

SUMMARY NOTIFICATION INFORMATION FORMAT FOR PRODUCTS CONTAINING GENETICALLY MODIFIED HIGHER PLANTS (GMHPs) IN ACCORDANCE WITH DIRECTIVE 2001/18/EC

A. GENERAL INFORMATION

1. Details of notification

(a) Member State of notification:

Spain

(b) Notification number:

C/ES/01/01

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

The product is *B.t.* Cry1F maize line 1507, referred to as 1507 maize, (no commercial names assigned yet). The product consists of maize products derived from seed of genetically modified 1507 maize expressing CRY1F and PAT proteins. The maize product also consists of progeny derived from conventional breeding between 1507 maize with any traditionally bred maize.

(d) Date of acknowledgment of notification:

11th July 2001

2. Notifier

(a) Name of notifier

Pioneer Hi-Bred International, Inc. as represented by Pioneer Overseas Corporation.

Mycogen Seeds, c/o Dow AgroSciences LLC.

This is a joint notification submitted by Pioneer Hi-Bred and Mycogen Seeds. PioneerHi-Bred is taking the lead for this submission.

(b) Address of notifier

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Avenue Tedesco, 7
B-1160 Brussels
Belgium

Pioneer Hi-Bred International, Inc.
400 Locust Street, Suite 800
Des Moines, IA 50309
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Dow AgroSciences Europe
European Development Centre
3 Milton Park, Abingdon
Oxon OX14 4RN
United Kingdom

Mycogen Seeds
c/o Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268-1054
U.S.A.

(c) Is the notifier

domestic manufacturer

Pioneer Hi-Bred and Mycogen Seeds are developers of the technology and producers of 1507 maize seed (inbreds and hybrids).

importer

Yes, as importer of seed

(d) In case of an import the name and address of the manufacturer shall be given

Same as notifier

3. 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.), which is extensively cultivated and has a long history of safe use. The 1507 maize has been genetically modified to express CRY1F protein, conferring resistance to certain lepidopteran insect pests, such as the European corn borer and *Sesamia* spp., and PAT protein, conferring tolerance to glufosinate-ammonium herbicide.

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

No, there is no specific form in which 1507 maize must not be placed on the market as a proposed condition of the authorisation applied for.

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

Use of 1507 maize will be consistent with current uses of commercial maize products and in accordance with the monitoring plan (**Section 5** of notification **C/ES/01/01**) and the insect resistance management strategy (**Section 4** of notification **C/ES/01/01**) proposed for the cultivation of 1507 maize. Use of 1507 maize will include cultivation and import of grain and grain products for storage and processing into food, animal feed and industrial uses. Approval for food use of 1507 maize is being considered separately in accordance with Regulation (EC) No. 258/97.

There are multiple categories of users of 1507 maize, e.g. animal feed and milling industry, agriculture, skilled trades and consumer use by public at large. Maize, together with rice and wheat, is one of the most important cereal crops in the world with total production of 596.4 million tonnes in 2000 (FAOSTAT Database, 2000). The FAO estimation for the EU maize production in 2000 was 38.4 million tonnes. Majority of grain and forage derived from maize is used for animal feeds, and about 8% of the grain is processed for human food products mainly by wet-milling or dry-milling. Maize grain is also processed into industrial products (11%), such as ethyl alcohol by fermentation and highly refined starch by wet-milling to produce starch and sweetener products. In addition to milling, the maize germ can be processed to obtain maize oil.

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

Use of 1507 maize will be consistent with current uses of maize products and in accordance with the monitoring plan (**Section 5** of notification C/ES/01/01) and the insect resistance management strategy (**Section 4** of notification C/ES/01/01) developed for the cultivation of 1507 maize. Labelling of 1507 products will be carried out in accordance with EU legislation. See **Point A.3.h**) below for labelling of 1507 maize.

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

Not applicable.

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

The 1507 maize would be particularly suitable in environments where there is infestation from lepidopteran insect pests, such as Southern and Central Europe, provided specific varietal germplasm with appropriate maturity is available.

(g) Any proposed packaging requirements

The packaging, handling, and storage systems that are currently used for maize will apply. The 1507 maize products will be packaged in the same manner as products from other commercial maize. See **Point A.3.h**) below for labelling of 1507 maize.

(h) Any proposed labelling requirements in addition to those required by law

Product information to indicate that genetic modification has been used in the development of 1507 maize will be provided on a label or in an accompanying document. A proposal for the labelling of products consisting of, or containing, genetically modified 1507 maize has been prepared in accordance with Annex IV of Directive 2001/18/EC (**Section 6** of notification C/ES/01/01). This will enable

1507 maize products to be labelled appropriately in accordance with Directive 2001/18/EC.

