

# Application for authorization of stacked Bt11 x MIR604 x GA21 maize in the European Union under Regulation (EC) No 1829/2003

## **PART II: SUMMARY**

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## A . GENERAL INFORMATION

### 1. Details of application

a) Member State of application
UK
b) Application number
Not available at the time of submission
c) Name of the product (commercial and other names)
Bt11 x MIR604 x GA21 maize
d) Date of acknowledgement of valid application
Not available at the time of submission

### 2. Applicant

a) Name of applicant
Syngenta Seeds S.A.S on behalf of Syngenta Crop Protection AG, Basel
b) Address of applicant
Syngenta Seeds S.A.S. 12, chemin de l'Hobit BP 27 F-31790 Saint-Sauveur
On behalf of Syngenta Crop Protection AG, Basel Switzerland and all affiliated companies Schwarzwaldallee 215 CH 4058 Basle Switzerland
c) Name and address of the person established in the Community who is responsible for the placing in 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))
Bt11 x MIR604 x GA21 maize will be imported and used as any other maize in the EU by operators currently involved in these processes.

**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 <input type="checkbox"/>	No <input checked="" type="checkbox"/>
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 <input checked="" type="checkbox"/> (for release in 2008)	No <input type="checkbox"/>
If <i>no</i> , refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC	

**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 <input type="checkbox"/>	No <input checked="" type="checkbox"/>
If yes, specify	

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

Yes <input checked="" type="checkbox"/>	No <input type="checkbox"/>
If yes, specify Bt11 x MIR604 x GA21 maize is approved for commercial cultivation in the USA and Canada. A number of other submissions have been made in other 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 MIR604 x GA21 maize is a stacked genetically modified (GM) product that has been produced by conventional breeding crosses of: <ul style="list-style-type: none"><li>- 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.</li><li>- Event MIR604 maize (MIR604 maize) which produces a modified Cry3A (mCry3A) protein for control of certain coleopteran pests and a phosphomannose isomerase (MIR604 PMI) protein, which acts as a selectable marker enabling transformed plant cells to utilize mannose as a primary carbon source.</li><li>- 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.</li></ul>
b) Types of products planned to be placed on the market according to the authorisation applied for The scope of the application includes all feed and food products containing, consisting or produced from Bt11 x MIR604 x GA21 maize including products from inbreds and hybrids obtained by conventional breeding of Bt11 x MIR604 x GA21 maize. The application also covers the import and industrial processing of Bt11 x MIR604 x GA21 maize for all potential uses as any other maize.
c) Intended use of the product and types of users It is intended that Bt11 x MIR604 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 MIR604 x GA21 maize and 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 MIR604 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 MIR604 x GA21 maize.

e) Any proposed packaging requirements

The characteristics of Bt11 x MIR604 x GA21 maize and products derived from it are not different from those of its conventional counterpart. Bt11 x MIR604 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 MIR604 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 MIR604 x GA21 maize and products derived from it 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 MIR604 x GA21 maize has been assigned in accordance with Commission Regulation (EC) 65/2004: SYN-BTØ11-1 x SYN-IR6Ø4-5 x MON-ØØØ21-9.

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

Bt11 x MIR604 x GA21 maize is 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 MIR604 x GA21 maize and products derived from it are not different from those of its conventional counterpart, apart from the intended effect of tolerance to certain lepidopteran and coleopteran insect pests and herbicide products containing glufosinate ammonium or glyphosate. Cultivation of Bt11 x MIR604 x GA21 maize in the EU is not within the scope of this application. In the unlikely event that small amounts of Bt11 x MIR604 x GA21 maize kernels accidentally found their way into the environment, this would represent extremely low levels of exposure and the survival of these kernels 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.

Bt11 x MIR604 x GA21 maize 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 <i>Zea</i>
c) Species <i>mays</i>
d) Subspecies <i>mays</i>
e) Cultivar/breeding line or strain Bt11 x MIR604 x GA21 maize
f) Common name Maize; corn

### 2 a. Information concerning reproduction

(i) Mode(s) of reproduction <p>Sexual reproduction: <i>Zea mays</i> is an allogamous plant that propagates through seed produced predominantly by cross-pollination and depends mainly on wind borne cross-fertilisation. <i>Z. mays</i> is a plant with protandrous inflorescence; however, decades of conventional selection and improvement have produced varieties of maize with protogynous traits. <i>Z. mays</i> has staminate flowers in the tassels and pistillate flowers on the ear shoots.</p> <p>Asexual reproduction: there is no asexually reproductive maize.</p>
(ii) Specific factors affecting reproduction <p>The key critical stages of maize reproduction are tasselling, silking, pollination and fertilization. Pollen dispersal is limited by several factors, including large size (0.1 mm diameter), rapid settling rate and short survivability. Most maize varieties are protoandrous so pollen shedding precedes silk emergence by up to five days. In general, over 98% of maize pollen falls to the ground within a radius of 25-50m of the source, although some grains can travel several hundred meters. Plants produce pollen for up to 13 days and shed pollen typically remains viable for 10 to 30 minutes, but may remain viable longer under refrigerated and humid conditions.</p>

(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

Maize has a polystichous (arranged in many rows) female inflorescence (flower), called the ear, on a stiff central spike (cob) enclosed in husks (modified leaves). Because of the structure of the ears, seed dispersal of individual kernels does not occur naturally. Maize is non-invasive of natural habitats.

