

## FACT SHEET

### APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF 305423 SOYBEAN FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK (S) 602-1/1/33

The objective of the Biosafety Act is to protect human, plant and animal health, the environment and biological diversity. Under the Biosafety Act, the National Biosafety Board (NBB) is currently assessing an application for approval submitted by DuPont Malaysia Sdn. Bhd. on behalf of Pioneer Hi-Bred International, Inc.

#### 1. What is the application for?

This application is to import and release genetically modified (GM) 305423 soybean and its products for use as food, feed and processing.

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

The purpose of the proposed authorization to import is to supply or offer for sale or placing in the market 305423 soybean and its products for direct use as food, feed and for processing.

#### 3. How has 305423 soybean been modified?

The 305423 soybean plants have been genetically modified by co-transformation, via microprojectile bombardment, with two separate cassettes: *gm-fad2-1* cassette that is responsible for the unique oil profile and the *gm-hra* gene cassette conferring tolerance to sulfonyleurea herbicides used as a selective agent following transformation.

#### 4. Characteristics of 305423 soybean

##### a. Details of the parent organism

Soybean (*Glycine max* (L.) Merr.) is grown as a commercial crop in over 35 countries worldwide and has a long history of safe use for both human food and stock feed (OECD, 2000). Soybean is grown primarily for the production of seed and has a multitude of uses in the food and industrial sectors. It represents one of the major sources of edible vegetable oil and of proteins for livestock feed use. A major food use in North America

and Europe is as purified oil, utilized in margarines, shortenings, and in cooking and salad oils. It is also used in various food products, including but not limited to, tofu, soy sauce, simulated milk, and meat products. Soybean meal is used as a supplement in feed rations for livestock. Industrial use of soybeans ranges from the production of yeasts and antibodies to the manufacture of soaps and disinfectants. Other fractions and derivatives of the seed have substantial economic importance in a wide range of industrial, food, pharmaceutical, and agricultural products.

#### **b. Details of the donor organism**

Soybean (*Glycine max* (L.) Mer) is the donor organism for all genetic elements comprising the *gm-fad2-1* and the *gm-hra* cassettes contained in two DNA fragments, PHP19340A and PHP17752A, respectively.

Fragment PHP17752A also contains sequences from *Saccharomyces cerevisiae* (Meyen) which is common budding yeast. *Saccharomyces cerevisiae* has an extensive history of use in the area of food processing. Known as baker's yeast or brewer's yeast, this organism has been used for centuries as leavening for bread, as a fermenter of alcoholic beverages, and for the production of macromolecular cellular components such as lipids, proteins including enzymes, and vitamins. *Saccharomyces cerevisiae* is ubiquitous in nature, being present on fruits and vegetables, and is not considered a pathogenic microorganism (OECD, 1999).

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

305423 soybean is a transgenic line with increased levels of monounsaturated (oleic) fatty acid and decreased levels of polyunsaturated fatty acids (linoleic and linolenic) and to a lesser extent, palmitic acid (hereafter referred to as high oleic phenotype).

The high oleic oil derived from 305423 soybean will be beneficial for both the food and industrial oil sectors. The food service industry and food processors will benefit from the availability of a highly stable vegetable oil suitable for uses including but not limited to spray and frying applications without the need for hydrogenation. In the industrial sector, high oleic soybean oil will offer an ingredient that is stable to oxidation for the formulation of cost effective, renewable, environmentally friendly industrial fluids.

### **5. Modification Method**

The 305423 soybean was produced by biolistic co-transformation with two linear DNA fragments, PHP19340A and PHP17752A, derived from plasmids PHP19340 and PHP17752, respectively (Stecca *et al.*, 2007). The fragment PHP19340A from plasmid PHP19340 contains the *gm-fad2-1* cassette. The fragment PHP17752A from plasmid PHP17752 contains the *gm-hra* gene cassette.

#### **a. Characterization of the Modification**

The molecular characterization of the inserted DNA within 305423 soybean was performed using Southern analyses, phenotypic segregation analyses, DNA sequencing, and bioinformatics analysis. These studies demonstrate that the introduced DNA segregate according to Mendel's law of inheritance, are stable across multiple generations, and are integrated at a single genetic locus with four insertions that contain several partial and complete copies of the *gm-fad2-1* cassette (in total, eight copies of the KTi3 promoter, seven copies of the *gm-fad2-1* fragment, and five copies of the KTi3 terminator) and a single copy of the intact *gm-hra* cassette. Southern blot analysis confirmed the absence of all functional elements from the plasmid backbone in 305423 soybean (the hygromycin resistance gene and the plasmid origin of replication) and the presence of a small non-functional fragment of the plasmid backbone DNA.

#### **b. Safety of the expressed protein**

The only novel protein expressed in 305423 soybean is GM-HRA.