(i) Estimated potential demand

(i) in the Community

Import of maize grain into the EU for 2000 was about 10.3 million tonnes and the EU maize production including cultivation was 38.4 million tonnes (FAOSTAT Database, 2002). The potential demand for 1507 maize in the Community is expected to be part of these imports and production.

(ii) in export markets for EC supplies

Maize grain is traded as a commodity and no specific demand for 1507 maize should be expected from export markets for EC supplies.

(j) Unique identification code of the GMO

In accordance with the OECD guidance for the designation of a unique identifier for transgenic plants (ENV/JM/MONO(2002)7), the unique identification code assigned to 1507 maize is DAS-Ø15Ø7-1.

4. Has the GMHP referred to in this product been notified under Part B of Directive 2001/18/EC and/or Directive 90/220/EEC?

Yes, 1507 maize has been notified in Italy, France and Spain for field trials under Part B of Directive 90/220/EEC.

<u>Year</u>	<u>Member State</u>	<u>Notification No</u>
1998	Italy	B/IT/98/19
1999	Italy	B/IT/98/19
1999	France	B/FR/99.03.09
2000	Italy	B/IT/98/19
2000	France	B/FR/99.03.09
2000	France	B/FR/00.03.04
2002	Spain	B/ES/02/11

If *no*, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC

Not applicable.

5. Is the product being simultaneously notified to another Member State?

Yes, a separate notification (Ref. C/NL/00/10) to import 1507 maize has been submitted to The Netherlands in accordance with Directives 90/220/EEC and 2001/18/EC. In addition a separate application has been submitted to The

Netherlands for food use of 1507 maize in accordance with Regulation (EC) No 258/97.

Or

Has the product being notified in a third country either previously or simultaneously?

Yes, an application for registration of 1507 maize was submitted to the US Environment Protection Agency (EPA). Also, an application for non-regulated status of 1507 maize to the US Department of Agriculture (USDA) was submitted in May 2000, and a notification concerning foods derived from 1507 maize to the US Food and Drug Administration (FDA) was submitted in July 2000. The corresponding permits were granted as follows: by US EPA and FDA on 18th May 2001 and by USDA on 14th June 2001.

In addition, applications have been submitted to Argentina, Australia/New Zealand, Canada, China, Japan, Korea, Mexico, South Africa, Switzerland and Taiwan. The necessary approvals for import, animal feed use and food safety of 1507 maize in Japan were obtained on 15th June, 28th May and 8th July of 2002, respectively. In Canada permits were granted by Health Canada for novel food use of 1507 maize on 10th October 2002 and by the Canadian Food Inspection Agency for animal feed use and environmental release on 11th October 2002. Approval for import of 1507 maize for animal feed and food use in South Africa was obtained on 12th December 2002.

6. Has the same GMHP been previously notified for marketing in the Community?

No.

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

Based on the conclusions from the environmental risk assessment (e.r.a.) for the placing on the market of 1507 maize in accordance to Annex II of Directive 2001/18/EC (**Section 4** of notification **C/ES/01/01**) no specific measures need to be taken in case of unintended release or misuse or for disposal and treatment.

In case of unintended release of 1507 maize, current agronomic measures taken to control unintended release or misuse of non-GM maize can be applied, such as cultivation, selective use of herbicides (with the exception of glufosinate-ammonium herbicide), and crop rotation.

B. NATURE OF THE GMHP CONTAINED IN THE PRODUCT INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

8. Complete name

(a) Family name:	Gramineae
(b) Genus:	<i>Zea</i>
(c) Species:	<i>Z. mays</i> L.
(d) Subspecies:	None
(e) Cultivar/breeding line:	Line Hi-II
(f) Common name:	Maize; corn

9. a) Information concerning reproduction

(i) Mode(s) of reproduction

As a wind-pollinated, monoecious grass species, self-pollination and fertilisation and, cross-pollination and fertilisation, are usually possible and frequencies of each are normally determined by proximity and other physical influences on pollen dispersal.

(ii) Specific factors affecting reproduction, if any

Tasselling, silking, and pollination are the most critical stages of maize development, and grain yield is greatly impacted by moisture and fertility stress. Dispersal of maize pollen tends to be limited, as it is influenced by the large size and rapid settling rate of the pollen.

(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.

9. b) Sexual compatibility with other cultivated or wild plant species

Maize will intra-pollinate and will not transfer genetic material to other plant species in the EU. The extent of pollination will depend upon prevailing wind patterns, humidity and temperature. It is generally considered that teosinte (*Zea mays* ssp. *mexicana*) is an ancestor of maize. Teosinte is an ancient wild grass found in Mexico and Guatemala and is not present in the EU.

10. Survivability

(a) Ability to form structures for survival or dormancy

During the domestication of maize, many agronomically significant attributes for cultivation have been gained whilst losing its ability to survive in the wild. 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, if any

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.

