Application for import and use (including cultivation) of genetically modified herbicide tolerant maize Event GA21 under Regulation (EC) No 1829/2003

PART II: SUMMARY

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) Maize Event GA21 In the USA, Event GA21 maize is marketed under the product name Agrisure GT[1]
d) Date of acknowledgement of valid application Not available at the time of submission

[1] http://www.syngenta-us.com/media/article.asp?article_id=749

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 France 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)) Event GA21 maize will be cultivated, 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 ☐	No ☒
If *yes*, specify	

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

Yes ☒	No ☐
If *no*, 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 ☒ By Monsanto	No ☒ By Syngenta

If *yes*, specify

An application for authorization and use of Event GA21 (excluding cultivation) was submitted, by Syngenta, in accordance with Articles 5 and 17 of Regulation (EC) No 1829/2003 on 29 July 2005 (Application EFSA-GMO-UK-2005-19). This application was assessed by the EFSA GMO Panel and the positive opinion may be found on the EFSA website (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620785956.htm.).
Leading on from this positive opinion, the import of GA21 maize for all uses was approved under Regulation (EC) No 1829/2003 on 28th March 2008. The Commission decision relating to this approval (Decision 2008/280/CE) may be found in the Official Journal of the European Union[2].

Applications for commercial approval were previously made by Monsanto under Directive 90/220/EEC but have been withdrawn. An application made by Monsanto under Regulation (EC) No 258/97 was approved on 13 January 2006[3] but this decision was repealed when the product was approved under the Regulation (EC) No 1829/2003[4].

[2] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:087:0019:0022:EN:PDF

[3] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:034:0029:0031:EN:PDF

[4] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:087:0017:0018:EN:PDF

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

Yes ☒	No ☐
If *yes*, specify Event GA21 maize is approved for cultivation in the USA, Canada, Argentina and Japan. It is approved for feed use in the EU, USA, Canada, Mexico, Argentina, Japan, South Africa, Russia, Korea, Philippines, Taiwan and China and food use in the EU, USA, Canada, Mexico, Argentina, Japan, South Africa, Russia, Australia, New Zealand, Korea, Philippines, Taiwan and China.	

8. General description of the product

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

Event GA21 is a genetically modified (GM) maize, which expresses a mutated maize 5-enolpyruvylshikimate-3-phosphate synthase enzyme (mEPSPS). EPSPS is a key enzyme in the shikimate pathway, involved in the biosynthesis of aromatic amino acids and is naturally found in all plants, fungi, and bacteria but absent in animals. EPSPS is highly sensitive to herbicide products containing glyphosate. Maize plants transformed with the mutated *epsps* (*mepsps*) gene, such as those derived from Event GA21, synthesize the mEPSPS protein that confers tolerance to herbicide products containing glyphosate.

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

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

c) Intended use of the product and types of users

It is intended that Event 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 Event GA21 maize and products derived from it are not different from those of its conventional counterpart, apart from the introduced trait of tolerance to herbicide products containing glyphosate. Event 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 Event GA21 maize.

e) Any proposed packaging requirements

The characteristics of Event GA21 maize and products derived from it are not different from those of its conventional counterpart. Event 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

Event GA21 maize grain will be labelled as "genetically modified maize" and products derived from it will be labelled as "containing (or produced from) genetically modified maize". Since Event GA21 maize and products derived from it are not different from those of its conventional counterpart, no additional labelling is required.

In addition to specifying the name of the GMO, containers of Event GA21 maize seed will be labelled using the commercial name of the product. A statement to specify "the product contains GMOs" will also be included, along with the name and full address of the person responsible for placing on the market. The labelling will also indicate how to access the information in the publicly accessible part of the register.

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 Event GA21 has been already assigned in accordance with Commission Regulation (EC) 65/2004: MON-∅∅∅21-9.

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

Event 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 Event GA21 maize and products derived from it are not different from those of its conventional counterpart, apart from the intended effect of tolerance to herbicide products containing glyphosate.

