FACT SHEET
APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF TC1507 MAIZE FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S) 602-1/1/11

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 the Du Pont Malaysia Sdn. Bhd.

1. What is this application for?

To import and release products of TC1507 maize (insect-resistant and herbicide-tolerant maize) for use as food, feed and for processing.

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

The aim of the import and release is to supply or offer to supply for sale or placing on the market - for direct use as food, feed and for processing (FFP).

3. How has the TC1507 maize been modified?

TC1507 maize has been genetically modified (GM) by incorporation of two bacterial genes: the *cry1F* gene (derived from the soil bacterium *Bacillus thuringiensis* var. *aizawai* strain PS811) which expresses an insect-specific protein toxin, Cry1F, and the *pat* gene (derived from the soil bacterium *Streptomyces viridochromogenes*) which expresses the enzyme phosphinothricin acetyltransferase (PAT), conferring tolerance to glufosinate-ammonium herbicides.

Cry1F protein, as expressed in TC1507 maize, is effective in controlling European Corn Borer (*Ostrinia nubilalis*), southwestern corn borer (*Diatraea grandiosella*), black cutworm (*Agrotis ipsilon*) and fall armyworms (*Spodoptera* sp.) that are common insect pests of maize in the USA where this product was first developed.

TC 1507 maize is also tolerant to glufosinate-ammonium herbicide through the expression of a bacterial gene from *Streptomyces viridochromogenes*, encoding an enzyme, phosphinothricin acetyltransferase (PAT). This enzyme is able to specifically break down the herbicide in the plant, converting it to an inactive form, thus allowing the plants to grow normally. The production of PAT in the plants allows selection of GM plants in the field as well as providing tolerance to the herbicide when used at agricultural levels.

4. Characteristics of the TC1507 maize

(a) Details of the parent organism

Maize (*Zea mays* L.), also known as corn is one of the most important cereal crops in the world with total production of 844 million tonnes in 2010 (FAOSTAT Database 2010). The majority of grain and forage derived from maize is used as animal feed, however maize also has a long history of safe use as food for human consumption. Maize grain is also processed into industrial products such as ethyl alcohol (by fermentation), and highly refined starch (by wet-milling) to produce starch and sweetener products. In addition to milling, the maize germ can be processed to obtain maize oil and numerous other more minor products (White and Pollak 1995).

The commercial production of maize has seen many improvements, particularly since the 1920's when maize varieties were developed by conventional breeding between progeny of two inbred lines to give hybrid varieties that were known to be superior to open-pollinated varieties in terms of their agronomic characteristics. In present agricultural systems, hybrid maize varieties are used in most developed countries for consistency of performance and production. In the case of maize line 1507 hybrids, the presence of the insect-protected and herbicide-tolerance traits will provide producers with additional improvements to the available genetic stock.

(b) Donor organisms

(i) *Bacillus thuringiensis*

The source of the *cry1F* gene is the common bacterium *Bacillus thuringiensis* subsp. *aizawai*. *Bacillus thuringiensis* are a diverse group of Gram-positive, spore-forming bacteria that were first isolated in 1901, and have proven to be a rich source of insecticidal proteins. Intensive research has identified a growing family of Bt proteins with different insecticidal specificities, including to coleopteran, dipteran and lepidopteran insect orders. The Bt organism has been used safely in spray form as a crop protective agent for at least 40 years (Schnepf *et al*. 1998; U.S. EPA 1996) as a useful alternative or supplement to synthetic chemical pesticide application in commercial agriculture, particularly in the organic farming industry, and in forest management. Several varieties of *B. thuringiensis* have been used as microbial insecticides since 1938 (Merritt 1998). The subspecies *aizawai* is

commercially used to control wax moth larvae and various caterpillars, especially the diamondback moth caterpillar (Cornell University 1996).

(ii) *Streptomyces viridochromogenes*

The *pat* gene is derived from the common soil bacterium *Streptomyces viridochromogenes*. The bacterium produces the tripeptide L-phosphinothricyl-L-alanyl-alanine (L-PPT), which was developed as a non-selective herbicide by Hoechst AG. Over the past decade, the *pat* gene has been introduced into several other genetically engineered food crops to confer tolerance to PPT and the synthetic form, glufosinate-ammonium.