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

Maize, which has very diverse morphological and physiological traits, is grown on approximately 145 million hectares worldwide. It is distributed over a wide range of conditions: from latitudes 50° North to 50° South, below sea level of the Caspian plains up to 3000 m in the Andes Mountains and from semi-arid regions to arid regions. The greatest maize production occurs where the warmest month isotherms range between 21° and 27° C and the freeze-free season lasts 120-180 days.

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 15<sup>th</sup> century by Columbus and is widely grown in the European Union Member States.

**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 MIR604 x GA21 maize described in this application has been produced by combining the genetically modified (GM) maize events: Bt11, MIR604 and GA21 through conventional breeding techniques. There was no further genetic modification to produce the stack.

The Bt11, MIR604 and GA21 maize events maize were produced by genetic modification as follows:

- Bt11 maize was produced using protoplast transformation/regeneration
- MIR604 maize was produced via *Agrobacterium*-mediated transformation
- GA21 maize was produced via microprojectile bombardment of maize suspension culture cells.

### 2. Nature and source of the vector used

The Bt11 x MIR604 x GA21 maize described in this application has been produced by combining the GM maize events: Bt11, MIR604 and GA21 through conventional breeding techniques.

The vectors used to produce Bt11 maize, MIR604 maize and GA21 maize are as follows:

- The Plasmid pZO1502, cut with a *NotI* restriction enzyme, was used to produce Bt11 maize. The plasmid is a derivative of the commercially available plasmid pUC18.
- The Plasmid pZM26, a binary vector used for *Agrobacterium* mediated plant transformation, was used to generate MIR604 maize.
- A *NotI* 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 MIR604 x GA21 maize described in this application has been produced by combining the GM maize events: Bt11, MIR604 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

Vector Component	Approx. Size (bp)	Description
35S 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.
<i>cryIAb</i>	1848	<i>cryIAb</i> gene, which encodes a CryIAb protein that confers resistance to certain lepidopteran insect pests. The <i>cryIAb</i> gene was originally cloned from <i>Bacillus thuringiensis</i> var. <i>kurstaki</i> HD-1
NOS	253	Polyadenylation region from the nopaline synthase gene from <i>Agrobacterium tumefaciens</i> .
35S 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.
<i>pat</i>	552	<i>Streptomyces viridochromogenes</i> gene encoding the selectable marker PAT. PAT confers resistance to herbicides containing glufosinate
NOS	253	Polyadenylation region from the nopaline synthase gene from <i>Agrobacterium tumefaciens</i> .

#### Event MIR604 maize

Vector component	Size (bp)	Description
MTL	2556	Promoter derived from the <i>Zea mays</i> (maize) metallothionein-like gene.
<i>mcry3A</i>	1797	A modified <i>cry3A</i> gene that confers tolerance to western corn rootworm ( <i>Diabrotica virgifera virgifera</i> ) and related <i>Diabrotica</i> species.
NOS	253	Polyadenylation region from the nopaline synthase gene from <i>Agrobacterium tumefaciens</i> .
ZmUbiInt	1993	Promoter from <i>Zea may</i> polyubiquitin genes
<i>pmi</i>	1176	<i>E. coli pmi</i> gene encoding the enzyme phosphomannose isomerase (PMI)
NOS	253	Polyadenylation region from the nopaline synthase gene from <i>Agrobacterium tumefaciens</i> .

### Event GA21 maize

Vector Component	Approx. Size (Kb)	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 <i>mepsps</i> 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 <i>epsps</i> gene, which confers resistance to herbicide products containing glyphosate.
Nos 3' end	0.3	Polyadenylation region from the nopaline synthase gene from <i>Agrobacterium tumefaciens</i> .

## D. INFORMATION RELATING TO THE GM PLANT

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

The Bt11 x MIR604 x GA21 maize described in this application has been produced by combining the GM maize events: Bt11, MIR604 and GA21 through conventional breeding techniques and produces the following proteins:

- a truncated Cry1Ab protein for control of certain lepidopteran pests.
- a phosphinothricin acetyltransferase (PAT) protein that confers tolerance to herbicide products containing glufosinate ammonium.
- a modified Cry3A (mCry3A) protein for control of certain coleopteran pests.
- a phosphomannose isomerase (MIR604 PMI) protein as a selectable marker.
- a modified maize EPSPS (mEPSPS) protein 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 MIR604 x GA21 maize described in this application has been produced by combining the GM maize events: Bt11, MIR604 and GA21 through conventional breeding techniques.

