PART II

SUMMARY

T25

GENETICALLY MODIFIED MAIZE WITH INCREASED GLUFOSINATE-AMMONIUM-TOLERANCE

- APPLICATION IN SUPPORT OF A REQUEST FOR
- AUTHORIZATION IN ACCORDANCE WITH ARTICLE 5 AND

- FOR RENEWAL OF AUTHORIZATIONS OF EXISTING PRODUCTS UNDER ARTICLES 8(1)(A) AND 20 (1) (A), (B) IN ACCORDANCE WITH ARTICLES 11AND 23

OF REGULATION (EC) NO 1829/2003

FOR FOOD AND FEED USES, AND FOR ENVIRONMENTAL RELEASE INCLUDING IMPORT AND PROCESSING, AND SEEDS FOR CULTIVATION IN EUROPE AS WELL AS FOR INDUSTRIAL USES

A. GENERAL INFORMATION

1. Details of application

a) Member State of application: The Netherlands
b) Application number: Not available at the date of application
c) Name of the product (commercial and other names): The product is genetically modified maize (*Zea mays*) with tolerance to the herbicide glufosinate ammonium, derived by traditional breeding methods from crosses between GM maize transformation event T25 (OECD code ACS-ZMØØ3-2) and non-GM maize cultivars. The product includes maize seed products for cultivation, for all food and feed uses, as well as for all food, feed and processed products derived from T25 maize. Glufosinate-ammonium tolerant maize plants are also referred to as LibertyLink® maize or LLmaize.
d) Date of acknowledgement of valid application: Not available at the date of application

2. Applicant

a) Name of applicant: Bayer CropScience AG
b) Address of applicant: Bayer CropScience AG Alfred-Nobel-Str.50 D-40789 Monheim am Rhein Germany E-mail address: info@bayercropscience.com
c) Name and address of the person established in the Community who is responsible for the placing on the market, whether it be the manufacturer, the importer or the distributor, if different from the applicant (Commission Decision 2004/204/EC Art 3(a)(ii)): T25 maize will be cultivated, imported and used in the European Union by the same growers and operators currently involved in the planting, trade and use of commercially available maize varieties.

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 T25 maize has been notified for field testing in the EU since 1994, under AgrEvo, Aventis CropScience, or Bayer CropScience notifications.	

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:

T25 maize has been authorized for all uses with the exception of food under Part C of Directive 90/220/EEC, Commission Decision 98/293/EC,

Derived products of T25 maize have also been authorized under Regulation (EC) No 258/97

Furthermore T25 has been notified under articles 8 and 20 of Regulation (EC) No 1829/2003 as an existing product.

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

Yes ☒	No ☐

If *yes*, specify:

Country	Food	Feed	Granted	Agency Name[1]
United States	X	X	1995	FDA[2], USDA[3]
South Korea	X		2003	KFDA
South Africa	X	X	2001	SA-DA
Argentina	X	X	1998	SENASA
Japan	X	X	1997	MHLW, MAFF
Aust./New Zea.	X	X	2002	FSANZ[4]
China	X	X	2004	MOA
Canada	X	X	1997	CFIA[5], CaH[6]
The Philippines	X	X	2003	PhDA
Russia	X	X	2001	MHCSP
Taiwan	X	X	2002	DOH

Abbreviations: FDA: Food and Drug Administration; **USDA:** United States Department of Agriculture; **KFDA:** Korea Food and Drug Administration; **SA-DA** -South Africa, Department of Agriculture, **SENASA:** Servicio Nacional de Sanidad Agraria; **MHLW:** Ministry of Health, Labour and Welfare: **MAFF:** Ministry of Agriculture, Forestry and Fisheries; **FSANZ**: Food Standards Australia New Zealand; **MOA:** Ministry of Agriculture; **CFIA:** Canadian Food Inspection Agency; **CaH-** Canada Health, **PhDA** - Philippines Department of Agriculture, **MHCSP-** Ministry of Health Care and Social Progress, **DOH:** Department of Health

1 link to risk assessment performed by agency
2 http://www.aphis.usda.gov/brs/aphisdocs2/94_35701p_com.pdf
3 http://www.cfsan.fda.gov/~rdb/bnfm029.html
4 http://www.foodstandards.gov.au/_srcfiles/A375%20Final%20AR.pdf,
5 http://www.inspection.gc.ca/scripts/priimp/priimppage.php?lang=e&ser=www.inspection.gc.ca&ref=/english/plaveg/bio/dd/dd9822e.shtml
6 http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/gmf-agm/32bg_agrevo-ct_agrevo_e.pdf

8. General description of the product

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

The recipient plant belongs to the species, *Zea mays* L. The genetic modification confers tolerance to the herbicide glufosinate-ammonium through the insertion of the P35S-pat-T35S gene cassette into the T25 maize. LibertyLink® maize hybrids are developed by traditional breeding methods from crosses between T25 and non-GM maize adapted for planting in the major maize production regions of the World.

Herbicide tolerance is based upon the *pat* gene derived from the soil microorganism *Streptomyces viridochromogenes*. The *pat* gene encodes for the production of the enzyme, Phosphinothricin-Acetyl-Transferase (PAT). The specific enzymatic action of the PAT protein is tolerance to glufosinate ammonium herbicide.

Agricultural production of maize requires control of weeds. Successful weed control depends upon combinations of management practices. For temperate maize production, farmers can use the following measures: planting of weed-free seed, crop rotations to break weed cycles, precision land levelling to aid irrigation, seed bed preparation, conservation tillage programs and the application of one or more herbicides.

