FACT SHEET
APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF DP915635 MAIZE FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET
NBB REF NO: JBK(S)600-2/1/32

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 Corteva Agriscience (Malaysia) Sdn. Bhd.

1. What is the application for?

This application is to import and release DP915635 maize and its products for supply or offer to supply for sale or placing in the market. The application does not cover deliberate environmental release (i.e. cultivation) in Malaysia and does not cover any subsequent maize products that result from the use of DP915635 maize for breeding purposes (stacked event[1]s).

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

The purpose of the import and release is for direct use as food, feed and processing (FFP) of DP915635 maize and its products. This means that DP915635 maize may enter Malaysia as grain, food ingredients for processing or packaging or as finished products ready for distribution, or as feed meal for animals. The DP915635 maize is not intended for cultivation in Malaysia.

3. How has DP915635 maize been modified?

The DP915635 maize was created by site-specific integration. DP915635 maize was genetically modified to express the IPD079Ea protein for control of susceptible corn rootworm (CRW) pests, the phosphinothricin acetyltransferase (PAT) protein for tolerance to glufosinate herbicide and the phosphomannose isomerase (PMI) protein that was used as a selectable marker. The PAT and PMI proteins present in DP915635 maize are found in several approved events that are currently in commercial use in Malaysia. More information about DP915635 maize can be found in the Biosafety Clearing House[2].

4. Characteristics of DP915635 maize

a. Details of parent organism

[1] An event in the context of a genetically modified organism is defined by the insertion of DNA into the plant genome as a result of a single transformation process. Multiple DNA sequences may be inserted during a single transformation process.

[2] https://bch.cbd.int/en/database/record?documentID=260914

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 associated 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 United States 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 donor organism

Characteristics of *Ophioglossum pendulum*: donor of the *ipd079Ea* gene

The IPD079Ea protein is encoded by the *ipd079Ea* gene from *Ophioglossum pendulum* (*O. pendulum*) or Daun rambu. *O. pendulum* is known as Old World adders-tongue fern because, like the other species in this family, the vascular stalk grows in the shape of a snake's tongue. Ferns are among the oldest living organisms on the planet and are globally distributed (Fernández, 2011), with the exception of Antarctica. The order Ophioglossales contains one family, Ophioglossaceae (the adder's-tongue family), which is divided into five genera (USDA-NRCS, 2020). The genus *Ophioglossum* L., containing approximately fifty species (Kew Science, 2020a), is native to many parts of North, Central, and South America, Africa, Europe, Asia, and Australia (Kew Science, 2020a). *O. pendulum* specifically has been introduced in the state of Florida in the United States (USDA-NRCS, 2020), and is native to India, Australia, parts of Africa, and southeast Asia (Kew Science, 2020b).

While there are some anecdotal accounts of *O. pendulum* ferns being used for medicinal applications or for food, these accounts are limited. There are no reports of *O. pendulum* being poisonous to humans or livestock.

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

The phosphinothricin acetyltransferase (PAT) protein is encoded by a maize-optimized version of the *phosphinothricin acetyltransferase* (*mo-pat*) gene from *Streptomyces viridochromogenes* (*S. viridochromogenes*) (Wohlleben *et al.,* 1988). *S. viridochromogenes* is a Gram-positive, saprophytic, aerobic bacterium commonly found in soil. *S. viridochromogenes* is not considered pathogenic to humans or animals and is not known to be an allergen or toxin. *S. viridochromogenes* produces the tripeptide L-phosphinothricyl-L-alanyl-alanine (L-PPT), which was developed as a non-selective herbicide (OECD, 1999).

Characteristics of *Escherichia coli*: donor of the *pmi* gene

The phosphomannose isomerase (PMI) protein is encoded by the *pmi* gene from *Escherichia coli* (*E. coli*). *E. coli* is a Gram-negative, facultatively anaerobic, rod-shaped bacterium. The strain *E. coli* K-12 is a strain which has been debilitated, does not normally

colonize the human intestine, and has a poor survival rate in the environment. *E. coli* K-12 has a history of safe use in human drug and specialty chemical production (US-EPA, 1997).

