APPLICATION FOR AUTHORISATION OF
GENETICALLY MODIFIED PLANTS
AND DERIVED FOOD AND FEED
IN ACCORDANCE WITH REGULATION (EC) No 1829/2003

4114 MAIZE

(DP-ØØ4114-3 MAIZE)

EFSA-GMO-NL-2014-123

PART VII – SUMMARY

Submitted by:

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PART VII – SUMMARY

1. GENERAL INFORMATION

1.1 Details of application

a) Member State of application The Netherlands
b) Application number [*To be provided*]
c) Name of the product (commercial and other names) The product described in this application is 4114 maize. In accordance with Commission Regulation (EC) 65/2004 and the OECD guidance for the designation of a unique identifier for transgenic plants (ENV/JM/MONO(2002)7), the unique identification code assigned to 4114 maize is: DP-ØØ4114-3
d) Date of acknowledgement of valid application [*To be provided*]

1.2. Applicant

a) Name of applicant	
Pioneer Hi-Bred International, Inc. as represented by Pioneer Overseas Corporation	
b) Address of applicant	As represented by:
Pioneer Hi-Bred International, Inc.	Pioneer Overseas Corporation
7100 NW 62nd Avenue	Avenue des Arts, 44
P.O. Box 1014	B-1040 Brussels
Johnston, IA 50131-1014 (U.S.A.)	Belgium
c) Name and address of the representative of the applicant established in the Union (if the applicant is not established in the Union)	
Same as applicant	

1.3. Scope of the application

(a) Genetically modified food X Food containing or consisting of genetically modified plants X Food produced from genetically modified plants or containing ingredients produced from genetically modified plants (b) Genetically modified feed X Feed containing or consisting of genetically modified plants X Feed produced from genetically modified plants (c) Genetically modified plants for food and feed use X Products other than food and feed containing or consisting of genetically modified plants with the exception of cultivation □ Seeds and plant propagating material for cultivation in the Union

1.4. Is the product or the uses of the associated plant protection product(s) already authorised or subject to another authorisation procedure within the Union?

Yes [x] Regulatory compliance in the framework of Article 10 of Regulation (EC) No 396/2005 on the establishment of a maximum residue levels (MRL) for the use of glufosinate in genetically modified maize containing the PAT gene is authorised according to Commission Regulation (EC) No 149/2008.	**No** [ ]

1.5. Has the GM plant been notified under Part B of Directive 2001/18/EC?

Yes [ ]	**No** [x] Agronomic performance, protein expression, composition, efficacy, yield and ecological studies on 4114 maize have been conducted in the US and Canada since 2006 in multiple locations. The risk assessment and risk characterisation of 4114 maize summarised in Part VII have been concluded on the basis of the data obtained from these studies.

1.6. Has the GM plant or derived products been previously notified for marketing in the Community under Part C of Directive 2001/18/EC?

Yes [ ]	**No** [x]

1.7. Has the product been subject to an application and/or authorised in a third country either previously or simultaneously to this application?

Yes [x]	**No** [ ]
Applications concerning all uses of 4114 maize, including cultivation of 4114 maize seed products,	

have been submitted in the US, Canada and Japan. Applications for an authorisation for food and feed use have been submitted in several other countries around the world where products of breeding stack combinations are regulated.

1.8. 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.), which is extensively cultivated and has a long history of safe use. The 4114 maize has been produced through *Agrobacterium*-mediated transformation and expresses the Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins.

The Cry1F protein is encoded by a plant codon-optimised gene from *Bacillus thuringiensis* and confers resistance against certain lepidopteran pests, such as the European corn borer (*Ostrinia nubilalis)* and the Mediterranean corn stalk borer (*Sesamia nonagrioides*). The binary Cry34Ab1 and Cry35Ab1 proteins are encoded by plant codon-optimised genes from *Bacillus thuringiensis*, and together confer protection against corn rootworm larvae (Coleoptera: Chrysomelidae; *Diabrotica* spp.). The PAT protein is encoded by a plant codon-optimised gene from *Streptomyces viridochromogenes*, and confers tolerance to the application of glufosinate-ammonium herbicides.

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

The types of products planned to be placed on the market according to the authorisation applied for include 4114 maize for all food and feed uses, and for all food, feed and processed products derived from 4114 maize in accordance with Regulation (EC) No 1829/2003. In addition, this application requests authorisation for import and processing of 4114 maize in accordance with Part C of Directive 2001/18/EC. However, this application does not include authorisation for the cultivation of 4114 maize seed products in the EU.

c) Intended use of the product and types of users

The 4114 maize products placed on the market will be used in a manner consistent with current uses of commercial maize grain and maize products. The 4114 maize will undergo existing methods of production and manufacturing used for commercial maize. No novel method of production and manufacturing is envisaged.