Growing LibertyLink maize T25 allows; 1) more options to rotate herbicides for weed resistance management programs, 2) control of less sensitive weeds, and 3) control of currently identified biotypes of herbicide resistant weeds. Thus more options for crop management, lesser impact on maize growing areas and potential implications for soil conservation through minimum tillage practices can be adopted.

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

T25 maize products include maize seed products for cultivation, all food and feed uses, and also for all food, feed, industrial and processed products derived from T25 maize.

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

T25 maize will be cultivated, traded and used in the European Union by the same growers and operators currently involved in the planting, trade and use of commercially available maize varieties.

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

No mandatory restrictions for use, storage and handling are proposed as a condition of the authorisation. All standard practices applicable to maize currently grown today are also applicable for the handling of glufosinate ammonium-tolerant, T25 varieties.

e) Any proposed packaging requirements:

T25 maize products are to be used in the same manner as other maize, therefore no specific packaging is required.

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: T25 maize does not have characteristics that require specific labelling. Hence, no additional labelling is proposed on top of the GM labelling requirements foreseen in regulations (EC) 1829/2003 and 1830/2003. Certified seeds of T25 maize will be commercialized for planting. Seed vendors shall be required to label seed bags containing T25 varieties with the words "genetically modified maize" or "contains genetically modified maize, as well as the unique identifier ACS-ZMØØ3-2. When genetically modified maize T25 is placed on the EU market (including co-mingled with non-genetically modified maize during use, storage and handling), the corresponding batch will be labelled and handled according to the legislation in application in the EU referred above Operators in the food-feed chain are fully aware of the traceability and labelling requirements for T25 maize, therefore no specific supplemental measures are to be taken by the applicant.
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): ACS-ZMØØ3-2.
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: No restrictions are necessary as T25 maize is suitable for food, feed and industrial uses in all regions of the European Union.

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

As the proposed use of T25 maize include all the uses of commercially available maize varieties its misuse is very unlikely.

Based on the conclusions of the Environmental Risk Assessment, T25 maze is substantially equivalent to commercially available maize varieties with the exception of an additional PAT protein conferring to tolerance to glufosinate-ammonium. T25 maize plants and T25 derived food and feed products as safe and as nutritious as commercially available maize varieties.

The 10 years history of practical cultivation of T25 maize in the Americas proves its environmental as well as food and feed safety.

In case of unintended release or misuse of T25 maize, current practices to control unintended release or misuse of non-GM maize can be applied, such as the use of herbicide other than glufosinate-ammonium, or any mechanical treatments.

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

1. Complete name

a) Family name:	*Poaceae / (Gramineae)*
b) Genus:	*Zea*
c) Species:	*Z. mays*
d) Subspecies:	Not applicable.
e) Cultivar/breeding line or strain:	HE/89
f) Common name:	maize / corn

2 a. Information concerning reproduction

(i) Mode(s) of reproduction

Maize is an annual, wind-pollinated, monoecious species, with separate staminate (tassels) and pistillate (silks) flowers. Maize is essentially cross-pollinating.

The tassel sheds pollen for 2 to 14 days. The major shed starts approximately on the 3rd day after the tassel is expended and last 5 to 8 days. The silks are receptive at emergence and can remain receptive for more than 10 days.

(ii) Specific factors affecting reproduction

Factors influencing tasselling, silking and pollination processes are critical for maize development and will therefore effect the grain yield.

(iii) Generation time

The generation time is 6 to 7 months in the primary areas of cultivation.

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

Maize is sexual compatible with the annual plant species Teosinte, grown in regions of Mexico and Guatemala.
In Europe, maize is only known as a cultivated species. No wild relatives are exist, thus the out-crossing is limited to other maize plants.
All maize is interpollinate, except for certain specialty varieties and hybrids that have one of the gametophyte factors of the allelic series Ga and ga on chromosome 4.

3. Survivability

Maize is an annual crop which dies at the end of the vegetative season. The plant is susceptible to frost, and cannot survive in nature for more than one season. Maize cannot reproduce asexually in

nature.
The grain is the only structure, which survive the growing season. During the ripening phase, the water content of the grain reduces from more than 50 % to 30-32 %. After the final ripening stage, the whole plant dries out and dies. When separated from the cob, the kernels cannot survive in the soil during winter, because they quickly rot. When complete ears fall down onto soil, some grains can survive and germinate in the following spring as volunteers, but do not persist as weeds.

b) Specific factors affecting survivability

The main factors affecting survivability of maize are related to soil microclimate such as temperature and humidity. Other factors are the moisture of the seeds, genotype or the husk protection.

4. Dissemination

a) Ways and extent of dissemination

The two differentiated reproductive structures possible for dispersal of maize genes in the environment are the seed and the pollen.

- **Seed dispersal** could occur during transport, but it is naturally restricted by the enclosed ear structure of the cob.
- **Pollen dispersal** occurs primarily over a short distance due to the large size of the pollen and the related settling rate of maize pollen (90 to 125 µm in diameter and 30-40 cm s^{-1}).

b) Specific factors affecting dissemination

Seed dispersal: Mechanical intervention, insects or wind can all lead to the isolation of seeds from the husk and can lead to dispersal of the isolated seeds. But as described earlier seeds/kernels separated from the cob cannot survive in the soil during winter, because they quickly rot.

