FACT SHEET

APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCT OF GMB151 SOYBEAN FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S) 600-2/1/27

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 BASF (Malaysia) Sdn. Bhd.

1. What is the application for?

The application is to import and release of GMB151 soybean 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 soybean products that result from the use GMB151 soybean for breeding purposes (stacked events).

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

The aim of the import and release is for direct use as food, feed and processing (FFP) of GMB151 soybean and its products. There will be no difference in use of product of GMB151 soybean compared to conventional soybeans already in the market. The GMB151 soybean is not intended for cultivation in Malaysia.

3. How has the GMB151 soybean been modified?

GMB151 soybean was developed through *Agrobacterium*-mediated transformation using the vector pSZ8832 containing the *cry14Ab-1.b* and *hppdPf-4Pa* gene cassettes. The *cry14Ab-1.b* gene is derived from *Bacillus thuringiensis* and produces the Cry14Ab-1 protein, a crystal protein, which confers resistance to nematode plant parasites, such as soybean cyst nematode. GMB151 also produces a modified 4-hydroxyphenylpyruvate dioxygenase (HPPD-4), The *hppdPf-4Pa* gene is derived from *Pseudomonas fluorescens*, which confers tolerance to HPPD-inhibiting herbicides such as isoxaflutole and mesotrione.

4. Characteristics of GMB151 Soybean

a) Details of the parent organism

The recipient or parental plant is *Glycine max* (L) Merr. (soybean). Soybean is widely cultivated and has a history of safe use. Historical and geographical evidence suggests that soybeans were first domesticated in eastern China, between the 17th and 11th century B.C. Soybean germplasm spread from China to other areas of Southeast and Southcentral Asia during the 1st through the 15-16th century AD (*OECD, 2000*).

The major soybean commodity products are seeds, oil, and meal. Whole soybeans are utilized to produce soy sprouts, baked soybeans, roasted soybeans, full fat soy flour and the traditional soy foods (miso, soy milk, soy sauce, and tofu). In addition to whole oil used for human consumption, refined soybean oil has many other technical and industrial applications. Glycerol, fatty acids, sterols and lecithin are all derived from soybean oil. Soy protein isolate is used as a source of amino acids in the production of infant food formula and other food products. Soybean meal is rich in essential amino acids, particularly lysine and tryptophan, which are required supplements in animal diets for optimum growth and health. Soybean meal is used in diets for poultry, swine, dairy cattle, beef cattle and pets. Legumes, and therefore also soybeans, possess several anti-nutritional factors such as phytic acid, protease inhibitors, lectins (hemagglutinins) and the oligosaccharides stachyose and raffinose. However, processing steps, including heating, inactivate anti-nutrient factors present in raw soya beans.

b) Details of donor organisms

Characteristics of *Bacillus thuringiensis*

The *cry14Ab-1.b* gene was originally derived from *Bacillus thuringiensis*. The properties of *Bacillus thuringiensis* (*Bt*) species were recognized many years before the bacterium was identified, with some references suggesting that Bt spores may have already been in use in ancient Egypt. The bacterium was isolated in 1901 by the Japanese biologist Shigetane Ishiwatari during an investigation into wilt disease in silkworms, and he named it *Bacillus soto*. Ten years later, the same bacterium was isolated by Ernst Berliner from a diseased Mediterranean flour moth (*Ephestia kuehniella*) in the German province of Thuringia and was named *Bacillus thuringiensis.*Bt is a Gram-positive, soil-dwelling bacterium. It also occurs naturally in the gut of caterpillars of various types of moths and butterflies, as well as on the dark surfaces of plants. *Bt* bacteria are common in the environment. *Bt* Cry proteins have an excellent track record in more than 50 years of use by organic and conventional farmers and are effective when expressed in genetically modified (GM) plants (Sanahuja,*et al.,* 2011).

Characteristics of *Pseudomonas fluorescens*

The *hppdPf-4Pa* gene was originally derived from *Pseudomonas fluorescens. P. fluorescens* has a long history of safe use in a wide variety of beneficial applications in agriculture, human health and bio-remediation. *P. fluorescens* is used in agriculture as growth promoting agent. It can enhance plant growth through production of siderophores, making it unavailable to other components of the soil microflora. In addition, *P. fluorescens* is used as biopesticide on certain crops and fruits to prevent the growth of frost-forming bacteria on leaves and blossoms. It is also used as seed treatment agent for damping off diseases caused by fungi and nematodes. Due to the metabolic diversity of *P. fluorescens*, it may be used in bio-remediation applications. *P. fluorescens* is able to degrade a wide variety of compounds, including 3-chlorobenzoic acid, naphthalene, phenanthrene, fluorene and fluoranthene, chlorinated aliphatic hydrocarbons, styrene, pure hydrocarbons and crude oil. *P. fluorescens* strains are generally classified as non-pathogenic bacteria or opportunistic pathogen in immuno-compromised patients in several national classifications for microorganisms. The virulence of *P. fluorescens* is low due to its inability to multiply rapidly at body temperatures and to compete with defense mechanisms of the host (OECD, 1997).

