

FACT SHEET
APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCT OF
T304-40 COTTON
FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK (S) 602-1/1/30

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 T304-40 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 T304-40 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 T304-40 cotton been modified?

T304-40 cotton (*Gossypium hirsutum*) plants express the insecticidal protein Cry1Ab (encoded by the *cry1Ab* gene) from the common soil bacterium *Bacillus thuringiensis* subsp. *berliner* (*B.t. berliner*) and the herbicide tolerance protein phosphinothricin acetyltransferase (PAT; encoded by the *bar* gene) from *Streptomyces hygroscopicus*. The Cry1Ab 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. The *cry1Ab* and *bar* genes have been stably integrated into the T304-40 cotton genome.

4. Characteristics of T304-40 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.</i>	Gossypol in cottonseed meal	Cotton is not considered to be allergenic, although there are rare, anecdotal reports of allergic reactions in the literature.

Center of Origin	Reproduction	Toxins	Allergenicity
	<i>hirsutum</i> , <i>G. barbadense</i> , and <i>G. tomentosum</i> .		

(b) Details of the donor organisms

Latin Name	Gene	Pathogenicity
<i>Bacillus thuringiensis</i> subsp Berliner	<i>cry1Ab</i>	While target insects are susceptible to oral doses of <i>Bt</i> proteins, no evidence of toxic effects in laboratory mammals given up to 10 µg protein/g body weight.
<i>Streptomyces hygroscopicus</i>	<i>bar</i>	<i>S. hygroscopicus</i> 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
<i>cry1Ab</i>	Cry1Ab delta endotoxin	IR	5'e1-Ps7s7 (subterranean clover stunt virus)	3'me1 (<i>Flaveria bidentis</i>)	1
<i>bar</i>	phosphinothricin acetyltransferase	HT	P35S3 (Cauliflower Mosaic Virus 35S)	3'nos (<i>Agrobacterium tumefaciens</i>)	1

5. Modification method

Agrobacterium-mediated transformation of Coker 315 cotton with the T-DNA vector pTDL008 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 T304-40 consists of one nearly complete copy of the T-DNA flanked by an inverted incomplete copy of the *cry1Ab* gene cassette and one additional 3' me1 terminator.

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 four generations, three environments and four genetic backgrounds. No differences were seen in any of the analyses.

Phenotypic stability was demonstrated by Mendelian segregation of the T304-40 insert. A T₀ plant was crossed with conventional cotton, and the resulting F₁ plants were evaluated in the greenhouse. The F₁ plants were crossed, resulting in the F₂ generation which was evaluated in three greenhouses for herbicide tolerance. The results in 3 different genetic backgrounds demonstrated Mendelian inheritance for a single gene locus and confirmed stability of the Event T304-40 cotton insert.

Expression analyses for Cry1Ab and PAT proteins were conducted on fuzzy seed, leaf, squares, pollen, stems, bolls, nectar, flowers and whole plants. Both proteins were detected in all tissues tested. The measured values, by ELISA, for Cry1Ab were below the limit of detection for all tissues except seed. For PAT, the values were highest in flowers (187 µg/g tissue) and lowest in seed (39.6 µg/g tissue). For Cry1Ab, the expression was highest in flowers (10.6 µg/g tissue) and lowest in bolls (0.37 µg/g tissue).

(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 Cry1Ab 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 Cry1Ab and PAT proteins expression in whole and processed fractions. Analyses were also conducted for naturally occurring toxicants and anti-nutrients (gossypol, cyclopropenoid fatty acids and phytic acid), as well as Vitamin E (tocopherol) for seed (whole

and delinted) and oil (crude and deodorized). T304-40 cotton was found to be substantially equivalent to other varieties of cotton.

(b) Toxicology

Since only the processed oil from transgenic T304-40 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 Cry1Ab 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 said proteins are not toxic to humans.

(c) Allergenicity

The low potential for allergenicity of the Cry1Ab and PAT proteins has been established through amino acid sequence comparisons to known allergens, digestibility in simulated gastric and intestinal fluids and presence of glycosylation. The Cry1Ab 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 T304-40 are available for human consumption, and the processing removes proteinaceous material, there are no additional allergenicity concerns regarding this product. The T304-40 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 T304-40 cotton 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 T304-40 cotton may be imported for processing. However, the T304-40 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 T304-40. As with conventional cotton, the plants from event T304-40 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 T304-40 cotton 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 T304-40 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 T304-40 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 T304-40.

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 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
No. 25, Persiaran Perdana, Precinct 4

62574 Putrajaya, MALAYSIA
E-mail: biosafety@nre.gov.my
Fax: 03-88904935

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