FACT SHEET

APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF DP202216 MAIZE FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S) 600-2/1/21

The objective of the Biosafety Act 2007 is to protect human, plant and animal health, the environment and biological diversity. Under the Biosafety Act 2007, the National Biosafety Board (NBB) is currently assessing an application for approval submitted by Du Pont Production Agriscience (Malaysia) Sdn. Bhd.

1. What is the application for?

This application is for the importation and release of genetically modified DP202216 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 genetically modified DP202216 maize and its products for direct use as food, feed and for processing. DP202216 maize products can be in the form of whole maize kernel utilized as direct human food, animal feed or processed into byproducts such as oil and starch. DP202216 maize is not intended for cultivation in Malaysia.

3. How has DP202216 maize been modified?

Genetically modified DP202216 maize was generated by the insertion of the *zmm28* gene isolated from maize and the phosphinothricin acetyltransferase gene (*mo-pat*) isolated from *Streptomyces viridochromogenes*. The introduced *zmm28* gene increases and extends expression of the ZMM28 protein resulting in plants with enhanced grain yield potential. The *mo-pat* gene encodes the PAT protein which confers tolerance to the herbicide glufosinate.

4. Characteristics of DP202216 maize

a. Details of the parent organism

The parent organism, *Zea mays* (maize) originates from the Meso-American region (middle South Mexico and Central America) (OECD, 2003). It is grown over a wide range of climatic conditions and is well-suited for warm, temperate climates. Maize grain and maize-derived products represent staple food and feed for a large portion of the global population (Shiferaw *et al*. 2011) 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 known major food

allergens described by the US Food and Drug Administration (FDA) (US-FDA, 2006). The biology and history of safe use of maize demonstrate that the parent organism is safe for human and animal consumption.

b. Details of the donor organism

***Zea mays*: donor of the *zmm28* gene**

According to the OECD, maize is the world's third leading cereal crop, following wheat and rice (OECD, 2002). It is grown as a commercial crop in over 25 countries worldwide. Field maize has been grown for 8,000 years in Mexico and Central America and for 500 years in Europe (OECD, 2002). Maize is cross-pollinated, and until about 1925 mainly open pollinated varieties were grown; today mainly hybrids are grown (OECD, 2002).

***Streptomyces viridochromogenes*: donor of the *mo-pat* gene**

Streptomyces viridochromogenes is a common soil bacterium that is not considered pathogenic to humans or animals 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 (PAT) protein, confers tolerance to the glufosinate.

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

DP202216 maize was genetically modified to increase and extend the expression of the *zmm28* gene. Both the introduced and native *zmm28* genes encode the ZMM28 protein, a MADS-box transcription factor. The increased and extended expression of the ZMM28 protein results in plants with enhanced grain yield potential. Molecular and biochemical characterization of zmm28 transgenic plants demonstrated that their enhanced agronomic traits are associated with elevated plant carbon assimilation, nitrogen utilization, and plant growth. Overall, these positive attributes are associated with a significant increase in grain yield relative to wild-type controls that is consistent across years, environments, and elite germplasm backgrounds (Wu, 2019). The PAT protein confers tolerance to glufosinate-ammonium, the active ingredient in phosphinothricin herbicides.

5. Modification Method

A proprietary corn cultivar PH17AW was transformed via Agrobacterium-mediated transformation with plasmid PHP40099 to produce DP202216 maize. The transfer DNA (T-DNA) region from plasmid PHP40099 contains two gene cassettes.

The first gene cassette (*zmm28* gene cassette) contains the *zmm28* gene from maize encoding the ZMM28 protein (Münster *et al.*, 2002; Pařenicová *et al.*, 2003). The ZMM28 protein is 251 amino acids in length and has a molecular weight of approximately 28 kDa.

The second gene cassette (*mo-pat* gene cassette) contains a maize-optimized version of the phosphinothricin acetyltransferase gene (*mo-pat*) from *Streptomyces viridochromogenes* (Wohlleben *et al.*, 1988). The *mo-pat* gene expresses the phosphinothricin acetyltransferase (PAT) enzyme that confers tolerance to phosphinothricin. The PAT protein is 183 amino acids in length and has a molecular weight of approximately 21 kDa.

a. Safety of the expressed protein

The safety of the ZMM28 protein was evaluated, based on the safety of the source of the *zmm28* gene, a history of exposure to transcription factors in food, homology of the introduced ZMM28 protein with the native ZMM28 protein in non-modified conventional maize, sweet corn, and other commonly consumed food crops.