11. Dissemination

(a) Ways and extent of dissemination

Maize has a polystichous female inflorescence (ear) on a stiff central spike (cob) enclosed in husks (modified leaves). As a result, seed dispersal of individual kernels does not occur naturally.

(b) Specific factors affecting dissemination, if any

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.

12. Geographical distribution of the plant

Maize is grown over a wide range of climatic conditions because of its many divergent types. 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. Maize has been cultivated in Europe starting in Southern Europe since the 16th century.

13. 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 has been cultivated in Europe since the 16th century.

14. Potentially significant interactions of the plant with other organisms in the ecosystem where it is usually grown, including information on toxic effects on humans, animals and other organisms

Maize is known to interact with other organisms in the environment including insects, birds, and mammals. It is susceptible to a range of fungal diseases and insect pests, as well as competition from surrounding weeds. Maize is extensively cultivated and has a history of safe use. Maize or derived products of maize are not considered to have harmful characteristics. Maize has no pathogenic characteristics.

15. Phenotypic and genetic traits

Maize (*Zea mays* L.) is the only species usually included in the genus *Zea*, of the family Gramineae. It is a highly domesticated agricultural crop with well-characterised phenotypic and genetic traits.

Controlled cross-pollination of inbred lines from chosen genetic pools combines desired genetic traits in a hybrid resulting in improved agronomic performance and yield increase. This inbred-hybrid concept and resulting yield response is the basis of the modern maize seed industry.

INFORMATION RELATING TO THE GENETIC MODIFICATION

16. Description of the methods used for the genetic modification

The particle acceleration method was used to introduce into maize cells a linear DNA fragment containing the *cry1F* and *pat* coding sequences and the necessary regulatory components (insert PHI8999A). Maize event 1507 expressing the CRY1F protein and the PAT protein was produced, referred to as 1507 maize.

17. Nature and source of the vector used

No vector was used for the transformation of 1507 maize. As described in the notification, the intended insert consists of a linear DNA fragment, containing the *cry1F* and *pat* coding sequences together with the necessary regulatory components only, which was introduced by particle acceleration for the transformation of 1507 maize. No additional DNA sequences were used for introduction of the insert into 1507 maize.

18. Size, source [name of donor organism(s)] and intended function of each constituent fragment of the region intended for insertion

The intended insert PHI8999A contains the plant optimised coding sequences for the *cry1F* and *pat* genes, together with the necessary regulatory components to drive their expression.

The *cry1F* gene (1818 bp; origin: *Bacillus thuringiensis* subsp. *aizawai*) is under the control of the ubiquitin promoter *ubiZM1(2)* (1986 bp; origin: *Zea mays*) and the ORF25PolyA terminator (714 bp; origin: *Agrobacterium tumefaciens* pTi15995). The function of the CRY1F protein in 1507 maize is to provide resistance against certain lepidopteran insect pests such as the European corn borer and *Sesamia* spp.

The *pat* gene (552 bp; origin: *Streptomyces viridochromogenes* strain Tü494) is under the control of the CaMV35S promoter and terminator (554 and 204 bp, respectively; origin: cauliflower mosaic virus). The function of the PAT protein in 1507 maize is to tolerate application of glufosinate-ammonium herbicide.

INFORMATION RELATING TO THE GMHP

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

The 1507 maize expresses CRY1F protein conferring resistance to certain lepidopteran insect pests, and PAT protein conferring tolerance to glufosinate-ammonium herbicide.

The *cry1F* gene is expressed constitutively by the *ubiZM1(2)* promoter. Expression of CRY1F protein provides control against lepidopteran insect pest damage to the above-ground parts of the maize plant including those parts which are beyond the reach of chemical insecticides. Specifically, the CRY1F protein confers season-long resistance against the European corn borer (*Ostrinia nubilalis*) and certain other lepidopteran pests such as the pink borer (*Sesamia* spp.). It is also highly effective against fall armyworm (*Spodoptera frugiperda*), black cutworm (*Agrotis ipsilon*) and southwestern corn borer (*Diatraea grandiosella*).

The *pat* gene is also expressed constitutively by the CaMV35S promoter. Expression of PAT protein confers tolerance to application of glufosinate-ammonium herbicide. Field trials show that 1507 maize will tolerate field application rates of 1600 g a.i./ha of glufosinate-ammonium herbicide without showing any phytotoxicity symptoms. Tolerance to glufosinate-ammonium herbicide provides for improved weed management.

No other new traits have been introduced into 1507 maize and, in particular, no trait for antibiotic resistance is present in 1507 maize. As discussed in detail throughout the notification (C/ES/01/01), these characteristics of 1507 maize have been confirmed by molecular characterization, protein expression analysis, agronomic performance, and comparison of composition data to other conventional non-GM maize.