Event 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

Zea

c) Species

mays

d) Subspecies

mays

e) Cultivar/breeding line or strain

Event GA21

f) Common name

Maize; corn

2 a. Information concerning reproduction

(i) Mode(s) of reproduction

Sexual reproduction: *Zea mays* is an allogamous plant that propagates through seed produced predominantly by cross-fertilisation which is effected mainly by on wind borne pollen. *Z.mays* is a plant with protoandrous inflorescence; however, decades of conventional

selection and improvement have produced varieties of maize with protogyny. *Z. mays* has staminate flowers in the tassels and pistillate flowers on the ear shoots.

Asexual reproduction: there is no asexually reproductive maize.

(ii) Specific factors affecting reproduction

The key stages of maize reproduction are tasselling, silking, pollination and fertilization. Outcrossing distances are limited by the rapid settling and limited viability of pollen. Most maize varieties are protoandrous with pollen shed preceding silk emergence by up to five days. As maize pollen is large and heavy it tends to be deposited close to the source plant and studies have indicated that most maize pollen falls within 5m of the field's edge. In general, such studies have shown that over 98% of maize pollen remains within a radius of 25-50m of the source, although some grains can travel several hundred metres. Shed pollen typically remains viable for 10 to 30 minutes, but may remain viable longer under refrigerated and humid conditions.

(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 be as short as 60 to 70 days and 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 mays*.

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 domestic cultivation and is non-invasive of natural habitats.

4. Dissemination

a) Ways and extent of dissemination

Maize dissemination may be accomplished through seed dispersal. Seed dispersal does not occur naturally due to the structure of the ear. Dissemination may also occur via pollen; however this is likely to be limited since maize pollen is large and heavy and tends to be deposited close to the source plant.

b) Specific factors affecting dissemination

Maize has a polystichous (arranged in many rows) female inflorescence (group of flowers), 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. The rate of dissemination via pollen will be influenced by the size of pollen, wind direction and speed, other weather conditions such as rainfall, the presence of barriers and the degree of synchrony of flowering. Maize pollen is large and heavy and tends to be deposited close to the source plant. In addition, most maize varieties are protoandrous so pollen shedding precedes silk emergence by up to five days.

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

Maize, which has very diverse morphology and physiology, is grown on approximately 147 million hectares worldwide. It is distributed over a wide range of conditions: from latitudes 50º North and 50º 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 21º and 27º 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 15th 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

Event GA21 was produced via microprojectile bombardment of maize suspension culture cells.

2. Nature and source of the vector used

A *Not*I restriction fragment from the Plasmid pDPG434, was used to transform Event GA21 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

Event GA21 is a genetically modified (GM) maize tolerant to herbicide products containing glyphosate. The DNA used for transformation that resulted in Event GA21, was contained within a *Not*I restriction fragment and the components are shown below. The *Not*I restriction fragment contains the modified 5-enolpyruvylshikimate-3-phosphate synthase (*mepsps*) expression cassette but does not contain the origin of replication, the *bla* gene or the partial *lacZ* sequence from the plasmid pDPG434.

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 *mepsps* gene in maize.
Optimised transit peptide	0.4	Optimised transit peptide sequence constructed based on transit peptide sequences from maize and sunflower ribulose-1,5-bis phosphate carboxylase oxygenase (RuBisCo) genes.
Modified maize EPSPS gene	1.3	Mutated maize *epsps* gene, which confers tolerance to herbicide products containing glyphosate.
Nos 3' end	0.3	Polyadenylation region from the nopaline synthase gene from *Agrobacterium tumefaciens*.

D. INFORMATION RELATING TO THE GM PLANT

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

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

In addition to sequencing, southern analysis has been performed to demonstrate the absence of further copies of the insert or vector sequence elsewhere in the genome.

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 of the insert in Event GA21 maize was investigated. Statistical analysis confirmed the expected Mendelian inheritance ratio for the *mepsps* gene, showing that insertion had taken place in the nuclear genome.

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

The entire insert and the 5' and 3' flanking regions were sequenced. The organisation of the insert is described in Section D2 (a) above.

