaires as a reporting format that provides relevant information. Farmer questionnaires should be designed to determine whether the farmer/manager/worker has noticed any differences between the GM plant and its management and that of similar non-GM plants growing on the farm, nearby or previously (EFSA, 2011a). The applicant and risk managers are advised to consider the new EFSA GMO Panel guidance document on PMEM (EFSA, 2011a) and the specific recommendations on the annual PMEM report of maize MON 810 cultivation in 2009 (EFSA, 2011b) when finalising their or evaluating monitoring plans.

While the EFSA GMO Panel considers the format and contents of the farmer questionnaire, as provided by the applicant, comprehensive, it proposes the following modifications:

- to add questions on the possible occurrence and observation of (GM) volunteer maize from previous crops (whenever relevant) and feral maize plants in field margins for the consideration of unanticipated effects on the persistence and invasiveness potential of maize 1507;
- to consider the occurrence of regionally important lepidopteran pests other than corn borers in maize 1507 fields and surrounding areas;
- in addition to the questions on pest and disease incidences on maize 1507, the farmer questionnaire should specifically request information on the occurrence of possible unexpected field damaged maize 1507 plants which might be associated with corn borer control failures, as this information will complement the CSM of resistance evolution to the Cry1F protein in target pests;
- to add questions on the proportion of non-Cry1F-expressing maize compared with maize 1507 on the farm, the distance between the refuge area and the monitored maize 1507 field in case the refuge is planted as a separate field adjacent to the *Bt*-maize field, the differences in pest management practices of the refuge.
- provide information on other GM plant events being grown at the same sites and farms;
- are adapted, where needed, to each GM plant monitoring on a case-by-case basis by considering additional data requirements relevant for each species/event, its management and its receiving environments;

Existing monitoring networks

Since farmer questionnaires focus mainly on the cultivation area of the GM plant and its surroundings, the EFSA GMO Panel supports the consideration of additional information sources for GS (EFSA, 2011a). In this respect, Directive 2001/18/EC proposed to make use

of established routine surveillance networks, in order to obtain data on environmental impacts in the landscape where GMOs are cultivated from a range of existing monitoring networks which observe changes in biota and production practices from farm up to regional level. EU Member States have various networks in place - some of which have a long history of data collection – that may be helpful in the context of GS of GM plant cultivations. Existing monitoring networks involved in routine surveillance offer recognised expertise in a specific domain and have the tools to capture information on important environmental aspects over a large geographical area. However, the EFSA GMO Panel recognises that existing monitoring networks fully meeting all the needs of the monitoring of GM plant cultivations can be limited (Schmidtke & Schmidt, 2007; Graef et al., 2008, ref EFSA 2011a). The development of harmonised criteria for the systematic identification, specification and analysis of existing surveillance networks across the EU is therefore considered important (EFSA, 2011a).

The EFSA GMO Panel agrees with the proposal of the applicant to describe the generic approaches for using existing monitoring networks. The applicant has also given consideration to the use of any future surveys of conservation goals as defined in the Directive 2004/35/EC on environmental liability (EC, 2004) in farming regions where maize 1507 will be cultivated and intends to investigate their suitability for providing data on potential changes in biota.

Knowing the limitations of existing monitoring networks, it is important to describe the processes and criteria that will be used for selecting and evaluating existing monitoring networks for supplying data related to the unanticipated adverse effects of GM plants in GS. Therefore, the applicant, in consultation with Member States, should consider the protection goals, the assessment endpoints and their indicators that could be monitored through existing monitoring programmes;

Monitoring and review of ongoing research and development, as well as scientific literature

- An additional approach to support GS is to review all new scientific, technical and other information pertaining to maize 1507, including information on GM plants with similar traits or characteristics, which has emerged during the reporting period. This will include reviewing of results from ongoing research and development studies (e.g., variety registration trials) and all publications including peer reviewed journal articles, conference proceedings, review papers and any additional studies or other sources of information relevant to the cultivation of the plant/trait combination for which the report is being drafted (EFSA, 2011a).

The EFSA GMO Panel recommends that the applicant:

- to cover all relevant peer-reviewed publications, including peer-reviewed journal articles, conference proceedings, review papers and any additional studies or other sources of information relevant to the cultivation of the plant/trait combination for which the report is being drafted;
- to describe the criteria for selecting and evaluating the scientific reliability of publications;

- to adhere to systematic literature review methodology to select relevant papers (EFSA 2010d).

8.3 Conclusion

VKM agrees with the conclusions and recommendations on general surveillance from the EFSA opinion (EFSA 2012b).

9 Uncertainty and data gaps

VKM finds that the application follows relevant guidance documents as developed by EFSA. These guidance documents address and seek to reduce measurement, parameter and sampling uncertainty. Moreover, they address various types of model uncertainty. VKM has not identified critical uncertainty remaining after previous assessments done by VKM and EFSA, or specific conditions in Norway that would require in-depth uncertainty analysis.

VKM acknowledge the ongoing work in EFSA that focus on developing guidance on systematic and transparent approaches to uncertainty analyses, and seeks to integrate such recommendations as they become available.

Examples of specific sources of uncertainty discussed for this application include the following:

- There are only a limited number of published scientific studies on the environmental effects of Cry1F protein.
- Few studies have been published examining potential effects of Cry1F protein on ecosystems in soil, mineralization, nutrient turnover and soil communities.
- 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. No specific lower-tier studies, assessing the impact of the Cry1F protein on non-target aquatic arthropods have been reported in the scientific literature so far.
- Acute toxicity tests do not provide enough information to conclude on possible adverse health effects of maize 1507

Adjuvanticity

There are many knowledge gaps related to assessment of adjuvants. Most of the immunologic adjuvant experiments have been performed using Cry1Ac. Whether the other Cry proteins have similar adjuvant properties is unknown. The quantities of Cry proteins in genetically modified maize and soya are marginal compared with the amounts of other adjuvants that are natural components of food. However, the extent to which these naturally occurring adjuvants and Cry proteins contribute to the development of allergies is largely unknown. Determination of their importance is hampered by the lack of validated methods for measuring adjuvant effects.

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

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

Herbicide residue levels

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

Since it is difficult to predict the toxicity of cocktails from the toxicity of the single components, there is uncertainty related to risk of confounding effects such as additive or synergistic effects between the residues in herbicide resistant plants. The transgene technology used can possibly lead to different metabolic products of the applied herbicides from what is expected from conventional usage. The risk assessment of herbicides should take into account plants with altered metabolism.

