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

DP4114xMON810xMIR604xNK603 MAIZE
and sub-combinations
(DP-ØØ4114-3xMON-ØØ81Ø-6xSYN-IR6Ø4-5xMON-ØØ6Ø3-6 MAIZE)

EFSA-GMO-NL-2018-1xx

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 DP4114xMON810xMIR604xNK603 maize and all sub-combinations, independently of their origin (MON810xMIR604xNK603; DP4114xMIR604xNK603; DP4114xMON810xNK603; DP4114xMON810xMIR604; DP4114xMON810; DP4114xMIR604; DP4114xNK603; MON810xMIR604; NK603xMON810 and MIR604xNK603) (hereafter referred to as "its sub-combinations").
d) Date of acknowledgement of valid application
[*Not available at the time of submission*]

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 and glyphosate in genetically modified maize is authorised according to Commission Regulation (EC) No 149/2008. In addition, NK603xMON810 maize has already been authorised by Commission Decision 2007/701/EC of 24 October 2007.	**No** [ ]

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

Yes [ ]	**No** [x] The scope of this application does not include authorisation for the cultivation of DP4114xMON810xMIR604xNK603 maize and its sub-combinations seed products in the EU. 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 the proposed uses of DP4114xMON810xMIR604xNK603 maize or any sub-combination of these events.

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 DP4114xMON810xMIR604xNK603 maize, including cultivation of DP4114xMON810xMIR604xNK603 maize seed products, have been submitted in the USA and Canada. 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

DP4114xMON810xMIR604xNK603 maize has been obtained by use of traditional breeding methods between genetically modified DP4114, MON810, MIR604 and NK603 maize. No new genetic modification has been introduced to obtain DP4114xMON810xMIR604xNK603 maize or its sub-combinations.

DP4114xMON810xMIR604xNK603 maize therefore confers i.) herbicide tolerance to glyphosate and glufosinate-ammonium herbicides due to the presence of the CP4 EPSPS and PAT proteins, respectively; ii.) protection against lepidopteran target pests based on the presence of the Cry1F, and Cry1Ab proteins, conferring independent modes of action for insect protection; and iii.) protection against coleopteran target pests based on the presence of the Cry34Ab1, Cry35Ab1, and mCry3A proteins, conferring independent modes of action for insect protection. Such pyramided stacks with independent modes of action against the same target pests can substantially enhance durability and delay the development of resistance by the insects.

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 DP4114xMON810xMIR604xNK603 maize and all its sub-combinations for all food and feed uses, and for all food, feed and processed products derived from DP4114xMON810x MIR604xNK603 maize in accordance with Regulation (EC) No 1829/2003. In addition, this application requests authorisation for import and processing of DP4114xMON810xMIR604xNK603 maize and its sub-combinations in accordance with Part C of Directive 2001/18/EC. The DP4114xMON810xMIR604xNK603 maize products placed on the market is expected to be used in a manner consistent with current uses of commercial maize grain and maize products. However, this application does not include authorisation for the cultivation of DP4114xMON810xMIR604xNK603 maize products in the EU.

c) Intended use of the product and types of users

The DP4114xMON810xMIR604xNK603 maize products placed on the market are expected to be used in a manner consistent with current uses of commercial maize grain and maize products. The stack 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 DP4114xMON810xMIR604xNK603 maize and its sub-combinations has shown that no specific instructions and/or recommendations for use, storage and handling of DP4114xMON810xMIR604xNK603 maize are necessary. Therefore, DP4114xMON810xMIR604xNK603 maize and all its sub-combinations can be used, stored and handled in the same way as is currently done for commercial maize. Labelling of DP4114xMON810xMIR604xNK603 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

DP4114xMON810xMIR604xNK603 maize will be used throughout the European Union as any other commercial maize products, with the exception of cultivation.

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

The application does not cover cultivation of DP4114xMON810xMIR604xNK603 maize and its sub-combinations in the European Union. The DP4114xMON810xMIR604xNK603 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 DP4114xMON810xMIR604xNK603 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.

