Application for authorization of stacked Bt11 x MIR162 x 1507 x GA21 maize in the European Union under Regulation (EC) No 1829/2003

PART II: SUMMARY

This document is complete as of March 2012. Since it is submitted as one part of a regulatory application, which is subject to an on-going regulatory review, it may be subject to later amendment or replacement. The information may also be supplemented with additional material requested by regulatory authorities. As such, it may only be considered properly with reference to those later amendments or supplementary materials and in the context of the dossier as a whole.

A. GENERAL INFORMATION

1. Details of application

a) Member State of application Germany
b) Application number Not available at the time of submission
c) Name of the product (commercial and other names) Bt11 x MIR162 x TC1507 x GA21 maize (hereafter referred to as Bt11 x MIR162 x 1507 x GA21 maize[1]) The commercial name assigned to seed products in the US market is Agrisure Viptera$^{(TM)}$ 3220.
d) Date of acknowledgement of valid application Not available at time of submission

2. Applicant

a) Name of applicant Syngenta Crop Protection AG, Basel Switzerland acting on its behalf and through its affiliated companies
b) Address of applicant Syngenta Crop Protection AG Schwarzwaldallee 215 CH 4058 Basel Switzerland
c) Name and address of the person established in the Community who is responsible for the placing on the market, whether it be the manufacturer, the importer or the distributor, if different from the applicant (Commission Decision 2004/204/EC Art 3(a)(ii)) The product will be imported and used as any other maize in the European Union Member States (EU) by operators currently involved in these processes

[1] Event TC1507 may be referred to as maize line 1507 in the European Union. It is normally referred to as Event TC1507 in applications submitted by Dow AgroSciences LLC to most countries other than the European Union. This applies also to the Detection Method published by the Community Reference Laboratory for Food and Feed in the EU.

3. Scope of the application

☒ GM plants for food use

☒ Food containing or consisting of GM plants

☒ Food produced from GM plants or containing ingredients produced from GM plants

☒ GM plants for feed use

☒ Feed containing or consisting of GM plants

☒ Feed produced from GM plants

☒ Import and processing (Part C of Directive 2001/18/EC)

☐ Seeds and plant propagating material for cultivation in Europe (Part C of Directive 2001/18/EC)

4. Is the product being simultaneously notified within the framework of another regulation (e.g. Seed legislation?)?

Yes ☐	No ☒
If *yes*, specify	

5. Has the GM plant been notified under Part B of Directive 2001/18/EC and/or Directive 90/220/EEC?

Yes ☐	No☒
A risk assessment has been performed according to the Directive 2001/18/EC and forms part of this application.	

6. Has the GM plant or derived products been previously notified for marketing in the Community under Part C of Directive 2001/18/EC or Regulation (EC) 258/97?

Yes ☐	No ☒
If *yes*, specify	

7. Has the product been notified in a third country either previously or simultaneously?

Yes ☒	No ☐
If *yes*, specify Submissions covering Bt11 x MIR162 x 1507 x GA21 maize have been made in third countries around the world and these are at different stages in the approval process.	

8. General description of the product

a) Name of the recipient or parental plant and the intended function of the genetic modification

Bt11 x MIR162 x 1507 x GA21 maize is a stacked genetically modified (GM) product that has been produced by conventional breeding crosses of:

- Event Bt11 maize (Bt11 maize) which produces a truncated Cry1Ab protein for control of certain lepidopteran pests and a phosphinothricin acetyltransferase (PAT) protein that confers tolerance to herbicide products containing glufosinate ammonium.
- Event MIR162 maize (MIR162 maize) is a GM product which expresses a Vip3Aa20 protein for control of certain lepidopteran pests and a phosphomannose isomerase (PMI) protein, which acts as a selectable marker enabling transformed plant cells to utilize mannose as the only primary carbon source.
- Maize line 1507 maize (1507[2] maize) expressing the Cry1F protein which confers protection against certain lepidopteran pests and a phosphinothricin acetyltransferase (PAT) protein that confers tolerance to herbicide products containing glufosinate ammonium.
- Event GA21 maize (GA21 maize) which produces a modified maize 5-enolpyruvylshikimate-3-phosphate synthase enzyme (mEPSPS) that confers tolerance to herbicide products containing glyphosate.

b) Types of products planned to be placed on the market according to the authorisation applied for

This application under Regulation (EC) 1829/2003 covers the import, food and feed use and processing of Bt11 x MIR162 x 1507 x GA21 maize. It does not cover cultivation.

[2] Maize line 1507 is normally referred to as Event TC1507 in applications submitted by Dow AgroSciences LLC to most countries other than the European Union

c) Intended use of the product and types of users

It is intended that Bt11 x MIR162 x 1507 x GA21 maize will be used as any other conventional maize which is cultivated or imported for all food, feed and industrial purposes.

d) Specific instructions and/or recommendations for use, storage and handling, including mandatory restrictions proposed as a condition of the authorisation applied for

The characteristics of Bt11 x MIR162 x 1507 x GA21 maizeand products derived from it are not different from those of its conventional counterpart, apart from the introduced traits of insect tolerance and tolerance to herbicide products containing glufosinate ammonium or glyphosate. Bt11 x MIR162 x 1507 x GA21 maize has been shown to be as safe and as wholesome as existing varieties of maize. Therefore there are no specific instructions or recommendations for use, storage and handling of Bt11 x MIR162 x 1507 x GA21 maize.

e) Any proposed packaging requirements

The characteristics of Bt11 x MIR162 x 1507 x GA21 maize and products derived from it are not different from those of its conventional counterpart. Bt11 x MIR162 x 1507 x GA21 maize has been shown to be as safe and as wholesome as existing varieties of maize. Therefore there are no specific instructions for packaging.

f) A proposal for labelling in accordance with Articles 13 and Articles 25 of Regulation (EC) 1829/2003. In the case of GMOs, food and/or feed containing or consisting of GMOs, a proposal for labelling has to be included complying with the requirements of Article 4, B(6) of Regulation (EC) 1830/2003 and Annex IV of Directive 2001/18/EC

A proposal for labelling has been included in the application following the guidance provided by EFSA. This includes the labelling requirements outlined by Regulation (EC) No 1829/2003 and Annex IV of Directive 2001/18/EC. Bt11 x MIR162 x 1507 x GA21 maize grain will therefore be labelled as “genetically modified maize” and products derived from it will be labelled as “containing (or produced from) genetically modified maize”. Since Bt11 x MIR162 x 1507 x GA21 maize and derived products are not different from those of its conventional counterpart, no additional labelling is required.

g) Unique identifier for the GM plant (Regulation (EC) 65/2004; does not apply to applications concerning only food and feed produced from GM plants, or containing ingredients produced from GM plants)

A unique identifier for Bt11 x MIR162 x 1507 x GA21 maize has been assigned in accordance with Commission Regulation (EC) 65/2004:
SYN-BT∅11-1 x SYN-IR162-4 x DAS-∅15∅7-1 x MON-∅∅∅21-9

The unique identifiers assigned to all the sub-combinations of SYN-BT∅11-1 x SYN-IR162-4 x DAS-∅15∅7-1 x MON-∅∅∅21-9 maize are the following:

- Bt11 x MIR162: SYN-BTØ11-1 x SYN-IR162-4
- Bt11 x 1507: SYN-BTØ11-1 x DAS-Ø15Ø7-1
- Bt11 x GA21: SYN-BTØ11-1 x MON-ØØØ21-9
- MIR162 x 1507: SYN-IR162-4 x DAS-Ø15Ø7-1
- MIR162 x GA21: SYN-IR162-4 x MON-ØØØ21-9
- 1507 x GA21: DAS-Ø15Ø7-1 x MON-ØØØ21-9
- Bt11 x MIR162 x GA21: SYN-BTØ11-1 x SYN-IR162-4 x MON-ØØØ21-9
- Bt11 x 1507 x GA21: SYN-BTØ11-1 x DAS-Ø15Ø7-1 x MON-ØØØ21-9
- MIR162 x 1507 x GA21: SYN-IR162-4 x DAS-Ø15Ø7-1 x MON-ØØØ21-9
- Bt11 x MIR162 x 1507: SYN-BTØ11-1 x SYN-IR162-4 x DAS-Ø15Ø7-1

h) If applicable, geographical areas within the EU to which the product is intended to be confined under the terms of the authorisation applied for. Any type of environment to which the product is unsuited

The Bt11 x MIR162 x 1507 x GA21 maize and derived productsis suitable for use as any other maize under the terms of the authorisation applied for.