20. Information on the sequences actually inserted/deleted/modified

(a) Size and structure of the insert and methods used for its characterisation, including information on any parts of the vector introduced in the GMHP or any carrier or foreign DNA remaining in the GMHP

The genetic modification in 1507 maize has been characterised in detail by Southern blot and DNA sequence analyses. The analyses have confirmed that the inserted genetic material is integrated into the nuclear genome of the maize plant and consists of an almost full-length copy of the linear fragment used in the transformation (*i.e.*, 6186 bp from the 6235 bp of insert PHI8999A, containing the *cry1F* and *pat* genes together with the regulatory sequences necessary for their expression). In addition, the plant insert contains the following non-functional fragments:

- one fragment (335 bp) of the *cry1F* gene, with no *ubiZM1(2)* promoter sequence, and one fragment (15 bp) of the *cry1F* gene, both located at the 5' end of the almost full-length insert;
- two fragments (201 bp and 138 bp long, respectively) of the *pat* gene, without regulatory sequences associated, located at the 5' border and, one fragment (188 bp) of the *pat* gene, located at the 3' border;
- one fragment (118 bp) of the polylinker region and *ubiZM1(2)* promoter sequence located at the 5' border;
- one fragment (550 bp) of the ORF25PolyA terminator sequence in inverted position located immediately at the 3' end of 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. 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.

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

Not applicable.

(c) Location of the insert in the plant cells (integrated in the chromosome, chloroplasts, mitochondrion, or maintained in a non-integrated form), and methods for its determination

The inserts are integrated into the maize plant genome as confirmed in the molecular characterisation of 1507 maize by detailed Southern blot analysis and DNA sequencing.

(d) Copy number and genetic stability of the insert

As discussed in **Point B.20.(a)** above, the insert integrated in 1507 maize contains one copy of the almost full-length linear fragment used in the transformation, which includes one functional copy of the complete *cry1F* gene and one functional copy of the complete *pat* gene, together with the regulatory sequences necessary for their expression. The insert also contains two non-functional fragments of the *cry1F* gene; three non-functional fragments of the *pat* gene; one non-functional fragment of the polylinker region and *ubiZM1(2)* promoter; and, one non-functional fragment of the ORF25PolyA terminator sequence.

The inserted genetic material in 1507 maize is stable for at least six generations, and the *cry1F* and *pat* genes are inherited as Mendelian dominant genes. Results from Southern blot and DNA sequencing analyses show that the additional non-functional fragments were present in the BC4 backcross generation, thus supporting the conclusion that they are genetically linked to the almost full-length insert containing the *cry1F* and *pat* genes.

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

Not applicable.

21. Information on the expression of the insert

- (a) Information on the expression of the insert and methods used for its characterisation**

Leaf, pollen, silk, stalk, whole plant, grain and senescent whole plant tissue samples from 1507 maize and control maize with comparable background genetics were obtained from field studies conducted during the growing seasons of 1998/99 in Chile, 1999 in France and Italy, and 2000 in France, Italy and Bulgaria. Expression levels of CRY1F and PAT proteins in these tissues were measured using specific Enzyme Linked Immunosorbent Assay (ELISA) developed for each protein. Results show that the CRY1F protein is expressed in all tissues and throughout the development of maize, while the PAT protein was measurable at the V9 developmental stage only.

The characteristics of the CRY1F and PAT proteins expressed in 1507 maize were further examined by Western blot analysis. The CRY1F protein was detected as two bands of approximately 65 and 68 kDa, respectively, which result from limited N-terminal processing of maize expressed CRY1F protein by a plant protease with trypsin-like specificity. No other bands indicative of a partial CRY1F protein or a fusion protein of greater molecular weight were observed.

The PAT protein is known to be a homodimer of approximately 43 kDa in its native form, and it is comprised of two components of approximately 22 kDa. The results of the Western blot analysis of 1507 maize confirmed the presence of the ~22 kDa PAT monomeric form and of the ~43 kDa PAT homodimer in leaf

tissue. No other bands indicative of a partial PAT protein or fusion protein of greater molecular weight were observed in 1507 maize.

The genetic modification in 1507 maize results in expression of CRY1F protein conferring resistance to certain lepidopteran insect pests, and PAT protein conferring tolerance to glufosinate-ammonium herbicide. Specifically, the CRY1F protein confers season-long resistance against the European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp. It is also highly effective against fall armyworm (*Spodoptera frugiperda*), black cutworm (*Agrotis ipsilon*) and southwestern corn borer (*Diatraea grandiosella*).

(b) Parts of the plant where the insert is expressed (e.g. roots, stem, pollen, etc.)

Addressed in **Point B.21.(a)** above.

22. Information on how the GMHP differs from the recipient plant in

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

No unexpected changes in pollen production, seed production, seed viability or germination compared to non-GM maize have been observed in field trials of 1507 maize.

(b) Dissemination

Maize hybrids have been domesticated to the extent that the seeds cannot be disseminated without human intervention. The 1507 maize plants show no difference in dissemination compared to non-GM maize.