Labelling of foods and feeds consisting of or containing DP4114xMON810xMIR604xNK603 maize

In accordance with Articles 12-14 and 24-26 of Regulation (EC) No 1829/2003, Article 13(2)f and Annex IV of Directive 2001/18/EC, and with Article 4 of Regulation (EC) No 1830/2003, operators shall be required to label products containing or consisting of DP4114xMON810xMIR604xNK603 maize with the words “genetically modified maize” or “contains genetically modified maize”, and operators shall be required to declare the unique identifier in the list of GMOs that have been used to constitute the mixture that contains or consists of this GMO.

Labelling of foods and feeds produced from DP4114xMON810xMIR604xNK603 maize

For food and feed products produced from DP4114xMON810xMIR604xNK603 that are not exempted according to Article 5(4) of Regulation (EC) No 1830/2003, operators shall be required to label foods and feeds derived from DP4114xMON810xMIR604xNK603with the words “produced from genetically modified maize”, in accordance with Articles 12-14 and 24-26 of Regulation (EC) No 1829/2003 and the requirements of Article 5 of Regulation (EC) No 1830/2003. In the case of products for which no list of ingredients exists, operators shall ensure that an indication that the food or feed product is produced from GMOs is transmitted in writing to the operator receiving the product.

Measures taken by the applicant

Although Pioneer Hi-Bred International, Inc. is the applicant under Regulation (EC) No 1829/2003 for authorisation to place DP4114xMON810xMIR604xNK603 maize on the market for all food and feed uses as any other maize in the EU, Pioneer Hi-Bred International, Inc. is not an operator handling or using the product in the EU.

Operators handling or using DP4114xMON810xMIR604xNK603 grain and derived foods and feeds in the EU are required to be aware of the legal obligations regarding traceability and labelling of these products. Given that explicit requirements for the traceability and labelling of GMOs and derived foods and feeds are laid down in Regulations (EC) No 1829/2003 and 1830/2003, and that authorised foods and feeds shall be entered in the Community Register, operators in the food/feed chain will be fully aware of the traceability and labelling requirements for DP4114xMON810xMIR604xNK603. Therefore, no further specific measures are to be taken by the applicant.

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 2016, a total of 11.4 million metric tonnes of maize were imported into the EU, with Ukraine being the largest supplier. Spain is the most important market for extra-EU maize imports with a share of 29% in 2016. Other significant import markets for extra-EU maize in 2016 were the Netherlands with a share of approximately 21.3 %, Italy and Portugal with a share of approximately 14.3 % and 10.9 %, respectively, followed by the United Kingdom with a share of approximately 5.2 %.

b) In EU export markets

The application does not cover cultivation of DP4114xMON810xMIR604xNK603 maize and its sub-combinations in the European Union.

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

DP-ØØ4114-3xMON-ØØ81Ø-6xSYN-IR6Ø4-5xMON-ØØ6Ø3-6

The unique identifiers of all sub-combinations covered in the application are:

DP-ØØ4114-3xMON-ØØ81Ø-6xSYN-IR6Ø4-5; DP-ØØ4114-3xMON-ØØ81Ø-6xMON-ØØ6Ø3-6; DP-ØØ4114-3xSYN-IR6Ø4-5xMON-ØØ6Ø3-6; MON-ØØ81Ø-6xSYN-IR6Ø4-5xMON-ØØ6Ø3-6; DP-ØØ4114-3xMON-ØØ81Ø-6; DP-ØØ4114-3xSYN-IR6Ø4-5; DP-ØØ4114-3xMON-ØØ6Ø3-6; MON-ØØ81Ø-6xSYN-IR6Ø4-5; and SYN-IR6Ø4-5xMON-ØØ6Ø3-6

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 DP4114xMON810xMIR604xNK603 maize and its sub-combinations, no specific measures need to be taken in case of unintended release or misuse or for disposal and treatment. There are no sexually compatible endogenous 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 DP4114xMON810xMIR604xNK603 maize or its sub-combinations, 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 and glyphosate).

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

Zea *mays* ssp. *mays* L.

e) Cultivar/breeding line

DP4114xMON810xMIR604xNK603

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 viable 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 endogenous 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

DP4114xMON810xMIR604xNK603 maize has been obtained by use of traditional breeding methods between genetically modified DP4114, MON810, MIR604 and NK603 maize. No new genetic modification has been introduced to obtain DP4114xMON810xMIR604xNK603 maize or its sub-combinations.