3. Information on the expression of the insert

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

To characterize the range of expression of mEPSPS in maize plants derived from Event GA21, the concentration of the protein was determined by ELISA at four growth stages (whorl, anthesis, seed maturity and senescence) in the USA and EU. In plants grown in the USA, quantifiable concentrations of mEPSPS protein were detected in most Event GA21-derived plant tissues. Across all growth stages, mean mEPSPS concentrations measured in leaves, roots and whole plants ranged from below the limit of quantification (<0.2 µg/gram fresh weight (gfw)) to *ca.* 15 µg/gfw. Mean mEPSPS concentrations measured in kernels ranged from *ca.* 4-7 µg/gfw and in pollen averaged *ca.* 168 µg/gfw. In the EU, the mean concentrations of mEPSPS measured in leaves and in roots on a fresh-weight basis ranged from 5.92-18.69 µg/g in leaves, and 2.09-5.48 µg/g in roots. The mean concentrations of mEPSPS ranged from 5.62-10.15 µg/g in whole-plants, and 5.85-6.78 µg/g in kernels on a fresh-weight basis. Concentrations of mEPSPS in pollen samples ranged from 99.81-101.58 µg/g on a fresh weight basis.

b) Parts of the plant where the insert is expressed

To characterize the range of expression of proteins in maize plants derived from Event GA21 maize, the concentrations of these proteins were determined by ELISA in several plant tissues (leaves, roots, kernels, pollen and whole plants) in the USA and EU. mEPSPS was quantifiable in most Event GA21-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-transgenic maize have been observed in agronomic assessments conducted with Event GA21 maize.

b) Dissemination

No changes in the dissemination compared to non-transgenic maize have been observed in agronomic assessments conducted with Event GA21 maize.

c) Survivability

No changes in the survivability compared to non-transgenic maize have been observed in agronomic assessments conducted with Event GA21 maize.

d) Other differences

No changes in the reproduction, dissemination or survivability compared to non-transgenic maize have been observed in agronomic assessments conducted with Event GA21 maize.

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

Genetic stability:

Southern analysis of DNA derived from several generations of Event GA21 plants was conducted to confirm a single insertion site. The hybridisation data demonstrate that the insert contained in Event GA21 maize is stable over several generations.

Phenotypic stability:

The stability of mEPSPS expression over multiple generations was evaluated. Seed from three backcross generations was grown under greenhouse conditions and leaf material was collected at anthesis for analysis of mEPSPS concentrations. Mean mEPSPS concentrations measured across all backcross generations were *ca.* 13—14 µg/gfw (82—96 µg/gdw). Overall, mEPSPS concentrations were similar across the three generations analyzed, demonstrating stable expression of mEPSPS across multiple generations.

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 and would occur primarily through homologous recombination in microbes[5]. The *mepsps* gene is under the control of a plant promoter and *epsps* genes are ubiquitous in plants and micro-organisms The specific changes present in the *mepsps* gene were introduced to specifically alter two amino acids in mEPSPS. These specific changes confer tolerance to herbicide products containing glyphosate and were not introduced to enhance recombination or gene transfer. Southern analysis of Event GA21 maize has been performed and has shown that the origin of replication in the original transformation vector has not been transferred to Event GA21 maize. The only sequence of bacterial origin in Event GA21 maize is the *nos* terminator, which has been derived from *Agrobacterium tumefaciens*. This sequence is commonly used in the production of genetically modified plants and there is no evidence to suggest that the presence of this sequence enhances the potential of intact horizontal gene transfer from GM

[5] EFSA, 2004c. Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants, The EFSAJournal 48, 1-18 http://www.efsa.eu.int/science/gmo/gmo_opinions/384_en.html

plants to bacteria.

b) Plant to plant gene transfer

The genetic modification in Event GA21 maize is not intended to change any of the typical crop characteristics of maize. Observations from field trials have confirmed that the agronomic characteristics of Event GA21 maize were not different from near-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 Event GA21 maize to other sexually compatible plant species is not possible since maize has no wild relatives in the EU.

