Application for import and use of genetically modified Event 3272 maize 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) Event 3272
d) Date of acknowledgement of valid application Not available at the time of submission

2. Applicant

a) Name of applicant Syngenta Seeds S.A.S on behalf of Syngenta Crop Protection AG, Basel
b) Address of applicant Syngenta Seeds S.A.S. 12, chemin de l'Hobit BP 27 F-31790 Saint-Sauveur On behalf of Syngenta Crop Protection AG, Basel Switzerland and all affiliated companies Schwarzwaldallee 215 CH 4058 Basle Switzerland
c) Name and address of the person established in the Community who is responsible for the placing on the market, whether it be the manufacturer, the importer or the distributor, if different form the applicant (Commission Decision 2004/204/EC Art 3(a)(ii)) Event 3272 maize derived products will be imported and used as any other maize in the EU by operators currently involved in these processes.

3. Scope of the application

☒ GM plants for food use

☒ Food containing or consisting of GM plants

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

☒GM plants for feed use

☒ Feed containing or consisting of GM plants

☒ Feed produced from GM plants

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

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

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

Yes ☐	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 Event 3272 will not be grown in the EU. Risk analysis data have been gathered by field trials outside of the EU.	

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

Yes ☐	No ☒
If *yes*, specify	

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

Yes ☒	No ☐
If *yes*, specify USA , China	

8. General description of the product

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

Event 3272 is a genetically modified (GM) maize developed to express a thermostable alpha-amylase, AMY797E. Alpha-amylases are key supplemental components in the production of ethanol derived from maize. They catalyse hydrolysis of starch into smaller and less complex carbohydrate molecules during the starch liquefaction step of the dry-grind ethanol process. Event 3272 also expresses a phosphomannose isomerase (PMI), acting as a selectable marker during the selection, that allows the plants to utilise mannose as a carbon source.

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

As explained in the next section (A.8.c), Event 3272 grain will be used for a specific processing application. However, as entry in the grain commodity cannot be excluded and import in the EU of by-products of the dry-grind ethanol processing will occur, the scope of the application includes all feed and food products containing, consisting or produced from the genetically modified Event 3272 maize including products from inbreds and hybrids obtained by conventional breeding of Event 3272. The application also covers the import and industrial processing of Event 3272 for all potential uses, as any other maize.

c) Intended use of the product and types of users

Event 3272 maize is intended to be cultivated outside the EU. The grain will be locally used in the dry-grind fuel ethanol process. The grain is not intended either to be used in other processing applications (e.g. wet milling and dry milling processes) or to be exported as a commodity crop. However, it cannot be excluded that extremely low levels of the grain originally intended to be used in the dry-grind fuel ethanol industry could finally enter international trade routes.
By-products of the dry-grind ethanol process produced from maize are used as feed and are exported to the EU (e.g. Distillers Dried Grains and Solubles). By-products of the dry-grind ethanol process produced from Event 3272 will be commingled with by-products from conventional maize and could therefore enter the trade routes.

Derived products from Event 3272 maize can be used in a manner similar to those products derived from conventional maize.

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 3272 maize and products derived from it are not different from those of its conventional counterpart. Event 3272 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 3272 maize.
e) Any proposed packaging requirements The characteristics of Event 3272 maize and products derived from it are not different from those of its conventional counterpart. Event 3272 has been shown to be as safe and as wholesome as existing varieties of maize. Therefore there are no specific instructions for packaging.
f) A proposal for labelling in accordance with Articles 13 and Articles 25 of Regulation ((EC) 1829/2003. In the case of GMOs, food and/or feed containing or consisting of GMOs, a proposal for labelling has to be included complying with the requirements of Article 4, B(6) of Regulation (EC) 1830/2003 and Annex IV of Directive 2001/18/EC A proposal for labelling has been included in the application following the guidance provided by EFSA. This includes the labelling requirements outlined by Regulation EC 1829/2003 and Annex IV of Directive 2001/18/EC. Event 3272 maize grain will therefore be labelled as "genetically modified maize" and products derived from it will be labelled as "containing (or produced from) genetically modified maize". Since Event 3272 maize and products derived from it are not different from those of its conventional counterpart, no additional labelling is required.
g) Unique identifier for the GM plant (Regulation (EC) 65/2004; does not apply to applications concerning only food and feed produced from GM plants, or containing ingredients produced from GM plants). SYN-E3272-5
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 Not applicable. Event 3272 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