Pollen dispersal is largely affected by the amount of pollen produced, the relative concentration of the pollen in the donor and receptor plot, the lengths of pollen shed, the viability of the pollen, the viability of the receptive silks (synchronization of flowering) and the wind speed and direction.

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

Although maize was domesticated in the Meso-American regions, today it is cultivated mainly in warm temperate regions. Corn is grown between the latitudes 35° and 55°, mainly in the northern hemisphere. In 2005, approximately 282 million tonnes were produced in the US, 140 million in China, 65 million in the EU-25, Bulgaria, Romania and Switzerland, followed by 35 million in Brazil and 18 million in Mexico.

In Europe, France produced approximately one fifth of the total EU production, followed by Italy and Romania accounting for one sixth, each. Hungary and Spain produce 14% and 6.3%, respectively. Other EU countries produce less than 6.3%.

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

The typical maize growing areas in Europe are extended from the Danube Basin in Southern Germany to the Black Sea, and from Southern France through the Poe Valley in Italy, Specific varieties are also grown even in Scandinavia.

Maize has a history of safe use and cultivation in Europe.

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 interacts in the environment with insects, birds, mammals. Maize is susceptible to a range of diseases caused by fungi, nematodes, insects and mite pests.

In the Mexican growing regions some fungi are reported to be associated with specific maize genotypes. There is a corn race in the Mixe Sierra of Oaxaca, Mexico with overdeveloped brace roots covered by a mucilaginous material that harbors species of nitrogen fixing free bacteria.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Description of the methods used for the genetic modification

The plasmid DNA was introduced into maize genotype HE/89 by polyethylene-glycol mediated protoplasts transformation.

2. Nature and source of the vector used

Plasmid, pUC/Ac was used for the transformation. For the construction of pUC/Ac vector, the synthetic *pat* gene was cloned into the Sal1 site, between the CaMV derived 35S gene promoter and terminator sequences of the pUC derived plasmid pDH51. The chimeric *pat* gene cassette can be isolated as a 1.3 kb EcoR1 fragment. The construct contains no other plant expressible genes. The pUC sequences include a β-lactamase (*bla*) gene and a bacterial origin of replication. The *bla* gene has regulatory signals recognized in bacteria but not functional in plant cells. The origin of replication is not known to function in eukaryotes.

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

Using the PEG mediated protoplast transformation, any part of the genetic element of the transformation plasmid can be inserted with the same probability into the recipient genome. In Table 1 the size, source and function is given to all the elements of the pUC/Ac. Please note only the P35S-pat-T35S cassette was intended to be inserted.

Table 1. Size, source and intended function of each constituent fragment of the region intended for insertion

Symbol	Definition	Source	Size (bp)	Function
T-35S	**Terminator**	Cauliflower Mosaic Virus	206	Stop signal
	Polylinker sequence	Synthetic	27	Plasmid cloning site
Pat	**Synthetic *pat* gene**	*Streptomyces viridochromogenes*	551	Herbicide tolerance and selectable marker
	Polylinker sequence	Synthetic	27	Plasmid cloning site
P-35S	**Promoter**	Cauliflower Mosaic Virus	529	High level constitutive expression
	Cloning vector sequences	pDH51	2642	Sequences were needed for the maintenance in the *E. coli* host

D. INFORMATION RELATING TO THE GM PLANT

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

The introduced trait confers herbicide tolerance. The characteristic of herbicide tolerance is based upon the *pat* gene, a phosphinothricin resistance gene, isolated from the soil micro-organism *Streptomyces viridochromogenes*. The *pat* gene encodes for the enzyme, phosphinothricin acetyl-transferase (PAT) that acetylates glufosinate to its inactive form. When the *pat* gene is expressed it confers tolerance to the herbicide glufosinate-ammonium.

While the basic characteristics of T25 maize remains unchanged the glufosinate-ammonium tolerant T25 LibertyLink® maize varieties will provide more flexibility in crop management practices for growers.

The introduced trait does not result in a tolerance to the elements of a biotic environment, therefore the identification of target and non- target organisms is not relevant in this case.

Several formulations of glufosinate-ammonium are commercially used in many regions of the world. Registered trade names include Liberty®, Ignite®, Finale® and Basta®. Registered uses in Europe include e.g. non-selective weed control in the floor of orchards and vineyards and desiccation of potatoes and oilseed rape prior to harvesting. The use of Liberty in LL Maize has also been registered in some member states. LibertyLink® crops currently on the market in certain areas include varieties of maize, cotton and canola.

2. Information on the sequences actually inserted or deleted

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

Southern-blot hybridization studies demonstrated that only one copy of the *pat* gene was integrated into the maize genome. Further analysis of the event T25 evidenced the insert consists of the P35S-pat-T35S expression cassette and at the 3' end of the insert a duplication of an internal fragment similar to part of the P35S promoter, linked to a fragment of the *bla* gene.

The duplicated P35S-like promoter fragment contains the sequences from bp 80 to bp 433 of P35S and the *bla* gene fragment contains the sequence from bp 196 until bp 861 of the bla gene. The first 5 bp of the *bla* gene (containing the transcription initiation codon ATG) are inserted at the 5' end of the insert.

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

Not relevant.

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

Based upon Southern blot and Mendelian segregation analysis, it was demonstrated that the DNA has integrated in a single genetic locus in the maize nuclear genome (chromosome).