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

Comparative assessment

Choice of the comparator

Event GA21 maize was compared with relevant control maize lines that had not been genetically modified. Results from Event GA21 maize and the non-transgenic near-isogenic control were compared to the range of responses reported for conventional maize hybrids in published databases.

7.2 Production of material for comparative assessment

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

To evaluate whether biologically significant changes in composition occurred in Event GA21-derived maize plants compared to the non-transgenic isolines, replicate trials of transgenic and corresponding isogenic controls were planted in the USA and EU. 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) produced from Event GA21-derived maize plants and simultaneously grown isogenic control plants. The mean values were also compared with the range of data published in the literature, where data was available.

7.3 Selection of materials and compounds for analysis

Based on guidance of the OECD, grain from transgenic Event GA21-derived maize plants and non-transgenic near-isogenic control plants were analysed for proximates (including fibre, and starch), minerals, amino acids and selected fatty acids, vitamins, anti-nutrients and secondary metabolites. Forage (whole plants) from transgenic Event GA21-derived maize plants and non-transgenic near-isogenic control plants were analysed for proximates (including fibre) 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 with or expression of the *mepsps* transgene.

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

7.4 Agronomic traits

Event GA21-derived hybrids were grown in the USA during 2004 and 2005 and in the EU in 2007. Up to 20 separate agronomic traits were assessed, although not all traits were recorded at all locations. The commercial Event GA21 hybrids and their non-transgenic near-isogenic hybrids were compared. While some differences between transgenic and controls were found to be significant, there were no consistent trends in the data across locations or hybrids that would indicate that any of these differences were due to the presence of the transgene. These differences were within the normal range of variation experienced in agronomic field trials conducted with transgenic events by Syngenta during the past years.

These data support the conclusion that there are no biologically significant differences between Event GA21-derived maize hybrids and their corresponding non-transgenic near-isogenic hybrids, apart from the introduced trait of herbicide tolerance.

7.5 Product specification

Maize 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 Event GA21 maize and products derived from it are not different from those of its conventional counterpart.

7.6 Effect of processing

Event GA21 maize will be produced and processed in the same way as any non-transgenic maize and there is no evidence to suggest that the expression of mEPSPS will influence this processing in any way.

Presence of mEPSPS was quantitatively analysed in maize grain and standard wet-milled and dry-milled processing fractions, corn oil and corn chips from the maize grain derived from Event GA21 maize and the corresponding non-transgenic near-isogenic controls.

Concentrations of mEPSPS were below the limit of detection in all of the wet-milled fractions. mEPSPS was quantifiable in the starting grain and all of the dry-milled fractions. The concentration of mEPSPS in both the corn chip and corn oil samples were below the limit of detection.

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 Event GA21 to the conventional maize supply. It is anticipated that Event 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 has been shown to be the case through extensive compositional assessment.

7.8 Toxicology

7.8.1 Safety assessment of newly expressed proteins

Event GA21 maize expresses a modified EPSPS protein (mEPSPS). mEPSPS is derived from *Zea mays* and is greater that 99.3% homologous to maize EPSPS. EPSPS proteins are ubiquitous in plants and micro-organsims and will naturally be present in foods derived from plant and microbial sources. EPSPS converts shikimate-3-phosphate and phosphoenolpyruvate to 5-enolpyruvylshikimate-3-phosphate, and has very high substrate specificity. To demonstrate the safety of this protein a series of studies was conducted. The data obtained showed that mEPSPS is not structurally and functionally related to proteins that have the potential to adversely affect human or animal health. It is sensitive to heat and is rapidly degraded in simulated gastric fluid. It shows no sequence homology with known toxins or allergens. In addition, an acute oral toxicity study of mEPSPS in mice confirmed that the protein is not acutely toxic to mice at the highest dose tested (2000 mg mEPSPS/kg

body weight).

Supplemental information was also provided by a 90-day whole food safety study in rodents and a poultry feeding study showing no adverse effects on rats and chickens.

7.8.2 Testing of new constituents other than proteins

Event GA21 maize has been modified to express the mEPSPS protein. No other new constituents apart from this protein are expected to be produced in Event GA21 maize and compositional analyses have confirmed the equivalence of Event GA21 maize and conventional maize. Therefore no testing of any other constituent is considered necessary.

