FACT SHEET

APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF SOYBEAN A2704-12 FOR SUPPLY OR OFFER TO SUPPLY

NBB REF NO: JBK(S) 602-1/1/7

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 this application for?

To import and release products of Soybean A2704-12 (herbicide-tolerant soybean)

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

The aim of the import and release is to supply or offer to supply for sale/placing on the market - for direct use as food, feed and/or for processing (FFP). The said soybean event is not intended for cultivation in Malaysia.

3. How has the Soybean A2704-12 been modified?

Soybean lines A2704-12 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).

Glufosinate is a short name for the ammonium salt, glufosinate ammonium. It is a broad spectrum contact herbicide and is used to control a wide range of weeds after the crop emerges or for total vegetation control on land not used for cultivation. Glufosinate is a natural compound isolated from two species of Streptomyces fungi. It inhibits the activity of an enzyme, glutamine synthetase, which is necessary for the production of glutamine and for ammonia detoxification. The application of glufosinate leads to reduced glutamine and increased ammonia levels in the plant tissues. This causes photosynthesis to stop and the plant dies within a few days. Glufosinate also inhibits the same enzyme in animals. It is highly biodegradable, has no residual activity, and very low toxicity for humans and wild fauna. The PAT enzyme detoxifies phosphinothricin via acetylation into an inactive compound.

4. Characteristics of the Soybean A2704-12

(a) Details of the parent organism

Characteristics of *Glycine max* (L.) Merr. (Soybean)

Center of Origin	Reproduction	Toxins	Allergenicity
Southeast Asia; wild soybean species endemic in China, Korea, Japan, Taiwan	self-pollinated; rarely displays any dormancy characteristics; does not compete well with other cultivated plants		

Soybean is widely cultivated and has a long history of safe use for consumption as food and feed. It is not considered harmful or pathogenic to humans. Soybean A2704-12 may enter Malaysia as raw agricultural commodity soya beans or legumes, whole and dried for direct use as food, feed or for processing and/or products from it.

(b) Donor organism

Donor Organism Characteristics

Latin Name	Gene	Pathogenicity
Streptomyces viridochromogenes	*pat*	*S. viridochromogenes* is ubiquitous in the soil. The spore chains are spirals and the spore surface is spiny. The spore mass is blue, the reverse is green and its pigments are pH sensitive. It exhibits very slight antimicrobial activity, is inhibited by streptomycin, and there have been no reports of adverse affects on humans, animals, or plants.

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

Summary of Introduced Genetic Elements

Code	Name	Type	Promoter, other	Terminator	Copies	Form
pat	phosphinothricin N-acetyltransferase *(S. viridochromogenes)*	HT	CaMV 35S NULL	CaMV 35S poly-A signal		Synthetic version of native gene

5. Modification Method

The soybean line A2704-12 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) Characteristics of the Modification

The Inserted DNA
Southern blot analysis of genomic DNA from line A2704-12 indicated the incorporation of two copies of the *pat* gene, inserted in a head-to-tail configuration, and that one copy of the 3' *bla* sequences and one copy of the 5' *bla* sequences were integrated between the two *pat* gene copies.

Expressed Material
The PAT enzyme was the only new protein expressed in these transgenic soybean lines. The levels of PAT protein expression were quantified using enzyme-linked immunosorbent assay (ELISA), which in the case of A2704-12 were found to correspond to 0.0031%, 0.0015% and 0.0005% of the total crude protein in samples of forage, hay and seed, respectively. As expected, no PAT protein was detected in refined oil, food grade oil, and crude lecithin. Although PAT enzyme activity could be detected in the hull fraction, no activity was detected in meal fractions prepared from A2704-12.

6. Assessment of Risks to Human Health - Food and/or Feed Safety Considerations

(a) Nutritional Data
Samples of hay, forage, seed, hulls, and toasted and non-toasted defatted soy meal from transgenic soybean were subjected to proximate analyses (moisture, crude protein, crude fat, ash, acid detergent fibre, neutral detergent fibre, carbohydrate). As well, seed samples were subjected to fatty acid analysis, amino acid analysis, and analyses of minerals (calcium, phosphorous, and potassium). Except in one case, there were no statistically significant differences between the values determined for transgenic plant samples and corresponding samples obtained from non-transgenic control plants. Statistical differences were seen in the levels of some amino acids between A2704-12 soybeans and non-transgenic soybean, but that the values were still within the normal range reported by the USDA for soybean.

(b) Toxicology
Samples of seed were analyzed for stachyose, raffinose, and phytic acid, and

additionally for trypsin inhibitor and lectins in seed. The concentrations of phytoestrogens, such as daidzein, genistein, and glycitein, were determined for samples of seed, and toasted and non-toasted soy meal. The levels of these compounds were not statistically different between samples from non-transgenic and transgenic soybeans.

(c) Allergenicity

The low potential for allergenicity of the PAT protein has previously been established through amino acid sequence comparisons with known protein allergens and digestibility studies using simulated gastric and intestinal fluids. Additionally, soybean seed extracts from line A2704-12 and from non-transgenic control plants were screened against a panel of sera from 16 soy-allergic individuals using the radioallergosorbent test (RAST). The results of this study did not reveal any qualitative or quantitative difference in endogenous soybean allergen content between transgenic and non-transgenic soybean.

7. Assessment of Risks to the Environment

The application does not cover an environmental release. The release is intended only to cover the import of the soybean A2704-12 products from countries where the corn 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 soybeans derived from event A2704-12 are intended to be imported for processing. The grain could be viable, but is not intended for planting as seeds. In case of doubt of the identity, specific detection tools are already developed and commercially available to enable the identification of a possible misuse of soybeans derived from event A2704-12. The identified plants could be easily eradicated by herbicides or mechanical destruction.

Soybeans derived from event A2704-12 are compositionally equivalent to conventional soybeans. The plants behave agronomically in the same way as conventional soybean except showing the intended tolerance to the herbicide Glufosinate-ammonium. As conventional soybeans, the soybeans plants from event A2704-12 are sensitive to all other herbicides and can be – if needed – inactivated by conventionally known herbicides except the ones, which contain glufosinate-ammonium as a single active ingredient. 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 for exposure to this product.

(b) Accidental Release Measure

During industrial processing, the soybean grain derived from event A2704-12 is undistinguishable from conventional soybean grain and needs no specific or additional treatment in regards to the conventional soybean commodity. Soybean seeds rarely display any dormancy characteristics. The soybean plant is not weedy in character and weedy soybean has not been reported growing naturally outside its centre of origin.

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.

(c) Handling and Storage

No special handling procedures are required for this product. For A2704-12 soybean and its products, the same storage and handling can be applied as for conventional soybeans and products already on the market. No special storage procedures are required for this product. Store as any soybean seed product.

(d) Disposal Considerations

The same measures for waste disposal and product treatment as for conventional soybeans are valid for soybeans derived from event A2704-12.There are no constraints to the disposal of this product. Empty containers should be discarded. Empty containers should not be used for other purposes. No materials used in this study should enter the food and feed chain, including unused soybeans material or derived feed, animal parts or carcasses. Disposal should be via incineration, autoclave or in the case of unused starting material return to the company in secure, clearly labeled double walled containers. Disposal should be managed in accordance with local, state or federal regulations.

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 **18 January 2011** 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, Persiaran Perdana, Precinct 4, 62574 Putrajaya, MALAYSIA. E-mail: biosafety@nre.gov.my. Fax: 03-88904935.

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