FACT SHEET

APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF DP4114 MAIZE FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S) 602-1/1/40

The objective of the Biosafety Act is to protect human, plant and animal health, the environment and biological diversity. Under the Biosafety Act, the National Biosafety Board (NBB) is currently assessing an application for approval submitted by DuPont Malaysia Sdn. Bhd. on behalf of Pioneer Hi-Bred International, Inc.

1. What is the application for?

This application is for the importation of DP4114 maize and its products for use as food, feed and processing.

2. What is the purpose of the import and release?

The purpose of the import and release is to supply or offer for sale or placing in the market DP4114 maize and its products for direct use as food, feed and for processing. DP4114 maize is not intended for cultivation in Malaysia.

3. How has DP4114 maize been modified?

Genetically modified (GM) DP4114 maize is a new event that has been transformed with a single genetic construct containing each of the proteins found in the previously approved maize events DAS-Ø15Ø7-1 (TC1507 maize; Cry1F and PAT proteins) and DAS-59122-7 (59122 maize; Cry34Ab1, Cry35Ab1, and PAT proteins).

DP4114 maize was generated by the insertion of a truncated *cry*1F gene, which was isolated from *Bacillus thuringiensis* (*Bt*) var. *aizawai*; *cry*34Ab1 and *cry*35Ab1 genes, which were both isolated from *Bt*; and a gene for phosphinothricin acetyltransferase (*pat*) which was isolated from *Streptomyces viridochromogenes*. The Cry1F protein, encoded by the *cry*1F gene, confers protection against certain lepidopteran pests, including the European corn borer (ECB, *Ostrinia nubilalis*). The Cry34Ab1 and Cry35Ab1 proteins, encoded by the *cry*34Ab1 and *cry*35Ab1 genes, together comprise an active insecticidal crystal protein that confers protection against certain coleopteran pests, including western corn rootworm (WCR, *Diabrotica virgifera virgifera*). The PAT protein, encoded by the *pat* gene, confers tolerance to the herbicidal active ingredient glufosinate.

4. Characteristics of DP4114 maize

a. Details of the parent organism

Maize is extensively cultivated worldwide and has a long history of safe use. Maize grain and maize-derived products represent staple food and feed for a large portion of the global population (CFIA, 1994). No significant toxicity or allergenicity has been ascribed to any food or feed uses of maize and has been described as a food that is likely to have low allergenicity (OECD, 2002). Maize is not included in the list of food allergy indication of the US Food and Drug Administration (FDA) (US-FDA, 2006). The biology and history of safe use of maize demonstrate that the unmodified organism is safe for human and animal consumption.

b. Details of the donor organism

Bacillus thuringiensis (*Bt*): donor of the *cry*1F, *cry*34Ab1 and *cry*35Ab1 genes

Bt is a diverse group of Gram-positive, spore-forming bacteria that has a history of safe use as a pesticide over several decades (US-EPA, 1998; US-EPA, 2001). It occurs ubiquitously in the soil and on plants including vegetables, cotton, tobacco, tree crops, and forest crops (Schnepf et al, 1998; Shelton, 2012). Several Cry proteins have been deployed as safe and effective pest control agents in microbial *Bt* formulations for almost 40 years.

Streptomyces viridochromogenes: donor of the *pat* gene

Streptomyces viridochromogenes is a common soil bacterium that is not considered pathogenic to humans or animals (OECD, 2007) and produces the tripeptide phosphinothricyl-L-alanyl-L-alanine, which was developed as a non-selective herbicide. The *pat* gene, encoding the phosphinothricin acetyl transferase, confers tolerance to the phosphinothricin herbicide application (OECD, 1999).

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

DP4114 maize produces the Cry proteins Cry1F, Cry34Ab1, and Cry35Ab1, as well as the herbicide tolerance protein PAT. The Cry1F protein confers resistance to certain lepidopteran pests, including European corn borer (*Ostrinia nubilalis*), a major maize pest. This protein is identical to that assessed in event DAS-Ø15Ø7-1 (referred to as TC1507 maize), which was authorized by Malaysian National Biosafety Board (NBB) in 2013. The Cry34Ab1 and Cry35Ab1 proteins together comprise an active binary

insecticidal crystal protein that confers resistance to corn rootworm pests, including western corn rootworm (*Diabrotica virgifera virgifera)*, also a major maize pest. This binary protein is identical to that in event DAS-59122-7 (referred to as 59122 maize), which was authorized by Malaysian National Biosafety Board (NBB) in 2016. Finally, the PAT protein confers tolerance to the herbicidal active ingredient glufosinate-ammonium at current labeled rates. The Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins present in DP4114 maize are identical to the corresponding proteins found in a number of approved events across several different crops that are currently in commercial use, including TC1507 maize and 59122 maize.

