FACT SHEET
APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF DAS1131 MAIZE FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET
NBB REF NO: JBK(S)600-2/1/29

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 DAS1131 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 for processing (FFP) of genetically modified DAS1131 maize and its product. This means that DAS1131 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 DAS1131 maize is not intended for cultivation in Malaysia.

3. How has DAS1131 maize been modified?

The DAS1131 maize was created by *Agrobacterium*-mediated transformation with plasmid PHP88492, which contains two gene cassettes and expresses the Cry1Da2 protein for protection against certain susceptible lepidopteran pests and the DGT-28 EPSPS protein for tolerance to glyphosate herbicide.

4. Characteristics of DAS1131 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 organisms

Characteristics of *Bacillus thuringiensis (Bt).*

The *cry*1Da2 gene is a chimeric gene comprised of sequences from the *cry*1Da2 gene encoding an insecticidal core toxin and a derivative of the *cry*1Ab gene, both derived from *Bacillus thuringiensis* (Bt).

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 including for genetically modified crops (ISAAA, 2019).

Characteristics of Streptomyces sviceus, Brassica napus* and *Brassica rapa

The DGT-28 EPSPS protein is encoded by the *dgt-28 epsps* (*5-enolpyruvylshikimate-3-phosphate synthase*) gene derived from *Streptomyces sviceus,* fused to a chimeric chloroplast transit peptide, TraP8, from *Brassica napus* and *Brassica rapa.* The chloroplast transit peptide of the precursor protein is expected to be cleaved upon transport into the chloroplast.

B. napus L. and *B. rapa* belong to the Brassicaceae family, also known as the mustard family. *B. napus* has dark bluish green foliage, branched stems, and yellow flowers (CFIA, 2017). The genus Brassica and its wild relatives are part of the tribe Brassiceae that has its origin in the Mediterranean basin and in south-western Asia (OECD, 2012). Early cultivars of *B. napus*. and *B. rapa* contain high levels of erucic acid and glucosinolates, which are a concern for humans and animal consumption (Eskin and Przybylski, 2003). Varieties with lower in erucic acid and glucosinolates have been developed to meet specific standards on the level of erucic acid and glucosinolates (OGTR, 2011).

Streptomyces sviceus is a Gram-positive, aerobic bacterium commonly found in soil. There are very few species within the genus *Streptomyces* that are considered pathogenic to plants (Bignell *et al.*, 2010) or animals (Kämpfer, 2006). *S. sviceus* is not known to be an allergen or toxin. The enzymatic properties of tannase gene from *S. sviceus* is being investigated for application in the food, feed, beverage and pharmaceutical industries (Wu *et al.*, 2015).

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

DAS1131 maize was genetically modified to produce the Cry1Da2 protein for protection against certain susceptible lepidopteran pests and the DGT-28 EPSPS protein for tolerance to glyphosate herbicide.

The expressed Cry1Da2 protein binds to receptors in the brush border membrane of susceptible lepidopteran pests and causes cell death through the formation of non-specific, ion conducting pores in the apical membrane of the midgut epithelial cells.

DGT-28 EPSPS is a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme in the shikimate pathway that catalyzes the reaction of shikimate-3-phosphate (S3P) with phosphoenolpyruvate (PEP) to produce 5-enolpyruvylshikimate 3-phosphate and inorganic phosphate. The expressed DGT-28 EPSPS protein is targeted to the maize chloroplasts through the TraP8 peptide to provide tolerance to glyphosate herbicide.

5. Modification Method

DAS1131 maize was created by *Agrobacterium*-mediated transformation with plasmid PHP88492. Public inbred line B104 (Hallauer *et al*.,1997) was transformed with plasmid PHP88492 to produce DAS1131 maize. B104 maize inoculated with *Agrobacterium tumefaciens* strain DAt13192 containing plasmid PHP88492. *Agrobacterium tumefaciens* strain DAt13192 is a disarmed strain that contains the *vir* genes and enables efficient transfer of the T-DNA region of the transformed plasmid to the inoculated host plant tissue.

a. Characterization of the modification

Characterization of the inserted DNA in DAS1131 maize was conducted using a Next Generation Sequencing (NGS) method known as Southern-by-Sequencing) SbS™ technology, hereafter refered to as SbS) to determine the insertion copy number and organization within the plant genome and to confirm the absence of plasmid backbone sequences. Southern blot analysis was performed to confirm stable genetic inheritance of the inserted *cry*1Da2 and *dgt-28 epsps* gene cassettes across multiple generations during the breeding process. Segregation analysis was conducted for five generations of DAS1131 maize to confirm stable Mendelian inheritance.

b. Safety of the expressed proteins

Cry1Da2 Protein

A weight-of-evidence approach was applied to determine the allergenic and toxic potential of the Cry1Da2 protein expressed in DAS1131 maize, including an assessment of the following: assessment of the Cry1Da2 protein source organism and history of safe; bioinformatic comparison of the amino acid sequence of Cry1Da2 protein to known or putative protein allergen and toxin sequences, evaluation of the stability of the Cry1Da2 protein using *in vitro* gastric and intestinal digestion models, determination of the Cry1Da2 protein glycosylation status, evaluation of the heat lability of the Cry1Da2 protein using a sensitive insect bioassay, and an evaluation of acute toxicity in mice following oral exposure to Cry1Da2 protein.

