FACT SHEET

APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF DAS-81419-2 SOYBEAN FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S) 602-1/1/36

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-81419-2 soybean 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 soybean event is not intended for cultivation in Malaysia.

3. **How has the LMO been modified?**
   DAS-81419-2 soybean carry the genes *cry1Ac (*synpro*), cry1F*v3 and a *pat*, which express theCry1Ac protein, Cry1F protein and phosphinothricin acetyl transferase (PAT), respectively. These newly introduced proteins provide protection against several lepidopteran pests and tolerance to glufosinate herbicides, respectively.

4. **Characteristics of LMO**

   a) **Details of the parent organism**
   Soybean is a highly domesticated agricultural crop with well-characterized phenotypic and genetic traits.

   The soybean is grown as a commercial crop in over 35 countries world-wide. The soybean, *Glycine max* (L.), dominates the major oilseeds traded in international markets. The major producers are U.S., Argentina, Brazil, and China, which account for 87% of the total production. Most soybean meal, 97%, is used in animal feed, with 46% going to poultry, 32% to swine, and 9% each going to dairy and beef cattle feed, respectively. A sizeable amount is also used in pet food.

   Cultivated soybean seed rarely displays any dormancy characteristics and only under certain environmental conditions grows as a volunteer in the year following cultivation. If this should occur, volunteers do not

compete well with the succeeding crop, and can easily be controlled mechanically or chemically. The soybean plant is not weedy in character. In managed ecosystems, soybean does not effectively compete with other cultivated plants or primary colonizers.

Soybean can only cross with other members of *Glycine* subgenus *Soja*. The potential for such gene flow is limited by geographic isolation and by the fact that they are highly self-pollinating species. Wild soybean species are endemic in China, Korea, Japan, Taiwan and the former USSR.

Centre of Origin	**Reproduction**	**Toxins**	**Allergenicity**
North and Central China	Soybean is considered a self-pollinated species and cross pollination is usually less than one percent	Soybean (*Glycine max*) and its derived products, are not considered to have toxic effects on humans, animals and other organisms	The soybean (*Glycine max)* is one of the top eight important allergenic foods.

b) Details of the donor organism

Bacillus thuringiensis: donor of: *cry1F*v3 and *cry1Ac (*synpro*)*

Bacillus thuringiensis (or Bt) is a Gram-positive, soil-dwelling bacterium, commonly used as a biological pesticide. *B. thuringiensis* also occurs naturally in the gut of caterpillars of various types of moths and butterflies, as well on leaf surfaces, aquatic environments, animal feces, insect-rich environments, and flour mills and grain-storage facilities. The Cry1Ac protein is derived from *Bacillus thuringiensis* subsp. *kurstaki* and the Cry1F protein from subsp. *aizawai*. Spectrum of activity of Cry1F and Cry1Ac proteins remains within Order Lepidoptera.

Streptomyces viridochromogenes: donor of the *pat* gene

Streptomyces viridochromogenes is a common soil bacterium that produces the tripeptide L-phosphinothricyl-L-alanyl-alanine (L-PPT), which was developed as a non-selective herbicide by Hoechst Ag. The

pat gene, encoding the phosphinothricin acetyl transferase, confers *S. viridochromogenes* tolerance to glufosinate ammonium herbicide.

Latin Name	Gene	Pathogenicity
*Bacillus thuringiensis (*or *Bt)*	*cry1Fv3* and *cry1Ac (synpro)*	*Bacillus thuringiensis* produces the Cry insecticidal proteins that attacks the gut of pests and kills them internally. The toxin is species-specific and has no known negative effects on humans, vertebrates, or plants. *Bt* is the most common environmentally-friendly insecticide used and is the basis of many of the pesticides available in the market today.
Streptomyces viridochromogenes	*pat*	*Streptomyces viridochromogenes* is a ubiquitous, gram-positive, soil bacterium and is not considered pathogenic.

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

DAS-81419-2 soybean was generated by *Agrobacterium*-mediated transformation using plasmid pDAB9582. The T-DNA insert in the plasmid contains two synthetic genes from *Bacillus thuringiensis*, *cry1Ac(synpro)* and *cry1Fv3,* as well as a *pat* gene from *Streptomyces viridochromogenes.*

The *cry1F*v3 gene is comprised of three parts; at the 5′ end, a toxin core that was optimized from the native *cry1Fa2* gene originally isolated from *Bacillus thuringiensis* subsp*. aizawai* strain PS811; in the middle, a very small portion of *cry1Ca3* which was originally isolated from *B. thuringiensis* subsp. *aizawai* strain PS811; and at the 3′ end, a tail that was optimized from the native *cry1Ab1* tail originally isolated from *B. thuringiensis* subsp. *berliner* 1715. The *cry1F*v3 gene encodes the Cry1F protein that is comprised of 1148 amino acids and has a molecular weight of ~130.2 kDa.

