Consolidated Technical Report of Dow Agrosciences' Soybean DAS68416-4 Application for Direct Use as Food, Feed or for Processing (FFP)

EXECUTIVE SUMMARY

On January 20, 2017, Dow AgroSciences B.V. - Philippine Branch submitted soybean DAS68416-4 application for direct use as food and feed, or for processing to the Bureau of Plant Industry (BPI) 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 (BAI), concurred that soybean DAS68416 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 that the conditions set by them 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 DAS68416-4 will not pose any significant risk to 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 DAS68416-4

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 AgroSciences B.V. - Philippine Branch.

Below is the summary of the evaluation conducted by the STRP and regulatory agencies.

A. STRP, PPSSD, BAI ASSESSMENT

After thorough review of the technical documents submitted by the applicant, the assessors' findings are as follows:

A. Host Organism

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).

On the other hand, it was reported that the most common anti-nutritional factors present in raw soybean or meal are the trypsin inhibitors. In the process of heating or roasting of the beans, the activity of the trypsin inhibitors are destroyed. Other common anti-nutrient factors present in soybeans are lectins, stachyose and raffinose oligosaccharides, and phytic acid. Soybean was also reported to be a common source of allergens. The allergenic effect is attributed to the globulin fraction of soybean proteins that comprise about 85% of total protein.

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. There have been no identified toxicants in soybean.

B. Transgenic Plant

DAS-68416-4 soybean has been reviewed and approved for food and/or feed use in many countries including Australia (Food, 2011), Brazil (Food and Feed, 2015), Canada (Food and Feed, 2012), Colombia (Food, 2016), Japan (Food and Feed, 2013), Mexico (Food, 2012), New Zealand (Food, 2011), Taiwan (Food, 2013), South Korea (Feed, 2014) and United States of America (Food and Feed, 2011).

Studies conducted relevant to the safety and nutritional component of the transgenic soybean lead to conclude that DAS-68416-4 is as safe as its conventional counterpart. Being so, the consumption pattern by people is not expected to be changed or altered.

C. 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 (DAS, 2016).

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 (DAS, 2016).

Delftia acidovorans and *Streptomyces viridochromogenes* are both not known to be toxic or allergenic.

D. Transformation System

DAS-68416-4 soybean was generated via Agrobacterium-mediated transformation. The T-DNA insert in the plasmid contains a synthetic, plantoptimized sequence of the *aad-12* gene from *Delftia acidovorans* and the *pat* gene from *Streptomyces viridochromogenes.*

The genetic modification was intended to express AAD-12 and PAT in soybean plants, thus provide tolerance to 2,4-D and glufosinate ammonium

herbicides. PAT was also used as a selectable marker during DAS-68416-4 soybean development.

No carrier DNA sequences known to affect gene expression are present in the T-DNA region of pDAB4468 used to transform event DAS-68416-4.

E. Inserted DNA

The molecular characterization of the event DAS-68416-4 was conducted by Southern blot analysis. The results clearly demonstrated that the transgene insert occurred as a simple integration of the T-DNA insert from plasmid pDAB4468, including a single intact copy of the aad-12 and pat expression cassettes.

The characterization of T-DNA insertion site revealed a 55bp deletion from the original locus and a 9bp insertion at 3' integration junction of DAS-68416-4. This was demonstrated through cloning the genomic fragment from the non-trasgenic soybean genome corresponding to the region of the identified flanking border sequence (Poorbaugh et al, 2009).

The T-DNA insert sequence, border sequence, and parental locus sequence were searched for potential putative reading frames using highly conservative criteria. All putative reading frames (stop to stop, greater than 8 amino acids) were then searched against databases for sequence similarity to known allergens or protein toxins. Bioinformatic results showed that putative reading frames did not have sequence similarity with known allergens and toxins.

F. Genetic Stability

The multigenerational stability of the introduced traits is assessed by Southern Blot Analysis of genetic samples from four generations (T2, T3, T4 and T5) of DAS-68416-4 soybean 4 (DAS, 2016). Results showed that the hybridization bands specific to the DAS-68416-4 insert were identical in lanes containing DNA from soybeans grown from 4 generations. No extraneous fragments of DAS-68416-4 insert were observed. These showed the stability of the DAS-68416-4 insert inherited from one generation to the next (DAS, 2016).

Single Segregating Generation (F2) was tested for the presence of aad-12 and pat genes. F2 generations was generated from crossing T4 plants with conventional soybean line. F1 plants was self pollinated to produce F2 seeds. Southern Blot Analysis using Nco I restriction enzyme and aad-12 and pat probes was used to determine the genetic equivalence of the inserted DNA among the same F2 individual plants. Through southern blot analysis and protein expression testing, the expression of aad-12 and pat genes in F2 generation of DAS-68416-4 were analyzed. This was used to determine the segregation ratios of aad-12 and pat. Chi Square Analysis of these segregation data was performed to determine if the ratio of aad-12 and pat genes follows the Mendelian Law of Segregation.

G. Expressed Material

The expression levels of AAD-12 protein from the different tissues of the plants collected from DAS-68416-4 are summarized in Table 1 while the

summary of PAT protein levels from various parts collected from DAS-68416-4 are summarized in Table 2.

