

FACT SHEET
APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF
MZIR098 CORN
FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK (S) 602-1/1/42

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 is for obtaining authorization to import genetically modified (GM) Event MZIR098 corn (hereafter referred to as MZIR098 corn) and its products for use as food, feed and processing.

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

The purpose of the import and release is to supply or offer for sale or placing in the market MZIR098 corn and its products for direct use as food, feed and for processing. MZIR098 corn is not intended for cultivation in Malaysia.

3. How has the MZIR098 corn been modified?

MZIR098 corn is genetically modified (GM) to produce insecticidal proteins eCry3.1Ab and mCry3A for corn rootworm control as well as PAT protein conferring tolerance to glufosinate-ammonium in herbicide products.

4. Characteristics of MZIR098 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 (OECD, 2002).

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

MZIR098 corn contains the transgenes *ecry3.1Ab* and *mcry3A* derived from *Bacillus thuringiensis*, a ubiquitous soil bacterium. The engineered protein eCry3.1Ab is a chimera of mCry3A and Cry1Ab. The native Cry3A protein is derived from *B. thuringiensis* subsp. *tenebrionis* whereas the native Cry1Ab protein is derived from *B. thuringiensis* subsp. *kurstaki*.

MZIR098 corn also contains the transgene *pat-08*, derived from *Streptomyces viridochromogenes*, a common nonpathogenic soil bacterium. Bacteria are not known to be sources of allergenic proteins (Taylor and Hefle 2001).

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

Syngenta transformed corn (maize; *Zea mays* L.) to produce MZIR098 corn, which offers dual modes of action for corn rootworm control in a single event. In addition, MZIR098 corn also offers tolerance to glufosinate-ammonium herbicide products.

MZIR098 corn contain the transgenes *ecry3.1Ab* and *mcry3A*, which encode the insecticidal proteins eCry3.1Ab and mCry3A, and the transgene *pat-08*, which encodes the enzyme phosphinothricin acetyltransferase (PAT). The native Cry3A from the soil bacterium *Bacillus thuringiensis* subsp. *tenebrionis* is active against certain coleopteran pests. The modified protein mCry3A produced by MZIR098 corn has enhanced activity against western corn rootworm (*Diabrotica virgifera virgifera*) and other related coleopteran pests of corn. The engineered protein eCry3.1Ab is a chimera of mCry3A and Cry1Ab that is also active against *D. virgifera virgifera* and other related pests of corn. The native Cry1Ab from *B. thuringiensis* subsp. *kurstaki* is active against certain lepidopteran pests; however, the portion of Cry1Ab included in eCry3.1Ab has not preserved the activity of Cry1Ab against lepidopterans. Although the proteins act by the same general mechanism (i.e., pore formation in the target pest gut), the evidence indicates that they have unique gut binding sites in the target pest, thus effectively representing different modes of action (Walters *et al.* 2010).

The transgene *pat-08* was derived from the soil bacterium *Streptomyces viridochromogenes*. PAT protein acetylates glufosinate-ammonium, thus inactivating it and conferring tolerance to glufosinate-ammonium in herbicide products. PAT was used as a selectable marker in the development of MZIR098 corn.

5. Modification Method

Transformation of *Z. mays* to produce MZIR098 corn was accomplished through the use of immature embryos of a proprietary corn line (NP2222) via *Agrobacterium tumefaciens*-mediated transformation, as described by Negrotto *et al.* 2000. By this method, genetic elements within the left and right border regions of the transformation plasmid were efficiently transferred and integrated into the genome of the target plant cell, while genetic elements outside these border regions were not transferred.

The transformation plasmid pSYN17629 was used to produce MZIR098 corn. The DNA region between the left and right borders of the transformation plasmid included gene-expression cassettes for *ecry3.1Ab*, *mcry3A*, and *pat-08*. The *ecry3.1Ab* expression cassette consisted of the *ecry3.1Ab* coding region regulated by a CMP promoter from cestrum yellow leaf curling virus (CMP-04) and the nopaline synthase (NOS) terminator sequence from *A. tumefaciens* (NOS-05-01), as well as the NOS enhancer sequence (NOS-02). The *mcry3A* expression cassette consisted of the *mcry3A* coding region regulated by a corn ubiquitin promoter (Ubi1-18) and NOS terminator (NOS-20). The *pat-08* expression cassette consisted of the *pat-08* coding region regulated by the 35S promoter from cauliflower mosaic virus (35S-04) and the NOS terminator (NOS-05-01).

