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 III – Cartagena Protocol

Submitted by:

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PART III – Cartagena Protocol

A. INFORMATION REQUIRED CONCERNING LIVING MODFIED ORGANISMS INTENDED FOR DIRECT USE AS FOOD OR FEED, OR FOR PROCESSING UNDER ANNEX II OF REGULATION (EC) No 1946/2003

(a) The name and contact details of the applicant for a decision for domestic use

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(b) The name and contact details of the authority responsible for the decision

European Commission
Health and Consumer Protection Directorate General DDG2.E.1
(DG SANCO)
Rue Belliard 232 03/100
1049 Brussels
Belgium

(c) Name and identity of the GMO

The name of the genetically modified organism (GMO) described in this application is 4114 maize.

(d) Description of the gene modification, the technique used, and the resulting characteristics of the GMO

4114 maize was produced by *Agrobacterium*-mediated transformation using a binary vector containing four gene expression casettes: the first cassette contains a truncated version of the *cry*1F gene from *Bacillus thuringiensis var. aizawai*; expression of Cry1F protein confers protection against certain Lepidoptera insect pests. The second and third cassettes contain the *cry*34Ab1 and *cry*35Ab1 genes, respectively, isolated from *Bacillus thuringiensis* strain PS149B1, which have been codon optimised for expression in plants; expression of the Cry34Ab1/Cry35Ab1 binary protein confers protection against corn rootworm infestation. The fourth gene cassette contains the phosphinothricin acetyl transferase (*pat*) gene from *Streptomyces viridochromogenes,* which has been codon optimised for expression in plants and confers tolerance to application of glufosinate herbicides.

(e) Any unique identification of the GMO

The unique identifier assigned to 4114 maize is DP-ØØ4114-3.

(f) Taxonomic status, common name, point of collection or acquisition, and characteristics of recipient organism or parental organisms related to biosafety

Taxonomy of recipient organism:

Family name: Poaceae
Genus: *Zea*
Species: *Zea maize* L.
Subspecies: N/A
Common name: maize, corn

Point of collection or acquisition: USA

Characteristics of recipient organism or parental organisms related to biosafety:

Maize has a history of safe use, being used in foods and feed. It is grown for the production of grain and forage (silage), and is mainly used as a feedstuff for livestock.

(g) Centres of origin and centres of genetic diversity, if known, of the recipient organism and/or the parental organisms and a description of the habitats where the organisms may persist or proliferate

Centre of origin and centre of genetic diversity of maize: Mexico

Description of the habitats where the organisms may persist or proliferate:

It is generally accepted that most crop plants, including maize, have undergone many years of selective breeding and domestication, and only function optimally under managed agricultural conditions, such as high soil fertility or low plant competition. These conditions rarely occur in natural habitats (including roadsides and ports), resulting in poor fitness of maize plants outside of a managed field. Reduced recruitment, low survivorship, poor competitive ability, and low seed production are common indicators of poor fitness of maize in natural situations.

(h) Taxonomic status, common name, point of collection or acquisition, and characteristics of the donor organism or organisms related to biosafety

The 4114 maize has been modified with a T-DNA vector that contains sequences from the following donor organisms (*Point of collectio*n: USA):

- *Bacillus thuringiensis*, donor of the *cry*1F, *cry*34Ab1 and *cry*35Ab1 genes, has a history of safe use as a biopesticide over several decades and occurs naturally in the soil and on plants including vegetables, cotton, tobacco, tree crops and forest crops.

- *Agrobacterium tumefaciens*, donor of the mannopine synthase (mas) ORF25 terminator and the T-DNA border sequences, is a Gram-negative, non-spore forming, rod-shaped bacterium commonly found in the soil. It is closely related to beneficial soil bacteria involved in nitrogen fixation by certain plants, e.g. *Rhizobium*. Removal of the native T-DNA genes on the Ti plasmid of *Agrobacterium tumefaciens* has resulted in non-pathogenic bacteria that are widely employed in plant genetic engineering.

- Cauliflower Mosaic Virus, the donor of the 35S promoter and terminator sequences. It is a DNA caulimovirus with a host range restricted primarily to cruciferous plants.

- *Solanum tuberosum* (potato), donor of the proteinase II gene terminator. Potato is one of the major starch crops and has a long history of safe use.

- *Streptomyces viridochromogenes* strain Tü494, donor of the *pat* gene, is a common gram-positive sporulating soil bacterium that produces the tripeptide L-phosphinothricyl- L-alanyl-alanine (L-PPT), which was developed as a non-selective herbicide. Few *Streptomyces* have been isolated from animal or human sources and pathogenicity is not a typical property of these organisms. A recent safety evaluation concluded that the inclusion of the PAT protein from *Streptomyces* in food or feed causes no harm to human or animal safety.

