Part II

SUMMARY OF THE APPLICATION FOR 1507x59122 MAIZE FOR IMPORT FOOD AND FEED USE, IN ACCORDANCE WITH REGULATION (EC) N^{O} 1829/2003, INCLUDING AUTHORISATION FOR CULTIVATION IN ACCORDANCE WITH DIRECTIVE 2001/18/EC

According to Articles 5(3)(l) and 17(3)(l) of Regulation (EC) 1829/2003, the application shall be accompanied by a summary of the dossier in a standardised form. This annex specifies the format of such summary for genetically modified plants and/or derived food and feed. Depending on the scope of the application, some of the specifications may not be applicable. The summary shall be presented in an easily comprehensible and legible form. It shall not contain parts which are considered to be confidential.

A. GENERAL INFORMATION

1. Details of application

a) Member State of application

b) Application number
[to be provided]

c) Name of the product (commercial and other names)
The product described in this application consists of 1507x59122 maize -including seed products for cultivation-: for all food and feed uses, and for all food, feed and processed products derived from 1507x59122 maize, produced by a traditional breeding cross between progeny of two genetically modified (GM) maize. The two GM maize are: *B.t* Cry1F maize, event DAS-∅15∅7-1, referred to as 1507 maize; and *B.t.* Cry 34Ab1 and Cry35Ab1 maize, event DAS-59122-7, referred to as 59122 maize. The commercial name assigned to 1507x59122 maize is Herculex Xtra[1].

d) Date of acknowledgement of valid application
Not available at the time of application.

2. Applicant

a) Name of applicant
This is a joint application submitted by Mycogen Seeds, c/o Dow AgroSciences LLC and Pioneer Hi-Bred International, Inc. as represented by Pioneer Overseas Corporation. Mycogen Seeds, c/o Dow AgroSciences LLC is the primary contact for this submission.

b) Address of applicant

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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))
Same as applicant

[1] Herculex Xtra Insect Protection by Dow AgroSciences and Pioneer Hi-Bred. Herculex is a trademark of Dow AgroSciences LLC.

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 ☐
1507x59122 maize has been notified and tested in Spain (B/ES/04/02), Hungary (12413/3/2004) and Bulgaria (107/1.04.2004) in 2004.	

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 ☐
Yes, a notification concerning foods derived from 1507x59122 maize was submitted to the US Food and Drug Administration (FDA) and an application was also submitted to the US Environmental Protection Agency (EPA) in June 2004. In addition a commodity clearance application has been submitted to the National Department of Agriculture (NDA) in South Africa in March 2005.	

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 is maize (*Zea mays* L.), a monoecious annual plant, which is extensively cultivated with a long history of safe use, and its taxonomy is well established. 1507x59122 maize is the result of the traditional breeding between progeny of two genetically modified maize: 1507 maize and 59122 maize. 1507 maize has been genetically modified to express the Cry1F and PAT proteins while, 59122 maize has been genetically transformed to express the Cry34Ab1, Cry35Ab1 and PAT proteins. Consequently, 1507x59122 maize has been bred to express Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins, conferring resistance to certain lepidopteran and coleopteran insect pests along with tolerance to glufosinate-ammonium herbicide.
b) Types of products planned to be placed on the market according to the authorisation applied for This application is for consent to place on the market 1507x59122 maize -including seed products for

cultivation-: for all food and feed uses, and for all food, feed and processed products derived from 1507x59122 maize, in accordance with Regulation (EC) No 1829/2003. Consequently, the scope of this application is for: use as food/feed ingredient, and use as food/feed material (Regulation (EC) No 1829/2003); and for import and processing (part C of Directive 2001/18/EC).

c) Intended use of the product and types of users

1507x59122 maize will be used in the European Union in the same manner, consistent with current uses of maize products, derived from conventional and commercial maize varieties, and by the same operators. There are several types of products and users, such as animal feed and milling industry, traders, agriculture, and consumer use by public at large.

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

1507x59122 maize and derived products will be used, stored, packaged, handled and transported in the same manner as maize products derived from conventional maize varieties. 1507x59122 maize and derived products will be labelled in accordance with Community law (See point 8.f); no other specific instructions and recommendations for use of 1507x59122 maize are required.

e) Any proposed packaging requirements

Since 1507x59122 maize and derived products are substantially equivalent to products derived from conventional maize varieties, no specific packaging requirements are necessary, other than proper labelling, described in point 8.f.

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

Proposal for the labelling of 1507x59122 maize food products

In accordance with articles 12 and 13 of regulation (EC) 1829/2003, foods containing, consisting of food stuffs, foods containing, consisting of, produced from or containing ingredients from 1507x59122 maize, in a proportion higher than 0.9%, should be labelled as:

(i) where the food consists of more than one ingredient, the words 'genetically modified' or 'produced from genetically modified maize' shall appear in the list of ingredients provided for in Article 6 of Directive 2000/13/ EC in parentheses immediately following the ingredient concerned;

(ii) where the ingredient is designated by the name of a category, the words 'contains genetically modified maize' or 'contains maize produced from genetically modified maize' shall appear in the list of ingredients;

(iii) where there is no list of ingredients, the words 'genetically modified' or 'produced from genetically modified maize' shall appear clearly on the labelling;

(iv) the indications referred to in (i) and (ii) may appear in a footnote to the list of ingredients. In this case they shall be printed in a font of at least the same size as the list of ingredients. Where there is no list of ingredients, they shall appear clearly on the labelling;

(v) where the food is offered for sale to the final consumer as non-pre-packaged food, or as pre-packaged food in small containers of which the largest surface has an area of less than 10 cm^2, the information required under this paragraph must be permanently and visibly displayed either on the food display or immediately next to it, or on the packaging material, in a font sufficiently large for it to be easily identified and read.

