

FACT SHEET

APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF
MZHG0JG CORN
FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S) 602-1/1/35

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 Syngenta Crop Protection Sdn. Bhd.

1. What is the application for?

This application seeks to allow the use of genetically modified corn (corn; *Zea mays* L.) derived from Event MZHG0JG corn (hereafter MZHG0JG corn) in Malaysia food industries.

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

The purpose of the import and release is to supply or offer for sale/ placing on the market of MZHG0JG corn for direct use as food and feed and for processing (FFP).

3. How has the MZHG0JG corn been modified?

Corn derived from insertion event MZHG0JG is genetically modified (GM) to produce a protein that provides tolerance to glyphosate herbicides. This protein, a modified 5-enolpyruvylshikimate-3-phosphate synthase (mEPSPS), is expressed from the gene *mepsps-02*, a variant of the native *epsps* from corn. In addition MZHG0JG corn produces phosphinothricin acetyltransferase (PAT), expressed from the gene *pat-09*, an enzyme that acetylates glufosinate-ammonium, thus inactivating it and confirming tolerance to glufosinate-ammonium in herbicide products. PAT was used as a selectable marker in the development of MZHG0JG corn.

MZHG0JG corn was produced by *Agrobacterium tumefaciens*-mediated transformation of an elite Syngenta inbred corn line, NP2222 with the transformation plasmid pSYN18857. The T-DNA region between the left and right borders of the transformation plasmid included gene expression cassettes for *mepsps-02* and *pat-09*. The *mepsps-02* expression cassette consisted of the *mepsps-02* coding region regulated by a corn ubiquitin promoter (Ubi158-02) and terminator (Ubi158-02), as well as the figwort mosaic virus (FMV-05), cauliflower mosaic virus 35S (35S-05), and tobacco mosaic virus (TMV-03) enhancer sequences and an optimized transit peptide (OTP-02). The *pat-09* expression cassette consisted of the *pat-09* coding region regulated by a 35S promoter from cauliflower mosaic virus (35S-19) and the nopaline synthase (NOS) terminator sequence from *A. tumefaciens* (NOS-05-01).

4. Characteristics of MZHG0JG corn

(a) Details of the parent organism

The recipient or parental plant is *Zea mays* L. (corn), also known as maize. Corn is one of the world's leading cereal crops, ranked after wheat and rice, and is grown in over 25 countries. Corn has a long history of safe use as food for consumption by humans and other animals. No significant native toxins are reported to be associated with the genus *Zea*. Corn is cultivated worldwide and represents a staple food for a significant proportion of the world's population. A major proportion of grain and forage derived from corn is used in animal feed. Corn-derived products are also routinely used in a large number and diverse range of foods for human consumption. Such products include flour, breakfast cereals, high fructose corn

syrup, and starch products. Corn grain is also used to produce industrial products, such as ethanol by fermentation.

(b) Details of the donor organism

The native *epsps* gene is derived from *Zea mays* L. spp. *mays*. No significant native toxins or allergens are reported to be associated with the genus *Zea*, the source organism for mEPSPS. The transgene *mepsps-02* in MZHG0JG corn is a modified version of *epsps* that was specifically engineered to encode the mEPSPS protein which confers tolerance to the herbicide glyphosate.

The gene *pat-09*, was derived from *Streptomyces viridochromogenes*, a common nonpathogenic soil bacterium. Bacteria are not known to be sources of allergenic proteins.

(c) Description of the trait(s) and characteristics which have been introduced or modified

Syngenta transformed corn (maize; *Zea mays* L.) to produce MZHG0JG corn, which exhibits tolerance to herbicides with two different modes of action. Specifically, MZHG0JG corn is tolerant to the herbicides glyphosate and glufosinate-ammonium.

MZHG0JG corn plants contain the transgene *mepsps-02*, which encodes the enzyme mEPSPS, and the transgene *pat-09*, which encodes the enzyme phosphinothricin acetyltransferase (PAT).

