

**FACT SHEET**  
**APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCT OF**  
**GHB119 COTTON**  
**FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET**

**NBB REF NO: JBK(S) 602-1/1/32**

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 GHB119 cotton 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 GHB119 cotton 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 GHB119 cotton been modified?**

GHB119 cotton (*Gossypium hirsutum*) plants express the insecticidal protein Cry2Ae (encoded by the *cry2Ae* gene) from the common soil bacterium *Bacillus thuringiensis* subsp. *dakota* (*B.t. dakota*) and the herbicide tolerance protein phosphinothricin acetyltransferase (PAT; encoded by the *bar* gene) from *Streptomyces hygroscopicus*. The Cry2Ae protein is effective in controlling lepidopteran larvae such as bollworm (CBW, *Helicoverpa zea*) and tobacco budworm (TBW, *Heliothis virescens*) larvae, which are common pests of cotton. The expression of the PAT (phosphinothricin acetyltransferase) protein provides tolerance to glufosinate ammonium herbicides by acetylating glufosinate ammonium, thus detoxifying it.

**4. Characteristics of GHB119 cotton**

**(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 <i>G. hirsutum</i> , <i>G. barbadense</i> , and <i>G. tomentosum</i> .	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
<i>Bacillus thuringiensis</i> subsp. Dakota	<i>cry2Ae</i>	Although target insects are susceptible to oral doses of <i>Bt</i> proteins, there is no evidence of toxic effects in laboratory mammals or bird given up to 10 µg protein/g body wt. There are no significant mammalian toxins or allergens associated with the host organism.
<i>Streptomyces hygroscopicus</i>	<i>bar</i>	<i>S. hygroscopicus</i> is ubiquitous in the soil and there have been no reports of adverse effects 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	Form
<i>cry2Ae</i>	Cry2Ae delta-endotoxin	IR	Cauliflower mosaic virus 35S	3'35S-RB, from the Cauliflower mosaic virus	1	<i>cry2Ae</i> coding sequence
<i>bar</i>	Phosphinothricin acetyl-transferase	HT	Cassava vein virus promoter sequence	3' untranslated region of nopaline synthase gene from <i>Agrobacterium tumefaciens</i>	1	Coding sequence of <i>bar</i> gene

## 5. Modification method

*Agrobacterium*-mediated transformation of Coker 312 cotton with the T-DNA vector pTEM12 was carried out using cotyledon explants. Following co-culture with *Agrobacterium*, callus formation was initiated on a specific medium containing glufosinate ammonium as the selective agent and cefotaxime to eliminate residual *Agrobacterium*. Selected tissues were transferred to the appropriate regeneration medium. The developed plantlets were transferred to the greenhouse to allow flowering and seed set.

### (a) Characterization of the modification

Southern blot analyses determined that the inserted transgenic sequence in cotton event GHB119 consists of one complete copy of the T-DNA.

The inserted genes are inherited as a single dominant trait. The stability of the gene insertion was demonstrated by Southern blot analyses and Mendelian inheritance analyses. Southern analyses were conducted across three generations and two genetic backgrounds. No differences were seen in any of the analyses.

Phenotypic stability was demonstrated by Mendelian segregation of the GHB119 insert. A T<sub>0</sub> plant was crossed with conventional cotton, and the resulting F<sub>1</sub> plants were evaluated in the greenhouse. The F<sub>1</sub> plants were crossed, resulting in the F<sub>2</sub> generation which was evaluated in the

greenhouse for herbicide tolerance. The results in 3 different genetic backgrounds demonstrate Mendelian inheritance for a single gene locus and confirm stability of the GHB119 cotton insert.

Expression analyses for Cry2Ae and PAT proteins were conducted on seed (grain), leaf, squares, pollen, stems, bolls, nectar, flowers, and whole plants. Both proteins were detected in all tissues tested. For Cry2Ae, the expression values were highest in young leaves (37.5 µg/g tissue) and lowest in grain (0.99 µg/g tissue). For PAT, the values were highest in pre-flower leaves (114 µg/g tissue) and lowest in grain (2.59 µg/g tissue). The absence of any detectable Cry2Ae and PAT proteins in crude and food grade oil produced from GHB119 cotton seeds has 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 products were not detectable in the refined oil produced from transgenic cotton, there will be no human exposure to these proteins based on normal consumption patterns. Furthermore, the amounts of Cry2Ae and PAT proteins present in seed meal fed to animals would be too low to cause concern.

### **6. Assessment of risks to human health**

#### **(a) Nutritional data**

Proximate analyses were carried out on seed, meal, toasted meal, hulls and linters, amino acid profiles of seed, meal and toasted meal, fatty acid profiles of seed, crude oil and deodorized oil, and levels of Cry2Ae and PAT proteins expression in whole and processed fractions. Analyses were also conducted for naturally occurring toxicants and antinutrients (gossypol, cyclopropenoid fatty acids and phytic acid), as well as Vitamin E (tocopherol) for seed (whole and delinted) and oil (crude and deodorized). GHB119 cotton was found to be substantially equivalent to other varieties of cotton.

#### **(b) Toxicology**

Since only the processed oil from transgenic GHB119 cotton 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 Cry2Ae and PAT proteins, no oral toxicity were demonstrated in mice at a very high dose of 2000 mg/kg bodyweight. The weight of evidence shows that the proteins are not toxic to humans.

### **(c) Allergenicity**

The low potential for allergenicity of the Cry2Ae and PAT proteins have been established through amino acid sequence comparisons to known allergens, digestibility in simulated gastric and intestinal fluids, presence of glycosylation and assessment of heat stability. The Cry2Ae and PAT proteins share no epitopes with known allergens, are not glycosylated or heat stable and degrade rapidly in simulated gastric and intestinal fluids.

Since only the processed oil from transgenic GHB119 cotton is available for human consumption, and the processing removes proteinaceous material, there are no additional allergenicity concerns regarding this product. The GHB119 cotton 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 GHB119 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 GHB119 may be imported for processing. However, the GHB119 cotton 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 GHB119. As with conventional cotton, the plants from event GHB119 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 GHB119 is compositionally equivalent to those from conventional cotton. The plants behave agronomically in the same way as conventional cotton except showing the intended insect resistance and 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 GHB119 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 GHB119 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 GHB119.

**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 8<sup>th</sup> November 2016 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  
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Please indicate your full name, address and contact details in your submission.