The characteristics of Event 3272 maize and products derived from it are not different from those of its conventional counterpart. Event 3272 has been shown to be as safe and as wholesome as conventional varieties of maize. Any unintended releases or misuse can be managed 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 (formely Graminaea)
b) Genus *Zea*
c) Species *mays*
d) Subspecies *mays*
e) Cultivar/breeding line or strain A Syngenta proprietary line of maize
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-pollination and depends mainly on wind borne cross-fertilisation. *Z. mays* is a plant with protandrous inflorescence; however, decades of conventional selection and improvement have produced varieties of maize with protogynous traits. *Z. mays* has staminate flowers in the tassels and pistillate flowers on the ear shoots. There is no asexually reproductive maize.
(ii) Specific factors affecting reproduction The key critical stages of maize reproduction are tasselling, silking, pollination and fertilization. Pollen dispersal is limited by several factors, including large size (0.1 mm diameter), rapid settling rate and short survivability. Most maize varieties are protoandrous so pollen shedding precedes silk emergence by up to five days. More than 98% of the pollen settles to the ground within a maximum distance of 25-50 meters of its source. 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 range from as short as 60 to 70 days to as long as 43 to 48 weeks from seedling emergence to maturity.

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

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

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

3. Survivability

a) Ability to form structures for survival or dormancy

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

b) Specific factors affecting survivability

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

4. Dissemination

a) Ways and extent of dissemination

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

b) Specific factors affecting dissemination

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

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

Maize, which has very diverse morphological and physiological traits, is grown on approximately 147 million hectares worldwide. It is distributed over a wide range of conditions: from 56º N Lat to 40º S Lat, 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

Transformation of Syngenta's Event 3272 maize, was conducted using immature maize embryos derived from a proprietary *Zea mays* line, *via Agrobacterium*-mediated transformation.

2. Nature and source of the vector used

Plasmid pNOV7013, a vector used for *Agrobacterium* mediated plant transformation, was used to generate Event 3272.

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

The intended insert contains the *amy797E* and *pmi* genes, including the necessary regulatory components to drive their expression in maize.

The source, size and function of each constituent are listed below.

Component	Size	Function and origin of the sequence
GZein promoter	677 bp	Promoter region from the *Zea mays* 27-kDa storage protein (*zein*) gene. Provides endosperm-specific expression in *Zea mays*.
amy797E	1383 bp	The gene includes the fusion of the 797GL3 alpha-amylase gene, derived from alpha-amylase genes from microorganisms of the archael order *Thermococcales* with the sequences encoding the maize gamma-zein signal sequence and the endoplasmic reticulum (ER) retention signal. The alpha-amylase enzyme catalyses the hydrolysis of starch by cleaving the internal α-1,4-glucosidic bonds into dextrins, maltose and glucose. The maize gamma-zein signal sequence and the ER retention signal provide signals for protein targeting to and retention in the endoplasmic reticulum of the cell, respectively.
PEPC9	108 bp	Intron #9 from the phosphoenolpyruvate carboxylase gene from *Zea mays*.
35S terminator	70 bp	Terminator sequence from the 35S RNA from the cauliflower mosaic virus genome.

ZmUbiIntron	1993 bp	Promoter region from *Zea mays* polyubiquitin gene, contains the first intron. Provides constitutive expression in monocots.
pmi	1176 bp	*E.coli manA* gene encoding phosphomannose isomerase. Catalyzes the isomerization of mannose-6-phosphate to fructose-6-phosphate. Serves as a plant selectable marker.
NOS	253 bp	Terminator sequence from the nopaline synthase gene of *Agrobacterium tumefaciens*.

D. INFORMATION RELATING TO THE GM PLANT

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

Event 3272 is a genetically modified (GM) maize that expresses a synthetic *amy797E* gene encoding the thermostable AMY797E alpha-amylase protein. Alpha-amylases are key additional components in the production of ethanol derived from maize. These enzymes catalyse hydrolysis of starch into smaller and less complex carbohydrate molecules during the starch liquefaction step of the dry-grind ethanol process. The expression of AMY797E is specifically targeted to the grain endosperm and the genetic modification has been engineered to retain the protein in the endoplasmic reticulum of the cells. Maize grain from Event 3272 expressing the AMY797E alpha-amylase enzyme will serve as the source of amylase enzyme in the dry-grind ethanol process, replacing the external addition of microbially produced enzyme. Event 3272 also expresses the PMI protein that allows transformed maize cells to utilize mannose as a sole carbon source and acts as a selectable marker.

