Application for authorisation of stacked GA21 x T25 maize in the European Union under Regulation (EC) No 1829/2003

PART VII: SUMMARY

This document is complete as of October 2016. 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.

PART VII

SUMMARY

APPLICATION FOR AUTHORISATION OF GA21 X T25 MAIZE UNDER REGULATION (EC) 1829/2003

1. GENERAL INFORMATION

1.1. Details of application

(a) Member State of application

Germany

(b) Application Number

Not available at time of submission

(c) Name of the product (commercial and other names)

GA21 x T25 maize

(d) Date of acknowledgement of valid application

Not available at time of submission

1.2. Applicant

(a) Name of applicant

Syngenta Crop Protection NV/SA, Brussels, Belgium acting on its behalf and for its affiliated companies.

(b) Address of applicant

Syngenta Crop Protection NV/SA
Avenue Louise 489
1050 Brussels
Belgium

(c) Name and address of the representative of the applicant established in the Union (if the applicant is not established in the Union)

Not applicable.

1.3. Scope of the application

(a) GM food

☒ Food containing or consisting of GM plants

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

(b) GM feed

☒ Feed containing or consisting of GM plants

☒ Feed produced from GM plants

(c) GM plants for food or feed use

☒ Products other than food and feed containing or consisting of GM plants with the exception of cultivation

☐ Seeds and plant propagating material for cultivation in the Union

1.4. Is the product or the uses of the associated plant protection product(s) already authorised or subject to another authorisation procedure within the Union?

No ☒

Yes ☐ (in that case, specify)

1.5. Has the GM plant been notified under Part B of Directive 2001/18/EC?

Yes ☐

No ☒ (in that case provide risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC)

Risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC is provided in the application.

1.6. Has the GM plant or derived products been previously notified for marketing in the Union under Part C of Directive 2001/18/EC?

No ☒

Yes ☐ (in that case, specify)

1.7. Has the product been subject to an application and/or authorised in a third country either previously or simultaneously to this application?

No ☐

Yes ☒ (In that case, specify the third country, the date of application and where available, and provide a copy of the risk assessment conclusions, the date of the authorisation and the scope of the application)

Submissions covering GA21 x T25 maize have been made in third countries

around the world and are at different stages in the approval process. GA21 x T25 maize is currently authorized for cultivation in the United States (U.S.), Canada; and is authorized for import in Mexico, Japan, South Korea, Taiwan, Philippines, Australia/New Zealand and South Africa.

1.8. General description of the product

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

GA21 x T25 maize is a GM maize that is produced by conventional breeding crosses of the following GM maize events: GA21 and T25.

- Event GA21 maize (GA21 maize) which produces a double-mutated maize 5-enolpyruvylshikimate-3-phosphate synthase enzyme (mEPSPS) for weed control by providing tolerance to herbicide products containing glyphosate.
- Event T25 maize expressing a phosphinothricin acetyltransferase (PAT) protein for weed control by providing tolerance to herbicide products containing glufosinate ammonium.

Where cultivated, the intended function of the genetic modification of GA21 x T25 maize is to facilitate the control of weeds by providing tolerance to herbicides with two different modes of action: conferring tolerance to glyphosate and glufosinate-ammonium in herbicide products.

(b) Types of products planned to be placed on the market according to the authorisation applied for and any specific form in which the product must not be placed on the market (seeds, cut-flowers, vegetative parts, etc.) as a proposed condition of the authorisation applied for

This application under Regulation (EC) No 1829/2003 covers the import, food and feed use and processing of GA21 x T25 maize. It does not cover cultivation.

The scope of the application includes all food and feed products containing, consisting of or produced from GA21 x T25 maize including products from inbreds and hybrids obtained by conventional breeding of this maize product. The application also covers the import and industrial processing of GA21 x T25 maize for all potential uses as any other maize.

(c) Intended use of the product and types of users

It is intended that GA21 x T25 maize will be used as any other conventional maize for all food, feed and industrial purposes.

(d) Any 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 GA21 x T25 maize and products derived from it are not different from those of its conventional counterpart, apart from the introduced traits of herbicide tolerance. GA21 x T25 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 GA21 x T25 maize.

(e) If applicable, geographical areas within the EU to which the product is intended to be confined under the terms of the authorisation applied for

The GA21 x T25 maize and derived products are suitable for use as any other maize under the terms of the authorisation applied for.