10 Conclusions

Food and feed safety evaluation (updating of the 2014 opinion)

Molecular characterisation

Appropriate analyses of the transgenic DNA insert, its integration site, number of inserts and flanking sequences in the maize genome, have been performed. The results show that one copy only of the insert is present in maize 1507. Homology searches with databases of known toxins and allergens have not indicated any potential production of harmful proteins or polypeptides caused by the genetic modification in maize 1507. Southern hybridisation analyses and segregation studies show that the introduced genes *cry1F* and *pat* are stably inherited and expressed over several generations along with the phenotypic characteristics of maize 1507. VKM considers the molecular characterisation of maize 1507 satisfactory.

Comparative assessment

Comparative analyses of maize 1507 to its non-GM conventional counterpart have been performed during multiple field trials located at representative sites and environments in Chile (1998/99), USA (1999) and in Europe (1999, 2000 and 2002). With the exception of small intermittent variations, no biologically significant differences were found between maize 1507 and the conventional maize. Based on the assessment of available data, the VKM GMO Panel concludes that maize 1507 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart, except for the introduced characteristics, and that its composition fell within the normal ranges of variation observed among non-GM varieties. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of 1507 compared to conventional maize.

Food and feed safety assessment

Whole food feeding studies on rats, broilers, pullets, pigs and cattle have not indicated any adverse health effects of maize 1507. These studies further indicate that maize 1507 is nutritionally equivalent to conventional maize. The PAT and Cry1F proteins do not show sequence resemblance to other known toxins or IgE allergens, nor have they been reported to cause IgE mediated allergic reactions. Some studies have however, indicated a potential role of Cry-proteins as adjuvants in allergic reactions.

Based on current knowledge, VKM concludes that maize 1507 is nutritionally equivalent to conventional maize varieties. It is unlikely that the PAT and Cry1F proteins will introduce a toxic or allergenic potential in food or feed based on maize 1507 compared to conventional maize.

Answers to the Terms of Reference from the Norwegian Environment Agency and the Norwegian Food Authority

Environmental risk assessment

Maize is the only representative of the genus *Zea* in Europe, and there are no cross-compatible wild or weedy relatives outside cultivated maize 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. Since maize 1507 has no altered agronomic and phenotypic characteristics, except for the specific target insect resistance and herbicide tolerance, the likelihood of unintended environmental effects as a consequence of spread of genes from maize 1507 is considered to be extremely low.

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.

There are a limited number of published scientific studies on environmental effects of Cry1F protein. Published scientific studies showed that the likelihood of negative effects of Cry1F protein on non-target arthropods that live on or in the vicinity of maize plants is low.

In Norway, the maize cultivation is marginal. The total crop area of forage maize is estimated to 2000-2800 decares, equivalent to less than 0.1 % of the areas with cereal crops. The area of individual fields is limited by the topography such that the quantity of maize pollen produced under flowering is also limited. The potential exposure of Cry1F-containing maize pollen on non-target lepidopteran species in Norway is therefore negligible.

Cultivation of maize 1507 is not considered to represent a threat to the prevalence of red-listed species in Norway.

Cry1F protein does not negatively affect honeybee larvae and adults in laboratory settings. Considering that the proportion of maize pollen as a total of all pollen collected and fed to larvae during a summer will be low, VKM does not consider that maize 1507 will cause reductions to pollinating insects that are significantly greater from those caused by cultivation of conventional maize in Norway.

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, and no specific lower-tier studies, assessing the impact of the Cry1F protein on non-target aquatic arthropods have been reported in the scientific literature so far. However, exposure of non-target organisms to Cry proteins in aquatic ecosystems is likely to be very low, and

potential exposure of Cry proteins to nontarget organisms in aquatic ecosystems in Norway is considered to be negligible.

Few studies have been published examining potential effects of Cry1F protein 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.

VKM concludes that, although the data on the fate of the Cry1F protein and its potential interactions in soil are limited, the relevant scientific publications analysing the Cry1F protein, together with the relatively broad knowledge about the environmental fate of other Cry1 proteins, do not indicate significant direct effects on the soil environment.

The PAT protein expressed in maize 1507 has been used as selectable marker to facilitate the selection process of transformed plant cells and is not intended for weed management purposes. As the scope of the notification does not cover the use of glufosinate-ammonium-containing herbicides, and maize 1507 will not be marketed in the EEA as a herbicide-tolerant crop, potential environmental adverse effects due to the applications of these herbicides and possible changes in weed management has not been assessed by VKM in this opinion.

As there are no pests which are controlled by Bt-based insecticides, cultivation of maize Bt11 will not lead to changes in cultivation practices in Norway

Post-market environmental monitoring plan

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 that the likelihood of adverse effects of Cry 1F protein on non-target arthropods that live on or in the vicinity of maize plants is low. Likewise, cultivation of maize is not considered to represent a threat to the prevalence of red-listed species in Norway. VKM therefore concludes that a case-specific monitoring plan is not necessary in Norway.

VKM is of the opinion that the general surveillance plan provided by the applicant, with the recommendations from EFSA, is sufficient to observe and register possible unanticipated adverse effects of maize 1507.

Management measures in the draft Annex to the Commission Implementing Decision concerning the placing on the market for cultivation of genetically modified maize 1507:

(a) Refuge areas to delay the development of resistance and limit exposure of non-target Lepidoptera species to maize pollen expressing Cry1F protein

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.

(b) Isolation distances from protected habitats

Cultivation of maize 1507 is not considered to represent a threat to the prevalence of red-listed Lepidoptera species in Norway.

(c) Teosinte control/eradication measures

Teosinte, the common name for a group of annual and perennial species of the genus *Zea*, is native to Mexico and Central America. In Europe, teosinte has been reported in maize fields in Spain and France. Teosinte control/eradication measures is not considered an issue in Norway.

Coexistence measures

VKM concludes that separation distances of 200 meters most likely will ensure an upper limit of 0.9 % of adventitious presence as a result of introgression via pollination in maize.

Overall conclusion

Based on current knowledge, VKM concludes that maize 1507 is nutritionally equivalent to conventional maize varieties. It is unlikely that the Cry1F and PAT proteins will introduce a toxic or allergenic potential in food or feed derived from maize 1507 compared to conventional maize.