2. Information on the sequences actually inserted or deleted

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

Data from Southern analysis and DNA sequencing demonstrated that single copies of the alpha-amylase (*amy797E*) gene, the phosphomannose isomerase (*pmi*) gene, the maize gamma-zein (GZein) promoter and the maize ubiquitin (ZmUbiInt) promoter derived from the transformation plasmid pNOV7013 are present in Event 3272 maize. Event 3272 does not contain any of the backbone sequences from the transformation plasmid pNOV7013.

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

Not applicable

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

The inheritance pattern of the T-DNA insert derived from pNOV7013 in Event 3272 maize was investigated; statistical analysis confirms the expected Mendelian inheritance ratio for *amy797E* gene. The results show that insertion had taken place in the nucleus.

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

The entire T-DNA insert and the 5’ and 3’ flanking regions of Event 3272 have been sequenced. The sequence data demonstrated the overall integrity of the insert and that contiguousness of the functional elements within the insert as intended in pNOV7013 have been maintained.

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 AMY797E and PMI proteins in maize plants derived from Event 3272, the concentrations of these proteins were determined by ELISA at five growth stages (whorl, anthesis, kernel dough, kernel maturity and senescence) in several plant tissues (leaves, roots, kernels, pollen and whole plants).

Expression of the AMY797E alpha-amylase protein in Event 3272 plants is driven by the maize gamma-zein promoter for endosperm-specific expression in the kernel. As expected, relatively high levels of AMY797E were measured in kernels across all plant stages in which kernels are present; mean AMY797E levels ranged from *ca*. 838 - 1627 µg/g fresh weight. Low levels of AMY797E, ranging from *ca*. <0.02 µg/g fw - *ca*. 3 µg/g fw, were detected in only some samples of whole-plant and roots at early stages of development and one sample of leaf at senescence stage. The protein was not detected in pollen.

PMI protein expression in Event 3272 plants is driven by the constitutive maize polyubiquitin promoter. PMI protein was detected in most of the Event 3272-derived plant tissues analysed across all plant stages; mean PMI levels measured ranged from not detectable to a maximum of ca 8.5µg /g fresh weight.

b) Parts of the plant where the insert is expressed

To characterize the range of expression of alpha-amylase (AMY797E) and phosphomannose isomerase (PMI) proteins in maize plants derived from Event 3272, the concentrations of these proteins were determined by ELISA in several plant tissues (leaves, roots, kernels, pollen and whole plants). This has been addressed in section D.3.(a) above.

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

a) Reproduction

No changes in the reproduction compared to conventional maize have been observed in agronomic assessments conducted with Event 3272.

b) Dissemination

No changes in the dissemination compared to conventional maize have been observed in agronomic assessments conducted with Event 3272.

c) Survivability

No changes in the survivability compared to conventional maize have been observed in agronomic assessments conducted with Event 3272.

d) Other differences

No changes in the reproduction, dissemination or survivability compared to conventional maize have been observed in agronomic assessments conducted with Event 3272.

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

Genetic stability:

Southern analysis was conducted to confirm the presence of a single copy of the *amy797E* gene over several generations. The hybridization data demonstrated that the T-DNA insert from pNOV7013 incorporated into Event 3272 is stable over several generations.

Phenotypic stability:

The stability of AMY797E and PMI protein expression over multiple generations was evaluated. Seed from four backcross generations was grown under field conditions. Grain was harvested at grain maturity for AMY797E analysis and leaf material was collected at anthesis for PMI analysis. Mean AMY797E concentrations measured across all backcross generations were *ca.* 1044 - 1264 µg/g fresh weight (1147 - 1389 µg/g dry weight). Mean PMI concentrations measured across all backcross generations were *ca.* 6.6 - 9.3 µg/g fresh weight (25.6 - 35.7 µg/g dry weight). Overall, concentrations were similar across the four generations analysed. There was no evidence of any significant trend either up or down, indicating that the expression of AMY797E protein and of the PMI protein is stable.

