

FACT SHEET

APPLICATION FOR APPROVAL FOR RELEASE OF MON 94313 SOYBEAN FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S) 600-2/1/33

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 MON 94313 soybean and all 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 of MON 94313 soybean for breeding purposes (stacked event's).

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 MON 94313 soybean and its products. This means that MON 94313 soybean 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 MON 94313 soybean is not intended for cultivation in Malaysia.

3. How has MON 94313 soybean been modified?

MON 94313 soybean was developed using *Agrobacterium*-mediated transformation to confer tolerance to herbicides dicamba (3,6-dichloro-2-methoxybenzoic acid), glufosinate (2-amino-4-(hydroxymethylphosphinyl) butanoic acid), 2,4-D (2,4-dichlorophenoxyacetate), and mesotrione (2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione).

Information on the inserted genes are as follows:

	Gene	Donor organism	Trait
1	<i>dmo</i>	<i>Stenotrophomonas maltophilia</i>	Dicamba monooxygenase protein confers tolerance to dicamba herbicide

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.

2	<i>pat</i>	<i>Streptomyces viridochromogenes</i>	PAT protein confers tolerance to glufosinate herbicide
3	<i>ft_t.1</i> (a modified version of the R-2,4-dichlorophenoxypropionate dioxygenase (<i>RdpA</i>) gene)	<i>Sphingobium herbicidovorans</i>	2,4-D and FOPs dioxygenase protein confers tolerance to 2,4-D herbicide
4	<i>TDO</i>	<i>Oryza sativa</i> (rice)	Triketone dioxygenase (TDO) protein of <i>Oryza sativa</i> confers tolerance to mesotrione herbicide.

The dicamba monooxygenase and PAT proteins are found in several approved events that are currently in commercial use in Malaysia. More information about MON 94313 soybean can be found in the Euginius² database.

4. Characteristics of MON 94313 soybean

a. Details of the parent organism

The recipient or parental plant is *Glycine max*, also known as soybean. Soybean is grown as a commercial crop in over 35 countries. Soybean is a largely self-pollinated species, although low levels of natural cross-pollination can occur. In studies with cultivated soybean where conditions have been optimized to ensure close proximity and flowering synchrony, natural cross-pollination generally has been found to be very low.

Soybean is the second most planted field crop in the U.S. after corn. According to data from the American Soybean Associations, soybean was planted on approximately 87.2 million acres in the U.S. in 2021, producing 120.7 MMT of soybean (ASA, 2021).

Soybean is used in various food products, including tofu, soy sauce, soymilk, energy bars, and meat products. A major food use for soybean is purified oil, for use in margarines, shortenings, cooking, and salad oils. Soybean oil generally has a smaller contribution to soybean's overall value compared to soybean meal because the oil constitutes just 18 to 19% of the soybean's weight. Nonetheless, soybean oil accounted for approximately 29% of all the vegetable oils consumed globally in 2021, and was the second largest source of vegetable oil worldwide, slightly behind palm oil at approximately 36% share (ASA, 2021).

Soybean meal is a high-value component obtained from processing of soybean, and is used as a supplement in feed rations for livestock. Industrial edible and industrial uses of soybean range from a carbon/nitrogen source in the production of yeasts via fermentation to the manufacture of soaps, inks, paints, disinfectants, and biodiesel. Industrial uses of soybean have been summarized by the American Soybean Association (ASA, 2021).

² https://euginius.eu/euginius/pages/gmo_detail.jsf?gmoname=MON94313

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 *Sphingobium herbicidovorans*

