FACT SHEET
APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF A5547-127 SOYBEAN
FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S) 602-1/1/14

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 A5547-127 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 A5547-127 soybean and its products. The said soybean event is not intended for cultivation in Malaysia

3. How has the A5547-127 soybean been modified?

A5547-127 soybean was developed through a specific genetic modification to allow for the use of glufosinate ammonium, the active ingredient in phosphinothricin herbicides (e.g. Liberty®) as a weed control option in soybean crops. The *pat* gene, conferring tolerance to glufosinate ammonium, was cloned from the common aerobic soil actinomycete, *Streptomyces viridochromogenes,* and encodes the enzyme phosphinothricin-N- acetyltransferase (PAT).

The herbicides bialaphos, phosphinothricin and its chemically synthesized form glufosinate ammonium are potent inhibitors of glutamine synthetase (GS), the enzyme that plays a central role in the assimilation of ammonia and in the regulation of the nitrogen metabolism in the plant. The *pat* gene codes for a PAT protein that metabolizes glufosinate to an inactive, acetylated derivative conferring the plant tolerant to glufosinate ammonium.

4. Characteristics of A5547-127 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 suggests 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, however natural cross-pollination can occur. In studies, natural cross-pollination has been found to be very low. Cultivated soybean seed rarely displays any dormancy characteristics and only under certain environmental conditions grows as a volunteer 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 organism

Characteristics of *Streptomyces viridochromogenes*, strain Tü 494

Streptomyces viridochromogenes belongs to the family *Streptomycetae*. *S. viridochromogenes* is a common saprophytic, gram positive, aerobic, sporulating bacterium naturally occurring in soil. *S. viridochromogenes* is not itself known to be a human pathogen nor has it been associated with other properties (e.g. production of toxins) known to affect human health. *Streptomyces viridochromogenes* is not known to be an allergen or toxin. Other members of the genus produce therapeutically useful antibiotics. In the family *Streptomycetae*, few *Streptomyces* species have been isolated from animal or human sources and pathogenicity is not a typical property of these organisms. *Streptomyces viridochromogenes* is not used as a food source itself, although it may be found unintentionally in food without any harm.

(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
pat	Phosphinothricin N-acetyltransferase	HT	CaMV 35S NULL	CaMV 35S poly(A) signal	1	Modified for transcription in plants
bla	beta lactamase	SM	bacterial promoter	NULL		Truncated, not expressed

5. Modification method

The soybean line A5547-127 was produced via biolistic transformation of soybean line with a pUC19 based plasmid containing a modified form of the *pat* gene under the control of promoter and termination sequences derived from the 35S transcript from cauliflower mosaic virus (CaMV). The plasmid was linearized prior to transformation in order to destroy the beta-lactamase (*bla*) encoding antibiotic resistance marker gene present in the plasmid backbone. The nucleotide sequence of the *pat* gene was altered via site-directed mutagenesis in order to reduce the high G:C content (typical for bacterial genes but atypical for plant genes) and generate plant-preferred codons. These sequence modifications did not result in changes to the predicted amino acid sequence of the PAT enzyme.

(a) Characterization of the modification

Southern blot analysis demonstrate that the transgenic glufosinate-ammonium tolerant A5547-127 soybean contains one copy of the *pat* gene cassette and parts of the *bla* gene sequences located at the 5' and 3' ends of the inserted DNA. The complete insert DNA of A5547-127 soybean was sequenced.

(b) Safety of the expressed protein

The PAT content of beans from glufosinate-ammonium tolerant A5547-127 soybean is very low making up an average of 10 µg/g fw or 2.8 x 10^{-3}% of the seed protein. The PAT protein was not detected in oil products. In oil products, the main soybean product consumed by humans, the PAT protein was below the detection level of 6ng/g. For hulls, defatted meal, toasted meal, and protein isolate from A5547-127 soya beans, PAT protein content of

9.5 µg/g fw, 0.07 µg/g fw, 0.01 µg/g fw and 0.08 µng/g fw was measured, respectively.

The PAT protein expressed in the transgenic soybean A5547-127 has a remote potential for toxicity. This was demonstrated by examining the amino acid sequence homology, chemical characteristics of the protein and by a toxicity study in rats. The nucleotide sequence of the *pat* gene and the deduced amino acid sequence of the PAT protein were compared with sequences available for known toxins in the GenBank database and showed no significant homology with any known toxins.

The PAT protein expressed in A5547-127 soybean does not possess characteristics typical of known protein allergens and is extremely unlikely to be allergenic. There were no regions of homology when the sequences of the introduced protein were compared to the amino acid sequences of known protein allergens. Unlike some known protein allergens, the PAT protein was rapidly degraded by acid and/or enzymatic hydrolysis when exposed to simulated gastric fluids. *In vitro* digestibility studies, under simulated mammalian gastric conditions, demonstrated that the PAT enzyme was rapidly degraded.

The (14-day) repeated dose oral toxicity feeding study in rats has shown the absence of treatment related adverse effect related to the PAT protein. No unscheduled mortality and no clinical signs were observed in any group of studied animals. Food consumption and body weights were not affected by the treatment.

6. Assessment of risks to human health

(a) Nutritional data

Compositional analyses of beans from A5547-127, conventional non-GM variety and current commercial soybean varieties were compared for compositional and nutritional parameters including moisture, crude fat, crude protein, crude fiber, ash, carbohydrate, mineral content (including calcium and phosphorous), amino acid profile, and fatty acid composition. In all cases protein content was within the normal range of soybean published in literature. Furthermore, an evaluation of the nutrient values determined that they were similar to the range of nutrient values reported for soya beans. The data and findings show that A5547-127 soybean is compositionally and nutritionally equivalent to currently grown conventional commercial soybean varieties.

(b) Toxicology

The low potential for toxicity of the PAT protein expressed in the transgenic soybean A5547-127 was demonstrated by examining the amino acid

sequence homology, chemical characteristics of the protein and by repeated dose oral toxicity study in rats. The nucleotide sequence of the *pat* gene and the deduced amino acid sequence of the PAT protein were compared with sequences available for known toxins in the GenBank database and showed no significant homology with any known toxins or allergens.

A repeated dose oral toxicity (14-day) feeding study in rats performed with the PAT protein encoded by the *pat* gene has also shown the absence of adverse effect. No adverse effect, no unscheduled mortality and no clinical signs were observed in any group of studied animals. Food consumption and body weights were not affected by the treatment.

(c) Allergenicity

The PAT enzyme expressed in A5547-127 soybean does not possess characteristics typical of known protein allergens and is extremely unlikely to be allergenic. There were no regions of homology when the sequences of the introduced protein were compared to the amino acid sequences of known protein allergens. There was no evidence found of post-translation modifications such as acetylation, glycosylation or phosphorylation of the PAT protein.

Unlike known protein allergens, the PAT protein was rapidly degraded by acid and/or enzymatic hydrolysis when exposed to simulated gastric fluids. *In vitro* digestibility studies, under simulated mammalian gastric conditions, demonstrated that the PAT enzyme was inactivated and was rapidly degraded. No adverse effects have been reported to be associated with this enzyme.

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 A5547-127 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 A5547-127 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 A5547-127. As with conventional soybean, the plants from event A5547-127 are sensitive to herbicides other than glufosinate-ammonium and can be controlled or eradicated

either by herbicides other than glufosinate ammonium or by mechanical destruction.

Bean derived from A5547-127 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 herbicide glufosinate ammonium. 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 A5547-127 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 A5547-127 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 A5547-127.

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 30 November 2013 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.