The native 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *Z. mays* is involved in the synthesis of aromatic amino acids and is inhibited by glyphosate. The mEPSPS enzyme is a variant of the native corn EPSPS, and has a lower affinity for glyphosate, thus conferring tolerance to glyphosate in herbicide products. The mEPSPS enzyme produced by MZHG0JG corn includes two amino acid substitutions, at amino acid position 102 (threonine to isoleucine) and 106 (proline to serine), that were introduced specifically to confer tolerance to the herbicide glyphosate.

The transgene *pat-09* was derived from the soil bacterium *Streptomyces viridochromogenes*. PAT acetylates glufosinate-ammonium, thus inactivating it and conferring tolerance to glufosinate-ammonium in herbicide products. PAT was also used as a selectable marker in the development of MZHG0JG corn.

5. Modification Method

MZHG0JG corn was produced by transformation of immature embryos of proprietary variety NP2222 via *Agrobacterium tumefaciens*-mediated transformation. The region of the plasmid vector pSYN18857, intended for insertion into the corn genome included gene-expression cassettes for *mepsps-02* and *pat-09*. The *mepsps-02* expression cassette consisted of the *mepsps-02* coding region regulated by a corn ubiquitin promoter (Ubi58-02) and terminator (Ubi158-02), as well as the figwort mosaic virus (FMV-05), cauliflower mosaic virus 35S (35S-05), and tobacco mosaic virus (TMV-03) enhancer sequences and an optimized transit peptide (OTP-02). The *pat-09* expression cassette consisted of the *pat-09* coding region regulated by a 35S promoter from cauliflower mosaic virus (35S-19) and the nopaline synthase (NOS) terminator sequence from *A. tumefaciens* (NOS-05-01).

(a) Characterisation of the Modification

Genetic characterization studies demonstrate that MZHG0JG corn contains, at a single locus within the corn genome, a single copy of each of the following functional elements: *mepsps-02*, *pat-09*, FMV-05 enhancer, 35S-05 enhancer, OTP-02 transit peptide, Ubi158-02 promoter, TMV-03 enhancer, Ubi158-02 terminator, 35S-19 promoter, and NOS-05-01 terminator. No extraneous DNA fragments of these functional elements occur elsewhere in the MZHG0JG corn genome. Similarly, plasmid backbone sequence from transformation plasmid pSYN18857 is not present in the MZHG0JG corn genome.

Analyses comparing the corn genomic sequence flanking the MZHG0JG insert with sequences in public databases indicate that the inserted DNA does not disrupt any known endogenous corn gene.

Southern blot analyses demonstrated that the MZHG0JG T-DNA insert is stably inherited from one generation to the next and that the MZHG0JG corn genome contains a single T-DNA insert.

(b) Safety of the expressed protein(s)

A comprehensive characterisation and safety assessment of the mEPSPS (Herouet-Guicheney *et al.* 2009) or PAT protein (Hérouet *et al.* 2005) are available in articles published in *Regulatory Toxicology and Pharmacology*.

Biotechnology-derived corn products that produce EPSPS and PAT have been available to farmers and in the food and feed supply for almost two decades. Variants of EPSPS and PAT occur in numerous commercial transgenic corn varieties, and have a long history of safe use in transgenic food crops.

6. Assessment of Risks to Human or Animal Health

A comprehensive characterisation and safety assessment of the mEPSPS (Herouet-Guicheney *et al.* 2009) or PAT protein (Hérouet *et al.* 2005) are available in articles published in *Regulatory Toxicology and Pharmacology*. Food and feed products derived from MZHG0JG corn are not materially different from food and feed products derived from conventional corn. The native EPSPS, the modified corn EPSPS and PAT proteins have a long history of safe use, as they are widely consumed in corn crop commodities. The mEPSPS protein is greater than 99% identical in amino acid sequence to the native corn EPSPS protein. The mEPSPS enzyme retains the native function of corn EPSPS, which is to enable the synthesis of aromatic amino acids via the shikimate pathway; this pathway does not occur in animals. No adverse effects associated with intake of EPSPS or PAT have been reported.