(c) Survivability

Cultivated maize has been domesticated to the extent that it can not survive outside managed agricultural environments. Lack of dormancy prevents maize seed to readily survive from one growing season to the next. The genetic modification in 1507 maize results in expression of CRY1F conferring resistance to certain lepidopteran insect pests and expression of PAT conferring tolerance to the herbicide glufosinate-ammonium. The survival characteristics of 1507 maize in the environment remain comparable to those of non-GM maize.

(d) Other differences

Maize does not exhibit any weedy tendencies and is non-invasive in natural ecosystems. Based on the agronomic data, there is no evidence for altered survival, multiplication, or dissemination of 1507 maize in the environment as compared to non-GM maize. In addition, the inserted traits do not alter the phenotype of maize in a way that would confer a fitness advantage for maize outside managed agricultural environments.

23. Potential for transfer of genetic material from the GMHP to other organisms

The potential for transfer of genetic material from 1507 maize to other organisms will be negligible as there are no sexually compatible wild or weedy relatives of *Zea mays* known to exist in the EU.

Transfer of genetic material from 1507 maize to bacteria is a negligible concern. There is no known mechanism for, or definitive demonstration of, DNA transfer from plants to microbes under natural conditions. Even if horizontal gene transfer were to take place, transfer of the *cry1F* or *pat* gene from 1507 maize does not represent a risk to human or animal health nor is it of consequence as a plant pest risk. The *nptII* gene coding for resistance to the antibiotic kanamycin is not present in 1507 maize.

24. Information on any harmful effects on human health and the environment, arising from the genetic modification

(i) Toxicity

The genetic modification in 1507 maize results in expression of CRY1F and PAT proteins. The CRY1F protein has specific toxicity against certain lepidopteran insect pests (target organisms). An acute toxicity study with CRY1F protein in mice has confirmed the safety of the CRY1F protein to human and animal health. No mortality, toxicity or adverse clinical signs were observed at the highest dose tested of 5050 mg of test material per kg of body weight which was equivalent to 576 mg of pure CRY1F protein per kg of body weight. In addition, there is no evidence for CRY proteins originating from *Bacillus thuringiensis* to have harmful effects on the health of humans and animals.

The safety in terms of toxicity for the PAT protein has already been determined in detail 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. Toxicity studies carried out on rats and mice containing up to 50000 and 5000 mg/kg body weight respectively, have confirmed the absence of any adverse treatment-related clinical signs.

In addition, a poultry feeding study over a period of 42 days has been carried out confirming that there are no statistically significant differences on mortality, body weight gain or feed conversion between chickens fed a diet containing grain from 1507 maize or from non-GM maize.

(ii) Allergenicity

The most important factor to consider in assessing allergenic potential is whether the source of the gene being introduced into plants is known to be allergenic. Neither *Bacillus thuringiensis* (the source of the *cry1F* gene) nor *Streptomyces viridochromogenes* (the source of the *pat* gene) have a history of causing allergy. Also, both donor organisms are common soil bacteria.

The assessment of the allergenic potential of the CRY1F and PAT proteins has been made following the recommendations and the application of the decision-tree from FAO/WHO. The analyses have consisted of amino acid sequence comparison with known allergens, rapid degradation in simulated gastric fluids, relatively low level of expression, lack of glycosylation and thermolability. The results confirm that CRY1F and PAT proteins do not pose any significant risk of being a potential allergen.

(iii) Ecotoxicity studies

The absence of toxicity of the CRY1F protein to non-target and beneficial organisms has been thoroughly assessed in multiple dietary toxicity studies including green lacewing larvae, ladybird beetle, beneficial parasitic Hymenoptera *Nasonia vitripennis*, Monarch butterfly larvae, honey bees, earthworm, collembola and daphnia. In addition, levels of beneficial arthropods in field plots of 1507 maize were found to be comparable to those observed in non-GM maize.

25. Information on the safety of the GMHP to animal health, where the GMHP is intended to be used in animal feedstuff, if different from that of the recipient/parental organism(s)

A detailed safety evaluation concerning possible feed applications of 1507 maize and feed products derived from 1507 maize (processed and non-processed) has been carried out (**Annex 1 to Section 2** of notification **C/ES/01/01**).

The conclusions obtained confirm that feed products from 1507 maize are substantially equivalent to, nutritionally equivalent to, and as safe as, feed products derived from commercially available (non-GM) maize. This is based on compositional analyses comprising protein, fiber, carbohydrates and ash of 1507 maize forage and on compositional analyses comprising protein, fiber, carbohydrates, ash, minerals, fatty acids, amino acids, vitamins, secondary metabolites and anti-nutrients in grain samples from 1507 maize; the nutritional equivalence shown in a poultry feeding study; and, the detailed safety evaluation of the CRY1F and PAT proteins expressed in 1507 maize.