The *cry1Ac(*synpro*)* gene is comprised of three parts; at the 5′ end, a toxin core that was optimized from the native *cry1Ac1* gene originally isolated from *B. thuringiensis* subsp*. kurstaki* strain HD73; in the middle, a very small portion of *cry1Ca3* which was originally isolated from *B. thuringiensis* subsp. *aizawai* strain PS811; and at the 3′ end, a tail that was optimized from the native *cry1Ab1* tail originally isolated from *B. thuringiensis* subsp. *berliner* 1715. The *cry1Ac(*synpro*)* gene encodes the Cry1Ac protein that is comprised of 1156 amino acids and has a molecular weight of ~130.7 kDa.

The *pat* gene originates from the common soil bacterium *Streptomyces viridochromogenes* (Wohlleben *et al.*, 1988). Expression of PAT protein in soybean plants confers tolerance to glufosinate and was used as a selectable marker during DAS-81419-2 soybean development. The *pat* gene encodes a protein of 183 amino acids that has a molecular weight of approximately 20.6 kDa. The *pat* gene has been widely used both as a selectable marker and herbicide tolerance trait in previously commercialized products.

No other traits have been introduced or modified in DAS-81419-2 Soybean.

5. Modification method

DAS-81419-2 soybean (*Glycine max*) was generated through *Agrobacterium*-mediated transformation of soybean cotyledonary node explants.

Soybean seeds (cv Maverick) were germinated on basal media and cotyledonary nodes were isolated and infected with the *Agrobacterium* EHA101 carrying plasmid pDAB9582. After infection with *Agrobacterium*, cotyledonary nodes were cultured on the cocultivation medium for 5 days before transferring to shoot initiation medium. All media, including shoot initiation, shoot elongation, and rooting media were supplemented with cefotaxime, timentin and vancomycin to inhibit the growth of *Agrobacterium*. Glufosinate-ammonium selection (3-8 mg/L) was also employed in those media to inhibit the growth of non-transformed shoots. Selected shoots were transferred to rooting medium for root development and then transferred to soil mix for acclimatization of plantlets.

Terminal leaflets of regenerated plantlets were painted with glufosinate-ammonium (0.05% - 2% w/v) to screen for putative transformants. Those plantlets exhibiting tolerance were transferred to the greenhouse, allowed to acclimate and then leaf painted again with glufosinate-ammonium (0.05% - 2% w/v) to reconfirm herbicide tolerance (not enough to be used commercially under field conditions but enough to detect it under experimental conditions).

The glufosinate-ammonium tolerant plants were sampled and analyzed at molecular level to confirm the presence of the selectable marker gene and/or the genes of interest. Specifically, for T0 plants, PCR analysis was performed to verify the absence of the spectinomycin resistance gene sequence as well as the presence of the *cry1Ac(*synpro*)* and *cry1F*v3 genes. Invader assay was carried out for copy number detection for *pat*, *cry1Ac(*synpro*)*, and *cry1F*v3 genes. Selected T0 plants were allowed to self-fertilize in the greenhouse to give rise to T1 seed. For T1 plants, Invader assay and Southern blot analysis were performed to detect copy number, integration number, and PTU integrity.