(f) Any type of environment to which the product is unsuited

This application under Regulation (EC) No 1829/2003 covers the import, food and feed use and processing of GA21 x T25 maize. It does not cover cultivation.

(g) Any proposed packaging requirements

The characteristics of GA21 x T25 maize and products derived from it are not different from those of its conventional counterpart. GA21 x T25 maize has been shown to be as safe and as wholesome as existing varieties of maize. Therefore, there are no specific instructions for packaging.

(h) Any proposed labelling requirements in addition to those required by other applicable EU legislation than regulation (EC) N° 1829/2003 and when necessary a proposal for specific labelling in accordance with Articles 13(2), and (3), Articles 25(2)(c), and (d) and Articles 25(3) of Regulation (EC) No 1829/2003.

In the case of products other than food and feed containing or consisting of genetically modified plants, a proposal for labelling which complies with the requirements of point A(8) of Annex IV to Directive 2001/18/EC must be included.

A proposal for labelling has been included in the application. This includes the labelling requirements outlined by Regulation (EC) No 1829/2003 and Annex IV of Directive 2001/18/EC. GA21 x T25 maize 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 GA21 x T25 maize and derived products are not different from those of its conventional counterpart, no additional labelling is required.

(i) Estimated potential demand

(i) In the EU

There are no anticipated changes to the intake/extent of use of maize as a result of the introduction of GA21 x T25 maize to the maize supply. It is anticipated that the introduction of GA21 x T25 maize will replace some of the maize in existing food and feed products.

(ii) In EU export markets

There are no anticipated changes to the extent of maize production in export markets for EU supplies as a result of the introduction of GA21 x T25 maize products.

(j) Unique identifier in accordance with Regulation (EC) No 65/2004

The unique identifier assigned to this product in accordance with Regulation (EC) No 65/2004 is MON-ØØØ21-9 X ACS-ZMØØ3-2 (also referred to as GA21 x T25 maize).

1.9. 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 GA21 x T25 maize and products derived from it are not different from those of its conventional counterpart, apart from the intended traits.

The scope of this application does not include cultivation of GA21 x T25 maize in the EU.

In the unlikely event that small amounts of seed from GA21 x T25 maize accidentally found their way into the environment, this would represent extremely low levels of exposure and the survival of these seeds 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, with the exception of herbicide products containing glyphosate and glufosinate-ammonium.

Exposure to the environment will be limited to unintended release of GA21 x T25 maize, which could occur for example via substantial losses during loading/unloading of the viable commodity including GA21 x T25 maize destined for processing into animal feed or human food products. In the event that small amounts of GA21 x T25 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. 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. In addition, volunteers could be easily controlled using

any of the current agronomic measures taken to control other commercially available maize.

The GA21 x T25 maize and derived products have 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.

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

2.1. Complete name

(a) Family name

Poaceae (formally Gramineae)

(b) Genus

Zea

(c) Species

Zea mays L

(d) Subspecies

mays

(e) Cultivar/breeding line

A proprietary Syngenta line

(f) Common name

Maize

2.2. Geographical distribution and cultivation of the plant, including the distribution within the Union

Maize is the world's most widespread cereal with very diverse morphological and physiological traits; it is grown on approximately 185 million hectares worldwide. 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 21ºC and 27ºC and the freeze-free season lasts 120-180 days.

In the EU, between 60 and 78 million tonnes of maize are produced annually. Another major maize product is silage maize produced on about 5.2 million hectares.

This application requests authorization for food and feed uses, and for import and processing and does not include cultivation in the EU.

2.3. Information concerning reproduction (for environmental safety aspects)

(a) Mode(s) of reproduction

Zea mays (*Z. 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.

(b) 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. 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.

(c) Generation time

Maize is an annual crop. The generation time from sowing to harvesting varies according to the genetic background and the climate; cultivars can range in maturity from 50 days to over a year from seedling emergence to maturity.

2.4. Sexual compatibility with other cultivated or wild plant species (for environmental safety aspects)

Other cultivated species: The sexual compatibility of maize with other cultivated plant species is limited to *Zea* species. However cross-pollination between maize volunteers and other maize crops, although possible, would only occur at very low levels.

Wild plant species: Species that are sexually compatible with maize are not native

to the EU and steps are already in place to control them, therefore, cross-hybridisation and introgression with these species are highly unlikely. Any vertical gene transfer would therefore be limited to other maize plants where cross-pollination between maize varieties under European cultivation conditions could occur.