26. Mechanism of interaction between the GMHP and target organisms (if applicable), if different from that of the recipient/parental organism(s)

The mechanism of interaction between CRY1F protein expressed in 1507 maize and target organisms can be summarized as follows:

Maize expressed CRY1F protein consists of residues 1 to 605 of the native CRY1F sequence from *B. thuringiensis* sbsp. *aizawai*, with a single and conservative amino acid substitution (F to L at position 604). Upon ingestion of 1507 maize tissue by susceptible insects (target pests) the maize expressed CRY1F protein will reach the alkaline conditions of the insect gut where proteolytic processing of CRY1F protein by trypsin-like proteases may occur before it binds to specific receptors on the apical microvilli of epithelial midgut cells of the insect and the

CRY1F protein undergoes a conformational change that allows insertion into the membrane of the cell. Protein oligomerization will then occur with formation of pores in the membrane of the midgut cells of the insect causing osmotic cell lysis leading to insect death.

27. Potentially significant interactions with non-target organisms, if different from the recipient or parental organism(s)

There are no potentially significant changes in the interactions of 1507 maize with non-target organisms resulting from the genetic modification. As discussed in detail in **Point D.10.** of **Section 2** and **Points C.1.c** and **D.5.** of **Section 4** of the notification (**C/ES/01/01**), the specific biological activity of CRY1F and PAT proteins expressed in 1507 maize together with the absence of toxicity of CRY1F protein to non-target and beneficial organisms provides strong evidence for the absence of any significant toxicity to non-target organisms which may arise from exposure to 1507 maize.

28. Description of detection and identification techniques for the GMHP, to distinguish it from the recipient or parental organism(s)

The 1507 maize can be detected by placing small amounts of the glufosinate-ammonium herbicide on leaves of maize plants. Maize plants with the expression of the PAT protein will be those with leaves that do not show any necrosis at point of herbicide application. Alternatively, maize plants can be sprayed with glufosinate-ammonium herbicide, and those that survive will be expressing the PAT protein.

Plant parts of 1507 maize can also be analysed by ELISA to detect the proteins expressed by the *cry1F* and *pat* genes. Additionally, an insect bioassay with sensitive lepidopteran insect species such as European corn borer (*Ostrinia nubilalis*) can be used to identify maize plants expressing the CRY1F protein.

A PCR detection method to confirm the molecular identity of 1507 maize has been developed. The PCR method can also be used to confirm presence of 1507 maize for the purposes of labelling products containing or derived from 1507 maize. The detection method for 1507 maize has been provided to the Spanish regulatory authority. The PCR detection method and reference material will be available to a central body operating under the auspices of the EU regulatory authorities and the European Commission.

A combination of the techniques described above can be used to identify 1507 maize.

INFORMATION ON THE POTENTIAL ENVIRONMENTAL IMPACT FROM THE RELEASE OF THE GMHP

29. Potential environmental impact from the release or the placing on the market of GMO(s) (Annex II, D2 of Directive 2001/18/EC), if different from a similar release or placing on the market of the recipient or parental organism(s)

A comparison of the characteristics of 1507 maize with those of commercially available (non-GM) maize under corresponding conditions of use has assisted in identifying any particular potential environmental impact from the placing on the market of 1507 maize. A detailed assessment has been carried out in accordance to Annex II, D2 of Directive 2001/18/EC (**Points D.1. to D.9. of Section 4** of notification **C/ES/01/01**), which has led to the following conclusions:

- There is negligible likelihood for 1507 maize to become environmentally persistent or invasive giving rise to any weediness;
- Expression of CRY1F and PAT proteins in 1507 maize does not confer any selective advantage outside the agricultural environment;
- There are no wild relatives of maize in the EU and the genetic modification in 1507 maize does not introduce any selective advantages to maize plants outside managed agricultural environments;
- The potential reduction of the control of certain lepidopteran insect pests if the target insect pests develop resistance to CRY1F protein as expressed in 1507 maize has been identified as the only potential risk resulting from the interaction of 1507 maize with target organisms;
- The placing on the market of 1507 maize will result in negligible immediate and/or delayed environmental impact resulting from direct and indirect interactions of 1507 maize with non-target organisms;
- The genetic modification in 1507 maize does not introduce any new compounds known to cause, or expected to cause, any potential immediate and/or delayed effects on human health;
- The genetic modification in 1507 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 consumption of 1507 maize and any animal feed products derived from it will result in no adverse consequences for the feed/food chain;
- The genetic modification in 1507 maize will not cause any possible immediate and/or delayed effects on biogeochemical processes; and,
- The specific cultivation, management and harvesting techniques used for the 1507 maize are identical to those used for other commercially available (non-GM) maize, with the exception of the application of the IRM strategy in the context of

product stewardship and of the monitoring plan proposed specifically for the cultivation of 1507 maize, thereby limiting the occurrence of any possible immediate and/or delayed, direct and indirect impacts to human and animal health or the environment.

