CONSOLIDATED REPORT ON OF DOW AGRO SCIENCES LLC SOYBEAN DAS44406-6 DIRECT USE AS FOOD, FEED OR FOR PROCESSING (FFP)

EXECUTIVE SUMMARY

On January 20, 2017, Dow-Agro Sciences LLC's Soybean DAS44406-6 for direct use as food and feed, or for processing, as original application under the DOST-DA-DENR-DOH-DILG Joint Department Circular (JDC) No. 1 Series of 2016. After reviewing the Risk Assessment Report and attachments submitted by the applicant, the assessors namely: Scientific and Technical Review Panel (STRP), BPI Plant Products Safety Services Division (BPI-PPSSD) and Bureau of Animal Industry- Biotech Team (BAI-BT), concurred that corn soybean DAS44406-6 is as safe for human food and animal feed as its conventional counterpart.

The Department of Environment and Natural Resources – Biosafety Committee (DENR-BC), after a thorough scientific review and evaluation of the documents related to Environmental Risk along with the submitted sworn statement and accountability of the proponent, recommended the issuance of a biosafety permit for this regulated event provided the conditions set by DENR are complied. Also, the Department of Health – Biosafety Committee (DOH-BC), after a thorough scientific review and evaluation of documents related to Environmental Health Impact, concluded that soybean DAS44406-6 will not pose any significant risk to the health and environment and that any hazards could be managed by the measures set by the department. DOH-BC also recommended for the issuance of biosafety permit for soybean DAS44406-6.

Furthermore, the Socio-economic, Ethical and Cultural (SEC) Considerations expert also recommended for the issuance of biosafety permit for this regulated article after assessing the socio-economic, social and ethical indicators for the adoption of Genetically Modified Organisms.

BACKGROUND

In accordance with Article VII. Section 20 of the JDC, no regulated article, whether imported or developed domestically, shall be permitted for direct use as food and feed, or for processing, unless: (1) the Biosafety Permit for Direct Use has been issued by the BPI; (2) in the case of imported regulated article, the regulated article has been authorized for commercial distribution as food and feed in the country of origin; and (3) regardless of the intended use, the regulated article does not pose greater risks to biodiversity, human and animal health than its conventional counterpart.

The BPI Biotech Office provided the assessors the complete dossier submitted by Dow-Agro Sciences LLC. Upon receipt of the individual reports from the assessors, the BPI Biotech Secretariat prepared this consolidated risk assessment report for the information of the public.

A. <u>STRP, BPI-PPSSD, BAI (Safety Assessment)</u>

After thorough review of the technical documents submitted by the applicants, the assesors' findings were as follows:

1. Host Organism

Soybean (Glycine max L.) has been grown world-wide as an important staple food for humans and feed ingredient for animals. Its major products are seeds, oil, and meal. Unprocessed soybeans are not suitable for food and their use for animal feed remains limited because they contain anti nutritional factors such as trypsin inhibitors and lectins which are inactivated by heat processing. Humans consume soybean mostly in processed form such as soy milk, milk curd/ tofu, whole cooked seed, edible soy oil, soy protein concentrate, isolated soy protein, hydrolyzed vegetable protein, textured soy protein and soy protein fibers. It is also being consumed by animals in the form of seed, forage/silage, hay, meal and hulls (OECD, 2012).

Soybean is a source of key nutrients such as proteins, fat, ash, acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber (TDF), crude fiber, carbohydrates, amino acids, fatty acids, minerals and vitamins (OECD, 2012). Anti-nutrients such as stachyose, raffinose, oligosaccharides, trypsin inhibitors, lectins and phytic acid (ILSI, 2010).

History of safe use was attributed to soybean. Based on OECD report, soybeans are commonly consumed in processed form and primary source of oil and protein. Heat processing eliminates the anti-nutritional factors in soybean. Toxicants are not commonly found in soybean.

2. Transgenic Plant

DAS-44406-6 soybean has been reviewed and approved for food and/or feed use in many countries including Argentina (Food and Feed, 2015), Australia (Food, 2013), Brazil (Food and Feed, 2015), Canada (Food and Feed, 2013), Colombia (Food, 2016), Japan (Food, 2014; Feed, 2013), Mexico (Food, 2014), New Zealand (Food, 2013), South Africa (Food and Feed, 2013), Taiwan (Food, 2014), South Korea (Feed, 2014) and United States of America (2014).