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

The insert is fully characterized and the DNA sequences are identified as part of the vector pUC/Ac. The characterisation of the inserted sequences in event T25 confirmed the presence of one copy of the *pat* gene cassette. There are no antibiotic resistance markers present in the event T25 maize.

The 5-prime and 3-prime flanking sequences were analysed and confirmed as native to the maize genome.

3. Information on the expression of the insert

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

Samples of leaf, stem and root of T25 maize plants were taken at different growth stages. The average values for concentration of the PAT protein as percent of total extractable protein from transgenic leaf and stem tissue, suggested a decrease in expression over time. The values ranged from 3.06 % to 1.58 % in leaf tissue, from 0.58 % to 0.28 % in stem tissue and from 0.67% to 0.54% in root tissue

b) Parts of the plant where the insert is expressed

Linked to the plant promoter, *35S*, the expression of the *pat* gene is targeted to green tissue of the plant.

PAT activity was measured in leaves, roots, stems and pollen by monitoring the N-acetylation of L-14C-Glufosinate to 14C-N- acetyl-glufosinate with HPLC. No PAT activity was detected in pollen. The highest enzyme activity was measured in stems followed by leaves, root and seed.

ELISA studies undertaken to determine the PAT content in grain of T25, and also in different fractions of the grain showed very low level of PAT as percent of the total crude protein.

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

a) Reproduction

The trait of herbicide tolerance had no effect on the reproduction of T25 maize.

Characters related to reproduction such as flowering time, plant height tested in field trials in the Europe and the North American maize growing region showed no difference in reproduction between T25 maiz the non GM counterpart.

b) Dissemination

The trait of herbicide tolerance had no effect on the dissemination of T25 maize.

Characters related to dissemination such seed production in yield, seed germination, moisture percent, st lodge tested in field trials in the European and the North American maize growing region showed no difference in dissemination between T25 maize and the non GM counterpart.

c) Survivability

The trait of herbicide tolerance had no effect on the survivability of T25 maize.

Characters related to survivability such disease and pest resistance tested in field trials in the European the North American maize growing region showed no difference in survivability between T25 maize an non GM counterpart.

d) Other differences

The only biologically significant difference observed in field evaluations of this T25 maize is the tolerance to Liberty® herbicide, active ingredient glufosinate ammonium.

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

The trait is inherited as a single dominant gene. To demonstrate the stability of the inserted DNA, Southern blot analysis was completed for the original transformant and for individual plants from the 3rd backcross generation.

The resulting Southern blots demonstrate the molecular stability of T25 maize at the genetic level over generations.

Phenotypic stability was demonstrated by Mendelian inheritance.

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

a) Plant to bacteria gene transfer

T25 does not contain any sequences responsible for an enhanced frequency of recombination.

The *pat* gene expressed in T25 maize is under the control of eukaryotic promoter, with very limited activity in prokaryotic organisms. Further *pat* gene under control of prokaryotic regulatory elements conferring the same trait as expressed in T25 maize is widespread in microorganisms in natural environment.

b) Plant to plant gene transfer

The genetic modification had not changed the ability of T25 maize to transfer its genetic material.

The reproductive, dissemination and survivability characteristics of T25 were found to be the same as for any other maize plants.

In addition the out-crossing rate of T25 maize studied in Farm Scale Evaluation in the UK remained in the range of out-crossing rate characteristic to maize.

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

Direct comparative assessments of corn derived from event T25 were made using the non transgenic isogenic lines as comparators (Cecillia/LLMoldova; Anjou 400/ LLAnjou400; Torino/ LLKingston ; LLTR7245xSG1847 / TR7245xSG1847). In addition, comparisons were also made to unrelated non-transgenic corn lines for which information was available in the literature.

7.2 Production of material for comparative assessment

a) Number of locations, growing seasons, geographical spread and replicates

Grain samples of T25 maize for compositional analyses were generated from 15 different field trials from Europe, performed in 1999 and 2000. The trials were located throughout the main maize growing areas of the EU northern and southern zones in order to cover different soil and climatic conditions. Trials were designed in randomized complete block with 4 replications. In all cases samples from non GM comparators treated with conventional herbicide (treatment A), were compared to samples from T25 plants treated with conventional herbicide (treatment B) and T25 plants with Liberty herbicide (treatment C).

The observation of the agronomic performance of T25 maize was undertaken in France. Trials were designed in randomized complete block with 4 replications. In all cases A, B and C treatment were used.

b) The baseline used for consideration of natural variations

Published literature was consulted to establish a range of values to be expected for each nutritional component and ranges built from values of the non-transgenic comparators.

7.3 Selection of material and compounds for analysis

The components selected for compositional and nutritional analyses for the assessment of substantial equivalence in maize, comprised the important, basic nutrients. These are the proximates, the amino acids, fatty acids, the micro-nutrients, such as minerals and vitamins including vitamin B, and the anti-nutrient phytic acid. Maize grain samples were not tested for their trypsin inhibition activity since previous studies showed that the activity in conventional maize varieties tested is very low.

7.4 Agronomic traits

From each site, 6 characteristics were quantitatively analyzed as follows: Time to anthesis, Plant height, Plant count, Yield, Length of ears and Diameter of ears.

For the agronomic characters assessed; first leaf shape of tip, time of silk emergence, anthocyan coloration of silks, susceptibility to pests and diseases, plant height, diameter of ears, shape of ears, type of grain, anthocyan coloration of glumes and colour of top of grain no differences were seen between the LLmaize event T25 plants and the non-transgenic plants at any trial site.