No other particulars such as those referred to in Article 13(2)(a) and (b) and Article 13(3) of Regulation No (EC) 1829/2003 would need to be specified on the label of 1507x59122 maize food products as 1507x59122 maize has been shown to be equivalent to non-GM maize in composition; nutritional value and nutritional effects; intended use; health characteristics; and, the genetic

modification in 1507x59122 maize does not give rise to any ethical or religious concerns.

Proposal for the labelling of 1507x59122 maize feed products

In accordance with Article 24(2) of Regulation No (EC) 1829/2003, labelling will apply to feed containing material which contains, consists of or is produced from 1507x59122 maize in a proportion higher than 0.9% of the feed and of each feed of which it is composed. In accordance with Article 25 of Regulation (EC) 1829/2003, and without prejudice to the other requirements of Community law concerning the labelling of feed, feed referred to in Article 15(1) of Regulation (EC) 1829/2003, *i.e.* 1507x59122 maize for feed use, and feed containing, consisting of or produced from 1507x59122 maize, should be labelled as follows:

(i) where the feed contains or consists of 1507x59122 maize, or where 1507x59122 maize is used for the purpose of feed use, the words 'genetically modified maize' will appear in parentheses immediately following the specific name of the feed;

Alternatively, these words may appear in a footnote to the list of the feed. It should be printed in a font of at least the same size as the list of feed;

(ii) where the feed is produced from 1507x59122 maize, the words 'produced from genetically modified maize' will appear in parentheses immediately following the specific name of the feed;

Alternatively, these words may appear in a footnote to the list of the feed. It should be printed in a font of at least the same size as the list of feed.

No other particulars such as those referred to in Article 25(2)(c) and Article 25(3) of Regulation No (EC) 1829/2003 would need to be specified on the label of 1507x59122 maize feed products as 1507x59122 maize has been shown to be equivalent to non-GM maize in composition; nutritional value and nutritional effects; intended use; health characteristics; and, the genetic modification in 1507x59122 maize does not give rise to any ethical or religious concerns.

Proposal for the labelling of products consisting of, or containing, 1507x59122 maize

As specified on Point **A.8** of Annex IV of Directive 2001/18/EC, the information provided on a label or in an accompanying document for the purpose of satisfying the labelling requirements regarding placing on the market of 1507x59122 maize will include the following:

i) *Commercial name of the product and the statement that 'this product contains genetically modified organisms';*

ii) *Name of the GMO;*

iii) *Information referred to in Point **A.2.** of Annex IV of Directive 2001/18/EC (name and full address of the person established in the Community who is responsible for the placing on the market);*

iv) *How to access the information in the publicly accessible part of the register.*

g) Unique identifier for the GM plant (Regulation (EC) 65/2004; does not apply to applications concerning only food and feed produced from GM plants, or containing ingredients produced from GM plants)

1507x59122 maize is uniquely identified as DAS-Ø15Ø7-1xDAS-59122-7 using the combination of unique identifiers of corresponding parental lines, 1507 maize (DAS-Ø15Ø7-1) and 59122 maize (DAS-59122-7).

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

1507x59122 maize is suitable for import, food and feed use throughout 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

Since 1507x59122 maize is as safe for human health, animal health and the environment, and as nutritious as conventional maize varieties, no specific measures, differing from those for any other commercial maize grain and derived products, need to be taken in case of unintended release or misuse or for disposal and treatment, such as the use of herbicides (with the exception of glufosinate-ammonium herbicide).

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

1. Complete name

a) Family name: Gramineae.
b) Genus: *Zea.*
c) Species: *Z. mays* L.
d) Subspecies: None.
e) Cultivar/breeding line or strain: Line Hi-II
f) Common name: Maize, corn.

2 a. Information concerning reproduction

(i) Mode(s) of reproduction Maize reproduces sexually by wind-pollination and being a monoecious species has separate male staminate (tassels) and female pistillate (silk) flowers. This gives natural outcrossing between maize plants but it also enables the control of pollination in the production of hybrid seed.
(ii) Specific factors affecting reproduction As a wind-pollinated, monoecious species, reproduction is by self-pollination and fertilisation and, cross-pollination and fertilisation, with frequencies of each normally determined by proximity and other physical influences on pollen dispersal. Reproductive factors such as tasseling (pollen production), silking and pollination are the most critical stages of maize development.
(iii) Generation time Maize is an annual crop with a cultural cycle ranging from as short as 10 weeks to as long as 48 weeks covering the period of seedling emergence to maturity. This variance in maturity allows maize to be grown over a range of climatic conditions.

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

There are no other cultivated or wild plant species sexually compatible with maize in the EU. Maize plants will intra-pollinate and transfer genetic material between maize except for certain popcorn varieties. The extent of pollination between maize will depend upon prevailing wind patterns, humidity and temperature.

3. Survivability

a) Ability to form structures for survival or dormancy Maize is a non-dormant annual crop and seeds are the only survival structures. Natural regeneration of maize from vegetative tissue is not known to occur.
b) Specific factors affecting survivability Commercial maize varieties cannot survive without human assistance. In addition, survival of maize seed is dependent upon temperature, moisture of seed, genotype, husk protection and stage of development. Maize seed can only survive under favourable climatic conditions. Freezing temperatures have an adverse effect on germination of maize seed and it has been identified as a major risk in limiting production of maize seed.

