FACT SHEET
APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCT OF LLCotton25
FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK (S) 602-1/1/31

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.

1. What is the application for?

The application is for import and release of LLCotton25 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 LLCotton25 and its products. The use pertains to the end products in food (essentially oil) and feed (essentially meal) derived from genetically modified cotton. The said cotton event is not intended for cultivation in Malaysia.

3. How has the LLCotton25 been modified?

LLCotton25 was developed to allow the use of glufosinate ammonium as a weed control option in cotton production. This genetically engineered cotton line expresses a protein, phosphinothricin-acetyl-transferase (PAT) that confers tolerance to the active ingredient L-phosphinothricin in glufosinate ammonium. The expression of PAT is due to the introduction of the *bar* gene. This gene was isolated from *Streptomyces hygroscopicus*, a gram-positive soil bacterium. L-phosphinothricin inhibits the activity of glutamine synthetase (GS) irreversibly by binding to its active sites. The inhibition of GS caused by the application of glufosinate ammonium to plants results in the accumulation of ammonia, the reduction in the levels of glutamine, and the inhibition of photosynthesis, all of which results in the death of the plant. Plants transformed with the *bar* gene express the enzyme phosphinothricin-acetyl-transferase (PAT) which acetylates L-phosphinothricin into a non- phytotoxic metabolite (N-acetyl-L-glufosinate) hence conferring tolerance to the said herbicide.

4. Characteristics of LLCotton25

(a) Details of the parent organism

Center of Origin	Reproduction	Toxins	Allergenicity
Believed to originate in Meso-America (Peruvian-Ecuadorian-Bolivian region).	Generally self-pollinating, but can be cross-pollinating in the presence of suitable insect pollinators (bees). In the U.S., compatible species include *G. hirsutum, G. barbadense*, and *G. tomentosum*.	Gossypol in cottonseed meal.	Cotton is not considered to be allergenic, although there are rare, anecdotal reports of allergic reactions in the literature.

(b) Details of the donor organism

Latin Name	Gene	Pathogenicity
Streptomyces hygroscopicus	*bar*	*S. hygroscopicus* is ubiquitous in the soil and there have been no reports of adverse affects on humans, animals, or plants.

(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
bar	phosphinothricin acetyltransferase	HT	P35S3 (Cauliflower Mosaic Virus 35S)	3'nos (*Agrobacterium tumefaciens*)	1

5. Modification method

LLCotton25 was produced by *Agrobacterium*-mediated transformation of plant cells from the cotton variety Coker 312. The plasmid vector pGSV71 used for transformation contained the following: the gene coding for glufosinate ammonium tolerance (*bar* gene), the promoter region (P35S) from the cauliflower mosaic virus, and the 3' untranslated end of the nopaline synthase gene (3' *nos*). The transformation was achieved by culturing cotton tissue, excised between the hypocotyl and the radicle of three-day old cotton seedlings, with a culture of *A. tumefaciens* harbouring the Ti plasmid pGV3000 and the plasmid vector pGSV71. Explants from this culture were then regenerated to whole plants using tissue culture techniques. Transformed plants expressing the *bar* gene were selected with glufosinate ammonium.

(a) Characterization of the modification

Southern blot analysis and Polymerase Chain Reaction (PCR) amplification of the genomic DNA of LLCotton25 demonstrated one site of integration of a

single copy of the T-DNA of pGSV71. Southern blot analysis also confirmed the integrity of the *bar* gene along with its promoter and terminator sequences and, that no sequences of the vector backbone were integrated into the genome of LLCotton25. The P35S promoter and the 3' *nos* terminator sequences were derived from plant pathogenic organisms. Data from observations on several generations of LLCotton25 confirmed that these non-coding sequences did not cause diseases in the plants.

The stability of the *bar* gene, along with its promoter and terminator sequences (collectively termed the "*bar* gene cassette"), were assessed over multiple generations of conventional breeding at several locations, and in different genetic backgrounds. The results of genomic DNA blot analysis confirmed the stable inheritance of the *bar* gene cassette. Mendelian segregation data confirmed the stable inheritance of a single integrated gene.