The inserts in Bt11 maize and MIR604 maize are present at a single locus and inherited as a single gene in a Mendelian fashion. The insert in GA21 maize is comprised of six contiguous regions derived from the 3.49 kb *NotI* 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 *NotI* 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 MIR604 x GA21 maize, additional Southern analysis was performed. The predicted DNA hybridization patterns from each individual event were confirmed in Bt11 x MIR604 x GA21 maize, demonstrating preservation of the integrity of the transgenic fragment from each individual event to Bt11 x MIR604 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, MIR604 and GA21 maize were analysed and the results showed that insertions had taken place in the nucleus.

The Bt11 x MIR604 x GA21 maize described in this application has been produced by combining the GM maize events: Bt11, MIR604 and GA21 through conventional breeding techniques. It therefore contains the inserts derived from the single events. The presence of the inserts from Bt11, MIR604 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 MIR604 x GA21 maize described in this application has been produced by combining the GM maize events: Bt11, MIR604 and GA21 through conventional breeding techniques. The organisation of the inserted genetic material in Bt11, MIR604 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.

**MIR604 maize**

Sequencing and southern data have demonstrated that MIR604 maize contains a single DNA insertion with one copy of both the *mcry3A* and the *pmi* genes.

**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 *NotI* restriction fragment from pDPG434 employed in the generation of Event GA21 (copies 1-6).

A molecular comparison of the Bt11 x MIR604 x GA21 maize with the single events Bt11, MIR604 and GA21 maize has shown that the inserts are preserved in Bt11 x MIR604 x GA21.

### 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 MIR604 x GA21 maize was produced by combining Bt11, MIR604 and GA21 maize through conventional breeding techniques. Therefore these maize plants produce the five transgenic proteins inherited from these single GM maize events: Cry1Ab, PAT, mCry3A, MIR604 PMI and mEPSPS.

Tissues from maize plants derived from Bt11 maize, MIR604 maize, GA21 maize and a conventional breeding stack containing these events (Bt11 x MIR604 x GA21) were analyzed by ELISA to compare the concentrations of Cry1Ab, PAT, mCry3A, MIR604 PMI 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.

Mean Cry1Ab and PAT concentrations were comparable in the tissues of Bt11 maize and Bt11 x MIR604 x GA21 maize. Similarly, mCry3A and MIR604 PMI concentrations were comparable in the tissues of MIR604 maize and Bt11 x MIR604 x GA21 maize and mEPSPS concentrations were comparable in the tissues of GA21 maize and Bt11 x MIR604 x GA21 maize. As expected, no Cry1Ab, PAT, mCry3A, MIR604 PMI or 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 support the conclusion that, as expected, transgenic protein expression in Bt11 x MIR604 x GA21 maize is not substantially different from that of the Bt11, MIR604 or GA21 single maize events.

#### b) Parts of the plant where the insert is expressed

To characterize the range of expression of Cry1Ab, PAT, mCry3A, MIR604 PMI and mEPSPS proteins in Bt11 x MIR604 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 MIR604 x GA21 maize. Very low levels of Cry1Ab expression were detected in the pollen of Bt11 maize and Bt11 x MIR604 x GA21 maize.

Quantifiable concentrations of PAT protein were detected in leaves and roots derived from Bt11 maize and Bt11 x MIR604 x GA21 maize at most stages of development, however no quantifiable levels could be detected in the kernels or pollen.

Quantifiable concentrations of mCry3A protein were detected in leaves, roots and kernels derived from MIR604 maize and Bt11 x MIR604 x GA21 maize. Very low levels of mCry3A expression were detected in the pollen of MIR604 maize and Bt11 x MIR604 x GA21 maize.

Quantifiable concentrations of MIR604PMI protein were detected in all MIR604 maize and Bt11 x MIR604 x GA21 maize derived plant tissues.

Quantifiable concentrations of mEPSPS protein were detected in all GA21 maize and Bt11 x MIR604 x GA21 maize derived plant tissues.

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

##### **a) Reproduction**

No changes in the reproduction compared to non-GM maize have been observed in agronomic assessments conducted with Bt11 x MIR604 x GA21 maize.

##### **b) Dissemination**

No changes in the dissemination compared to non-GM maize have been observed in agronomic assessments conducted with Bt11 x MIR604 x GA21 maize.

##### **c) Survivability**

No changes in the survivability compared to non-GM maize have been observed in agronomic assessments conducted with Bt11 x MIR604 x GA21 maize.

##### **d) Other differences**

No changes in the reproduction, dissemination or survivability compared to non-GM maize have been observed in agronomic assessments conducted with Bt11 x MIR604 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, MIR604 and GA21 maize.

Bt11 x MIR604 x GA21 maize F<sub>1</sub> seed is produced through conventional breeding involving Bt11, MIR604 and GA21 lines. Bt11 x MIR604 x GA21 seed once planted by growers produces grain (F<sub>2</sub>) which is harvested for food, feed or industrial use. Such grain or products entering the commodity chain are not kept for further sowing.