(b) Nature and source of the vector used

The DP4114xMON810xMIR604xNK603 maize was produced by means of conventional breeding between DP4114 maize, MON810 maize, MIR604 maize, and NK603 maize. No vector has been used to produce this maize hybrid.

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

DP4114xMON810xMIR604xNK603 maize has been obtained by use of traditional breeding methods between genetically modified DP4114, MON810, MIR604 and NK603 maize. No new genetic modification has been introduced to obtain DP4114xMON810xMIR604xNK603 maize or its sub-combinations.

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

DP4114xMON810xMIR604xNK603 maize therefore confers i.) herbicide tolerance to glyphosate and glufosinate-ammonium herbicides due to the presence of the CP4 EPSPS and PAT proteins, respectively; ii.) protection against lepidopteran target pests based on the presence of the Cry1F, and Cry1Ab proteins, conferring independent modes of action for insect protection; and iii.) protection against coleopteran target pests based on the presence of the Cry34Ab1, Cry35Ab1, and mCry3A proteins, conferring independent modes of action for insect protection. Such pyramided stacks with independent modes of action against the same target pests can substantially enhance durability and delay the development of resistance by the insects.

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 DP4114xMON810xMIR604xNK603 maize contains four separate copies of inserted DNA from DP4114 maize, MON810 maize, MIR604 and NK603 maize. Southern blot analysis demonstrated that DP4114xMON810xMIR604xNK603 maize does not contain other fragments from the inserts than those that were present in the respective single lines.

Information on the elements present in the single events can be found in the following EFSA opinions:

EFSA, 2003. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safety of foods and food ingredients derived from herbicide-tolerant genetically modified maize NK603, for which a request for placing on the market was submitted under Article 4 of the Novel Food Regulation (EC) No. 258/97 by Monsanto. EFSA Journal 1149, [85 pp.].

EFSA, 2009a. Applications (EFSA-GMO-RX-MON810) for renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON810; (2) feed consisting of and/or containing maize MON810, including the use of seed for cultivation; and of (3) food and feed additives, and feed materials produced from maize MON810, all under Regulation (EC) No 1829/2003 from Monsanto EFSA Journal 1149.

EFSA, 2009b. Applications (references EFSA-GMO-NL-2005-22, EFSA-GMO-RX-NK603) for the placing on the market of the genetically modified glyphosate tolerant maize NK603 for cultivation, food and feed uses, import and processing and for renewal of the authorisation of maize NK603 as existing products, both under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 1137, [pp 50].

EFSA, 2009c. Scientific Opinion of the Panel on Application (Reference EFSA-GMO-UK-2005-11) for the placing on the market of insect-resistant genetically modified maize MIR604 event, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Seeds S.A.S on behalf of Syngenta Crop Protection AG. The EFSA Journal 7, 1193 [pp.26]

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

Not applicable

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

The DP4114, MON810, MIR604, and NK603 maize inserts are all integrated into different loci in the maize nuclear genome as confirmed by the inheritance of the inserts through conventional crosses and by the molecular characterisation of DP4114xMON810xMIR604xNK603 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 that the copy number, structure and organisation of the inserts in DP4114xMON810xMIR604xNK603 maize are equivalent to those found in the parental breeding lines DP4114, MON810, MIR604 and NK603 maize. The

organisation of the inserted material in the parental lines is as follows:

For DP4114 maize, consists of four gene cassettes. The first cassette contains a synthetic truncated *cry*1F gene from *Bacillus thuringiensis* var. *aizawai*. The second and third cassettes contain the *cry*34Ab1 and *cry*35Ab1 genes isolated from *Bacillus thuringiensis* strain PS149B1. The fourth cassette contains a version of the phosphinothricin acetyl transferase (*pat*) gene from *Streptomyces viridochromogenes* that has been optimized for expression in plants.

MON810 maize contains the enhanced 35S promoter (e35S), the maize hsp70 intron (*Zmhsp70*), the *cry*1Ab coding sequence, and a portion of the 3' end of the e35S promoter as well as a portion of the 5' end of the *cry*1Ab coding sequence. No other portion of the plasmid PV-ZMBK07, including the *npt*II gene, has been integrated in MON810 maize.

MIR604 maize contains a T-DNA insertion with one copy of both the m*cry*3A and *pmi* genes.

