FACT SHEET

APPLICATION FOR APPROVAL FOR RELEASE OF KWS20-1 SUGAR BEET FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S)600-2/1/37

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 Bayer Co. (Malaysia) Sdn. Bhd.

1. What is the application for?

This application is to import and release genetically modified herbicide tolerant KWS20-1 sugar beet 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 sugar beet products that result from the use of KWS20-1 sugar beet 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 KWS20-1 sugar beet and its products. This means that KWS20-1 sugar beet pure and raw sugar (sucrose) may enter Malaysia as food ingredients for processing or packaging or as finished products ready for distribution, or as dried pulp and molasses for use as feed meal for animals. The KWS20-1 sugar beet is not intended for cultivation in Malaysia.

3. How has KWS20-1 sugar beet been modified?

KWS20-1 sugar beet was developed by insertion of a single T-DNA containing demethylase (*dmo*), phosphinothricin N-acetyltransferase (*pat*) and *cp4 epsps* genes into the sugar beet genome using *Agrobacterium*-mediated transformation method to confer tolerance to the herbicides dicamba, glufosinate-ammonium and glyphosate.

4. Characteristics of KWS20-1 sugar beet

a. Details of the parent organism

The recipient or parental plant is *Beta vulgaris,* also known as sugar beet. Sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima*) belongs to the family *Chenopodiaceae*

[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.

and the genus *B. vulgaris* comprises several cultivated forms of *B. vulgaris* subsp. *vulgaris*. Cultivars include leaf beet (i.e., Swiss chard) and beetroot (i.e., red table beet). Sugar beet is cultivated worldwide, but primarily in warm and temperate climates with sufficient precipitation. Sugar beet root is seldom used directly for food or feed, but is processed into refined sugar for food and molasses and pulp for feed uses.

b. Details of donor organism

Characteristics of *Stenotrophomonas maltophilia*

The dicamba monooxygenase protein is encoded by the *dmo* gene derived from *Stenotrophomonas maltophilia. Stenotrophomonas maltophilia* is an aerobic, gram-negative bacterium ubiquitously present in the environment, including in water and dairy products (An and Berg, 2018; Mukherjee and Roy, 2016; Okuno et al., 2018; Todaro et al., 2011). These bacteria have been used as effective biocontrol agents in plant and animal pathogenesis (Mukherjee and Roy, 2016), and have antibacterial activity against both gram-positive and gram-negative bacteria (Dong et al., 2015). These bacteria can form biofilms that become resistant to antibiotics (Berg and Martinez, 2015; Brooke et al., 2017). *S. maltophilia* has been found in healthy individuals without any hazard to human health. Other than the potential to become an opportunist pathogen in immunocompromised hosts, *S. maltophilia* is not known for human or animal pathogenicity (Heller et al., 2016; Lira et al., 2017).

Characteristics of *Streptomyces viridochromogenes*

The PAT protein is encoded by the *pat* gene derived from *Streptomyces viridochromogenes. Streptomyces viridochromogenes* is a saprophytic, soil-borne bacterium with no known safety issues. *Streptomyces* species are widespread in the environment and present no known allergenic or toxicity issues (Kämpfer, 2006; Kutzner, 1981), though human exposure is quite common (Goodfellow and Williams, 1983). *S. viridochromogenes* is widespread in the environment and the history of safe use is discussed in Hérouet *et al*. (2005).

Characteristics of *Agrobacterium* sp.

Agrobacterium is a gram-negative, motile, soil-dwelling plant pathogen. *Agrobacterium* sp. strain CP4 is the source of the *cp4 epsps* gene. *Agrobacterium* species are not known for human or animal pathogenicity and are not commonly allergenic (FAO-WHO, 1991; Mehrotra and Goyal, 2012; Nester, 2015).

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

KWS20-1 sugar beet contains *dmo*, *pat* and *cp4 epsps* genes that expresses DMO, PAT and CP4 EPSPS proteins respectively, that confer tolerance to the herbicides dicamba, glufosinate and glyphosate.

