FACT SHEET
APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF T25 MAIZE FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S) 602-1/1/10

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 the application for?

To import product of T25 maize/corn (herbicide-tolerant maize) for food, feed and processing.

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

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

3. How has the T25 maize been modified?

T25 maize was developed through 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 maize 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)

The herbicides bialaphos, phosphinothricin and its chemically synthesized form glufosinate ammonium are potent inhibitors of glutamine synthetase (GS), the enzyme that plays a central role in the assimilation of ammonia and in the regulation of the nitrogen metabolism in the plant. The *pat* gene codes for a PAT protein that metabolizes glufosinate to an inactive, acetylated derivative conferring the plant tolerant to glufosinate ammonium. The plants not carrying the transgene can be recognized and destroyed by using the herbicide at an early stage of plant development.

4. Characteristics of T25 maize

(a) Details of the parent organism

Characteristic of Zea mays L. (Maize)

Maize, Zea mays L., is the world's third largest cereal crop, following wheat and rice and 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 over 500 years in North America and in Europe. Maize has lost the ability to survive in the wild due to its long process of domestication and needs human intervention to disseminate its seed.

Maize plants are non-invasive in natural habitats and are incapable of sustained reproduction outside of domestic cultivation (OECD 2003 M-257582-01-1).

Center of Origin	Reproduction	Toxins	Allergenicity
Mesoamerican region, now Mexico And Central America	Cross-pollination via wind-borne pollen is limited; pollen viability is about 30 minutes. Hybridization reported with *Teosinte* species and rarely with members of the genus *Tripsacum.*	No endogenous toxins or significant levels of anti-nutritional factors.	Although some reported cases of maize allergy, protein (s) responsible have not been identified.

(b) Details of the donor organism

Characteristics of *Streptomyces viridochromogenes***, strain Tü 494**

Latin	Gene	Pathogenicity
Streptomyces viridochromogenes	*Pat*	S. *viridochromogenes* is a common saprophytic, gram positive, aerobic, sporulating bacterium naturally occurring in soil. The spore chains are spiral and 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 and is not itself known to be a human pathogen nor has it been associated with other properties (e.g. production of toxins) known to affect human health.

(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
pat	Phosphinothricin N-acetyltransferase	HT	CaMV 35S NULL	CaMV 35S poly(A) signal	1	Modified for transcription in plants
bla	beta lactamase	SM	bacterial promoter	NULL		Truncated, not expressed

5. Modification Method

The maize transformation event T25 contains a synthetic version of the phosphinothricin acetyltransferase (*pat*) gene derived from *Streptomyces viridochromogenes* strain Tü 494.

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 modification did not result in changes to the predicted amino acid sequence of the PAT enzyme.

The *pat* gene was inserted into the Sal1 site, between the cauliflower mosaic virus (CaMV) derived 35S promoter and terminator sequences of the pUC18 derived plasmid pDH51, resulting in the construct of a pUC/Ac vector. DNA from the resulting pUC/Ac construct was then purified and used for polyethylene glycol-mediated direct gene transfer into maize protoplasts.

Transformed cell colonies were selected and regenerated on medium containing glufosinate ammonium. Fertile maize plants are then regenerated from maize protoplasts.

The transformed T25 contain fragments of the beta lactamase (*bla*) gene. This marker was used during the plasmid construction to identify transformed bacterial cell colonies during the cloning of the recombinant *pat* gene. The *bla* gene codes for a beta-lactamase enzyme that confers resistance to some beta-lactam antibiotics, including the moderate-spectrum penicillin and ampicillin antibiotics. The *bla* gene fragments are not functional in the modified maize lines, and its promoter is active in bacteria.

(a) Characterization of the Modification

Southern blot analysis of genomic DNA from the maize line T25 indicated the incorporation of one copy of the *pat* gene cassette and two fragments of the *bla* sequences. A part of the cauliflower mosaic virus (35S, CaMV) promoter was duplicated during insertion. Northern Blot analysis confirmed the lack of transcripts from the *bla* fragments. The complete insert DNA of T25 was sequenced.

