FACT SHEET

APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF DAS-40278-9 MAIZE FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S) 602-1/1/34

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 Dow AgroSciences (M) Sdn Bhd.

1. What is the application for?

Importation of DAS-40278-9 maize for use as food, feed and for processing.

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

The aim of the import is to supply or offer to supply for sale/placing on the market – for direct use as food, feed and processing (FFP). The said maize event is not intended for cultivation in Malaysia.

3. How has the LMO been modified?

DAS-40278-9 maize has been genetically modified to express the AAD-1 protein. Expression of the AAD-1 protein, confers tolerance to application of 2,4-D and to certain aryloxyphenoxypropionate (AOPP) herbicides, providing growers with greater flexibility in selection of herbicides for the improved control of economically important weeds. It also allows an increased application window for effective weed control.

4. Characteristics of LMO

a) Details of the parent organism

Maize (*Zea mays* subsp. *mays*.) is the only domesticated species included in the genus *Zea*, of the family Poaceae. It is a highly domesticated agricultural crop with well-characterised phenotypic and genetic traits.

Maize has long history of safe use and is extensively cultivated worldwide. Maize is the world's third leading cereal crop, following wheat and rice. It is grown as a commercial crop in over 25 countries. Field maize has been grown for 8000 years in Mexico and Central America and for 500 years in Europe. Maize is naturally cross-pollinated and until about 1925 mainly open pollinated varieties were grown. Today mainly hybrids are grown.

Because of its highly domesticated nature, maize cannot survive without human assistance in non-agricultural habitats. Maize seed requires the

semi-uniform soil conditions resulting from cultivation in order to germinate and establish.

Centre of Origin	Reproduction	Toxins	Allergenicity
Mexico and Central America	Wind movements across the maize field cause pollen from the tassel to fall on the silks of the same or adjoining plants	Maize grain and forage, or derived products of maize, are not considered to have toxic effects on humans, animals and other organisms	Although there have been reports of allergenic reactions to maize, maize is not considered as a major allergenic food and maize allergies have mainly been caused by pollen

b) Details of the donor organism

Sphingobium herbicidovorans, the source organism for the *aad*-1 gene, is a gram-negative soil bacterium. As with other soil dwelling bacteria, *Sphingobium herbicidovorans* has evolved over time the ability to use phenoxy auxin and AOPP herbicides as carbon sources for growth, thus affording the bacterium a competitive advantage in soil. *Sphingobium* spp. are commonly isolated from soil. Sphingomonads are widely distributed in nature and have been isolated from land and water habitats, as well as from plant root systems, clinical specimens, etc. Due to their biodegradative and biosynthetic capabilities, the sphingomonads have been used for a wide range of biotechnological applications, including bioremediation of environmental contaminants and production of extracellular polymers such as sphingans which are used extensively in the food industry.

Latin Name	Gene	Pathogenicity
Sphingobium herbicidovorans	*aad*-1	There are no reports of *S. herbicidovorans* being implicated as a human pathogen or producing any allergens. Out of the ~20 recognized species of *Sphingobium*, only one, *S. yanoikuyae* has been isolated from a clinical environment. Other related genera however, are known to cause infrequent infections which are generally limited in virulence. Because of their ubiquity and adaptability, sphingomonads are often found in clinical settings, but usually not associated with infection. There are reports of sphingomonads producing antigenic glycolipids that may have use as therapeutics.

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

DAS-40278-9 maize was developed by inserting a linear *Fsp* I fragment from plasmid pDAS1740, using direct Whiskers-mediated transformation to stably incorporate the *aad-1* gene from the soil bacterium, *Sphingobium herbicidovorans*, into maize.