VKM concludes that cultivation of maize 1507 is unlikely to have any adverse effect on the environment and agriculture in Norway.

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12 Appendix 1

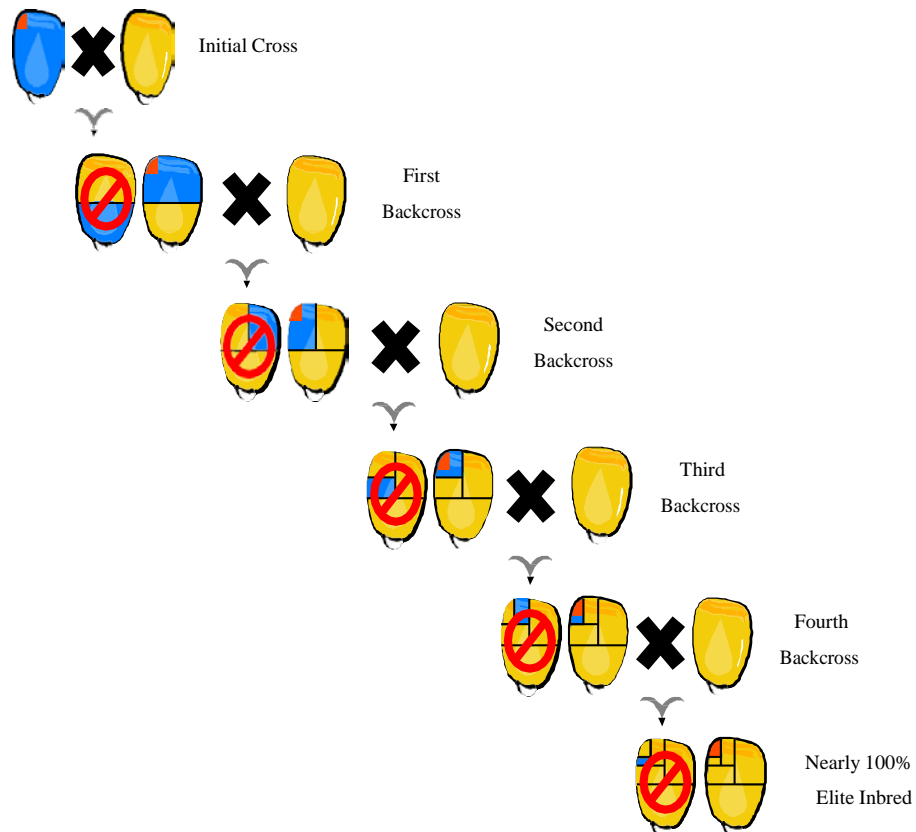
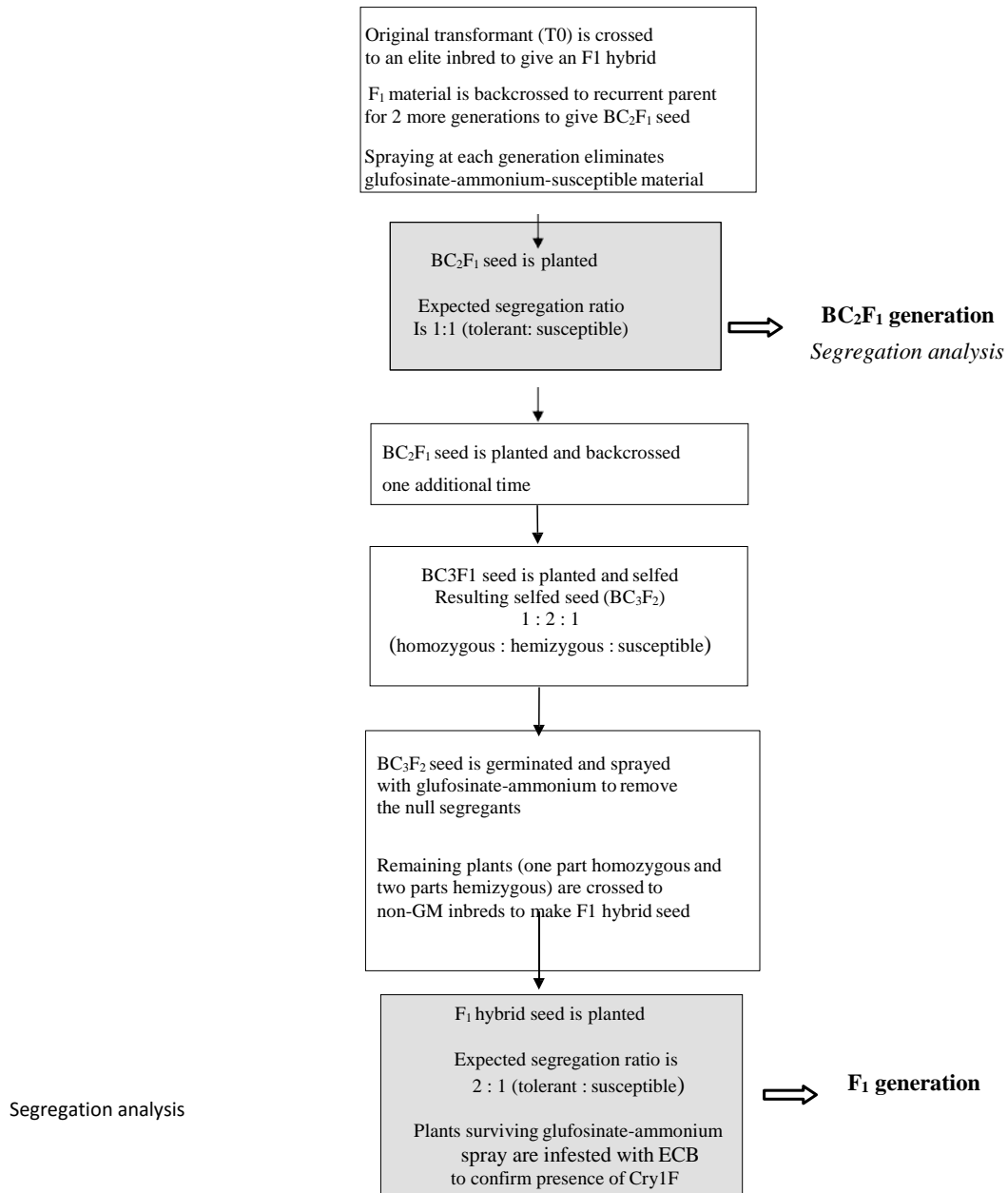


Figure 1 Integration of desired genes into elite inbreds through backcrossing, selection and selfing. The inserted genetic material is represented by the red portion at the top and left of the kernels. The blue and yellow colours represent the portion of genetic material that is identical to that of the original transformant and elite inbred, respectively.