4. Dissemination

a) Ways and extent of dissemination Maize dissemination occurs via kernel (seed/grain) and pollen. Maize has been domesticated for thousands of years, and as a result maize dispersal of individual kernels does not occur naturally.
b) Specific factors affecting dissemination Mechanical harvesting and transport are ways of disseminating grain and insect or wind damage may cause mature ears to fall to the ground and avoid harvest. Regardless of these routes of dissemination, maize cannot survive without human assistance in non-agricultural habitats in the EU.

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

Because of its many divergent types, maize is grown over a wide range of climatic conditions, however, survival and reproduction in maize is limited by cool conditions. The greatest maize production occurs where the warmest month isotherms range between 21 and 27°C and the freeze-free season lasts 120 to 180 days. Summer rainfall of 15 cm is the lower limit for maize production without irrigation.

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

Not applicable as maize is widely grown throughout the EU.

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 extensively cultivated in the EU and has a long history of safe use. Maize grain and forage, or derived products of maize, are not considered to have toxic effects on humans, animals and other organisms.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Description of the methods used for the genetic modification

No new genetic modification has been implemented in the production of 1507x59122 maize, produced through a traditional breeding cross between 1507 maize and 59122 maize. While 1507 maize was obtained by insertion of a linear DNA fragment (insert PHI8999A) using the particle acceleration method, the 59122 maize was genetically modified using *Agrobacterium*-mediated transformation of the maize line Hi II, using binary vector PHP17662.

2. Nature and source of the vector used

No vector was used in the production of 1507x59122 maize. Traditional breeding techniques were used in the sexual crossing between 1507 maize with 59122 maize, to generate 1507x59122 maize. Therefore, there is no new genetic modification in 1507x59122 maize.

No vector was used in the transformation of 1507 maize. The insert in 1507 consists of a linear DNA fragment, containing the *cry*1F and *pat* coding sequences together with the necessary regulatory components only. The insert integrated in the 59122 maize genome is the T-DNA region of the binary vector PHP17662. No additional DNA sequences were used in the introduction of the respective inserts into 1507 maize or 59122 maize.

3. Source (name) of donor organism(s), size and intended function of each constituent fragment of the region intended for insertion

The individual components and the function of the inherited DNA sequences are listed below

Genetic elements in insert PHI8999A used in the transformation of 1507 maize

Location on plasmid PHP8999 (bp to bp incl.)	**Genetic element**	**Size (bp incl.)**	**Function**
21 – 100	Polylinker region	80	Contains restriction sites required for cloning of the genetic elements
101 – 2086	*ubi*ZM1(2)	1986	The ubiquitin promoter (plus 5' untranslated region) from *Zea mays* (Christensen *et al*., 1992)
2087 – 2109	Polylinker region	23	Contains restriction sites required for cloning of the genetic elements
2110 – 3927	*cry*1F	1818	A synthetic version of truncated *cry*1F from *Bacillus thuringiensis* sbsp. *aizawai* (plant optimized) (Chambers *et al.*, 1991)
3928 – 3973	Polylinker region	46	Contains restriction sites required for cloning of the genetic elements
3974 – 4687	ORF25PolyA	714	A terminator from *Agrobacterium tumefaciens* extrachromosomal plasmid pTi15955 (Barker *et al.*, 1983)
4688 – 4743	Polylinker region	56	Contains restriction sites required for cloning of the genetic elements

4744 – 5297	CaMV 35S promoter	554	35S promoter from Cauliflower Mosaic Virus (Odell *et al.*, 1985)
5298 – 5849	*pat*	552	The synthetic glufosinate-ammonium tolerance gene (plant optimized), based on a phosphinothricin acetyltransferase gene sequence from *Streptomyces viridochromogenes* (Wohlleben *et al*., 1988; Eckes *et al*., 1989)
5850 – 5866	Polylinker region	17	Contains restriction sites required for cloning of the genetic elements
5867 – 6070	CaMV 35S terminator	204	35S terminator from Cauliflower Mosaic Virus (Pietrzak *et al.*, 1986)
6071 – 6255	Polylinker region	185	Contains restriction sites required for cloning of the genetic elements

Genetic elements in the T-DNA region of binary vector PHP17662 used in the transformation of 59122 maize

Location on PHP17662 T-DNA (bp to bp)	**Genetic element**	**Size (bp)**	**Function**
1 – 177	RB	177	Right T-DNA border region from Ti plasmid of *Agrobacterium tumefaciens*. T-DNA right border 25 bp repeat region located from bp 1 to bp 25.
178 – 248	Polylinker region	71	Region required for the cloning of the genetic elements.
249 – 2241	*ubi*1ZM promoter	1993	Ubiquitin promoter from *Zea mays* including 5'UTR (bp 1149 to bp 1231) and intron (bp 1232 to bp 2241) (Christensen *et al*., 1992).
2242 – 2269	Polylinker region	28	Region required for the cloning of the genetic elements.
2270 – 2641	*cry*34Ab1	372	Maize-optimised *cry*34Ab1 gene encoding the 14 kDa delta-endotoxin parasporal crystal protein from *Bacillus thuringiensis* strain PS149B1 (Ellis *et al*., 2002). Coding region from start codon through stop codon.
2642 – 2664	Polylinker region	23	Region required for cloning of the genetic elements.