Since for most characters good correspondence between the LLMaize event T25 plants and their conventional counterparts was found the result of the comparison of the agronomic characters in the context of the study confirmed that the T25 maize plants show the same agronomic behaviour and performance as their commercially available maize varieties.

7.5 Product specification

The product is glufosinate-ammonium tolerant maize line, T25.

T25 maize line has been introgressed by traditional breeding into an array of germplasm.

The intended use of the product is the same as any other commercially available maize varieties.

7.6 Effect of processing

Maize harvested from T25 plants will be produced and processed in the same manner as maize produced with conventional breeding methods. The genetic modification was not aimed at changing the processing method.

T25 specific i.) DNA sequences and the ii.) PAT protein content of processed commodities from wet and dry milling were analysed. Dry milled fractions of field maize grain included bran (hull), germ, combined grits, flour, defatted germ meal, crude oil and refined, bleached and deodorized oil. Wet milled products analysed were milled germ, hulls, 1st starch, 2nd starch, gluten, crude and refined oil cake and meal.

The results confirmed that

i.) -No DNA could be detected in the refined oil samples. The degradation of the DNA occurs during the processing when the crude oil enters the refinery process.

ii.) -Crude Oil and Refined, Bleached and Deodorized Oil from transgenic Grain did not contain detectable amounts of PAT protein.

7.7 Anticipated intake/extent of use

There are no anticipated changes in the intake and extend of use of maize or derived products as a result of the presence of T25 maize in the supply chain. T25 maize is expected to replace a portion of current maize products, therefore the intake and use of T25 maize will represent some part of the total products derived from maize.

7.8 Toxicology

7.8.1 Safety assessment of newly expressed proteins

The PAT protein is not toxic for mammals and does not possess any of the characteristics associated with food allergens. Findings to support this conclusion include:

- The coding sequence of the *pat* gene is derived from a common soil microbe not known to be a

pathogen.
- The PAT protein is quickly degraded and denatured in gastric and intestinal fluids of domestic animals and humans.
- The PAT enzyme is highly substrate specific. It acts on its target, glufosinate ammonium but it does not act on glutamate, the closest structural analogue of L-glufosinate.
- There were no adverse effects found in mice, even at a high dose level of the PAT protein, after intravenous administration.

7.8.2 Testing of new constituents other than proteins

No other constituent than the PAT protein is novel and no changes in composition of maize were discovered by chemical analysis.

7.8.3 Information on natural food and feed constituents

Natural constituents of maize have not been changed in T25. Extensive compositional analysis was undertaken, taking into consideration the OECD consensus document on "compositional considerations for new varieties of maize: key food and feed nutrients and anti-nutrients". Equivalence in field maize grain was demonstrated for all proximates, fibre compounds, and the total amino acids. Good agreement between the findings for T25, the comparator and the baseline support the conclusion of compositional equivalence to maize currently in commerce.

7.8.4 Testing of the whole GM food/feed

The compositional and nutritional equivalence of field maize from T25 and commercially available maize varieties were established by compositional analysis. In addition the wholesomeness and safety of T25 maize have been confirmed by animal feeding studies, which did not indicate adverse effects associated with T25 maize.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein

The PAT protein does not possess any of the characteristics associated with food allergens.

The PAT protein has no homology with any known allergens, toxins or antinutrients.

The PAT protein has no glycosylation sites present on certain food allergens.

The PAT protein forms only an extremely minor part of the crude protein fraction in T25, making it unlikely to become a food allergen, which tend to be major proteins**.**

7.9.2 Assessment of allergenicity of the whole GM plant or crop

Maize (*Zea mays* L.) is not considered an allergenic food crop and the introduce protein does not have allergenic potential.

It has been concluded that the use of T25 maize for cultivation and for food and feed does not lead to an increased risk for allergenic reactions compared to the equivalent uses of commercially available maize varieties.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

Glufosinate-ammonium tolerance is an agronomic interest, the genetic modification did not change the nutritional value of the maize, and therefore there is no reason to assume that anticipated intake will change in the future. Consequently no nutritional imbalances are expected as a result of increased consumption of T25 derived maize products.

7.10.2 Nutritional assessment of GM feed

Extensive compositional analysis proved the equivalence between T25 and commercially available maize varieties. In order to confirm the unchanged nutritional value of T25 maize two animal feeding studies were performed.

One feeding study was performed with male broiler chickens. Poultry were selected to evaluate the effects of a feed component over an entire life span and under conditions of rapid growth, thus the assay is highly sensitive for nutritional deficiencies or toxic effects. No negative impacts of the nutritional quality of the event T25 were observed on poultry.

A ruminant feeding study was performed on T25 maize silage material partly to determine the nutritional quality of the feeding material. The nutritive value, fermentation characteristics, mineral content, and amino acid composition of all the studied silages were similar.

7.11 Post-market monitoring of GM food/feed

No post-market monitoring plan is required for GM food/feed produced from T25 maize. Traditional comparators were used in the comparative analysis. The intent of the genetic modification was for agronomic benefit, not for the change in the nutritional composition or value was intended and no change was identified. No health claims are intended and T25 food and feed products will not be marketed as an alternative to or replacement for commercially available maize varieties. T25 has no specific properties that might increase the dietary intake compared to traditional cotton maize. There is no evidence that the short or long term nutritional and health status of the European population could be impacted by the marketing of T25 food and feed products.