The source of *zmm28* gene is maize and the safety of maize for the food and feed uses is well established (OECD, 2002). The *zmm28* gene is a transcription factor. Transcription factors are present in commonly consumed foods and are a common component of human and animal diets (Parrott *et al.*, 2010). The amino acid sequence alignment confirms that native and introduced ZMM28 proteins from DP202216 maize are identical. The amino acid sequence alignment also confirms the introduced ZMM28 protein in DP202216 maize is identical to the ZMM28 protein from conventional maize (represented by the B73 reference genome; Genbank accession no: NP_001105155.1). The amino acid sequence of the ZMM28 protein in DP202216 maize is identical to the amino acid sequence of the ZMM28 protein in several commonly consumed varieties of sweet corn, and shares homology with proteins in many other food crops, fruits, and vegetables (Anderson *et al.,* 2019a). The safety of the *zmm28* gene is supported by the history of safe use, its source, the history of exposure to transcription factors in food and the presence of the ZMM28 protein in sweet corn supports, in part, the evaluation of history of safe use, which can be leveraged in the safety assessment of the ZMM28 protein.

The PAT protein is unlikely to present significant risks to the environment, human, or animal health (CERA - ILSI Research Foundation, 2011; CERA - ILSI Research Foundation, 2016; Hérouet *et al.*, 2005). Bioinformatic analyses, heat liability, digestibility, and acute protein toxicity studies (Hérouet *et al.*, 2005) of the PAT protein support that the PAT protein is unlikely to be allergenic or toxic to humans or animals and consumption of the PAT protein is unlikely to cause an adverse effect on humans or animals.

6. Assessment of Risks to Human Health

a. Nutritional Data

Compositional assessments of DP202216 maize were evaluated in comparison to concurrently grown non-modified maize (referred to as control maize) to identify statistical differences and subsequently were evaluated in the context of normal ranges of variation

established from multiple sources of non-GM, commercial maize data. Nutrient composition analysis of DP202216 maize included proximates, fiber, minerals, fatty acids, amino acids, vitamins, secondary metabolites, and anti-nutrients.
Overall, the results of the compositional analysis showed that nutrient composition of forage and grain derived from DP202216 maize was comparable to that of conventional maize (Anderson *et al.,* 2019b).

b. Toxicology

The safety of the ZMM28 protein was evaluated, based on the safety of the source of the *zmm28* gene, a history of exposure to transcription factors in food, homology of the introduced ZMM28 protein with the native ZMM28 protein in non-modified conventional maize, sweet corn and other commonly consumed food crops. This protein is also present in conventional maize, including sweet corn. The increased and extended expression of the ZMM28 protein in DP202216 maize is unlikely to present an increased risk for adverse health effects due to consumption (Anderson *et al.*, 2019a).

The PAT protein is unlikely to present significant risks to the environment, human, or animal health (CERA - ILSI Research Foundation, 2011; CERA - ILSI Research Foundation, 2016; Hérouet et al., 2005). Bioinformatic analyses, heat liability, digestibility, and acute protein toxicity studies (Hérouet et al., 2005) support the conclusion that the PAT protein is unlikely to be allergenic or toxic to humans or animals.

c. Pathogenicity

Streptomyces viridochromogenes are ubiquitous in the environment and has not been reported of causing allergies.

7. Assessment of Risks to the Environment

The application does not cover an environmental release or cultivation. The application is intended only for approval to import DP202216 maize and its products and that it may enter Malaysia as grain, food ingredients for processing or packaging or as finished products ready for distribution, or as feed meal for animals.

8. What is the Emergency Response Plan?

As the scope of this application does not include authorization for the cultivation of DP202216 maize, any exposure to the environment from the import of DP202216 maize is limited and may be due to unintended release via spillage during transportation of the grain.

Any 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

Any exposure to the environment from the import of DP202216 maize will be limited to unintended release via spillage during transportation of the grain. However, survival and reproduction of maize is limited by extreme environmental conditions (heat stress, drought, excessive rainfall, etc.) (OECD, 2003). Populations of maize are unlikely to survive outside managed agricultural environments (OECD, 2003). Although plants may occasionally grow in uncultivated fields or occur as volunteers, maize generally does not sustain reproduction outside of cultivation (OECD, 2003).

Any 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.

Spilled grains should be swept, scooped or vacuumed in a manner that avoids dust generation and dust-related hazards.

c. Handling and Storage

No special handling procedures are required for this product. DP202216 maize and its products may be handled and stored as any conventional maize product.

d. Disposal Consideration

The same measures for waste disposal and treatment as for conventional maize are valid for DP202216 maize.

9. 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 **20 May 2021** and written submissions are required by that date. Submissions must be addressed to:

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

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