Based on the documents provided by the developer, the consumption patterns by population subgroups are not expected to be altered. Analyses had identified that the only introduced trait that is being expressed in DAS-44406-06 was the tolerance to 2,4 D, glyphosate and glufosinate herbicides (Dow AgroSciences, 2016).

3. **Donor Organism**

Delftia acidovorans is the donor organism of aad-12 gene. History of safe use was attributed to D. acidovorans since it is being used in the transformation of ferulic acid into vanillin and related flavor metabolites (Yoon et al., 2005).

Zea mays is the donor organism for 2mepsps gene. History of safe use was attributed to Z. mays since it is widely being consumed as food and feed in several countries worldwide (OECD, 2002, Section I, pp. 12-18).

Streptomyces viridochromogenes is the donor organism of pat gene. History of safe use is being attributed to S. viridochromogenes since it is a common soil bacterium known to produce

tripeptide L-phosphinothricyl-L-alanyl-alanine (L-PPT) which was developed as a non-selective herbicide.

No food safety concern with regards to the other donor organism used in the transformation including Arabidopsis thaliana, Nicotiana tabacum, Agrobacterium tumefaciens, Cassava Vein Mosaic Virus and Helianthus annuus since the genetic elements derived from these organisms are not detected in DAS-44406-6. The developer provided sufficient information on the donor organisms which are not known to be toxic. History of safe use was attributed to all donor organisms.

4. Transformation System

The event, DAS-44406-6 was developed to express AAD-12 protein derived from *Delftia acidovorans*, 2mEPSPS protein derived from *Zea mays* L. and PAT protein derived from *Streptomyces viridochromogenes*. AAD-12 protein confers tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase (ACCase) inhibitors ("fop" herbicides). The 2mEPSPS and PAT proteins confer tolerance to glyphosate-containing herbicides and glufosinate ammonium-containing herbicides, respectively. The transformation method is through *Agrobacterium*-mediated transformation with plasmid vector pDAB8264. The plasmid vector, is composed of three (3) gene expression cassettes for *aad-12, 2mepsps* and *pat*.

The *2mepsps* gene expression cassette is composed of histone promoter H4A748 3' UTR from *Arabidopsis thaliana*, *2mepsps* gene from *Zea mays* L., optimized chloroplast transit peptide (Tpotp C) from maize and sunflower RuBisCO and intervening sequences.

The *aad-12* gene expression cassette is composed of histone promoter H4A748 from *Arabidopsis thaliana* including an intron from the Histone 3 gene from *A. thaliana*, promoter along with the 5' untranslated region and an intron from the *A. thaliana* polyubiquitin 10 (UBQ10) gene (AtUbi10), *aad-12* gene from *Delftia acidovorans*, 3' untranslated region (UTR) comprising the transcriptional terminator and polyadenylation site of open reading frame 23 (ORF23) of plasmid pTi15955 from *Agrobacterium tumefaciens* (AtuORF23 3' UTR) and intervening sequences.

The *pat* gene expression cassette is composed of CsVMV promoter from Cassava Vein Mosaic Virus, *pat* gene from *Streptomyces viridochromogenes*, 3' untranslated region (UTR) comprising the transcriptional terminator and polyadenylation site of open reading frame 1 (ORF1) of plasmid pTi15955 from *Agrobacterium tumefaciens* (AtuORF1 3' UTR) and intervening sequences.

5. Inserted DNA Genetic Stability

Southern blot analysis demonstrated that there is only one insertion site showing one intact copy of the t-DNA insert at a single locus. Southern blot analysis also showed that there are no specific hybridization bands detected in the negative control samples in any of the restriction enzyme and probe combinations. This indicates that the single insert in DAS-44406-6 soybean contains an intact single copy of each of the PTUs for 2mepsps, aad-12 and pat.

A 3-bp insertion at the 5' integration junction of the T-DNA insert in DAS-44406-6 was detected. There was also a deletion of 4383 bp identified from the Maverick genome in DAS-44406-6. For the observed 3'bp insertion, sequence analysis using BLASTn against the soybean scaffold sequence collection did not reveal any novel open reading frames (>=450 bp, 150 aa) spanning the junctions resulting from the T-DNA insertion. For the 4383 bp deletion, an open reading frame (ORF) of 666 bp was identified. Based on the dossier provided by the developer , BLASTp search of the ORF did not identified any significant homology to known proteins.

There are no any plasmid backbone sequences present as demonstrated by Southern Blot Analysis. Six probes covering nearly the entire backbone region of pDAB8264 are used to hybridize the Southern blots containing genomic DNA samples digested with MscI/EcoRI, HindIII and PstI/XhoI. The results showed that no hybridization bands were detected in any samples tested and confirming that no vector backbone sequences from pDAB8264 were integrated into DAS-44406-6 soybean.