## 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 MIR604 x GA21 maize, Bt11, MIR604 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, MIR604 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 characteristics of Bt11 maize, MIR604 maize, GA21 maize and Bt11 x MIR604 x GA21 maize have not changed in comparison with isogenic controls, and therefore, there is no increase or decrease in the potential for plant-to-plant gene transfer compared to traditional maize. Gene transfer from Bt11 x MIR604 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 of Bt11 x MIR604 x GA21 maize, 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, MIR604 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 confirm that Bt11 x MIR604 x GA21 maize plants are substantially equivalent to the non-transgenic near-isogenic lines, replicate trials of transgenic and corresponding isogenic controls were conducted. 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 the transgenic and simultaneously grown isogenic control plants. The mean values are 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 significant changes in composition or nutritive value of the grain or forage had occurred as an unintended result of transformation or expression of the transgenes in Bt11 x MIR604 x GA21 maize.

These data support the conclusion that Bt11 x MIR604 x GA21 maize is compositionally equivalent to conventional maize, apart from the introduced traits of insect and herbicide tolerance.

### 7.4 Agronomic traits

The scope of the application does not include cultivation, however measurement and observation of agronomic characteristics can add to the assessment of unintended effects of the genetic modification.

Bt11 x MIR604 x GA21 maize were evaluated in a series of trials across the USA in 2006. Up to 18 separate agronomic traits were assessed, although not all traits were recorded at all locations. The Bt11 x MIR604 x GA21 maize and their near-isogenic hybrids were compared. Results of these trials support the conclusion that Bt11 x MIR604 x GA21 maize is agronomically 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 MIR604 x GA21 maize and products derived from it are not different from those of its conventional counterpart.

## 7.6 Effect of processing

Bt11 x MIR604 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 this maize (Cry1Ab, PAT, mCry3A, MIR604 PMI 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 MIR604 x GA21 maize to the conventional maize supply. It is anticipated that the introduction of Bt11 x MIR604 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 and this been shown to be the case through extensive compositional assessment.

## 7.8 Toxicology

### 7.8.1 Safety assessment of newly expressed proteins

Bt11 x MIR604 x GA21 maize was produced by combining Bt11, MIR604 and GA21 maize through conventional breeding and therefore produces the five proteins inherited from the single GM maize events. Potential adverse effects to human and animal health arising from Cry1Ab, PAT, mCry3A, MIR604 PMI 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. A summary is provided below:

- 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, mCry3A, MIR604 PMI and mEPSPS have no significant amino acid homology to known mammalian protein toxins.
- Cry1Ab, PAT, mCry3A, MIR604 PMI and mEPSPS are unlikely to be allergenic
- Cry1Ab, PAT, mCry3A, PMI and mEPSPS are readily degraded in *in vitro* digestibility assays.
- Cry1Ab, PAT, mCry3A, MIR604 PMI 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 MIR604 x GA21 maize has been modified to produce the Cry1Ab, PAT, mCry3A, MIR604 PMI and mEPSPS proteins. No other new constituents apart from these proteins are expected to be produced in Bt11 x MIR604 x GA21 maize and compositional analyses have confirmed the substantial equivalence of Bt11 x MIR604 x GA21 maize compared to conventional maize. Therefore no testing of any other constituent is considered necessary.

### 7.8.3 Information on natural food and feed constituents

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 significant changes in composition or nutritive value of the grain or forage had occurred as an unintended result of the expression of the transgenes.

### 7.8.4 Testing of the whole GM food/feed

In addition to the compositional analysis, the wholesomeness and safety of Bt11 x MIR604 x GA21 maize was confirmed in a poultry feeding study. The study showed that the transgenic corn had no deleterious effects on broiler chickens.

## 7.9 Allergenicity

### 7.9.1 Assessment of allergenicity of the newly expressed protein

Bt11 x MIR604 x GA21 maize was produced by combining Bt11, MIR604 and GA21 maize through conventional breeding and therefore expresses the five proteins inherited from these three single GM maize events. Potential allergenic potential arising from Cry1Ab, PAT, mCry3A, MIR604 PMI and mEPSPS have previously been assessed and it was concluded that the potential allergenic potential to humans and animals of these proteins could be considered negligible. A summary is provided below:

- None of the five transgenic proteins produced by Bt11 x MIR604 x GA21 (Cry1Ab, PAT, mCry3A, MIR604 PMI and mEPSPS) come from donors known to be a significant cause of food allergy.
- The Cry1Ab, PAT, mCry3A, MIR604 PMI and mEPSPS proteins have no biologically significant amino acid homology to known allergens
- The Cry1Ab, PAT, mCry3A, PMI and mEPSPS are readily degraded in *in vitro* digestibility assays.

From these data, it can be concluded that the Cry1Ab, PAT, mCry3A, MIR604 PMI and mEPSPS proteins produced by Bt11 x MIR604 x GA21 maize plants 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 Bt11 x MIR604 x GA21 maize produces five new proteins that are very unlikely to be allergenic.