Figure 2. Backcrossing generations used to determine Mendelian segregation ratios for 1507 maize.



13 Appendix 2

Table 1

The means and p-values for the proximate analysis of grain from maize line 1507 and the control hybrid for samples collected in the 1998/1999 field trial. The range of values found in the literature for each trait is listed.

Proximate Analysis of Grain			
Response Variable^a	Treatment Estimates (Mean)	Treatment Effect p-value	Range of Values in Literature
Fat %	Line 1507 mean = 3.83 Control line mean = 3.94	0.046	3.1 - 5.7 ^b
Protein %	Line 1507 mean = 11.20 Control line mean = 11.32	0.611	6.0 - 12 ^b
ADF %	Line 1507 mean = 3.55 Control line mean = 3.68	0.250	3.0 - 4.3 ^c
NDF %	Line 1507 mean = 10.47 Control line mean = 10.08	0.315	8.3 - 11.9 ^b
Carbohydrates % ^d	Line 1507 mean = 83.45 Control line mean = 83.23	0.352	63.3 - 89.7 ^c
Ash %	Line 1507 mean = 1.51 Control line mean = 1.50	0.335	1.1 - 3.9 ^c

a - Percentage on a dry weight basis.

b - Watson, 1987.

c - Watson, 1982.

d - Carbohydrates are calculated as the percentage of dry weight = 100% - % protein - % fat - % ash. Fiber (ADF and NDF) is included in the carbohydrates.

Table 2.

The means and p-values for the mineral composition of grain from maize line 1507 and the control hybrid for samples collected in the 1998/1999 field trial. The range of values found in the literature for each trait is listed.

Mineral Analyses of Grain			
Response Variable ^a	Treatment Estimates	Treatment Effect p-value	Range of Values in Literature ^c
Calcium %	Line 1507 mean = 0.0036 Control line mean = 0.0031	0.620	0.01 - 0.10 0.002 - 0.011 ^d
Phosphorous %	Line 1507 mean = 0.33 Control line mean = 0.32	0.161	0.26 - 0.75
Copper ppm	Line 1507 mean = 2.03 Control line mean = 2.11	0.845	0.9 - 10
Iron %	Line 1507 mean = 0.0025 Control line mean = 0.0025	0.549	0.0001 - 0.01
Magnesium %	Line 1507 mean = 0.12 Control line mean = 0.13	0.524	0.09 - 1.0
Manganese %	Line 1507 mean = 0.0005 Control line mean = 0.0006	0.0003	0.00007 - 0.0054
Potassium %	Line 1507 mean = 0.40 Control line mean = 0.36	0.023	0.32 - 0.72
Sodium %	Line 1507 mean = < LOQ ^b Control line mean = < LOQ	-	0.0 - 0.15
Zinc %	Line 1507 mean = 0.002 Control line mean = 0.002	0.141	0.0012 - 0.0030

^a - Percentage on a dry weight basis or parts per million (ppm) on a dry weight basis.

^b - Below the level of quantitation for sodium of 0.01%

^c - Watson, 1982.

^d - Data from analysis of 22 commercial Pioneer[®] Brand Hybrids.

Table 3.

The means and p-values for the fatty acid composition of grain from maize line 1507 and the control hybrid for samples collected in the 1998/1999 field trial. The range of values found in the literature for each trait is listed.

Fatty Acid Analyses of Grain			
Response Variable ^a	Treatment Estimates	Treatment Effect p-value	Range of Values in Literature ^b
Palmitic %	Line 1507 mean = 11.07 Control line mean = 10.92	0.091	7 - 19
Stearic %	Line 1507 mean = 2.28 Control line mean = 2.44	0.007	1 - 3
Oleic %	Line 1507 mean = 30.61 Control line mean = 32.53	0.002	20 - 46
Linoleic %	Line 1507 mean = 53.10 Control line mean = 51.16	0.002	35 - 70
Linolenic %	Line 1507 mean = 1.29 Control line mean = 1.21	0.0001	0.8 - 2

a - Value of fatty acid is % of total lipid.

b - Watson, 1982.

c - "Within range" refers to whether the treatment estimate for maize line 1507 is within the range given for maize.

Table 4.

The means and p-values for the amino acid composition of grain from maize line 1507 and the control hybrid for samples collected in the 1998/1999 field trial. The range of values found in the literature for each trait is listed.

Amino Acid Analyses of Grain			
Essential Amino Acids			
Response Variable ^a	Treatment Estimates	Treatment Effect p-value	Range of Values in Literature
Glycine	Line 1507 mean = 0.39 Control line mean = 0.40	0.150	0.26 - 0.47 ^b 0.24 - 0.41 ^c
Cysteine	Line 1507 mean = 0.21 Control line mean = 0.23	<0.0001	0.12 - 0.16 ^b 0.13 - 0.27 ^c
Methionine	Line 1507 mean = 0.19 Control line mean = 0.20	0.020	0.10 - 0.21 ^b 0.12 - 0.26 ^c
Tryptophan	Line 1507 mean = 0.08 Control line mean = 0.08	0.065	0.05 - 0.12 ^b 0.05 - 0.10 ^c

a - Percentage on a dry weight basis.

b - Watson, 1982.

c - Data from analysis of 22 commercial Pioneer[®] Brand Hybrids.

d - Iowa Gold Catalog, 1994.

Table 5

The means and p-values for the vitamin composition of grain from maize line 1507 and the control hybrid for samples collected in the 1998/1999 field trial. The range of values found in the literature for each trait is listed.