7.8.3 Information on natural food and feed constituents

Maize is a common source of food and feed and has a long history of safe use. 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 in Event GA21 maize and compared with non-transgenic 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 transformation process or expression of the transgene.

7.8.4 Testing of the whole GM food/feed

In addition to the compositional analysis, the wholesomeness and safety of maize Event GA21 maize was confirmed in a 90-day whole food safety study in rats and a 49-day poultry feeding study.

The 90-day whole food safety study was conducted to observe potential effects of Event GA21 grain on rats. The incorporation of Event GA21 maize grain in diets fed to rats for at least 90 consecutive days produced no deleterious effects that were considered to be attributable to the inclusion of Event GA21 maize grain in the diet.

The 49-day poultry feeding study was also conducted to evaluate whether standard poultry diets prepared with Event GA21 grain had any adverse effect on male or female broiler chickens as compared to diets prepared with non-transgenic near-isogenic control grain and a commercial source of corn. 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

Maize derived from Event GA21 maize expresses a modified EPSPS protein (mEPSPS). mEPSPS is derived from *Zea mays* and is greater than 99.3% homologous to maize EPSPS.

EPSPS proteins are ubiquitous in plants and micro-organisms and will naturally be present in foods derived from plant and microbial sources. To assess the potential for allergenicity of the mEPSPS protein a series of studies were conducted. The data obtained showed that the mEPSPS

- has no homology to known or putative allergens
- is rapidly degraded in simulated gastric fluid
- is sensitive to heat
- is not derived from a source known to be a significant cause of food allergy

From these data, it can be concluded that mEPSPS expressed in Event GA21 maize plants is 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 Event GA21 maize does not express any new proteins with allergenic characteristics.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

Event GA21 maize is not intended to have changed the nutritional status of individuals of populations or to be processed into 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

Event GA21 maize is not intended to have changed 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 Event 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)

Event GA21 expresses a mEPSPS protein that confers tolerance to herbicide products containing glyphosate. Since the only modified trait expressed by the Event GA21 maize is that of herbicide tolerance there are no target organisms in this case.

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

9.1 Persistence and invasiveness

Maize plants do not possess characteristics required for persistence or invasiveness in the environment. The expression of the mEPSPS protein does not affect the agronomic characteristics or weediness potential of Event GA21 maize, as demonstrated in field trials conducted to compare the agronomic performance of this event and isogenic controls.

Therefore the probability of the genetically modified plants becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats as a result of cultivation and use of this event in the EU can be considered negligible.

9.2 Selective advantage or disadvantage

No biologically significant unintended changes were observed in Event GA21 maize when compared with non-transgenic, near-isogenic lines in agronomic field trials in the United States and Europe. Many traits that could affect fitness were measured. It is highly unlikely, therefore, that the expression of mEPSPS in Event GA21 maize would provide a selective advantage or disadvantage over non-transgenic maize in natural habitats, or in agriculture where glyphosate herbicides are not used.

Event GA21 maize is intended to be tolerant of herbicides containing glyphosate. Therefore in fields sprayed with glyphosate herbicides at recommended rates, Event GA21 maize will survive and produce seed, whereas all non-transgenic maize would be likely to die before flowering. In such situations, greater seed production in Event GA21 maize does not confer a selective advantage over non-transgenic maize. Most seed will be harvested and removed; and any Event GA21 seedlings produced from seed spilled during harvest are unlikely to survive to flowering because they will be killed by frost, or removed by standard agronomic practice, as are seedlings of non-transgenic maize.

Therefore the potential risk of the expression of the mEPSPS protein in Event GA21 maize resulting in a selective advantage or disadvantage to the plants can be considered negligible.

9.3 Potential for gene transfer

Gene transfer from Event GA21 maize to wild relatives is not possible since no wild relatives of maize exist in the EU.