## 7.10 Nutritional assessment of GM food/feed

### 7.10.1 Nutritional assessment of GM food

Bt11 x MIR604 x GA21 maize is not intended to change the nutritional status of individuals of populations or to be processed 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.

### 7.10.2 Nutritional assessment of GM feed

Bt11 x MIR604 x GA21 is not intended to change the nutritional status of livestock animals. 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.

## 7.11 Post-market monitoring of GM food/feed

As described in sections 7.1 to 7.10 above, the presence of Bt11 x MIR604 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 MIR604 x GA21 maize was produced by combining Bt11, MIR604 and GA21 maize through conventional breeding. Therefore Bt11 x MIR604 x GA21 maize plants produce the five transgenic proteins inherited from each of the single GM maize events: Cry1Ab, PAT, mCry3A, MIR604 PMI and mEPSPS. The Cry1Ab protein confers protection against certain lepidopteran pests and it is well known for its specificity to insects from the order Lepidoptera. The mCry3A protein confers protection against certain coleopteran pests and it is well known for its specificity to insects from the order Coleoptera. The other three transgenic proteins produced by Bt11 x MIR604 x GA21 maize, PAT, MIR604 PMI and mEPSPS are not known to have any effects on organisms. Therefore the target organisms for Bt11 x MIR604 x GA21 maize are limited to certain species of Coleoptera and Lepidoptera.

The cultivation of Bt11 x MIR604 x GA21 maize is not within the scope of this application, therefore plant interactions with target organisms are highly unlikely in the EU. In the unlikely event that small amounts of Bt11 x MIR604 x GA21 maize grain could accidentally find 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 Bt11 x MIR604 x GA21 maize is extremely unlikely to germinate and survive outside agricultural environments and its potential to interact with target species is very low.

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

### 9.1 Persistence and invasiveness

Cultivation of Bt11 x MIR604 x GA21 maize in the EU is not within the scope of this application. In the unlikely event that small amounts of maize kernels of Bt11 x MIR604 x GA21 maize accidentally found their way into the environment their survival would be very unlikely as maize is highly domesticated and cannot survive without human intervention, especially under normal European climatic conditions. The expression of the Cry1Ab, PAT, mCry3A, MIR604 PMI and mEPSPS does not affect the agronomic characteristics or weediness potential of Bt11 x MIR604 x GA21 maize, as demonstrated in field trials conducted to evaluate the agronomic performance in comparison with the isogenic control. In the unlikely event that these maize plants were to survive they could be easily controlled using any of the current agronomic measures taken to control other commercially available maize.

In summary, the likelihood that Bt11 x MIR604 x GA21 maize becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats as a result of importing maize kernels of this maize into the EU can be considered negligible.

### 9.2 Selective advantage or disadvantage

Bt11 x MIR604 x GA21 maize was produced by combining Bt11, MIR604 and GA21 maize through conventional breeding. No new genetic modification has therefore taken place in Bt11 x MIR604 x GA21 maize and, as intended, the plants produce the five proteins inherited from these three single GM events: Cry1Ab, PAT, mCry3A, MIR604 PMI and mEPSPS.

Expression of Cry1Ab and mCry3A, conferring resistance to certain species of Lepidoptera or Coleoptera, 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 and mCry3A 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 MIR604 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 MIR604 PMI cannot be considered a factor that would confer selective advantage to maize.

Cultivation of Bt11 x MIR604 x GA21 maize in the EU is not within the scope of this application. In the unlikely event that small amounts of grain from Bt11 x MIR604 x GA21 maize could accidentally find their way into the environment in the EU, the survival of this grain would be very unlikely for the reasons stated above. In addition, any plants germinating

from this grain could be easily controlled using any of the current agronomic measures taken to control other commercially available maize.

In summary, the likelihood that the expression of the Lepidoptera and Coleoptera pest protection traits, the herbicide tolerant traits or the selectable marker in Bt11 x MIR604 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

Gene transfer from Bt11 x MIR604 x GA21 maize to other sexually compatible plant species is not possible since there are not any maize wild relatives in the EU.

Gene transfer from Bt11 x MIR604 x GA21 maize to other maize could occur through pollen dispersal during the cultivation of the crop. However, cultivation of Bt11 x MIR604 x GA21 maize is not within the scope of this application. It is therefore highly unlikely that this maize could grow in significant quantities in the EU. In the unlikely event that small amounts of grain from Bt11 x MIR604 x GA21 maize accidentally found their way into the environment in the EU, this would represent extremely low levels of exposure, and the survival of the grain would be very unlikely. In addition, plants resulting from spilled grain could be easily controlled using any of the current agronomic measures taken to control other commercially-available maize. Therefore Bt11 x MIR604 x GA21 maize is extremely unlikely to flower and fertilise other varieties of maize in Europe.

In summary, the potential for gene transfer between Bt11x MIR604 x GA21 maize plants and other maize plants or sexually compatible wild relatives in the EU can be considered negligible under the scope of this application.

