

FACT SHEET

APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF DP73496 CANOLA FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S) 602-1/1/41

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 DuPont Malaysia Sdn. Bhd. on behalf of Pioneer Hi-Bred International, Inc.

1. What is the application for?

This application is for the importation of DP73496 canola 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 DP73496 canola and its products for direct use as food, feed and for processing. DP73496 canola is not intended for cultivation in Malaysia.

3. How has DP73496 canola been modified?

DP73496 canola was produced by Pioneer Hi-Bred International, Inc. (Pioneer, Johnston, IA, USA). DP73496 canola expresses the GAT4621 protein for tolerance to glyphosate herbicides. The GAT4621 protein, encoded by the *gat4621* gene, confers tolerance to the herbicidal active ingredient glyphosate through the conversion of glyphosate into non-phytotoxic *N*-acetylglyphosate through acetylation of glyphosate

4. Characteristics of DP73496 canola

a. Details of the parent organism

B. napus L. belongs to the Brassicaceae family, also known as the mustard family. Biology documents on the unmodified plant species, canola (*Brassica napus* L.), have been published by the Australia Office of the Gene Technology Regulator (OGTR, 2011), Canadian Food Inspection Agency (CFIA, 1994) and by the Organization for Economic Co-operation and Development (OECD, 1997a; OECD, 2012).

B. napus was considered unsuitable as a source of food for either humans or animals, because the seed naturally contains erucic acid and glucosinolates, which are toxic to humans and other organisms. However, it was widely used as an edible oil in Asia for thousands of years (OECD, 1997a). In the 1970s, intensive breeding programs

produced high quality varieties that were significantly lower in erucic acid and glucosinolates. The term 'canola' refers to those varieties of *B. napus* that meet specific standards on the level of erucic acid and glucosinolates OGTR (2011).

b. Details of the donor organism

***Bacillus licheniformis*: donor of the *gat4621* gene**

Bacillus licheniformis, the source organism for the *gat4621* gene, is a Gram-positive saprophytic bacterium that is ubiquitous in soil. It has been used widely in the detergent industry for production of a number of enzymes (*e.g.*, proteases and amylases) that have wide applications and in the fermentation industry for production of food enzymes (*e.g.*, alpha-amylase, cyclodextrin glycosyltransferase, hemicellulase, proteases, and pullulanase; Rey *et al.* (2004)). *B. licheniformis* is widespread in the environment; therefore, animals and humans are regularly exposed without adverse consequences to this organism. All *B. licheniformis* cultures available from the American Type Culture Collection (ATCC) are classified as Biosafety Level 1, which have no known history of causing disease in humans or animals (US-EPA, 1997). The glyphosate *N*-acetyltransferase (GAT) proteins are members of the GCN 5-related family of *N*-acetyltransferases (also known as the GNAT family). The GNAT superfamily is one of the largest enzyme superfamilies recognized to date with over 10,000 representatives from plants, animals, and microbes. The GAT4621 protein is 75-78% identical and 90-91% similar at the amino acid level to each of the three native GAT enzymes derived from strains 401, B6, and DS3 of *B. licheniformis*.

5. Modification Method

DP73496 canola was produced by microprojectile bombardment with the *Hind* III/*Not* I-digested fragment PHP28181A (2,112 bp) from plasmid PHP28181. The transformation cassette in fragment PHP28181A contains a *gat4621* gene derived from the glyphosate acetyltransferase gene of *Bacillus licheniformis* (Castle *et al.*, 2004; Siehl *et al.*, 2007). The expression of the *gat4621* gene is regulated by the promoter region of the ubiquitin 10 gene (*UBQ10* promoter region) from *Arabidopsis thaliana*, including promoter, 5' untranslated region (UTR), and intron (Norris *et al.*, 1993). The terminator for the *gat4621* gene is the terminator sequence of the proteinase inhibitor II gene (*pinII* terminator) from *Solanum tuberosum* (An *et al.*, 1989; Keil *et al.*, 1986). The GAT4621 protein, encoded by the *gat4621* gene, confers tolerance to the herbicidal active ingredient glyphosate through the conversion of glyphosate to the inactive acetylated form (Castle *et*

al., 2004). The GAT4621 protein is comprised of 147 amino acids and has a molecular weight of approximately 17 kDa (Castle *et al.*, 2004; Siehl *et al.*, 2007).

a. Characterization of the Modification

The molecular characterization of the inserted genes of DP73496 canola was performed using DNA sequencing, and bioinformatics analysis. These studies demonstrate that the introduced genes were integrated at a single point of insertion. The introduced genes in DP73496 canola express the GAT4621 protein for tolerance to glyphosate herbicide. The GAT4621 protein, encoded by the *gat4621* gene, confers tolerance to the herbicidal active ingredient glyphosate through the conversion of glyphosate into non-phytotoxic *N*-acetylglyphosate through acetylation of glyphosate.

b. Safety of the expressed protein

The GAT4621 protein was evaluated using a series of studies, including bioinformatics, *in vitro* digestibility, glycosylation analyses, acute protein toxicity, and heat lability analyses. The results of these studies demonstrate that the proteins are unlikely to be allergens or toxins.