(a) Characterisation of the Modification

Genetic characterization studies demonstrated that MZIR098 corn contains, at a single locus within the corn genome, a single copy of each of the following functional elements: *ecry3.1Ab*, *mcry3A*, *pat-08*, NOS-02 enhancer, CMP-04 promoter, Ubi1-18 promoter, NOS-20 terminator, 35S-04 promoter, and two copies of the NOS-05-01 terminator as expected. It does not contain any extraneous DNA fragments of

these functional elements elsewhere in the MZIR098 corn genome, and it does not contain the plasmid backbone sequence from transformation plasmid pSYN17629.

Nucleotide sequence analysis determined that the MZIR098 insert consists of the intact T-DNA region of the pSYN17629. The results of the Southern blot analyses are consistent with the results of the nucleotide sequence analysis. Sequence analysis of the MZIR098 insertion site demonstrated that 24-bp from the corn genomic sequence was deleted during the integration of the MZIR098 insert.

The observed segregation ratios for *ecry3.1Ab*, *mcry3A*, and *pat-08* in three generations of MZIR098 corn plants were as expected for a gene inherited according to Mendelian principles. The data indicate that the insert is inherited as a single locus in the corn nuclear genome. These data and the results of Southern blot analyses of five generations of Event MZIR098 corn indicate that the transgenic locus is stably inherited during conventional breeding

6. Assessment of Risks to Human or Animal Health

The mode of action for insecticidal Cry proteins from *B. thuringiensis* is highly specific within narrow ranges of related insect species and are not relevant to mammals or other vertebrates.

PAT belongs to the class of acetyltransferase enzymes common in plants and animals, and it shares similar three-dimensional structure, molecular weight, and functional properties with other acetyltransferase enzymes, which are present as natural components of human and animal diets. It is likely that small amounts of acetyltransferase enzymes from various sources have always been present in the food and feed supply, because of the ubiquitous occurrence of PAT proteins in nature.

Food and feed products derived from MZIR098 corn are not materially different from food and feed products derived from conventional corn.

(a) Nutritional Data

MZIR098 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 MZIR098 corn and nontransgenic, near-isogenic control corn, and that those levels that did differ fell within ranges considered to be normal for conventional corn.

(b) Toxicology

eCry3.1Ab, mCry3A and PAT proteins have been demonstrated to be nontoxic in acute toxicity tests in mammals. Bioinformatic analyses demonstrated that eCry3.1Ab, mCry3A and PAT have no significant amino acid sequence similarity to known or putative toxins.

(c) Allergenicity

eCry3.1Ab, mCry3A or PAT proteins are unlikely 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, eCry3.1Ab, mCry3A, and PAT 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

Corn is not weedy in character, and the agronomic and phenotypic characteristics of MZIR098 corn were compared to that of conventional corn. No differences indicative of increased weediness potential were observed in plant growth habit, vegetative vigor, flowering characteristics or yield.

This application is for obtaining authorization to import MZIR098 corn and its products for use as food, feed and processing. MZIR098 corn may enter Malaysia as food ingredients for processing or packaging, as finished products ready for distribution, or as animal feed meal or pellets. There are no intentions to cultivate MZIR098 corn in Malaysia.

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 is not weedy in character. Corn has lost the ability to survive without cultivation and is very uncompetitive against perennial vegetation. This unlikely unintended release can be controlled with current agronomic measures taken to control other commercially available corn, such as selective use of herbicides (with the exception of glufosinate-ammonium), and manual or mechanical removal of plants.

(c) Handling and Storage

Recommendations for storage and handling of MZIR098 corn will be no different from that of conventional corn.

(d) Disposal Considerations

Measures for waste disposal and treatment of MZIR098 corn will be no different from that of conventional 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 30 December 2017 and written submissions are required by that date. Submissions must be addressed to:

Director General, Department of Biosafety

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