Event GA21 maize will behave as any other maize with respect to outcrossing among varieties. Maize is an outcrossing species and almost all varieties are interfertile. Therefore Event GA21 maize could cross-fertilize other maize varieties; however the extent of cross fertilization will be limited. As maize pollen is large and heavy it tends to be deposited close to the source plant and studies have indicated that most maize pollen falls within 5m of the field's edge. In general, such studies have shown that over 98% of maize pollen remains within a radius of 25-50m of the source, although some grains can travel several hundred metres

The relatively few seeds produced by cross-fertilisation of other maize with Event GA21 pollen are highly unlikely to have a selective advantage or disadvantage compared with other maize. No significant unintended effects that could confer a selective advantage or disadvantage have been detected in comparisons of Event GA21 maize and non-transgenic, near-isogenic lines. Cross-fertilisation with Event GA21 pollen will produce seeds that could germinate to produce plants tolerant of herbicides containing glyphosate. If such seeds were spilled during harvest or transport, they are very unlikely to have a selective advantage or disadvantage as tolerance of glyphosate is not known to increase survival or reproduction of maize, except in cases where glyphosate is used. Most seed however, will be harvested and removed; and any GA21 seedlings produced from seed spilled during harvest are unlikely to survive to flowering because they will be killed by frost, or removed by standard agronomic practice, as are seedlings of non- transgenic maize.

In summary, the potential for gene transfer between Event GA21 maize plants and wild plants is negligible in the EU. Relatively few seeds will be produced by cross-fertilisation of non-GA21 maize by Event GA21 pollen. Any plants resulting from such seeds are highly unlikely to have a competitive advantage or disadvantage compared with other maize varieties.

9.4 Interactions between the GM plant and target organisms

As the only modified trait expressed by the Event GA21 maize is that of herbicide tolerance there are no target organisms in this case.

9.5 Interactions of the GM plant with non-target organisms

Event GA21 maize expresses a mEPSPS protein that confers tolerance to herbicide products containing glyphosate. As such the interaction of Event GA21 maize with non-target organisms in the field is likely to be the same as non-transgenic maize.

EPSPS proteins are specifically involved in the production of aromatic amino acid synthesis and are found only in plants and micro-organisms. They are not found in animals. Maize plants derived from Event GA21 express a modified EPSPS protein (mEPSPS). The mEPSPS protein is derived from *Zea mays* and is greater that 99.3% homologous to maize

EPSPS. EPSPS proteins are ubiquitous in plants and micro-organisms and will naturally be present in foods derived from plant and microbial sources.

Based on the specificity of the EPSPS reaction and on the ubiquitous nature of EPSPS proteins in the environment it is highly unlikely that mEPSPS will be hazardous to non-target organisms.

Therefore immediate or delayed effects in the environment due to direct or indirect interactions between Event GA21 maize plants and non-target organisms can be considered highly unlikely.

9.6 Effects on human health

Event GA21 maize will be cultivated and imported in the EU and could enter the food chain. Human exposure to this maize could therefore occur through food consumption or worker exposure. However, the recipient organism, maize, has a history of safe use throughout the world. Event GA21 maize expresses a mEPSPS protein conferring tolerance to herbicide products containing glyphosate. Studies comparing the composition of Event GA21 maize plants and non-transgenic maize have been performed. These studies lead to the conclusion that this maize is equivalent to conventional maize. mEPSPS is sensitive to heat and rapidly degraded in simulated gastric fluids. It shows no sequence homology with known toxins or allergens.

In addition, the acute oral toxicity study conducted with mEPSPS in mice, confirmed that the protein is not acutely toxic to mice at the highest dose tested (2000mg mEPSPS/kg body weight). No test substance related mortalities occurred during the study and no clinical signs attributable to the test substance were observed. There were no treatment related effects on body weight, food consumption or organ weights, nor were any treatment related effects observed following macroscopic or microscopic examination of tissues. Given these results and the low levels at which humans will be exposed to mEPSPS the risk of toxicity effects can be considered negligible.

An allergenicity assessment has been conducted showing that this protein is unlikely to be allergenic.

The inserted DNA and the flanking regions have been sequenced. A detailed open reading frame analysis could find no evidence for sequences of ORFs with homology to known toxins or allergens.