9. Measures suggested by the applicant to take in case of unintended release or misuse as well as measures for disposal and treatment

Maize is incapable of sustained reproduction outside domestic cultivation and is non-invasive of natural habitats. The characteristics of Bt11 x MIR162 x 1507 x GA21 maize, and derived products are not different from those of its conventional counterpart, apart from the intended effect of tolerance to certain lepidopteran insect pests and herbicide products containing glufosinate ammonium or glyphosate.

Cultivation of Bt11 x MIR162 x 1507 x GA21 maize in the EU is not within the scope of this application. In the unlikely event that small amounts of Bt11 x MIR162 x 1507 x GA21 grain accidentally found their way into the environment, this would represent extremely low levels of exposure and the survival of this grain to produce flowering plants would be very unlikely. In addition, volunteers could be easily controlled using any of the current agronomic measures taken to control other commercially available maize.

The Bt11 x MIR162 x 1507 x GA21 maize and derived products has been shown to be as safe and as wholesome as existing varieties of maize. Any unintended releases or misuse can be dealt with in the same way as any other conventional maize.

B. INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

1. Complete name

a) Family name Poaceae (formerly Gramineae)
b) Genus *Zea*
c) Species *mays*
d) Subspecies *mays*
e) Cultivar/breeding line or strain Bt11 x MIR162 x 1507 x GA21
f) Common name Maize; corn

2 a. Information concerning reproduction

(i) Mode(s) of reproduction Sexual reproduction: *Zea mays* is an allogamous plant that propagates through seed produced predominantly by wind-borne cross-pollination. Self pollination of up to 5% may be observed. Male and female flowers are separated on the plant by about 1–1.3m. *Z. mays* has staminate flowers in the tassels and pistillate flowers on the ear shoots. *Z. mays* is a plant with protoandrous inflorescence; however, decades of conventional selection and breeding have produced varieties of maize with protogyny. Asexual reproduction: There is no asexual reproduction in maize.
(ii) Specific factors affecting reproduction The key critical stages of maize reproduction are tasselling, silking, pollination and fertilization. Climatic and drought stress affect pollen viability and silk longevity thus potentially limiting the period of possible cross-pollination. Maize pollen is very sensitive to dehydration as it loses water rapidly. Other factors like rainfall or irrigation inhibit pollen emission because the anther dehiscence is limited by the mechanical layer. In general, maize pollen is only viable for a few hours after

emission. As maize pollen is large and heavy it tends to be deposited close to the source plant and studies have indicated that most maize pollen falls within 5m of the field's edge. In general, such studies have shown that over 98% of maize pollen remains within a radius of 25-50m of the source, although some grains can travel several hundred meters. Climatic conditions also affect grain and seed production, especially under drought conditions during flowering, tasseling and silking. If severe drought occurs during these phenological stages, the grain yield is reduced.

(iii) Generation time

Maize is an annual crop. The generation time from sowing to harvesting varies according to the genetic background and the climate, it can range from as short as 60 to 70 days to as long as 43 to 48 weeks from seedling emergence to maturity.

2 b. Sexual compatibility with other cultivated or wild plant species

Other cultivated plant species: The sexual compatibility of maize with other cultivated plant species is limited to *Zea* species.

Wild plant species*:* No wild relatives of maize are present in Europe. Therefore, maize cannot exchange genes with any other wild species in the EU.

3. Survivability

a) Ability to form structures for survival or dormancy

Maize is an annual crop. Seeds are the only survival structures; they cannot be dispersed without mechanical disruption of the cobs and show little or no dormancy. Natural regeneration from vegetative tissue is not known to occur.

b) Specific factors affecting survivability

Survival of maize is dependent upon temperature, seed moisture, genotype, husk protection and stage of development. Maize cannot persist as a weed. Maize seed can only survive under a narrow range of climatic conditions. Volunteers are killed by frost or easily controlled by current agronomic practices including cultivation and the use of selective herbicides. Maize is incapable of sustained reproduction outside of domestic cultivation and is non-invasive of natural habitats.

4. Dissemination

a) Ways and extent of dissemination

Maize dissemination can only be accomplished through seed dispersal. Seed dispersal does not occur naturally due to the structure of the ear.

b) Specific factors affecting dissemination

Compared to other wind-pollinated species, maize pollen grains are relatively large and therefore settle to the ground rapidly and have usually a short flight range. Although vertical wind movements or gusts during pollen shedding can lift pollen up high in the atmosphere and distribute it over significant distances, concentrations of viable pollen considerably decrease with height and distance from the source. Hence, only low levels of cross-pollination could occur over longer distances under suitable climatic conditions.

5. Geographical distribution and cultivation of the plant, including the distribution in Europe of the compatible species

Maize is the world's most widespread cereal with very diverse morphological and physiological traits; it is grown on approximately 161 million hectares worldwide (2008). Maize is distributed over a wide range of conditions: from latitudes 50º North to 50º South, below sea level of the Caspian plains up to 3000m in the Andes Mountains and from semi-arid regions to arid regions. The greatest maize production occurs where the warmest month isotherms range between 21º and 27º C and the freeze-free season lasts 120-180 days.

The EU is the fourth largest grain maize producer in the world, after the US, China and Brazil. In the EU-27, grain maize was cultivated on about 8.4 million hectares (2009) with a production of 57 million tonnes (2009). Another major maize product is silage maize produced on about 5.1 million hectares (2008).

There are no wild relatives of maize in Europe.

6. In the case of plant species not normally grown in the Member State(s), description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts

Maize was introduced into Europe in the 15th century by Columbus and is widely grown in the EU.

7. Other potential interactions, relevant to the GM plant, of the plant with organisms in the ecosystem where it is usually grown, or used elsewhere, including information on toxic effects on humans, animals and other organisms

Maize is known to interact with other organisms in the environment including insects, birds, and mammals. It is susceptible to a range of fungal diseases and insect pests, as well as to competition from surrounding weeds. Maize is extensively cultivated and has a history of safe use for human food and animal feed. No significant native toxins are reported to be associated with the genus *Zea*.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Description of the methods used for the genetic modification

The Bt11 x MIR162 x 1507 x GA21 maize described in this application has been produced by combining the GM maize events: Bt11, MIR162, 1507 and GA21 through conventional breeding techniques. There was no further genetic modification to produce the stack.

The Bt11, MIR162, 1507 and GA21 maize events maize were produced by genetic modification as follows:

- Bt11 maize was produced using protoplast transformation/regeneration
- MIR162 maize was produced by transformation of immature maize embryos derived from a proprietary *Zea mays* line *via Agrobacterium tumefaciens*-mediated transformation.
- 1507 maize was produced by insertion of a DNA fragment into the maize genome using microprojectile bombardment.
- GA21 maize was produced via microprojectile bombardment of maize suspension culture cells.

2. Nature and source of the vector used

The Bt11 x MIR162 x 1507 x GA21 maize described in this application has been produced by combining the GM maize events: Bt11, MIR162, 1507 and GA21 through conventional breeding techniques.