The AAD-1 protein (encoded by the *aad*-1 gene) is an alpha-ketoglutarate-dependent dioxygenase enzyme and has been shown to facilitate a one-step metabolic detoxification of 2,4-D to the herbicidally-inactive compound, dichlorophenol (DCP). AAD-1 is able to degrade the *R*-enantiomers (herbicidally active isomers) of the chiral phenoxy auxins (e.g., dichlorprop and mecoprop) in addition to achiral phenoxy auxins (e.g., 2,4-D, MCPA, 4-chlorophenoxyacetic acid). AAD-1 also catalyzes the degradation reaction of the general class of herbicides known as aryloxyphenoxypropionates (AOPPs), such as quizalofop, to their corresponding inactive phenols.

No other traits have been introduced or modified in DAS-40278-9 maize.

5. Modification method

The recipient maize line Hi-II was transformed using direct insertion of the DNA fragment from plasmid pDAS1740 via Whiskers-mediated transformation. The vector DNA fragment was isolated by digesting the whole plasmid pDAS1740 DNA with the restriction enzyme *Fsp* I which resulted in 5 fragments: a 6236 bp fragment containing the *aad*-1 expression cassette, two fragments (1023 bp and 1235 bp respectively) each containing a portion of the ampicillin resistance gene sequence from the plasmid backbone, and two minor fragments (9 bp each). The two smaller ampicillin resistance gene fragments and the two minor fragments were separated from the larger desired *aad*-1 expression cassette fragment via column chromatography. The final transformation fragment was a 6236 bp linear DNA carrying the *aad*-1 expression cassette for insertion into the plant genome. The isolated fragment, pDAS1740/*Fsp* I, contained the following elements: RB7 MAR, maize ZmUbi1 promoter, *aad*-1 gene, maize ZmPer5 3' UTR, RB7 MAR.

1. Characterization of the modification

Genetic elements of the linear Fsp I fragment delivered into the maize cells

Location on pDAS1740	Genetic Element	Size (base pairs)	Description
1-164	Intervening sequence	164 bp	Sequence used for DNA cloning
165-1330	RB7 MAR v3	1166 bp	Matrix attachment region (MAR) from *Nicotiana tobacum*
1331-1459	Intervening sequence	129 bp	Sequence used for DNA cloning
1460-3450	ZmUbi1 promoter	1991 bp	Ubiquitin promoter from Zea mays
3451-3472	Intervening sequence	22 bp	Sequences used for DNA cloning
3473-4363	aad-1	891 bp	Synthetic, plant-optimized version of an aryloxyalkanoate dioxygenase gene from *Sphingobium herbicidovorans*
4364-4397	Intervening sequence	34 bp	Sequence used for DNA cloning
4398-4762	ZmPer5 3' UTR	365 bp	3’ untranslated region from *Zea mays* peroxidase gene
4763-4801	Intervening sequence	39 bp	Sequence used for DNA cloning
4802-5967	RB7 MAR v4	1166 bp	Matrix attachment region (MAR) from *Nicotiana tobacum*
5968-6236	Intervening sequence	269 bp	Sequence used for DNA cloning

A thorough evaluation of the safety of the AAD-1 protein establishes that it is highly unlikely that this protein would cause any toxic effects on human or animal health and is considered to have a low risk of allergenic potential. Field expression of DAS-40278-9 maize (unsprayed or sprayed with 2,4-D, quizalofop or both herbicides) ranged from 2.08 ng/mg dry weight (R6 whole plant) to 102 ng/mg dry weight (pollen tissue). Expression values were similar for all AAD-1 treatments irrespective of the herbicide regime.

DAS-40278-9 maize is substantially equivalent to conventional maize, except for the introduced herbicide tolerance trait and is as safe and nutritious as conventional maize. DAS-40278-9 also has a history of safe

use. No adverse effects were brought forward during extensive field trials conducted in the U.S.A. and it has been authorized for use in 10 key maize cultivation and import countries.