(a) Nutritional Data

MZHG0JG corn grain is compositionally equivalent to nontransgenic corn grain in terms of proximate composition, key nutrients, vitamins, minerals, and anti-nutrients. The levels of the majority of nutritional components did not differ between MZHG0JG corn and nontransgenic, near-isogenic control corn, and that those levels that did differ fell within ranges considered to be normal for conventional corn. Consumption of poultry diets containing MZHG0JG maize grain did not cause any adverse effects on broiler chickens.

(b) Toxicology

EPSPS and PAT proteins have been demonstrated to be nontoxic in acute toxicity tests in mammals. Bioinformatic analyses demonstrated that mEPSPS and PAT has no significant amino acid sequence similarity to known or putative toxins.

(c) Allergenicity

Neither mEPSPS nor PAT is likely to be allergenic, based on their derivation from nonallergenic sources, a long history of safe use, and lack of biologically relevant sequence similarity to known or putative allergens based on bioinformatics analyses. Additionally, both proteins are rapidly digested in simulated gastric and/or intestinal fluids, or inactivated by heating and/or processing.

7. Assessment of Risks to the Environment

There are no intentions to cultivate MZHG0JG corn in Malaysia. The release is intended only to cover the import of MZHG0JG corn products from countries where the corn is approved for commercial cultivation purposes. MZHG0JG corn may enter Malaysia as food ingredients for processing or packaging, as finished products ready for distribution, or as animal feed meal or pellets.

8. What is the Emergency Response Plan?

(a) First Aid Measures

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

(b) Accidental Release Measures

It is possible for seed to be accidentally released during transport, however, corn (*Zea mays*) is not weedy in character. Corn has lost the ability to survive without cultivation and is very uncompetitive against perennial vegetation. The agronomic and phenotypic characteristics of MZHG0JG corn were compared to those of conventional corn. No differences indicative of increased weediness potential were observed in plant growth habit, vegetative vigor, flowering characteristics, or yield.

(c) Handling and Storage

There are no specific instructions or recommendations for use, storage and handling of MZHG0JG corn. The characteristics of MZHG0JG corn and products derived from it are not different from those of conventional corn, apart from the introduced traits. The same measures for handling and storage for conventional corn are valid for MZHG0JG corn.

(d) Disposal Considerations

MZHG0JG corn has been assessed as being as safe as its conventional non-GM corn counterpart. Waste grain and processed products from MZHG0JG corn may be disposed of and treated in the same way as grain and processed products of non-GM corn.

9. How can I comment on this application?

Any member of the public may submit their comments or queries on publicly notified information about the application. Before submission of comments or queries, the person should review the information provided. 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 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 or 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 12 January 2017 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, Periaran Perdana, Presinct 4, 62574
Putrajaya, MALAYSIA.
E-mail: biosafety@nre.gov.my.

Fax: 03-88904935.

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

Reference

- Hérouet C, Esdaile DJ, Mallyon BA, Debruyne E, Schulz A, Currier T, Hendrickx K, van der Klis RJ, Rouan D. 2005. Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the *pat* and *bar* sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants. *Regulatory Toxicology and Pharmacology* 41:134–149.
- Herouet-Guicheney C, Rouquié D, Freyssinet M, Currier T, Martone A, Zhou J, Bates E E.M, Ferullo JM, Hendrickx K, Rouan D. 2009. Safety evaluation of the double mutant 5-enol pyruvylshikimate-3-phosphate synthase (2mEPSPS) from maize that confers tolerance to glyphosate herbicide in transgenic plants. *Regulatory Toxicology and Pharmacology* 54:143–153