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

a) Plant to bacteria gene transfer

The horizontal gene transfer from GM plants to bacteria with subsequent expression of the transgene is regarded as a highly unlikely event under natural conditions, especially in the absence of selective pressure as discussed in details by EFSA (2004)[1]. The inserted DNA has been evaluated for possible enhancement of gene transfer potential. Southern analysis showed that the origins of replication of the pNOV7013 plasmid have not been transferred to Event 3272 and can therefore be excluded from the analysis. The *pmi* gene is from bacterial origin but adapted for optimal expression in plants. The *amy797E* gene is derived from alpha-amylase genes from microorganisms of the archael order *Thermococcales* and was optimised

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

for maize expression. No changes in *pmi* and *amy797E* genes were introduced to enhance recombination or gene transfer. The *nos* terminator, derived from *Agrobacterium tumefaciens*, 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 plants to bacteria.
Therefore no change in the ability of the Event 3272 to transfer genetic material to other organism is perceived compared to conventional maize.

b) Plant to plant gene transfer

The scope of this application does not include authorization for the cultivation of Event 3272 maize in the EU.

The genetic modification was not intended to change any of the typical crop characteristics of maize. Observations from field trials have confirmed that the agronomic characteristics of Event 3272 have not changed in comparison with controls, and therefore, that there is no increase or decrease in the potential for plant-to-plant gene transfer.

In the unlikely event that small amounts of grain of maize Event 3272 accidentally found their way into the 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. Gene transfer from Event 3272 maize to other sexually compatible plant species is not possible since maize has no wild relatives in the EU. Dissemination of pollen to other cultivated maize plants is not likely to occur since these plants are not intended for cultivation in the EU. In the highly unlikely event that cross-pollination did occur this would not lead to establishment of the transgene in the maize genetic pool as commercially grown hybrids are used only for grain production and volunteers can easily be controlled using standard agronomic practices.

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

7.1 Comparative assessment

Choice of the comparator

Event 3272 was compared with relevant control maize lines that had not been genetically modified. Commercial varieties were included in the comparison whenever possible.

7.2 Production of material for comparative assessment

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

To confirm that Event 3272-derived maize plants are substantially equivalent to the non-transgenic isolines, replicate trials of transgenic and corresponding isogenic controls were planted for grain analysis and for forage analysis in 12 and 13 locations respectively over two growing seasons. 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 3272-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 material and compounds for analysis

Based on guidance of the OECD, grain from transgenic Event 3272-derived maize plants and isogenic non-transgenic control plants were analysed for proximates (including starch), minerals, amino acids and selected fatty acids, vitamins, anti-nutrients and secondary metabolites. Forage (whole plants) from transgenic Event 3272-derived maize plants and isogenic non transgenic control plants were analysed for proximates and minerals.

No consistent pattern has emerged to suggest that biologically significant changes in composition or nutritive value of the grain or forage had occurred as an unintended result of the transformation process or expression of the transgenes.

7.4 Agronomic traits

Event 3272-derived hybrids were grown in 25 locations in 7 states during 2003 and 2004. Up to 26 separate agronomic parameters were assessed. The outcome of these studies indicates that the agronomic performance of Event 3272-derived hybrids is similar, and for most parameters, equivalent to their non-transgenic control counterparts. Although certain genotype and variate combinations showed indications of genotype effects, there was no indication of a consistent effect on any observed agronomic characteristic across the hybrids tested.

7.5 Product specification

Maize as a product has a history of safe use for human food and animal feed. No significant native toxins are reported to be associated with the genus *Zea*. The information presented in this application confirms that Event 3272 maize and products derived from it are not different from those of its conventional counterpart.

7.6 Effect of processing

Event 3272 maize expressing a thermostable alpha-amylase enzyme (AMY797E) in grain has been developed for use in the dry-grind fuel ethanol process. During this process, the starch contained in the maize grain is hydrolysed into glucose which is then used to produce ethanol by fermentation. Maize grain from Event 3272 expressing the AMY797E alpha-amylase enzyme will serve as the source of amylase enzyme in the dry-grind ethanol process, replacing the external addition of microbially produced enzyme. Event 3272 maize grain will be mixed with conventional maize at the processing plant and will be processed in the same way as any non-GM maize. There is no evidence that the expression of the AMY797E alpha-amylase and PMI proteins modifies in any way the characteristics of the end products compared to the use of conventional maize with added microbially produced alpha-amylase.