Results of bioinformatic analyses of the GM-HRA amino acid sequence indicate the lack of both amino acid identity and immunologically relevant similarities between the GM-HRA protein and known protein allergens and protein toxins or antinutrients. The GM-HRA protein was rapidly hydrolyzed in both simulated gastric and intestinal fluids. In addition, the GM-HRA protein is not glycosylated. There was also no evidence of acute toxicity in mice at a target dose of 2000 mg purified protein preparation per kg of body weight (equivalent to approximately 436 mg of full-length GM-HRA protein per kg of body weight). Taken together, these data support the conclusion that the GM-HRA protein is not a potential allergen or toxin.

Similarly, safety data has been submitted to regulatory agencies globally.

### **6. Assessment of Risks to Human Health**

#### **a. Nutritional Data**

Extensive nutrient composition analysis of forage and grain was conducted to compare the composition of 305423 soybean to that of a non-transgenic near isolate and four conventional soybean varieties. Compositional analysis of 305423 soybean was used to evaluate any changes in the levels of key nutrients, isoflavones, or anti-nutrients.

Based on the compositional evaluation of 52 different analytical components, the grain and forage of 305423 soybean were comparable to conventional soybean except for the intended changes in fatty acid composition of the grain due to the introduction of the *gm-fad2-1* fragment. The content of oleic acid was increased while the content of the linoleic acid, linolenic acid, and to a lesser extent, palmitic acid was decreased.

#### **b. Toxicology**

The only novel protein expressed in 305423 soybean is GM-HRA. The toxicity potential of the GM-HRA protein was evaluated using a series of studies, including bioinformatics, *in vitro* digestibility, glycosylation analyses, acute protein toxicity, and heat lability analyses. The results of these studies demonstrate that the GM-HRA protein is not a potential toxin or anti-nutrient.

#### **c. Allergenicity**

The only novel protein expressed in 305423 soybean is GM-HRA. The allergenic potential of the GM-HRA protein was assessed using guidance from the Codex Alimentarius Commission by assessing the gene source; conducting a bioinformatics comparison of the amino acid sequence of the protein with known protein allergen sequences; evaluating the stability of the protein using *in vitro* gastric and intestinal digestion models; and determining the protein glycosylation status.

The results of these studies demonstrate that the GM-HRA protein is not a potential allergen. In addition, comparing 305423 soybean to its non-transgenic counterpart by immunoblot and ELISA inhibition studies using sera from soy-allergic individuals demonstrating that concentrations of endogenous allergens in 305423 soybean are comparable to those seen in the control soybean, indicating that the transformation process did not alter the allergenic potential of 305423 soybean. Based on the food and

feed safety information of 305423 soybean, no adverse effects to animal or human health are expected.

## **7. Assessment of Risks to the Environment**

This application is for obtaining authorization to import 305423 soybean and its products for use as food, feed and processing. There are no intentions to cultivate 305423 soybean in Malaysia.

## **8. What is the Emergency Response Plan?**

This application is for obtaining authorization to import and release genetically modified 305423 soybean products for food, feed and processing use in Malaysia. The proposed uses of grain and other products of 305423 soybean, arising from imports, will be the same as for any other soybean. Given that the scope of this application does not include authorization for the cultivation of 305423 soybean, any exposure to the environment from the import of 305423 soybean will be limited 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 soybean, such as selective use of herbicides (with the exception of acetolactate synthase (ALS) herbicide), 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**

This application is for obtaining authorization to import 305423 soybean and its products for use as food, feed and processing. Accidental release to the environment from the import of 305423 soybean will be limited to unintended spillage during transportation of the grain.

This unlikely unintended release can be controlled with current agronomic measures taken to control other commercially available soybean, such as selective use of herbicides [with the exception of acetolactate synthase (ALS) herbicide], and manual or mechanical removal of plants.

**c. Handling and Storage**

Recommendations for storage and handling of 305423 soybean will be no different from conventional, non-transgenic soybean.

**d. Disposal Consideration**

Measures for waste disposal and treatment of 305423 soybean will not be different from conventional, non-transgenic 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. 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 of 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 17 February 2017 and written submissions are required by that date. Submissions must be addressed to:

Director General

Department of Biosafety

Ministry of Natural Resources and Environment

Level 1, Podium 2, Wisma Sumber Asli No. 25, Periaran Perdana, Precinct 4, 62574 Putrajaya, MALAYSIA.

E-mail: [biosafety@nre.gov.my](mailto:biosafety@nre.gov.my)

Fax: 03-88904935

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

**References**

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 (2000) Consensus Document on the Biology of *Glycine max* (L.) Merr. (Soybean). Organisation for Economic Co-operation and Development, ENV/JM/MONO(2000)9

Stecca K, Meyer K, Henderson N, Cressman RJ, Hunt S, Young J, Barden K, Crowgey E, Zhong C (2007) Sequence Characterization of Inserts and Genomic Border Regions of Soybean Event DP-3Ø5423-1. Pioneer Hi-Bred International, Inc, Study No. PHI-2006-010/041