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

Safety evaluation of 4114 maize has shown that no specific instructions and/or recommendations for use, storage and handling of 4114 maize are necessary. Therefore, 4114 maize can be used, stored and handled in the same way as is currently done for commercial maize. Labelling of 4114 maize products will be carried out in accordance with Community law.

e) Geographical areas within the Union to which the product is intended to be confined under the terms of the authorisation applied for

4114 maize will be used throughout the European Union as any other commercial maize products.

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

The application does not cover cultivation of 4114 maize in the European Union. The 4114 maize will be used throughout the European Union as any other commercial maize products.

g) Any proposed packaging requirements

The packaging, handling, and storage systems that are currently used for commercial maize will apply. The 4114 maize products will be packaged in the same manner as other commercial maize products.

h) Any proposed labelling requirements in addition to those required by other applicable EU legislation than Regulation (EC) No 1829/2003 and when necessary a proposal for specific labelling in accordance with Article 13(2) and (3), Article 25(2)(c) and (d) and Article 25(3) of Regulation (EC) No 1829/2003. In the case of products other than food and feed containing or consisting of genetically modified plants, a proposal for labelling which complies with the requirements of point A(8) of Annex IV to Directive 2001/18/EC must be included.

See proposal below.

1.- PROPOSAL FOR THE LABELLING OF 4114 MAIZE FOOD PRODUCTS ACCORDING TO ARTICLES 12 AND 13 OF REGULATION (EC) No 1829/2003

In accordance with Article 12(2) of Regulation (EC) No 1829/2003, labelling will apply to foods containing material which contains, consists of or is produced from 4114 maize in a proportion at or higher than 0,9 per cent of the food ingredients considered individually or of the entire food if consisting of a single ingredient.

In accordance with Article 13 of Regulation (EC) No 1829/2003, and without prejudice to the other requirements of Community law concerning the labelling of foodstuffs, foods containing, consisting of, produced from, or containing ingredients produced from 4114 maize should be labelled as follows:

(a) where the food consists of more than one ingredient, the words 'genetically modified' or 'produced from genetically modified maize' will appear in the list of ingredients provided for in Article 6 of Directive 2000/13/EC in parentheses following the ingredient concerned;
(b) where the ingredient is designated by the name of a category, the words 'contains genetically modified maize' or 'contains (name of ingredient) produced from genetically modified maize' will appear in the list of ingredients;
(c) where there is no list of ingredients, the words 'genetically modified' or 'produced from genetically modified maize' will appear clearly on the labelling;
(d) the indications referred to in (a) and (b) 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 will appear clearly on the labelling;
(e) 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 referred to above will 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 (EC) No 1829/2003 would need to be specified on the label of 4114 maize food products as 4114 maize has been shown to be equivalent to non-GM control maize in composition; nutritional value and nutritional effects; intended use; health characteristics; and, the genetic modification in 4114 maize does not give rise to any safety concerns.

2.- PROPOSAL FOR THE LABELLING OF 4114 MAIZE FEED PRODUCTS ACCORDING TO ARTICLES 24

AND 25 OF REGULATION (EC) No 1829/2003

In accordance with Article 24(2) of Regulation (EC) No 1829/2003, labelling will apply to feed containing material which contains, consists of or is produced from 4114 maize in a proportion at or higher than 0,9 per cent of the feed and of each feed of which it is composed.

In accordance with Article 25 of Regulation (EC) No 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) No 1829/2003, *i.e.* 4114 maize for feed use, and feed containing, consisting of or produced from 4114 maize, should be labelled as follows:

(a) where the feed contains or consists of 4114 maize, or where 4114 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.
(b) where the feed is produced from 4114 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 (EC) No 1829/2003 would need to be specified on the label of 4114 maize feed products as 4114 maize has been shown to be equivalent to non-GM control maize in composition; nutritional value and nutritional effects; intended use; health characteristics; and, the genetic modification in 4114 maize does not give rise to any safety concerns.

3.- PROPOSAL FOR THE LABELLING OF PRODUCTS CONSISTING OF, OR CONTAINING, 4114 MAIZE ACCORDING TO ARTICLE 4, B(6) OF REGULATION (EC) No 1830/2003 AND ANNEX IV OF DIRECTIVE 2001/18/EC

As specified in Section **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 4114 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 Section **A.2.** of Annex IV of Directive 2001/18/EC (name and full address of the notifier established in the Community who is responsible for the placing on the market);
iv) An indication on how to access the information in the publicly accessible part of the register.

i) Estimated potential demand

a) In the EU

Extra-EU maize imports vary from year to year depending on annual EU maize harvest yields; maize import figures for the current and following year are generally influenced by maize harvested in the EU in that given year. In 2013, a total of 6.4 million metric tonnes of maize were imported into the EU, 63% from Ukraine and 20% from Brazil. Spain is the most important market for extra-EU maize imports with a share of 25% in 2013. It is estimated that the Netherlands followed with a share around 23%, then Portugal and Italy with a share around 14% and 12%, respectively, while other countries individually contribute less than 7% to imports.

b) In EU export markets

The application does not cover cultivation of 4114 maize in the European Union.

g) Unique identifier in accordance with Regulation (EC) No 65/2004

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

Based on the conclusions from the environmental risk assessment of 4114 maize, no specific measures need to be taken in case of unintended release or misuse or for disposal and treatment. There are no sexually compatible wild plant species in Europe with which maize can cross-hybridise and maize plants cannot survive as a weed outside agricultural fields. The establishment of maize volunteer plants is therefore very unlikely.