The wholesomeness and safety of maize Event GA21 was confirmed in a 90-day toxicity study in rats and a poultry feeding study, where no treatment related effects were observed and no differences with the non-transgenic controls were detected.

In summary, it can be concluded that grain from maize Event GA21 maize is safe for food consumption and no differences in wholesomeness are expected with comparable non-transgenic maize varieties.

9.7 Effects on animal health

Event GA21 maize will be cultivated and imported in the EU and could enter the feed chain. Animal exposure to this maize could therefore occur though feed consumption

An assessment was conducted to evaluate the possible adverse effects on animal health resulting from exposure to Event GA21. The assessment was based on an extensive characterisation of the expressed protein and the genetically modified plant (see section D.9.6 above).

In summary, it can be concluded that grain from Event GA21 maize is safe for feed consumption and no differences in wholesomeness are expected with comparable non-transgenic maize varieties.

9.8 Effects on biogeochemical processes

EPSPS is ubiquitous in soil as it is an enzyme essential to aromatic amino acid biosynthesis in microbes. mEPSPS is present in low concentrations in roots of Event GA21 maize and therefore cultivation of Event GA21 maize is unlikely to significantly increase the load of EPSPS in soil. 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 and the expression of the *mepsps* gene in Event GA21 maize is not expected to enhance this in any way. However, if horizontal gene transfer occurred, it is unlikely to lead to production of mEPSPS in soil microbes because mEPSPS is transcribed from a plant promoter, which is unlikely to function in soil bacteria. In the unlikely event of acquisition and expression of *mepsps* by soil microbes, significant adverse effects on biochemical processes are highly improbable. If this unlikely event were to occur, it is possible that mEPSPS could enhance the tolerance of a microbe to glyphosate; however, native microbial EPSPSs are already ubiquitous and tend to be relatively insensitive to glyphosate, as indicated by the degradation of glyphosate in soil.

In summary, there is negligible likelihood of immediate or delayed adverse impacts on biogeochemical processes resulting from cultivation of Event GA21 maize.

9.9 Impacts of the specific cultivation, management and harvesting techniques

There are no cultivation, management or harvesting techniques recommended specifically for Event GA21 maize since these will be comparable to those used for conventional maize. It should be noted that most maize cultivated in the EU is sprayed with herbicides which are selected for effective weed control. Event GA21 maize merely provides the farmer with an additional tool for weed control.

10. Potential interactions with the abiotic environment

Event GA21 maize has been shown to be equivalent to non-transgenic maize apart from the introduced herbicide tolerant trait. EPSPS proteins are specifically involved in the production of aromatic amino acid synthesis and are found only in plants and micro-organisms. Event GA21 plants express a mEPSPS derived from *Zea mays* which is greater that 99.3% homologous to maize EPSPS. EPSPS proteins are ubiquitous in plants and micro-organisms and will naturally be present in plant and microbial sources.

Based on the specificity of the EPSPS reaction and on the ubiquitous nature of EPSPS proteins in the environment it is highly unlikely that expression of mEPSPS will have any adverse effect on the abiotic environment.

Therefore potential interactions with the abiotic environment are likely to be the same as those of conventional maize and will not represent any new adverse effects in the environment.

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 Event 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.

The scope of this application covers all uses of Event GA21 maize, including cultivation. An environmental risk assessment (e.r.a.) has been conducted as recommended by the Guidance document of the EFSA Scientific Panel of Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed.

The conclusions of this e.r.a. confirm that the effects to the environment arising from the use of Event GA21 maize can be considered as negligible as 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 to evaluate potential adverse effects of Event 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 Event 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 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 Event 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 Event 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 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.

The scope of this application includes import, cultivation and use of Event GA21 maize.

It is intended that Event GA21 maize should be used as any other maize in the EU, hence background information relating to conventional practices in the import, cultivation, movement and processing have been considered in the development of the monitoring plan.

In relation to the cultivation of Event GA21 maize, farmers are considered to be the most valuable source of information because they are regularly in contact with that part of the receiving environment occupied by the GM crop.

