FACT SHEET
APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF DAS24236 COTTON FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REFERENCE NO.: JBK(S)600-2/1/35

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 DAS24236 cotton 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 cotton products that result from the use of DAS24236 cotton for breeding purposes (stacked events[1]).

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 DAS24236 cotton and its products. This means that DAS24236 cotton 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 DAS24236 cotton is not intended for cultivation in Malaysia.

3. How has DAS24236 cotton been modified?

DAS24236 cotton was genetically modified using *Agrobacterium*-mediated transformation to express the Cry1F and PAT proteins. The Cry1F protein is encoded by the *cry1F* gene isolated from *Bacillus thuringiensis* (*Bt*) and confers protection against certain susceptible lepidopteran pests. The PAT protein is encoded by the *pat* gene isolated from *Streptomyces viridochromogenes,* conferring tolerance to the herbicide glufosinate. However, the main purpose of the PAT protein expressed in DAS24236 cotton was for use as a selection marker for initial transformants during development and not as a herbicide-tolerance trait in the field. The PAT protein present in DAS24236 cotton is found in several events that have been approved by the NBB for the purpose of food, feed and processing in Malaysia.

[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.

4. Characteristics of DAS24236 cotton

a. Details of the parent organism

The genus Gossypium, to which cotton belongs, includes approximately 50 known species with distributions across tropical and subtropical regions (*Viot and Wendel, 2023*). Two primary species, *Gossypium hirsutum* and *Gossypium barbadense*, are the predominantly grown species of cotton today, with cultivation occurring on every continent except Antarctica. Cotton is thought to have been cultivated for over 5000 years (OECD, 2015). Cotton fiber is used to produce textiles which account for approximately 40% of the total textile industry globally (Wang and Memon, 2020). There are additional uses for refined products originating from cotton seed (OECD, 2015).

b. Details of donor organisms

Characteristics of *Bacillus thuringiensis (Bt)*

Bacillus thuringiensis is a diverse group of Gram-positive, spore-forming bacteria that has a history of safe use as an insecticide for several decades (US-EPA, 1998; US-EPA, 2001). It is found naturally in soil and in plants including vegetables, cotton, tobacco, tree crops and forest crops. *(Schnepf et al., 1998; Shelton, 2012*). Several Cry proteins have been used as safe and effective pest control agents in microbial formulations of *Bt* for almost 40 years including for genetically modified crops (ISAAA, 2019).

Characteristics of *Streptomyces viridochromogenes*

Streptomyces 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 a toxin. *S. viridochromogenes* produces the tripeptide L-phosphinotricyl-L-alanyl-alanine (L-PPT), which was developed as a non-selective herbicide (OECD, 1999).

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

DAS24236 cotton has been genetically modified to express the Cry1F protein and the PAT protein making it tolerant to the herbicide glufosinate and resistant to lepidopteran insects.

The Cry1F protein is encoded by the *cry1F* gene for protection against certain susceptible lepidopteran pests. The Cry1F protein binds to receptors in the brush border membrane of certain 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 (Schnepf *et al.* 1998). The Cry1F protoxin is a chimeric, full-length δ-endotoxin comprised of the core toxin of Cry1F from *Bacillus thuringiensis* var. aizawai strain PS81I and nontoxic portions

of Cry1Ca3 and Cry1Ab1 proteins. Together, the portions of Cry1Ca3 and Cry1Ab1 that comprise the chimeric C-terminal domain are approximately those removed by alkaline proteases during the formation of the active Cry1F core toxin.

The PAT protein is encoded by the *pat* gene which inactivates glufosinate and provides tolerance to the herbicide glufosinate-ammonium. It is mainly used as a selectable marker during transformation of DAS24236 cotton.

5. Modification Method

DAS24236 cotton was produced by processing with *Agrobacterium*-mediated transformation with the plasmid pAGM281. The pAGM281 plasmid T-DNA contains two complete gene expression cassettes, the *cry1F* target gene expression cassette and the *pat* marker gene expression cassette. The *cry1F* (synpro) gene was synthesized based on the peptide structure of the Cry1F protein. The protoxin Cry1F is a protein with a core toxin originally identified in *Bacillus thuringiensis var. aizawai* strain PS811. DNA was inserted into the cotton genome through an *Agrobacterium*-mediated transformation. Plant transformation using unarmed *A. tumefaciens* technology is widely used and has been well described (Ooms *et* al., 1982; Zambryski, 1988).

a. Safety of the expressed proteins

Cry1F Protein

A weight-of-evidence approach was applied to determine the allergenic and toxic potential of the Cry1F protein expressed in DAS24236 cotton, including assessment of the following: bioinformatic analyses of the Cry1F protein source organism and history of safe use; bioinformatic comparison of the amino acid sequence of the Cry1F protein to known or putative allergen and toxin sequences, evaluation of the stability of the Cry1F protein using thermal lability, *in vitro* gastric and intestinal digestibility, glycosylation, and acute protein toxicity studies supporting conclusions that the Cry1F protein is unlikely to be a potential allergen or toxin for humans and animals.