2665 – 2979	PINII terminator	315	Terminator sequence from *Solanum tuberosum* proteinase inhibitor II gene (An *et al*., 1989).
2980 – 3005	Polylinker region	26	Region required for cloning of the genetic elements.
3006 – 4303	TA peroxidase promoter	1298	*Triticum aestivum* peroxidase promoter (wheat peroxidase); (Hertig *et al*., 1991).
4304 – 4319	Polylinker region	16	Region required for cloning of the genetic elements.
4320 – 5471	*cry*35Ab1	1152	Maize-optimised *cry*35Ab1 gene encoding the 44 kDa delta-endotoxin parasporal crystal protein from *Bacillus thuringiensis* strain PS149B1 (Ellis *et al*., 2002). Coding region from start codon through stop codon.
5472 – 5494	Polylinker region	23	Region required for cloning of the genetic elements.
5495 – 5809	PINII terminator	315	Terminator sequence from *Solanum tuberosum* proteinase inhibitor II gene (An *et al*., 1989).
5810 – 5811	Polylinker region	2	Region required for cloning of the genetic elements.
5812 – 6341	CaMV 35S promoter	530	35S promoter from Cauliflower Mosaic Virus, Strasbourg strain (Hohn *et al*., 1982; Pietrzak, *et al*., 1986).
6342 – 6360	Polylinker region	19	Region required for cloning of the genetic elements.
6361 – 6912	*pat*	552	Plant-optimised phosphinothricin acetyltransferase coding sequence from *Streptomyces viridochromogenes*. Coding region from start codon through stop codon (Wohlleben *et al*., 1988).
6913 – 6931	Polylinker region	19	Region required for cloning of the genetic elements.
6932 – 7125	CaMV 35S terminator	194	35S terminator from Cauliflower Mosaic Virus (Hohn *et al*., 1982).
7126 – 7308	Polylinker region	183	Region required for cloning of the genetic elements.
7309 – 7390	LB	82	Left T-DNA border region from Ti plasmid of *Agrobacterium tumefaciens*. T-DNA Left Border 25 bp repeat region located from bp 7366 to bp 7390.

D. INFORMATION RELATING TO THE GM PLANT

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

1507x59122 maize has been produced by crossing, progeny of genetically modified, 1507 maize with 59122 maize, using traditional breeding techniques. Hence, no new genetic modification has been implemented in the production of 1507x59122 maize.

1507 maize has been genetically modified to express the proteins Cry1F and phosphinothricin-N-acetyltransferase (PAT). Expression of the Cry1F protein confers season-long resistance against certain lepidopteran pests, such as the European corn borer (*Ostrinia nubilalis*) and the pink borer (*Sesamia* spp.). It is expressed constitutively by the *ubi*ZM1(2) promoter and provides control against insect pest damage when cultivated. Constitutive expression of the PAT protein, also used as a selectable marker, confers tolerance to application of glufosinate-ammonium herbicide.

59122 maize has been genetically modified to express the Cry34Ab1 and Cry35Ab1 proteins - conferring resistance against certain coleopteran insect pests: *Diabrotica spp.* which are important maize pests - and the PAT protein conferring tolerance to application of glufosinate-ammonium herbicide.

2. Information on the sequences actually inserted or deleted

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

Both, 1507 maize and 59122 maize, include a single DNA insert containing one single copy of the respective inserted DNA fragment, at different loci in the maize genome. As shown by the Mendelian segregation pattern of the inserts of 1507 maize and 59122 maize, in their corresponding progeny, the inserts are stably inherited, as single dominant genes. Given that 1507 maize and 59122 maize are homozygous, their DNA inserts are inherited by 1507x59122 maize.

1507 maize

The results of the detailed molecular characterization of 1507 maize supported the conclusion that 1507 maize contains an almost full-length copy of the DNA insert used in the transformation (i.e., 6186 bp from the 6235 bp fragment of insert PHI8999A containing the *cry*1F and *pat* genes) and a limited number of non-functional sequence rearrangements linked to the almost full length insert. The 1507 maize does not contain the *nptII* gene nor any other detectable fragments from the portion of plasmid PHP8999 that was not intended for transformation of 1507 maize. Maize genomic DNA flanking regions at both the 5' and 3' borders of the 1507 maize insert have been sequenced and characterised in detail. In addition, analysis by PCR amplification has confirmed the presence of both maize genomic flanking regions in non-GM Hi-II maize used in the transformation of 1507 maize.

59122 maize

The results of the molecular characterisation of 59122 maize, support the conclusion that 59122 maize contains a single intact copy of the T-DNA region from binary vector PHP17662. Southern blot analysis demonstrated that 59122 maize does not contain fragments from the vector backbone portion of binary vector PHP17662. In addition, PCR amplification and sequence analysis have confirmed that the 5' and 3' regions flanking the 59122 maize insert are of maize genomic origin.

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 respective analyses of the segregation of 1507 maize and 59122 maize are consistent with single active sites of integration of these inserts into the genomic DNA of 1507 maize, 59122 maize. Southern blot analysis on 1507 maize, 59122 maize and 1507x59122 maize, further demonstrated the stability of the inserted sequences from 1507 maize (containing *cry*1F and *pat* genes) along with those from 59122 maize (containing *cry*34Ab1, *cry*35Ab1 and *pat* genes) inherited by 1507x59122 maize.

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

Results of the extensive molecular characterisation provide clear evidence that 1507x59122 maize has maintained the number, structure and organization of 1507 maize and 59122 maize inserts, inherited by conventional breeding.

3. Information on the expression of the insert

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

1507x59122 maize expresses the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins. Grain samples were taken from plots that were sprayed with glufosinate-ammonium herbicide or unsprayed. Expression levels of Cry1F and PAT proteins were comparable between 1507 and 1507x59122 maize and expression levels of the Cry34Ab1, Cry35Ab1 and PAT proteins were comparable between 59122 and 1507x59122 maize.

b) Parts of the plant where the insert is expressed

The Cry1F, Cry34Ab1 and Cry35Ab1 proteins were expressed in leaf, pollen, silk, stalk, whole plant, grain, and senescent whole plant tissue samples from 1507x59122 maize throughout the growing season.