7.7 Anticipated intake/extent of use

It is expected that the introduction of Event 3272 will replace some of the maize in existing feed products (Distillers Dried Grains and Solubles). Event 3272 is expected neither to be used for processing applications other than for the production of ethanol, nor to enter commodity trade. Although we cannot exclude that Event 3272 grain will finally enter international trade routes, the presence of Event 3272 maize in the feed chain (other feed products than DDGS) and in the food chain would be at extremely low level.

The genetic modification was not intended to change any of the compositional parameters in food and feed (as shown in Section 7.3), therefore no nutritional changes are expected from this presence. No impact on the extent of use of maize can be expected.

7.8 Toxicology

7.8.1 Safety assessment of newly expressed proteins

Maize derived from Event 3272 expresses two new proteins: the AMY797E and the PMI proteins. Alpha-amylases and phosphomannose isomerase proteins have a history of safe use. To demonstrate the safety of the AMY797E and PMI proteins expressed in Event 3272, a series of studies were conducted. The data obtained showed that both newly expressed proteins are not structurally and functionally related to proteins which have the potential to adversely affect human or animal health. They are rapidly degraded in simulated gastric fluid and neither of them showed any significant sequence homology with known toxins. In addition, acute oral toxicity studies of AMY797E and PMI in mice confirmed that these proteins are not acutely toxic to mice at the highest dose tested.

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

Maize is a common source of food and feed and has a long history of safe use. Event 3272 has been modified to express two proteins: the AMY797E and the PMI proteins. No other new constituents apart from these two proteins are expected to be produced in Event 3272 and compositional analyses have confirmed the substantial equivalence of Event 3272 compared to conventional maize.

7.8.3 Information on natural food and feed constituents

The presence and levels of natural food and feed constituents such as macro- and micronutrients, secondary plant metabolites as well as natural toxins and antinutritional factors have been analysed in Event 3272 maize and compared with non-genetically modified 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 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 the transformation process or expression of the transgenes.

7.8.4 Testing of the whole GM food/feed

In addition to the compositional analysis, the wholesomeness and safety of maize Event 3272 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 the effect of Event 3272 on rats. The incorporation of Event 3272 transgenic maize grain in diets fed to rats for 90 consecutive days produced no deleterious effects that were considered to be attributable to the inclusion of Event 3272 transgenic maize grain in the diet.

A 49-day poultry feeding study was also conducted to evaluate whether standard poultry diets prepared with Event 3272 grain had any adverse effect on male or female broiler chickens as compared to diets prepared with non-transgenic isoline control grain and a commercial source of maize. The study showed that the transgenic maize 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 3272 expresses two proteins: the AMY797E and the PMI proteins. Alpha-amylases and phosphomannose isomerase proteins are ubiquitous in nature and will naturally be present in foods derived from plant and microbial sources. To assess the potential for allergenicity of the AMY797E and PMI proteins a series of studies were conducted. The data obtained showed that:

- these proteins are not derived from known allergenic sources
- these proteins have no significant amino acid homology to known or putative allergenic protein sequences that are biologically relevant or have implications for the allergenic potential.
- these proteins are rapidly degraded in simulated gastric fluid
- the AMY797E alpha-amylase protein was selected for its increased thermostability and activity during the high temperatures required for starch hydrolysis in the dry-grind ethanol processing from maize. Microbially produced thermostable alpha-amylases are commonly used in the dry-grind ethanol process. The PMI protein is inactivated at high temperature.

From these data, it can be concluded that the AMY797E and PMI proteins expressed in Event 3272 maize plants are very 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 an allergenic food crop and maize Event 3272 expresses two new proteins that are very unlikely to be allergenic.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

Presence of Event 3272 in food products cannot be excluded but will be at extremely low levels. Genetically modified Event 3272 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.

7.10.2 Nutritional assessment of GM feed

Genetically modified Event 3272 is not intended to change the nutritional status of livestock animals. Compositional analysis and whole food safety tests have demonstrated that no unexpected alterations in nutrients and other food components have occurred and that no nutritional imbalances were introduced.

7.11 Post-market monitoring of GM food/feed

As described in sections 7.1 to 7.10 above, the presence of Event 3272 in food and feed will not result in any nutritional changes, therefore post-market monitoring is not considered appropriate.

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

Event 3272 maize plants have been modified to contain the *amy797E* gene that expresses an alpha-amylase, along with a marker gene, *pmi*, that expresses PMI, an enzyme that allows the plant cells to use mannose as a carbon source. Neither of these newly expressed proteins have toxic modes of action, therefore discussions about the interactions between Event 3272 and target organisms are not applicable.