NK603 maize contains two adjacent copies of the CP4 *epsps* gene fused to chloroplast transit peptide (CTP) sequences, with both ctp2-CP4 *epsps* gene cassettes being intact in NK603 maize. The sequence of the CP4 *epsps* gene of the first cassette in NK603 maize is identical to that in the original plasmid, whilst in the second inserted cassette the sequence of the CP4 *epsps* gene differs by two nucleotides from that in the original plasmid.

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 DP4114xMON810xMIR604xNK603 maize of these events in comparison with the expression levels in the GM parental lines. 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 expression of the Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1Ab, mCry3A, PMI and CP4 EPSPS proteins in various tissues of DP4114xMON810xMIR604xNK603 maize was comparable to the expression of these proteins in the corresponding GM parental lines.

b) Parts of the plant where the insert is expressed

As summarised above, studies to evaluate the range of expression of the proteins Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1Ab, mCry3A, PMI and CP4 EPSPS in different tissues of DP4114xMON810xMIR604xNK603 maize have been conducted.

These results obtained confirm that, as expected, transgenic protein expression in DP4114xMON810xMIR604xNK603 maize tissues is not substantially different from that of the respective single maize events.

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

Genetic and phenotypic stability of the inserts in DP4114xMON810xMIR604xNK603 maize was confirmed by molecular analysis of DP4114xMON810xMIR604xNK603 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

DP4114xMON810xMIR604xNK603 maize does not differ from conventional maize in this respect.

(b) Dissemination

DP4114xMON810xMIR604xNK603 maize does not differ from conventional maize in this respect.

(c) Survivability

DP4114xMON810xMIR604xNK603 maize does not differ 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 potential transfer and impact of plant to bacteria gene transfer has been assessed for all the parental single events.

b) Plant to plant gene transfer

There are no other cultivated or endogenous 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 DP4114xMON810xMIR604xNK603 maize and its sub-combinations do not introduce any selective advantages to maize plants outside the agricultural environment.

It should be noted that this application is for authorisation of DP4114xMON810xMIR604xNK603 maize and its sub-combinations for all food and feed uses, and for all food, feed and processed products derived from DP4114xMON810xMIR604xNK603 maize and its sub-combinations, and not for cultivation of DP4114xMON810xMIR604xNK603 maize or its sub-combinations 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 DP4114xMON810xMIR604xNK603 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 DP4114xMON810xMIR604xNK603 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 2015 growing season at sites in North America, which were selected on the basis of their inclusion in the commercial maize-growing regions. Each site utilized a randomized complete block design and contained four blocks. Each block contained the following entries: conventional herbicide-treated (CHT) DP4114xMON810xMIR604xNK603 maize, intended herbicide-treated (IHT) DP4114xMON810xMIR604xNK603 maize, non-genetically modified (non-GM) near-isoline CHT control maize (referred to as control maize), and non-GM CHT commercial reference maize lines. Samples were collected for nutrient composition analysis at 8 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

DP4114xMON810xMIR604xNK603 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 DP4114xMON810xMIR604xNK603 maize and non-GM control maize with comparable genetic background.

It should be noted that this application is for authorisation of DP4114xMON810xMIR604xNK603 maize and its sub-combinations for all food and feed uses, and for all food, feed and processed products derived from DP4114xMON810xMIR604xNK603 maize, and not for cultivation of DP4114xMON810xMIR604xNK603 maize seed products.

4.5 Effect of processing

As discussed in this application, food and animal feed products derived from DP4114xMON810xMIR604xNK603 maize and its sub-combinations 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 DP4114xMON810xMIR604xNK603 maize and its sub-combinations is equivalent to that of food and animal feed products derived from commercial maize.

5. Toxicology

a) Toxicological testing of newly expressed proteins

DP4114xMON810xMIR604xNK603 maize was produced by combining the maize events DP4114, MON810, MIR604 and NK603 through conventional breeding and therefore produces the insert-encoded proteins inherited from their parents. Potential adverse effects to human and animal health from expression of the Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1Ab, mCry3A, PMI and CP4 EPSPS proteins have previously been assessed taking into account the following considerations:

- the recipient organism and the donor organisms 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 oral toxicity to mammals.