In case of unintended release of 4114 maize, current agronomic measures taken to control other commercially available maize can be applied, such as use of mechanical means and selective use of herbicides (with exception of glufosinate-ammonium).

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

1. Complete name

a) Family name

Poaceae

b) Genus

Zea

c) Species

Z. mays L.

d) Subspecies

None

e) Cultivar/breeding line

4114

f) Common name

Maize, corn

2.2. Geographical distribution and cultivation of the plant, including the distribution within the Union

Maize is one of the most important crops worldwide with an annual cultivation area of more than 177 million hectares and an annual harvest of over 872 million tonnes (MT) of grain in 2012 (FAOSTAT, 2014). The cropping area within the 28 Member States of the European Union (EU-28) reached 9.8 million hectares for grain maize in 2012 and 5.0 million hectares for silage maize. The annual production quantity in the EU-28 was 59.9 MT of grain. By far the largest maize producer in the EU-28 is France (15.6 MT), followed by Italy (8.2 MT), Romania (5.9 MT), and Germany (5.5 MT) (EUROSTAT, 2014).

2.3. Information concerning reproduction

(i) Mode(s) of reproduction

Maize (*Zea mays* L.) is the only species usually included in the subspecies *mays* of the genus *Zea*, belonging to the Poaceae family. It is a highly domesticated annual crop with well-characterised phenotypic and genetic traits. It reproduces sexually by wind-pollination and being a monoecious species has separate male staminate (tassels) and female pistillate (silk) flowers. This allows natural outcrossing between maize plants but also enables the control of pollination in the production of hybrid seed. Typical for wind-pollinated plants, a large amount of excess maize pollen is produced for each successful fertilisation of an ovule on the ear. Wind movements across the maize field cause pollen from the tassel to fall on the silks of the same or adjoining plants. Measuring about 90 µm in diameter, maize pollen is the largest of any pollen normally disseminated by wind from a comparably low level of elevation.

(ii) Specific factors affecting reproduction

As a wind-pollinated, monoecious species, reproduction takes place by both self- and cross-pollination and fertilisation, with frequencies of each normally determined by proximity and other physical influences on pollen dispersal. Reproductive factors such as tasselling (pollen production), silking, and pollination are the most critical stages of maize development. Repeated cycles of self-pollination lead to homogeneity of the genetic characteristics within a single maize plant (inbred). Controlled cross-pollination of inbred lines from chosen genetic pools combines desired genetic traits resulting in a hybrid with improved agronomic performance and yield increase (heterosis effect). This inbred-hybrid concept and improved yield response is the basis of the modern maize seed industry. Maize varieties planted by EU farmers are almost entirely hybrid plants.

(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.

2.4. Sexual compatibility with other cultivated or wild plant species

In the EU, there are no other cultivated or wild plant species that are sexually compatible with maize. Maize plants intra-pollinate and transfer genetic material between maize except for certain popcorn varieties. The extent of pollination between maize depends upon wind patterns, humidity and temperature. Low humidity and high temperatures cause the pollen to become desiccated and unviable.

2.5. Survivability

a) Ability to form structures for survival or dormancy

During the domestication of maize, many significant agronomic attributes for cultivation have been gained, whilst maize has lost the 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

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 this has been identified as a major risk in limiting production of maize seed. Furthermore, maize is a C_4 plant and therefore its vegetative growth is sensitive to low temperatures. Chlorosis will occur at temperatures below 15 °C. The generative phase of maize is supported by short day conditions. The minimum temperature for germination of 8 to 10 °C restricts maize survival and reproduction capabilities mainly to the Central and Southern European geographical zones.

2.6. 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.
Pollen shedding from the tassels takes place over a period of 10 to 15 days. Pollen grains are round, heavy and contain a large amount of water, characteristics that limit their dispersal and attachment to plant surfaces, such as leaves. Generally, viability of shed pollen is 10 to 30 minutes, although it can remain viable for longer time under favourable conditions. However, dispersal of maize pollen tends to be limited as it is influenced by the large size and rapid settling rate of the pollen. Deposition of maize pollen has been found to rapidly decline within 30 m from the source, with very low dispersal remaining at distances farther than 30-50 m from the source.

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. Because of its highly domesticated nature, maize seed requires the semi-uniform soil conditions resulting from cultivation in order to germinate and establish in agricultural habitats.