The scope of this application does not cover the cultivation of GA21 x T25 maize. Therefore, any outcrossing between GA21 x T25 maize and cultivated *Z. mays* varieties is highly unlikely.

2.5. Survivability (for environmental safety aspects)

(a) Ability to form structures for survival or dormancy

Maize is a highly domesticated plant and cannot survive without human intervention. Maize is an annual crop and 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, and stage of development. Maize is not a persistent 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.

2.6. Dissemination (for environmental safety aspects)

(a) Ways and extent of dissemination

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

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

(b) Specific factors affecting dissemination

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. Most maize pollen falls within 5m of the field's edge. In general, these studies have shown that over 98% of maize pollen remains within a radius of 25 – 50m of the source, although some pollen grains can travel several hundred meters.

2.7. Geographical distribution within the Union of the sexually compatible species (for environmental safety aspects)

The only sexually compatible species in the EU is other cultivated maize.

2.8. In the case of plant species not normally grown in the Union, description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts (for environmental safety aspects)

Not applicable, as maize is commercially cultivated in the European Union.

2.9. 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 (for environmental safety aspects)

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 and nematode pests, as well as to competition from surrounding weeds. Maize is extensively cultivated and has a history of safety for environmental safety aspects.

3. MOLECULAR CHARACTERISATION

3.1. Information relating to the genetic modification

(a) Description of the methods used for the genetic modification

The GA21 x T25 maize is a GM maize that is produced by conventional breeding crosses of the GM maize events: GA21 and T25 maize. No further genetic modification to produce this stack has taken place.

The GA21 and T25 maize events maize were produced by genetic modification as follows:

- GA21 maize was produced via microprojectile bombardment of maize suspension culture cells.
- T25 maize was produced through polyethylene-glycol mediated

protoplast transformation

(b) Nature and source of the vector used

The GA21 x T25 maize described in this application has been produced by combining the GM maize events: GA21 and T25 through conventional breeding techniques.

The vectors used to produce GA21 and T25 maize are as follows:

- 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.
- The purified plasmid vector pUC/Ac was used for the transformation of T25 maize through protoplast transformation. The plasmid pUC/Ac was constructed by cloning the synthetic *pat* gene between the Cauliflower Mosaic Virus (CaMV)-derived 35S gene promoter and terminator sequences of the pUC derived plasmid pDH51.

(c) Source of donor DNA used for transformation, size and intended function of each constituent fragment of the region intended for insertion

The GA21 x T25 maize described in this application has been produced by combining the GM maize events: GA21 and T25 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:

Table 1. Event GA21 maize (transformation vector pDPG434)

Vector component	Size (bp)	Description
Actin promoter complex	1424	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	393	Optimised transit peptide sequence construct based on transit peptide sequences from maize and sunflower ribulose-1,5-bis phosphate carboxylase oxygenase (RuBisCo) genes.
mepsps gene	1338	Doube-mutated *epsps* gene, which confers tolerance to herbicide products containing glyphosate.
NOS terminator	272	Polyadenylation region from the nopaline synthase gene from *Agrobacterium tumefaciens*.

Table 2. Event T25 maize (transformation vector pUC/Ac)

Vector component	Size (bp)	Description
35S terminator	206	Stop signal, CaMV 35S terminator
Polylinker sequence	27	Synthetic plasmid cloning site
pat	551	*Streptomyces viridochromogenes* gene encoding the selectable marker PAT (phosphinothricin acetyltransferase). PAT confers resistance to herbicides containing glufosinate
Polylinker sequence	27	Synthetic plasmid cloning site
35S promoter	529	CaMV Promoter
Cloning vector sequences	2642	Sequences from pDH51 needed for the maintenance in the *E. coli* host

3.2. Information relating to the GM plant

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

The GA21 x T25 maize described in this application has been produced by combining the GM maize events: GA21 and T25 through conventional breeding techniques and produces the following proteins:

1. The mEPSPS protein that confers tolerance to herbicide products containing glyphosate; and
2. The PAT protein that confers tolerance to herbicide products containing glufosinate-ammonium.

3.2.2. Information on the sequences actually inserted or deleted

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

The GA21 x T25 maize described in this application has been produced by combining the GM maize events: GA21 and T25 through conventional breeding techniques.

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

Not applicable.

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

Not applicable

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

The genetic stability of each of the single maize inserts in GA21 x T25 maize has been assessed by Southern blot analysis, concluding that each transformation event in the stacked event has the same molecular properties as the single transformation event. Furthermore, sequence comparisons of GA21, and T25 inserts in GA21 x T25 showed that no nucleotide changes were identified.