Table 1. Summary of AAD-12 protein levels in tissues collected from DAS-68416-4 produced in the U.S. and Canada during 2008

		AAD-12 ng/mg Tissue Dry Weight		
Tissue	**Treatment**	**Mean**	**Std. Dev.**	**Range**
V5 Leaf	DAS-68416-4 Unsprayed	51.42	25.22	26.37 - 97.66
	DAS-68416-4 + Glufosinate	50.63	23.69	28.03 - 94.00
	DAS-68416-4 + 2,4-D	51.68	25.41	27.16 - 100.79
	DAS-68416-4 + Glufosinate and 2,4-D	66.08	37.82	25.14 - 164.58
V10 Leaf	DAS-68416-4 Unsprayed	53.95	20.85	29.83 - 90.89
	DAS-68416-4 + Glufosinate	56.06	21.95	25.06 - 91.95
	DAS-68416-4 + 2,4-D	55.24	20.62	30.84 - 91.80
	DAS-68416-4 + Glufosinate and 2,4-D	57.07	22.97	32.02 - 95.16
Root	DAS-68416-4 Unsprayed	17.10	5.68	8.80 - 27.62
	DAS-68416-4 + Glufosinate	15.48	4.58	6.30 - 23.08
	DAS-68416-4 + 2,4-D	16.01	6.64	3.16 - 27.91
	DAS-68416-4 + Glufosinate and 2,4-D	16.66	6.81	1.84 - 26.50
Forage	DAS-68416-4 Unsprayed	41.11	25.72	5.70 - 91.17
	DAS-68416-4 + Glufosinate	39.35	24.47	5.49 - 87.96
	DAS-68416-4 + 2,4-D	40.56	25.58	5.02 - 88.02
	DAS-68416-4 + Glufosinate and 2,4-D	39.65	22.41	4.96 - 69.62
Grain	DAS-68416-4 Unsprayed	16.47	3.55	9.40 - 21.86
	DAS-68416-4 + Glufosinate	16.94	3.15	11.9 - 22.74
	DAS-68416-4 + 2,4-D	16.47	3.78	9.71 - 21.95
	DAS-68416-4 + Glufosinate and 2,4-D	16.21	3.62	9.91 - 23.40

Table 2. Summary of PAT protein levels in tissues collected from DAS-68416-4 produced in the U.S. and Canada during 2008.

		PAT ng/mg Tissue Dry Weight		
Tissue	**Treatment**	**Mean**	**Std. Dev.**	**Range**
V5 Leaf	DAS-68416-4 Unsprayed	9.17	2.99	4.33 - 13.75
	DAS-68416-4 + Glufosinate	9.83	2.66	3.67 - 13.78
	DAS-68416-4 + 2,4-D	9.01	3.03	4.87 - 13.92
	DAS-68416-4 + Glufosinate and 2,4-D	10.05	3.76	3.00 - 15.03
V10 Leaf	DAS-68416-4 Unsprayed	10.94	1.31	8.43 - 13.35
	DAS-68416-4 + Glufosinate	11.51	1.69	9.08 - 14.44
	DAS-68416-4 + 2,4-D	11.76	2.02	7.49 - 14.81
	DAS-68416-4 + Glufosinate and 2,4-D	11.58	1.45	9.26 - 14.15
Root	DAS-68416-4 Unsprayed	1.73	0.51	0.47 - 2.84
	DAS-68416-4 + Glufosinate	1.92	0.45	1.01 - 2.67
	DAS-68416-4 + 2,4-D	1.73	0.68	0.42 - 2.83
	DAS-68416-4 + Glufosinate and 2,4-D	1.93	0.55	0.36 - 2.68
Forage	DAS-68416-4 Unsprayed	3.63	2.88	0.06 - 12.54
	DAS-68416-4 + Glufosinate	4.81	3.75	0.40 - 12.10
	DAS-68416-4 + 2,4-D	5.28	4.20	0.12 - 12.13
	DAS-68416-4 + Glufosinate and 2,4-D	4.73	3.63	0.45 - 12.35
Grain	DAS-68416-4 Unsprayed	2.73	0.34	1.96 - 3.37
	DAS-68416-4 + Glufosinate	2.74	0.28	2.29 - 3.39
	DAS-68416-4 + 2,4-D	2.79	0.26	2.21 - 3.13
	DAS-68416-4 + Glufosinate and 2,4-D	2.82	0.23	2.43 - 3.25

H. Toxicological Assessment and Allergenicity Assessment

The safety assessment of novel proteins, AAD-12 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 (DAS, 2016).

Digestibility study using Simulated Gastric Fluid (SGF) with pepsin demonstrated that AAD-12 is readily degraded within 30 seconds of incubation with SGF.

Heat stability of AAD-12 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 (Schafer, 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 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). Enzymatic activity of AAD-12 was determined through monitoring the phenol production resulted from the conversion of Dichlorprop (2-(2,4-dichlorophenoxy) propanoic acid to 2,4-dichlorophenol (DCP). Immunoreactivity was determined through ELISA using an immobilized anti-AAD-12 polyclonal antibody. 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.