30. Potential environmental impact of the interaction between the GMHP and target organisms (if applicable), if different from that of the recipient or parental organism(s)

The environmental risk assessment (e.r.a.) for the placing on the market of 1507 maize has concluded that there might be a limited potential environmental impact derived from the interaction between 1507 maize and target organisms consisting of the potential development of resistance to CRY1F protein as expressed in 1507 maize within the target insect pest population. However, and in order to ensure that placing on the market of 1507 maize poses negligible risk to the environment, appropriate monitoring and risk management proposals have been developed for application following approval of 1507 maize.

31. Possible environmental impact resulting from potential interactions with non-target organisms, if different from that of the recipient or parental organism(s)

a) Effects on biodiversity in the area of cultivation

Placing on the market of 1507 maize will result in negligible immediate and/or delayed environmental impact resulting from potential interactions of 1507 maize with non-target organisms. In particular, no significant adverse effects on biodiversity will occur in the area of cultivation. This conclusion is based on the information presented in **Section 2** and **Section 4** of the notification (C/ES/01/01), and, in particular, on the results obtained from:

i) ecotoxicity studies showing no toxic effects of CRY1F protein on a range of non-target organisms and beneficial insects, such as green lacewing larvae (*Chrysoperla carnea*), the ladybird beetle (*Hippodamia convergens*), the beneficial parasitic Hymenoptera *Nasonia vitripennis*, honey bee larvae (*Apis mellifera*), earthworms (*Eisenia foetida*), collembola (*Folsomia candida*), the cladoceran aquatic invertebrate *Daphnia magna*, and, the non-target Lepidoptera Monarch butterfly;

ii) field studies showing no significant adverse effects of 1507 maize in the population of non-target arthropods;

iii) a detailed non-target exposure and risk assessment for the placing on the market of 1507 maize.

Such evidence, together with the specificity of the biological and biochemical activities of the CRY1F and PAT proteins expressed in 1507 maize, confirms that 1507 maize will have negligible effects on the dynamics or biodiversity of populations of non-target organisms in the area of cultivation.

b) Effects on biodiversity in other habitats

There will be negligible effects on the dynamics or biodiversity of populations of non-target organisms in other habitats. This is based on the fact that maize has no wild relatives in the EU and the genetic modification in 1507 maize has not altered the lack of potential for dispersal or weediness of maize. In particular, the genetic modification in 1507 maize has not introduced any selective advantages outside managed agricultural habitats. Also, the lack of ability by the genetic modification in 1507 maize to transfer genetic material to other organisms has not been altered, and, the genetic modification in 1507 maize has not introduced any new compounds known to be toxic, allergenic or harmful to human or animal health.

c) Effects on pollinators

As mentioned in **Point B.31.b** above, expression of CRY1F protein in 1507 maize has no toxic effects on non-target organisms and beneficial insects and it is highly specific against certain lepidopteran insect pests that feed on maize plant tissues, such as the European corn borer and *Sesamia* spp. As a result, cultivation of 1507 maize will have negligible effects on pollinators.

d) Effects on endangered species

The geographic distribution, habitat preferences and host plants of endangered insect species are not predominantly associated with agricultural environments such as those used for maize cultivation. The habitat preferences for the non-target endangered species include marshy, arid, semiarid or alpine (> 1000 m elevation) environments. Such habitat preferences and distribution confirm that endangered species will not be significantly affected from cultivation of 1507 maize.

In addition, a recent publication by Hellmich *et al.* (2001) has confirmed that CRY1F protein from 1507 maize is relatively non-toxic to Monarch larvae compared to other CRY proteins and that pollen from 1507 maize will have no acute effects on Monarch butterfly larvae in field settings. These results further confirm the high degree of specificity of the biological activity of CRY1F protein expressed in 1507 maize against certain target lepidopteran insect pests.

C. INFORMATION RELATING TO PREVIOUS RELEASES

32. History of previous releases 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/IT/98/19

(b) Conclusions of post-release monitoring

The 1507 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics. In particular, there was no evidence of enhanced weediness of 1507 maize.

(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.

(a) Notification number

B/FR/99.03.09

(b) Conclusions of post-release monitoring

The 1507 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics. In particular, there was no evidence of enhanced weediness of 1507 maize.

(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.

(a) Notification number

B/ES/02/11

(b) Conclusions of post-release monitoring

The 1507 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics. In particular, there was no evidence of enhanced weediness of 1507 maize.

(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.

33. History of previous releases carried out inside or outside the Community by the same notifier

(a) Release country

Argentina.

(b) Authority overseeing the release

Secretary of Agriculture.