2.7. Geographical distribution within the Union of the sexually compatible species

Because of its many available cultivars, maize can grow in a wide range of climatic conditions. However, survival and reproduction in maize is limited by cool conditions. Practically no maize can be cultivated where the mean mid-summer temperature is <19 °C or where the average night temperature is <13 °C. The majority of maize is produced between latitudes 30 and 55 degrees, with a relatively small amount grown at latitudes higher than 47 degrees anywhere in the world. Summer rainfall of 15 cm is the lower limit for maize production without irrigation. There is no upper limit of rainfall for growing maize, although excess rainfall will decrease yields. There are no wild plant species that are sexually compatible with maize in the EU.

2.8. 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 normally grown in the EU.

2.9. 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 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 or derived products of maize are not considered to have toxic effects on humans, animals and other organisms.

3. MOLECULAR CHARACTERISATION

3.1. Information relating to the genetic modification

(a) Description of the methods used for the genetic modification

4114 maize was produced by *Agrobacterium tumefaciens*-mediated transformation of a Pioneer proprietary maize line.

(b) Nature and source of the vector used

The plasmid PHP27118 used for transformation intended for development of 4114 maize was constructed using the plasmid pSB1 (Komari *et al.*, 1996) as a backbone. The number of nucleotides in the plasmid pSB1 is 36,909 bp. The plasmid PHP27118 is a binary vector that includes the cassettes for expression of *cry*1F, *cry*34Ab1, *cry*35Ab1, and *pat* genes, which were isolated from the corresponding plasmids used for the development of maize events 1507 and 59122.

(c) Source of donor DNA, size and intended function of each constituent fragment of the region intended for insertion

*cry*1F, *cry*34Ab1, *cry*35Ab1 and *pat* genes and regulatory elements inserted in 4114 maize are the same as the genes inserted in 1507 and 59122. These genes have been present in commercial maize varieties such as 1507, 59122, and 1507x59122 maize since 2003, 2006, and 2006, respectively. These commercial lines are currently licensed broadly across the seed industry without reported adverse effects.

3.2. Information relating to the genetically modified plant

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

The Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins expressed in 4114 maize are identical to the proteins expressed in previously approved maize events 1507, 59122, and 1507x59122 maize, conferring resistance to certain lepidopteran and coleopteran pests and tolerance to the use of glufosinate-ammonium herbicides. The PAT protein was also used as a selectable marker during transformation.

- The Cry1F protein acts to control certain lepidopteran insect pests, such as the European corn borer (*Ostrinia nubilalis*) and the pink borer (*Sesamia* spp.);
- The binary Cry proteins Cry34Ab1 and Cry35Ab1 act together in the control of corn rootworm larvae (Coleoptera: Chrysomelidae; *Diabrotica* spp.);
- Expression of the PAT protein, a phosphinotricine acetyltransferase, confers tolerance to the application of glufosinate-ammonium herbicide through acetylation of the herbicide.

No other new traits have been introduced into 4114 maize and, in particular, no trait for antibiotic resistance is present in 4114 maize. As discussed in detail throughout the application, these characteristics of 4114 maize have been confirmed by molecular characterisation, protein expression analysis, agronomic performance and comparison of 4114 maize compositional data to non-GM control maize.

3.2.2. Information on the nucleic acid(s) sequences actually inserted or deleted

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

The results of the molecular characterisation described in this application support the conclusion that 4114 maize contains a single copy of intact inserted DNA. Southern blot analysis demonstrated that 4114 maize does not contain other fragments from the vector.

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

Based on Southern blot analysis and sequencing, the single-copy insert in 4114 was determined to contain the whole T-DNA region of plasmid PHP27118 with minor truncations at the border regions. No functional sequences have been deleted during T-DNA integration in 4114 maize.

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

The 4114 maize insert is integrated into the maize nuclear genome as confirmed by the inheritance of the insert through conventional crosses and by the molecular characterisation of 4114 maize by Southern blot and characterisation of the flanking sequences through BLAST searches.

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

A detailed molecular characterisation by Southern blot analysis has confirmed the copy number, structure and organisation of the insert in 4114 maize. Based on Southern blot analysis and sequencing, the single-copy insert in 4114 was determined to contain the whole T-DNA region of plasmid PHP27118 with minor truncations at the border regions. No functional sequences have been deleted during T-DNA integration in 4114 maize.

e) In the 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.

3.2.3. Information on the expression of the insert

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

Field studies have been carried out in order to estimate the level of expression of the insert-encoded proteins in 4114 maize. Key plant tissues were collected from the plants at different developmental stages across the growing season. Protein concentrations were measured using Enzyme Linked Immunosorbent Assay (ELISA) systems developed for each protein. The results of the field studies have shown that the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins were generally expressed throughout the different plant tissues and developmental stages of 4114 maize; the PAT protein was not detected in grain.

b) Parts of the plant where the insert is expressed

Field tests have shown that the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins are expressed in different plant tissues throughout maize development; the PAT protein was not detected in grain.