(e) In case of modifications other than insertion or deletion, describe function of the modified genetic material before and after the modification as well as direct changes in expression of genes as a result of the modification

Not applicable.

3.2.3. Information on the expression of the insert

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

GA21 x T25 maize was produced by conventional breeding and express the genes *mepsps* and *pat*. Therefore these GA21 x T25 hybrid maize plants produce the transgenic proteins mEPSPS and PAT, respectively.

Several tissue types from GA21 x T25 maize plants were analyzed by enzyme-linked immunosorbent assay (ELISA) to compare the concentrations of mEPSPS and PAT. The concentrations of the proteins measured in tissues from plants of the GA21 x T25 maize hybrid were then compared to those measured in the corresponding single-event maize hybrids. Samples from plants of a corresponding conventional counterpart grown concurrently were included with analyses as analytical controls.

The concentrations of mEPSPS and PAT in tissues of the GA21 x T25 hybrid were compared to those measured in the corresponding component single event hybrid. Overall, the concentrations of mEPSPS and PAT in tissues of the GA21 x T25 maize hybrid were similar to those of the corresponding single-event maize hybrids; GA21 and T25. Two significant differences were observed out of thirteen statistical comparisons conducted in this study; however, these differences were not consistently observed across tissue types or developmental stages.

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

Concentrations of mEPSPS and PAT were quantifiable in most GA21 × T25 maize tissue types analysed (leaves, roots, whole plants and kernels), apart from pollen where concentration of both proteins was below the limit of quantification.

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

The genetic and phenotypic stability of the insert in GA21 x T25 maize has been assessed by Southern blot and protein expression analyses across several generations. The results concluded that each transformation event in the stacked

event has the same molecular properties as the single transformation event. The phenotypic stability was confirmed and demponstrated that expression of the transgenic proteins in GA21 x T25 maize is not substantially different from the expression in the GA21 and T25 single maize events.

3.2.5. Information (for environmental safety aspects) on how the GM plant differs from the recipient plant in:

(a) Mode(s) and/or rate of reproduction

No changes in the reproduction compared to the conventional counterpart have been observed in agronomic assessments conducted with GA21 x T25 maize.

(b) Dissemination

No changes in the dissemination compared to the conventional counterpart have been observed in agronomic assessments conducted with GA21 x T25 maize.

(c) Survivability

No changes in the survivability compared to the conventional counterpart have been observed in agronomic assessments conducted with GA21 x T25 maize.

(d) Other differences

No changes in the reproduction, dissemination or survivability compared to the conventional counterpart have been observed in agronomic assessments conducted with GA21 x T25 maize.

In summary, the results of these studies indicate that the genetic modification to produce GA21 x T25 maize does not result in any biologically relevant agronomic or phenotypic differences related to reproduction, dissemination or survivability of GA21 x T25 maize.

3.2.6. Any change to the ability of the GM plant to transfer genetic material to other organisms (for environmental safety aspects)

(a) Plant to bacteria gene transfer

The probability of horizontal gene transfer (HGT) between the GA21 x T25 insert and micro-organisms was investigated *in silico*, and no sequences were identified as being able to promote homologous recombination.

The HGT 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 GA21 x T25 maize to transfer genetic material to other organisms 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 GA21 x T25 maize is not intended to change any of the typical crop characteristics of maize (except for the tolerance to herbicide products). Observations from field trials have confirmed that the agronomic and phenotypic characteristics of GA21 x T25 maize have not changed in comparison with the conventional counterpart, and therefore, there is no increase or decrease in the potential for plant-to-plant gene transfer of GA21 x T25 maize compared to traditional maize. Gene transfer from GA21 x T25 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.

4. COMPARATIVE ANALYSIS

4.1. Choice of the conventional counterpart and additional comparators

GA21 x T25 maize plants were compared with the conventional counterpart that had not been genetically modified. Commercial varieties were also included in the comparison where possible.

4.2. Experimental design and statistical analysis of data from field trials for comparative analysis

The experimental design for comparative analysis was in accordance with EFSA guidance. To evaluate whether biologically significant changes occurred in GA21 x T25 maize plants compared to the conventional counterpart, trials were planted at ten locations in the USA in 2012. The locations of the trial sites were selected to be representative of the agricultural regions suitable for the cultivation of the selected maize hybrids. Entries within each trial were grown in a randomized complete block design with four replicates.

