FACT SHEET
APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCT OF
FG72 SOYBEAN
FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S) 602-1/1/16

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 Bayer Co. (Malaysia) Sdn. Bhd. (Bayer CropScience).

1. What is the application for?

The application is for import and release of FG72 soybean and its products for supply or offer to supply for sale or placing in the market.

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 FG72 soybean and its products. The said soybean event is not intended for cultivation in Malaysia

3. How has the FG72 soybean been modified?

FG72 soybean was developed through a specific genetic modification to allow for the use of isoxaflutole and glyphosate herbicides as weed control options in soybean crops. The *hppdPf W336* gene which encodes the modified 4-hydroxyphenylpyruvate dioxygenase (HPPD W336) conferring tolerance to isoxaflutole and *2mepsps* gene which encodes a double mutant 5-enolpyruvylshikimate 3-phosphate synthase (2mEPSPS) conferring tolerance to glyphosate were cloned from *Pseudomonas fluorescens* and *Zea mays* (corn), respectively.

4. Characteristics of FG72 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 long history of safe use for consumption as food and feed. The crop is grown primarily for the production of beans, has a multitude of uses in the food and industrial sectors, and represents one of the major sources of edible vegetable oil and of proteins for livestock feed use. Historical and geographical evidence suggest that soybeans were first domesticated in eastern China between the 17th and 11th century B.C.

Today soybean is grown as a commercial crop in over 35 countries without any detrimental effect on the environment. The soybean plant is not weedy in character. Soybean is a largely self-pollinated species and studies have found natural cross-pollination to be very low. Cultivated soybean seeds rarely display any dormancy characteristics and only under certain environmental conditions grow as volunteers in the year following cultivation. If this should occur, volunteers do not compete well with the succeeding crop.

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 the donor organisms

Characteristics of *Pseudomonas fluorescens*

Pseudomonas 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 as biopesticide on certain crops and fruits to prevent the growth of frost-forming bacterial on leaves and blossoms. It is also used as seed treatment agent for damping off diseases caused by fungi and nematodes. Due to metabolic diversity, it may be used in bioremediation applications because of its being able to degrade a variety of compounds. *P. fluorescens* strains are generally classified as non-pathogenic bacteria or non-opportunistic pathogen in immune-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.

Characteristics of *Zea mays*

Assessments indicate that corn is not pathogenic, allergenic, nor toxic to mammals. Corn is one of the few major crops grown in nearly all areas of the world over a wide range of climatic conditions. Literally, thousands of food/feed

and industrial products depend on corn-based ingredients. Because of its high levels of starch, protein, oil and other nutritionally valuable substances, corn is an important crop in human and animal nutrition. Over the years, corn has demonstrated and excellent record of safe use.

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

Summary of introduced genetic elements

Code	Name	Type	Promoter, other	Terminator	Copies	Form
hppdPf W33	4-hydroxyphenylpyruvate dioxygenase	HT	Ph4a748At ABBC	3' nos	2	Modified for sensitivity to isoxaflutole
2mepsps	5-enolpyruvylshikimate 3-phosphate synthase	HT	Ph4a748At	3'histonAt	2	Double mutant

5. Modification method

The soybean line FG72 was produced by means of direct gene transfer with purified *Sal*I fragment from plasmid pSF10 into soybean line Jack. The plasmid pSF10 contains the *hppdPf W336* gene cassette encoding the 4-hydroxyphenylpyruvate dioxygenase of *Pseudomonas fluorescens* and *2mepsps* gene cassette coding for the double-mutant 5-enolpyruvylshikimate-3-phosphate synthase of *Zea mays*. No intermediary host was used during the genetic modification. No antibiotic resistance marker gene was used in the transformation process. Instead, the *hppdPf W336* and *2mepsps* genes that confer tolerance to the herbicides isoxaflutole and glyphosate, respectively, were used as selectable marker genes.

(a) Characterization of the modification

Southern blot and sequence analyses of genomic DNA from FG72 soybean demonstrated that the transgenic event contains two copies of the transferred DNA of plasmid pSF10 integrated in recipient chromosomal DNA. The complete insert DNA of FG72 soybean was sequenced. Using bioinformatics analysis, no relevant known functional genes interrupted upon transformation could be identified.

(b) Safety of the expressed protein

HPPD protein is ubiquitous in nature across all kingdoms: bacteria, fungi, plants, and animals including mammals. In particular, HPPD protein has been characterized in organisms present in human food from plant, fungal or animal origin with good safety records. HPPD proteins have a history of safe consumption. No toxicity or allergenicity findings were found associated with

HPPD proteins. Since the HPPD W336 protein has only one amino acid modification, the safety profile of the novel protein is expected to remain unchanged relative to its wild-type counterpart.