Vitamin Analyses of Grain			
Response Variable ^a	Treatment Estimates	Treatment Effect p-value	Range of Values in Maize ^b
Thiamine Hydrochloride (B1) ppm	Line 1507 mean = 3.64 Control line mean = 4.06	0.002	3.0 – 8.6
Riboflavin (B2) ppm	Line 1507 mean = 1.67 Control line mean = 1.66	0.827	0.25 – 5.6
Folic Acid ppm	Line 1507 mean = 0.151 Control line mean = 0.144	0.676	0.3 ^c
Total tocopherols ppm	Line 1507 mean = 48.2 Control line mean = 41.6	0.0005	42 - 87

a – Parts per million (ppm) on a dry weight basis.

b – Watson, 1982

c – Watson, 1987 reports an average value for folic acid in grain as 0.3 mg/kg.

Table 6. Mean values of proximate composition of forage (France and Italy, 1999)

Proximate Analysis of Forage				
Response Variable	1507lp (unsprayed)	1507s (sprayed)	2722 (control)	Range of Values in Literature*
Fat %	2.48 ^a ± 0.30	2.42 ^a ± 0.30	2.48 ^a ± 0.30	0.7 - 6.7
Protein %	8.72 ^a ± 0.29	9.27 ^a ± 0.31	8.87 ^a ± 0.29	3.5 - 15.9
ADF %	28.07 ^a ± 1.55	28.46 ^a ± 1.58	28.68 ^a ± 1.54	30**
NDF %	50.62 ^a ± 1.93	50.15 ^a ± 2.03	50.83 ^a ± 1.96	51**
Carbohydrates %***	84.25 ^a ± 0.63	83.50 ^a ± 0.65	84.00 ^a ± 1.31	66.9 – 94.5
Ash %	4.56 ^a ± 0.28	4.81 ^a ± 0.29	4.63 ^a ± 0.28	1.3 - 10.5

Estimated mean values (across all sites) ±SE followed by different letters are significantly different ($\alpha = 0.05$). Data presented as % of dry weight.

* Watson, 1982

** Watson, 1982 reports an average value for ADF of 30% and NDF of 51%.

*** Carbohydrates are calculated as the percentage of dry weight = 100% - % protein - % fat - % ash. Fiber (ADF and NDF) is included in the carbohydrates.

Table 7. Mean values of proximate composition of grain (France and Italy, 1999)

Proximate Analysis of Grain				
Response Variable	1507lp (unsprayed)	1507s (sprayed)	2722 (control)	Range of Values in Literature*
Fat %	4.21 ^a ± 0.12	4.41 ^a ± 0.14	4.41 ^a ± 0.12	3.1 – 5.7*
Protein %	11.73 ^a ± 0.24	12.04 ^a ± 0.28	10.98 ^b ± 0.24	6.0 – 12*
ADF %	2.37 ^a ± 0.17	2.52 ^a ± 0.18	2.29 ^a ± 0.17	3.0 – 4.3**
NDF %	10.16 ^a ± 0.30	10.54 ^a ± 0.35	10.13 ^a ± 0.30	8.3 – 11.9*
Carbohydrates % ***	82.46 ^{ab} ± 0.57	81.97 ^b ± 0.25	83.00 ^a ± 0.28	63.3 – 89.7**
Ash %	1.60 ^a ± 0.04	1.67 ^a ± 0.05	1.56 ^a ± 0.04	1.1 – 3.9**

Estimated mean values (across all sites) ±SE followed by different letters are significantly different ($\alpha = 0.05$). Data presented as % of dry weight.

* Watson, 1987.

** Watson, 1982.

*** Carbohydrates are calculated as the percentage of dry weight = 100% - % protein - % fat - % ash. Fiber (ADF and NDF) is included in the carbohydrates.

Table 8. Mean values of vitamin composition of grain (France and Italy, 1999)

Vitamin Analyses of Grain				
Response Variable	1507lp (unsprayed)	1507s (sprayed)	2722 (control)	Range of Values in Literature*
Thiamine Hydrochloride (B1) ppm	3.502 ^a ± 0.184	3.874 ^a ± 0.208	3.818 ^a ± 0.184	3.0 – 8.6
Riboflavin (B2) ppm	1.208 ^b ± 0.037	1.199 ^b ± 0.045	1.314 ^a ± 0.037	0.25 – 5.6
Folic Acid ppm	0.158 ^a ± 0.005	0.161 ^a ± 0.007	0.154 ^a ± 0.005	0.3**
Total tocopherols ppm	28.51 ^a ± 1.15	29.30 ^a ± 1.40	29.24 ^a ± 1.15	42 - 87

Estimated mean values (across all sites) ±SE followed by different letters are significantly different ($\alpha = 0.05$). Data presented as parts per million (ppm) on a dry weight basis.

* Watson, 1982

** Watson, 1987 reports an average value for folic acid in grain as 0.3 mg/kg.

Table 9. Mean values of amino acid composition of grain (France and Italy, 1999), cont.

Response Variable	1507lp (unsprayed)	1507s (sprayed)	2722 (control)	Range of Values in Literature
Cysteine	0.22 ^a ± 0.004	0.23 ^a ± 0.005	0.22 ^a ± 0.004	0.12 - 0.16* 0.13 - 0.27**
Methionine	0.20 ^a ± 0.0035	0.21 ^a ± 0.0041	0.20 ^a ± 0.0035	0.10 - 0.21* 0.12 - 0.26**
Tryptophan	0.10 ^a ± 0.0035	0.10 ^a ± 0.0037	0.09 ^a ± 0.0035	0.05 - 0.12* 0.05 - 0.10**
Non-essential Amino Acids				
Serine	0.55 ^a ± 0.012	0.56 ^a ± 0.014	0.50 ^b ± 0.012	0.42 - 0.55* 0.25 - 0.46**
Alanine	0.83 ^a ± 0.018	0.85 ^a ± 0.022	0.74 ^b ± 0.018	0.64 - 0.99* 0.37 - 0.81**
Glutamic Acid	2.12 ^a ± 0.050	2.18 ^a ± 0.060	1.90 ^b ± 0.050	1.24 - 1.96* 0.89 - 2.02**
Proline	1.00 ^a ± 0.0212	1.04 ^a ± 0.0258	0.92 ^b ± 0.0217	0.66 - 1.03* 0.43 - 1.01**
Aspartic Acid	0.79 ^a ± 0.0157	0.81 ^a ± 0.0186	0.71 ^b ± 0.0157	0.58 - 0.72* 0.37 - 0.80**
Tyrosine	0.21 ^a ± 0.0048	0.21 ^a ± 0.0057	0.19 ^b ± 0.0048	0.29 - 0.47* 0.17 - 0.31***

Estimated mean values (across all sites) ±SE followed by different letters are significantly different ($\alpha = 0.05$). Data presented as % of dry weight.