Information on the inserted genes are as follows:

	Gene	Donor organism	Trait
1	*dmo*	*Stenotrophomonas maltophilia*	confers tolerance to dicamba herbicide
2	*pat*	*Streptomyces viridochromogenes*	confers tolerance to glufosinate herbicide
3	*cp4 epsps*	*Agrobacterium* sp.	confers tolerance to glyphosate herbicide

5. Modification method

KWS20-1 sugar beet was produced by insertion of *dmo, pat* and *cp4 epsps* genes into the sugar beet genome using *Agrobacterium*-mediated transformation method to confer tolerance to herbicides dicamba, glufosinate and glyphosate, respectively.

a. Characterization of the modification

KWS20-1 sugar beet contains a demethylase gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein. The DMO protein enzymatically catalyzes the demethylation of the broadleaf herbicide dicamba to the non-herbicidal compound 3,6-dichlorosalicylic acid (DCSA) and formaldehyde, thus conferring dicamba tolerance (Chakraborty et al., 2005).

The PAT protein produced in KWS20-1 sugar beet is from the *pat* gene, and is identical to the wild type PAT protein encoded by *S. viridochromogenes*, except for the first methionine that is removed due to co-translational processing in KWS20-1 sugar beet. The PAT protein in KWS20-1 sugar beet is identical to the PAT protein expressed in several commercially available glufosinate-tolerant products (Hérouet et al., 2005; ILSI-CERA, 2011).

KWS20-1 sugar beet contains a codon optimized coding sequence of the *aroA* gene from the soil bacterium *Agrobacterium* sp. strain CP4 that expresses the CP4 EPSPS protein (Barry et al., 2001; Padgette et al., 1996). The CP4 EPSPS protein is structurally similar and functionally equivalent to endogenous plant EPSPS enzymes, but has a much-reduced affinity for glyphosate relative to endogenous plant EPSPS (Barry et al., 2001; Padgette et al., 1996). The presence of this protein renders the plant tolerant to glyphosate.

b. Safety of the expressed proteins

Information and data from studies demonstrate that the DMO, PAT and CP4 EPSPS proteins are unlikely to be allergens or toxins or other biologically active proteins. This is based on the assessment of the donor organisms, *Stenotrophomonas maltophilia* strain DI-6, *Streptomyces viridochromogenes* and *Agrobacterium* sp. which are not known for human or animal toxicity, and are not commonly allergenic (FAO-WHO, 1991; Heller et al., 2016; Kämpfer, 2006; Lira et al., 2017). Bioinformatics analysis was used to compare the DMO, PAT and CP4 EPSPS amino acid sequences against known allergens and toxins, and the results showed a lack of significant structural similarity between the DMO, PAT and CP4 EPSPS proteins and known allergens or toxins (Gu, 2022; Jin, 2022; Skottke, 2022). In addition, studies using the DMO, PAT and CP4 EPSPS proteins have demonstrated that the proteins were digested rapidly in simulated digestive fluids (Chen and Wang, 2019; Fang and Wang, 2022; Leach et al., 2002), and ingestion of the proteins did not cause acute toxicity in mice (Good, 2022; Landin, 2016; Naylor, 1994). These data support the safety for DMO, PAT and CP4 EPSPS proteins.

6. Utilization of sugar beet

Sugar beet is used for the production of sugar and sugar is mainly used as a food ingredient. By-products of sugar production as pulp and molasses are used as animal feed. When sugarbeet is grown in areas of livestock production, leaves of the plant may also be used for fodder. Feed products from sugar beet are high in fibre and energy. Therefore, they are primarily used in feeding ruminants (dairy cows, beef cattle, sheep), but can also be fed to non-ruminants. Sugar beet tops are usually ploughed under. In rare cases tops are ensiled or directly used in ruminant feeding. Wet pulp is typically pressed and dried for feeding purposes. In some regions mixtures of pulp and molasses are used for animal feed. Molasses is mainly used in animal feed or as a fermentation substrate (yeast, citric acid, alcohol, etc.). To a minor extent molasses is used for various industrial purposes such as fuels, rubber, printing, chemical and construction industries.