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

Summary of introduced genetic elements

Gene introduced	Gene source	Function of the gene	Product
cry14Ab-1.b	*Bacillus thuringiensis*	Nematode resistance	Cry14Ab-1 protein
hppdPf-4Pa	*Pseudomonas fluorescens* - hydroxyphenyl-pyruvate dioxygenase	Herbicide tolerance	modified p-hydroxyphenyl-pyruvate dioxygenase (HPPD) enzyme

5. Modification Method

GMB151 soybean was developed through *Agrobacterium*-mediated transformation using the vector pSZ8832 containing the *cry14Ab-1.b* and *hppdPf-4Pa* gene cassettes. GMB151 soybean produces the Cry14Ab-1 protein, a crystal protein derived from *Bacillus thuringiensis*, which confers resistance to soybean cyst nematode. GMB151 also produces a modified 4 -hydroxyphenylpyruvate dioxygenase (HPPD-4), derived from *P. fluorescens*, which confers tolerance to HPPD-inhibiting herbicides such as isoxaflutole and mesotrione.

a) Characterization of the modification

The inserted sequences of GMB151 soybean were characterized by means of NGS/JSA on genomic DNA (gDNA) prepared from seeds (Schilling, *et al.,* 2018). The results demonstrated that GMB151 soybean contains a single transgenic locus which consists of a single T - DNA copy, lacking the 5’ part of the P2X35S promoter.

The absence of vector backbone sequences in GMB151 soybean was investigated by NGS/JSA on GMB151 soybean, generation T2 (Schilling, *et al.,* 2018). JSA did identify 2 unique junction regions for GMB151 soybean. The bioinformatics junction sequence analysis did not detect any additional vector backbone sequence in GMB151 soybean.

The presence of the *cry14Ab-1b* and *hppdPf-4Pa* genes was confirmed over generations and showed that it was inherited in a predictable and stable manner (Cisneros, 2018). The data are consistent with Mendelian principles and support the conclusion that the GMB151 event consists of a single insert integrated at a single locus within the soybean genome that is stably inherited over generations.

b) Safety of the expressed protein

Cry14Ab-1 Protein

Cry14Ab-1 protein has been evaluated and shown to be safe for human food and animal feed uses. There are neither allergenic nor toxicological *in silico* findings associated with the Cry14Ab-1 protein (Ranjan, 2018). An acute toxicity study was conducted on groups of female and male mice to evaluate the potential impact of Cry14Ab-1 protein. The treatment with the Cry14Ab-1 protein at 2000 mg/kg body weight via the oral route did not produce any signs of systemic toxicity in the male and female C57BL/6J mice (Muhamedi, 2016).

A study was conducted to determine the phenotypic stability of the Cry14Ab -1 and HPPD-4 proteins over five generations of GMB151 soybean (Cisneros, 2017). The concentrations of Cry14Ab-1 protein ranged from 24.24 to 92.84 µg/g DW in GMB151 soybean grain and hull. The concentration of Cry14Ab-1 protein in not treated and treated grain were 81.48 and 92.84 µg/g DW, respectively. The Cry14Ab-1 protein in toasted meal, refined, bleached, and deodorized (RBD) oil, and protein isolated were below the LLOQ (Jeffries, *et al.,* 2018).

HPPD-4 Protein

HPPD-4 protein is derived from the wild-type HPPD protein from *P. fluorescens* with only 4 amino acid substitutions (Porée, 2014). The HPPD proteins have a well-characterized enzymatic activity, and are ubiquitous in nature across all kingdoms. No toxicity or allergenicity findings associated with HPPD proteins were found. Therefore, the HPPD-4 protein is unlikely to be toxic or allergenic (OECD, 1997).

The concentration of HPPD-4 protein ranged from 0.27 to 5.57 µg/g DW in GMB151 soybean grain, protein isolate, and hull. The concentration of HPPD-4 protein in GMB151 not treated and treated grain were 3.79 and 5.57 µg/g DW, respectively. The HPPD-4 protein concentrations in toasted meal and RBD oil were below the LLOQ (Jeffries, *et al*., 2018)

6. Assessment of risks to human health

a) Nutritional data

Composition analysis was conducted to determine the levels of nutrients and anti-nutrients in grain and processed fraction samples from GMB151 soybean, not treated and treated with trait-specific herbicide, and the non-GM counterpart. Soybean grain samples were processed to generate toasted meal, RBD oil, protein isolate, and hull samples.