(iii) Cauliflower mosaic virus

The 35S promoter and transcription termination sequences used in the genetic construct are derived from the commonly occurring cauliflower mosaic virus (CaMV), a DNA plant virus with a host range restricted primarily to cruciferous plants (ICTV Database 1998) that are common in the food supply. The DNA sequences originating from this virus have no pathological characteristics, other than in association with their target plant species (USDA 1995).

(iv) *Agrobacterium tumefaciens*

The species *Agrobacterium tumefaciens* is a Gram-negative, non-spore forming, rod-shaped bacterium commonly found in the soil. It is closely related to other soil bacteria involved in nitrogen-fixation by certain plants. *Agrobacterium* naturally contains a plasmid (the *Ti* plasmid) with the ability to enter plant cells and insert a portion of its genome into plant chromosomes. Normally therefore, *Agrobacterium* is a plant pathogen causing root deformation mainly with sugar beets, pome fruit and viniculture crops. However, adaptation of this natural process has now resulted in the ability to transform a broad range of plant species without causing adverse effects in the host plant.

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

(i) Method used in the genetic modification

TC1507 maize was generated by transformation of embryogenic Hi-II maize cells, using a particle acceleration method. A purified linear DNA segment containing the *cry1F* and *pat* coding sequences, together with essential regulatory elements, was used in the transformation process. The DNA segment of 6235 bp was derived from plasmid PHP8999, and contained only the genes of interest. No additional plasmid DNA was used in the transformation event.

(ii) *cry1F gene*

The bacterial *cry1F* gene sequence has been shown to provide high levels of protection against certain insect pests when it is expressed in plants. The gene encodes one of the families of Bt insecticidal proteins, Cry1F, that specifically inhibits European and southwestern corn borers, black cutworm and fall armyworms.

(iii) *pat gene*

Tolerance to the herbicide phosphinothricin (glufosinate-ammonium) has been introduced to a variety of plant species using molecular techniques to insert a copy of the *pat* gene which enables the plant to produce the PAT enzyme. Expression of PAT within the plant cell inactivates L-PPT thereby conferring tolerance to the herbicide (OECD 1999).

(iv) Function and regulation of the novel genes

The purified linear segment PH18999A, was used in the transformation. The 6235 bp DNA segment comprised two adjacent gene cassettes for expression of the two novel proteins, Cry1F and PAT. The *cry1F* gene is under the regulation of the ubiquitin promoter [ubiZM1(2)] from maize, and a 3' regulatory element derived from *Agrobacterium tumefaciens* (ORF25PolyA). The *pat* gene is regulated by the 35S promoter and the 35S transcription terminator, both from the Cauliflower Mosaic Virus (CaMV). The inserted DNA does not contain an antibiotic resistance gene or bacterial origin of replication sequences.

(d) Safety of the Expressed Proteins

Mammalian toxicity studies conducted using Btk and PAT test material did not reveal any harmful effects. The amino acid sequence of the truncated Btk protein expressed in TC1507 maize is closely related to the sequence of the same proteins that are present in strains of *B. thuringiensis* that have been used as commercial organic microbial insecticides. An analysis of the amino acid sequences of the inserted Btk and PAT proteins did not show homologies with known mammalian protein toxins and they are assessed to have no risk for human toxicity. The truncated *Btk* and PAT proteins expressed in TC1507 maize do not possess characteristics typical of known protein allergens. There were no regions of homology when the sequences of these introduced proteins were compared to the amino acid sequences of known protein allergens. Unlike known protein allergens, both of these proteins are rapidly degraded by acid and/or enzymatic hydrolysis when exposed to simulated gastric fluids. The Btk and PAT proteins are extremely unlikely to be allergenic.

5. Assessment of Risks to Human Health

No significant health hazards are currently associated with this product. Potential health effects associated with the product or exposure to its dusts include those described below.