Similarly, safety data has been submitted to regulatory agencies in other jurisdictions and authorizations have been obtained for cultivation and/or food and feed uses of DP73496 canola in nine countries.

6. Risks to Human Health

a. Nutritional Data

A compositional comparison was conducted between DP73496 canola treated with glyphosate (referred to as herbicide-treated DP73496 canola) and non-GM near-isoline control canola (referred to as control canola) to determine if herbicide-treated DP73496 canola is as safe and nutritious as other canola varieties, as well as whether it can be used interchangeably with its comparator without negatively affecting human and animal health. A total of 99 analytes were measured for the comparative assessment based on the recommendations of the OECD consensus document on compositional considerations for new varieties of canola (OECD, 2001; OECD, 2011): proximates, fiber, fatty acids, amino acids, vitamins, minerals, glucosinolates, secondary metabolites, phytosterols, and anti-nutrients.

The GAT4621 protein is known to acetylate certain free amino acids (L-aspartate, L-glutamate, glycine, L-serine, and L-threonine) resulting in the respective production of acetylated amino acids *N*-acetylaspartate, *N*-acetylglutamate, *N*-acetylglycine, *N*-acetylserine, and *N*-acetyllethreonine (NAA, NAG, NAGly, NAS, and NAT, respectively). Based on the known activity of the GAT4621 enzyme, these 5 acetylated amino acid analytes in seed and processed fraction samples, as well as 26 free amino acid analytes in seed samples, were analyzed in addition to the 99 standard compositional analytes.

The results of the compositional assessment demonstrated that the nutrient composition of seed derived from herbicide-treated DP73496 is comparable to that of conventional canola represented by non-GM near-isoline control canola and non-GM commercial canola, with the exception of two acetylated amino acids (NAA and NAG) which were elevated in DP73496 canola relative to the control canola.

b. Toxicology

The toxicity potential of GAT4621 protein was evaluated using a series of studies, including bioinformatics, in vitro digestibility, glycosylation analyses, acute protein toxicity, and heat lability analyses. The results of these studies demonstrate that the protein is unlikely to be a toxin and not acutely toxic in mice.

c. Allergenicity

The allergenicity potential of the GAT4621 protein was evaluated using a series of studies, including bioinformatics, in vitro digestibility, glycosylation analyses, and heat lability analyses. The results of these studies demonstrate that the protein is unlikely to be an allergen.

7. Assessment of Risks to the Environment

Not applicable. This application does not cover an environmental release. This application is for the importation of DP73496 canola and its products for use as food, feed and processing.

8. What is the Emergency Response Plan?

This notification is for consent to allow the marketing of genetically modified DP73496 canola products for food/feed/processing use in Malaysia. The proposed uses of seeds

and other products of DP73496 canola, arising from imports, is anticipated to be the same as for any other canola. Given that the scope of this application does not include authorization for the cultivation of DP73496 canola, any exposure to the environment from the import of DP73496 canola is anticipated to be limited to unintended release via spillage during transportation of the seed.

The response plan for reports of unintended release into the environment likely to result in persistence in the environment will include a multiparty investigation of the report together with the competent national agencies and the implementation of mitigating measures jointly agreed upon for confirmed cases. This unlikely unintended release can be controlled with current agronomic measures taken to control other commercially available canola, such as selective use of herbicides (with the exception of glyphosate), and manual or mechanical removal of plants.

a. First Aid Measures

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

b. Accidental Release Measure

This application is for the importation of DP73496 canola and its products for use as food, feed and processing.

Any exposure to the environment from the import of DP73496 canola will be limited to unintended release via spillage during transportation of the seed. Populations of canola are unlikely to survive outside managed agricultural environments (OGTR, 2002). Canola is a poor competitor and is not regarded as a colonizing species. Unless the habitat is regularly disturbed or seeds are replenished from outside, canola will be displaced by other plants (Salisbury, 2002).

This unlikely unintended release can be controlled with current agronomic measures taken to control other commercially available canola, such as selective use of herbicides (with the exception of glyphosate), and manual or mechanical removal of plants.

c. Handling and Storage

On the basis of rigorous testing and multiple comprehensive evaluations, DP73496 canola has been demonstrated to be as safe to humans and animals, and the seeds of DP73496 canola are as safe and nutritious as conventional canola. Considering this,

recommendations for storage and handling of DP73496 canola are not anticipated to be different from conventional canola.

d. Disposal Consideration

On the basis of rigorous testing and multiple comprehensive evaluations, DP73496 canola has been demonstrated to be as safe to humans and animals, and the seeds of DP73496 canola are as safe and nutritious as conventional canola. Considering this, measures for waste disposal and treatment of DP73496 canola are not anticipated to be different from conventional canola.

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. 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 to 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 8th December 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.

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