The vectors used to produce Bt11, MIR162, 1507 and GA21 maize are as follows:

- The Plasmid pZO1502, cut with a *Not*I restriction enzyme, was used to produce Bt11 maize. The plasmid is a derivative of the commercially available plasmid pUC18.
- The plasmid pNOV1300 was used for transformation of MIR162 maize.
- No vector was used for the transformation of 1507 maize.
- A *Not*I restriction fragment from the Plasmid pDPG434, was used to transform GA21 maize via microprojectile bombardment transformation. The plasmid is derived from a pSK- vector which is commonly used in molecular biology and is derived from pUC19.

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

The Bt11 x MIR162 x 1507 x GA21 maize described in this application has been produced by combining the GM maize events: Bt11, MIR162, 1507 and GA21 through conventional breeding techniques. There was no further genetic modification to produce the stacked product. The size, source and intended function of each constituent fragment of the regions intended for insertion in each of the single events is described below:

Event Bt11 maize (pZ01502)

Vector component	Size (bp)	Description
CaMV35S promoter	509	Promoter from the cauliflower mosaic virus.
IVS6-ADH1	471	Maize intron sequence from the maize alcohol dehydrogenase gene used to enhance gene expression in maize.
cry1Ab	1848	Modified *cry1Ab* gene, which encodes a Cry1Ab protein that confers resistance to certain lepidopteran insect pests. The *cry1Ab* gene was originally cloned from *Bacillus thuringiensis* var. *kurstaki* HD-1
NOS	253	Polyadenylation region from the nopaline synthase gene from *Agrobacterium tumefaciens*.
Selectable marker cassette		
CaMV35S promoter	418	Promoter from the cauliflower mosaic virus.
IVS2-ADH1	180	Maize intron sequence from the maize alcohol dehydrogenase gene used to enhance gene expression in maize.
pat	552	*Streptomyces viridochromogenes* gene encoding the selectable marker PAT (phosphinothricin acetyltransferase). PAT confers resistance to herbicides containing glufosinate
NOS	253	Polyadenylation region from the nopaline synthase gene from *Agrobacterium tumefaciens*.
Vector backbone component		
Component present on the 6.2 kb Not1 restriction fragment of pZ01502 used for transformation		
ColE1	674	Origin of replication that permits replication of plasmids in *Escherichia coli (E. coli)*

3. Source of donor DNA, size and intended function of each constituent fragment of the region intended for insertion (cont`d)

Event MIR162 maize (pNOV1300)

Vector component	Size (bp)	Description
ZmUbiInt promoter	1993	Promoter derived from the maize (*Zea mays*) polyubiquitin gene
vip3Aa19	2370	A modified version of the native *vip3Aa1* gene from *Bacillus thuringiensis* that confers resistance to certain lepidopteran pest species.
iPEPC9	108	Intron from the phosphoenolpyruvate carboxylase gene from maize *(Zea mays).*
35S 3' nontranslated region	70	3' nontranslated region sequence from the 35S DNA from the cauliflower mosaic virus (CaMV).
Selectable marker cassette		
ZmUbiInt promoter	1993	Promoter from the maize (*Zea mays*) polyubiquitin gene
pmi	1176	*E. coli pmi* gene encoding the enzyme phosphomannose isomerase (PMI). This gene is also known as *manA.*
NOS	253	Polyadenylation region from the nopaline synthase gene from *Agrobacterium tumefaciens.*

Event 1507 maize (PHI8999A)

Active ingredient cassette	Size (bp)	Description
cry1F	1818	The *cry1F* gene was originally cloned from *Bacillus thuringiensis* subsp. *aizawai*. gene. It provides resistance against certain lepidopteran insect pests such as the European corn borer and *Sesamia* spp.
*ubi*ZM1	1986	Ubiquitin promoter derived from *Zea mays*
ORF25Poly A terminator	714	Terminator from *Agrobacterium tumefaciens* pTi15995
Selectable marker cassette		
CaMV35S	418	Promoter from the cauliflower mosaic virus.
pat	552	*Streptomyces viridochromogenes* gene encoding the selectable marker PAT (phosphinothricin acetyltransferase). PAT confers resistance to herbicides containing glufosinate
terminator	204	Terminator from the cauliflower mosaic virus.

Event GA21 maize (pDPG434)

Vector component	Size (bp)	Description
Rice actin promoter, exon and intron	1.4	5' region of the rice actin 1 gene containing the promoter and first exon and intron provides constitutive expression of the *mepsps* gene in maize.
Optimised transit peptide	0.4	Optimised transit peptide sequence constructed based on transit peptide sequences from maize and sunflower ribulose-1,5-bis phosphate carboxylase oxygenase (RuBisCo) genes.
Modified maize EPSPS gene	1.3	Mutated *epsps* gene, which confers resistance to herbicide products containing glyphosate.
Nos 3' end	0.3	Polyadenylation region from the nopaline synthase gene from *Agrobacterium tumefaciens*.

D. INFORMATION RELATING TO THE GM PLANT

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

The Bt11 x MIR162 x 1507 x GA21 maize described in this application has been produced by combining the GM maize events: Bt11, MIR162, 1507 and GA21 through conventional breeding techniques and produces the following proteins:

1. A truncated Cry1Ab protein for control of certain lepidopteran pests like the common European maize pests: ECB (*Ostrinia nubilalis*) and Mediterranean corn borer; MCB (*Sesamia nonagrioides*).
2. A PAT protein that confers tolerance to herbicide products containing glufosinate ammonium.
3. A Vip3Aa20 protein for control of certain lepidopteran pests like corn earworm (*Heliothis zea.*), black cutworm (*Agrotis ipsilon*), fall armyworm (*Spodoptera frugiperda*), and western bean cutworm (*Striacosta albicosta*) or other lepidopteran pests of the order Noctuidae.
4. A PMI protein as a selectable marker. PMI allows transformed maize cells to utilize mannose as the only primary carbon source while maize cells lacking this protein fail to grow.
5. A Cry1F insecticidal protein, which confers protection against certain lepidopteran pests such as European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp.
6. A modified mEPSPS enzyme that confers tolerance to herbicide products containing glyphosate.

2. Information on the sequences actually inserted or deleted

a) The copy number of all detectable inserts, both complete and partial

The Bt11 x MIR162 x 1507 x GA21 maize described in this application has been produced by combining the GM maize events: Bt11, MIR162, 1507 and GA21 through conventional breeding techniques.

The inserts in Bt11, MIR162 and 1507 maize are present at a single locus and inherited as a single gene in a Mendelian fashion.

The insert integrated in 1507 maize contains one copy of the almost full-length linear fragment (6186 bp from the 6235 bp of insert PHI8999A) used in the transformation, which includes one functional copy of the complete *cry*1F gene and one functional copy of the complete *pat* gene, together with the regulatory sequences necessary for their expression. In addition, the 1507 maize insert contains the following non-functional fragments: one fragment (335 bp) of the *cry*1F gene, with

no *ubi*ZM1(2) promoter sequence; one fragment (15 bp) of the *cry*1F gene, both located at the 5' end of the almost full-length insert; two fragments (201 bp and 138 bp long, respectively) of the *pat* gene, without regulatory sequences associated, located at the 5' border and, one fragment (188 bp) of the *pat* gene, located at the 3' border; one fragment (118 bp) of the polylinker region and *ubi*ZM1(2) promoter sequence located at the 5' border; one fragment (550 bp) of the ORF25PolyA terminator sequence in inverted position located immediately at the 3' end of the almost full-length insert.

