FACT SHEET

APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF DAS-444Ø6-6 SOYBEAN FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S) 602-1/1/37

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 and MS Technologies LLC.

1. **What is the application for?**
   Importation of DAS-444Ø6-6 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-444Ø6-6 soybean carry the genes *aad-12, 2mepsps* and *pat* which express the proteins aryloxyalkanoate dioxygenase 12 (AAD-12), double mutant maize 5-enolpyruvylshikimate-3-phosphate synthase (2mEPSPS) and and phosphinothricin acetyl transferase (PAT), respectively. These newly introduced proteins provide tolerance to 2,4-D,glyphosate and 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. 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.

Zea mays: donor of the *2mepsps* gene

The donor organism, *Zea mays*, (commonly referred to as corn or maize) is a major cereal crop grown for food and feed. The 2mEPSPS contains two amino acid substitutions compared with the wild-type EPSPS.

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
Zea mays	*2mepsps*	*Zea mays*, (commonly referred to as corn or maize) is a major cereal crop grown for food and feed and is not considered pathogenic.
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-444Ø6-6 soybean was generated by *Agrobacterium*-mediated transformation using the plasmid pDAB8264. The T-DNA insert in the plasmid contains the *2mepsps* gene from *Zea mays*, a synthetic, plant-optimized sequence of the *aad-12* gene from *Delftia acidovorans*, and the *pat* gene from *Streptomyces viridochromogenes*.

DAS-444Ø6-6 soybean expresses a double mutant maize 5-enolpyruvylshikimate-3-phosphate synthase (2mEPSPS) protein providing tolerance to glyphosate, and the aryloxyalkanoate dioxygenase-12 (AAD-12) and phosphinothricin acetyl transferase (PAT) proteins which, when expressed in plants, degrades 2,4-D into herbicidally-inactive 2,4-dichlorophenol (DCP) and inactivates glufosinate, respectively.

No other traits have been introduced or modified in DAS-444Ø6-6 soybean.

5. Modification method

DAS-444Ø6-6 soybean was generated through *Agrobacterium*-mediated transformation of soybean (*Glycine max*) cotyledonary node explants. The disarmed *Agrobacterium tumefaciens* strain EHA101, carrying the binary vector with the *2mepsps, aad-12* and *pat* within the T-DNA region, was used to initiate transformation.

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 to inhibit the growth 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 painted with glufosinate to screen for putative transformants. The glufosinate-resistant plantlets were transferred to the greenhouse, allowed to acclimate and then painted with glufosinate to reconfirm tolerance. Surviving plantlets were deemed to be putative transformants. The screened plants were sampled and analyzed at the molecular level for the presence of the T-DNA insert and the absence of the vector backbone DNA. Specifically, for T0 plants, PCR analysis was performed to verify the absence of the spectinomycin resistance gene in the vector backbone as well as the presence of the *aad-12* coding region and *2mepsps* plant transcription unit (PTU). A PCR-based zygosity assay was conducted for copy number detection for *2mepsps, aad-12* and *pat* genes. Selected T0 plants were allowed to self-fertilize in the greenhouse to give rise to T1 seed. For T1 plants, PCR analysis, zygosity assay, and Southern blot analysis were performed to detect copy number, number of integration sites, and PTU integrity.

a) Characterization of the modification

Table 1: Genetic elements of the T-DNA insert from plasmid pDAB8264

Feature Name	Feature Start	Feature Stop	Feature Length	Description
T-DNA Border B	1	24	24	Required for transfer of T-DNA insert from *Agrobacterium tumefaciens* into plant cells (Barker *et al.*, 1983)
Intervening sequence	25	160	136	Non-specific DNA sequences necessary for cloning
RB7 MAR	161	1326	1166	Matrix attachment region from the *Nicotiana tabacum* rb-7-5A gene (Hall *et al.*, 1991)
Intervening sequence	1327	1365	39	Non-specific DNA sequences necessary for cloning
Histone H4A748 3′ UTR	1366	2026	661	3′ untranslated region (UTR) comprising the transcriptional terminator and polyadenylation site of the histone H4A748 gene from *Arabidopsis thaliana* (Chaboute *et al.*, 1987)
Intervening sequence	2027	2049	23	Non-specific DNA sequences necessary for cloning
2mepsps	2050	3387	1338	Native 5-enolpyruvylshikimate-3-phosphate synthase gene from *Zea mays* with two mutations providing glyphosate tolerance (Lebrun *et al.*, 1996; Lebrun *et al.*, 2003)
TPotp C	3388	3759	372	Optimized chloroplast transit peptide derived from maize and sunflower RuBisCO (Lebrun *et al.*, 1996; Lebrun *et al.*, 2003)
Intervening sequence	3760	3763	4	Non-specific DNA sequences necessary for cloning
Histone H4A748 promoter	3764	5193	1430	Promoter along with the 5′ untranslated region of the Histone H4A748 gene from *Arabidopsis thaliana* including an intron from the Histone 3 gene from *Arabidopsis thaliana* (Chaboute *et al.*, 1987)
Intervening sequence	5194	5285	92	Non-specific DNA sequences necessary for cloning
AtUbi10 promoter	5286	6607	1322	Promoter along with the 5′ untranslated region and intron from the *Arabidopsis thaliana* polyubiquitin 10 (UBQ10) gene (Norris *et al.*, 1993)
Intervening sequence	6608	6615	8	Non-specific DNA sequences necessary for cloning
aad-12	6616	7497	882	Plant-optimized version of an aryloxyalkanoate dioxygenase gene from *Delftia acidovorans* encoding an enzyme with an alpha ketoglutarate-dependent dioxygenase activity which results in metabolic inactivation of the herbicide(s) (Wright *et al.*, 2009; Wright *et al.*, 2010)
Intervening sequence	7498	7599	102	Non-specific DNA sequences necessary for cloning
AtuORF23 3′ UTR	7600	8056	457	3′ untranslated region (UTR) comprising the transcriptional terminator and polyadenylation site of open reading frame 23 (ORF23) of plasmid pTi15955 from *Agrobacterium tumefaciens* (Barker *et al.*, 1983)
Intervening sequence	8057	8170	114	Non-specific DNA sequences necessary for cloning
CsVMV promoter	8171	8687	517	Promoter along with the 5′ untranslated region derived from the Cassava Vein Mosaic virus (Verdaguer *et al.*, 1996)
Intervening sequence	8688	8694	7	Non-specific DNA sequences necessary for cloning

