FACT SHEET

APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF DAS-68416-4 SOYBEAN FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S) 602-1/1/38

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-68416-4 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-68416-4 soybean has been genetically modified to express the AAD-12 and PAT proteins. The *aad*-12 gene encodes the aryloxyalkanoate dioxygenase-12 (AAD-12) enzyme which, when expressed in plants, degrades 2,4-D into herbicidally-inactive 2,4-dichlorophenol (DCP). The *pat* gene encodes the enzyme phosphinothricin acetyl transferase (PAT) that inactivates glufosinate. The availability of DAS-68416-4 soybean is expected to have a beneficial impact on weed control practices by providing growers with another tool to address their weed control needs.

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. Of the major oilseeds traded in international markets, the soybean, *Glycine max* (L.), dominates. The major producers, U.S., Argentina, Brazil, and China, 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

Delftia acidovorans: donor of the *aad-12* gene

Delftia acidovorans, which has previously been identified as *Pseudomonas acidovorans* and *Comamonas acidovorans*, is a non glucose-fermenting, gram-negative, non-spore-forming rod present in soil, fresh water, activated sludge, and clinical specimens. *Delftia acidovorans* can be used to transform ferulic acid into vanillin and related flavor metabolites. This utility has led to a history of safe use for *D. acidovorans* in the food processing industry. For example, US Patent 5,128,253 “Bioconversion process for the production of vanillin” was issued on July 7, 1992 to Kraft General Foods. This strain also produces polyhydroxyalkanoates that are being developed as biomaterials for medical applications.

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
Delftia acidovorans	*aad-12*	*Delftia acidovorans* is an aerobic, nonfermenting Gram-negative bacillus. It is usually a nonpathogenic environmental organism and is rarely clinically significant. *D. acidovorans* has a history of safe use in the food processing industry
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

Transgenic soybean (*Glycine max*) DAS-68416-4 was generated through *Agrobacterium*-mediated transformation, using the disarmed *Agrobacterium tumefaciens* strain EHA101 carrying the binary vector pDAB4468 that lead to the transfer and insertion of its T-DNA into the genome of cells from soybean cotyledonary node explants. The T-DNA insert in the plasmid contains the plant-optimized sequence of the *aad-12* gene from *Delftia acidovorans* and the *pat* gene from *Streptomyces viridochromogenes*. The *aad*-12 gene encodes the aryloxyalkanoate dioxygenase-12 (AAD-12) enzyme which, when expressed in plants, degrades 2,4-D into herbicidally-inactive 2,4-dichlorophenol (DCP). The *pat* gene encodes the enzyme phosphinothricin acetyl transferase (PAT) that inactivates glufosinate.

No other traits have been introduced or modified in DAS-68416-4 soybean.

5. Modification method

Transgenic soybean (*Glycine max*) DAS-68416-4 was generated through *Agrobacterium*-mediated transformation, using the disarmed *Agrobacterium tumefaciens* strain EHA101 carrying the binary vector pDAB4468 that lead to the transfer and insertion of its T-DNA into the genome of cells from soybean cotyledonary node explants.

Briefly, soybean seeds (cv Maverick) were germinated on basal media and cotyledonary nodes were isolated and infected with *Agrobacterium.* Shoot initiation, shoot elongation, and rooting media were supplemented with

cefotaxime, timentin and vancomycin for removal of *Agrobacterium*. Glufosinate selection was employed 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 selected plantlets were leaf painted with glufosinate to screen for putative transformants. The screened plantlets were transferred to the greenhouse, allowed to acclimate and then leaf-painted with glufosinate to reconfirm tolerance. Surviving plantlets were deemed to be putative transformants. The screened plants were sampled and molecular analyses for the confirmation of the selectable marker gene and/or the gene of interest were carried out. T_0 plants were allowed to self fertilize in the greenhouse to give rise to T_1 seed.

a) Characterization of the modification

Table 1: Genetic elements of the T-DNA insert from plasmid pDAB4468

Location on T-DNA insert of pDAB4468[1]	***Genetic Element***	***Size (base pairs)***	***Description***
1−24	T-DNA Border B	24	Transferring DNA sequences
25−160	Intervening sequence	136	Sequence from Ti plasmid pTi15955 (Barker *et al.*, 1983)
161−1326	RB7-MAR	1166	Matrix attachment region (MAR) from *Nicotiana tabacum* (Hall *et al.*, 1991)
1327−1421	Intervening sequence	95	Sequence from plasmid pENTR/D-TOPO (Invitrogen Cat. No. A10465) and multiple cloning sites
1422−2743	AtUbi10	1322	*Arabidopsis thaliana* polyubiquitin UBQ10 comprising the promoter, 5' untranslated region and intron (Norris *et al.*, 1993)
2744−2751	Intervening sequence	8	Sequence used for DNA cloning
2752−3633	aad-12	882	Synthetic, plant-optimized version of an aryloxyalkanoate dioxygenase gene from *Delftia acidovorans* (Wright *et al.*, 2007)
3634−3735	Intervening sequence	102	Sequence used for DNA cloning
3736−4192	AtuORF23	457	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)
4193−4306	Intervening sequence	114	Sequence from plasmid pENTR/D-TOPO (Invitrogen Cat. No. A10465) and multiple cloning sites
4307−4823	CsVMV	517	Promoter and 5’ untranslated region derived from the cassava vein mosaic virus (Verdaguer *et al.*, 1996)
4824−4830	Intervening sequence	7	Sequence used for DNA cloning
4831−5382	pat	552	Synthetic, plant-optimized version of phosphinothricin N-acetyl transferase (PAT) gene, isolated from *Streptomyces viridochromogenes* (Wohlleben et al., 1988)
5383−5484	Intervening sequence	102	Sequence from plasmid pCRI2.1(Invitrogen Cat. No. K205001) and multiple cloning sites