4.3. Selection of materials and compounds for analysis

The selected materials for analysis were forage and grain (raw material). Maize grain from transgenic plants and conventional counterpart plants were analysed for proximates and starch, minerals, vitamins, amino acids, selected fatty acids, anti-nutrients and secondary metabloites. Forage (above ground portion) from

transgenic maize plants and conventional counterpart plants were analysed for proximates and minerals.

The vast majority of nutritional components in GA21 x T25 maize are equivalent to those in the reference lines, and are not significantly different from those in conventional counterpart maize. When differences did occur, levels were within ranges considered to be normal for conventional maize, and therefore are expected to have no impact on the health or nutrition of consumers.

These data support the conclusion that GA21 x T25 maize is compositionally equivalent to conventional maize.

4.4. Comparative analysis of agronomic and phenotypic characteristics

To confirm that GA21 x T25 maize plants are equivalent in agronomic characteristics compared to the conventional counterpart, apart from the introduced traits, selected agronomic and phenotypic characteristics were assessed and compared. Data were collected for multiple agronomic characteristics: early stand count; days to 50% pollen shed; days to 50% silking; plant height; root-lodged plants; stalk-lodged plants; final stand count; grain yield; grain moisture; grain test weight. The results of these trials showed that GA21 x T25 maize is agronomically and phenotypically equivalent to conventional maize, apart from the introduced traits.

4.5. Effect of processing

GA21 x T25 maize will be produced and processed in the same way as any conventional counterpart maize and there is no evidence to suggest that the expression of the proteins produced by GA21 x T25 maize (mEPSPS and PAT) will influence this processing in any way.

5. TOXICOLOGY

(a) Toxicological testing of newly expressed proteins

GA21 x T25 hybrid maize plants produce the proteins mEPSPS and PAT, that confer tolerance to herbicide products containing glyphosate and glufosinate-ammonium respectively. The mEPSPS and PAT proteins produced in GA21 x T25 maize are identical to the mEPSPS protein produced in GA21 maize and the PAT protein produced in T25 maize. Both of these proteins have been previously assessed by EFSA.

Neither of the newly expressed proteins, mEPSPS and PAT, are structurally or functionally related to proteins which have the potential to adversely affect human or animal health; they are rapidly degraded in *in vitro* digestibility assays; have no biologically relevant sequence simliarity to known or putative mammalian protein toxins; and show no acute oral

toxicity in mammalian studies.

(b) Testing of new constituents other than proteins

Maize is a common source of food and feed and has a long history of safe use. GA21 x T25 maize has been modified to produce the mEPSPS and PAT proteins. No other new constituents apart from these proteins are expected to be produced in GA21 x T25 maize and compositional analyses have confirmed the compositional equivalence of GA21 x T25 maize to conventional maize. Therefore, no testing of any other constituent is considered necessary.

(c) Information on natural food and feed constituents

GA21 x T25 maize grain and forage have been found to be compositionally equivalent to conventional maize varieties.

These analyses showed that the levels of the components measured had not changed beyond the natural variation in maize. No significant differences emerged to suggest that biologically relevant changes in composition or nutritive value of the maize grain or forage had occurred as an unintended result of the expression of the novel proteins in GA21 x T25 maize.

(d) Testing of the whole genetically modified food or feed

Although there is no reason to believe that consumption of GA21 and T25 maize would lead to any toxicity as a result of unintended effects, 90-day feeding studies with GA21 and T25 maize grain in rodents have been performed and submitted in the context of previous applications. These studies have been previously assessed by EFSA. A 90-day feeding study with GA21 x T25 maize has also been performed.

Dietary administration of GA21 x T25 transgenic maize grain to rats for at least 91 consecutive days was well tolerated. There were no toxicological effects noted on body weight, food consumption, clinical condition (including neurotoxicity assessments), ophthalmoscopy, haematology, coagulation, blood chemistry or macroscopic and microscopic pathology at inclusion levels up to and including 41.5%. The highest dose used in the 90-day rat study is the maximum achievable without causing nutritional imbalance and the lowest dose is above the anticipated human/target animal intake level.

6. ALLERGENICITY

(a) Assessment of allergenicity of the newly expressed protein

The weight-of-evidence indicates that the newly expressed proteins produced by GA21 x T25 maize are not likely to be food allergens because:

1. the mEPSPS and PAT proteins are not derived from allergenic sources,
2. mEPSPS and PAT do not have biologically relevant amino acid sequence similarity to known or putative allergenic proteins,
3. mEPSPS and PAT are readily degraded in *in vitro* digestibility assays.