a) Characterization of the modification

Table 1. Genetic elements of plasmid pDAB9582

Feature Name	Feature Start	Feature Stop	Feature Length	Description
T-DNA Border B	1	24	24	T-DNA Border B sequence required for transfer of DNA from *Agrobacterium tumefaciens* into plant cells (Barker *et al.*, 1983)
Intervening sequence	25	295	271	Non-specific DNA sequences necessary for cloning
AtUbi10 promoter	296	1617	1322	AtUbi10 promoter along with the 5' untranslated region and intron from *Arabidopsis thaliana* polyubiquitin 10 (UBQ10) gene (Norris *et al.*, 1993)
Intervening sequence	1618	1625	8	Non-specific DNA sequences necessary for cloning
cry1F v3	1626	5072	3447	*cry1F v3* (synthetic version of the *cry1F* gene from *Bacillus thuringiensis* subsp. *aizawai* strain PS811) (Cardineau *et al.*, 2001; Gao *et al.*, 2006)
Intervening sequence	5073	5174	102	Non-specific DNA sequences necessary for cloning
AtuORF23 3' UTR	5175	5631	457	AtuORF23 3' UTR (3' untranslated region (UTR) comprising the transcriptional terminator and polyadenylation site of open reading frame 23 (ORF23) of *Agrobacterium tumefaciens* pTi15955) (Barker *et al.*, 1983)
Intervening sequence	5632	5694	63	Non-specific DNA sequences necessary for cloning
CsVMV promoter	5695	6211	517	CsVMV promoter along with the 5' untranslated region derived from Cassava Vein Mosaic virus (Verdaguer *et al.*, 1996)
Intervening sequence	6212	6220	9	Non-specific DNA sequences necessary for cloning
cry1Ac(synpro)	6221	9691	3471	*cry1Ac (synpro)* (synthetic version of the *cry1Ac* gene from *Bacillus thuringiensis* subsp. *kurstaki* strain HD73) (Adang *et al.*, 1985; Gilroy and Wilcox, 1992; Cardineau *et al.*, 2001)
Intervening sequence	9692	9724	33	Non-specific DNA sequences necessary for cloning
AtuORF23 3' UTR	9725	10181	457	AtuORF23 3' UTR (3' untranslated region (UTR) comprising the transcriptional terminator and polyadenylation site of open reading frame 23 (ORF23) of *Agrobacterium tumefaciens* pTi15955) (Barker *et al.*, 1983)

Feature Name	Feature Start	Feature Stop	Feature Length	Description
Intervening sequence	10182	10295	114	Non-specific DNA sequences necessary for cloning
CsVMV promoter	10296	10812	517	CsVMV promoter along with the 5' untranslated region derived from Cassava Vein Mosaic virus (Verdaguer *et al.*, 1996)
Intervening sequence	10813	10819	7	Non-specific DNA sequences necessary for cloning
pat	10820	11371	552	*pat* (synthetic version of the phosphinothricin acetyl transferase gene from *Streptomyces viridochromogenes*) (Wohlleben *et al.*, 1988)
Intervening sequence	11372	11473	102	Non-specific DNA sequences necessary for cloning
AtuORF1 3' UTR	11474	12177	704	AtuORF1 3' UTR (3' untranslated region (UTR) comprising the transcriptional terminator and polyadenylation site of open reading frame 1 (ORF1) of *Agrobacterium tumefaciens* pTi15955) (Barker *et al.*, 1983)
Intervening sequence	12178	12405	228	Non-specific DNA sequences necessary for cloning
T-DNA Border A	12406	12429	24	T-DNA Border A sequence required for transfer of T-DNA insert from *Agrobacterium tumefaciens* into plant cells (Barker *et al.*, 1983)
Intervening sequence	12430	12448	19	Non-specific DNA sequences necessary for cloning
T-DNA Border A	12449	12472	24	T-DNA Border A sequence required for transfer of T-DNA insert from *Agrobacterium tumefaciens* into plant cells (Barker *et al.*, 1983)
Intervening sequence	12473	12759	287	Non-specific DNA sequences necessary for cloning
T-DNA Border A	12760	12783	24	T-DNA Border A sequence required for transfer of T-DNA insert from *Agrobacterium tumefaciens* into plant cells (Barker *et al.*, 1983)
Intervening sequence	12784	13162	379	Plasmid backbone sequences from gram-negative bacteria broad host-range RK2 plasmid (Stalker *et al.*, 1981)
ori	13163	14182	1020	*ori* (Replication origin sequences from gram-negative bacteria broad host-range RK2 plasmid) (Stalker *et al.*, 1981)
Intervening sequence	14183	14727	545	Plasmid backbone sequences from gram-negative bacteria broad host-range RK2 plasmid (Stalker *et al.*,