- *Triticum aestivum* (wheat), donor of the wheat peroxidase promoter, is grown as a commercial food crop in over 120 countries worldwide.

- *Zea mays* L. (maize), host plant and donor of the ubiquitin (*ubi*ZM1) constitutive promoter, has a long history of cultivation and safe food and feed use. It has a multitude of uses in the food and industrial sectors, representing one of the major sources of edible vegetable oil and of proteins for animal feed use.

(i) Approved uses of the GMO

Regulatory submissions and reviews are in progress in selected countries around the world. The intended use of 4114 maize includes all uses of 4114 maize for food and feed purposes, and for all food, feed and processed products derived from 4114 maize, as with any other commercial maize, excluding cultivation of 4114 maize seed products in the EU.

(j) A risk assessment report consistent with Annex II to Directive 2001/18/EC

A risk assessment report consistent with Annex II to Directive 2001/18/EC is included below.

(k) Suggested methods for the safe handling, storage, transport and use, including packaging, labelling, documentation, disposal and contingency procedures, where appropriate

The handling, storage, transport and use of 4114 maize, including packaging, documentation, disposal and contingency procedures, will be done as for any other commercial maize. Labelling of 4114 maize products will be carried out in accordance with Community law.

B. A RISK ASSESSMENT REPORT CONSISTENT WITH ANNEX II TO DIRECTIVE 2001/18/EC

RISK ASSESSMENT

The objective of this risk assessment is to identify and evaluate the potential adverse effects of genetically modified 4114 maize on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account risks to human health. The risk assessment may be used by competent authorities to make informed decisions regarding living modified organisms.

This risk assessment for genetically modified 4114 maize has been carried out in accordance with Commission Decision 2002/623/EC establishing the guidance notes supplementing Annex II of Directive 2001/18/EC.

The risk assessment of 4114 maize follows a stepwise approach, taking into account the following technical and scientific details regarding the characteristics of the following subjects:

(a) *Recipient organism or parental organisms:* See **Point A.(f)**.

(b) *Donor organism or organisms:* See **Point A.(h)**.

(c) *Vector:* See **Point A.(d)**.

(d) *Insert or inserts and/or characteristics of modification:* See **Point A.(d)**.

(e) *Living modified organism:* See **Point A.(c)**.

(f) *Detection and identification of the living modified organism:* A PCR-based quantitative event-specific detection method for 4114 maize has been developed and is under validation by the European Union Reference Laboratory (EURL) for GM Food and Feed, established at the EC Joint Research Centre in Italy.

(g) *Information relating to the intended use:* See **Point A.(i)**.

(h) *Receiving environment:* Cultivation is outside the scope of this application. See **Point A.(g)**.

STEPS IN THE ENVIRONMENTAL RISK ASSESSMENT

Step 1: Identification of characteristics which may cause adverse effects

1. Characteristics of the GMO linked to the genetic modification

The characteristics of 4114 maize linked to the genetic modification have been described in **Point A.(d)**. 4114 maize was produced by *Agrobacterium*-mediated transformation using a binary vector containing four gene expression casettes conferring insect resistance and herbicide tolerance. However, 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 herbicides).

2. Potential adverse effects of the GMO(s)

a) Disease to humans including toxic or allergenic effects

The 4114 maize expresses the Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins. The safety assessment of the individual proteins has been conducted in the frame of previous submissions (among others, applications on 1507, 59122 and 1507x59122 maize) and was reported in several EFSA scientific opinions. The safety assessment has been based on a broad body of evidence, including previous use of the proteins; their modes of action; specificity of their biological activity; relatedness to other proteins with a history of safe use; absence of toxicity to mammals; absence of adverse effects on fast growing species; lack of homology to known toxins or allergens; lack of resistance to proteolysis; and lack of stability to heating.

Analysis of the concentration of the proteins in various tissues revealed that expression of the inserted-related proteins in 4114 maize was as expected and comparable to that in the single event lines 1507 and 59122 maize, which contain the same expression cassettes.

The potential occurrence of interaction effects between the encoded proteins, and their impact, if any, on food or feed safety has been evaluated and no concerns are identified. On the basis of the natural occurrence of combinations of Cry proteins in *Bt* strains and biopesticide formulations produced from such strains without reported health effects, their target specificity, their mode of action, the lack of interaction in target insects, and the absence of unexpected effects in experimental studies, it is concluded that there is no evidence for any interactions between the Cry proteins, or between Cry and PAT proteins, that would affect food or feed safety of 4114 maize.

Detailed compositional analyses and nutritional assessment of 4114 maize have confirmed that whole food and feed consisting of or derived from 4114 maize is nutritionally equivalent to whole food and feed consisting of or derived from commercial maize.