6. Genetic Stability

The multigenerational stability of the introduced traits is assessed by Southern Blot Analysis of genetic samples from five generations (T2, T3, T4, T6 and F2) of DAS-44406-6 (Dow AgroSciences, 2016). Segregation is assessed by Event-Specific PCR. One population of F2 and three populations of BC1F2 are assessed. Chi-square analysis indicated that the segregation ratio of the plants with positive transgene insert versus negative transgene insert is consistent with the 3:1 segregation ratio characteristic of Mendelian inheritance pattern of a single dominant trait.

7. Expressed Material

AAD-12 protein has specific mode of action on 2,4-dichlorophenoxyacetic acid (2,4-D) and arylophenoxypropionate (AOPP) acetyl coenzyme A carboxylase (ACCase) inhibitors ("fop" herbicides). The protein have no metabolic role in plants (Dow AgroSciences, 2016).

The 2mEPSPS is an enzyme involved in the shikimic biosynthesis of aromatic amino acids and is present in plants, bacteria and fungi, but not in animals (Padgette et al., 1996). It catalyzes the reversible reaction of shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP) to produce EPSPS and inorganic phosphate (Pi) (Alibhai and Stallings, 2001). History of safe use has been attributed to *2mepsps* as it was being

used to induce glyphosate tolerance in other approved single events as listed in ISAAA GM ApprovalDatabase (2017).

PAT protein is involved in the acetylation of L-phosphinothricin, the active isomer of the glufosinate-ammonium herbicide, resulting in tolerance of transgenic plants to post-emergent application of the non-selective herbicide (Herouet et al., 2005).

The expression of novel protein in different plant parts is measured using ELISA methods. The measurements are in dry weight basis (ng/mg dry weight). The expression level of AAD-12, 2mEPSPS and PAT in grain is 27.3 ng/mg dry weight, 21.97 ng/mg dry weight and 2.12 ng/mg dry weight, respectively.

8. Toxicological and Allergenicity Assessment

The safety assessment of novel proteins, AAD-12, 2mEPSPS and PAT, includes digestibility, heat inactivation, oral toxicity and amino acid sequence comparison studies to determine its potential to cause toxicity or allergenicity to humans.

Digestibility study using Simulated Gastric Fluid (SGF) with pepsin demonstrated that AAD-12 and 2mEPSPS is readily degraded within 30 seconds and I minute of incubation with SGF, respectively, in presence of 0.32% w/v pepsin at pH 1.2, a characteristic of most non-toxic proteins. According to OECD (1999) PAT proteins are rapidly digested n SGF and is readily denatured by heat.

Heat stability of AAD-12 and 2mEPSPS was evaluated by monitoring the change in protein bands in SDS-PAGE of the protein solutions heated for 30 minutes at 4 °C, 50°C, 70°C and 95°C for AAD-12 and at 25 °C, 37°C, 55°C, 75°C and 95°C for 2mEPSPS (Schafer, 2012; Embrey, 2012). Results of the SDS-PAGE analysis indicated no change in protein bands on all heated proteins at 30 minutes incubation.

Heat inactivation of AAD-12 and 2mEPSPS was evaluated through monitoring the enzymatic activity and immunoreactivity of the proteins subjected to heat for 30 minutes at the assigned temperature variants (Schafer, 2012; Embrey, 2012). For AAD-12, enzymatic activity was determined through monitoring the phenol production resulted from the conversion of Dichlorprop (2-(2,4-dichlorophenoxy) propanoic acid to 2,4-dichlorophenol (DCP). For 2mEPSPS enzymatic activity was determined through determining the presence or absence of inorganic phosphate.

Immunoreactivity of the two (2) proteins was determined through ELISA using an immobilized anti-AAD-12 and anti-2mEPSPS polyclonal antibodies. Complete loss of enzymatic activity and immunoreactivity was observed in AAD-12 upon subject to temepratures at 50°C, 70°C and 95°C for 30 minutes. At 55°C, 90% loss in immunoreactivity was observed for 2mEPSPS.

BLASTp search algorithm against the GenBank showed that AAD-12, 2mEPSPS and PAT has no biologically relevant identities to toxic proteins.