### 9.4 Interactions between the GM plant and target organisms

The scope of this application does not include cultivation of Bt11 x MIR604 x GA21 maize in the EU, therefore interactions between Bt11 x MIR604 x GA21 maize and target organisms are highly unlikely.

Bt11 x MIR604 x GA21 maize was produced by combining Bt11, MIR604 and GA21 maize through conventional breeding and therefore Bt11 x MIR604 x GA21 maize produces the five transgenic proteins inherited from the three single GM maize events: Cry1Ab, PAT, mCry3A, MIR604 PMI and mEPSPS. The Cry1Ab protein confers protection against certain lepidopteran pests and it is well known for its specificity to insects from the order Lepidoptera. The mCry3A protein confers protection against certain coleopteran pests and it is well known for its specificity to insects from the order Coleoptera. The other three proteins produced by Bt11 x MIR604 x GA21 maize, PAT, MIR604 PMI and mEPSPS are not known to have adverse effects on organisms.

In summary, immediate or delayed effects in the environment due to direct or indirect interactions between Bt11 x MIR604 x GA21 maize plants and target organisms can be considered highly unlikely under the scope of this application.

### 9.5 Interactions of the GM plant with non-target organisms

The scope of this application does not include the cultivation of Bt11 x MIR604 x GA21 maize in the EU, therefore interactions between Bt11 x MIR604 x GA21 maize and non-target organisms are highly unlikely.

Bt11 x MIR604 x GA21 maize was produced by combining Bt11, MIR604 and GA21 maize through conventional breeding and therefore Bt11 x MIR604 x GA21 maize produces the five transgenic proteins inherited from the three single GM maize events: Cry1Ab, PAT, mCry3A, MIR604 PMI and mEPSPS. The Cry1Ab protein confers protection against certain lepidopteran pests and it is well known for its specificity to insects from the order Lepidoptera. The mCry3A protein confers protection against certain coleopteran pests and it is well known for its specificity to insects from the order Coleoptera. The other three proteins produced by Bt11 x MIR604 x GA21 maize, PAT, MIR604 PMI and mEPSPS are not known to have adverse effects on organisms.

In the unlikely event that small amounts of Bt11 x MIR604 x GA21 maize grain 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 Bt11 x MIR604 x GA21 maize is extremely unlikely to germinate and survive outside agricultural environments and its potential to interact with non-target species is very low.

In summary, immediate or delayed effects in the environment due to direct or indirect interactions between Bt11 x MIR604 x GA21 maize plants and non-target organisms can be considered highly unlikely under the scope of this application.

### 9.6 Effects on human health

The scope of this application does not include cultivation of Bt11 x MIR604 x GA21 maize in the EU, therefore exposure to this maize is most likely to occur through ingestion of food containing Bt11 x MIR604 x GA21 maize. Bt11 x MIR604 x GA21 maize was produced by combining Bt11 maize, MIR604 maize and GA21 maize through conventional breeding. The potential for adverse effects on human health of Bt11 maize or MIR604 maize have been previously assessed in risk assessments and it has been concluded that the potential for adverse effects on human health from consumption of Bt11 maize, MIR604 maize or GA21 maize are negligible.

In addition, compositional analysis and broiler feeding studies have confirmed that the Bt11 x MIR604 x GA21 maize is equivalent in composition to conventional maize and as safe and nutritious as conventional maize.

There is no reason to anticipate that conventional breeding of Bt11, MIR604 and GA21 maize would result in a stacked product that differs in toxicity or allergenic potential to humans or animals. None of the proteins produced by Bt11, MIR604 and GA21 maize are known to be toxic or allergenic to humans or animals and there are not known precedents where

interactions between non-toxic proteins lead to toxic effects. Throughout all the tests conducted by Syngenta with Bt11 x MIR604 x GA21 no evidence of interaction between the five proteins produced by these plants (Cry1Ab, PAT, mCry3A, MIR604 PMI 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 MIR604 x GA21 maize.

#### 9.7 Effects on animal health

The scope of this application does not include cultivation of Bt11 x MIR604 x GA21 maize in the EU, therefore exposure to this maize is most likely to occur through ingestion of feed containing Bt11 x MIR604 x GA21 maize. Bt11 x MIR604 x GA21 maize was produced by combining Bt11, MIR604 and GA21 maize through conventional breeding. The potential for adverse effects on animal health of each of the single GM maize events (Bt11, MIR604 and GA21) have been previously assessed in risk assessments and it has been concluded that the potential for adverse effects on animal health from consumption of Bt11, MIR604 or GA21 maize are negligible.

In addition, compositional analysis and broiler feeding studies have confirmed that the Bt11 x MIR604 x GA21 maize is equivalent in composition to conventional maize and as safe and nutritious as conventional maize.

There is no reason to anticipate that conventional breeding of Bt11, MIR604 and GA21 would result in a stacked product that differs in toxicity or allergenic potential to humans or animals. None of the proteins produced by these single GM maize events (Bt11, MIR604 and GA21) are known to be toxic or allergenic to humans or animals and there are not known precedents where interactions between non-toxic proteins lead to toxic effects. Throughout all the tests conducted by Syngenta with Bt11 x MIR604 x GA21 plants, no evidence of interaction between the five proteins produced by these plants (Cry1Ab, PAT, mCry3A, MIR604 PMI and mEPSPS) has been observed.

