FACT SHEET
APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCT OF GHB614 COTTON
FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK (S) 602-1/1/29

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 GHB614 cotton and itsproducts 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) ofGHB614 cottonand 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 GHB614 cottonbeen modified?

Glyphosate normally exerts herbicide activity by binding and inactivating EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), an enzyme that is essential for the synthesis of proteins in plants. Cotton line GHB614 has been genetically modified (GM) for tolerance to glyphosate herbicides by expression in the plant of a modified *epsps* gene from corn (*Z. mays*), *2mepsps*, which introduces two amino acid changes in the enzyme. The amino acid changes in the 2mEPSPS protein significantly lower the sensitivity to glyphosate, allowing the enzyme to continue to function in the presence of the herbicide.

4. Characteristics of GHB614 cotton

(a) Details of the parent organism

Cotton has a history of safe cultivation and consumption since the tenth century. Plants of the tribe Gossypiae originated in the tropics and subtropics. Except as a cultivated crop, they are essentially excluded from temperate climates. They also tend to be plants of the southern hemisphere. Geographical distribution for the cotton crop is located between 42° Latitude N. (Central Asia, China) and 30° Latitude S. (Australia, Northern Argentina). Thus, cotton is a plant of tropical origin, but presently more than 50% of world-wide production is grown in temperate zones above 30° Latitude N., where three of the four (India, USA, China and Pakistan) major producers are located.

Cotton is widely cultivated and has a history of safe use. Cotton is not considered harmful or pathogenic to humans; however the plant does produce gossypol and cyclopropenoid fatty acids (CPFA), which are natural toxicants.

Gossypol is a terpenoid substance found naturally in many *Gossypium* species including cotton and is located in glands throughout the plant, including the seed. Terpenoid aldehydes in pigment glands are an important source of resistance to herbivores and insects. Damage by many insect pests, rodents and birds is more extensive on glandless cotton. The levels of gossypol in food and feed products of cottonseed must be minimized as it can cause toxicity problems, *e.g.* depressed appetite, body weight loss and dyspnea. Animal sensitivity to gossypol is considerably different between species and classes of animals, with ruminants being the least sensitive. The amount of free gossypol has been the guide used by many nutritionists to make recommendations on feeding of cottonseed products, as free gossypol is the toxic compound. Refined cottonseed oil that goes into the human food chain is free of gossypol.

The cyclopropenoid fatty acids (0.1 - 1.3% of cottonseed oil), sterculic (C19) and malvalic acid (C18), are unique fatty acids common in cotton: these acids are responsible for giving a positive Halphen test, which has been used to characterize cottonseed oil for almost 100 years. The levels of cyclopropenoid fatty acids must also be minimized due to undesirable effects, which result in unsafe food and feed products. The cyclopropenoid fatty acids inhibit the desaturation of stearic to oleic acid, which alters membrane permeability. These acids are largely deactivated or removed from the oil by hydrogenation or during deodorization at 230-235°C.

(b) Details of the donor organisms

The cotton event GHB614 contains the *2mepsps* gene derived from maize, *Zea mays*. Maize (corn, *Zea mays* L.), is the world's third largest cereal crop, following wheat and rice and is grown in over 25 countries worldwide. Maize has a long history of safe use for consumption as food and feed. Field maize has been grown for 8000 years in Mexico and Central America and for 500 years in Europe.Maize has been regarded as beneficial and safe to eat for animals and humans for several generations, raw or after processing. It is concluded that maize has a long history of safe use. It can be deemed safe as a source of the gene

Maize naturally contains a few anti-nutrients and allergens. When compared to other cereals, the anti-nutritional compounds in maize are few. The two relevant anti-nutrients known are phytic acid and enzyme inhibitors. Phytin is concentrated in the bran fraction. The levels of the trypsin- and chymotrypsin-inhibitors in maize grains are very low and they are subject to heat denaturation.The insignificant amounts of a few anti-nutrients present in maize grains are removed by commonly practiced processing steps.