The insert in GA21 maize is comprised of six contiguous regions derived from the 3.49 kb *Not*I restriction fragment from pDPG434 employed in the generation of GA21 maize (copies 1-6). Copy 1 contains the rice actin promoter that has a 5' deletion of 696 bp, the actin first exon and intron, the optimized transit peptide, the *mepsps* gene and the NOS terminator. Copies 2, 3 and 4 are intact versions of the 3.49 kb *Not*I restriction fragment from pDPG434. Copy 5 contains a complete rice actin promoter, the actin first exon and intron, the optimized transit peptide and the first 288 bp of the *mepsps* gene which ends in a stop codon and does not contain the NOS terminator. Copy 6 contains the rice actin promoter and a truncated actin first exon only and contains no other elements from pDPG434.

In addition to sequencing, Southern analysis performed on each of the single events demonstrate the absence of further copies of the insert or vector sequence elsewhere in the genome. In order to assess the integrity of the insert from each individual event during conventional breeding to produce Bt11 x MIR162 x 1507 x GA21 maize, additional Southern analysis was performed. The predicted DNA hybridization patterns from each individual event were confirmed in Bt11 x MIR162 x 1507 x GA21 maize, demonstrating preservation of the integrity of the transgenic fragment from each individual event to Bt11 x MIR162 x 1507 x GA21 maize.

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

Not applicable

c) Chromosomal location(s) of insert(s) (nucleus, chloroplasts, mitochondria, or maintained in a non-integrated form), and methods for its determination

The inheritance pattern of the inserts in Bt11, MIR162, 1507 and GA21 maize were analysed and the results showed that insertions had taken place in the nucleus.

The Bt11 x MIR162 x 1507 x GA21 maize described in this application has been produced by combining the GM maize events: Bt11, MIR162, 1507 and GA21 through conventional breeding techniques. It therefore contains the inserts derived from the single events. The presence of the inserts from Bt11, MIR162, 1507 and GA21 maize in the stacked product was confirmed by Southern blot analyses.

d) The organisation of the inserted genetic material at the insertion site

The Bt11 x MIR162 x 1507 x GA21 maize described in this application has been produced by combining the GM maize events: Bt11, MIR162, 1507 and GA21 through conventional breeding techniques. The organisation of the inserted genetic

material in Bt11, MIR162, 1507 and GA21 maize is as follows:

Bt11 maize

Sequencing and southern data have demonstrated that Bt11 maize contains a single DNA insertion with one copy of both the *cry1Ab* and the *pat* genes. The sequence analysis confirmed that the insert is intact and that the contiguousness of the functional elements within the insert as intended in pZO1502 has been maintained

MIR162 maize

Sequencing and southern data have demonstrated that MIR162 maize contains a single DNA insertion with one copy of both the *vip3Aa20* and the *pmi* genes. The sequence analysis confirmed that the insert is intact and that the contiguousness of the functional elements within the insert as intended in pNOV1300 has been maintained.

1507 maize

Sequencing and southern data have demonstrated that 1507 maize contains a single DNA insertion with one copy of the almost full-length linear fragment used in the transformation, which includes one functional copy of the complete *cry*1F gene and one functional copy of the complete *pat* gene, together with the regulatory sequences necessary for their expression. The insert also contains two non-functional fragments of the *cry*1F gene, three non-functional fragments of the *pat* gene, one non-functional fragment of the polylinker region and *ubi*ZM1(2) promoter, and one non-functional fragment of the ORF25PolyA terminator sequence. See section D.2.a for further detail. The sequence analysis confirmed that the insert is intact and that the contiguousness of the functional elements within the insert as intended in PHI18999A has been maintained.

GA21 maize

Sequence analysis of the GA21 maize insert demonstrates that the insert is comprised of six contiguous regions derived from the 3.49 kb *Not*I restriction fragment from pDPG434 employed in the generation of Event GA21 (copies 1-6). See section D.2.a for further detail. The sequence analysis confirmed that the insert is intact and that the contiguousness of the functional elements within the insert as intended in pDPG434 has been maintained.

Molecular comparisons of the Bt11 x MIR162 x 1507 x GA21 maize with the single events Bt11, MIR162, 1507 and GA21 maize have shown that the inserts are preserved in Bt11 x MIR162 x 1507 x GA21 maize.

3. Information on the expression of the insert

a) Information on developmental expression of the insert during the life cycle of the plant

Bt11 x MIR162 x 1507 x GA21 maize was produced by combining Bt11, MIR162, 1507 and GA21 maize through conventional breeding techniques. Therefore these maize plants produce the transgenic proteins inherited from these single GM maize events: Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS.

Tissues from maize plants derived from Bt11, MIR162, 1507 and GA21 maize and a breeding stack containing these events (Bt11 x MIR162 x 1507 x GA21) were analyzed by enzyme-linked immunosorbent assay (ELISA) to compare the concentrations of Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS.

The analyses were performed on key plant tissues collected from transgenic hybrid plants at different sampling times across the growing season. To control for background effects, the corresponding tissues from a near-isogenic control maize were also analyzed.

The concentrations of Cry1Ab, Vip3Aa20, PMI, Cry1F and mEPSPS were, in general, statistically similar in the Bt11 x MIR162 x 1507 x GA21 maize hybrid and the corresponding individual event hybrids. Bt11 x MIR162 x 1507 x GA21 maize however contains two functional copies of the *pat* gene and is therefore not expected to contain the same PAT concentrations as the single event parents.

Mean Cry1Ab concentrations were comparable in the tissues of Bt11 maize and Bt11 x MIR162 x 1507 x GA21 maize. PAT concentrations were higher in the Bt11x MIR162 x 1507 x GA21 stack than in Bt11 maize. Vip3Aa20 and PMI concentrations were comparable in the tissues of MIR162 maize and Bt11 x MIR162 x 1507 x GA21 maize; Cry1F and PAT concentrations were comparable in the tissues of 1507 maize and Bt11 x MIR162 x 1507 x GA21 maize and mEPSPS concentrations were comparable in the tissues of GA21 maize and Bt11 x MIR162 x 1507 x GA21 maize. As expected, no Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS proteins were detected in the near-isogenic control samples. Although some statistically significant differences were seen, these differences were small or not consistent across the growing season. These results confirm that, as expected, transgenic protein expression in Bt11 x MIR162 x 1507 x GA21 maize is not substantially different from that of the Bt11, MIR162, 1507 or GA21 single maize events.

b) Parts of the plant where the insert is expressed

To characterize the range of expression of Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS proteins in Bt11 x MIR162 x 1507 x GA21 maize plants and the single events, the concentrations of these proteins were determined by ELISA in several plant tissues (leaves, roots, kernels and pollen).

Quantifiable concentrations of Cry1Ab protein were detected in leaves, roots and kernels derived from Bt11 maize and Bt11 x MIR162 x 1507 x GA21 maize. Very

low levels of Cry1Ab expression were detected in the pollen of Bt11 maize and Bt11 x MIR162 x 1507 x GA21 maize.

Quantifiable concentrations of PAT protein were detected in leaves, roots and whole plants derived from Bt11 maize, 1507 maize and Bt11 x MIR162 x 1507 x GA21 maize, however no quantifiable levels could be detected in the kernels or pollen.

Quantifiable concentrations of Vip3Aa20 protein were detected in leaves, roots, whole plants, kernels and pollen derived from MIR162 maize and Bt11 x MIR162 x 1507 x GA21 maize.

Quantifiable concentrations of PMI were detected in leaves, roots, whole plants, kernels and pollen derived from MIR162 maize and Bt11 x MIR162 x 1507 x GA21 maize.

Quantifiable concentrations of Cry1F were detected in leaves, roots, whole plants, kernels and pollen in 1507 maize and Bt11 x MIR162 x 1507 x GA21 maize derived plant tissue.

Quantifiable concentrations of mEPSPS protein were detected in leaves, roots, whole plants and pollen derived from GA21 maize and Bt11 x MIR162 x 1507 x GA21 maize.

These results confirm that, as expected, transgenic protein expression in Bt11 x MIR162 x 1507 x GA21 maize is not substantially different from that of the Bt11, MIR162, 1507 or GA21 single maize events.