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 MIR604 x GA21 maize.

#### 9.8 Effects on biogeochemical processes

The scope of this application does not include cultivation of Bt11 x MIR604 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 MIR604 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 Bt11 x MIR604 x GA21 maize plants 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 MIR604 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 MIR604 x GA21 maize plants in the EU; therefore there are no specific cultivation, management and harvesting techniques for the use of Bt11 x MIR604 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 MIR604 x GA21 maize in the EU; therefore interactions of maize Bt11 x MIR604 x GA21 with the abiotic environment are highly unlikely. In the unlikely event that small amounts of grain of Bt11 x MIR604 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 Bt11 x MIR604 x GA21 maize plants survived, the potential effects on the abiotic environment are likely to be the same as those effects resulting from cultivation of non-modified maize.

In summary, environmental impacts as a result of interactions between Bt11 x MIR604 x GA21 and the abiotic environment can be considered negligible within the scope of this application.

**11. Environmental monitoring plan** (not if application concerns only food and feed produced from GM plants, or containing ingredients produced from GM plants and if the applicant has clearly shown that environmental exposure is absent or will be at levels or in a form that does not present a risk to other living organisms or the abiotic environment)

**11.1 General (risk assessment, background information)**

As required by Article 5(5)(b) and 17(5)(b) of Regulation (EC) No. 1829/2003 the proposed monitoring plan for Bt11 x MIR604 x GA21 maize has been developed according to the principles and objectives outlined in Annex VII of Directive 2001/18/EC and Decision 2002/811/EC establishing guidance notes supplementing Annex VII to Directive 2001/18/EC. The structure of the monitoring plan also takes into account the guidance on presentation of applications provided in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed.

An environmental risk assessment (e.r.a) for each of the individual GM maize used to produce Bt11 x MIR604 x GA21 maize has previously been conducted as part of the application for the import and use under Regulation (EC) No 1829/2003. These risk assessments were conducted as recommended by the Guidance document of the Scientific Panel of Genetically Modified Organisms for the risk assessment of GM plants and derived food and feed and taking into account the scope of the applications. The conclusion of these risk assessments was that the adverse effects to the environment arising from the import and use of either Bt11, MIR604 or GA21 maize could be considered as negligible as those from any other commercial maize.

A risk assessment for the stacked Bt11 x MIR604 x GA21 maize has also been conducted following the same Guidance document and taking into account the recent Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of GM plants containing stacked transformation events. Risk assessment concepts described in recent publications have also been used.

The conclusions of the e.r.a. confirm that the effects to the environment arising from the use of Bt11 x MIR604 x GA21 maize will be no different to those from any other commercial maize.

**11.2 Interplay between environmental risk assessment and monitoring**

In general two types of environmental monitoring can be described:

- a. case-specific monitoring, designed to evaluate potential adverse effects linked to the genetic modification, identified in the e.r.a.
- b. general surveillance, which is aimed to identify adverse unforeseen effects that were not anticipated in the environmental risk assessment.

An environmental risk assessment (e.r.a.) has been conducted in accordance with Annex II of Directive 2001/18/EC and takes into account the recent Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of GM plants containing

stacked transformation events to evaluate potential adverse effects of Bt11 x MIR604 x GA21 maize on human and animal health and the environment. The conclusions of this e.r.a. confirmed that the potential risks to human and animal health or the environment arising from the placing on the market of Bt11 x MIR604 x GA21 maize can be considered negligible. Therefore, a case-specific monitoring plan is not considered necessary under the scope of this application. However, a general surveillance plan based on Annex II of the Directive 2001/18/EC has been developed by EuropaBio and is outlined below.

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

An environmental risk assessment (e.r.a.) has been conducted in accordance with Annex II of Directive 2001/18/EC to evaluate potential adverse effects of Bt11 x MIR604 x GA21 maize on human and animal health and the environment. The conclusions of this e.r.a. confirm that the potential risks to human and animal health or the environment arising from the placing on the market of Bt11 x MIR604 x GA21 maize can be considered negligible, under the scope of this application. Therefore, a case-specific monitoring plan is not considered necessary under the scope of this application. However, a general surveillance plan based on Annex II of the Directive 2001/18/EC has been developed by EuropaBio and is outlined below.

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

In accordance with Council Decision 2002/811/EC, 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 e.r.a.

Exposure to the environment will be limited to unintended release of Bt11 x MIR604 x GA21 maize, which could occur for example via substantial losses during loading/unloading of the viable commodity including Bt11 x MIR604 x GA21 maize destined for processing into animal feed or human food products. However, such exposure is highly unlikely to give rise to an adverse effect and can be easily 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 (with the exception of herbicides containing glyphosate or glufosinate). Furthermore, unintended environmental effects due to the unintended release of Bt11 x MIR604 x GA21 maize will be no different than that of other commercial maize.