6. Assessment of risks to human health

a) Nutritional data

Compositional analyses on grain samples of DAS-40278-9 maize and non-GM control maize, grown alongside, in replicated plots at the same field sites, were performed. Samples of maize forage and grain were analyzed for nutrient content with a variety of tests. The analyses performed for forage included ash, fat, moisture, protein, carbohydrate, acid detergent fiber, neutral detergent fiber, calcium and phosphorus. The analyses performed for grain included proximates (ash, fat, moisture, protein, carbohydrate), total dietary fiber, acid detergent fiber (ADF), neutral detergent fiber (NDF), minerals, amino acids, fatty acids, vitamins, secondary metabolites and anti-nutrients. In addition, wherever possible, publicly available data on commercial maize were also used in the comparisons with DAS-40278-9 maize. Evaluation of the nutrient composition data of DAS-40278-9 maize confirms that it is substantially equivalent to the non-GM control maize as well as to commercial maize.

b) Toxicology

The low potential toxicity of the AAD-1 protein expressed in DAS-40278-9 maize was demonstrated in a number of ways:

- Bioinformatics analysis of the AAD-1 protein using a BLASTp search against an up-to-date NCBI non-redundant protein database did not identify any sequence similarity with any known toxins that are harmful to humans or animals.
- An acute oral toxicity study with AAD-1 protein was conducted in mice at a level of 2000 mg AAD-1/kg after adjustment for purity. All animals survived and no clinical signs were observed during the study.
- The thermal stability of the AAD-1 protein was evaluated by heating protein solutions for 30 min at 50, 70 and 95 ^{o}C in a phosphate based buffer. Data indicates that industrial processing of the grain would significantly degrade the tertiary structure of the AAD-1 protein, reduce its immunoreactivity, and eliminate its enzymatic activity.

c) Allergenicity

An amino acid sequence comparison to known allergens showed that AAD-1 does not share any significant amino acid sequence similarity with known protein allergens. Further to this, the results of an *in vitro,* simulated gastric fluid (SGF) study demonstrated that the AAD-1 protein was readily digested (not detectable at 30 seconds) in SGF. Finally, the immunoaffinity-purified,

plant-derived AAD-1 protein was analyzed for evidence of glycosylation by eletrophoresis. No covalently-linked carbohydrates were detectable on the plant-derived or the microbe-derived AAD-1 proteins.

7. Assessment of risks to the environment

Because the application is for consent to import and use DAS-40278-9 maize grain, as any other maize, not including the cultivation of DAS-40278-9 maize hybrids, environmental release would be more likely to occur during import, storage and processing of DAS-40278-9 maize grain. However, modern methods of grain handling minimize losses of grain, so there is little chance of germination of spilt grain resulting in the development of mature plants of DAS-40278-9 maize. Moreover, the information presented in the application established that DAS-40278-9 maize is unlikely to be different from other maize and, therefore, is unlikely to pose any threat to the environment or to require special measures for its containment.

8. What is the emergency response plan?

Grain from DAS-40278-9 maize is intended to be imported for food, feed and/or processing use only and is not intended for planting as seed. In the event of plants establishing, they can be easily controlled either mechanically or by the use of selective herbicides.

As previously stated, DAS-40278-9 maize is substantially equivalent to conventional maize, except for the introduced herbicide tolerance trait and is as safe and nutritious as conventional maize. DAS-40278-9 also has a history of safe use. No adverse effects were brought forward during extensive field trials conducted in the U.S.A. and it has been authorized for use in 10 key maize cultivation and import countries.

a) First aid measures

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

b) Accidental release measures

In the event of incidental spillage, the establishment of volunteer plants would be unlikely, since maize cannot survive without human assistance and is not capable of surviving as a weed. Maize volunteers, if they occurred, could be easily controlled by the use of selective herbicides.

c) Handling and storage

DAS-40278-9 maize is substantially equivalent to other maize varieties except for its tolerance to application of 2,4-D, which is a trait of agronomic interest. Therefore no specific instructions are warranted or required for the storage and handling of DAS-40278-9 maize and derived products as it will be stored, packaged, transported, handled and used in the same manner as the commercial maize products.

d) Disposal considerations

Measures for waste disposal and treatment of DAS-40278-9 maize will be the same as for conventional, non-transgenic maize.

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, which 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 5 January 2016 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.