4. Information on how the GM plant differs from the recipient plant in

a) Reproduction

No changes in the reproduction compared to near-isogenic conventional maize have been observed in agronomic assessments conducted with Bt11 x MIR162 x 1507 x GA21 maize.

b) Dissemination

No changes in the dissemination compared to near-isogenic conventional maize have been observed in agronomic assessments conducted with Bt11 x MIR162 x 1507 x GA21 maize.

c) Survivability

No changes in the survivability compared to near-isogenic conventional maize have been observed in agronomic assessments conducted with Bt11 x MIR162 x 1507 x GA21 maize.

d) Other differences

No changes in the reproduction, dissemination or survivability compared to near-isogenic conventional maize have been observed in agronomic assessments

conducted with Bt11 x MIR162 x 1507 x GA21 maize.

In summary, the results of these studies indicate that the combination of Bt11, MIR162, 1507 and GA21 maize using conventional breeding techniques does not result in any biologically relevant agronomic or phenotypic differences related to reproduction, dissemination or survivability of Bt11 x MIR162 x 1507 x GA21 maize.

5. Genetic stability of the insert and phenotypic stability of the GM plant

Molecular analyses showed that the inserts have been stably integrated into the plant's genome in Bt11, MIR162, 1507 and GA21 maize.

In addition, the genetic and phenotypic stability of each of the single maize inserts in Bt11 x MIR162 x 1507 x GA21 maize has been assessed by Southern blot and protein expression analyses. The results confirmed that the single events are present and that the structure of each insert is retained in the stacked product .

6. Any change to the ability of the GM plant to transfer genetic material to other organisms

a) Plant to bacteria gene transfer

The horizontal gene transfer from GM plants to bacteria with subsequent expression of the transgene is regarded as a highly unlikely event under natural conditions, especially in the absence of selective pressure. No changes in the ability of the Bt11 x MIR162 x 1507 x GA21, Bt11, MIR162, 1507 or GA21 maize to transfer genetic material to other organism are expected compared to conventional maize since no sequences have been introduced to allow this to occur.

b) Plant to plant gene transfer

The genetic modification in the single maize events (Bt11, MIR162, 1507 and GA21) is not intended to change any of the typical crop characteristics of maize (except for the tolerance to insect and herbicide products). Observations from field trials have confirmed that the agronomic and phenotypic characteristics of Bt11, MIR162, 1507, GA21 and Bt11 x MIR162 x 1507 x GA21 maize have not changed in comparison with near-isogenic controls, and therefore, there is no increase or decrease in the potential for plant-to-plant gene transfer of Bt11 x MIR162 x 1507 x GA21 maizecompared to traditional maize. Gene transfer from Bt11 x MIR162 x 1507 x GA21 maize to other sexually compatible plant species is not possible since maize has no wild relatives in the EU. In addition, since the scope of this application does not include authorisation for the cultivation, the likelihood of dissemination of pollen to other plants (including cultivated maize plants) is considered to be negligible.

7. Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed

7.1 Comparative assessment

Choice of the comparator

Stacked maize plants containing Bt11, MIR162, 1507 and GA21 maize were compared with relevant control maize lines that had not been genetically modified. Commercial varieties were also included in the comparison where possible.

7.2 Production of material for comparative assessment

a) number of locations, growing seasons, geographical spreading and replicates

To evaluate whether biologically significant changes in composition occurred in Bt11 x MIR162 x 1507 x GA21 maize plants compared to near-isogenic conventional maize, trials were planted at six locations in the US in 2008. The locations of the trial sites were selected to be representative of the range of environmental conditions under which the hybrid varieties are expected to be grown. At each location, three replicate plots of each genotype were planted.

b) the baseline used for consideration of natural variations

The levels of multiple nutritive components were compared in maize kernels (grain) or whole plants (forage) from Bt11 x MIR162 x 1507 x GA21 maize and near-isogenic conventional maize plants grown concurrently. The mean values were also compared with the range of data published in the literature, where data was available.

7.3 Selection of materials and compounds for analysis

Based on guidance of the OECD, grain from transgenic plants and isogenic control plants were analysed for proximates (including starch), minerals, amino acids and selected fatty acids, vitamins, anti-nutrients and secondary metabolites. Forage (whole plants) from transgenic maize plants and isogenic control plants were analysed for proximates and minerals.

No consistent pattern has emerged to suggest that biologically relevant changes in composition or nutritive value of the grain or forage have occurred as an unintended result of the combination of the single events or expression of the transgenes in Bt11 x MIR162 x 1507 x GA21 maize.

These data support the conclusion that Bt11 x MIR162 x 1507 x GA21 maize are compositionally equivalent to conventional maize, apart from the introduced traits of insect and herbicide tolerance.

7.4 Agronomic traits

To confirm that the stack maize plants are equivalent in agronomic characteristics compared to corresponding near-isogenic conventional maize, apart from the introduced traits, Bt11 x MIR162 x 1507 x GA21 maize plants were grown concurrently with near-isogenic conventional maize plants at 13 US locations in 2008. Selected agronomic and phenotypic traits were assessed and compared. The results of these trials showed that Bt11 x MIR162 x 1507 x GA21 maize is agronomically and phenotypically equivalent to conventional maize, apart from the introduced traits.

7.5 Product specification

Maize as a product has a history of safe use for human food and animal feed. No significant native toxins are reported to be associated with the genus *Zea*. The information presented in this application confirms that Bt11 x MIR162 x 1507 x GA21 maize and derived products are not different from those of its conventional counterpart.

7.6 Effect of processing

Bt11 x MIR162 x 1507 x GA21 maize will be produced and processed in the same way as any non-GM maize and there is no evidence to suggest that the expression of the proteins produced by Bt11 x MIR162 x 1507 x GA21 maize (Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS) will influence this processing in any way.

7.7 Anticipated intake/extent of use

There are no anticipated changes to the intake/extent of use of maize as a result of the introduction of Bt11 x MIR162 x 1507 x GA21 maize to the conventional maize supply. It is anticipated that the introduction of Bt11 x MIR162 x 1507 x GA21 maize will replace some of the maize in existing food and feed products. However, the genetic modification was not intended to change any of the compositional parameters in food and feed as confirmed by the results obtained from the extensive compositional assessment.

Furthermore, the expected levels of intake of the proteins Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS through consumption of Bt11 x MIR162 x 1507 x GA21 maize in the EU will be very low. Margins of exposure exceed a factor of at least 5,000, supporting the conclusion that the risk to consumers from Bt11 x MIR162 x 1507 x GA21 maize is negligible and confirming the results previously obtained for the single events.

7.8 Toxicology

7.8.1 Safety assessment of newly expressed proteins

Bt11 x MIR162 x 1507 x GA21 maize is produced by combining Bt11, MIR162, 1507 and GA21 maize through conventional breeding. No new genetic modification has therefore taken place in Bt11 x MIR162 x 1507 x GA21 maize and, as intended, the Bt11 x MIR162 x 1507 x GA21 maize plants produce the six proteins inherited from these four single GM events: Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS.

Potential adverse effects to human and animal health arising from Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS have previously been assessed and it was concluded that the potential toxic effects to humans and animals of these proteins could be considered negligible. In summary:

- The recipient organism, maize, has a history of safe use throughout the world.
- None of the gene sequences or their donors are known to be pathogenic to humans and no pathogenic sequences have been introduced.
- Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS have no significant amino acid homology to known mammalian protein toxins.
- Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS are unlikely to be allergenic
- Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS are readily degraded in *in vitro* digestibility assays.
- Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS show no acute oral toxicity in mammalian studies.