(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
epsps	5-enolpyruvyl shikimate-3-phosphate synthase	HT	Promoter region of the histone H4 gene from *Arabidopsis thaliana*.	3' untranslated region of the histone H4 gene from *A. thaliana*	1	double-mutated *epsps* gene from *Z. mays*

5. Modificationmethod

Cotton event GHB614 was developed through *Agrobacterium*-mediated transformation of the cotton variety Coker 312, using the transformation vector pTEM2. Cotton explants were exposed to a culture of disarmed *Agrobacterium tumefaciens* containing plasmid pTEM2. After co-culture, the cotton cells were regenerated to whole plants using the appropriate regeneration media with 500 mg/L claforan to eliminate residual *Agrobacterium*, and then selected with glyphosate.

The shoots that developed were transferred to the greenhouse, further tested for tolerance to glyphosate, and allowed to flower and set seed. The transformation was confirmed by 2mEPSPS enzyme activity assay, by glyphosate application to leaves, and by polymerase chain reaction (PCR) and Southern blot analyses.

The transformation vector used to generate cotton line GHB614, pTEM2, contains one gene expression cassette within the left and right border segments (T-DNA). The sequence of the 2mepsps gene is derived from the wildtype *epsps* gene from corn (*Zea mays*) with two single nucleotide mutations introduced by site directed mutagenesis. A methionine codon has been added to the N-terminal end of the 2mEPSPS protein sequence in order to restore the cleavage site of the optimized plastid transit peptide. The double mutant produces a 47 kDa protein with normal enzyme function and reduced affinity for glyphosate.

The Ph4a748At promoter and h3At intron are regulatory elements used to control expression of the *2mepsps* gene in cotton and are derived from the histone H4 gene of the plant *Arabidopsis thaliana*. The use of these elements directs high level constitutive expression, particularly in rapidly growing plant tissues. TPotpC encodes the optimized transit peptide derived from genes of corn and sunflower and targets the mature protein to the plastids where it is normally located in the cell. The 3'histonAt terminator from *Arabidopsis thaliana* corresponds to the polyadenylation signal which is essential to end transcription of the introduced gene.

(a) Characterization of the modification

Southern blot analysis was conducted to determine the insert number, the copy number, the integrity of the inserted 2mepsps gene cassette, and evaluate the presence or absence of plasmid backbone sequences. Isolated genomic DNA samples from GHB614 cotton and conventional cotton were digested with nine different restriction enzymes, separated on agarose gels and then subjected to Southern blot analysis. To determine the insert and copy number of the introduced DNA, the separated DNA fragments were transferred to a membrane and sequentially hybridized with different radioactively labelled probes: four probes containing each single genetic element present in the pTEM2 vector used for the transformation, and the complete T-DNA probe.

Based on a comparison of the size and pattern of observed fragments with the expected fragment sizes from digestion of genomic DNA, a single and unique site of insertion of the transgenic sequences is present in cotton line GHB614.

The organization of the genetic elements within the insert in GHB614 cotton was further characterized using PCR analysis by amplifying three overlapping regions of DNA spanning the entire length of the insert. The PCR products generated, following PCR of genomic DNA from GHB614 cotton, were all of the expected sizes.

(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 amounts of 2mEPSPS protein present in seedmeal fed to animals would be too low to cause concern.

6. Assessment of risks to human health

(a) Nutritional data

Compositional analyses were done on fuzzy seed collected from GHB614 cotton and the non-GM counterpart, Coker 312, grown in field trials typical of commercial agricultural production.

Nine field trials were conducted in 2005 at sites representing primary cotton-growing regions of the south-eastern United States. At each test site, six plots of transgenic event GHB614 cotton and three nontransgenic plots of Coker 312 were planted. Three of the six plots containing GHB614 cotton were sprayed three times with glyphosate herbicide (0.75 pounds active ingredient/acre). Compositional analysis of the cottonseed samples included

proximates (protein, fat, ash and moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), minerals (calcium, iron, magnesium, phosphorus, potassium and zinc), amino acids, fatty acids, vitamin E (alpha tocopherol) and carbohydrates by calculation. In addition, three known anti-nutrients found in cotton (gossypol, cyclopropenoid fatty acids and phytic acid) were analysed. Methods of analysis were based on internationally recognised procedures (e.g. AOAC International methods) or other published methods.