5. Modification Method

DP4114 maize was produced by *Agrobacterium*-mediated transformation of a maize line with plasmid PHP27118. The gene expression cassettes contained within the T-DNA region of plasmid PHP27118 are exactly the same as used in the production of TC1507 maize and 59122 maize, and are briefly described below.

The first cassette contains a truncated version of the *cry*1F gene from *Bt* var. *aizawai.* Transcriptional control is provided by the maize ubiquitin gene 1 (*ubi*ZM1) promoter (Christensen *et al.*, 1992), resulting in constitutive expression of the Cry1F protein in maize. The second cassette contains the *cry*34Ab1 gene isolated from *Bt* strain PS149B1 (Ellis *et al.*, 2002; Herman *et al.*, 2002; Moellenbeck *et al.*, 2001). The expression of the *cry*34Ab1 gene is controlled by a second copy of the maize *ubi*ZM1 promoter with 5' UTR and intron (Christensen *et al*., 1992). The third gene cassette contains the *cry*35Ab1 gene, also isolated from *Bt* strain PS149B1 (Ellis *et al.*, 2002; Herman *et al.*, 2002; Moellenbeck *et al.*, 2001). The expression of the *cry*35Ab1 gene is controlled by the *Triticum aestivum* (wheat) peroxidase promoter (TA peroxidase promoter) and leader sequence (Hertig *et al.*, 1991). The fourth and final gene cassette contains a version of the phosphinothricin *N*-acetyltransferase *(pat)* gene from *Streptomyces viridochromogenes* that has been optimized for expression in plants. Expression of the *pat* gene is controlled by the promoter and terminator regions from the cauliflower mosaic virus (CaMV) 35S transcript (Franck *et al*., 1980; Guilley *et al*., 1982; Odell *et al*., 1985).

a. Characterization of the Modification

The molecular characterization of the inserted genes of DP4114 maize was performed using phenotypic segregation analyses, DNA sequencing, and

bioinformatics analysis. These studies demonstrate that the introduced genes segregate according to Mendel's law of inheritance, are stable across multiple generations, and were integrated at a single point of insertion. The introduced genes in DP4114 maize express the Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins.

b. Safety of the expressed protein

DP4114 maize was evaluated by examining the allergenic potential of maize as a crop and by assessing the allergenic and toxic potential of the Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins. Maize is not a common allergenic food and the modification in DP4114 maize is not expected to alter the allergenic potential of maize. The Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins have been assessed previously for TC1507 maize and 59122 maize and have been determined to be unlikely to be potential allergens or toxins to humans and animals. Previous assessments of these proteins included bioinformatic analyses, digestibility studies, and acute protein toxicity studies and are relevant for the assessment of DP4114 maize. Bioinformatic analyses support the original conclusions that the Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins are unlikely to be allergens or toxins. These data support the conclusion that the Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins in DP4114 maize are therefore safe for the food and feed supply.

Similarly, safety data has been submitted to regulatory agencies globally, resulting in the authorization of DP4114 maize for cultivation and/or food and feed uses in 10 countries. The first authorizations of DP4114 maize occurred in 2013.

6. Assessment of Risks to Human Health

a. Nutritional Data

Compositional analysis of DP4114 maize grain was used to evaluate any changes in the levels of proximates, vitamins and minerals, fatty acids, amino acids, vitamins and minerals, and key anti-nutrients, and secondary metabolites compared to near-isoline control maize. For this analysis, grain was selected because of its use in both food and feed. These analyses provided an indication whether DP4114 maize is as nutritious as conventional maize.