3.2.4. Genetic stability of the insert and phenotypic stability of the genetically modified plant

The genetic stability of the insert and phenotypic stability and segregation of the introduced traits was evaluated on five maize generations. Event-specific PCR analysis and phenotypic evaluation

data demonstrated Mendelian inheritance of the 4114 insert in the five generations and confirmed the stability of the transgenic insertion in 4114 maize.

3.2.5. Information on how the genetically modified plant differs from the recipient plant in:

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

4114 maize does not differs from conventional maize in this respect.

(b) Dissemination

4114 maize does not differs from conventional maize in this respect.

(c) Survivability

4114 maize does not differs from conventional maize in this respect.

(d) Other differences

Not applicable.

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

a) Plant to bacteria gene transfer

The genetic modification in 4114 maize does not change the inability of maize to transfer genetic material to bacteria. In particular, no sequences are present on the inserted regions that could potentially be involved in transfer of genetic material between maize and bacteria.

b) Plant to plant gene transfer

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. The extent of pollination between maize will depend upon wind patterns, humidity and temperature. Potential for gene transfer is therefore limited to other maize grown in culture. In addition, the genetic modification in 4114 maize does not introduce any selective advantages to maize plants outside the agricultural environment.

It should be noted that this application is for authorisation of 4114 maize for all food and feed uses, and for all food, feed and processed products derived from 4114 maize, and not for cultivation of 4114 maize seed products. Any plant to plant gene transfer is therefore limited to only occasional unintentional releases.

4. COMPARATIVE ANALYSIS

4.1 Choice of the conventional counterpart and additional comparators

The comparator chosen for the safety evaluation of 4114 maize consists of non-GM near-isogenic control maize. Wherever possible, data on other commercial non-GM maize hybrids have also been used in the comparisons with 4114 maize.

4.2 Experimental design and statistical analysis of data from field trials for comparative analysis

The field phase of this study was conducted during the 2011 growing season at six sites in the United States, and during the 2012 growing season at four sites (three sites in the United States and one site in Canada), which were selected on the basis of their inclusion in the commercial maize-growing regions of North America. Each site utilized a randomized complete block design

and contained four blocks. Each block contained the following entries: conventional herbicide-treated (CHT) 4114 maize, intended herbicide-treated (IHT) 4114 maize, non-genetically modified (non-GM) near-isoline CHT control maize (referred to as control maize), and three of six non-GM CHT commercial reference maize lines per study (12 lines in total over the two field studies). Samples were collected for nutrient composition analysis at all sites, and consisted of forage (R4 growth stage) and grain (R6 growth stage).

Statistical analysis was done according to the EFSA Guidelines using difference and equivalence testing.

4.3 Selection of material and compounds for analysis

Samples were analyzed for the following key nutritional components in accordance with OECD guidelines for the assessment of genetically modified maize: the forage assessment included proximates, fiber, and mineral analytes; the grain assessment included proximates, fiber, fatty acid, amino acid, mineral, vitamin, secondary metabolite, and anti-nutrient analytes. There were only a few analytes that showed statistically significant differences or non-equivalences, however all of the data fell within the range of natural variation.

4.4 Comparative analysis of agronomic and phenotypic characteristics

4114 maize has been tested at different locations across key maize growing regions of North America for the major agronomic and phenotypic characteristics in maize The agronomic data obtained support the conclusion that there are no unexpected agronomic differences between 4114 maize and non-GM control maize with comparable genetic background.

It should be noted that this application is for authorisation of 4114 maize for all food and feed uses, and for all food, feed and processed products derived from 4114 maize, and not for cultivation of 4114 maize seed products.

4.5 Effect of processing

As discussed in this application, food and animal feed products derived from 4114 maize can be considered to be as safe as and nutritionally equivalent to food and animal feed products derived from commercial maize. Therefore, the specification of food and animal feed products from 4114 maize is equivalent to that of food and animal feed products derived from commercial maize.

5. Toxicology

a) Toxicological testing of newly expressed proteins

4114 maize contains the same transgenic proteins as expressed in 1507x59122 maize. Potential adverse effects to human and animal health from expression of the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins have previously been assessed taking into account the following considerations:

- the recipient organism and the donor organism for each protein have a history of safe use;
- the molecular and biochemical characteristics of the proteins do not indicate toxicity risks;
- the proteins have no significant amino acid sequence homology to known toxins or other biologically active proteins that could cause adverse effects to humans or animals;
- the proteins show no acute or repeated-dose oral toxicity to mammals.