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

a) Reproduction

Comparative assessments of phenotypic and agronomic characteristics of 1507x59122 maize versus conventional maize have been conducted at multiple sites in the EU in 2004. The observations have shown that, except from the combined tolerance to glufosinate-ammonium herbicide and protection against certain lepidopteran and coleopteran insect pests, there are no biologically significant differences between 1507x59122 maize and non-GM control maize when observing pollen production, seed production, seed viability or germination

b) Dissemination

Same as point 4.a.

c) Survivability

Same as point 4.a.

d) Other differences

Same as point 4.a.

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

Results from the Southern analysis, agronomic characteristics and protein expression analysis of 1507x59122 maize plants have confirmed the stable inheritance of *cry*1F, *cry*34Ab1, *cry*35Ab1 and *pat* genes along with preserving the expression of Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins.

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

a) Plant to bacteria gene transfer

In comparison with the possible transfer of genetic material between 1507x59122 maize and traditional maize, no changes are expected in the ability of 1507x59122 maize to exchange genetic material with bacteria. In addition, there is no known mechanism for, or definitive demonstration of, DNA transfer from plants to microbes under natural conditions. In general, microbial transfers of certain genes between compatible donors and recipients occurs at frequencies of 10^{-1} to 10^{-3} transferrants per recipient (usually from conjugation); but in the environment, transfer frequencies occur at 10^{-8} to 10^{-9} transferrants per recipient. Moreover, horizontal gene transfer to enteric bacteria of the human or animal gut or direct transfer to animal or human tissues is an equally remote possibility.

b) Plant to plant gene transfer

There are no sexually compatible wild or weedy relatives of *Zea mays* known to exist in the EU, which eliminates the possibility of potential gene transfer to such species.

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

The comparator chosen for the safety evaluation of 1507x59122 maize consisted of non-GM control maize with comparable genetic background. In addition, publicly available data on commercial maize was also used in the comparisons with 1507x59122 maize.

7.2 Production of material for comparative assessment

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

A field study was conducted at several field locations located in the major maize growing regions of the EU in 2004. Each location included a randomized block design containing four blocks (or replicates), three out of the four blocks were used for compositional analysis of 1507x59122 maize and the remaining one for protein expression. Each block contained the 1507x59122 maize and a non-GM control for comparison. Plots of 1507x59122 maize were treated with herbicide containing glufosinate-ammonium or were unsprayed.

b) The baseline used for consideration of natural variations

Publicly available data on commercial maize along with a comparative assessment with non-GM control maize of comparable genetic background was used to define the baseline used for consideration of natural variations.

7.3 Selection of material and compounds for analysis

Compositional analyses on 1507x59122 maize were performed on grain and forage samples. The compounds selected for analysis consisted of proximates along with fatty acids, minerals, amino acids, vitamins, secondary metabolites and anti-nutrients, following the recommendations of the OECD guidance document. The results obtained showed that 1507x59122 maize is compositionally comparable to non-GM control maize.

7.4 Agronomic traits

The evaluation of the agronomic characteristics of 1507x59122 maize, tested in several locations of the EU, confirmed that it is comparable to non-GM control maize, regardless of herbicide treatment.

7.5 Product specification

The product described in this application consists of products from 1507x59122 maize -including seed products for cultivation-: for all food and feed uses, and for all food, feed and processed products derived from 1507x59122 maize derived from traditional breeding between progeny of genetically modified 1507 maize and 59122 maize. No new genetic modification has been introduced in 1507x59122 maize.

7.6 Effect of the production and processing

In the EU, most of the maize is used for animal feed, and about 8% is processed into food products such as highly refined starch by the wet-milling process and maize flour by the dry-milling process. Since 1507x59122 maize is as safe and nutritious as conventional maize, it will undergo existing production processes used for conventional maize varieties.

7.7 Anticipated intake/extent of use

The evidence presented throughout this application confirms that the nutritional value and compositional characteristics of 1507x59122 maize are comparable and substantially equivalent to those of commercial maize. Therefore, the anticipated uses of 1507x59122 maize derived food and feed products will be no different from those corresponding to traditionally-bred maize.

The food products derived from 1507x59122 maize, will replace or be mixed with products derived from other commercial maize. The replacement of a proportion of the food and feed products derived from commercial maize can be expected, however, since 1507x59122 maize is of equivalent nutritional value to traditionally-bred maize, any replacement of food and feed products with traditionally-bred 1507x59122 maize will be of no nutritional consequence.

7.8 Toxicology

7.8.1 Safety assessment of newly expressed proteins

1507x59122 maize expresses the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins at low level, throughout the development of the 1507x59122 maize plants. The safety of the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins for animal and human health has already been demonstrated as part of

