FACT SHEET
APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF DP910521 MAIZE FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET
NBB REF NO: JBK(S)600-2/1/28

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 of genetically modified insect resistant and herbicide tolerant DP910521 maize and its products for supply or offer to supply for sale or placing in the market.

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 genetically modified DP910521 maize and its products. This means that DP910521 maize 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. The DP910521 maize is not intended for cultivation in Malaysia.

3. How has DP910521 maize been modified?

The DP910521 maize was created by site-specific integration. The DP910521 maize was genetically modified to express the Cry1B.34 protein for protection against certain lepidopteran insect pests, as well as 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 DP910521 maize are identical to the corresponding proteins found in a number of approved events across several different crops that are currently in commercial use.

4. Characteristics of DP910521 maize

a. Details of 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 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 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 donor organism

Characteristics of *Bacillus thuringiensis (Bt).*

The cry1B.34 gene cassette contains the cry1B.34 gene, a chimeric gene comprised of sequences from a cry1B-class gene, the cry1Ca1 gene, and the cry9Db1 gene, all derived from Bacillus thuringiensis (WO Patent 2016061197 Izumi Wilcoxon and Yamamoto, 2016)]; GenBank accession CAA30396.1; (US Patent 7541517 Flannagan and Abad, 2009), respectively). *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. Several Cry proteins have also been effectively deployed as safe and effective pest control agents and have a history of safe use in genetically modified crops (ISAAA, 2019).

Characteristics of *Streptomyces viridochromogenes*

The *mo-pat* gene cassette contains a maize-optimized version of the *phosphinothricin acetyltransferase* (*mo-pat*) gene from *Streptomyces viridochromogenes* (Wohlleben et al., 1988). *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 non-selective herbicide glufosinate).

Characteristics of *Escherichia coli*

The *pmi* gene cassette contains the *phosphomannose isomerase* (*pmi*) gene from *Escherichia coli* (Negrotto et al., 2000). *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

DP910521 maize was genetically modified to express the Cry1B.34 protein for protection against certain susceptible lepidopteran pests, the phosphinothricin acetyltransferase (PAT) protein for tolerance to the glufosinate herbicide, and the phosphomannose isomerase (PMI) protein that was used as a selectable marker. The PAT and PMI proteins present in DP910521 maize are identical to the corresponding proteins found in a number of approved events across several different crops that are currently in commercial use.

5. Modification Method

DP910521 maize was developed by site-specific integration (SSI) using two sequential transformation steps to (1) insert an integration site sequence (referred to as a "landing pad" sequence) at a specific location of the maize genome using microprojectile bombardment, and (2) insert the intended expression cassettes from the plasmid PHP79620 recombination fragment region into the landing pad in the maize genome using microprojectile bombardment. 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 concerns potential for insertional effects.

a. Characterization of the modification

Characterization of the inserted DNA in DP910521 maize was conducted using a Next Generation Sequencing (NGS) method known as Southern-by-Sequencing (SbS™ technology, hereafter referred to as SbS) to determine the insertion copy number and organization within the plant genome and to confirm the absence of plasmid backbone and other unintended plasmid sequences. Southern blot analysis was performed to confirm stable genetic inheritance of the inserted *cry*1B.34, *mo-pat*, and *pmi* gene cassettes across multiple generations during the breeding process. Segregation analysis was conducted for five generations of DP910521 maize to confirm stable Mendelian inheritance.

b. Safety of the expressed proteins

Cry1B.34 Protein

A weight-of-evidence approach was applied to determine the allergenic and toxic potential of the Cry1B.34 protein expressed in DP910521 maize, including an assessment of the following: assessment of the Cry1B.34 protein source organism and history of safe; bioinformatic comparison of the amino acid sequence of Cry1B.34 protein to known or putative protein allergen and toxin sequences, evaluation of the stability of the Cry1B.34 protein using *in vitro* gastric and intestinal digestion models, determination of the Cry1B.34 protein glycosylation status, evaluation of the heat lability of the Cry1B.34 protein using a sensitive insect bioassay, and an evaluation of acute toxicity in mice following oral exposure to Cry1B.34 protein.

Overall, the data and information from these assessments support the conclusion that consumption of the Cry1B.34 protein is unlikely to cause an adverse effect on humans or animals.

PAT Protein

The amino acid sequence of the PAT protein present in DP910521 maize was demonstrated to be 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 DP910521 maize supports a weight of evidence 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.

PMI Protein

The PMI protein present in DP910521 maize is identical to the corresponding proteins found in a number of approved GM events that are currently in commercial use. The history of safe use of the PMI protein supports the weight of evidence that the PMI protein expressed in DP910521 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 support the original conclusions that the PMI protein is unlikely to be allergenic or toxic to humans or animals. Overall, consumption of the PMI protein is unlikely to cause an adverse effect on humans or animals.

c. Utilization of maize

Maize has been a staple of the human diet for centuries, and its processed fractions are consumed in a multitude of food and animal feed products.