However and in order to safeguard against any adverse effects on human and animal health or the environment that were not anticipated in the e.r.a., general surveillance on Bt11 x MIR604 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 Bt11 x MIR604 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 Bt11 x MIR604 x GA21 maize. The operators will be provided

with guidance to facilitate reporting of any unanticipated adverse effect from handling and use of viable Bt11 x MIR604 x GA21 maize.

#### 11.5 Reporting the results of monitoring

In accordance with Regulation (EC) No 1829/2003, the authorisation holder is responsible to inform the European Commission of the results of the general surveillance.

If information that confirms an adverse effect of Bt11 x MIR604 x GA21 maize and that alters the existing risk assessment becomes available, the authorisation holder will immediately investigate and inform the European Commission. The authorisation holder, in collaboration with the European Commission and based on a scientific evaluation of the potential consequences of the observed adverse effect, will define and implement management measures to protect human and animal health or the environment, as necessary. It is important that the remedial action is proportionate to the significance of the observed effect.

The authorisation holder will submit an annual monitoring report including results of the general surveillance in accordance with the conditions of the authorisation. The report will contain information on any unanticipated adverse effects that have arisen from handling and use of viable Bt11 x MIR604 x GA21 maize .

The report will include a scientific evaluation of the confirmed adverse effect, a conclusion of the safety of Bt11 x MIR604 x GA21 maize and, as appropriate, the measures that were taken to ensure the safety of human and animal health or the environment.

The report will also clearly state which parts of the provided information are considered to be confidential, together with a verifiable justification for confidentiality in accordance with Article 30.

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

The Bt11 x MIR604 x GA21 maize described in this application has been produced by combining the GM maize events: Bt11, MIR604 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, MIR604 and GA21 maize have been developed by Syngenta. 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 MIR604 maize and GA21 maize have been validated by the DG JRC-CRL. A method for detection of Bt11 maize has been submitted to the DG JRC-CRL for validation. In addition, information relating to the applicability of the single event methods for use on the stacked product has been submitted to the DG JRC-CRL.

## **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**

Syngenta has not previously carried out field trials of Bt11 x MIR604 x GA21 in the EU.

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

a) Release country	Bt11 x MIR604 x GA21 is approved for cultivation in the USA and Canada. Syngenta has conducted research field trials with this maize in the USA and Argentina.
b) Authority overseeing the release	US EPA, USDA, Conabia
c) Release site	Various release sites across the USA and Argentina.
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 MIR604 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 have 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):**

<p>a) Status/process of approval</p> <p>The status and process of approval can be found on the EFSA website: <a href="http://www.efsa.europa.eu/EFSA/ScientificPanels/GMO/efsa_locale-1178620753812_GMOApplications.htm">http://www.efsa.europa.eu/EFSA/ScientificPanels/GMO/efsa_locale-1178620753812_GMOApplications.htm</a></p>
<p>b) Assessment Report of the Competent Authority (Directive 2001/18/EC)</p> <p>An application for approval of Bt11 x MIR604 x GA21 under the Directive 2001/18/EC has not been made by Syngenta.</p>
<p>c) EFSA opinion</p> <p>An EFSA opinion on Bt11 x MIR604 x GA21 maize was not available at the time of submission. EFSA opinions, once available can be found at <a href="http://www.efsa.europa.eu/EFSA/ScientificPanels/GMO/efsa_locale-1178620753812_GMOApplications.htm">http://www.efsa.europa.eu/EFSA/ScientificPanels/GMO/efsa_locale-1178620753812_GMOApplications.htm</a></p>
<p>d) Commission Register (Commission Decision 2004/204/EC)</p> <p>The Commission register of GM Food and Feed can be found at <a href="http://ec.europa.eu/food/food/biotechnology/authorisation/index_en.htm">http://ec.europa.eu/food/food/biotechnology/authorisation/index_en.htm</a></p>
<p>e) Molecular Register of the Community Reference Laboratory/Joint Research Centre</p> <p>The Community Reference Laboratory webpage is <a href="http://gmo-crl.jrc.it/">http://gmo-crl.jrc.it/</a></p>
<p>f) Biosafety Clearing-House (Council Decision 2002/628/EC)</p> <p>Information relating to the Biosafety clearing house can be found at: <a href="http://bch.biodiv.org/">http://bch.biodiv.org/</a></p>
<p>g) Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC)</p> <p>An application for approval of Bt11 x MIR604 x GA21 maize under the Directive 2001/18/EC has not been made by Syngenta, however a link to this Summary under Regulation (EC) No 1829/2003, should be found at: <a href="http://www.efsa.europa.eu/EFSA/ScientificPanels/GMO/efsa_locale-1178620753812_GMOApplications.htm">http://www.efsa.europa.eu/EFSA/ScientificPanels/GMO/efsa_locale-1178620753812_GMOApplications.htm</a>.</p>