(a) Toxicological Information

There are no known health hazards associated with the product. It is not known to be capable of causing allergic sensitization. Studies have shown no toxicity toward mammals. In addition, there are no amino acid sequence similarities to known mammalian toxins.

(b) Carcinogenicity

This product does not contain any substances that are considered by the US Occupational Safety and Health Administration (OSHA), National Toxicology Program (NTP), or the International Agency for Research on Cancer (IARC) to be probable or suspected human carcinogens.

(c) Pathogenicity

Bacillus thuringiensis var *kurstaki* has no known pathogenicity and allergenicity to humans, animals and non-target organisms. *Streptomyces viridochromogenes* also has no known pathogenicity and allergenicity to humans, animals and non-target organisms.

6. Assessment of Risks to the Environment

(a) Environmental Assessment

The application does not cover an environmental release. The release is intended only to cover the import of the TC1507 maize products from countries where the maize is already approved and commercially grown, and that may enter Malaysia as food, feed and for further processing (FFP).

(b) Nutritional Composition (Compositional Analysis)

The grain analysis, protein, oil, starch and fiber content of TC 1507 maize lines were shown to be substantially equivalent to the untransformed maize. The proximate analysis (protein, fat, fiber and starch.) of the insect-resistant maize hybrids gave values well within the published range for traditional maize cultivars. Under the same agronomic condition, the analysis of nutrients (the levels of protein, calcium, magnesium, phosphorus and potassium) from TC1507 maize and its conventional counterpart did not reveal any significant differences.

(c) Anti-Nutritional Factors

Few anti-nutrients have been reported to occur in maize which has no relevance for its food use. Regarding the feed use of maize, phytic acid reduces the availability of phosphorus, especially in mono-gastric animals. There are no toxic or anti-nutritional factors present in maize which would need to be controlled by a specification. Though trypsin inhibitor, phytic acid, and secondary metabolites such as raffinose, ferulic acid and p-coumaric acid have been established as anti-nutrients in maize, they are present in very low amount and are below the thresholds considered to raise a food safety concern. The amount of anti-nutrients present in TC1507 maize fell within the range found in non-transgenic maize. Results of animal feeding studies have demonstrated similar performance between animals fed with TC1507 maize and conventional maize. Feeding studies done with cattle showed that there was no effect on dry matter intake, milk production, milk composition or a number of rumen parameters relating to feed utilization. Similarly, there were no significant differences observed for feed intake, bodyweight, egg production and egg weight in laying hens.

7. What is the Emergency Response Plan?

An emergency response plan is considered to be not relevant for TC1507 maize which has been demonstrated to be substantially equivalent to conventional, non-transgenic maize. TC1507 maize and food and feed products derived from it have

been assessed as being safe as its conventional non-GM counterparts and there are no reports of adverse effects since its commercialization in 2003. 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 for exposure to this product.

(b) Accidental Release Measure

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.

(c) Handling and Storage

On the basis of rigorous testing and multiple comprehensive evaluations, TC1507 maize has been demonstrated to be safe to humans, animals, non-target organisms and beneficial insects, and that the forage and grain of TC1507 maize are as safe and nutritious as conventional maize varieties. TC1507 maize has received full food, feed and environmental approval by Argentina, Brazil, Canada, Colombia, Honduras, Japan and the United States. In addition, it has also been approved for import by the respective regulatory bodies in Australia/New Zealand, China, European Union, Korea, Mexico, Philippines, Singapore, South Africa, Taiwan and Uruguay. These regulatory agencies concluded that TC1507 maize does not pose risks to human and animal health and is not materially different from conventional, non-transgenic maize that is already on the market. Considering this, recommendations for storage and handling of TC1507 maize will be no different from conventional, non-transgenic maize.

(d) Disposal Considerations

Measures for waste disposal and treatment of TC1507 maize will not be different from conventional, non-transgenic maize. Disposal should be managed in accordance with local, state or federal regulations.

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. 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 _____ and written submissions are required by that date. Submissions must be addressed to:

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