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

9.1 Persistence and invasiveness

Cultivation of maize derived from Event 3272 in the EU is not within the scope of this application. In the unlikely event that small amounts of maize kernels of Event 3272 could accidentally find their way into the environment their survival would be very unlikely as maize is highly domesticated and cannot survive without human intervention, especially under normal European climatic conditions. The expression of the AMY797E and PMI proteins does not affect the agronomic characteristics or weediness potential of Event 3272, as demonstrated in field trials conducted to evaluate the agronomic performance of this event in comparison with the isogenic control. In the unlikely event that these maize plants were to survive they could be easily controlled using any of the current agronomic measures taken to control other commercially available maize.

Conventional maize is not an invasive or persistent crop in the EU, and Event 3272 maize is no different from conventional maize in agronomic performance. 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 importing maize kernels of this event into the EU can be considered negligible.

9.2 Selective advantage or disadvantage

Event 3272 plants have been modified to contain the *amy797E* gene that expresses an alpha-amylase (AMY797E), an enzyme that allows the hydrolysis of starch into smaller and less complex carbohydrate molecules specifically during the dry-grind ethanol process, along with a marker gene, *pmi*, that expresses PMI, an enzyme that allows plant cells to use mannose as a carbon source.

Both transgenic enzymes do not play a functional role in the plant metabolism. The genetic modification has been engineered to retain the AMY797E protein in the endoplasmic

reticulum where no starch is present. It is highly unlikely that the expression of alpha-amylase AMY797E will result in selective advantage or disadvantage of Event 3272 plants compared with conventional maize. Extensive field trials conducted by Syngenta, wherein the agronomic performance of Event 3272 and isogenic control maize was compared, demonstrated that there were no consistent differences in any agronomic characteristics between Event 3272 and control maize.
PMI is a selectable marker. Its expression could confer an advantage to Event 3272 plant cells growing under conditions where mannose is the only source of carbon. However, these conditions are limited to *in vitro* uses and do not normally occur in soils. Therefore, expression of PMI cannot be considered a factor that would confer selective advantage to Event 3272 maize plants.

Cultivation of Event 3272 in the EU is not within the scope of this application. In the unlikely event that small amounts of grain from Event 3272 maize could accidentally find their way into the environment in the EU the survival of this grain would be very unlikely for the reasons stated above. In addition, any plants germinating from this grain could be easily controlled using any of the current agronomic measures taken to control other commercially available maize.

In summary, the likelihood that the expression of AMY797E and PMI will result in a selective advantage or disadvantage to Event 3272 compared with conventional maize, under the scope of this application, can be considered negligible.

9.3 Potential for gene transfer

Gene transfer from Event 3272 maize to other sexually compatible plant species is not possible since there are no maize wild relatives in the EU.

Gene transfer from Event 3272 maize to other maize could occur through pollen dispersal during the cultivation of the crop. Maize is predominantly wind pollinated. Plants produce pollen for 10-13 days and shed pollen typically remains viable only a short time. 98% of pollen settles to the ground within 25-50 meters of its source. Cultivation of Event 3272 maize is not within the scope of this application. It is therefore highly unlikely that this maize could grow in significant quantity in the EU. In the unlikely event that small amounts of grain from Event 3272 maize accidentally find their way into the environment in the EU, this would represent extremely low levels of exposure, and the survival of the grain would be very unlikely for the reasons stated above. In addition, plants resulting from spilled grain could be easily controlled using any of the current agronomic measures taken to control other commercially-available maize. Therefore Event 3272 maize is extremely unlikely to flower and fertilise other varieties of maize in Europe.

In summary, the potential for gene transfer between Event 3272 maize plants and other maize plants or sexually compatible wild relatives in the EU can be considered negligible under the scope of this application.

9.4 Interactions between the GM plant and target organisms

The newly expressed proteins in maize Event 3272 are not intended to interact with other organisms.

9.5 Interactions of the GM plant with non-target organisms

The scope of this application does not include cultivation of Event 3272 maize in the EU, therefore exposure to non-target organisms in the environment is unlikely. Both phosphomannose isomerases and alpha-amylases are well described in literature and no harmful effects on non-target organisms have been described to date. This suggests that even if small amounts of grain from Event 3272 maize could accidentally find their way into the environment in the EU this would represent extremely low risk to non-target organisms.