7.8.2 Testing of new constituents other than proteins

Maize is a common source of food and feed and has a long history of safe use. Bt11 x MIR162 x 1507 x GA21 maize has been modified to produce the Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS proteins. No other new constituents apart from these proteins are expected to be produced in Bt11 x MIR162 x 1507 x GA21 maize and compositional analyses have confirmed the compositional equivalence of Bt11 x MIR162 x 1507 x GA21 maize to conventional maize. Therefore no testing of any other constituent is considered necessary.

7.8.3 Information on natural food and feed constituents

Grain from Bt11 x MIR162 x 1507 x GA21 maize and forage from Bt11 x MIR162 x 1507 x GA21 maize have been found to be compositionally equivalent to conventional maize varieties except for the presence of the intended traits. In particular, the presence and levels of natural food and feed constituents such as macro- and micronutrients, secondary plant metabolites as well as natural toxins and antinutritional factors have been analysed and compared with non-GM isolines and data from the literature.

These analyses showed that the levels of the components measured had not changed beyond the natural variation in maize. No consistent patterns emerged to suggest that biologically relevant changes in composition or nutritive value of the grain or forage had occurred as an unintended result of the expression of the transgenes in Bt11 x MIR162 x 1507 x GA21 maize.

7.8.4 Testing of the whole GM food/feed

Grain and forage from Bt11 x MIR162 x 1507 x GA21 maize have been found to be compositionally equivalent to conventional maize varieties except for the presence of the intended traits. In addition, the transgenic proteins produced in Bt11 x MIR162 x 1507 x GA21 maize are digested rapidly, show a lack of acute toxicity and show no significant homology to known protein toxins. Also, the respective function and mode of action of these newly expressed proteins are known and there is no evidence of interaction of safety concern between the newly expressed proteins expressed in Bt11 x MIR162 x 1507 x GA21 maize. Therefore no additional testing of the whole GM food/feed is considered necessary.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein

Bt11 x MIR162 x 1507 x GA21 maize is produced by combining Bt11, MIR162, 1507 and GA21 maize through conventional breeding and therefore expresses the proteins inherited from these three single GM maize events.

The allergenic potential arising from Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS have previously been assessed and it was concluded that the allergenic potential to humans and animals of these proteins could be considered negligible. In summary:

- None of the transgenic proteins produced by Bt11 x MIR162 x 1507 x GA21 maize (Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS) come from donors known to be a significant cause of food allergy.
- Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS have no biologically significant amino acid homology to known allergens
- Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS are readily degraded in *in vitro* digestibility assays.

From these data, it can be concluded that Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS produced by Bt11 x MIR162 x 1507 x GA21 maize are highly unlikely to be allergenic.

7.9.2 Assessment of allergenicity of the whole GM plant or crop

Maize has been extensively cultivated and has a history of safe use for human food and animal feed. Maize is not considered to be a food crop which causes significant food allergy and the newly expressed proteins in Bt11 x MIR162 x 1507 x GA21 maize are very unlikely to be allergenic.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

Bt11 x MIR162 x 1507 x GA21 maize, or any of the sub-combinations, is not intended to change the nutritional status of individuals of populations or to result in products with enhanced functionality. Compositional analysis and whole food safety tests have demonstrated that no unexpected alterations in nutrients and other food components have occurred and that no nutritional imbalances were introduced in Bt11 x MIR162 x 1507 x GA21 maize, or any of the sub-combinations.

7.10.2 Nutritional assessment of GM feed

Bt11 x MIR162 x 1507 x GA21 maize is not intended to change the nutritional status of livestock animals. Compositional analysis and whole food and feed safety tests have demonstrated that no unexpected alterations in nutrients and other food or feed components have occurred and that no nutritional imbalances were introduced in Bt11 x MIR162 x 1507 x GA21 maize.

7.11 Post-market monitoring of GM food/feed

As described in sections 7.1 to 7.10 above, the presence of Bt11 x MIR162 x 1507 x GA21 maize, in food and feed will not result in any nutritional changes, therefore post-market monitoring is not considered necessary.

8. Mechanism of interaction between the GM plant and target organisms (if applicable)

Bt11 x MIR162 x 1507 x GA21 maize is produced by combining Bt11, MIR162, 1507 and GA21 maize through conventional breeding. Therefore Bt11 x MIR162 x 1507 x GA21 maize plants produce the six transgenic proteins inherited from each of the single GM maize events: Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS. The resistance to corn borers (ECB and MCB) is achieved through the expression of Cry1Ab and Cry1F insecticidal proteins well known for its specificity to insects from the order Lepidoptera. Resistance to the other Lepidopteran pests is achieved through the expression of VIP3Aa20, an insecticidal protein with specific activity to insects from the order Noctuidae.
The other transgenic proteins produced by Bt11 x MIR162 x 1507 x GA21 maize, PAT, PMI and mEPSPS, are not known to have any effects on organisms. Therefore

the target organisms for Bt11 x MIR162 x 1507 x GA21 maize are limited to certain species of Lepidoptera.

In any case, the scope of this application does not include cultivation of Bt11 x MIR162 x 1507 x GA21 maizein the EU. Therefore, any direct or indirect interactions between plants of Bt11 x MIR162 x 1507 x GA21 maize and target organisms are highly unlikely.

9. Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification

9.1 Persistence and invasiveness

Potential changes in persistence and invasiveness in Bt11 x MIR162 x 1507 x GA21 maize compared to conventional maize have been assessed.

For the comparative assessment of phenotypic and agronomic characteristics of Bt11 x MIR162 x 1507 x GA21 maize and its corresponding near-isogenic conventional maize, multiple field trials were grown in the US. One of the aims of these trials was to test the hypothesis of no greater persistence or invasiveness of Bt11 x MIR162 x 1507 x GA21 maize compared with conventional maize. The endpoints measured in these trials were selected to study significant unintended changes in seed dispersal or other traits that might affect the ability of maize to survive without human intervention (such as seedling emergence, plant height, failure to produce an ear, dropping of ears before harvest, grain yield and disease incidence).

These investigations showed that, while some differences between Bt11 x MIR162 x 1507 x GA21 maize and near-isogenic controls for some of the measured endpoints were statistically significantly different at some locations, there were no consistent trends in the data across locations or hybrids that would indicate that any of these differences were due to the presence of the transgenes. Therefore the results indicate that Bt11 x MIR162 x 1507 x GA21 maize will not be more persistent or invasive than conventional maize.

In summary, the likelihood that Bt11 x MIR162 x 1507 x GA21 maize will become more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats can be considered negligible.

9.2 Selective advantage or disadvantage

Bt11 x MIR162 x 1507 x GA21 maize was produced by combining Bt11, MIR162, 1507 and GA21 maize through conventional breeding. No new genetic modification has therefore taken place in Bt11 x MIR162 x 1507 x GA21 maize and, as intended, the Bt11 x MIR162 x 1507 x GA21 maize plants produce the six proteins inherited

from these four single GM events: Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS.

Expression of Cry1Ab, Cry1F and Vip3Aa20, conferring resistance to certain species of Lepidoptera, in areas of Europe where these are important maize pests, could be considered an advantage over conventional maize. However maize is a highly domesticated plant and cannot survive without human intervention, even in areas without pressure from these target pests. Therefore, expression of Cry1Ab, Cry1F and Vip3Aa20 will not increase the chances of maize survival under European conditions and would not confer any selective advantage.

Expression of PAT and mEPSPS could confer advantage to maize plants when herbicide products containing glufosinate ammonium or glyphosate are applied. However this rarely happens outside agricultural environments. Therefore, expression of PAT and mEPSPS is highly unlikely to confer selective advantage to maize plants.

Expression of PMI could only confer an advantage to maize plants growing under conditions where mannose was the only source of carbon, conditions that are highly unlikely in normal soils. Therefore, expression of PMI cannot be considered a factor that would confer selective advantage to maize.

In summary, the likelihood that the expression of the Lepidoptera pest protection traits, the herbicide tolerant traits or the selectable markers in Bt11 x MIR162 x 1507 x GA21 maize will result in a selective advantage or disadvantage compared with conventional maize, under the scope of this application, can be considered negligible.