The majority of imported Event GA21 material will be used for feed purposes. Therefore, traders and processors as well as the European feed industry serve as a good focal point to address questions related to any unanticipated effects that might be associated with the use of Event GA21 maize.

Syngenta is committed to inform farmers, traders, processors and the European feed and food industry with details on the safety of Event GA21 maize and to establish a communication network where unforeseen effects can be reported. If unusual observations are reported, more focussed in-depth studies can be carried out in order to determine cause and relationship with the specific event. Final decisions on whether any identified effects are significant can only be made if causality is clear and endpoints are determined.

Although not a formal part of the surveillance plan, there is an extensive information network, with global reach, which will provide additional information on possible adverse effects arising from the use of GM crops. These include new and rapid means of access to information from across the globe through telecommunications, the media and Internet access. Through these means, many groups, including agronomists, ecologists, health professionals, and the general public now have unprecedented access to reports on the use world-wide of GM crops. In addition, electronic discussion sites, for example those of WHO, OECD, FAO, and consumer organisations, are valuable sources of information and communication for professionals and, in many cases, the general public.

11.5 Reporting the results of monitoring

The notifier/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 notifier will submit a General Surveillance Report containing information related to the monitoring on an annual basis.

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

A method for detection of Event GA21 has been developed by Syngenta and validated by the DG JRC CRL. For further information please see http://gmo-crl.jrc.it

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 carried out field trials of Event GA21 in Spain, Czech Republic, Romania and France.
a) Notification number Spain: B/ES/06/04, B/ES/07/32 France:B/FR/06/01/12 , B/FR/06/12/01 Czech Republic: B/CZ/05/02 Romania: 108378/20.12.2005/64265/A.B./20.12.2005; B/RO/07/02 B/RO/07/11
b) Conclusions of post-release monitoring No unexpected effects or observations have been detected to date. No adverse effects on human health or the environmental have been observed or reported during these releases The results of these field trials confirm the safety of the deliberate release of Event GA21 into the environment in the E.U.
c) Results of the release in respect to any risk to human health and the environment (submitted to the Competent Authority according to Article 10 of Directive 2001/18/EC) No unexpected effects or observations have been detected. No adverse effects on human health or the environmental have been observed or reported during these releases Final reports of the releases can be found at the JRC web page http://gmoinfo.jrc.it/

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

Event GA21 maize is approved for cultivation in the USA, Canada, Argentina and Japan. Syngenta has grown Event GA21 commercially in the USA and Canada since 2005 and in Argentina in 2007. Additionally Event GA21 field trials have been carried out in South Africa, Brazil, Chile and the Philippines.

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

a) Status/process of approval The status and process of approval can be found on the EFSA website: http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html
b) Assessment Report of the Competent Authority (Directive 2001/18/EC) An application for approval of Event GA21 maize under the Directive 2001/18/EC has not been made by Syngenta.
c) EFSA opinion A positive EFSA opinion on the import and use of Event GA21 (Application EFSA-GMO-UK-2007-19) was given in September 2007 and is available at http://www.efsa.eu.int/science/gmo/gmo_opinions/catindex_en.html
d) Commission Register (Commission Decision 2004/204/EC) The Commission register of GM Food and Feed can be found at http://europa.eu.int/comm/food/dyna/gm_register/index_en.cfm
e) Molecular Register of the Community Reference Laboratory/Joint Research Centre The Community Reference Laboratory webpage is http://gmo-crl.jrc.it/
f) Biosafety Clearing-House (Council Decision 2002/628/EC) Information relating to the Biosafety clearing house can be found at: http://bch.biodiv.org/
g) Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC) An application for approval of Event GA21 maize under the Directive 2001/18/EC has not been made by Syngenta, however a link to this Summary and the Summary supplied as part of the Application EFSA-GMO-UK-2005-19 under Regulation (EC) No 1829/2003 may be found at:

http://www.efsa.europa.eu/EFSA/ScientificPanels/GMO/efsa_locale-1178620753812_GMOApplications.htm