There were no statistical differences in nutrient composition between DP4114 maize and control maize except for ash, phosphorus, potassium, and eicosenoic acid (C20:1);

however all individual data points for these four analytes were within the respective tolerance intervals. Considering the compositional equivalence between DP4114 maize and conventional maize it was concluded that DP4114 maize is comparable to near-isoline and reference maize hybrids.

b. Toxicology

The toxicity potential of the Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins were evaluated using a series of studies, including bioinformatics, in vitro digestibility, glycosylation analyses, acute protein toxicity, and heat lability analyses. The results of these studies demonstrate that the proteins are unlikely to be toxins and not acutely toxic in mice.

c. Allergenicity

The allergenicity potential of the Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins were evaluated using a series of studies, including bioinformatics, in vitro digestibility, glycosylation analyses, and heat lability analyses. The results of these studies demonstrate that the proteins are unlikely to be allergens.

6. Assessment of Risks to the Environment

Not applicable. This application does not cover an environmental release. This application is for the importation of DP4114 maize and its products for use as food, feed and processing.

7. What is the Emergency Response Plan?

This notification is for consent to allow the marketing of genetically modified DP4114 maize products for food/feed/processing use in Malaysia. The proposed uses of grain and other products of DP4114 maize, arising from imports, is anticipated to be the same as for any other maize. Given that the scope of this application does not include authorization for the cultivation of DP4114 maize, any exposure to the environment from the import of DP4114 maize is anticipated to be limited to unintended release via spillage during transportation of the grain.

The response plan for reports of unintended release into the environment likely to result in persistence in the environment will include a multiparty investigation of the report together with the competent national agencies and the implementation of mitigating measures jointly agreed upon for confirmed cases. This unlikely unintended release can be controlled with current agronomic measures taken to control other commercially available maize, such as selective use of herbicides (with the exception of glufosinate-ammonium), and manual or mechanical removal of plants.

a. First Aid Measures

No special first aid measures are required in response to exposure to this product.

b. Accidental Release Measure

This application is for the importation of DP4114 maize and its products for use as food, feed and processing.

Any exposure to the environment from the import of DP4114 maize will be limited to unintended release via spillage during transportation of the grain. We are not aware of any study done in Malaysia to assess the extent of accidental spillage of maize grain during transport and its potential germination and establishment. However, survival and reproduction of maize is limited by extreme environmental conditions (heat stress, frost, drought, excessive rainfall, etc.) (Shaw, 1988). Populations of maize are unlikely to survive outside managed agricultural environments (Shaw, 1988). Although plants may occasionally grow in uncultivated fields or occur as volunteers, maize generally does not sustain reproduction outside of cultivation (CFIA, 1994).

This unlikely unintended release can be controlled with current agronomic measures taken to control other commercially available maize, such as selective use of herbicides (with the exception of glufosinate-ammonium), and manual or mechanical removal of plants.

c. Handling and Storage

On the basis of rigorous testing and multiple comprehensive evaluations, DP4114 maize and grain produced from DP4114 maize has been demonstrated to be as safe to humans and animals as conventional maize.

Considering this, recommendations for storage and handling of DP4114 maize are not anticipated to be different from conventional maize.

d. Disposal Consideration

On the basis of rigorous testing and multiple comprehensive evaluations, DP4114 maize and grain produced from DP4114 maize has been demonstrated to be as safe to humans and animals as conventional maize.

Considering this, measures for waste disposal and treatment of DP4114 maize are not anticipated to be different from conventional maize.

8. How can I comment on this application?

Any member of the public may submit their comments or queries on publicly notified information about the application. Before submission of comments or queries, the person should review the information provided. Your comments and queries on any possible impacts/risks to the health and safety of the people and the environment that may be posed by the proposed release are appreciated. The submission to the comments or queries should be prepared carefully as it will be given the same scrutiny as the application by the NBB. The submission of comments and clarifications of queries should contribute to the NBB's assessment. Even if the submission is not science-based, and focuses on cultural or other values, it should still be developed in the form of a well-founded argument. Please note that the consultation period closes on 8 December 2017 and written submissions are required by that date. Submissions must be addressed to:

Director General

Department of Biosafety

Ministry of Natural Resources and Environment

Level 1, Podium 2, Wisma Sumber Asli No. 25, Periaran Perdana, Presinct 4, 62574 Putrajaya, MALAYSIA.

E-mail: biosafety@nre.gov.my

Fax: 03-88904935

Please include your full name, address and contact details in your submission.

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