The 2,4-D and FOPs dioxygenase protein is encoded by the *ft_t.1* (a modified version of the R-2,4-dichlorophenoxypropionate dioxygenase (RdpA) gene from *Sphingobium herbicidovorans*. *Sphingobium herbicidovorans* is a common gram-negative, rod-shaped, non-motile, non-spore-forming soil bacterium (Takeuchi *et al.*, 2001; Zipper *et al.*, 1996), which is strictly aerobic and chemo-organotrophic, and not known to be associated with human disease. Members of the genus *Sphingobium* have been isolated from a wide variety of habitats including soil and freshwater (Chaudhary *et al.*, 2017). *Sphingobium* species have also been isolated from food products such as maize (Rijavec *et al.*, 2007), papaya (Thomas *et al.*, 2007) and tomato (Enya *et al.*, 2007). The biosynthesis and biodegrading properties of this genus have been exploited in the food industry (Fialho *et al.*, 2008; Pozo *et al.*, 2007), bioremediation (Alarcón *et al.*, 2008; Jin *et al.*, 2013), and biofuel industry (Varman *et al.*, 2016).

Characteristics of *Oryza sativa*

The *TDO* gene is derived from Asian (*japonica*) rice, *Oryza sativa* (Maeda *et al.*, 2019), which is a crop plant with a long history as food and feed. It is one of the most important

crops in the world serving as a primary food source for more than half of the world's population (Khush, 1997). *Oryza sativa* has two subspecies, *indica* and *japonica*, which account for almost all global rice production (Khush, 1997). Brown, milled, polished and parboiled rice are the major rice products consumed by humans in the form of grain after being cooked. Rice is also consumed as food ingredients which are part of food products. For example, rice flour is used in cereals, baby food, and snacks. The primary nutrients provided by rice are carbohydrates and proteins (OECD, 2016).

Rice is also widely used as feed for livestock. This is fed in various forms such as rice grain, hulls, bran, straw, polishings, and whole crop silage (OECD, 2016).

Generally, rice is considered to be a safe source of food and feed, and is not considered to be a common source of allergens. There are very few compounds in rice which are considered unfavourable for human or animal food/feed, and these compounds have not been observed to exist at levels in rice-based foods that would be a concern for food or feed safety (OECD, 2016).

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

MON 94313 soybean contains *dmo*, *pat*, *ft_t.1*, and *TDO* genes that express DMO, PAT, FT_T.1 and TDO proteins, respectively that confer tolerance to the herbicides dicamba, glufosinate, 2,4-D and mesotrione.

5. Modification method

MON 94313 soybean was produced by insertion of *dmo*, *pat*, *ft_t.1*, and *TDO* genes into the soybean genome using *Agrobacterium*-mediated transformation method to confer tolerance to herbicides dicamba, glufosinate, 2,4-D and mesotrione.

a. Characterization of the modification

In the construction of the plasmid vector used in the development of MON 94313, PV-GMHT529103, a CTP coding sequence from *Arabidopsis thaliana* (*APG6*) was joined to the *dmo* coding sequence; Data from N-terminal sequencing analysis of the MON 94313-produced DMO indicate that processing of the DMO precursor protein expressed in MON 94313 produced a single isoform of the mature MON 94313 DMO protein with no additional N-terminal residues remaining from partial processing of the *APG6* transit peptide. Except for an additional leucine at position two, the MON 94313 DMO protein has an identical sequence to the wild-type DMO protein from the DI-6 strain of *S. maltophilia* (Herman *et al.*, 2005).

The PAT protein produced in MON 94313 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 MON 94313. The PAT protein in MON 94313 is identical to the PAT protein expressed in several commercially available glufosinate tolerant products.

FT_T.1 in MON 94313 soybean is a modified version of the FT_T protein present in MON 87429 maize. FT_T was created through modifications to the RdpA amino acid sequence to improve the enzyme activity towards herbicide substrates, including 2,4-D. FT_T was further modified to enhance its enzymatic activity towards 2,4-D, providing the 2,4-D tolerance in MON 94313 soybean. The FT_T.1 protein in MON 94313 soybean differs from the FT_T protein in MON 87429 maize by 3 amino acids.

