

FACT SHEET
APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF
3272 CORN
FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S) 602-1/1/25

The objective of the Biosafety Act is to protect human, plant, 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 to import and release 3272 corn (*Zea mays* L.) and its products.

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 3272 corn for direct use as food and feed and for processing (FFP).

3. How has the 3272 corn been modified?

Corn derived from insertion event 3272 (marketed under the brand name Enogen®) is genetically modified (GM) to produce the thermostable AMY797E alpha-amylase enzyme. The alpha-amylase AMY797E protein (AMY797E) is encoded by the *amy797E* gene. This precursor protein undergoes cleavage of the N-terminal signal sequence yielding the mature AMY797E. Additionally, a marker transgene, *pmi*, has been added to allow the selection of transformed plants during development. The gene *pmi* was isolated from the common bacterium *Escherichia coli*. The protein produced by this gene is the enzyme phosphomannose isomerase (PMI).

3272 corn was produced by *Agrobacterium tumefaciens*-mediated transformation of a proprietary line of corn with the transformation plasmid vector pNOV7013 containing a synthetic, corn optimized *amy797E* transgene, derived from alpha-amylase genes from three hyperthermophilic microorganisms of the archael order *Thermococcales*.

4. Characteristics of 3272 corn

(a) Details of the parent organism

The recipient or parental plant is *Zea mays* L. also known as corn. 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 AMY797E alpha-amylase enzyme is a chimeric enzyme derived from three wild-type alpha-amylases from the archaeal order *Thermococcales*. Alpha-amylase enzymes from fungal and bacterial sources (*Aspergillus niger*, *A. oryzae*, *Rhizopus oryzae*, *Bacillus licheniformis*, *B. amyloliquefaciens*, *B. stearothermophilus*) have a long history of safe use for starch processing in the food processing industry.

The transgene *pmi*, also known as *manA*, was derived from *E. coli* strain K-12, a non-pathogenic strain. *E. coli* is a normal inhabitant of the intestinal flora of humans and other animals, where it doesn't normally cause disease.

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

Syngenta has developed a thermostable alpha-amylase enzyme (AMY797E) expressed 3272 corn for use in the dry grind fuel ethanol production industry of the United States of America. In general, alpha-amylases enzyme catalyzes the hydrolysis of starch by cleaving the internal α -1,4-glucosidic bonds into dextrin, maltose and glucose. Microbial produced alpha-amylases are commonly used commercially in the starch-processing step during corn dry grind and wet milling processing. 3272 corn produces a synthetic thermostable alpha-amylase enzyme in the endosperm with improved enzyme characteristics for the starch processing step of dry grind ethanol production. The product concept is that the corn amylase grain (Event 3272) will serve as the source of amylase enzyme in the dry grind ethanol process, replacing the addition of microbial produced enzyme. The corn grain expressing the AMY797E alpha amylase enzyme will be mixed at a low percentage with conventional corn at the processing plant.

3272 also contain the *pmi* transgene, also known as *manA*, which encodes the enzyme phosphomannose isomerase. PMI was utilized as a plant selectable marker during development of 3272 corn. The PMI enzyme catalyzes the isomerization of mannose-6-phosphate to fructose-6-phosphate. It allows transformed corn cells to utilize mannose as a primary carbon source in culture media, whereas cells that lack *pmi* expression will fail to proliferate.

(d) Safety of the expressed protein

Alpha-amylase enzymes from fungal and bacterial sources such as *Aspergillus niger*, *A. oryzae*, *Rhizopus oryzae*, *Bacillus licheniformis*, *B. amyloliquefaciens*, *B. stearothermophilus* have a long history of safe use for starch processing in the food processing industry. Alpha-amylases, of varying degrees of amino acid homology with AMY797E, occur widely in nature among prokaryotes and eukaryotes, including many plants and animals. Some of the alpha-amylase genes and enzymes that have been identified and characterized include those from barley, rice and corn, and are also found in human saliva.

The safety of PMI has been established previously, and it is exempt from food and feed tolerances in all crops in the USA (US Code of Federal Regulations 40 CFR §174.527). Small amounts of PMI enzymes from various sources have always been present in the food and feed supply as a result of their ubiquitous occurrence in nature, including in food plants and animals. PMI enzymes occur in mammals, humans, yeast, fungi and bacteria, and have been found in such diverse plant species as tobacco, walnut and *Brassica* species as well as in seeds of soybeans and other legumes.