The allergenicity of the newly expressed proteins in 4114 maize has been assessed using a weight-of-the-evidence approach. The newly expressed proteins in 4114 maize are not known to be allergenic, they do not have the characteristics of known allergenic proteins, and they are not derived from organisms with a known allergenic potential. Therefore, it can be concluded that expression of the insert-related proteins in 4114 maize is unlikely to alter the overall allergenicity of maize.

Similarity searches using up-to-date databases have also confirmed the absence of any similarity of the insert-encoded proteins in 4114 maize to toxins, bioactive proteins or allergenic proteins that may raise any safety concerns.

Furthermore, a poultry feeding study has been conducted over a 42-day period with diets containing 4114 maize untreated or treated with glufosinate. For comparison, diets containing non-genetically modified maize with comparable genetic background and diets containing commercial maize were also fed to the broiler chickens. No statistically significant differences were observed in mortality, weight gain, feed efficiency and carcass yields between broilers consuming diets produced with 4114 maize untreated or treated with glufosinate and those consuming diets produced with non-genetically modified control maize. In addition, all response variables evaluated fell within the tolerance intervals of the values observed in broilers fed with diets produced with the commercial reference maize grains. Based on the results from these studies, it was concluded that 4114 maize is

nutritionally equivalent to non-genetically modified maize with comparable genetic background and to commercial maize.

In conclusion, 4114 maize is as safe to human and animal health as any other commercially available maize.

b) Disease to animals and plants including toxic, and where appropriate, allergenic effects

The safety of 4114 maize to animal health is comparable to that of any other commercially available maize. Please refer to **Section 2.a)** above.

c) Effects on the dynamics of populations of species in the receiving environment and the genetic diversity of each of these populations

The scope of 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 does not include cultivation of 4114 maize seed products in the EU. Therefore, any potential exposure of 4114 maize to a potential receiving environment will be restricted to limited unintentional release, *e.g.* accidental spillage of grain during loading/unloading of vessels, trains or trucks, or during transportation. Any unintentional release or misuse of 4114 maize would be limited and highly unlikely to have any adverse effect. Furthermore and if necessary, such limited release can be controlled by management practices currently applied to control unintentional releases of any other commercially available maize, such as selective use of herbicides (with the exception of glufosinate-ammonium) and manual or mechanical removal.

In conclusion, negligible effects are expected on the dynamics of populations in the receiving environment and the genetic diversity of each of these populations.

d) Altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors

There have been no signs observed of any altered susceptibility of 4114 maize to pathogens in field trials with this maize. The assessment of the agronomic characteristics of 4114 maize has confirmed that it is comparable to other commercially available maize except for the expression of the insert-encoded proteins conferring herbicide tolerance and insect resistance traits. Therefore, no adverse effects are expected to human or animal health or to the environment as a result of an altered susceptibility of 4114 maize to pathogens.

e) Compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments

Expression of the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in 4114 maize does not compromise prophylactic or therapeutical medical, veterinary, or plant protection treatments. No genetic material coding for genes conferring resistance to antibiotics, including those used in human or veterinary medicine, is present in 4114 maize.

f) Effects on biogeochemistry (biogeochemical cycles), particularly carbon and nitrogen recycling through changes in soil decomposition of organic material

As the scope of this application does not cover cultivation of 4114 maize in the EU, any effects on biogeochemical processes are not expected.

g) Other potential adverse effects

Adverse effects may occur directly or indirectly through mechanisms that may include:

- The spread of the GMO in the environment;
- The transfer of the inserted genetic material to other organisms, or the same organism, whether genetically modified or not;
- Phenotypic and genetic instability;
- Interactions with other organisms;
- Changes in management, including, where applicable, in agricultural practices.

An evaluation to identify any potential adverse effects on human and animal health or the environment that may occur through these mechanisms has been carried out and the results obtained are presented below.

The spread of the GMO in the environment: there is negligible likelihood for 4114 maize to become environmentally persistent or invasive giving rise to weediness. Maize does not possess any trait for weediness and the expression of the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in 4114 maize does not introduce new traits for weediness.

The transfer of the inserted genetic material to other organisms, or the same organism, whether genetically modified or not: there are no sexually compatible wild or weedy relatives of maize known to exist in the EU, which eliminates the possibility of potential gene transfer to such species. The potential for gene transfer is therefore limited to other maize grown in agricultural systems. In addition, there is negligible likelihood for 4114 maize plants to become environmentally persistent or invasive giving rise to weediness. Furthermore, expression of the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in 4114 maize does not provide a significant selective advantage outside the agricultural environment.