No differences of biological significance were observed between GHB614 cotton and its conventional counterpart. Statistically significant differences in some of the key constituents were noted, however the differences observed were minor, and in each case the levels observed were within the range of literature values reported for conventional cotton varieties. The composition of cotton is known to vary significantly with the site, agricultural conditions and season of production, and differences reported here most likely reflect normal biological variability. Food from GHB614 cotton is therefore considered to be compositionally equivalent to food from conventional cotton varieties.

(b) Toxicology

Since only the processed oil from transgenic GHB614 are available for human consumption, and the processing removes proteinaceous material, there are no additional toxicity concerns regarding this product.

Bioinformatics studies with the 2mEPSPS protein sequence has confirmed the absence of any significant amino acid sequence similarity to known protein toxins. Furthermore, in the study conducted GHB614 cotton, the 2mEPSPS protein was administered by a single oral gavage dose of 2000 mg protein/kg bodyweight to 5 female OF1 mice. A second group of female mice received the same dose of bovine serum albumin as a negative control. All animals were observed for clinical signs daily for fifteen days and body weights were measured weekly. At termination, all animals were subjected to necropsy including macroscopic examination. There were no clinical signs, mortalities or treatment related effects on bodyweight in female OF1 mice observed during this study. Based on these findings, it was concluded that no oral toxicity was demonstrated in mice at a very high dose of 2000 mg/kg bodyweight.

The weight of evidence shows that the 2mEPSPS protein is not toxic to humans.

(c) Allergenicity

Bioinformatics studies with the 2mEPSPS protein sequence has confirmed the absence of any significant amino acid sequence similarity to known protein allergens. Additionally, one of the criteria for assessing potential

allergenicity is to determine the stability of novel proteins in conditions that simulate human digestion. Proteins that are rapidly degraded in such conditions are considered less likely to be involved in eliciting an allergic response. The 2mEPSPS protein was subjected to digestibility studies using simulated human gastric fluid (SGF) containing pepsin and simulated intestinal fluid (SIF) containing porcine pancreatin, which is a mixture of enzymes including amylase, trypsin, lipase, ribonuclease and protease. In SGF (with pepsin), there was no full length or partially degraded 2mEPSPS protein observed at 30 seconds and at subsequent time points. In SIF, the 2mEPSPS protein band was only faintly visible after scanning the gel even at time zero. At all subsequent incubation times, there was no full length or partially degraded 2mEPSPS protein observed.The weight of evidence shows that the 2mEPSPS protein is unlikely to be allergenic in humans.

Since only the processed oil from transgenic GHB614are available for human consumption, and the processing removesproteinaceousmaterial,therearenoadditionalallergenicity concerns regarding this product. The GHB614 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 applicationis intended only to coverthe import of GHB614 cottonproducts 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 GHB614 cottonmay be imported for processing. However, the GHB614 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 cottonseeds grow, specific detection tools are already developedand commercially available to enable the identification ofproductsderived from event GHB614. As with conventionalcotton, the plants from event GHB614 are sensitive to herbicides other than glyphosate and can be controlled or eradicated either by herbicides other than glyphosateor by mechanical destruction.

Seed derived from GHB614 cottonis compositionally equivalent to those from conventionalcotton. The plants behave agronomically in the same way as conventional cotton except showing the intended tolerance to the herbicide glyphosate. Should adverseeffectsbereportedandverified,appropriatefollow upaction would be takentoinvestigate theseandifverifiedappropriate actiontaken.

(a) First aid measures

No special first aid measures are required with exposure to this product.

(b) Accidental release measures

Nospecialmeasuresarerequiredinresponsetoanaccidentalrelease.Spilledseed should beswept,scoopedorvacuumedin amannerthat avoidsdustgeneration and dust-relatedhazards. During industrial processing, the seed derived from GHB614 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 GHB614 cotton 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 GHB614.

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 1st August2016 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.