* Watson, 1982.

** Data from analysis of 22 commercial Pioneer® Brand Hybrids.

*** Iowa Gold Catalog, 1994.

Table 10. Mean values of mineral composition of grain (France and Italy, 1999)

Mineral Analyses of Grain				
Response Variable	1507lp (unsprayed)	1507s (sprayed)	2722 (control)	Range of Values in Literature**
Calcium %	0.008 ^a ± 0.001	0.007 ^a ± 0.001	0.007 ^a ± 0.001	0.01 - 0.10 0.002 - 0.011***
Phosphorous %	0.33 ^{ab} ± 0.008	0.34 ^a ± 0.010	0.31 ^b ± 0.008	0.26 - 0.75
Copper ppm	1.88 ^a ± 0.52	1.53 ^a ± 0.57	1.35 ^a ± 0.52	0.9 - 10
Iron %	0.0021 ^{ab} ± 0.00005	0.0021 ^a ± 0.00006	0.0019 ^b ± 0.00005	0.0001 - 0.01
Magnesium %	0.114 ^a ± 0.0036	0.117 ^a ± 0.0041	0.106 ^a ± 0.0036	0.09 - 1.0
Manganese %	0.0008 ^a ± 0.00004	0.0008 ^a ± 0.00050	0.0008 ^a ± 0.00004	0.00007 - 0.0054
Potassium %	0.416 ^a ± 0.016	0.417 ^a ± 0.012	0.380 ^b ± 0.016	0.32 - 0.72
Sodium* %	0.0015 ^a ± 0.0	0.0015 ^a ± 0.0	0.0015 ^a ± 0.0	0.0 - 0.15
Zinc %	0.0018 ^a ± 0.0001	0.0017 ^a ± 0.0001	0.0019 ^a ± 0.0001	0.0012 - 0.0030

Estimated mean values (across all sites) ±SE followed by different letters are significantly different ($\alpha = 0.05$). Data presented as % of dry weight.

* All values were below the level of quantitation for sodium of 0.01%

** Watson, 1982.

*** Data from analysis of 22 commercial Pioneer[®] Brand Hybrids.

Table 11. Summary analysis for minerals in forage, year 2000

Response Variable ¹	Range of Values in Literature ²	Means ³		
		1507	1507 Sprayed	Control
Phosphorus	0.15 – 0.55	0.223 ± 0.005	0.228 ± 0.005	0.229 ± 0.005
Calcium	0.2 – 0.6	0.335 ± 0.013	0.301 ± 0.013	0.334 ± 0.013

¹ Percent of dry weight

² Watson, 1982

³ Least square means; values followed by an asterisk are significantly ($p < 0.05$) different from the control

Table 12. Single site analysis of mineral composition in forage, site BU1, Bulgaria

Response Variable ¹	Range of Values in Literature ²	Means ³		
		1507	1507 Sprayed	Control
Phosphorus	0.15 – 0.55	0.175 * ± 0.006	0.177 * ± 0.006	0.208 ± 0.006
Calcium	0.2 – 0.6	0.323 ± 0.025	0.288 ± 0.025	0.346 ± 0.025

¹ Percent of dry weight

² Watson, 1982

³ Least square means; values followed by an asterisk are significantly ($p < 0.05$) different from the control

Table 13. Summary analysis of proximates in maize grain from the year 2000 study

Response Variable ¹	Range of Values in Literature ²	Means ³		
		1507	1507 Sprayed	Control
Fat	3.1 – 5.7	3.57 ± 0.04	3.53 ± 0.04	3.54 ± 0.04
Protein	6 – 12	10.9 * ± 0.1	10.2 ± 0.1	10.1 ± 0.1
Fiber (crude)	2.0 – 5.5 ⁴	2.6 ± 0.0	2.6 ± 0.0	2.5 ± 0.0
ADF	3.0 – 4.3 ⁴	3.6 ± 0.1	3.6 ± 0.1	3.6 ± 0.1
NDF	8.3 – 11.9	10.1 ± 0.1	9.9 ± 0.1	9.9 ± 0.1
Ash	1.1 – 3.9	1.64 * ± 0.02	1.63 * ± 0.02	1.54 ± 0.02
Carbohydrates	78.4 – 89.8	83.9 * ± 0.1	84.6 ± 0.1	84.9 ± 0.1

¹ Percent of dry weight

² Watson, 1987

³ Least square means; values followed by an asterisk are significantly ($p < 0.05$) different from the control

⁴ Watson, 1982

Table 14. Summary analysis of amino acids in grain (2000)

Response Variable ¹	Range of Values in Literature ²	Means ³		
		1507	1507 Sprayed	Control
Essential Amino Acids⁴				
Methionine	0.10 – 0.21	0.19 ± 0.00	0.18 * ± 0.00	0.19 ± 0.00
Cysteine	0.12 – 0.16 0.16 – 0.22 ⁵	0.21 * ± 0.00	0.20 ± 0.00	0.20 ± 0.00
Lysine	0.20 – 0.38	0.29 * ± 0.00	0.28 ± 0.00	0.28 ± 0.00
Tryptophan	0.05 – 0.12	0.10 ± 0.00	0.09 ± 0.00	0.09 ± 0.00
Threonine	0.29 – 0.39	0.36 * ± 0.00	0.34 ± 0.00	0.34 ± 0.00
Isoleucine	0.26 – 0.40	0.35 * ± 0.00	0.33 ± 0.00	0.32 ± 0.00
Histidine	0.20 – 0.28	0.27 * ± 0.00	0.26 ± 0.00	0.26 ± 0.00
Valine	0.21 – 0.52	0.47 * ± 0.00	0.45 ± 0.00	0.44 ± 0.00
Leucine	0.78 – 1.52	1.31 * ± 0.02	1.23 ± 0.02	1.19 ± 0.02
Arginine	0.29 – 0.59	0.36 * ± 0.00	0.35 ± 0.00	0.34 ± 0.00
Phenylalanine	0.29 – 0.57	0.52 * ± 0.01	0.49 * ± 0.01	0.47 ± 0.01
Glycine	0.26 – 0.47	0.36 ± 0.04	0.35 ± 0.04	0.42 ± 0.04
Nonessential Amino Acids				
Alanine	0.64 – 0.99	0.80 * ± 0.01	0.76 * ± 0.01	0.73 ± 0.01
Aspartic Acid	0.58 – 0.72	0.69 * ± 0.01	0.65 ± 0.01	0.64 ± 0.01
Glutamic Acid	1.24 – 1.96 0.89 – 2.02 ⁶	1.99 * ± 0.03	1.88 ± 0.03	1.82 ± 0.03
Proline	0.66 – 1.03	0.79 * ± 0.01	0.76 ± 0.01	0.75 ± 0.01
Serine	0.42 – 0.55	0.49 * ± 0.01	0.46 ± 0.01	0.45 ± 0.01
Tyrosine	0.29 – 0.47 0.17 – 0.31 ⁷	0.16 * ± 0.00	0.16 * ± 0.00	0.15 ± 0.00