Table 1 (cont.). Genetic elements of the T-DNA insert from plasmid pDAB4468

Location on T-DNA insert of pDAB4468[1]	Genetic Element	Size (base pairs)	Description
5485−6188	AtuORF1	704	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)
6189−6416	intervening sequence	228	Sequence from Ti plasmid C58 (Zambryski *et al.*, 1982; Wood *et al.*, 2001)
6417−6440	T-DNA border A	24	Transferring DNA sequences
6441−6459	intervening sequence	19	Sequence from Ti plasmid C58 (Zambryski et al., 1982; Wood et al., 2001,),
6460−6483	T-DNA border A	24	Transferring DNA sequences
6484−6770	intervening sequence	287	Sequence from Ti plasmid pTi15955 (Baker et al., 1983)
6771−6794	T-DNA border A	24	Transferring DNA sequences
6795-7173	Plasmid backbone sequences	379	Plasmid backbone sequences from RK2 plasmid (Stalker *et al.*, 1981)
7174-8193	Ori Rep	1020	Replication origin sequences from RK2 plasmid (Stalker *et al.*, 1981)
8194-8738	Plasmid backbone sequences	545	Plasmid backbone sequences from RK2 plasmid (Stalker *et al.*, 1981)
8739-9887	Trf A	1149	Plasmid replication sequences for Trf A protein from RK2 plasmid (Stalker *et al.*, 1981)
9888-11091	Plasmid backbone sequences	1204	Plasmid backbone sequences from RK2 plasmid (Stalker *et al.*, 1981)
11092-11880	Spec R	789	Sequences for Spectinomycin resistance gene (Dagert and Ehrlich, 1979)
11881-12154	Plasmid backbone sequences	274	Plasmid backbone sequences for cloning

1 Base pair position.

b) Safety of the expressed protein

A thorough evaluation of the safety of the AAD-12 and PAT proteins establishes that it is highly unlikely that these proteins would cause any toxic effects on human or animal health and are considered to have a low risk of allergenic potential. Field expression of DAS-68416-4 soybean (unsprayed or sprayed with 2,4-D and glufosinate) ranged from 17.94 ng/mg dry weight (R3 stage root) to 78.52 ng/mg dry weight (V5 leaf tissue) for the AAD-12 protein and 1.85 ng/mg dry weight (R3 stage root) to 7.80 ng/mg (V10-12 leaf tissue) for the PAT protein. Expression values were similar for all AAD-12 and PAT treatments irrespective of the herbicide regime.

DAS-68416-4 soybean is substantially equivalent to conventional soybean, except for the introduced herbicide tolerance trait and is as safe and nutritious as conventional soybean. DAS-68416-4 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 9 key soybean cultivation and import countries.

6. Assessment of risks to human health

a) Nutritional data

Compositional analyses, including proximates, minerals, amino acids, fatty acids, vitamins, isoflavones, and anti-nutrients were conducted to investigate the equivalency of DAS-68416-4 soybean (with or without 2,4-D and/or glufosinate treatments) to the control. Mean composition levels for DAS-68416-4 soybean samples were all statistically indistinguishable from the control line and/or within reference or literature ranges for conventional soybean. In conclusion, DAS-68416-4 soybean was compositionally equivalent to conventional soybean.

b) Toxicology

The low potential toxicity of the AAD-12 and PAT proteins expressed in DAS-68416-4 soybean was demonstrated in a number of ways:

- Bioinformatics analysis of the AAD-12 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 AAD-12 and PAT proteins were conducted in mice at a level of 2000 mg AAD-12/kg and 5000 mg PAT/kg after adjustment for purity. All animals survived and no clinical signs were observed during the study.
- The thermal stability of the AAD-12 protein was evaluated by heating protein solutions for 30 min at various temperatures. Data indicates that industrial processing of the grain would significantly degrade the tertiary structure of the AAD-12 protein, reducing its immunoreactivity, and significantly diminish its enzymatic activity. Given that 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

Amino acid sequence comparisons to known allergens showed that AAD-12 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) study demonstrated that the AAD-12 protein was readily digested (not detectable at 30 seconds) in SGF. Finally, the AAD-12 protein was analyzed for evidence of glycosylation. No covalently-linked carbohydrates were detectable on the plant-derived or the microbe-derived protein. The PAT protein has a long history of commercial use and been thoroughly risk assessed worldwide and it has already been established that the PAT protein is readily degradable in simulated digestive juice and lacks glycosylation when expressed in soybeans.

7. Assessment of risks to the environment

Because the application is for consent to import and use DAS-68416-4 soybean grain, as any other soybean, not including the cultivation of DAS-68416-4 hybrids, environmental release would be more likely to occur during import, storage and processing of DAS-68416-4 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-68416-4 soybean. Moreover, the information presented in the application established that DAS-68416-4 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-68416-4 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-68416-4 soybean is substantially equivalent to conventional soybean, except for the introduced herbicide tolerance trait and is as safe and nutritious as conventional soybean. DAS-68416-4 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 9 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-68416-4 soybean is substantially equivalent to other soybean varieties except for its herbicide tolerance trait, which is a trait of agronomic interest. Therefore no specific instructions are warranted or required for the storage and handling of DAS-68416-4 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-68416-4 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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