the safety evaluation of 1507 maize and 59122 maize.
The Cry1F, Cry34Ab1 and Cry35Ab1 proteins have very specific modes of action and selective toxicity against certain lepidopteran and coleopteran insect pests (target organisms). Additionally, there is no evidence for Cry proteins originating from *Bacillus thuringiensis* to have any harmful effects on the health of humans and animals. The potential toxicity of the Cry1F protein to humans and animals was specifically examined in an acute oral toxicology study where Cry1F protein was evaluated for acute toxicity in mice. The relatively high dose tested did not give rise to any toxicity. Concomitantly, no sub-chronic adverse effects were observed in a thirteen-week feeding study in rats conducted with diets prepared with 1507 maize. The mixture of Cry34Ab1 and Cry35Ab1 proteins was evaluated for acute oral toxicity from gavage administration to five male and five female CD-1 mice. The relative high dose tested did not give rise to any toxicity. All mice survived and there were no adverse effects in terms of body weights, detailed clinical observations, and gross pathological lesions during the two-week observation period. Concomitantly, no sub-chronic adverse effects were observed in a 90-day study were rats were fed with diets prepared with 59122 maize.
The PAT protein has already been found to be safe to human health during the assessment of glufosinate-ammonium tolerant maize. The *pat* gene was originally obtained from *Streptomyces viridochromogenes* strain Tü494 which has no known toxic or pathogenic potential. The PAT protein is enzymatically active but it has high substrate specificity to L-phophinothricin (L-PPT), the active ingredient of glufosinate-ammonium. A toxicity study consisting of feeding rats with the PAT protein has been carried out. Results from the range of doses tested showed no adverse effects on the growth or histopathology of the animals. The PAT protein has also been tested in an additional acute toxicity study in mice. As before, the relatively high dose tested did not give rise to any toxicity.
The lack of toxicity of the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins was anticipated since they all have a long history of safe use, and they all lack a toxic mechanism in mammals.

7.8.2 Testing of new constituents other than proteins

Not applicable, since 1507x59122 maize has shown to be substantially equivalent to non GM commercial maize.

7.8.3 Information on natural food and feed constituents

Detailed compositional analyses of 1507x59122 maize demonstrated that the composition of natural food and feed constituents of 1507x59122 maize is equivalent to that of non-GM control maize with comparable genetic background. Hence, no particular natural constituents of maize are considered to be of significant concern to require additional information.

7.8.4 Testing of the whole GM food/feed

Evaluation of the nutrient composition of 1507x59122 maize has confirmed its equivalency to non-GM control maize with comparable genetic background. Additionally, the wholesomeness and safety of 1507x59122 maize has been confirmed in a 42-day feeding study using broiler chickens.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein

In accordance with the ILFSI-IFBC allergenicity assessment decision tree and WHO/FAO recommendations, the newly expressed proteins -Cry1F, Cry34Ab1, Cry35Ab1 and PAT-, as intended by the genetic modification and the subsequent conventional cross, in 1507x59122 maize, were assessed for their allergenic potential through: i) assessing the allergenicity potential of the source of the gene; ii) homology searches with known allergens; iii) *in vitro* simulated digestibility studies; iv) evaluation of glycosylation, and; v) assessment of heat stability. Results of the above studies indicate that the newly expressed proteins in 1507x59122 maize lack any allergenic and toxic potential, which could harm human or animal health.

7.9.2 Assessment of allergenicity of the whole GM plant or crop

Maize is not considered as a major allergenic food. Based on the characteristics of the introduced genetic material and of the newly expressed proteins - Cry1F, Cry34Ab1, Cry35Ab1 and PAT - together with the evidence provided throughout this application, we conclude that the sexual cross between 1507 maize and 59122 maize, to produce 1507x59122 maize, does not introduce any new allergens and does not alter the inherent characteristics of maize regarding its allergenic potential.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

Composition analyses of 1507x59122 maize have shown that the contents of protein, fiber, carbohydrates, fat, ash, minerals, fatty acids, amino acids, vitamins, secondary metabolites and anti-nutrients are all equivalent to that found in non-GM control maize with comparable genetic background and to the published range of values in the literature. Also, spraying with glufosinate-ammonium does not have any significant effect on the nutrient composition of 1507x59122 maize. The comparable composition and nutritional value of 1507x59122 maize together with the results of the assessment of dietary intake and nutritional impact confirm that food products derived from 1507x59122 maize are nutritionally equivalent to food products derived from commercial maize.

7.10.2 Nutritional assessment of GM feed

Feed products derived from 1507x59122 maize are expected to be comparable to those obtained from non-GM maize since they are compositionally equivalent to products derived from conventional maize and there are no known toxic, allergenic or any other harmful effects arising from the genetic modification in 1507 maize and 59122 maize. The nutritional equivalency of feed derived from 1507x59122 maize has been further confirmed by a feeding study involving broiler chickens, conducted to compare the nutritional value of 1507x59122 maize and non-GM control maize. The results did reveal no biologically significant differences between broilers fed on diets including 1507x59122 maize or conventional non-GM maize.

7.11 Post-market monitoring of GM food/feed

Since no risks to human and animal health and the environment have been identified from the use of 1507x59122 maize in food, animal feed and industrial products, and the use of the food and feed products derived from 1507x59122 maize will not be different from that of food and feed products derived from commercial maize, post-market monitoring of GM food/feed products derived from 1507x59122 maize is not necessary.

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

When cultivated, expression of Cry1F, Cry34Ab1 and Cry35Ab1 proteins in 1507x59122 maize provides resistance against certain lepidopteran and coleopteran pests that attack maize plants, such as European corn borer, *Sesamia* spp.and corn rootworms *Diabrotica* spp.

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

9.1 Persistence and invasiveness

1507x59122 maize: i) does not possess any traits for weediness; ii) does not give rise to traits for weediness; and, iii) commercial maize hybrids are highly domesticated. Consequently, the likelihood of imported or commercially cultivated 1507x59122 maize having a significant increased persistence and invasiveness on the environment, is negligible, with a corresponding negligible risk.