9.3 Potential for gene transfer

Bt11 x MIR162 x 1507 x GA21 maize is produced by combining Bt11, MIR162, 1507 and GA21 maize through conventional breeding. No new genetic modification has therefore taken place in Bt11 x MIR162 x 1507 x GA21 maize and, as intended, the Bt11 x MIR162 x 1507 x GA21 maize plants produce the six proteins inherited from these four single GM events: Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS.

Given the characteristics of these genes and the constructs used, the likelihood that genes from Bt11 x MIR162 x 1507 x GA21 maize would become established in the genome of microorganisms in the environment or human and animal digestive tract is very low. In the very unlikely event that such a horizontal gene transfer would take place, no adverse effects on human and animal health or the environment are expected.

Gene transfer from Bt11 x MIR162 x 1507 x GA21 maize to other sexually compatible plant species is not possible since there are no wild relatives of maize in the EU.

9.4 Interactions between the GM plant and target organisms

The scope of this application does not include cultivation of Bt11 x MIR162 x 1507 x GA21 maize in the EU. Therefore, immediate or delayed effects in the environment due to direct or indirect interactions between Bt11 x MIR162 x 1507 x GA21 maize plants and target organisms are highly unlikely.

Interactions of the target pests with Bt11 x MIR162 x 1507 x GA21 maize have been described in the application in Section D.8.

9.5 Interactions of the GM plant with non-target organisms

The scope of this application does not include the cultivation of Bt11 x MIR162 x 1507 x GA21 maize in the EU, therefore interactions between Bt11 x MIR162 x 1507 x GA21 maize and non-target organisms are highly unlikely. In the unlikely event that small amounts of grain from Bt11 x MIR162 x 1507 x GA21 maize accidentally found their way into the environment this would represent extremely low levels of exposure and the survival of this grain would be very unlikely. Any plants germinating from this grain could be easily controlled using any of the current agronomic measures taken to control other commercially available maize. Therefore grains from Bt11 x MIR162 x 1507 x GA21 maize are extremely unlikely to germinate and survive outside agricultural environments and its potential to interact with non-target species is very low.

Bt11 x MIR162 x 1507 x GA21 maize was produced by combining Bt11, MIR162, 1507 and GA21 maize through conventional breeding. No new genetic modification has therefore taken place in Bt11 x MIR162 x 1507 x GA21 maize and, as intended, the Bt11 x MIR162 x 1507 x GA21 maize plants produce the proteins inherited from these four single GM events: Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS.

Previous environmental risk assessment (ERA) conducted for Bt11, MIR162, 1507 and GA21 maize concluded that the cultivation of these individual maize events will pose low risk to non-target organisms. It is therefore unlikely that the combination of these individual maize events through conventional breeding techniques will result in a maize product with adverse effects in the environment.

9.6 Effects on human health

The scope of this application does not include cultivation of Bt11 x MIR162 x 1507 x GA21 maize in the EU. Therefore, exposure to this maize is most likely to occur through ingestion of food containing Bt11 x MIR162 x 1507 x GA21 maize.

In addition, the compositional analysis of Bt11 x MIR162 x 1507 x GA21 maize has confirmed that the Bt11 x MIR162 x 1507 x GA21 maize is equivalent in

composition to conventional maize, except for the newly expressed proteins.

There is no reason to anticipate that Bt11 x MIR162 x 1507 x GA21 maize would result in product that differs in toxicity or allergenic potential to humans. None of the proteins produced by Bt11 x MIR162 x 1507 x GA21 maize are known to be toxic or allergenic to humans and there are no known precedents where interactions between non-toxic proteins lead to toxic effects. Throughout all the tests conducted by Syngenta with Bt11 x MIR162 x 1507 x GA21 maize no evidence of interaction between the transgenic proteins produced by these plants (Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS) has been observed.

In summary, no adverse effects on human health or adverse consequences for the food chain are expected following consumption of food consisting or containing Bt11 x MIR162 x 1507 x GA21 maize.

9.7 Effects on animal health

The scope of this application does not include cultivation of Bt11 x MIR162 x 1507 x GA21 maize in the EU. Therefore exposure to these maize is most likely to occur through ingestion of feed containing Bt11 x MIR162 x 1507 x GA21 maize. Bt11 x MIR162 x 1507 x GA21 maize was produced by combining Bt11, MIR162, 1507 and GA21 maize through conventional breeding and therefore produces the proteins expressed in these single maize events. The potential for adverse effects on animal health of each of the newly expressed proteins Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS has been assessed in risk assessments and it has been concluded that the potential for adverse effects on animal health from consumption of Bt11 x MIR162 x 1507 x GA21 maize is negligible.

In addition, the compositional analysis of Bt11 x MIR162 x 1507 x GA21 maize has confirmed that the Bt11 x MIR162 x 1507 x GA21 maize is equivalent in composition to conventional maize.

There is no reason to anticipate that Bt11 x MIR162 x 1507 x GA21 maize would result in product that differs in toxicity or allergenic potential to humans. None of the proteins produced by Bt11 x MIR162 x 1507 x GA21 maize are known to be toxic or allergenic to humans and there are no known precedents where interactions between non-toxic proteins lead to toxic effects.

In summary, no adverse effects on animal health or adverse consequences for the feed chain are expected following consumption of feed consisting or containing Bt11 x MIR162 x 1507 x GA21 maize.

9.8 Effects on biogeochemical processes

The scope of this application does not include cultivation of Bt11 x MIR162 x 1507 x GA21 maize in the EU. Interactions with target or non-target organisms that could lead to effects on biogeochemical processes are therefore highly unlikely.

In the unlikely event that small amounts of grain of Bt11 x MIR162 x 1507 x GA21 maize accidentally found their way into the EU environment, their survival would be very unlikely, as maize is a highly domesticated plant and cannot survive without human intervention, especially under normal European climatic conditions. Moreover, these plants could be easily controlled using any of the current agronomic measures taken to control other commercially available maize. In the unlikely event that some plants of Bt11 x MIR162 x 1507 x GA21 maize survived, the potential effects on biogeochemical processes as a result of interactions with target and non-target organisms are likely to be the same as those effects resulting from cultivation of non-modified maize.

In summary, the risk of adverse effects on biogeochemical processes resulting from interactions of Bt11 x MIR162 x 1507 x GA21 maize and target or non-target organisms can be considered negligible under the scope of this application.

9.9 Impacts of the specific cultivation, management and harvesting techniques

The scope of this application does not include cultivation of Bt11 x MIR162 x 1507 x GA21 maize in the EU; therefore there are no specific cultivation, management and harvesting techniques for the use of Bt11 x MIR162 x 1507 x GA21 maize in the EU.

10. Potential interactions with the abiotic environment

The scope of this application does not include cultivation of Bt11 x MIR162 x 1507 x GA21 maize in the EU; therefore interactions of Bt11 x MIR162 x 1507 x GA21 maize with the abiotic environment are highly unlikely. In the unlikely event that small amounts of grain of Bt11 x MIR162 x 1507 x GA21 maize accidentally found their way into the EU environment, their survival would be very unlikely, as maize is a highly domesticated plant and cannot survive without human intervention, especially under normal European climatic conditions. Moreover, these plants could be easily controlled using any of the current agronomic measures taken to control other commercially available maize. In the unlikely event that some plants of Bt11 x MIR162 x 1507 x GA21 maize survive, the potential effects on the abiotic environment are likely to be the same as those effects resulting from cultivation of non-modified maize.