Maize is grown globally and is the largest grain crop in the world in total metric ton production, ahead of both wheat (*Triticum sp*.) and rice (*Oryza sativa L*.) (FAOSTAT, 2020). In the 2019/2020 marketing year, world maize area was approximately 192 million hectares (USDA-FAS, 2020).

Food uses of maize include processed products from field maize and direct consumption of sweet maize and popcorn. Food products derived from the wet milling process include starch and sweetener products (e.g., high fructose maize syrup) (May, 1987). Food products derived from the dry milling process include maize grits, maize meal, and maize flour (Watson, 1988). Maize oil may be derived from both wet and dry milling processes (Watson, 1988).

Maize is used extensively as a livestock feed for reasons that include its palatability, digestibility, and metabolizable energy (Loy and Lundy, 2019) and its relatively low cost (OECD, 2002). Animal feed products include maize gluten feed, maize gluten meal, and hominy feed (Loy and Lundy, 2019). Ethanol production from dry milled maize provides distillers grains, another source of animal feed (Loy and Lundy, 2019). Maize can also be fed as a whole plant silage.

Products of DP910521 maize are expected to be used as food, feed, and for processing. The type of expected use of products from DP910521 maize in Malaysia will be the same as the expected usage for products derived from conventional corn. Potential users of products from DP910521 maize are feed millers, food processors and other industrial users.

6. Assessment of risks to human health

a. Nutritional Data

A compositional equivalence assessment demonstrated that the nutrient composition of DP910521 maize forage and grain is comparable to that of conventional maize. Samples were analysed for the following key nutritional components in accordance with OECD guidelines 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 DP910521 maize is comparable to the conventional counterpart and commercial reference maize lines, taking into account biological variation.

b. Toxicology

Evaluation of the potential toxicity of the expressed the Cry1B.34, PAT, and PMI proteins expressed in DP910521 maize support that consumption of DP910521 maize is unlikely to cause an adverse effect on humans or animals.

The Cry1B.34, PAT, and PMI proteins are derived from the bacterial species *B thuringiensis, S. viridochromogenes*, and *E. coli* respectively, which have a long history of safe use,are present in the environment and have no adverse safety reports. The proteins have no structural similarity to known toxins or other biologically active proteins that could cause adverse effects in humans or animals; The proteins are rapidly digested by proteases found in the mammalian gastrointestinal systems. The proteins are lacks of glysoylation. Moreover, the Cry1B.34 protein was assessed for heat liability and acute protein toxicity and the data generated support the conclusion that consumption of the Cry1B.34 is unlikely to cause an adverse effect on humans or animals. It should be further noted that the safety of the PAT, and PMI proteins and their donor organisms was reviewed by numerous global regulatory agencies, including the Ministry of Environment and Water

In addition, the low concentration of these proteins in maize tissues provides further assurance for the safety of the consumed DP910521 maize products. It is therefore highly unlikely that the newly expressed proteins will cause any adverse effects to human and animal health.

c. Pathogenicity

Bacillus thuringiensis

Bacillus thuringiensis is not a known human or animal pathogen and there are no known reports of allergies derived from the organism (Hammond et al. 2004; OECD, 2010).

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 was conducted. The assessment demonstrated that it is unlikely that the Cry1B.34, PAT and PMI proteins will cause allergenicity concerns due to the following considerations: The Cry1B.34, PAT and PMI proteins are derived from the bacterial species which have a long history of safe use,are present in the environment and have no adverse safety reports. Bioinformatic comparison of the amino acid sequences of the Cry1B.34, PAT and PMI proteins with known or putative allergen sequences using an annually updated database from the Comprehensive Protein Allergen Resource (COMPARE) indicated that all three proteins do not share structural similarities with known allergen. The proteins are rapidly digested by proteases found in the human gastrointestinal tract. The proteins are lacks of glysoylation. Moreover, the Cry1B.34 protein was assessed for heat liability and acute protein toxicity and the data generated support the conclusion that consumption of the Cry1B.34 is unlikely to cause an adverse effect on humans or animals. It should be further noted that the safety of the PAT, and PMI proteins and their donor organisms was reviewed by numerous global regulatory agencies, including the Ministry of Environment and Water.

Overall, data and information contained herein support the conclusion that DP910521 maize containing the Cry1B.34, PAT, and PMI proteins is as safe and nutritious as non-GM maize for food and feed uses.

e. Herbicide residue

A herbicide tolerant crop 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 residual level established by the Ministry of Health.

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 DP910521 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 DP910521 maize, any exposure to the environment from the import of DP910521 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 DP910521 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. DP910521 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 DP910521 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 26 January 2023 and written submissions are required before/by that date. Submissions must be addressed to:

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Department of Biosafety
Ministry of Environment and Water
Level 4, Block F11, Complex F
Lebuh Perdana Timur, Precinct 1
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Please include your full name, address and contact details in your submission.

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