From these data, it can be concluded that mEPSPS and PAT produced by GA21 x T25 maize are highly unlikely to be allergenic.

(b) Assessment of allergenicity of the whole genetically modified plant

Maize grain has a history of safe use throughout the world and it is not considered to be a major allergenic food source. Although rare cases of occupational allergy to maize dust or maize pollen allergy have been reported and IgE-binding proteins have been identified in maize flour, the prevalence of maize allergy is exceedingly low amongst the human population. Equivalence of GA21 x T25 maize (with the exception of the introduced traits) to the conventional comparator, was been demonstrated on the basis of compositional analysis. Therefore, no increased allergenicity is anticipated for GA21 x T25 maize.

There is no evidence to suggest that GA21 x T25 maize will have a greater allergenic potential compared to conventional counterpart maize varieties.

7. NUTRITIONAL ASSESSMENT

(a) Nutritional assessment of the genetically modified food

GA21 x T25 maize is not intended to change the nutritional status of individuals or 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 in GA21 x T25 maize, and derived food products.

(b) Nutritional assessment of the genetically modified feed

GA21 x T25 maize is not intended to change the nutritional status of livestock animals or to be processed in products with enhanced functionality. Compositional analysis has demonstrated that no unexpected alterations in nutrients and other food or feed components have occurred and that no nutritional imbalances were introduced in GA21 x T25 maize, and derived feed products.

8. EXPOSURE ASSESSMENT – 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 GA21 x T25 maize to the conventional maize supply. It is anticipated that the introduction of GA21 x T25 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 mEPSPS and PAT, through maximum consumption and exposure assumptions considered in GA21 x T25 maize in the EU, will be very low. The dietary exposure assessment performed took into consideration a maximum exposure assumption leading to margins of exposure that greatly exceed a factor of 100, supporting the conclusion that the risk to humans and animal livestock from GA21 x T25 maize is negligible. The dietary exposure assessment supports the conclusion that the risk to consumers from GA21 x T25 maize is negligible.

9. RISK CHARACTERISATION

Maize food and feed products have a long history of safe use. No significant native toxins are reported to be associated with the genus *Zea*.

The information presented in the application confirms that GA21 x T25 maize and derived food and feed products are not different from those of its conventional counterpart. The molecular characterization of GA21 x T25 maize did not raise any safety concerns nor identified any unintended changes as a result of the genetic modification. Compositional analysis concluded that the levels of the vast majority of nutritional components in GA21 x T25 maize are equivalent to those in the nontransgenic reference lines, and are not significantly different from those in the nontransgenic, conventional counterpart maize. The agronomic and phenotypic characteristics of GA21 x T25 maize plants, except for the introduced traits, are not different to those of its conventional counterpart comparator, taking into account natural variation. Characterisation of mEPSPS and PAT proteins, and evidence of history of safe use, continue to confirm that these proteins are safe for human and animal consumption, and that no adverse effects on human and animal health can be expected. The genetic modification in GA21 x T25 maize is not intended to improve the nutritional status of individuals or populations or to be processed in products with enhanced functionality. The exposure assessment in humans and animals did not indicate any safety concerns, and dietary role of GA21 x T25 maize is intended to be the same as the dietary role of conventional maize.

10. POST-MARKET MONITORING ON THE GENETICALLY MODIFIED FOOD OR FEED

As described in Sections 4 to 9 above, the presence of GA21 x T25 maize or its derived products in food and feed will not result in any nutritional changes. Therefore, post-market monitoring of GA21 x T25 maize food/feed is not considered necessary.

11. ENVIRONMENTAL ASSESSMENT

11.1. Mechanism of interaction between the GM plant and target organisms

GA21 x T25 maize has been developed to confer tolerance to certain herbicides. However, the scope of this application covers the import and food and feed use of GA21 x T25 maize and derived products in the EU. Cultivation of these maize products in the EU is not included in the scope. Therefore, exposure of target organisms to maize leaves and roots of GA21 x T25 maize will be highly unlikely.