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

Not applicable. There are no target organisms.

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

9.1 Persistence and invasiveness

Studies have been undertaken to evaluate biological features that affect fitness and environmental sensitivity that might be associated with a change of persistence and invasiveness of T25 maize. No changes in fertility, floral morphology or pollen dissemination have been observed by plant breeders in the course of the variety development program. Reproduction and dissemination studies including flowering time, plant height, seed production, seed germination, moisture percent, stalk lodge, disease and pest resistance shown no difference in the listed characters between T25 maize and its traditional counterparts.

9.2 Selective advantage or disadvantage

T25 was developed to express tolerance to glufosinate-ammonium herbicide applications only. There is no other advantage or disadvantage conferred to T25 plants. As described in earlier there is no indication for an altered ecological fitness of T25 in comparison to conventionally bred hybrids with similar genetic background.

Corn derived from event T25 has a seasonal advantage over weed competition only with the recommended use of a glufosinate-ammonium herbicide to control weeds growing in the same field. T25 corn, as all corn, is an annual cultivated crop without weedy characteristics and without wild relatives in the European Union. Except for tolerance to glufosinate-ammonium, there are no significant phenotypic, genotypic or reproductive biology differences between the event T25 and commercial corn varieties developed solely through conventional breeding practices. In the absent of the use of glufosinate –ammonium T25 maize have no selective advantage over conventionally developed maize.

9.3 Potential for gene transfer

Potential gene transfer pathways for T25 are the transfer of the DNA through the transfer of the pollen through cross-pollination to other corn plants or the occurrence of gene transfer to microorganisms.

Contact of microorganisms to DNA of T25 plants can take place in the environment during the natural decay of T25 plants or in the digestive tract of human or animals consuming T25 food or feed.

Pollen from T25 plants can only cross-pollinate maize plants or compatible wild relatives of maize, which are not present in Europe.

Gene transfer from plant to bacteria: Gene transfer from GM plants to bacteria under natural conditions occurs, but is extremely rare. It occurs primarily through homologous recombination in microbes. The pat gene expressed in T25 maize is under the control of a eukaryotic promoter, with limited activity in prokaryotic organisms. Further on the *pat* gene is present in abounded soil microorganisms living in the environment.

Without selection pressure the uptake of the *pat* gene would not provide a selective advantage in the environment.

In the unlikely case that such a gene transfer would take place, no adverse effect on human and animal health or the environment are expected, because principally no new properties would be introduced into the microorganisms.

Gene transfer from plant to plant: The extent of cross-pollination to conventionally bred hybrids depends on the proximity, synchronic flowering and the scale of the area. T25 maize will be primarily imported to Europe. The amount of the imported commodity is estimated to be in the range of 0.1 million tones T25 maize yearly.

In Europe no wild relatives of maize exists, which could serve as crossing partners. Most of the maize varieties are hybrids, which are grown under strict pollination control. The production of maize seed follows special cultivation requirements and is grown respecting isolation distances, which guarantee the requested purity of the variety.

9.4 Interactions between the GM plant and target organisms

The introduced trait is not a pesticidal trait. There are no target organisms.

9.5 Interactions of the GM plant with non-target organisms

The genetic modification, tolerance to the herbicide glufosinate-ammonium, does not change the interaction of GM corn varieties with other organisms in the absence of herbicide application.

The interaction with non target organisms associated with the use of glufosinate-ammonium based herbicides in T25 maize is described in Section 9.9.

9.6 Effects on human health

No immediate and / or delayed effects on human health are indicated for people working with, coming into contact with or in the vicinity of T25 maize fields. No toxic or allergic effect from handling T25 has been observed on workers in the field since 1994, year of its first field of release in Europe.

9.7 Effects on animal health

No immediate and / or delayed effects on animal health have been observed associated with the consumption of T25 derived feed since its authorization.

9.8 Effects on biogeochemical processes

No immediate and / or delayed effects were observed that would indicate an effect on biogeochemical processes resulting from the potential direct interactions of the T25 and non target organisms in the vicinity of the environmental release of T25 maize.

However a beneficial indirect effect was observed in the UK Farm Scale Evaluation study that is associated effect with the use of glufosinate ammonium herbicides than with the T25 maize itself.

The collembolan (springtail) detrivores are known to have an important role for the cycling of nutrients within the soil. The later application and relative abundance of weeds in glufosinate ammonium tolerant T25 maize farming system resulted in a greater capture if these insects compared to conventional maize herbicide system.

9.9 Impacts of the specific cultivation, management and harvesting techniques

The herbicide tolerant crops, like T25 maize, provide flexible weed management systems for growers. The system allows the reduction of the use of residual or pre-emergence herbicides in maize. The reduction in field management practices contribute to an improvement in soil structure and moisture conservation. Unlike with a conventional practice, in a field of herbicide tolerant GM maize, weeds are present in the crop before spraying. Because glufosinate-ammonium is rapidly inactivated in the soil, any new flushes of weeds are free to establish. Thus, except for the early developmental stages when maize is sensitive to competition from weeds, there are weeds often present in the field for most of the season. Since maize forms a canopy over the field, new weed flushes have no effect on the growth of the crop. The weeds provide food and shelter to insects and to other farm wildlife organisms, like for birds and mammals.

This way of managing weed populations does not reduce the yield or quality of the maize.