Grain was analyzed for proximates and fiber, amino acids, fatty acids, minerals, vitamins, and anti-nutrients. Toasted meal was analyzed for proximates and fiber (except total dietary fiber), amino acids, minerals, vitamins (except for alpha-tocopherol and Vitamin K1), and anti-nutrients. RBD oil was analyzed for fatty acids, alpha-tocopherol and Vitamin K1. Protein isolate was analyzed for proximates and amino acids. Hulls were analyzed for proximates and fiber (except for total dietary fiber). Comparable levels of all analytes were observed between the three samples: GMB151 not treated and treated, and the non-GM counterpart (Jeffries *et al.,* 2018).

b) Toxicology

An overall amino acid sequence homology study was carried out by comparing the complete amino acid sequence of the Cry14Ab-1 protein with all protein sequences present in public protein sequence databases. *In silico* analyses results indicate it is unlikely that Cry14Ab-1 protein could exhibit toxic properties. In addition, acute studies in mice demonstrated no clinical signs, mortalities, or treatment related effects after acute oral administration of the Cry14Ab-1 protein at 2,000 mg/kg body weight. It is thus concluded that the Cry14Ab-1 protein is very unlikely to be a toxin even under conditions of maximum oral exposure at a very high dose.

Similarly, another overall amino acid sequence homology study was carried out by comparing the complete amino acid sequence of the HPPD-4 protein with all protein sequences present in public protein sequence databases. *In silico* analyses found no identity with known toxins. Only high similarities to other HPPD proteins and to other enzymes from various organisms, which have good safety records, were found. Therefore, it is unlikely that the HPPD-4 protein could exhibit toxic properties. In addition, an acute study in mice demonstrated no clinical signs, mortalities, or treatment related effects after acute oral administration of the HPPD-4 protein at 2,000 mg/kg body weight. It is thus concluded that the HPPD-4 protein is very unlikely to be a toxin even under conditions of maximum oral exposure at a very high dose (Muhamedi, 2016).

c) Allergenicity

The potential amino acid sequence similarity of Cry14Ab-1 protein with known allergens was evaluated by using several in silico approaches. The overall identity search was carried out to compare the complete query sequence with all protein sequences present in the public allergen database COMPARE (www.comparedatabase.org). In addition, the 8-mer search showed no 100% identity with known allergenic proteins. Therefore, it is unlikely that the Cry14Ab-1 protein possesses allergenic properties. Furthermore, *in vitro* digestibility assays demonstrated that the Cry14Ab-1 protein is degraded in simulated human gastric and intestinal fluids, minimizing the likelihood that this protein could survive in the digestive tract and pose little risks to human and animal health.

The potential amino acid sequence similarity of HPPD-4 protein with known allergens was evaluated by using several in silico approaches. The overall identity search was carried out to compare the complete query sequence with all protein sequences present in the public allergen database COMPARE (www.comparedatabase.org). In addition, the 8-mer search showed no 100% identity with known allergenic proteins. Furthermore, HPPD-4 protein is degraded in the simulated gastric or intestinal fluids indicates a minimal likelihood that the protein could survive and be absorbed through the gastrointestinal system. Consequently, this rapidly digested protein would likely pose no or little risks to human and animal health.

d) 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 environment release. The application is intended only to cover the import of the GMB151 soybean products from countries where the said soybean event is already approved and commercially grown, and that may enter Malaysia as foodstuffs or as feed or for further food processing.

8. What is the emergency response plan?

The seed derived from GMB151 soybean may be imported for processing. The bean could be viable, but is not intended for planting as seed. Specific detection tools are already developed and commercially available to enable the identification of products derived from event GMB151. As with conventional soybean, the plants from event GMB151 are sensitive to herbicides other than HPPD inhibitor such as isoxaflutole and can be controlled or eradicated either by herbicides other than isoxaflutole or by mechanical destruction.

Bean derived from GMB151 soybean is compositionally equivalent to those from conventional soybean. The plants behave agronomically in the same way as conventional soybean except its resistance to nematode plant parasites such as soybean cyst nematode and the intended tolerance to the HPPD inhibitor herbicides such as isoxaflutole and mesotrione. Should adverse effects be reported and verified, appropriate follow up action would be taken to investigate these and if verified appropriate action taken.

(a) First aid measures

No special first aid measures are required with exposure to this product.

(b) Accidental release measures

No special measures are required in response to an accidental release. Spilled seed should be swept, scooped or vacuumed in a manner that avoids dust generation and dust-related hazards. During industrial processing, the seed derived from GMB151 soybean is indistinguishable from conventional soybean and needs no specific or additional treatment compared to conventional soybean.

(c) Handling and storage

No special handling procedures are required for this product. For GMB151 seeds and its products, the same storage and handling can be applied as for conventional soybean. No special storage procedures are required for this product. Seed is stored as any soybean product.

(d) Disposal considerations

The same measures for waste disposal and treatment as for conventional soybean are valid for seed derived from GMB151 soybean.

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. If you require any additional information/ clarifications or would like to check any references provided, you can contact 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 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 **16 November 2022** and written submissions are required by that date. Submissions must be addressed to:

Director General,
Department of Biosafety
Ministry of Environment and Water
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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