(b) Safety of the expressed protein

The PAT content of grains from glufosinate-ammonium tolerant T25 is very low making up 0.000022% of the total protein in maize grain. No PAT protein was detected in starch, crude or refined oil. Based on the greatest amount of PAT detected and the lowest level of crude protein reported in literature, PAT protein could represent the following percent of crude protein in the fractions indicated: 0.000029% (dry milled hulls), 0.00005% (grits), 0.000045% (flour), 0.000035% (meal), 0.00008%(germ), and 0.0000023% (coarse gluten starch).

T25 maize is proven as safe as its conventional counterpart. Maize T25 has already been in the market for over 15 years. It shows no different allergenic or toxic potential compared to conventional maize currently in the market.

6. Assessment of Risks to Human Health

(a) Nutritional Data

Compositional analyses of grains from T25 maize and current commercial maize varieties were compared for compositional and nutritional parameters including moisture, crude fat, crude protein, crude fiber, ash, carbohydrate, mineral content (including calcium and phosphorous), amino acid profile, and fatty acid composition. In all cases protein content was within the normal published range of maize. Similarly, an evaluation of the nutrient values determined that they were similar to the range of nutrient values reported for maize grain. The data and findings show that T25 maize is compositionally and nutritionally equivalent to currently grown conventional commercial maize varieties.

(b) Toxicology

The low potential for toxicity of the PAT protein expressed in the transgenic maize T25 was demonstrated by examining the amino acid sequence homology, chemical characteristics of the protein and by acute oral toxicity testing in rats. The nucleotide sequence of the *pat* gene and the deduced amino acid sequence of the PAT protein were compared with sequences available for known toxins in the GenBank database and showed no significant homology with any known toxins or allergens.

An acute oral toxicity study demonstrated no evidence of toxicity for PAT protein when administered to rats at dietary concentrations up to 50,000 ppm for 14 days, which represents concentration 6 orders of magnitude greater than that in grain from T25 maize.

(c) Allergenicity

The PAT enzyme expressed in T25 maize does not possess characteristics typical of known protein allergens and is extremely unlikely to be allergenic. There were no regions of homology when the sequences of the introduced protein were compared to the amino acid sequences of known protein allergens. There was no evidence found of post-translation modifications such as acetylation, glycosylation or phosphorylation of the PAT protein.

Unlike known protein allergens, the PAT protein was rapidly degraded by acid and/or enzymatic hydrolysis when exposed to simulated gastric fluids. *In vitro* digestibility studies, under simulated mammalian gastric conditions, demonstrated that the PAT enzyme was inactivated and was rapidly degraded. No adverse effects have been reported to be associated with this enzyme.

7. Assessment of Risks to the Environment

The application does not cover an environment release. The release is intended only to cover the import of the T25 maize products from countries where the maize 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 grain derived from T25 maize is intended to be imported for processing. The grain could be viable, but is not intended for planting as seeds. In case of the identification specific detection tools are already developed and commercially available to enable the identification of grain derived from event T25. The identified plants could be easily eradicated by herbicides or mechanical destruction.

Grain derived from T25 maize is compositionally equivalent to conventional maize. The plants behave agronomically in the same way as conventional maize except showing the intended tolerance to the herbicide glufosinate ammonium. As with conventional maize, the plants from event T25 are sensitive to herbicides other than glufosinate and can be controlled by herbicides other than glufosinate ammonium. T25 maize has been approved and used in other countries for more than 15 years and no adverse effects have been known to be reported. However, should adverse effects occur these will be investigated 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 Measures

During industrial processing, the grain derived from event T25 is indistinguishable from conventional maize grain and needs no specific or additional treatment compared to conventional maize. Maize grain rarely displays any dormancy characteristics. The maize plant is not weedy in character and weedy maize has not been reported growing naturally outside its center of origin. In the occasion that accidental release like spillage into the environment happens, Bayer CropScience will cooperate with traders and the relevant Government agencies, including the Department of Biosafety in providing guidance on the measures of controlling dissemination and will assist as needed.

(c) Handling and Storage

No special handling procedures are required for this product. For T25 maize and its products, the same storage and handling can be applied as for conventional maize. No special storage procedures are required for this product. Grain is stored as any maize grain product.

(d) Disposal Considerations

The same measures for waste disposal and treatment as for conventional maize are valid for grain derived from event T25.

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 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 7 August 2012 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.