The beneficial effects of the cultivation of T25 forage maize compared to conventional maize farming with respect to presence of farm wildlife organisms was confirmed by the 3 years Farm Scale Evaluation study in the UK (2000-2003). The relative abundance of weeds in T25 maize fields supports many soil dwelling and surface active invertebrates, which have important ecological and conservation functions for providing food for mammals, birds and other invertebrates.

10. Potential interactions with the abiotic environment

T25 maize plants have no direct potential interaction with the abiotic environment. Indirect interactions however can be identified that relate to the tillage methods the glufosinate ammonium tolerant T25 maize system offers.

Today, minimum tillage and conservation tillage systems are becoming more prevalent, especially in areas of highly erodible soils. Glufosinate-ammonium herbicide application together with T25 maize will likely become an important component of minimum tillage and conservation tillage systems. The facts from one side that glufosinate ammonium herbicide is used on a needs basis after weed emergence, thus will be no need for the incorporation of pre-planting herbicides and therefore less superficial soil treatments will be necessary. Furthermore the roots of weeds even after being dried contribute to the soil retention and have important abiotic environmental consequences. The use of glufosinate ammonium tolerant T25 corn can lead to soil enrichment in mineral nutrients, to lesser soil erosion and the lesser fuel use could result in a reduction of CO_2 emission.

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 the scope of this application under Regulation (EC) No 1829/2003 includes the cultivation of T25 maize in the EU, a general surveillance plan according to Annex VII of Directive 2001/18/EC was introduced, as required by Articles 5(5) and 17(5) of the said Regulation and by the EFSA guidance document on renewals.

11.2 Interplay between environmental risk assessment and monitoring

An environmental risk assessment (e.r.a.) on T25 maize was conducted as required by Articles 5(5) and 17(5) of Regulation (EC) No 1829/2003. Hazard analysis related to T25 maize has shown that the risk for potential adverse effects on human and animal health and the receiving environment resulting from the proposed use of T25 maize in the EU is consistently negligible. Consequently the overall environmental risk posed by the use of T25 is negligible. Therefore no case specific strategies for risk management and no case specific post-market monitoring plans are considered to be necessary.

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

Since no risk has been identified, there is no need for a case-specific monitoring plan.

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

Any potential adverse effects of T25 maize on human and animal health and the environment, which were not anticipated in the environmental risk assessment, are addressed by the general surveillance plan according to the principles of Directive 2001/18/EC, Annex VII.

General surveillance is largely based on routine observations and implies the collection, scientific evaluation and reporting of reliable scientific evidences, in order to be able to identify whether unanticipated, direct or indirect, immediate or delayed adverse affects have been caused by the placing on the market of a genetically modified (GM) crop in its receiving environment.

In order to identify the broadest possible range of unanticipated adverse effect, general surveillance is performed by either selected, existing networks, by company stewardship programmes, and by a combination of both. The authorization holder will ensure that appropriate technical information on T25 maize under relevant legislation will be available, post marketing for the network identified, in addition to further relevant information from a number of sources, including industry and government websites, official registers and government publication.

Following the re-approval of the cultivation of T25 maize in the EU, Bayer CropScience will approach key stakeholders and key network of stakeholders of the products (including European farmers and their organizations, international grain traders, maize processors and users of maize grain for animal feed) and inform them that the product has been re-authorized and may be present in European maize production. Bayer CropScience will ask key stakeholders and networks to participate in the general surveillance of the placing on the market of T25 maize according to the provisions of Directive 2001/18/EC. Stakeholders will be asked to be aware of the uses of this maize and to inform Bayer CropScience in case of occurrence of any unanticipated adverse to human and animal health and the environment, which might be associated with the use of this product. Appropriate technical and safety information will be provided to them. As growers are constantly present in the environments where T25 maize will be released, they are well placed to ensure good stewardship in the cultivation of the GM crop, as well as being valuable sources of surveillance information. Therefore, in addition to already existing stewardship programmes, a number of farmers who have experience with the cultivation of T25 maize will be contacted and requested to participate in regular environmental surveys. Information revealed by these farmers will be examined by Bayer CropScience (including where possible the use of statistical data analysis) and include this information in the annual general surveillance reports. In addition to the above described general surveillance actions directed to T25 maize growers, international traders, grain processors, users of grain and other stakeholders, the experts of Bayer CropScience will actively monitor existing information sources such as official websites and experts reports on GMOs in order to identify, collate and follow up on potentially adverse observations on this maize and any other relevant information, in particular with respect to occupational health, animal feed safety or ecological effects of the release of T25 maize.

Where scientifically evidenced potential adverse effects (direct or indirect) linked to the genetic modification are reported, further science based evaluation of the consequences of that effect will be compared to available baseline information. Relevant baseline information will reflect prevalent use practice and the associated impact of these practices on the environment. Where scientific valuation of the observations confirms the possibility of an adverse effect, this would be investigated further to establish a correlation, if present between the use of T25 maize and the observed effect. The evaluation should consider the consequence of the adverse effect and the remediation actions, if necessary, should be proportionate to the significance of the observed affect.

11.5 Reporting the results of monitoring

Bayer CropScience will collates annual General Surveillance Reports containing information obtained from participating networks, and/or in case of an effect that was confirmed. If information that confirms an adverse effect which alters the existing environmental risk assessment becomes available, Bayer CropScience will submit a report, consisting of the scientific evaluation of the potential adverse affect and the conclusion on the safety of the product. The report will also include, where appropriate, the measures taken to ensure the safety of (human and livestock health and) the environment.