Phenotypic and genetic instability: the 4114 maize is phenotypically and genetically stable. This has been confirmed through multiple studies including molecular and compositional analyses, and evaluation of the agronomic characteristics and expression levels of the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in 4114 maize. In addition, the genetic material inserted in 4114 maize is integrated as a single copy in the nuclear genome of the plant and is inherited in a Mendelian way.

Interactions with other organisms: considering the scope of this application, which does not include authorisation for the cultivation of 4114 maize seed products in the EU, any exposure to the environment from the import of 4114 maize will be limited to accidental releases of this maize during loading/unloading or during processing, or to the indirect exposure through manure or faeces from animals fed on this maize. In general, any proteins present in maize, including the insert-encoded proteins, are readily degraded as a result of the processes applied during harvesting, storage and processing of maize materials.

Furthermore, the insert-encoded proteins have been shown to be susceptible to rapid degradation by simulated gastrointestinal fluids, and in the digestive tract of animals fed on silage from *Bt* maize.

In addition, the natural ubiquity of the transgenes and of the encoded proteins in the environment, together with the absence of toxicity and the specific biochemical activity of the proteins expressed in 4114 maize indicates that there will be no adverse effects that may occur through interactions of 4114 maize with other organisms.

Changes in management, including, where applicable, in agricultural practices: Not applicable, as the scope of this application does not include authorisation for the cultivation of 4114 maize seed products in the EU.

Step 2: Evaluation of the potential consequences of each adverse effect, if it occurs

On the basis of the available weight of evidence (**Step 1**), no adverse effects of 4114 maize could be identified on the conservation and sustainable use of biological diversity or on human or animal health, resulting from the transboundary movement of this maize for direct use as food or feed, or for processing.

Accordingly, no consequences of such effects are conceivable.

Step 3: Evaluation of the likelihood of the occurrence of each identified potential adverse effect

As mentioned in **Step 1**, there are no identified adverse effects to human and animal health or the environment arising from 4114 maize.

Therefore, we can conclude that the relative likelihood of occurrence of any potential adverse effect to human and animal health or the environment arising from 4114 maize is as negligible as for any other commercial maize.

Step 4: Estimation of the risk posed by each identified characteristic of the GMO

An estimation of the risk to human and animal health or the environment posed by any identified characteristic of 4114 maize which has the potential to cause adverse effects has been made by combining the magnitude of the consequences and the likelihood of the adverse effect, if it occurs, on the basis of the conclusions reached in **Steps 2** and **3**, respectively:

- No potential adverse effects have been identified and therefore the magnitude of the potential consequences is as negligible as for any other commercial maize; and,

- The likelihood of occurrence of potential adverse effects is as negligible as for any other commercial maize.

As a result, the potential risk to human and animal health or the environment arising from 4114 maize is negligible, *i.e.* as insignificant as for any other commercial maize.

The overall uncertainty underlying the conclusion that negligible risk will arise from 4114 maize is very low, *i.e.* it is comparable to the overall uncertainty related to any potential risks that might arise from the food and feed use and the import and processing of any other commercial maize.

Step 5: Application of management strategies for risks from the deliberate release or marketing of the GMO

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 accidental releases of commercially available maize, such as use of mechanical means and selective use of herbicides (with the exception of glufosinate-ammonium).

Furthermore, the conclusions obtained from **Steps 1** to **4** of this risk assessment have not identified any risks to human and animal health or the environment arising from 4114 maize. Therefore, the same management strategies for safeguarding apply to 4114 maize as for any other commercial maize.

Step 6: Determination of the overall risk of the GMO

The overall risk to human and animal health or the environment arising from 4114 maize has been evaluated by taking into account the conclusions obtained from the consecutive steps followed in this risk assessment.

*Conclusions from **Step 1** of this risk assessment:*

There are no identified adverse effects to human and animal health or the environment arising from 4114 maize.

*Conclusions from **Step 2** of this risk assessment:*

The magnitude of the potential consequences arising from 4114 maize will be as negligible as for any other commercial maize.

*Conclusions from **Step 3** of this risk assessment:*

The likelihood of the occurrence of potential adverse effects to human and animal health or the environment arising from 4114 maize is as negligible as for any other commercial maize.

*Conclusions from **Step 4** of this risk assessment:*

The potential risk to human and animal health or the environment arising from 4114 maize is negligible, *i.e.* as insignificant as for any other commercial maize.

*Conclusions from **Step 5** of this risk assessment:*

The conclusions obtained from **Steps 1** to **4** of this risk assessment have not identified any risks to human and animal health or the environment arising from 4114 maize. Therefore, the same management strategies apply to 4114 maize for safeguarding as for any other commercial maize.

Overall risk

Based on the above conclusions, we conclude that there is negligible overall risk to human and animal health or the environment arising from the use of 4114 maize or any sub-combination of these events for all food and feed purposes and the import and processing of 4114 maize.