Amino acid sequence comparison of AAD-1, 2mEPSPS and PAT protein to toxins and allergens was conducted using BLASTp search algorithm against the GenBank and FASTA program (Dow AgroSciences, 2016). Results of bioinformatics analyses indicated that AAD-1 protein is not homologous to any toxin and allergen. Bioinformatics tools and comparison to FARRP Allergen Database Version 12 indicates that the AAD-12, 2mEPSPS and PAT has no amino acid sequence similarity to known allergens.

An acute oral gavage studies for AAD-12 and 2mEPSPS were included in the dossier indicating that the No Observed Effect Level of AAD-12 and 2mEPSPS is > 2000 mg/kg body weight and >5000 mg/kg body weight, respectively. However, the actual data of the studies were not provided by the developer. Weight of evidences approach indicates that AAD-12 and 2mEPSPS are not likely to cause toxicity to humans and animals. Hence, the data on acute oral gavage study are no longer required.

The safety of PAT by Acute oral gavage was already assessed. No mortality is observed and there are no observable adverse or non-adverse effects in male and female treated mice at 5000 mg/kg body weight (OECD, 1999).

The AAD-12 and 2mEPSPS proteins used for the toxicological studies were obtained from *Pseudomonas* and were found biochemically and functionally equivalent to AAD-12 and 2mEPSPS expressed in DAS-44406-6 soybean. PAT protein used for toxicological study was obtained from *Streptomyces viridochromogenes* and is 100% identical in amino acid sequence to the PAT protein expressed in several transgenic plants that has been previously deregulated.

The novel proteins are expressed independently of each other. List of genetic elements provided by the developer indicated that the three novel proteins are being regulated by different promoters. They are expressed in same plant tissues as indicated in the specific ELISA method of determining the level of expression of the proteins in different plant parts. They do not interact to express the phenotype.

The percent of total protein of AAD-12, 2mEPSPS and PAT is estimated to be <0.003%, <0.002% and <0.0002%, respectively.

Results of the toxicological and allergenicity assessment indicate that AAD-1, 2mEPSPS and PAT protein being expressed in DAS-44406-6 soybean are not toxic or allergenic to humans.

9. Nutritional Data

Compositional analysis provided by the developer indicating the nutritional data of DAS-44406-6 in comparison with the non-transgenic soybean (Maverick), commercial varieties and range of literature values. The trials were conducted at ten sites located in Georgia, Iowa, Illinois, Indiana, Michigan, Missouri and Nebraska at same environmental conditions. Results of the analysis indicated that there is no differences in the proximate, fiber, mineral, amino acid, fatty acid, vitamins, anti-nutrient and secondary metabolite levels of DAS-44406-6 and the non-transgenic soybean that can be considered biologically relevant.

10. Recommendation

For the transgenic DAS-44406-6 soybean, enough evidence is provided to support the equivalence of the genetically modified crop, in terms of the nutritional composition and food safety, with the conventional soybean other than tolerance to 2,4-dichlorophenoxyacetic acid- (2,4-D), glyphosate- and glufosinate ammonium-containing herbicides. After reviewing the provided material of the applicant, it is therefore concluded that DAS-44406-6 soybean is as safe as its conventional counterpart.

B. DENR Biosafety Committee (Environmental Safety)

Upon extensive review and evaluation of the application, including scientific evidences from provided references, literature and other related studies, as well as the submitted sworn statement and accountability, the DENR-BC accepts that the direct use of the regulated article does not pose any harm to the environment and that the data provided supports the conclusion that the regulated articles is as safe as its conventional counterpart. The DENR-BC has determined that the the protein produced will immediately degrade upon exposure to the natural environment and that the protein product will not increase the weediness potential of the transgenic crop.

Upon evaluation of the submitted Project Description Report, the committee notes that the chances of unintended release or planting of the regulated article is very minimal and will not cause any damaging and lasting effects to the environment.

The DENR-BC hereby recommends the approval of the this application.

C. DOH Biosafety Committee (Environmental Health Safety)

After a thorough scientific review and evaluation of the documents, with the submitted sworn statement and accountability of the applicant, the DOH-BC finds sufficient evidence that the regulated article applied for direct use will not pose any significant risk to health and the environment and that any hazards could be managed by the measures set by DOH. The regulated article does not require changes in the usual practices in unloading, loading, hauling, transport, storage and processing

D. <u>SEC Expert</u>

The applicant has provided sufficient answers to the queries made by the SEC Expert in relation to their initial responses. It is not expected that the regulated article will affect local consumption patterns. The SEC Expert recommends that approval and issuance of biosafety permits for direct use as food, feed and for processing of Soybean DAS 44406-6.