In summary, immediate or delayed effects in the environment due to direct or indirect interactions between Event 3272 maize plants and non-target organisms can be considered highly unlikely under the scope of this application.

9.6 Effects on human health

Presence of Event 3272 in food products cannot be excluded but will be at extremely low levels. An assessment was conducted to evaluate the possible adverse effects on human health resulting from exposure to Event 3272.

The recipient organism, maize, has a history of safe use throughout the world. Event 3272 expresses an alpha–amyalse AMY797E for use in starch hydrolysis during ethanol production and a PMI protein that allows the plants to utilise mannose as a carbon source. Alpha-amylases and PMI proteins are ubiquitous in nature and will naturally be present in foods derived from plant and microbial sources. Both proteins, AMY797E and PMI show no significant sequence homology with known toxins. In addition, the acute oral toxicity studies conducted with AMY797E and PMI in mice, confirmed that these proteins are not acutely toxic to mice at the highest dose tested. Both proteins are rapidly degraded in simulated gastric fluids. They have no significant amino acid homology to known or putative allergenic protein sequences that are biologically relevant or have implications for the allergenic potential. The allergenicity assessment conducted shows that these proteins are not likely to be allergenic.

The inserted DNA has been sequenced. An open reading frame analysis could find no evidence for sequences of any other proteins.

Studies comparing the composition of Event 3272 maize plants and non-modified maize lead to the conclusion that this maize is substantially equivalent to conventional maize.

The wholesomeness and safety of Event 3272 maize 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-GM controls were detected.

In summary, it can be concluded that grain from Event 3272 maize is safe for food consumption and no differences in wholesomeness are expected with comparable non-GM maize varieties.

9.7 Effects on animal health

An assessment was conducted to evaluate the possible adverse effects on animal health resulting from exposure to Event 3272. The assessment was based on an extensive characterisation of the expressed proteins and of the genetically modified plant (see Section D.9.6 above).

In summary, it can be concluded that grain from Event 3272 maize is safe for feed consumption and no differences in wholesomeness are expected with comparable non-GM maize varieties.

9.8 Effects on biogeochemical processes

Since the scope of this application does not include cultivation of Event 3272 maize, effects on biogeochemical processes are highly unlikely. In the unlikely event that small amounts of maize kernels of Event 3272 could accidentally find their way into the environment their survival would be very unlikely as maize is highly domesticated and cannot survive without human intervention, especially under normal European climatic conditions. Moreover, if these plants were to survive they could be easily controlled using any of the current agronomic measures taken to control other commercially available maize.

In summary, effects on biogeochemical processes resulting from the use of Event 3272 under the scope of this application are highly unlikely.

9.9 Impacts of the specific cultivation, management and harvesting techniques

The scope of this application does not include cultivation of maize plants of Event 3272 in the EU.

10. Potential interactions with the abiotic environment

The scope of this application does not include cultivation of Event 3272 maize in the EU, therefore interactions of Event 3272 maize with the abiotic environment are highly unlikely. In the event of unintentional release, these plants will not have different interactions with the abiotic environment compared to non-GM plants.

In summary, environmental impacts as a result of interactions between Event 3272 and the abiotic environment can be considered negligible within the scope of this application.

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

11.1 General (risk assessment, background information)

The scope of this application does not include cultivation of Event 3272 maize in the EU. Environmental exposure to Event 3272 could only occur in the unlikely event that small amounts of grain of Event 3272 maize accidentally found their way into the environment in the EU. However, the survival of this grain would be very unlikely as maize is a highly domesticated plant and cannot survive without human intervention, especially under normal European climatic conditions. This grain, if germinated could be easily controlled using any of the current agronomic measures taken to control other commercially available maize.

An environmental risk assessment (e.r.a.) has been conducted as recommended by the

Guidance document of the Scientific Panel of Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. According to this guidance the e.r.a. should follow the principles outlined in Directive 2001/18/EC (Annex II). The risk assessment is described as *"a process of evaluation, including the identification of the attendant uncertainties, of the likelihood and severity of an adverse effect(s)/event(s) occurring to man or the environment following exposure under defined conditions to a risk source(s)"*. This risk assessment has been conducted taking into account that cultivation of Event 3272 is not included in the scope of the application. A sequential approach to the e.r.a., as recommended by Commission Decision 2002/623/EC has been followed, so the characteristics of Event 3272 that may cause an adverse effect have been identified, their potential consequences evaluated, the likelihood of their occurrence assessed and the overall risk posed by each characteristic identified. Comparison of Event 3272 maize with conventional maize has been used as a baseline.