Overall, the data and information from these assessments support the conclusion that consumption of the Cry1Da2 protein is unlikely to cause an adverse effect on humans or animals.

DGT-28 EPSPS Protein

Similarly, a weight-of-evidence approach was applied to determine the allergenic and toxic potential of the DGT-28 EPSPS protein expressed in DAS1131 maize, including an assessment of the following: assessment of the Cry1Da2 protein source organism and history of safe; bioinformatic comparison of the amino acid sequence of DGT-28 EPSPS protein to known or putative protein allergen and toxin sequences, evaluation of the stability of the DGT-28 EPSPS protein using *in vitro* gastric and intestinal digestion models, determination of the DGT-28 EPSPS protein glycosylation status, evaluation of the heat lability of the DGT-28 EPSPS protein using an enzymic assay, and an evaluation of acute toxicity in mice following oral exposure to DGT-28 EPSPS protein.

Overall, the data and information from these assessments support the conclusion that consumption of the DGT-28 EPSPS 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 feedand 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 DAS1131 maize are expected to be used as food, feed, and for processing. The type of expected use of products from DAS1131 maize in Malaysia will be the same as the expected usage for products derived from conventional corn. Potential users of products from DAS1131 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 DAS1131 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 DAS1131 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 Cry1Da2 and DGT28 EPSP proteins in DAS1131 maize support that consumption of DAS1131 maize is unlikely to cause an adverse effect on humans or animals. The Cry1Da2 and DGT28 EPSP proteins are derived from the bacterial species *B thuringiensis* and *Streptomyces sviceus*. 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 Cry1Da2 and DGT28 EPSP proteins were assessed for heat liability and acute protein toxicity and the data generated support the conclusion that consumption of the Cry1Da2 and DGT28 EPSP proteins is unlikely to cause an adverse effect on humans or animals. In addition, the low concentration of these proteins in maize tissues provides further assurance for the safety of the consumed DAS1131 maize products. Based on this weight of evidence, it is therefore highly unlikely that the newly expressed proteins in DAS1131 maize, Cry1Da2 and DGT28 EPSP proteins, will cause any adverse effects to human and animal health.

c. Pathogenicity

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).

There are very few species within the genus *Streptomyces* that are considered pathogenic to plants (Bignell *et al.*, 2010) or animals (Kämpfer, 2006).

d. Allergenicity

Following the guidelines adopted by the Codex Alimentarius, an assessment of the allergenic potential of the CryDa2 and DGT28-EPSPS proteins expressed in DAS1131 maize was conducted. The assessment demonstrated that it is unlikely that the CryDa2 and DGT28-EPSPS proteins will cause allergenicity concerns due to the following consideration. The Cry1Da2 and DGT28 EPSP proteins are derived from the bacterial species *B thuringiensis* and *Streptomyces sviceus*, respectively, which have a long history of safe use, are non-allergenic sources and have no adverse safety reports.

A weight-of-evidence approach was applied to determine the allergenic potential of the Cry1Da2 protein expressed in DAS1131 maize, including an assessment of the following: a bioinformatic comparison of the amino acid sequence of Cry1Da2 protein to known or putative protein allergen and toxin sequences, evaluation of the stability of the Cry1Da2 protein using *in vitro* gastric and intestinal digestion models, determination of the Cry1Da2 protein glycosylation status, evaluation of the heat lability of the Cry1Da2 protein using a sensitive insect bioassay, and an evaluation of acute toxicity in mice following oral exposure to Cry1Da2 protein.

Similarly, a weight-of-evidence approach was applied to determine the allergenic potential of the DGT-28 EPSPS protein expressed in DAS1131 maize, including an assessment of the following: a bioinformatic comparison of the amino acid sequence of DGT-28 EPSPS protein to known or putative protein allergen and toxin sequences, evaluation of the stability of the DGT-28 EPSPS protein using *in vitro* gastric and intestinal digestion models, determination of the DGT-28 EPSPS protein glycosylation status, evaluation of the heat lability of the DGT-28 EPSPS protein using an enzymic assay, and an evaluation of acute toxicity in mice following oral exposure to DGT-28 EPSPS protein.

Overall, the data and information generated from these studies support the conclusion that DAS1131 maize containing the Cry1Da2 and DGT-28 EPSPS proteins is as safe and nutritious as non-GM maize for food and feed uses and that it is unlikely to cause an adverse effect on humans or animals.

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 DAS1131 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 DAS1131 maize, any exposure to the environment from the import of DAS1131 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 glyphosate), 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 DAS1131 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 glyphosate), 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. DAS1131 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 DAS1131 maize.

9. How can I comment on this application?

Any member of the public may submit their comments or queries on this public notice about the application. Before submission of comments or queries, the person should review the information provided in this Fact Sheet. Your comments or 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. The submission of comments and clarifications of queries should contribute to the NBB's assessment. Your submission will be given the same scrutiny as the application by the NBB.

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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Level 4, Block F11, Complex F
Lebuh Perdana Timur, Precinct 1
Federal Government Administrative Centre
62000 Putrajaya, MALAYSIA
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Please include your full name, address and contact details in your submission.

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