Feature Name	Feature Start	Feature Stop	Feature Length	Description
pat	8695	9246	552	Plant-optimized version of phosphinothricin acetyltransferase (PAT) gene, isolated *from Streptomyces viridochromogenes*, encoding a protein that confers tolerance to glufosinate (Wohlleben *et al.*, 1988)
Intervening sequence	9247	9348	102	Non-specific DNA sequences necessary for cloning
AtuORF1 3′ UTR	9349	10052	704	3′ untranslated region (UTR) comprising the transcriptional terminator and polyadenylation site of open reading frame 1 (ORF1) of plasmid pTi15955 from *Agrobacterium tumefaciens* (Barker *et al.*, 1983)
Intervening sequence	10053	10280	228	Sequence from Ti plasmid C58 (Zambryski *et al.*, 1982; Wood *et al.*, 2001)
T-DNA Border A	10281	10304	24	Required for transfer of T-DNA insert from *Agrobacterium tumefaciens* into plant cells (Barker *et al.*, 1983)
Intervening sequence	10305	10323	19	Sequence from Ti plasmid C58 (Zambryski *et al.*, 1982; Wood *et al.*, 2001)
T-DNA Border A	10324	10347	24	Required for transfer of T-DNA insert from *Agrobacterium tumefaciens* into plant cells, aiming to prevent vector DNA being transferred into plant genome (Barker *et al.*, 1983)
Intervening sequence	10348	10634	287	Sequence from Ti plasmid pTi15955 (Barker *et al.*, 1983)
T-DNA Border A	10635	10658	24	Required for transfer of T-DNA insert from *Agrobacterium tumefaciens* into plant cells, aiming to prevent vector DNA being transferred into plant genome (Barker *et al.*, 1983)

b) Safety of the expressed protein

A thorough evaluation of the safety of the 2mEPSPS, 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 is considered to have a low risk of allergenic potential. Field expression of DAS-444Ø6-6 soybean (unsprayed or sprayed with 2,4-D, glufosinate, and/or glyphosate) ranged from 22.20 ng/mg dry weight in grain to 2323.94 ng/mg in V10-12 leaf tissue (2mEPSPS), 25.75 ng/mg dry weight in R3 stage root to 116.73 ng/mg in V10-12 leaf tissue (AAD-12) and 1.74 ng/mg dry weight in R3 stage root to 10.22 ng/mg in V10-12 leaf tissue (PAT). Expression values were similar for all 2mEPSPS, AAD-12 and PAT treatments irrespective of the herbicide regime.

DAS-444Ø6-6 soybean is substantially equivalent to conventional soybean, except for the introduced herbicide tolerance traits and is as safe and nutritious as conventional soybean. DAS-444Ø6-6 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 seed samples of DAS-444Ø6-6 soybean and non-GM control soybean, grown alongside, in replicated plots at the same field sites, were performed. Samples of soybean forage and seed 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-444Ø6-6 soybean. Evaluation of the nutrient composition data of DAS-444Ø6-6 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 2mEPSPS, AAD-12 and PAT proteins expressed in DAS-444Ø6-6 soybean was demonstrated in a number of ways:

- Bioinformatics analysis of the 2mEPSPS, 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 2mEPSPS, AAD-12 and PAT proteins was conducted in mice at levels of 5000 mg 2mEPSPS/kg, 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 studies.
- The thermal stability of the 2mEPSPS and AAD-12 proteins 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 2mEPSPS and AAD-12 proteins, reduce their immunoreactivity, and significantly diminish their 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 2mEPSPS and AAD-12 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 2mEPSPS, and AAD-12 proteins were readily digested (not detectable at 1 minute and 30 seconds respectively) in SGF. Finally, the 2mEPSPS and AAD-12 proteins were analyzed for evidence of glycosylation. No covalently-linked carbohydrates were detectable on the plant-derived or the microbe-derived proteins. 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-444Ø6-6 soybean grain, as any other soybean, not including the cultivation of DAS-444Ø6-6 hybrids, environmental release would be more likely to occur during import, storage and processing of DAS-444Ø6-6 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-444Ø6-6 soybean. Moreover, the information presented in the application established that DAS-444Ø6-6 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-444Ø6-6 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-444Ø6-6 soybean is substantially equivalent to conventional soybean, except for the introduced herbicide tolerance traits and is as safe and nutritious as conventional soybean. DAS-444Ø6-6 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-444Ø6-6 soybean is substantially equivalent to other soybean varieties except for its herbicide tolerance, which are traits of agronomic interest. Therefore no specific instructions are warranted or required for the storage and handling of DAS-444Ø6-6 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-444Ø6-6 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:

Director General
Department of Biosafety
Ministry of Natural Resources and Environment
Level 1, Podium 2, Wisma Sumber Asli
No. 25, Persiaran Perdana, Precinct 4
62574 Putrajaya, MALAYSIA
Email: biosafety@nre.gov.my
Fax: 03-88904935

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