Feature Name	Feature Start	Feature Stop	Feature Length	Description
				1981)
trfA	14728	15876	1149	*trfA* (Plasmid replication sequences for Trf A protein from gram-negative bacteria broad host-range RK2 plasmid) (Stalker *et al.,* 1981)
Intervening sequence	15877	17080	1204	Plasmid backbone sequences from gram-negative bacteria broad host-range RK2 plasmid (Stalker *et al.,* 1981)
*Spec*R	17081	17869	789	*Spec*R (spectinomycin resistance gene from *Escherichia coli* Tn7 transposon) (Fling *et al.,* 1985)
Intervening sequence	17870	18143	274	Plasmid backbone sequences for cloning

b) Safety of the expressed protein

A thorough evaluation of the safety of the Cry1Ac, Cry1F and PAT proteins establishes that it is highly unlikely that these proteins 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-81419-2 soybean ranged from 0.39 ng/mg dry weight in root to 25.44 ng/mg in V5 leaf tissue (Cry1Ac), 5.23 ng/mg dry weight in root to 56.75 ng/mg in V5 leaf tissue (Cry1F) and 0.63 ng/mg dry weight in root to 5.60 ng/mg in V10-12 leaf tissue (PAT).

DAS-81419-2 soybean is substantially equivalent to conventional soybean, except for the introduced insect resistance trait and is as safe and nutritious as conventional soybean. DAS-81419-2 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 11 key soybean cultivation and import countries.

6. Assessment of risks to human health

a) Nutritional data

Compositional analyses on grain samples of DAS-81419-2 soybean and non-GM control soybean, grown alongside, in replicated plots at the same field sites, were performed. Samples of soybean 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 and bioactives. In addition, wherever possible, publicly available data on commercial soybean were also used in the

comparisons with DAS-81419-2 soybean. Evaluation of the nutrient composition data of DAS-81419-2 soybean confirms that it is substantially equivalent to the non-GM control soybean as well as to commercial soybean.

b) Toxicology

The low potential toxicity of the Cry1Ac, Cry1F and PAT proteins expressed in DAS-81419-2 soybean was demonstrated in a number of ways:

- Bioinformatics analysis of the Cry1Ac, Cry1F and PAT proteins 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.
- Acute oral toxicity studies with Cry1Ac, Cry1F and PAT proteins was conducted in mice at levels of 700 mg Cry1Ac/kg body weight, 600 mg Cry1F/kg body weight and 5000 mg PAT/kg body weight after adjustment for purity. All animals survived and no clinical signs were observed during the studies.
- The thermal stability of the Cry1Ac and Cry1F proteins was evaluated by heating protein solutions in a phosphate based buffer. Data indicates that industrial processing of the grain would significantly degrade the tertiary structure of the Cry1Ac and Cry1F proteins, reduce their immunoreactivity, and eliminate their enzymatic activity. The PAT protein has a long history of commercial use and been thoroughly risk assessed worldwide. It has already been established that the PAT protein is readily denatured by heat

c) Allergenicity

An amino acid sequence comparison to known allergens showed that Cry1Ac, Cry1F and PAT do not share any significant amino acid sequence similarities with known protein allergens. Further to this, the results of *in vitro* using simulated gastric fluid (SGF) studies demonstrated that the Cry1Ac and Cry1F proteins were readily digested in SGF. PAT protein is readily degradable in simulated digestive juice. Finally, the immunoaffinity-purified, plant-derived Cry1Ac, Cry1F and PAT proteins were analyzed for evidence of glycosylation by eletrophoresis. No covalently-linked carbohydrates were detectable on the plant-derived or the microbe-derived Cry1Ac, Cry1F and PAT proteins.

7. Assessment of risks to the environment

Because the application is for consent to import and use DAS-81419-2 soybean grain, as any other soybean, not including the cultivation of DAS-81419-2 hybrids, environmental release would be more likely to occur during import, storage and processing of DAS-81419-2 soybean 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-81419-2 soybean. Moreover, the information presented in the application established that DAS-81419-2 soybean is unlikely to be different from other soybean 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-81419-2 is intended to be imported for food, feed and 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 with the use of selective herbicides.

As previously stated, DAS-81419-2 soybean is substantially equivalent to conventional soybean, except for the introduced insect resistance trait and is as safe and nutritious as conventional soybean. DAS-81419-2 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 11 key soybean 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 soybean cannot survive without human assistance and is not capable of surviving as a weed. Soybean volunteers, if they occurred, could be easily controlled by the use of selective herbicides.

c) Handling and storage

DAS-81419-2 soybean is substantially equivalent to other soybean varieties except for its resistance to certain pests. Therefore no specific instructions are warranted or required for the storage and handling of DAS-81419-2 and derived products as it will be stored, packaged, transported, handled and used in the same manner as the commercial soybean products.

d) Disposal considerations

Measures for waste disposal and treatment of DAS-81419-2 soybean will be the same as for conventional, non-transgenic soybean.

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 10 August 2017 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.