PAT Protein

The amino acid sequence of the PAT protein present in DAS24236 cotton was demonstrated to be identical to the corresponding protein found in a number of authorized GM events across several different crops that are currently commeralized and have a history of safe use, including the NBB.

The history of safe use of the PAT protein expressed in DAS24236 cotton supports a weight of evidence that the PAT protein is unlikely to present significant risks to the environment, human, or animal health.

6. Assessment of risks to human health

a. Nutritional Data

Compositional analyses were performed on the seed and processed seed products including almond (pip), seed husks, roasted flour and refined oil. Cottonseed samples were analyzed individually and results from all sites were averaged.

The results of the compositional analyses of cottonseed demonstrate that the insertion of the *cry1F* and *pat* genes and expression of the Cry1F and PAT proteins in DAS24236, has not altered the levels of proximal, minerals, amino acids, fatty acids or antinutrients naturally present with respect to non-transgenic cotton. DAS24236 cotton and its products have compositional and nutritional values that are comparable with both control plants and representative values from the published literature (Berberich et al., 1996; Codex, 2009; Forster and Calhoun, 1995).

Detailed DAS24236 cotton composition analysis information can be obtained from the Department of Biosafety.

b. Toxicology

Evaluation of the potential toxicity of the Cry1F and PAT proteins expressed in DAS24236 cotton support that consumption of DAS24236 cotton is unlikely to cause an adverse effect in humans or animals.

The Cry1F and PAT proteins are derived from the bacterial species *B thuringiensis* and *S. viridochromogenes*, 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. Both proteins are rapidly digested by proteases found in the mammalian gastrointestinal systems and lack glycosylation. Moreover, the Cry1F protein was assessed for heat lability and acute protein toxicity and the data generated support the conclusion that consumption of the Cry1F is unlikely to cause an adverse effect on humans or animals. The safety of the PAT protein and its donor organism have also been reviewed by numerous global regulatory agencies, including the NBB.

In addition, the low concentration of these proteins in cotton seed provides further assurance for the safety of the consumed DAS24236 products. Therefore, the likelihood that the newly expressed proteins will cause any adverse effects to human and animal health is low.

Detailed toxicology information about DAS24236 cotton can be obtained from the Department of Biosafety.

c. Pathogenicity

Bacillus thuringiensis (Bt)

Bacillus thuringiensis is a diverse group of Gram-positive, spore-forming bacteria that has a history of safe use as an insecticide for several decades (US-EPA, 1998; US-EPA, 2001). It 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). It occurs naturally in soil and in plants including vegetables, cotton, tobacco, tree crops and forest crops. (Schnepf *et al.*, 1998; Shelton, 2012). Several Cry proteins have been used as safe and effective pest control agents in microbial formulations of *Bt* for almost 40 years (Sanahuja *et al*., 2011; Sanchis, 2011).

Streptomyces viridochromogenes

Streptomyces 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 a toxin. *S. viridochromogenes* produces the tripeptide L-phosphinotricyl-L-alanyl-alanine (L-PPT), which was developed as a non-selective herbicide (OECD, 1999).

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 Cry1F and PAT proteins will cause allergenicity concerns due to the following considerations: the 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 with known or putative allergen sequences using an annually updated database indicated that both proteins do not share structural similarities with known allergens. The proteins are rapidly digested by proteases found in the human gastrointestinal tract. The proteins lacks glycosylation. Moreover, the Cry1F protein was assessed for heat lability and the data generated support the conclusion that consumption of the Cry1F protein is unlikely to cause an adverse effect on humans or animals. The safety of the PAT proteins and its donor organism have also been reviewed by numerous global regulatory agencies, including the NBB.

Detailed allergenicity information about DAS24236 cotton can be obtained from the Department of Biosafety.

7. Assessment of Risks to the Environment

The application does not cover environmental release or cultivation. The application is intended only to cover the import of the DAS24236 cotton and its products from countries where it is already approved and commercially grown, and that 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 DAS24236 cotton, exposure to the environment from the import of DAS24236 cotton 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 cotton, 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 DAS24236 cotton is anticipated to be limited to unintended release via spillage during transportation of the grain. However, survival and reproduction of cotton is limited by extreme environmental conditions (cold and dry stress, etc.) (OECD, 2008). Populations of cotton are unlikely to survive outside managed agricultural environments (OECD, 2008). Although plants may occasionally grow in uncultivated fields or occur as volunteers, cotton generally does not sustain reproduction outside of cultivation (OECD, 2008).