(c) Release site

Pergamino area, 3 sites; Buenos Aires Province.

(d) Aim of the release

Efficacy trials and hybrid registration.

(e) Duration of the release

One season.

(f) Aim of post-release monitoring

Control of potential volunteers.

(g) Duration of post-release monitoring

One season.

(h) Conclusions of post-release monitoring

The 1507 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics. In particular, there was no evidence of enhanced weediness of 1507 maize.

(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.

(a) Release country

Brazil

(b) Authority overseeing the release

CTNBio

(c) Release site

One site.

(d) Aim of the release

Research.

(e) Duration of the release

One season.

(f) Aim of post-release monitoring

Control of potential volunteers.

(g) Duration of post-release monitoring

One season.

(h) Conclusions of post-release monitoring

The 1507 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics. In particular, there was no evidence of enhanced weediness of 1507 maize.

(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.

(a) Release country

Chile.

(b) Authority overseeing the release

Ministry of Agriculture.

(c) Release site

Four sites.

(d) Aim of the release

Research.

(e) Duration of the release

One season.

(f) Aim of post-release monitoring

Control of potential volunteers.

(g) Duration of post-release monitoring

One season.

(h) Conclusions of post-release monitoring

The 1507 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics. In particular, there was no evidence of enhanced weediness of 1507 maize.

(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.

(a) Release country

South Africa.

(b) Authority overseeing the release

Ministry of Agriculture.

(c) Release site

One site.

(d) Aim of the release

Research.

(e) Duration of the release

One season.

(f) Aim of post-release monitoring

Control of potential volunteers.

(g) Duration of post-release monitoring

One season.

(h) Conclusions of post-release monitoring

The 1507 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics. In particular, there was no evidence of enhanced weediness of 1507 maize.

(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.

(a) Release country

U.S.A.

(b) Authority overseeing the release

USDA and EPA.

(c) Release site

Multiple sites.

(d) Aim of the release

Research.

(e) Duration of the release

Five seasons.

(f) Aim of post-release monitoring

Control of potential volunteers.

(g) Duration of post-release monitoring

One season.

(h) Conclusions of post-release monitoring

The 1507 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics. In particular, there was no evidence of enhanced weediness of 1507 maize.

(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.

D. INFORMATION RELATING TO THE MONITORING PLAN – IDENTIFIED TRAITS, CHARACTERISTICS AND UNCERTAINTIES RELATED TO THE GMO OR ITS INTERACTION WITH THE ENVIRONMENT THAT SHOULD BE ADDRESSED IN THE POST COMMERCIALISATION MONITORING PLAN

A monitoring plan has been considered appropriate as part of the risk management strategy in order to minimize any potential risks from the placing on the market of 1507 maize including cultivation. The monitoring plan has been developed (**Section 5** of notification **C/ES/01/01**) based on the conclusions obtained from the environmental risk assessment (e.r.a.) for the placing on the market of 1507 maize, which has been elaborated as a separate document (**Section 4** of notification **C/ES/01/01**) in accordance to Annex II of Directive 2001/18/EC. The monitoring plan will be applied following approval for the placing on the market of 1507 maize.

As summarized in **Point B.29.** above, the conclusions from the e.r.a. confirm that there is no risk to human and animal health or the environment arising from the placing on the market of 1507 maize. In addition, there is no significant risk to non-target organisms. However, the e.r.a. identified a limited potential risk posed by the cultivation of 1507 maize due to the potential development of resistance to CRY1F protein as expressed in 1507 maize within the target insect pest population. Therefore, and in order to ensure that placing on the market of 1507 maize poses negligible risk, appropriate monitoring and risk management plans have been developed and proposed in the context of product stewardship.

The case-specific monitoring plan for 1507 maize will form part of the Insect Resistance Management (IRM) proposal entitled ‘Resistance management proposal for genetically modified insect-protected 1507 maize expressing the Cry1F insecticidal protein from *Bacillus thuringiensis* sbsp. *aizawai*’ and described in **Annex 3** of the e.r.a (see **Section 4** of notification **C/ES/01/01**). The IRM proposal has been developed in order to maintain the efficacy of the CRY1F protein in 1507 maize, thereby sustaining the environmental benefits of the *Bacillus thuringiensis* (Bt) technology. It is based on the following five principles: (i) deploying products with an effective dose of Bt protein; (ii) maintaining adequate refuges; (iii) monitoring product performance; (iv) educating seed distributors and farmers; and, (v) continuing to conduct research.

In addition and in the framework of general surveillance, the notifiers will encourage growers to report any observed adverse effects on non-target arthropod populations. Subject to further discussion with the relevant national Competent Authorities and associated bodies, the co-ordination of a surveillance network, where appropriate and necessary, within the framework of their national routine surveillance programmes, for example the monitoring of agricultural cultivars or plant protection products, may also take place.