¹ Percent of dry weight

² Watson, 1982

³ Least square means; values followed by an asterisk are significantly ($p < 0.05$) different from the control

⁴ Listed in approximate order of decreasing importance in feed formulation (Watson 1982)

⁵ Iowa Gold Catalog, 1997

⁶ Pioneer Commercial Hybrids: range from 22 commercial Pioneer[®] Brand hybrids

⁷ Iowa Gold Catalog, 1994

Table 15. Summary analysis of fatty acids in grain, year 2000

Response Variable ¹	Range of Values in Literature ²	Means ³		
		1507	1507 Sprayed	Control
Palmitic acid	7 – 19	11.0 ± 0.1	11.0 ± 0.1	11.0 ± 0.1
Stearic acid	1 – 3	1.62 ± 0.05	1.68 ± 0.05	1.64 ± 0.05
Oleic acid	20 – 46	32.6* ± 0.3	31.9 ± 0.3	31.6 ± 0.3
Linoleic acid	35 – 70	52.2* ± 0.3	52.9 ± 0.3	53.3 ± 0.3
Linolenic acid	0.8 – 2	1.32 ± 0.02	1.34 ± 0.02	1.30 ± 0.02
Arachidic acid	0.1 – 2	0.38 ± 0.00	0.37 ± 0.00	0.37 ± 0.00

¹ Percent relative total fatty acids

² Watson, 1982

³ Least square means; values followed by an asterisk are significantly ($p < 0.05$) different from the control

Table 16. Summary analysis of vitamin content in grain (2000)

Response Variable ¹	Range of Values in Literature ²	Means ³		
		1507	1507 Sprayed	Control
Vitamin A ⁴	0.3 – 5.4	2.39 ± 0.18	2.68 ± 0.18	2.24 ± 0.18
Vitamin B1	3.0 – 8.6	4.34 ± 0.11	4.31 ± 0.11	4.62 ± 0.11
Vitamin B2	0.25 – 5.6	1.79* ± 0.05	1.72 ± 0.05	1.63 ± 0.05
Folic Acid	0.3 (mean) ⁵	0.270 ± 0.015	0.273 ± 0.015	0.283 ± 0.015
Vitamin E ⁶	3.0 – 12.1	3.96 ± 0.72	4.81 ± 0.72	5.16 ± 0.72
Tocopherols (total) ⁷	42 – 87	48.9 ± 2.3	49.0 ± 2.3	45.3 ± 2.3

¹ mg/kg dry weight

² Watson, 1982

³ Least square means; values followed by an asterisk are significantly ($p < 0.05$) different from the control

⁴ Measured as beta-carotene

⁵ Watson, 1987

⁶ Measured as alpha-tocopherol

⁷ Sum of alpha-tocopherol, beta-tocopherol, gamma-tocopherol and delta-tocopherol

Table 17. Summary analysis of secondary metabolites and antinutrients in grain (2000)

Response Variable ^{1,2}	Range of Values In Literature ³	Means ⁴		
		1507	1507 Sprayed	Control
Secondary Metabolites				
Inositol	NR ⁵	0.155 ± 0.003	0.153 ± 0.003	0.148 ± 0.003
Raffinose	0.08 – 0.30	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Furfural	NR	0.0006* ± 0.0000	0.0009 ± 0.0000	0.0009 ± 0.0000
P-Coumaric Acid	NR	0.032 ± 0.001	0.031* ± 0.001	0.033 ± 0.001
Ferulic Acid	NR	0.295 ± 0.004	0.290 ± 0.004	0.287 ± 0.004
Anti-nutrients				
Phytic acid	0.7 – 1.0	0.698 ± 0.021	0.683 ± 0.021	0.686 ± 0.021
Trypsin Inhibitor (TIU/g)	NR	<LOQ ⁶	<LOQ	<LOQ

¹ Percent of dry weight

² Abbreviation: TIU, trypsin inhibitor units

³ Watson, 1982

⁴ Least square means; values followed by an asterisk are significantly ($p < 0.05$) different from the control

⁵ NR: Not reported

⁶ <LOQ: below the level of quantitation (2,000 TIU/g d.w.)

Table 18. Comparative efficacy for 1507 maize and non-GM control maize from field trials in Spain in 2002

Entry No.	Event	Montañana ¹		Calatorao ²	
		Rep. 1	Rep. 2	Rep. 1	Rep. 2
1	1507 maize A	1.5	0.5	4.3	4.9
2	Non-GM maize A	16.2	13.6	64.3	45.2
3	1507 maize B	0.6	1.1	7.8	9.3
4	Non-GM maize B	23.7	18.0	65.5	64.8
5	1507 maize C	5.2	13.7	17.4	35.8
6	Non-GM maize C	16.1	22.1	66.9	88.0
7	1507 maize D	4.8	0.9	5.2	7.2
8	Non-GM maize D	26.2	16.3	63.1	63.0
9	Non-GM maize E	21.8	14.9	64.5	73.2
10	Non-GM maize F	19.1	28.1	61.4	62.5
Mean 1507		3.5		11.5	
Mean non-GM		19.0		65.1	

¹ Mean insect tunnelling length (cm) in the stalks of samples of 10 plants per plot; at Montañana 50% of the larvae were identified as ECB and 50% as *Sesamia* spp.