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

(a) Persistence and invasiveness

Taking into account the results obtained in agronomic comparisons and the fact that the scope of this application does not include cultivation of GA21 x T25 maize, the growth of any maize plants outside cultivated areas is very unlikely, which means that environmental exposure in the EU would be very low and localised. It can be concluded that the genetic modification introduced in GA21 x T25 maize has not altered agronomic and phenotypic characteristics of GA21 x T25 maize associated with persistence or invasiveness potential compared to conventional maize. In addition, the genes introduced in GA21 x T25 maize will not confer any selective advantage or disadvantage to GA21 x T25 maize compared to conventional maize, apart from the intended modifications. Therefore GA21 x T25 maize will not differ in persistence and invasiveness from conventional maize.

In summary, the likelihood that GA21 x T25 maize will become more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats as a result of import, processing or food and feed use, in the EU can be considered negligible.

(b) Selective advantage or disadvantage

An assessment of whether the transfer of the newly introduced genes in GA21 x T25 maize (*mepsps* and *pat*) could confer any selective advantage or disadvantage to other maize plants or to sexually compatible wild relatives and the potential consequences of this transfer has been conducted. Taking into account the results obtained from the Environmental Risk Assessment (ERA), the results of the comparative safety assessment and the fact that the scope of this application does not include cultivation of GA21 x T25 maize in the EU, the conclusion from the assessment is that the expression of *mepsps* and *pat* will not confer any selective advantage or disadvantage to GA21 x T25 maize.

(c) Potential for gene transfer

The scope of this application covers the import, processing, and food and feed use of GA21 x T25 maize and derived products in the EU. Cultivation of these maize products in the EU is not included in the scope. Therefore, it is highly unlikely that GA21 x T25 maize plants will grow in the EU.

There is also no change in the ability of GA21 x T25 maize to transfer genetic material to other organisms when compared to conventional maize. The HGT from GM plants to bacteria with subsequent expression of the transgenes is regarded as highly unlikely under natural conditions, especially in the absence of selective pressure.

Gene transfer from GA21 x T25 maize to other sexually compatible plant species is not possible since there are no wild relatives of maize in the EU and vertical gene transfer would be limited to other maize plants. Therefore, it is highly unlikely that the import, processing, and food and feed use of GA21 x T25 maize and derived products in the EU would lead to any adverse environmental effects due to plant-to-plant gene transfer.

Given the low levels of exposure to micro-organisms that could arise from the import, processing, and food and feed use of GA21 x T25 maize in the EU and the characteristics of the transgenes, *mepsps* and *pat*, it is highly unlikely that HGT will occur. If gene transfer did occur, it is unlikely that the transgenes would become established in the genome of micro-organisms in the environment or human and animal digestive tract.

In the very unlikely event that any of the genes were established in the genome of micro-organisms, no adverse effects on human and animal health or the environment are expected.

(d) Interactions between the GM plant and target organisms

The introduced traits confer tolerance to herbicides, therefore there are no target organisms associated with this event.

(e) Interactions of the GM plant with non-target organisms

The scope of this application does not include cultivation of GA21 x T25 maize in the EU. Therefore, potential immediate or delayed effects in the environment due to direct or indirect interactions between GA21 x T25 maize plants and non-target organisms as a result of the import, processing or products for food and feed use of GA21 x T25 maize in the EU can be considered highly unlikely.

(f) Effects on human health

Compositional analysis with GA21 x T25 maize have confirmed that GA21 x T25 maize is equivalent in composition to conventional maize and is as safe and nutritious as conventional maize.

There is no reason to anticipate that GA21 x T25 maize would result in a product that differs in toxicity or allergenic potential to humans. Neither of the proteins (mEPSPS and PAT) produced by GA21 x T25 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. The results of the toxicological and allergenicity assessment indicate that consumption of GA21 x T25 maize food products will be as safe as consuming equivalent products from conventional maize, regardless of the anticipated intake level.

In summary, no adverse effects on human health or adverse consequences for the food chain are expected following consumption of food consisting, containing or derived from GA21 x T25 maize.

(g) Effects on animal health

The potential adverse effects of importing GA21 x T25 maize or derived products into the EU on animal health have been assessed. Studies conducted with mEPSPS and PAT show that these proteins are unlikely to be toxic to humans or animals. None of these proteins shows significant sequence identity to known protein toxins. In addition, mEPSPS and PAT are unlikely to be allergenic.

The results obtained from the comparative analysis of composition of GA21 x T25 maize with conventional maize have shown that the levels of natural food and feed constituents have not changed beyond the natural variation in maize and no evidence of unintended effects has been observed. The conclusion of this assessment is that feed derived from GA21 x T25 maize is as safe for animal consumption as feed derived from conventional maize.