TDO is a codon-optimized version of the *HPPD INHIBITOR SENSITIVE 1 (HIS1)* gene from rice that has the same amino acid sequence as the HIS1 protein. Expression of the codon-optimized TDO gene in MON 94313 soybean results in soybean plants that are capable of tolerating in-crop applications of mesotrione through oxidation of the mesotrione molecule.

b. Safety of the expressed proteins

Information and data from studies demonstrate that the DMO, PAT, FT_T.1 and TDO 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*, *Sphingobium herbicidovorans* and *Oryza sativa* which are not known for human or animal toxicity, and are not commonly allergenic (Heller *et al.*, 2016; Lira *et al.*, 2017; Kämpfer, 2006; Takeuchi *et al.*, 2001; Chaudhary *et al.*, 2017; OECD, 2016). Bioinformatics analysis was used to compare the DMO, PAT, FT_T.1 and TDO amino acid sequences against known allergens and toxins, and the results showed a lack of significant structural similarity between the DMO, PAT, FT_T.1 and TDO proteins and known allergens or toxins (Kessenich, 2022; Skottke, 2022). In addition, studies using the DMO, PAT, FT_T.1 and TDO proteins have demonstrated that the proteins were digested rapidly in simulated digestive fluids (Chen and Wang, 2022a; Chen and Wang, 2019; Bretsnyder and Wang, 2021; Chen and Wang, 2022b), and ingestion of the proteins did not cause acute toxicity in mice (Good, 2022; Blanck, 2014; Good, 2021a; Good, 2021b). These data support the safety for DMO, PAT, FT_T.1 and TDO proteins.

6. Assessment of risks to human health

a. Nutritional data

Data obtained from compositional analyses conducted on the grain and forage of MON 94313 soybean showed that there were no statistically significant differences in 48 of the 55 comparisons made between MON 94313 and conventional control. The mean values for the 7 components that showed statistically significant difference between MON 94313 and the conventional control were found to be within the natural variability

of these components as published in scientific literature and/or the AFSI Crop Composition Database (AFSI- CCDB) (Klusmeyer *et al.*, 2022). Therefore, these statistically significant differences are not considered biologically relevant. This data supports the statement that MON 94313 soybean is compositionally equivalent to conventional soybean.

Detailed MON 94313 soybean 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, FT_T.1 and TDO proteins produced in MON 94313 have shown no toxicity toward mammals (Good, 2022; Blanck, 2014; Good, 2021a; Good, 2021b). Additionally, there are no amino acid sequences similarities of MON 94313 to known toxins that would raise a safety concern for human (Kessenich, 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).

Sphingobium herbicidovorans

The ubiquitous presence of *Sphingobium* species in the environment has resulted in widespread human and animal exposure without any known adverse safety or allergenicity reports.

Oryza sativa

Oryza sativa is considered to be a safe source of food and feed, and is not considered by allergists to be a common source of allergens. There are very few compounds in rice which are considered unfavourable for human or animal food/feed, and these compounds have not been observed to exist at levels in rice-based foods that would be

a concern for food or feed safety (OECD, 2016).

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, FT_T.1 and TDO proteins sequences were used as a query for a FASTA search of the AD_2022 database (Kessenich, 2022; Skottke, 2022). Furthermore, no short (eight amino acid) polypeptide matches were shared between the DMO, PAT, FT_T.1 and TDO proteins sequences and proteins in the allergen database. These data show that DMO, PAT, FT_T.1 and TDO proteins sequences lacks both structurally and immunologically relevant similarities to known allergens, gliadins, and glutenins (Kessenich, 2022; Skottke, 2022).

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

e. Herbicide residue

A herbicide tolerant crop (GM) 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 herbicide and pesticide residue limits permitted by the Ministry of Health, Malaysia (Jabatan Pertanian, 2018).

7. Assessment of risks to the environment

The application does not cover an environmental release. The application is intended only to cover the import of MON 94313 soybean from countries where soybean 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?

MON 94313 soybean 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 seeds 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. MON 94313 soybean and its products may be handled and stored as any conventional soybean seed products.

d. Disposal considerations

The same measures for waste disposal and treatment as for conventional soybean are valid for MON 94313 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 in this Fact Sheet. Detailed safety studies for MON 94313 soybean 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 **21 February 2024** 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
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Please include your full name, address and contact details in your submission.

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