No reports have appeared in the scientific literature up to now that invalidate these conclusions, nor did a re-analysis of the similarity searches with updated databases reveal any safety concerns. Furthermore, there is no evidence of potential interactions between the different insert-encoded

proteins in 4114 maize that would affect the safety of this combined trait maize. In addition, the low concentration of these proteins in maize tissues and their rapid digestibility in simulated digestive fluids provide further assurance for the safety of the consumed 4114 maize products. It is therefore highly unlikely that the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins will cause any adverse effects to human and animal health.

b) Testing of new constituents other than proteins

Not applicable as the genetic modification in 4114 maize does not give rise to the expression of any new constituents other than the transgenic proteins.

c) Information on natural food and feed constituents

Detailed analyses of 4114 maize have demonstrated that the composition of 4114 maize is equivalent to that of control maize. In addition, the results obtained in 90-day oral toxicity feeding studies with 4114 maize in rats and those obtained in a 42-day poultry feeding study with 4114 maize provide further confirmation of the safety of the natural food and feed constituents from 4114 maize and nutritional equivalence between 4114 maize and non-GM control maize.

d) Testing of the whole GM food/feed

The evaluation of the nutrient composition of 4114 maize has confirmed that it is equivalent to non-GM control maize with comparable genetic background. A 90-day oral toxicity feeding study in rats fed 4114 maize has not revealed any adverse effects.

A poultry feeding study over a period of 42 days has confirmed that that there are no diet-related differences in mortality, body weight gain, feed efficiency, carcass yield and organ yield between chickens fed a diet containing grain from 4114 maize or a diet containing grain from non-GM control maize.

6. ALLERGENICITY

a) Assessment of allergenicity of the newly expressed proteins

In accordance with a weight-of-evidence approach, which accounts for a variety of factors and experimental approaches for an overall assessment of the allergenic potential of the new proteins, the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins were evaluated for their allergenic potential through:

- assessing the allergenicity potential of the source of the genes;
- homology searches against allergen databases;
- *in vitro* simulated digestibility studies;
- analysis of protein glycosylation and heat stability.

No reports have appeared in the scientific literature up to now that invalidate these conclusions, nor did a re-analysis of the similarity searches with updated allergen databases reveal any concerns. The results obtained confirm that the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins expressed in 4114 maize are highly unlikely to be allergenic.

b) Assessment of allergenicity of the whole GM plant

Maize has a long history of safe use as food and feed in the EU and is not considered to cause significant food allergies. Furthermore, the newly expressed proteins in 4114 maize are highly unlikely to be allergenic.

7. NUTRITIONAL ASSESSMENT

a) Nutritional assessment of GM food

Composition analysis of grain from 4114 maize has shown that the content of protein, fiber, carbohydrates, fat, ash, minerals, fatty acids, amino acids, vitamins, secondary metabolites and anti-nutrients is equivalent to that found in grain from non-GM control maize with comparable genetic background. Therefore, 4114 maize can be considered nutritionally equivalent to non-GM control maize. Nutritional equivalence between 4114 maize and non-GM control maize with comparable genetics has also been shown in a poultry feeding study where chickens were fed either maize grain over a 42-day period.

In conclusion and taking into account the anticipated dietary intake of 4114 maize products, consumption of 4114 maize foods or feed will not have any adverse nutritional impact.

b) Nutritional assessment of GM feed

As evaluated in Section **7.b)** above, consumption of 4114 maize feed will not give rise to any adverse nutritional impact.

8. EXPOSURE ASSESSMENT – ANTICIPATED INTAKE/EXTENT OF USE

The nutritional assessment has concluded that 4114 maize is nutritionally equivalent to non-GM control maize. In addition, the use of 4114 maize food and feed will not be different from that of commercially available maize food and feed. Exposure of animals and humans to the transgenic proteins in 4114 maize was shown to be negligible.

Therefore, post-market monitoring of GM food and GM feed products containing, consisting of or derived from 4114 maize is not necessary.

9. RISK CHARACTERISATION

The interaction between the transgenic proteins expressed in 4114 maize and target organisms is restricted to the interaction between Cry1F, and Cry34Ab1/Cry35Ab1 proteins and certain lepidopteran and coleopteran pests, respectively. The mode of action of CRY proteins is well known and is based on the formation of ion channels in the membrane of insect epithelial midgut cells, following specific receptor binding. This leads to cell homeostasis and insect death.

Any significant interactions with these insects are, however, limited to those countries where cultivation of the 4114 maize will be authorised. The cultivation of 4114 maize in the EU is not part of the scope of this application.

10. POST-MARKET MONITORING ON THE GENETICALLY MODIFIED FOOD OR FEED

A thorough risk assessment has confirmed that 4114 maize is comparable to any commercial maize and no safety concerns are identified. Therefore, post-market monitoring of GM food and GM feed products containing, consisting of or derived from 4114 maize is not necessary.

11. ENVIRONMENTAL ASSESSMENT

11.1 Mechanism of interaction between the GM plant and target organisms

The scope of this application does not include authorisation for the cultivation of 4114 maize seed products in the EU, therefore any interactions between the GM plant and target insects will be limited. Exposure to the environment from the import of 4114 maize will be limited to unintended release of 4114 maize, e.g. via spillage during transportation of the grain.