² Mean insect tunnelling length (cm) in the stalks of samples of 10 plants per plot; at Calatorao 5% of the larvae were identified as ECB and 95% as *Sesamia* spp

Table 19. Agronomic data obtained from field trials of 1507 maize in Spain in 2002. Summary of germination, time to pollen shed, time to silking, stalk lodging, root lodging, plant height, and ear height. The data was obtained from three locations and total number of replicates was n = 9

	Germination/ early population ¹	GDU ⁸ 50% pollen shed ²	GDU ⁸ 50% silking ³	Stalk lodging ⁴ (%)	Root lodging ⁵ (%)	Plant height ⁶ (cm)	Ear height ⁷ (cm)
Montaña (Aragón, Spain)							
1507 maize	41	814.5	888.7	1	0	191	63
Non-GM control	38	788.7	858.5	17	0	180	57
Cogullada (Aragón, Spain)							
1507 maize	45	815.5	890.5	2	0	193	64
Non-GM control	49	774.6	845	32	0	186	60
Calatorao (Aragón, Spain)							
1507 maize	56	840.9	915.9	2	0	208	85
Non-GM control	58	788.7	855.8	49	0	193	76
Average							
1507 maize	47	823.6*	898.4*	2*	0	197*	71*
Non-GM control	48	784.0	853.1	33	0	186	64

¹ Number of plants emerged per 60 seed planted

² Number of accumulated heat units when approximately 50% of the plants are shedding pollen

³ Number of accumulated heat units when approximately 50% of the plants are silking

⁴ Percent of plants broken below the primary ear

⁵ Percent of plants leaning $\geq 30^\circ$ in the first 1/2 meter above the soil surface

⁶ Measured from the soil surface to the tip of tassel, n=10

⁷ Measured from the soil surface to the base primary ear, n=10

⁸ GDU: Growing Degree Units or accumulated heat units

* Statistically significant differences (P-value <0.05)

Table 20. Agronomic data obtained from field trials of 1507 maize in Spain in 2002. Summary of final population, stay green, disease incidence, insect damage, and grain moisture. The data was obtained from three locations and total number of replicates was n = 9

	Final population¹	Stay green²	Disease incidence³	Insect damage⁴	Grain moisture⁵
Montañana (Aragón, Spain)					
1507 maize	14	3	8	9	29
Non-GM control	15	2	9	6	29
Cogullada (Aragón, Spain)					
1507 maize	19	1	8	8	26
Non-GM control	11	1	9	3	26
Calatorao (Aragón, Spain)					
1507 maize	15	2	9	8	34
Non-GM control	11	1	9	4	32
Average					
1507 maize	16	2	8	8*	30
Non-GM control	12	1	9	4	29

¹Total number of viable plants (per plot) remaining at maturity

²Overall plant health at maturity evaluated on a 1 to 9 scale where 1 is completely dead and 9 is very green

³Level of disease resistance at maturity evaluated on a 1 to 9 scale where 1 is poor resistance and 9 is high resistance or no visible disease

⁴Level of destructive insect resistance at maturity evaluated on a 1 to 9 scale where 1 is poor resistance and 9 is high resistance or no damage

⁵Percent water content of grain at typical harvest maturity

* Statistically significant differences (P-value < 0.05)

Table 21. Agronomic data obtained from field trials at two locations in Spain in 2002.

Ent. No.	Event	Yield (kg/ha)	Yield (% of mean) ¹	Moisture at harvest (%)	Root lodging ²	Stalk lodging ³	ECB + <i>Sesamia</i> damage ⁴
1	1507 maize A	9696.8	120.2	28.4	0	2	8.7
2	Non-GM maize A	7380.9	92.0	27.5	0	21	4.7
3	1507 maize B	9445.8	117.3	27.8	8	2	8.0
4	Non-GM maize B	7851.6	97.6	27.6	5	19	5.7
5	1507 maize C	8987.6	112.0	26.7	1	1	7.5
6	Non-GM maize C	8134.0	102.0	28.1	0	20	4.7
7	1507 maize D	8203.1	102.3	28.1	4	9	6.7
8	Non-GM maize D	7437.4	93.0	25.6	2	15	5.5
9	Non-GM maize E	6470.8	80.8	28.3	2	22	5.0
10	Non-GM maize F	6621.4	82.8	26.0	13	21	4.0

¹ Yield expressed as percent of overall experiment mean; overall mean is 8022.9 kg/ha

² Count of early (before flowering) root lodged plants in two central rows

³ Number of stalk lodged plants in two central rows; considered plants broken at or below ear node

⁴ Damage scores are recorded visually based on a 1 to 9 scale (1 = completely susceptible, and 9 = completely resistant)

Table 22. Mean agronomic data from 1507 maize and non-GM control in comparable genetic backgrounds, collected during field trials in the USA in 1999

Trait	1507 maize	Non-GM control	Number of locations	Number of replicates	LSD ¹
Yield (kg per hectare)	11510.8	11171.9	15	41	470.7
Moisture (%)	18.8	18.6	15	41	0.07
Accumulated maize growing degree days to reach 50% pollen shed	1351	1353	4	12	18.4
Accumulated maize growing degree days to reach 50% silking	1343	1337	4	12	18.9
Grain density ²	26.47	26.42	9	27	0.32
Plant height (metres)	2.52	2.50	9	19	0.07
Ear height (metres)	1.16	1.13	9	19	0.06
Early stand count establishment (average number of plants emerging per plot)	74.5	71.7	4	12	26.5
Visual rating of emergence vigour from spike to one-leaf stage ³	6.1	6.0	4	12	0.6
Visual rating of vigour at three- to five-leaf stage ³	6.1	6.3	4	12	0.6
Stalk lodging ⁴	0.3	0.6	11	33	0.8
Root lodging ⁴	1.1	1.5	10	30	1.4
Dropped ears per plot	0.0	0.0	10	30	0.1
Top integrity ⁵	7.9	7.6	9	27	1.1

1:Least Significant Difference at the 0.05 level

2:Weight (in kg) of a bushel of grain at 15.5% moisture

3:Scores are recorded visually based on a 1 to 9 scale (1 = worst, and 9 = best)

4:Average number of plants per plot that showed lodging of the specified type

5:1-9 visual scale that describes how well the stalks remain intact above the ear, (1 = worst, and 9 =best)