5. Assessment of Risks to Human Health

3272 corn are substantially equivalent in composition to other commercial corn hybrids and are as safe and nutritious for food and feed use. Usage of 3272 corn grain is intended to be within a closed-loop processing system during the production of ethanol. Therefore, dietary exposure to 3272 corn grain is expected to be negligible.

(a) Nutritional Data

3272 corn grain is compositionally equivalent to nontransgenic corn grain in terms of proximate composition, key nutrients, vitamins, minerals, and anti-nutrients. There are no biologically significant differences (including nutritional differences) between 3272 corn and nontransgenic corn, with the exception of the intended traits conferred by expression of the AMY797E and PMI proteins.

(b) Toxicology

AMY797E and PMI are both nontoxic enzymes. AMY797E catalyzes the hydrolysis of starch to sugars, and PMI interconverts fructose and mannose. Both AMY797E and PMI were demonstrated to be nontoxic at high doses in acute oral toxicity tests in mammals. Dietary exposure to AMY797E and PMI is expected to be negligible due to the intended usage of 3272 corn grain in a closed loop processing system for ethanol production. The dried distillers grain by-product does not have measureable ANY797E α -amylase protein or activity, indicating extremely low or non-existent levels of AMY797E in this potential animal feed. Both AMY797E and PMI proteins have been demonstrated to be rapidly digested under conditions of the human gastrointestinal tract. In addition, 3272 corn grain was not toxic in animal feeding studies, and is compositionally equivalent to conventional corn grain.

(c) Allergenicity

Neither AMY797E nor PMI is likely to be allergenic, based on their derivation from nonallergenic source organisms, a lack of biologically relevant sequence similarity to known allergens, minimal exposure following rapid digestion, and lack of glycosylation (a characteristic observed in some allergens). Exposure to AMY797E and PMI is expected to be negligible due to the intended usage of 3272 corn grain in a closed loop processing system for ethanol production. The dried distillers grain by-product does not have measureable ANY797E α -amylase protein or activity, indicating extremely low or non-existent levels of AMY797E in this potential animal feed. Both AMY797E and PMI proteins have been demonstrated to be rapidly digested under conditions of the human gastrointestinal tract.

6. Assessment of Risks to the Environment

There are no intentions to cultivate 3272 corn outside of North America. It is intended use, is in the dry-grind ethanol fuel process which is to be carried out in the United States. 3272 corn is not considered a commodity crop, but rather a high value specialty grain produced in a closed loop system which includes contracts with farmers and ethanol plants for sole use in dry grind ethanol production, under control by compliance measures. Thus the risk that these grains would enter the typical grain channel is small. This application will cover unintended Food and Feed use of co-products of the bioethanol production such as Dried Distiller's Grains and Solubles (DDGS) for Feed use. The dried distillers grain by-product does not have measureable ANY797E α -amylase protein or activity, indicating extremely low or non-existent levels of AMY797E in this potential animal feed.

The application does not cover an environmental release. The release is intended only to cover the import of the 5307 corn and its products from countries where the corn is already approved and commercially grown, and that may enter Malaysia as food, feed and for further processing (FFP).

7. 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

3272 corn is a high value specialty grain produced in a closed loop system which includes contracts with farmers and ethanol plants in the USA for sole use in dry grind ethanol production, under control by compliance measures. As mentioned in response to question 6, it would be highly unlikely that 3272 corn grains would enter the typical grain channel. However, if seed was accidentally released during transport, 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 characteristic of 5307 corn was compared to that of conventional maize. No differences indicative of increased weediness potential were observed in plant growth habit, vegetative vigor, flowering characteristics or yield. Further, the environmental safety of the introduced AMY797E and PMI proteins in 3272 corn has been established. Exposure in laboratory studies of mammals to AMY797E and PMI at concentrations equal to or greater than estimated environmental concentrations via cultivation of 3272 corn showed no adverse effects.

(c) Handling and Storage

There are no specific instructions or recommendations for use, storage and handling of 3272 corn. The characteristics of 3272 corn and products derived from it are not different from those of its conventional counterpart, apart from the introduced trait. The same measures for handling and storage for conventional corn are valid for 3272 corn.

(d) Disposal Considerations

3272 corn has been assessed as being as safe as its conventional non-GM counterparts. Waste grain and processed products from 3272 corn may be disposed of and treated in the same way as grain and processed products on non-GM corn.

8. 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 **30th October 2015** 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
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E-mail: biosafety@nre.gov.my.
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Please include your full name, address and contact details in your submission.

References

US Code of Federal Regulations 40 CFR §174.527