In summary, no adverse effects on animal health or adverse consequences for the feed chain are expected following consumption of feed consisting, containing or derived from GA21 x T25 maize.

(h) Effects on biogeochemical processes

The scope of this application does not include cultivation of GA21 x T25 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 GA21 x T25 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, except for the use of trait specific herbicides. In the unlikely event that some plants of GA21 x T25 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 changes in management practises or interactions of GA21 x T25 maize and target or non-target organisms can be considered negligible under the scope of this application.

(i) Impacts of the specific cultivation, management and harvesting techniques

Not applicable since the scope of this application does not include cultivation of GA21 x T25 maize in the EU.

11.3. Potential interactions with the abiotic environment

The scope of this application does not include cultivation of GA21 x T25 maize in the EU. Therefore, interactions of GA21 x T25 maize with the abiotic environment are highly unlikely. In the unlikely event that small amounts of GA21 x T25 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, except for the use of trait specific herbicides. In the unlikely event that some plants of GA21 x T25 maize survive, the potential effects on the abiotic environment will be negligible.

11.4. Risk characterisation for the environmental risk assessment

Cultivation of maize has a long history of environmental safety. Maize has no weedy characteristics and there are no significant native toxins associated with the genus *Zea*. The information presented in this application confirms that the environmental safety of GA21 x T25 maize is not different from the conventional counterpart.

12. ENVIRONMENTAL MONITORING PLAN

(a) General (risk assessment, background information)

The scope of this application does not include cultivation of GA21 x T25 maize. Environmental exposure to GA21 x T25 maize could only occur in the unlikely event that small amounts of GA21 x T25 maize accidentally found their way into the environment in the EU. However, the survival of this maize would be very unlikely as maize is a highly domesticated plant and cannot survive without human intervention, especially under normal European climatic conditions. If germinated, GA21 x T25 maize could easily be controlled using any of the current agronomic measures taken to control other commercially available maize.

An ERA has been conducted for GA21 x T25 maize as recommended in the EFSA Guidance for risk assessment of food and feed from genetically modified plants and the EFSA Guidance on the ERA of GM plants, and taking into account the scope of this application. Risk assessment concepts described in recent scientific publications have also been used.

The overall conclusion of the ERA confirms that the import and food and feed use of GA21 x T25 maize will not result in harmful effects on human or animal health or to the environment in the EU.

(b) Interplay between environmental risk assessment and monitoring

An ERA has been conducted for GA21 x T25 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 GA21 x T25 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.

(c) 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 GA21 x T25 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 GA21 x T25 maize can be considered negligible, under the scope of this application. Therefore, a case-specific monitoring plan is not considered necessary.

(d) 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 GM plant 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 GA21 x T25 maize. Therefore, exposure to the environment will be limited to unintended release of GA21 x T25 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 GA21 x T25 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 GA21 x T25 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 GA21 x T25 maize. The operators will be provided with guidance to facilitate reporting of any unanticipated adverse effect from handling and use of viable GA21 x T25 maize.

(e) 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.

13. DETECTION AND EVENT-SPECIFIC IDENTIFICACION TECHNIQUES FOR THE GM PLANT

GA21 x T25 hybrid maize is detectable using the event-specific real-time quantitative PCR methods for GA21 and T25 maize events, respectively. These detection methods have been validated by the European Union Reference Laboratory for GM Food and Feed (EURL GMFF) of the Joint Research Centre of the European Commission as part of this application.

14. INFORMATION RELATING TO PREVIOUS RELEASES OF THE GM PLANT (FOR ENVIRONMENTAL SAFETY ASPECTS)

14.1. 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 GA21 x T25 maize have been carried out in the EU.

14.2. History of previous releases of the GM plant carried out outside the Union by the same notifier

(a) Release country

U.S.

(b) Authority overseeing the release

Not applicable.

GA21 and T25 are deregulated by United States Department of Agriculture (USDA).

GA21 X T25 maize is not regulated in the U.S.

(c) Release site

Various sites across the U.S.

(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 GA21 x T25 maize 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.

(a) Release country

Argentina

(b) Authority overseeing the release

SAGyP: Secretariat of Agriculture, Livestock and Fisheries

(c) Release site

One location in Argentina.

(d) Aim of the release

Research and development.

(e) Duration of the release

5 months.

(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 GA21 x T25 maize field trials was no different to other maize.

(i) Results of the release in respect to any risk to human health and the environment

Out of the scope of the trial – however no differences were registered compared to conventional material.