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

T25 maize is detectable in food and feed using the validated event specific PCR method.

The validation report and further information on the method are provided at 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

a) Notification number

B/BE/95/VSP9, B/DE/94/16, B/DE/94/17, B/DE/94/18, B/DE/94/19, B/DE/94/21, B/DE/95/22, B/DE/95/23, B/DE/95/24, B/DE/95/25, B/DE/95/33, B/DE/96/42, B/DE/97/85, B/DK/98/03, B/ES/03/38-CON, B/ES/96/01, B/ES/97/01, B/ES/98/29, B/FR/95/01/04, B/FR/96/04/18, B/FR/98/03/02, B/FR/98/03/04/A, B/FR/98/04/22, B/FR/99/03/11/B, B/GB/95/R19/6, B/GB/96/R19/12, B/GB/97/R19/15, B/GR/96/02, B/GR/98/01, B/IT/95/03, B/IT/96/09, B/IT/96/55, B/IT/98/11, B/NL/95/13, B/NL/97/02, B/PT/97/01

b) Conclusions of post-release monitoring

The T25 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics.

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 adverse effects on human health and the environment observed.

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

a) Release country

USA

b) Authority overseeing the release

USDA Petition 94-357-01p Non-regulated Status Granted July 1995

c) Release site

multiple sites:

Tests have occurred at approximately 78 sites in the primary com growing regions of the US under field release authorizations granted by APHIS (USDA authorizations: permits 92-017-04, 92-043-01, 93-021-10, 93-021-11; notifications 93-120-17, 93-120-27, 94-074-03). More field studies at 95 additional sites in the US were done under notifications 95-053-05, 95-068-26, 95-068-27, 95-068-28, 95-068-29, 95-068-30, 95-068-31, 95-068-32, 95-068-33, 95-068-34, 95-068-35; and 95-079-04.

d) Aim of the release

Research and development.

e) Duration of the release

5 years (commercialized since 1997)

f) Aim of post-releases monitoring

Volunteers

g) Duration of post-releases monitoring

One season.

h) Conclusions of post-release monitoring

The T25 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics.

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

No adverse effects on human health and the environment observed.

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

Glufosinate-ammonium tolerant maize, T25 was notified to be placed on the market in 1998 in accordance with article 5 on the basis of article 3 of Regulation (EC) No 258/97 for i.) starch and all its derivatives, ii.) crude and refined oil, and iii.) all heat processed and fermented products obtained from hominy, grits and flour (dry milled fragments).

http://europa.eu.int/comm/food/food/biotechnology/novelfood/notif_list_en.pdf

T25 was authorized in the same year pursuant to the Part C consent of Directive 90/220/EEC for all uses with the exception of food.

http://europa.eu.int/eur-lex/pri/en/oj/dat/1998/l_131/l_13119980505en00300031.pdf

b) Assessment Report of the Competent Authority (Directive 2001/18/EC)

Scientific Committee on Plants of 10/02/1998,
Opinion of the Scientific Committee on Plants Regarding "Submission for Placing on the Market of

Glufosinate Tolerant Corns (*Zea Mays*) Transformation Event T25" by the Agrevo Company

http://europa.eu.int/comm/food/fs/sc/scp/out04_en.html

Scientific Committee on Plants of 05/09/2001,
Opinion of the Scientific Committee on Plants Regarding "Submission for Placing on the Market of Glufosinate Tolerant Corns (*Zea Mays*) Transformation Event T25" by the Agrevo Company

http://ec.europa.eu/food/fs/sc/scp/out108_gmo_en.pdf

CGB (Commission de Genie Biomoleculaire), 1996;
MAFF (Ministry of Agriculture, Fisheries and FOOD), 1997;
ACNFP (Advisory Committee on Novel Foods and Processes),
http://www.food.gov.uk/multimedia/pdfs/acnfp_app_i-vi.pdf,

Additional information is available on:

UK Defra (Department for Environment, Food and Rural Affairs),

http://www.defra.gov.uk/environment/gm/regulation/t25safety.htm

c) EFSA opinion

Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Austrian invoke of Article 23 of Directive 2001/18/EC, The EFSA Journal (2004) 78, 1-13.
http://www.efsa.europa.eu/etc/medialib/efsa/science/gmo/gmo_opinions/507.Par.0003.File.dat/opinion_gmo_safeguard_clauses_austria_en1.pdf
Opinion of the Scientific Panel GMO related to genetically modified crops (Bt176 maize, MON810 maize, T25 maize, Topas 19/2 oilseed rape and Ms1xRf1 oilseed rape) subject to safeguard clauses invoked according to Article 16 of Directive 90/220/EEC.
http://www.efsa.europa.eu/en/science/gmo/gmo_opinions/1439.html

d) Commission Register (Commission Decision 2004/204/EC)

T25 was notified as existing product under articles 8 and 20 of Regulation (EC) No 1829/2003 and entered in the Community Register of GM Food and Feed.

http://ec.europa.eu/food/dyna/gm_register/index_en.cfm

e) Molecular Register of the Community Reference Laboratory/Joint Research Centre

The validation report and further information on the method are provided at http://gmo-crl.jrc.it.

f) Biosafety Clearing-House (Council Decision 2002/628/EC

www.bch.biodiv.org/

g) Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC)

www.gmoinfo.jrc