No reports have appeared in the scientific literature up to now that would 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 DP4114xMON810xMIR604xNK603 maize and sub-combinations of these events 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 DP4114xMON810xMIR604xNK603 maize products. It is therefore highly unlikely that the Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1Ab, mCry3A, PMI and CP4 EPSPS 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 DP4114xMON810xMIR604xNK603 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 DP4114xMON810xMIR604xNK603 maize have demonstrated that the composition of DP4114xMON810xMIR604xNK603 maize is equivalent to that of control maize. In addition, the results obtained in 90-day oral toxicity feeding studies with the parental single maize events in rats and those obtained in 42-day poultry feeding studies with the parental single maize events indicate the safety of the natural food and feed constituents from DP4114xMON810xMIR604xNK603 maize and nutritional equivalence between DP4114xMON810xMIR604xNK603 maize and non-GM control maize.

d) Testing of the whole GM food/feed

The evaluation of the nutrient composition of DP4114xMON810xMIR604xNK603 maize has confirmed that it is equivalent to non-GM control maize with comparable genetic background.

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, PAT, Cry1Ab, mCry3A, PMI and CP4 EPSPS 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, PAT, Cry1Ab, mCry3A, PMI and CP4 EPSPS proteins expressed in DP4114xMON810xMIR604xNK603 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 DP4114xMON810xMIR604xNK603 maize are highly unlikely to be allergenic.

7. Nutritional assessment

a) Nutritional assessment of GM food

Composition analysis of grain from DP4114xMON810xMIR604xNK603 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, DP4114xMON810xMIR604xNK603 maize can be considered nutritionally equivalent to non-GM control maize.

In conclusion and taking into account the anticipated dietary intake of DP4114xMON810xMIR604xNK603 maize products, consumption of DP4114xMON810xMIR604xNK603 maize (or its sub-combinations) foods or feed will not have any adverse nutritional impact.

b) Nutritional assessment of GM feed

As evaluated in Section **7.a)** above, consumption of DP4114xMON810xMIR604xNK603 maize (or its sub-combinations) feed will not give rise to any adverse nutritional impact.

8. Exposure assessment – anticipated intake/extent of use

The nutritional assessment has concluded that DP4114xMON810xMIR604xNK603 maize is nutritionally equivalent to non-GM control maize. In addition, the use of DP4114xMON810xMIR604xNK603 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 DP4114xMON810xMIR604xNK603 maize was shown to be negligible.

Therefore, post-market monitoring of GM food and GM feed products containing, consisting of or derived from DP4114xMON810xMIR604xNK603 maize (and its sub-combinations) is not necessary.

9. Risk characterisation

Maize food and feed products have a long history of safe use. The information presented in this application confirms that DP4114xMON810xMIR604xNK603 maize (and its sub-combinations) and derived food and feed products are not different from those of its conventional counterpart.

10. Post-market monitoring on the genetically modified food or feed

A thorough risk assessment has confirmed that DP4114xMON810xMIR604xNK603 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 DP4114xMON810xMIR604xNK603 maize (or its sub-combinations) 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 DP4114xMON810xMIR604xNK603 maize (and its sub-combinations) 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 DP4114xMON810xMIR604xNK603 maize will be limited to unintended release of DP4114xMON810xMIR604xNK603 maize (or its sub-combinations), 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 DP4114xMON810xMIR604xNK603 maize and its sub-combinations to become environmentally persistent or invasive giving rise to any weediness. The cultivation of DP4114xMON810xMIR604xNK603 maize (and its sub-combinations) 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 DP4114xMON810xMIR604xNK603 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 DP4114xMON810xMIR604xNK603 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, PAT, Cry1Ab, mCry3A, PMI and CP4 EPSPS proteins in DP4114xMON810xMIR604xNK603 maize does not confer any selective advantage outside the agricultural environment.

c) Potential for gene transfer

There are no sexually compatible endogenous 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 DP4114xMON810xMIR604xNK603 maize or its sub-combinations 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 include cultivation of DP4114xMON810xMIR604xNK603 maize or its sub-combinations 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 include cultivation of DP4114xMON810xMIR604xNK603 maize (and its sub-combinations) 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 DP4114xMON810xMIR604xNK603 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, PAT, Cry1Ab, mCry3A, PMI and CP4 EPSPS proteins as expressed in DP4114xMON810xMIR604xNK603 maize, has been carried out. As a result and in conclusion, DP4114xMON810xMIR604xNK603 maize and its sub-combinations do not express any known toxic or allergenic proteins. Therefore, consumption of DP4114xMON810xMIR604xNK603 maize and its sub-combinations or derived food products will result in no adverse effects on human health.