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

The DP915635 maize was genetically modified to produce the IPD09Ea protein for control of susceptible corn rootworm (CRW) pest, the phosphinothricin acetyltransferase (PAT) protein for tolerance to the herbicidal active ingredient glufosinate-ammonium, and the phosphomannose isomerase (PMI) protein that was used as a selectable marker.

5. Modification Method

DP915635 maize was developed by site-specific integration (SSI; Anand *et al.,* 2019) using two sequential transformation steps to (i) insert an integration site sequence (referred to as a "landing pad" sequence) from plasmid PHP73878 at a specific location of the maize genome using microprojectile bombardment plant transformation and a clustered regularly interspaced short palindromic repeats-Cas9 (CRISPR-Cas9)-mediated targeted insertion process, and (ii) insert, via recombination, the intended expression cassettes from the plasmid PHP83175 T-DNA region into the landing pad in the maize genome using *Agrobacterium*-mediated transformation. After each transformation step, a line containing only the intended insertion with no unintended plasmid-derived sequences was selected for the next step in the process. The use of SSI for targeted transgene insertion has advantages compared to random transformation by allowing the ability to pre-select the insertion location to avoid endogenous gene disruption and pre-test the genomic location for agronomic neutrality (Gao *et al.*, 2020). Thus, the SSI approach can simplify risk assessment of the *event* intended for commercialization as it addresses the concern of potential for insertional effects. The molecular characterization analyses conducted on DP915635 maize demonstrated that the introduced genes are integrated at a single locus, stably inherited across multiple generations, and segregate according to Mendel's law of genetics.

a. Safety of the expressed proteins

<u>IPD079Ea Protein</u>

The allergenic and toxic potential of the IPD079Ea protein expressed in DP915635 maize was assessed by doing a bioinformatic comparison of the amino acid sequence of IPD079Ea protein to known or putative protein allergen and toxin sequences, evaluation of the stability of the IPD079Ea protein using *in vitro* gastric and intestinal digestion models, determination of the IPD079Ea protein glycosylation status, evaluation of the heat lability of the IPD079Ea protein using a sensitive insect bioassay, and an evaluation of acute toxicity in mice following oral exposure to IPD079Ea protein. Collectively these studies indicate that IPD079Ea is unlikely to present a hazard. A summary of the safety assessment for IPD079Ea protein has recently been published (Carlson *et al.,* 2022).

Detailed safety studies of the expressed IPD079Ea protein information can be obtained from the Department of Biosafety.

PAT Protein

The amino acid sequence of the PAT protein present in DP915635 maize is identical to the corresponding protein found in a number of authorized GM events across several different crops that are currently commercialized and have a history of safe use.

The history of safe use of the PAT protein expressed in DP915635 maize supports that the PAT protein is unlikely to present significant risks to the environment, human, or animal health. Updated bioinformatics comparisons of the PAT protein sequence to known or putative allergen and toxin sequences support the original conclusions that the PAT protein is unlikely to be allergenic or toxic to humans or animals. Overall, consumption of the PAT protein is unlikely to cause an adverse effect on humans or animals.

Detailed safety studies of the expressed PAT protein information can be obtained from the Department of Biosafety.

PMI Protein

The PMI protein present in DP915635 maize is identical to the corresponding proteins in a previously approved GM event that is currently in commercial use in Malaysia. The history of safe use of the PMI protein supports that the PMI protein expressed in DP915635 maize is unlikely to present significant risks to the environment, human, or animal health. Updated bioinformatics comparisons of the PMI protein sequence to known or putative allergen and toxin sequences further supports that the PMI protein is unlikely to be allergenic or toxic to humans or animals. Overall, the consumption of the PMI protein is unlikely to cause an adverse effect on humans or animals.

Detailed safety studies of the expressed PMI protein information can be obtained from the Department of Biosafety.

6. Assessment of risks to human health

a. Nutritional Data

A compositional equivalence assessment demonstrated that the nutrient composition of DP915635 maize is comparable to that of conventional maize. Samples were analysed for the following key nutritional components in accordance with OECD guidelines (OECD, 2002) for the assessment of genetically modified maize: proximate, fiber and mineral composition in forage and proximate, fiber, mineral, fatty acid, amino acid, vitamin, secondary metabolite and anti-nutrient composition in grain. The compositional data obtained support the conclusion that DP915635 maize is comparable to the

conventional counterpart and commercial reference non-GM maize lines, taking into account biological variation.