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

a) Persistence and invasiveness

There is negligible likelihood for 4114 maize to become environmentally persistent or invasive giving rise to any weediness. The cultivation of 4114 maize in the EU is not within the scope of this application.

Furthermore, cultivated maize does not possess any trait for weediness and the expression of the insert-encoded proteins in 4114 maize does not introduce new traits for weediness. Maize is a highly domesticated crop and cannot survive without human intervention.

b) 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 introduced by the genetic modification in 4114 maize do not confer any selective advantage to the plants in the natural environment, *i.e.* outside the agricultural environment.

In conclusion, expression of the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in 4114 maize does not confer any selective advantage outside the agricultural environment.

c) 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 such species. Potential for gene transfer is therefore limited to other maize grown in culture. Cultivation of 4114 maize is, however, not part of the scope of this application. The potential for gene transfer to other cultivated maize is, therefore, limited and the environmental risk of such gene transfer is negligible.

d) Interactions between the GM plant and target organisms

Considering the scope of this application, which does not included cultivation of 4114 maize in the EU, it is unlikely that any target organisms will be significantly exposed to the Cry proteins expressed in this maize. In the eventual case of such exposure, the environmental risks are limited.

e) Interactions of the GM plant with non-target organisms

Considering the scope of this application, which does not included cultivation of 4114 maize in the EU, it is unlikely that any non-target organisms will be significantly exposed to the Cry proteins

expressed in this maize. In the eventual case of an accidental release in the environment, the absence of any toxicity to humans or non-target animals of the insert-encoded proteins in 4114 maize, whether alone or in combination, indicates that any adverse effects on non-target organisms are highly unlikely.

f) Effects on human health

Maize has a long history of safe use in human food and animal feed. A detailed evaluation of the potential toxicity and allergenicity to humans of the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins as expressed in 4114 maize, has been carried out. As a result and in conclusion, 4114 maize does not express any known toxic or allergenic proteins. Therefore, consumption of 4114 maize or derived food products will result in no adverse effects on human health.

g) Effects on animal health

Consumption of 4114 maize or any derived food, feed and processed products will not result in any adverse effects on human or animal health. Therefore, use of 4114 maize as feed and consumption of any food, feed and processed products derived from 4114 maize will not result in adverse effects on animal health or the food/feed chain.

h) Effects on biogeochemical processes

Because of the natural ubiquity of the *cry* and *pat* genes and of the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in the soil environment, the specific biochemical activity of these proteins, and taking into account the scope of this application, which does not include cultivation, 4114 maize will not cause any significant immediate and/or delayed effects on biogeochemical processes.

i) Impacts of the specific cultivation, management and harvesting techniques

Not applicable as cultivation is not part of the scope of this application.

11.3 Potential interactions with the abiotic environment

The scope of this application does not include authorisation for the cultivation of 4114 maize seed products in the EU. Exposure to the environment from the import of 4114 maize will be limited to unintended release of 4114 maize. This can be controlled with current measures used to control unintended release of commercially available maize, such as use of mechanical means and selective use of herbicides (with the exception of glufosinate-ammonium). Moreover, maize cannot survive in the environment without human intervention. Therefore, the likelihood of adverse interactions with the abiotic environment is negligible.

11.4 Risk characterisation

The scope of this application does not include authorisation for the cultivation of 4114 maize seed products in the EU. Exposure to the environment from the import of 4114 maize will be limited to unintended release of 4114 maize. This can be controlled with current measures used to control unintended release of commercially available maize, such as use of mechanical means and selective use of herbicides (with the exception of glufosinate-ammonium). Moreover, maize cannot survive in the environment without human intervention. Therefore, the likelihood of adverse interactions with the abiotic environment is negligible.

12. ENVIRONMENTAL MONITORING PLAN

a) General (risk assessment, background information)

The scope of this application does not include authorisation for the cultivation of 4114 maize seed products in the EU. Exposure to the environment from the import of 4114 maize will be limited to unintended release of 4114 maize which can be controlled with current measures used to control unintended release of commercially available maize, such as use of mechanical means and selective use of herbicides (with the exception of glufosinate-ammonium).

A proposal for an environmental monitoring plan for 4114 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, and following the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified organisms and derived food and feed (EFSA, 2006).

b) 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.) carried out for this application for authorisation of genetically modified 4114 maize and derived food and feed in accordance with Regulation (EC) No 1829/2003.

The e.r.a. 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 4114 maize. Therefore, the risk to human and animal health or the environment from 4114 maize and any derived products is as negligible as for any commercial maize and any derived products.

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

In accordance with Annex VII of Directive 2001/18/EC and Council Decision 2002/811/EC establishing guidance notes supplementing Annex VII to Directive 2001/18/EC, case-specific monitoring should only be carried out in those cases where potential adverse effects have been identified in the e.r.a.