9.2 Selective advantage or disadvantage

Maize is highly domesticated, to the extent that it cannot become established as a feral species outside the agricultural environment. The specific advantages –insect resistance and herbicide tolerance- present in 1507x59122 maize, do not confer any selective advantage to the plants in the natural environment, *i.e.* outside the agricultural environment. Insect attack is only one of the multiple biotic and abiotic factors that prevent growth of maize outside heavily managed agricultural environments, and therefore expression of Cry1F, Cry34Ab1 and Cry35Ab1 proteins conferring resistance to certain lepidopteran and coleopteran insect pests cannot be considered as a selective advantage outside the agricultural environment. Conversely, application of a broad-spectrum herbicide, such as glufosinate-ammonium, does not commonly occur outside the agricultural environment, and therefore expression of the PAT protein in 1507x59122 maize does not confer a selective advantage outside the agricultural environment.

9.3 Potential for gene transfer

There are no sexually compatible wild or weedy relatives of *Zea mays* known to exist in the EU, which eliminates any potential for gene transfer to other species.

In addition, there is negligible likelihood for 1507x59122 maize plants to become environmentally persistent or invasive giving rise to any weediness. Furthermore, expression of the proteins Cry1F,

Cry34Ab1, Cry35Ab1 and PAT does not present any selective advantage outside the agricultural environment.

9.4 Interactions between the GM plant and target organisms

Expression of the proteins Cry1F, Cry34Ab1 and Cry35Ab1 in 1507x59122 maize provides growers with a highly effective and environmentally beneficial tool to control certain lepidopteran insect pests, European corn borer *Ostrinia nubilalis* and the pink borer *Sesamia* spp. and coleopteran insect pests such as *Diabrotica virgifera virgifera, Diabrotica barberi* and *Diabrotica undecimpunctata howardi* commonly known respectively as western corn rootworm, northern corn rootworm and southern corn rootworm.

However, this benefit would be reduced if the target insect pests develop resistance to Cry1F, Cry34Ab1 and Cry35Ab1 proteins as expressed in 1507x59122 maize seed products during cultivation. In the light of current thinking and existing experience with *Bacillus thuringiensis* (Bt) maize products, a detailed proposal for insect resistance management (IRM) has been developed in the context of product stewardship

9.5 Interactions of the GM plant with non-target organisms

There are no potential changes in the interactions of 1507x59122 maize with non-target organisms resulting from expression of the Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins. This has been confirmed by the specificity of the biological activities of Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins and by thoroughly assessing the absence of toxicity of Cry1F, Cry34Ab1 and Cry35Ab1 proteins to non-target and beneficial organisms through multiple studies.

9.6 Effects on human health

Maize is not considered to have harmful effects on human health. A very detailed evaluation for the potential toxicity to humans of Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins expressed in 1507 maize and 59122 maize has been carried out clearly demonstrating that 1507x59122 maize does not express any known toxic or allergenic proteins. Therefore, we conclude that expression of Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in 1507x59122 maize is not expected to be toxic to humans.

9.7 Effects on animal health

The genetic modifications in 1507x59122 maize does not introduce any new compounds known to cause, or expected to cause, any possible immediate and/or delayed effects on animal health, and therefore consumption of 1507x59122 maize and any animal feed products derived from it will result in no adverse consequences for the feed/food chain.

9.8 Effects on biogeochemical processes

The expression of Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in 1507x59122 maize will not cause any significant possible immediate and/or delayed effects on biogeochemical processes, the effects on biogeochemical processes are considered to be negligible.

9.9 Impacts of the specific cultivation, management and harvesting techniques

The specific cultivation, management and harvesting techniques used for 1507x59122 maize are comparable to those used for other commercially available maize, with the exception of the herbicide regime and the environmental monitoring plan proposed specifically for the cultivation of 1507x59122 maize seed products.

10. Potential interactions with the abiotic environment

Expression of Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in 1507x59122 maize does not alter the natural interactions of maize plants with the abiotic environment. In addition to the very limited exposure, there is very limited persistence of the ubiquitous microbially-derived Cry1F, Cry34Ab1 and Cry35Ab1 proteins in the soil environment.

11. Environmental monitoring plan (not if application concerns only food and feed produced from GM plants, or containing ingredients produced from GM plants)

11.1 General (risk assessment, background information)

The proposal for an environmental monitoring plan for 1507x59122 maize, has been developed according to the principles and objectives outlined in Annex VII of Directive 2001/18/EC and Council Decision 2002/811/EC establishing guidance notes supplementing Annex VII to Directive 2001/18/EC.

11.2 Interplay between environmental risk assessment and monitoring

The design of the environmental monitoring plan is based on the conclusions of the environmental risk assessment (e.r.a.) for authorisation of 1507x59122 maize for food and feed use.

The e.r.a. for this application for authorisation of genetically modified 1507x59122 maize and derived food and feed has been carried out in accordance with Annex II of Directive 2001/18/EC and Commission Decision 2002/623/EC establishing guidance notes supplementing Annex II to Directive 2001/18/EC. The overall conclusion obtained from the e.r.a. confirms that there are no identified adverse effects to human and animal health or the environment arising from 1507x59122 maize and any derived products. However, the e.r.a. has indicated that there is a limited potential for development of resistance within the target pest population to the Cry1F, Cry34Ab1 and Cry35Ab1 proteins as expressed in 1507x59122 maize plants. Therefore a case-specific monitoring is considered appropriate as part of the risk management strategy, to ensure that cultivation of 1507x59122 maize seed products poses negligible risk and that the efficacy of the Cry1F, Cry34Ab1 and Cry35Ab1 expressed in 1507x59122 maize will be maintained, sustaining the environmental benefits of the *Bt* technology.