Detailed DP915635 maize composition analysis information can be obtained from the Department of Biosafety.

b. Toxicology

Evaluation of the potential toxicity of the expressed IPD079Ea, PAT, and PMI proteins expressed in DP915635 maize support that consumption of DP915635 maize is unlikely to cause an adverse effect on humans or animals. The IPD079Ea protein derived from the fern plant, *O. pendulum* which is present in the environment and not reported to have any adverse effect. The toxic potential of the IPD079Ea protein was evaluated, and the IPD079Ea protein was found no structural similarity to known toxins that could cause adverse effects in humans or animals. PAT and PMI proteins are derived from bacterial species *S. viridochromogenes*, and *E. coli*, respectively, which are present in the environment, have no adverse safety reports, and have a history of safe use. The PAT and PMI proteins present in DP915635 maize are found in several approved events that are currently in commercial use. The history of safe use of the PAT and PMI proteins expressed in DP915635 maize supports that these proteins are unlikely to present significant toxicity risks to the environment, human, or animal health.

Detailed DP915635 maize toxicology information can be obtained from the Department of Biosafety.

c. Pathogenicity

Ophioglossum pendulum

While there are some anecdotal accounts of *O. pendulum* ferns being used for medicinal applications or for food, these accounts are limited. There are no reports of *O. pendulum* being poisonous to humans or livestock.

Streptomyces viridochromogenes

Streptomyces. viridochromogenes is not considered pathogenic to humans or animals and is not known to be an allergen or toxin. *S. viridochromogenes* produces the tripeptide L-phosphinothricyl-L-alanyl-alanine (L-PPT), which was developed as a non-selective herbicide (OECD, 1999).

Escherichia coli

Escherichia coli (*E. coli*) K-12 is a strain which has been debilitated, does not normally colonize the human intestine and has a poor survival rate in the environment. *E. coli* K-12 has a history of safe use in human drug and specialty chemical production (US-EPA, 1997).

d. Allergenicity

Following the guidelines adopted by the Codex Alimentarius, an assessment of the allergenic potential of the newly expressed proteins were conducted. The allergenic potential of the IPD079Ea protein were evaluated, and the IPD079Ea protein was found unlikely to be allergenic to humans or animals. The PAT and PMI proteins present in DP915635 maize are found in several approved events that are currently in commercial use in Malaysia. The history of safe use of the PAT and PMI proteins expressed in DP915635 maize supports that these proteins are unlikely to present significant allergenicity risks to the environment, human, or animal health.

Overall, data and information contained herein support the conclusion that DP915635 maize containing the IPD079Ea, PAT, and PMI proteins is as safe and nutritious as non-GM maize for food and feed uses.

Detailed DP915635 maize allergenicity information can be obtained from the Department of Biosafety.

e. Herbicide residue

A herbicide tolerant crop (GM) may have an altered application pattern of the herbicide to the crop as compared to its conventional non-GM counterpart. The safety of the active ingredient (independent of formulation and specific crop applications) and the safety of the formulation being applied to a given crop plant under particular regime is subject to the legislations and accepted agricultural practices of the country of cultivation. However, any agricultural crop (GM and non-GM) that is placed in the market for consumption are required to be compliant to the acceptable maximum herbicide and pesticide residue limits permitted by the Ministry of Health, Malaysia (Jabatan Pertanian, 2018).

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 DP915635 maize and its products from countries where it has been approved to be planted commercially and tit 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 DP915635 maize, exposure to the environment from the import of DP915635 maize is anticipated to be 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 DP915635 maize is anticipated to 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. DP915635 maize and its products may be handled and stored as any conventional maize product.

d. Disposal

The same measures for waste disposal and treatment as for conventional maize are valid for DP915635 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 in this Fact Sheet. Detailed DP915635 safety study for DP915635 maize can be obtained from the Department of Biosafety. 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 of the comments or queries should be prepared carefully to express your concerns 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. The submission of comments and clarifications of queries should contribute to the NBB's assessment.

Please note that the consultation period closes on 15 November 2023 and written submissions are required before/by that date. Submissions must be addressed to:

Director General
Department of Biosafety
Ministry of Natural Resources, Environment and Climate Change
Level 4, Block F11, Complex F
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Please include your full name, address and contact details in your submission.

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