The conclusions of the e.r.a. confirm that the effects to the environment arising from the use of Event 3272 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 in accordance with Annex II of Directive 2001/18/EC to evaluate potential adverse effects of Event 3272 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 3272 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)

The main objective of case-specific monitoring is to determine the significance of any adverse effects identified in the e.r.a. The e.r.a. conducted for Event 3272 maize use under the scope of this application, confirmed that the potential for adverse effects on human and animal health or the environment can be considered negligible. Consequently, in this case, case-specific monitoring is not considered necessary.

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

The objective of general surveillance is to identify unforeseen adverse effects of the GM plant or its use, on human health and the environment, which were not predicted in the risk assessment.

The scope of this application is limited to import of Event 3272 and excludes cultivation

practices.

The provisions concerning traceability and labelling for placing on the market of Event 3272 maize will allow the prompt identification of products containing or consisting of this maize, and thus enable any unanticipated adverse effects to be effectively traced.

The majority of imported Event 3272 maize 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 3272.

Syngenta is committed to informing traders, processors and the European feed and food industry with details on the safety of Event 3272 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, it is appropriate to note that 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 3272 has been developed. The proposed method is a real-time quantitative TaqMan® PCR which specifically detects Event 3272 genomic DNA.

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

a) Notification number No release of Event 3272 under Part B of Directive 2001/18/EC and under Directive 90/220/EEC was performed.
b) Conclusions of post-release monitoring Not applicable
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) Not applicable

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

a) Release country Japan
b) Authority overseeing the release Ministry of Agriculture, Forestry and Fisheries of Japan Ministry of the Environment
c) Release site National Institute of Livestock and Grassland Science (Nasu), Nasu, Tochigi, JAPAN
d) Aim of the release Assessment of Adverse Effect on Biological Diversity
e) Duration of the release Six months (from June to November 2005)
f) Aim of post-releases monitoring Control of volunteer plant carrying the event to avoid any accidental gene release
g) Duration of post-releases monitoring A half year after harvest
h) Conclusions of post-release monitoring Ongoing
i) Results of the release in respect to any risk to human health and the environment No evidence for adverse effects on human health or the environment was found

a) Release country

US

b) Authority overseeing the release

Event 3272 hybrids have been planted in several states under USDA-APHIS comprehensive permit and notification since 2002.

c) Release site

Year	USDA Notification or Permit No.	Trial Sites by State
2002	02-022-02 r/m	HI
2003	03-021-01 r/m	FL,IA,IL,MN,PR,SD,WI
	03-021-02 r/m	HI
2004[1]	04-051-08n	IA
	04-064-04n	FL,HI,IA,ID,IL,IN,KY,MN,NE,PA,PR,SD,WI
	04-082-03n	IA
	04-126-03n	NE
	04-203-03n	PR
	04-216-02n	HI
2005[1]	05-042-09n	HI, NE
	05-049-10n	CO,FL,HI,IA,ID,IL,IN,KY,MN,MO,NE,PR,SD,WI
	05-102-02n	IL
	05-104-08n	HI

1.Trial sites may be approved but not all trial sites planted

d) Aim of the release

Agronomy and compositional analysis trials, seed and grain production

e) Duration of the release

Various depending on location.

f) Aim of post-releases monitoring

Control of volunteers.

g) Duration of post-releases monitoring

Various, typically one year

h) Conclusions of post-release monitoring

No differences between Event 3272 and the isogenic controls in occurrence of volunteers, persistence or weediness were observed.

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

No evidence for adverse effects on human health or the environment was found.

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

a) Status/process of approval The status and process of approval can be found on the EFSA website: http://www.efsa.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 3272 under the Directive 2001/18/EC has not been made.
c) EFSA opinion EFSA opinions, once available can be found at http://www.efsa.eu.int/science/gmo/gmo_opinions/catindex_en.html
d) Commission Register (Commission Decision 2004/204/EC[2]) 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[3]) 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 3272 under the Directive 2001/18/EC has not been made, however a link to this Summary under Regulation (EC) No 1829/2003, should be found at: http://www.efsa.eu.int/science/gmo/gm_ff_applications/more_info/catindex_en.html