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

The introduced traits, characteristics and interaction with the environment of 1507x59122 maize will not give rise to any identified adverse effects to human and animal health or the environment. As a result, use of 1507x59122 maize is considered as safe as any commercially available maize regarding human and animal health or the environment. However, the e.r.a. has also indicated that there is a limited potential for development of resistance within the target pest population to the Cry1F, Cry34Ab1 and Cry35Ab1 proteins as expressed in 1507x59122 maize plants. Consequently, the case-specific monitoring plan for cultivation of 1507x59122 maize seed products consists of the Insect Resistance Management Plan (IRM) described in Annex VII to this application in accordance with Annexes II, III, IV and VII of Directive No 2001/18/EC.

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

In accordance with Council Decision 2002/811/EC, general surveillance is not based on a particular hypothesis and it should be used to identify the occurrence of unforeseen adverse effects of the GMO or its use for human health and the environment that were not predicted in the risk assessment. Hence, in order to safeguard against any adverse effect on human health and the environment that was not anticipated in the e.r.a., the applicants will implement a general surveillance plan for 1507x59122 maize throughout the period of validity of the consent.

11.5 Reporting the results of monitoring

The applicants will inform the European Commission, without delay, of any adverse effects arising from the handling and use of 1507x59122 maize reported to them. Furthermore, the applicants will investigate such reports and inform the outcome to the European Commission.

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

The 1507x59122 maize has been derived from traditional breeding methods between progeny of 1507 maize (DAS-Ø15Ø7-1), and 59122 maize (DAS-59122-7), consequently it can be detected using the identification techniques developed for 1507 maize and 59122 maize. The PCR detection methods to confirm the molecular identity of: 1507 maize has already been validated and 59122 maize has been developed and provided to the EC Joint Research Centre -Community Reference Laboratory (CRL)- in Ispra (Italy). In addition, complementary information and samples of 1507x59122 maize and non-GM maize have also been made available to the CRL.

E. INFORMATION RELATING TO PREVIOUS RELEASES OF THE GM PLANT AND/OR DERIVED PRODUCTS

1. History of previous releases of the GM plant notified under Part B of the Directive 2001/18/EC and under Part B of Directive 90/220/EEC by the same notifier

a) Notification number (B/ES/04/02)-Spain.
b) Conclusions of post-release monitoring 1507x59122 maize plants performed as expected. No evidence of unintentional morphological or phenotypical characteristics was observed.
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 environment were observed.

a) Notification number (12413/3/2004)-Hungary.
b) Conclusions of post-release monitoring 1507x59122 maize plants performed as expected. No evidence of unintentional morphological or phenotypical characteristics was observed.
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 environment were observed.

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

a) Release country Bulgaria
b) Authority overseeing the release Biosafety Committee
c) Release site Multiple sites.
d) Aim of the release Research.
e) Duration of the release One season.
f) Aim of post-releases monitoring Control of potential volunteers.
g) Duration of post-releases monitoring One season.
h) Conclusions of post-release monitoring 1507x59122 maize plants performed as expected. No evidence of unintentional morphological or phenotypical characteristics was observed.
i) Results of the release in respect to any risk to human health and the environment No adverse effects on human health and environment were observed.

a) Release country USA.
b) Authority overseeing the release EPA.
c) Release site

d) Aim of the release Research.
e) Duration of the release One season.
f) Aim of post-releases monitoring Control of potential volunteers.
g) Duration of post-releases monitoring One season.
h) Conclusions of post-release monitoring 1507x59122 maize plants performed as expected. No evidence of unintentional morphological or phenotypical characteristics was observed.
i) Results of the release in respect to any risk to human health and the environment No adverse effects on human health and environment were observed.

a) Release country Canada.
b) Authority overseeing the release Not regulated.
c) Release site Multiple sites.
d) Aim of the release Research.
e) Duration of the release One season.
f) Aim of post-releases monitoring Control of potential volunteers.
g) Duration of post-releases monitoring One season.
h) Conclusions of post-release monitoring 1507x59122 maize plants performed as expected. No evidence of unintentional morphological or phenotypical characteristics was observed.
i) Results of the release in respect to any risk to human health and the environment No adverse effects on human health and environment were 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 The EFSA website http://www.efsa.eu.int/science/gmo/gm_ff_applications/ catindex_en.html provides information related to the applications submitted under Regulation (EC) No 1829/2003 on genetically modified food and feed.
b) Assessment Report of the Competent Authority (Directive 2001/18/EC) Not relevant.
c) EFSA opinion An EFSA opinion for 1507x59122 maize was not available at the time of the submission.
d) Commission Register (Commission Decision 2004/204/EC[1]) http://europa.eu.int/comm/food/food/biotechnology/authorisation/commun_registe r_en.htm.
e) Molecular Register of the Community Reference Laboratory/Joint Research Centre Information on the detection protocols for 1507 maize can be found, and for 59122 will be posted at:

[1] Commission Decision of 23 February 2004 laying down detailed arrangements for the operation of the registers for recording information on genetic modifications in GMOs, provided for in Directive 2001/18/EC of the European Parliament and of the Council. Official Journal of the European Communities L 65: 20 – 22.

http://gmo-crl.jrc.it/statusofdoss.htm
f) Biosafety Clearing-House (Council Decision 2002/628/EC[2]) The publicly accessible portal site of the Biosafety Clearing-House (BCH) can be found at http://bch.biodiv.org/.
g) Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC) Not relevant.

[2] Council Decision of 25 June 2002 concerning the conclusion, on behalf of the European Community, of the Cartagena Protocol on Biosafety. Official Journal of the European Communities L 201: 48 – 49.