Any unintended release can be controlled with current agronomic measures taken to control other commercially available cotton, 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. DAS24236 cotton and its products may be handled and stored as any conventional cotton product.

d. Disposal

The same measures for waste disposal and treatment as for conventional cotton are valid for DAS24236 cotton.

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 **20th June 2025** and written submissions are required before/by that date. Submissions must be addressed to:

Director General
Department of Biosafety
Ministry of Natural Resources and Environmental Sustainability
Level 4, Block F11, Complex F
Lebuh Perdana Timur, Precinct 1
62000 Putrajaya, MALAYSIA
E-mail: dob@biosafety.gov.my

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

References

Berberich SA, Ream JE, Jackson TL, Wood R, Stipanovic R, Harvey P, Patzer S, Fuchs RL (1996) The Composition of Insect-Protected Cottonseed Is Equivalent to That of Conventional Cottonseed. Journal of Agricultural and Food Chemistry 44: 365-371

Codex Alimentarius Commission (2001) Codex Standard for Named Vegetable Oils. Codex Alimentarius, STAN-210-1999

Forster LA, Calhoun MC (1995) Nutrient values for cottonseed products deserve new look. In Feedstuffs, Vol 67, pp 16-18

Hammond BG, Campbell KW, Pilcher CD, Degooyer TA, Robinson AE, McMillen BL, Spangler SM, Riordan SG, Rice LG, Richard JL (2004) Lower Fumonisin Mycotoxin Levels in the Grain of Bt Corn Grown in the United States in 2000-2002. Journal of Agricultural and Food Chemistry 52: 1390-1397

ISAAA (2019) GM Approval Database. International Servide for the Acquisition of Agri-Biotech Applications, https://www.isaa.org/gmapprovaldatabase/default.asp

OECD (1999) Consensus document on general information concerning the genes and their enzymes that confer tolerance to phosphinothricin herbicide. Organisation for Economic Co-operation and Development, ENV/JM/MONO(99)13

OECD (2008) Consensus Document on the Biology of Cotton (Gossypium spp.). Organisation for Economic Co-operation and Development, ENV/JM/MONO(2008)33

OECD (2010), "Section 1 - Safety information on transgenic plants expressing Bacillus thuringiensis - Derived insect control protein", in Safety Assessment of Transgenic Organisms, Volume 3: OECD Consensus Documents, OECD Publishing, Paris.

OECD (2015) Safety assessment of Foods and Feeds Derived from Transgenic Crops, Volume 2, Novel food and Feed Safety, OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264180338-en.

Ooms G, Hooykaas PJJ, Van Veen RJM, Van Beelen P, Regensburg-Tuïnk TJG, Schilperoort RA (1982) Octopine Ti-plasmid deletion mutants of Agrobacterium tumefaciens with emphasis on the right side of the T-region. Plasmid. 7:15-29

Sanahuja G, Banakar R, Twyman RM, Capell T, Christou P (2011) Bacillus thuringiensis: a century of research, development and commercial applications. Plant Biotechnology Journal 9: 283-300

Sanchis V (2011) From microbial sprays to insect-resistant transgenic plants: history of the biospesticide Bacillus thuringiensis. A review. Agronomy for Sustainable Development 31: 217-231

Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, Zeigler DR, Dean DH (1998) Bacillus thuringiensis and its Pesticidal Crystal Proteins. Microbiology and Molecular Biology Reviews, 62: 775-806

Shelton A (2012) Bacteria. Biological Control: A Guide to Natural Enemies in North America,

*http://www.biocontrol.entomology.cornell.edu/pathogens/*bacteria.html

US-EPA (1998) R.E.D. Facts: Bacillus thuringiensis. United States Environmental Protection Agency, EPA-738-F-98-001

US-EPA (2001) Overview. In Biopesticides Registration Action Document: Bt Plant-Incorporated Protectants. United States Environmental Protection Agency, pp I1-I27, https://www3.epa.gov/pesticides/chem_search/reg_actions/pip/bt_brad2/1-overview.pdf

Viot CR, Wendel JF (2023) Evolution of the Cotton Genus, Gossypium, and Its Domestication in the Americas. Critical reviews in Plant Sciences 42(1), 1-33. https://doi.org/10.1080/07352689.2022.2156061

Wang H, Memon H (2020) Cotton Science and Processing Technology, Gene, Ginning, Garment and Green Recycling. Textile and Clothing Technology. Springer Nature Singapore Pte Ltd. Singapore.

Zambryski P (1988) Basic processes underlying Agrobacterium-mediated DNA transfer to plant cells. Annual Review of Genetics 22:1-30