g) Effects on animal health

Consumption of DP4114xMON810xMIR604xNK603 maize (or its sub-combinations) or any derived food, feed and processed products will not result in any adverse effects on human or animal health. Therefore, use of DP4114xMON810xMIR604xNK603 maize as feed and consumption of any food, feed and processed products derived from DP4114xMON810xMIR604xNK603 maize and its sub-combinations is not expected to 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, PAT, Cry1Ab, mCry3A, PMI and CP4 EPSPS 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, DP4114xMON810xMIR604xNK603 maize and its sub-combinations 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 DP4114xMON810xMIR604xNK603 maize (or its sub-combinations) seed products in the EU. Exposure to the environment from the import of DP4114xMON810xMIR604xNK603 maize and its sub-combinations will be limited to unintended release of DP4114xMON810xMIR604xNK603 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 and glyphosate). 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 DP4114xMON810xMIR604xNK603 maize (and its sub-combinations) seed products in the EU. Exposure to the environment from the import of DP4114xMON810xMIR604xNK603 maize (and its sub-combinations) will be limited to unintended release of DP4114xMON810xMIR604xNK603 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 and glyphosate). 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 DP4114xMON810xMIR604xNK603 maize (and its sub-combinations) seed products in the EU. Exposure to the environment from the import of DP4114xMON810xMIR604xNK603 maize (and its sub-combinations) will be limited to unintended release of DP4114xMON810xMIR604xNK603 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 and glyphosate).

A proposal for an environmental monitoring plan for DP4114xMON810xMIR604xNK603 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 on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants (EFSA, 2011).

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 DP4114xMON810xMIR604xNK603 maize and its sub-combinations 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 DP4114xMON810xMIR604xNK603 maize (or its sub-combinations). Therefore, the risk to human and animal health or the environment from DP4114xMON810xMIR604xNK603 maize or its sub-combinations 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 DP4114xMON810xMIR604xNK603 maize and its sub-combinations 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 DP4114xMON810xMIR604xNK603 maize or its sub-combinations for all food and feed purposes and the import and processing of DP4114xMON810xMIR604xNK603 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 DP4114xMON810xMIR604xNK603 maize and its sub-combinations 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 DP4114xMON810xMIR604xNK603 maize and its sub-combinations in accordance with Part C of Directive 2001/18/EC. In this application we are not seeking approval for cultivation of DP4114xMON810xMIR604xNK603 maize (or its sub-combinations) 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 DP4114xMON810xMIR604xNK603 maize (or its sub-combinations). 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 and glyphosate 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 DP4114xMON810xMIR604xNK603 maize and its sub-combinations 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 DP4114xMON810xMIR604xNK603 maize and its sub-combinations for all food and feed purposes and the import and processing of DP4114xMON810xMIR604xNK603 maize and its sub-combinations. As a result, no case-specific monitoring is proposed for this application for authorisation of DP4114xMON810xMIR604xNK603 maize and its sub-combinations.

The applicant will inform the European Commission, without delay, of any adverse effects reported arising from the handling and use of imported DP4114xMON810xMIR604xNK603 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, if any, a conclusion of the safety of DP4114xMON810xMIR604xNK603 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 DP4114; MON810; MIR604; and NK603 maize. In addition, an in-house validation study on the performance of the single event detection methods on the DP4114xMON810xMIR604xNK603 maize stacked product has been submitted to the European Union Reference Laboratory (EURL) for GM Food and Feed (Joint Research Centre, Italy) for verification, in accordance with the requirements of the EURL/ENGL Guidance document “Definition of minimum performance requirements for analytical methods of GMO testing”.

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
United States (USA), Chile
b) Authority overseeing the release
USA - EPA (Environmental Protection Agency); Chile - SAG (Agricultural and Livestock Service)
c) Release site
Multiple sites, selected to represent typical growing regions for maize.
d) Aim of the release
Regulatory trials
e) Duration of the release
One or more growing season for maize
f) Aim of post-releases monitoring
Monitoring of volunteers
g) Duration of post-releases monitoring
One season or year
h) Conclusions of post-release monitoring
The DP4114xMON810xMIR604xNK603 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.