The e.r.a. concluded that the risk to human and animal health or to the environment from 4114 maize and any derived products is as negligible as for any commercial maize and any derived products. As a result, case-specific monitoring is not applicable for the use of 4114 maize for all food and feed purposes and the import and processing of 4114 maize.

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

In accordance with Council Decision 2002/811/EC, general surveillance is not based on a particular hypothesis and it should be used to identify the occurrence of unanticipated adverse effects of the GMO or its use for human and animal health and the environment that were not predicted in the risk assessment.

The scope of this application is for the authorisation of 4114 maize for all food and feed uses in accordance with Articles 3(1) and 15(1) of Regulation (EC) No 1829/2003 and for import and processing of 4114 maize in accordance with Part C of Directive 2001/18/EC. In this application we are not seeking approval for cultivation of 4114 maize seed products in the EU.

As discussed in detail in the e.r.a., exposure to the environment will be limited to unintended release of 4114 maize. However, such limited exposure is highly unlikely to give rise to any adverse effect and, if necessary, can be controlled with current measures used to control unintended release of commercially available maize, such as use of mechanical means and selective use of

herbicides (with the exception of glufosinate herbicides).

However and in order to safeguard against any adverse effects on human and animal health or the environment that were not anticipated in the e.r.a., general surveillance on 4114 maize will be undertaken for the duration of the authorisation.

e) Reporting the results of monitoring

Case-specific monitoring is not applicable for the use of 4114 maize for all food and feed purposes and the import and processing of 4114 maize. As a result, no case-specific monitoring is proposed for this application for authorisation of 4114 maize.

The applicant will inform the European Commission, without delay, of any adverse effects reported arising from the handling and use of imported 4114 maize. Furthermore, the applicant will submit an annual monitoring report to the European Commission including results of the general surveillance in accordance with the conditions of the authorisation. The report will include a scientific evaluation of the confirmed adverse effect, a conclusion of the safety of 4114 maize and, as appropriate, any measures that were taken to ensure the safety of human and animal health or the environment.

13. DETECTION AND IDENTIFICATION TECHNIQUES FOR THE GM PLANT

A PCR-based quantitative event-specific detection method is available for 4114 maize and has been submitted for validation to the European Union Reference Laboratory (EURL) for GM Food and Feed (Joint Research Centre, Italy). This method was *in-house* validated by the applicant to demonstrate its performance on 4114 maize, in accordance with the requirements of the EURL/ENGL Guidance document "Definition of minimum performance requirements for analytical methods of GMO testing" of 13 October 2008.

Certified reference materials for calibration of this method or to serve as control materials will be produced by the Institute for Reference Materials and Measurements (IRMM) of the JRC at Geel.

14. INFORMATION RELATING TO PREVIOUS RELEASES OF THE GM PLANT

14.1. 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

Not applicable – no previous releases in the EU.

b) Conclusions of post-release monitoring

Not applicable.

c) Results of the release in respect to any risk to human health and the environment (submitted to the Competent Authority according to Article 10 of Directive 2001/18/EC)

Not applicable.

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

a) Release country

Field testing of 4114 Maize has been carried out in the US since 2006 and Canada since 2008.
b) Authority overseeing the release US: United States Department of Agriculture (USDA) Canada: Canadian Food Inspection Agency (CFIA)
c) Release site Multiple sites, selected to represent typical growing regions for Maize in the US and Canada.
d) Aim of the release Breeding, agronomic performance, efficacy, yield, ecological observations, product development, and regulatory data generation.
e) Duration of the release Included one growing season for Maize in the US and Canada
f) Aim of post-releases monitoring Monitoring of volunteers.
g) Duration of post-releases monitoring 12 months.
h) Conclusions of post-release monitoring The 4114 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics.
i) Results of the release in respect to any risk to human health and the environment No adverse effects on human health and the environment observed.

a) Release country Chile
b) Authority overseeing the release Servicio Agrícola y Ganadaro (SAG), Dpto. Protección Agricola
c) Release site Multiple locations
d) Aim of the release Research trials
e) Duration of the release Multiple years
f) Aim of post-releases monitoring

Control of potential volunteers
g) Duration of post-releases monitoring One season
h) Conclusions of post-release monitoring The 4114 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics.
i) Results of the release in respect to any risk to human health and the environment No adverse effects on human health and the environment observed.

a) Release country Argentina
b) Authority overseeing the release Conabia
c) Release site Multiple locations
d) Aim of the release Research and regulatory trials
e) Duration of the release One year
f) Aim of post-releases monitoring Control of potential volunteers
g) Duration of post-releases monitoring One season
h) Conclusions of post-release monitoring The 4114 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics.
i) Results of the release in respect to any risk to human health and the environment No adverse effects on human health and the environment observed.