The PAT protein was expected to be expressed in all tissues of the LLCotton25 plant since the *bar* gene was linked to a constitutive promoter (P35S from cauliflower mosaic virus). The concentrations of PAT protein were determined on a fresh weight basis. In plant tissues other than the seed and lint, PAT protein averaged 7.97 µg/g in roots, 59.2 µg/g in leaves, 38.8 µg/g in stems, and 19.2 µg/g in pollen. The average expression of PAT protein in cleaned seed was 127 µg/g and 69.9 µg/g in fuzzy seed. PAT protein was detected in all fractions of the seed *(i.e*., hulls, solvent extracted meal, toasted meal). The fiber components (*i.e.,* lint coat and lint fractions) contained less than 1.5% of the total PAT protein expressed in the plant. The absence of any detectable PAT protein amounts in crude and food grade oil produced from LLCotton25 seeds had been confirmed.

(b) Safety of the expressed protein

The human consumption of cotton products is limited to the refined oil. Because virtually no protein is present in the oil extracted from the seeds, the potential for human exposure is exceedingly low. As the introduced gene product was not detectable in the refined oil produced from transgenic cotton, there will be no human exposure to this protein based on normal consumption patterns. Furthermore, the amount of PAT protein present in seed meal fed to animals would be too low to cause concern.

6. Assessment of risks to human health

(a) Nutritional data

The nutritional components of LLCotton25 cottonseed were determined analytically and compared to those of its parental line, 'Coker 312.' These components included proximates [moisture, ash, fat (ether extract), crude

protein, crude fiber, acid detergent fiber (ADF), neutral detergent fiber (NDF), and carbohydrates], amino acids, fatty acids, minerals (calcium, phosphorus, iron, magnesium, potassium, and zinc), and vitamin E. Lint samples were analyzed for crude protein, fat, ash, crude fiber, ADF and NDF. The nutritional composition of the products of cottonseed processing (linters, delinted seed, meal, toasted meal, hull, crude oil and deodorized oil) was also determined, as appropriate for the particular fraction. The data from the nutritional analyses demonstrated that the composition of LLCotton25 cottonseed is comparable to that of the non-modified parental line 'Coker 312,' and seed of other commercial cotton varieties. The composition of the refined (deodorized) oil fraction was also comparable to Coker 312 and commercial cottonseed oil.

The levels of the anti-nutritional factors phytic acid, gossypol (total and free), and cyclopropenoid fatty acids, were determined in cottonseed of LLCotton25 and Coker 312. The levels of these anti-nutrients in LLCotton25 were comparable to those of Coker 312, and to values published in the literature, except for levels of free gossypol, which were higher in both LLCotton25 and its parental line Coker 312. The higher level of gossypol was therefore attributed to genotype, and not to any unintended effect of the genetic modification in LLCotton25.

(b) Toxicology

Since only the processed oil from transgenic LLCotton25 are available for human consumption, and the processing removes proteinaceous material, there are no toxicity concerns regarding this product. Bioinformatics studies have confirmed the absence of any significant amino acid sequence similarity to known protein toxins. Furthermore, in the study conducted with PAT protein, no oral toxicity was demonstrated in mice at a very high dose of 2000 mg/kg bodyweight. The weight of evidence shows that the protein is not toxic to humans.

(c) Allergenicity

The low potential for allergenicity of the PAT protein has been established through amino acid sequence comparisons to known allergens, digestibility in simulated gastric and intestinal fluids and presence of glycosylation. The PAT protein shares no epitopes with known allergens, is not glycosylated and degrades rapidly in simulated gastric and intestinal fluids.

Since only the processed oil from transgenic LLCotton25 is available for human consumption, and the processing removes proteinaceous material, there are no additional allergenicity concerns regarding this product. The LLCotton25 is considered as safe as its non-GM counterpart.

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 LLCotton25 products from countries where the said cotton 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 seed derived from LLCotton25 may be imported for processing. However, the LLCotton25 products that likely enter into Malaysia are in highly processed forms like refined oil or cottonseed meal. The seed may be viable but would not thrive as Malaysia conditions are not optimum for cotton growth. In the rare cases that cotton seeds grow, specific detection tools are already developed and commercially available to enable the identification of products derived from event LLCotton25. As with conventional cotton, the plants from event LLCotton25 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.

Seed derived from LLCotton25 is compositionally equivalent to those from conventional cotton. The plants behave agronomically in the same way as conventional cotton except showing the intended herbicide tolerance. 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 seed derived from LLCotton25 is indistinguishable from conventional cotton and needs no specific or additional treatment compared to conventional cotton.

(c) Handling and storage

No special handling procedures are required for this product. For LLCotton25 and its products, the same storage and handling can be applied as for conventional cotton. No special storage procedures are required for this product. Seed is stored as any cotton product.

(d) Disposal considerations

The same measures for waste disposal and treatment as for conventional cotton are valid for seed derived from LLCotton25.

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 _____ 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.