11. Environmental monitoring plan (not if application concerns only food and feed)

11.1 General (risk assessment, background information)

The scope of this application does not include cultivation of Bt11 x MIR162 x 1507 x GA21 maize in the EU. Environmental exposure to Bt11 x MIR162 x 1507 x GA21 maize could only occur in the unlikely event that small amounts of grain of Bt11 x MIR162 x 1507 x GA21 maize accidentally found their way into the environment in the EU. However, the survival of this grain would be very unlikely as maize is a highly domesticated plant and cannot survive without human intervention, especially under normal European climatic conditions. This grain, if germinated, could be easily controlled using any of the current agronomic measures taken to control other commercially available maize.

An ERA has been conducted as recommended by the Guidance document of the Scientific Panel of Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed, and taking into account the scope of this application. Risk assessment concepts described in recent scientific publications have also been used. The conclusion of this risk assessment was that the effects to the environment arising from the import and use of Bt11 x MIR162 x 1507 x GA21 maize could be considered as negligible as those from any other commercial maize.

The conclusions of the ERA confirm that the effects to the environment arising from the use of Bt11 x MIR162 x 1507 x GA21 maize will be no different to those from any other commercial maize.

11.2 Interplay between environmental risk assessment and monitoring

An ERA has been conducted for Bt11 x MIR162 x 1507 x GA21 maize according to the principles laid down in Annex II to Directive 2001/18/EC and Decision 2002/623/EC establishing guidance notes supplementing Annex II to Directive 2001/18/EC. The scientific evaluation of the characteristics of Bt11 x MIR162 x 1507 x GA21 maize in the ERA has shown that the risk for potential adverse effects on human and animal health or the environment is negligible in the context of the intended uses of this GM maize relative to:

- Persistence and invasiveness
- Selective advantage or disadvantage
- Potential for gene transfer
- Interactions between the GM plant and target organisms
- Interactions of the GM plant with non-target organisms
- Effects on human health
- Effects on animal health
- Effects on biogeochemical processes
- Impacts of the specific cultivation, management and harvesting techniques
- Potential interactions with the abiotic environment.

11.3 Case-specific GM plant monitoring (approach, strategy, method and analysis)

An ERA has been conducted in accordance with Annex II of Directive 2001/18/EC to evaluate potential adverse effects of Bt11 x MIR162 x 1507 x GA21 maize on human and animal health and the environment. The conclusions of this ERA confirm that the potential risks to human and animal health or the environment arising from the placing on the market of Bt11 x MIR162 x 1507 x GA21 maize can be considered negligible, under the scope of this application. Therefore, a case-specific monitoring plan is not considered necessary.

However, a general surveillance plan based on Annex II of the Directive 2001/18/EC has been developed and is outlined below.

11.4 General surveillance of the impact of the GM plant (approach, strategy, method and analysis)

General surveillance is not based on a particular hypothesis and it should be used to identify the occurrence of unanticipated adverse effects of the viable GMO or its use for human and animal health or the environment that were not predicted in the ERA.

The scope of this application does not include authorisation for the cultivation of Bt11 x MIR162 x 1507 x GA21 maize. Therefore, exposure to the environment will be limited to unintended release of grain from Bt11 x MIR162 x 1507 x GA21 maize, which could occur for example via substantial losses during loading/unloading of the viable commodity destined for processing into animal feed or human food products. Exposure can be controlled by clean up measures and the application of current

practices used for the control of any adventitious maize plants, such as manual or mechanical removal and the application of herbicides.

However and in order to safeguard against any adverse effects on human and animal health or the environment that were not anticipated in the ERA, general surveillance on grain from Bt11 x MIR162 x 1507 x GA21 maize will be undertaken for the duration of the authorisation. The general surveillance will take into consideration, and be proportionate to, the extent of imports of grain from Bt11 x MIR162 x 1507 x GA21 maize and use thereof in the Member States.

In order to increase the possibility of detecting any unanticipated adverse effects, a monitoring system will be used, which involves the authorisation holder and operators handling and using viable grain from Bt11 x MIR162 x 1507 x GA21 maize. The operators will be provided with guidance to facilitate reporting of any unanticipated adverse effect from handling and use of viable grain from Bt11 x MIR162 x 1507 x GA21 maize.

11.5 Reporting the results of monitoring

The applicant/consent holder is responsible, under Regulation (EC) No 1829/2003, to inform the Commission of the results of the surveillance. Consistent with the EFSA guidance, the applicant will submit a General Surveillance Report containing information related to the monitoring on an annual basis.

12. Detection and event-specific identification techniques for the GM plant

The Bt11 x MIR162 x 1507 x GA21 maize described in this application has been produced by combining the GM maize events: Bt11, MIR162, 1507 and GA21 through conventional breeding techniques. There was no further genetic modification to produce the stack. As such the detection methods developed for the single events should be appropriate for use on the stacked event.

Methods for detection of Bt11, MIR162, and GA21 maize have been developed by Syngenta and for 1507 by Dow AgroSciences LLC. The proposed methods are real-time quantitative TaqMan® PCR based on specific detection of the genomic DNA of these events. The methods for detection of Bt11, MIR162, 1507 and GA21 maize have been validated by the DG-JRC-CRL. There is no reason to suspect that the detection methods developed for the single events should not work on Bt11 x MIR162 x 1507 x GA21 maize. However, in order to confirm the applicability of these methods, Syngenta has tested the methods on Bt11 x MIR162 x 1507 x GA21 maize.

E. INFORMATION RELATING TO PREVIOUS RELEASES OF THE GM PLANT AND/OR DERIVED PRODUCTS

1. History of previous releases of the GM plant notified under Part B of the Directive 2001/18/EC and under Part B of Directive 90/220/EEC by the same notifier

No trials of Bt11 x MIR162 x 1507 x GA21 maize have been carried out in the EU

2. History of previous releases of the GM plant carried out outside the Community by the same notifier

a) Release country US.
b) Authority overseeing the release EPA and USDA.
c) Release site Various sites across the US.
d) Aim of the release Research and development.
e) Duration of the release Varied depending on the aim of the trial.
f) Aim of post-releases monitoring Control of volunteers.
g) Duration of post-releases monitoring Varied depending on the aim of the trial, typically one year.
h) Conclusions of post-release monitoring The occurrence of volunteers after planting Bt11 x MIR162 x 1507 x GA21 field trials was no different to other maize.
i) Results of the release in respect to any risk to human health and the environment No evidence of adverse effects to human health or the environment has been found.

3. Links (some of these links may be accessible only to the competent authorities of the Member States, to the Commission and to EFSA):

a) Status/process of approval The status and process of approval can be found on the EFSA website: http://www.efsa.europa.eu/EFSA/ScientificPanels/GMO/efsa_locale-1178620753812_GMOApplications.htm
b) Assessment Report of the Competent Authority (Directive 2001/18/EC) An application for approval of Bt11 x MIR162 x 1507 x GA21 under the Directive 2001/18/EC has not been made by Syngenta.
c) EFSA opinion An EFSA opinion on Bt11 x MIR162 x 1507 x GA21 maize was not available at the time of submission. EFSA opinions, once available can be found at http://www.efsa.europa.eu/EFSA/ScientificPanels/GMO/efsa_locale-1178620753812_GMOApplications.htm
d) Commission Register (Commission Decision 2004/204/EC) The Commission register of GM Food and Feed can be found at http://ec.europa.eu/food/food/biotechnology/authorisation/index_en.htm
e) Molecular Register of the Community Reference Laboratory/Joint Research Centre The Community Reference Laboratory webpage is http://gmo-crl.jrc.it/
f) Biosafety Clearing-House (Council Decision 2002/628/EC) Information relating to the Biosafety clearing house can be found at: http://bch.biodiv.org/
g) Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC) An application for approval of Bt11 x MIR162 x 1507 x GA21 maize under Directive 2001/18/EC has not been made by Syngenta, however a link to this Summary may be found at: http://www.efsa.europa.eu/EFSA/ScientificPanels/GMO/efsa_locale-1178620753812_GMOApplications.htm