EPSPS proteins are ubiquitous in nature, widely expressed in food and feed crops (such as soybean, tomato, corn, grapes, etc.). No health-related adverse effects have been associated with these plants. Since the 2mEPSPS protein is derived from corn and has only two amino acid modifications, the safety profile of the novel protein is expected to remain unchanged relative to its wild-type counterpart. The 2mEPSPS protein is highly homologous to, and shares similar molecular weight and functionalities with other shikimate synthase proteins, which have been demonstrated to be non-toxic and non-allergenic over the years through consumption. EPSPS proteins have a very well-known and specific biochemical role in plants. The biochemical properties and metabolic effects of the 2mEPSPS activity in plants are comparable to those of endogenous EPSPS proteins except for the insensitivity to glyphosate.

6. Assessment of risks to human health

(a) Nutritional data

Compositional analyses of beans from FG72 and those from conventional non-GM variety Jack and commercial cultivars were compared for compositional and nutritional parameters including moisture, crude fat, crude protein, crude fiber, ash, carbohydrate, mineral content, vitamins, amino acid profile, and fatty acid composition. Based on statistical evaluation of the data and an assessment of the nutritional impact of the different observations, the beans from FG72 soybean are found to be nutritionally equivalent to beans from the traditional non-transgenic comparator, the variety Jack. There is no impact on the nutritional value of the beans as a result of the genetic modifications or the treatment with the test herbicides. The data and findings show that FG72 soybean is compositionally and nutritionally equivalent to conventional counterpart and to currently grown commercial soybean varieties.

(b) Toxicology

An overall amino acid sequence homology study was carried out by comparing the complete amino acid sequence of the HPPD W336 protein with all protein sequences present in public protein sequence databases. *In silico* analyses results indicate it is unlikely that the HPPD W336 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 W336 protein at 2,000 mg/kg body weight. It is thus concluded that the HPPD W336 protein is very unlikely to be a toxin even under conditions of maximum oral exposure at a very high dose.

Similarly, an overall amino acid sequence homology study was carried out by comparing the complete amino acid sequence of the 2mEPSPS 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 EPSPS proteins and to other enzymes from various organisms, which have good safety records, were found. Therefore, it is unlikely that the 2mEPSPS 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 2mEPSPS protein at 2,000 mg/kg body weight. It is thus concluded that the 2mEPSPS protein is very unlikely to be a toxin even under conditions of maximum oral exposure at a very high dose.

(c) Allergenicity

The potential amino acid sequence similarity of HPPD W336 protein with known allergens was evaluated by using several *in silico* approaches. The overall and 80-mer identity searches showed no relevant sequence similarity with any known allergen from the AllergenOnline database. In addition, the results of the epitope homology search showed no identity with epitopes from known allergenic proteins. Therefore, it is unlikely that the HPPD W336 protein possesses allergenic properties. Furthermore, *in vitro* digestibility assays demonstrated that the HPPD W336 protein is rapidly 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 2mEPSPS protein with known allergens was evaluated by using several *in silico* approaches. The overall and 80-mer identity searches showed no relevant sequence similarity with any known allergens from the AllergenOnline database. In addition, the results of the epitope homology search showed no identity with epitopes from known allergenic proteins. Therefore, it is unlikely that the 2mEPSPS protein possesses allergenic properties. Furthermore, rapid degradation of the 2mEPSPS protein 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.

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 FG72 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 bean derived from FG72 soybean is intended to 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 FG72. As with conventional soybean, the plants from event FG72 are sensitive to herbicides other than isoxaflutole and glyphosate and can be controlled or eradicated either by herbicides other than isoxaflutole and glyphosate or by mechanical destruction.

Bean derived from FG72 soybean is compositionally equivalent to those from conventional soybean. The plants behave agronomically in the same way as conventional soybean except showing the intended tolerance to the herbicides isoxaflutole and glyphosate. 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 bean derived from event FG72 is indistinguishable from conventional soya beans and needs no specific or additional treatment compared to conventional soybeans.

(c) Handling and storage

No special handling procedures are required for this product. For FG72 soybean and its products, the same storage and handling can be applied as for conventional soybean. No special storage procedures are required for this product. Bean is stored as any soya bean product.

(d) Disposal considerations

The same measures for waste disposal and treatment as for conventional soybean are valid for bean derived from event FG72.

9. How can I comment on this application?

Any member of the public may submit their comment 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 19th March 2014 and written submissions are required by that date. Submissions must be addressed to:

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