KWS20-1 sugar beet may enter Malaysia as pure and raw sugar (sucrose) as food ingredients for processing or packaging or as finished food products ready for distribution, or as or dried pulp and molasses for use as feed meal for animals

7. Assessment of risks to human health

a. Nutritional data

Data obtained from compositional analyses conducted on the KWS20-1 sugar beet showed that there were no statistically significant differences in 25 of the 33 comparisons made between KWS20-1 sugar beet and non-genetically modified sugar beet. The mean values for the eight (8) components that showed statistically significant difference between KWS20-1 sugar beet and the non-

genetically modified sugar beet were found to be within the range of values for conventional reference sugar beet and/or the AFSI Crop Composition Database (AFSI-CCDB) (Taylor et al., 2022). Therefore, these statistically significant differences are not considered biologically relevant. These data support the statement that KWS20-1 sugar beet is compositionally equivalent to conventional sugar beet.

Detailed KWS20-1 sugar beet composition analysis information can be obtained from the Department of Biosafety.

b. Toxicology

There are no known health hazards associated with the product. Studies conducted using the DMO, PAT and CP4 EPSPS proteins produced in KWS20-1 sugar beet have shown no toxicity toward mammals (Good, 2022; Landin, 2016; Naylor, 1994). Additionally, there are no amino acid sequences similarities of KWS20-1 sugar beet to known toxins that would raise a safety concern for human (Gu, 2022; Jin, 2022; Skottke, 2022).

Detailed information on the toxicology studies can be obtained from the Department of Biosafety.

c. Pathogenicity

Stenotrophomonas maltophilia

Other than the potential to become an opportunistic pathogen in immune-compromised hosts, *Stenotrophomonas maltophilia* is not known for human or animal pathogenicity (Heller et al., 2016; Lira et al., 2017).

Streptomyces viridochromogenes

Streptomyces viridochromogenes is not considered pathogenic to plants, humans or other animals. *S. viridochromogenes* is widespread in the environment and the history of safe use is discussed in Hérouet *et al*. (2005).

Agrobacterium sp.

Agrobacterium sp. strain CP4 is ubiquitous in the environment and has lack of reports of allergies derived from the organism.

d. Allergenicity

The Codex Alimentarius guidelines for the evaluation of the allergenicity potential of introduced proteins (Codex Alimentarius, 2009) are based on the comparison of amino acid sequences between introduced proteins and allergens, where allergenic cross- reactivity may exist if the introduced protein is found to have at least 35% amino acid identity with an allergen over any segment of at least 80 amino acids.

The bioinformatic results demonstrated there were no biologically-relevant sequence similarities to allergens when the DMO, PAT and CP4 EPSPS proteins sequences were used as a query for a FASTA search of the AD_2022 database (Gu, 2022; Jin, 2022; Skottke, 2022). Furthermore, no short (eight amino acid) polypeptide matches were shared between the DMO, PAT and CP4 EPSPS proteins sequences and proteins in the allergen database. These data show that DMO, PAT and CP4 EPSPS proteins sequences lack both structurally and immunologically-relevant similarities to known allergens, gliadins, and glutenins (Gu, 2022; Jin, 2022; Skottke, 2022).

Detailed information on the allergenicity studies can be obtained from the Department of Biosafety.

8. Assessment of risks to the environment

The application does not cover an environmental release. The application is intended only to cover the import of KWS20-1 sugar beet products from countries where sugar beet is already approved and commercially grown, and that may enter Malaysia as foodstuffs or as feed or raw commodities (i.e., sugar, pulp and molasses) for further processing.

9. What is the Emergency Response Plan?

KWS20-1 sugar beet and food and feed products derived from it have been assessed as being as safe as its conventional non-genetically modified counterparts. Should adverse effects be reported and verified, appropriate follow-up action would be taken to investigate these, and if verified, appropriate actions taken.

a. First aid measures

No special first aid measures are required in response for exposure to this product.

b. Accidental release measures

No special measures are required in response to an accidental release. Spilled processed commodities of sugar, molasses and pulp should be swept, scooped or vacuumed in a manner that avoids dust generation and dust-related hazards.

c. Handling and storage

No special handling and storage procedures are required for this product. KWS20-1 sugar beet and its products may be handled and stored as any conventional sugar beet product.

d. Disposal considerations

The same measures for waste disposal and treatment as for conventional sugar beet are valid for KWS20-1 sugar beet.

10. 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 safety studies for KWS20-1 sugar beet can be obtained from the Department of Biosafety. 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 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 **30 July 2025** and written submissions are required before/by that date. Submissions must be addressed to:

Director General
Department of Biosafety
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.

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