Heat stability of PAT protein has been evaluated in literatures including Hérouet et al., (2005). According to the study mentioned in the dossier provided by the developer, the PAT protein encoded by bar and [pat genes remained detectable by SDS-PAGE upon incubation at temperatures up to 90^0C for 60 minutes. However, Wehrmann et al (1996) indicated that protein degradation does not directly correlates to the thermo-inactivity of the protein. Their study showed that PAT protein was completely thermo-inactivated after 10 minutes of incubation at 55^0C and higher temperatures. This was despite of the fact that the protein was not degraded.

BLASTp search algorithm against the GenBank non-redundant protein database showed that PAT has no biologically relevant identities to toxic proteins (DAS, 2016).

Amino acid sequence comparison of AAD-1 and PAT protein to toxins and allergens was conducted using BLASTp search algorithm against the GenBank and FASTA program (DAS, 2016). Results of bioinformatics analyses indicated that AAD-12 in DAS-68416-4 soybean has significant homologies with few major proteins with enzyme activity (DAS, 2016). Significant homologies were found on taurine dioxegenases that degrade taurine, clavaminic acid synthetases or “CAS-like”, tolC proteins which are known efflux pumps, a (S)-2-(2,4-dichlorophenoxy)propionate, 2-oxoglutarate dioxygenase, a pvcB protein which is a known “CAS-like” protein, an inosine-uridine preferring nucleoside hydrolase and a hypothetical protein with no functional annotation. The percentage sequence similarities ranges from 20 to 100%. According to the analysis, none of these proteins showed any safety concerns with regards to the expression of AAD-12 in plants (DAS, 2016).

Bioinformatics tools and comparison to FARRP Allergen Database Version 12 indicates that the AAD-12 and PAT has no amino acid sequence similarity to known allergens (DAS, 2016).

An acute oral gavage study for AAD-12 were included in the dossier indicating that the No Observed Effect Level of AAD-12 is > 2000 mg/kg body weight. Hérouet et al., (2005) indicated that there was no evidence of acute toxicity for the PAT protein when administered intravenously to mice up to 10mg/kg body weight confirming that the PAT proteins encoded by pat or bar gene are not acutely toxic.

The AAD-12 protein used for the toxicological studies were obtained from Pseudomonas fluorescens and were found biochemically and functionally equivalent to AAD-12 expressed in DAS-68416-4 soybean (DAS, 2016). For PAT protein, no structural and functional equivalency study specific for DAS-68416-4 was provided by the developer since information regarding the safety of PAT protein was from published references such as Hérouet et al., (2005), OECD (1999), etc.

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 and PAT is estimated to be <0.00004% and <0.000007%, respectively.

Results of the toxicological and allergenicity assessment indicate that AAD-1 and PAT protein being expressed in DAS-68416-4 soybean are not toxic or allergenic to humans (DAS, 2016).

I. Nutritional Data

Compositional Analysis of Soybean Forage

An analysis of the protein, fat, ash, moisture, carbohydrate, acid detergent fiber (ADF), neutral detergent fiber (NDF), calcium and phosphorus in soybean forage samples from the control, unsprayed AAD-12, AAD-12 + glufosinate, AAD-12 + 2,4-D and AAD-12 + both herbicides was performed.

the proximate levels in DAS-68416-4 forage are not significantly different from the proximate levels in the non-transgenic soybean forage except for fat content which was significantly higher than the non-transgenic control. Though the fat content was significantly higher, the means still fell well within the comparative literature ranges, and therefore the differences are not considered biologically relevant.

Compositional Analysis of Soybean Grain

Analysis of proximate, fiber, minerals, fatty acids, amino acids, vitamins, isoflavones, and antinutrients in soybean grain samples from the control, unsprayed AAD-12, AAD-12 + glufosinate, AAD-12 + 2,4-D and AAD-12 + both herbicides was performed.

Results of the analysis indicated that based on these compositional constituents, the grain from DAS-68416-4 soybean was substantially equivalent to that of non-transgenic soybean.

The assessors find scientific evidence that the regulated article applied for human food and animal feed use is as safe as its conventional counterpart and shall not pose greater risk to human and animal health

B. <u>DENR BC (for Safety of Event to the Environment)</u>

After a comprehensive review and evaluation of the documents including the scientific evidences from provided references and literature submitted by Dow AgroSciences B.V. - Philippine Branch on its application for Direct Use as FFP of **soybean DAS68416-4, h**ereunder are the observations and recommendation:

1. Upon extensive review and evaluation of the application submitted by the proponent, including the scientific evidences from provided references, literature, and other related studies, the Committee accepts that the direct use of the regulated article whether for food feed and for processing will not cause any significant adverse effect on the environment (land, air and soil) and non-target organisms, to wit:
   a) Before planting, the genetic stability in the transgenic crop is ensured such that no unintended horizontal gene transfer shall occur to unrelated species.
   b) The protein product produced by the transgenic crop will immediately degrade upon exposure to the natural environment.
   c) Characterization of the inserted gene has shown that the protein product will not increase the weediness potential of the transgenic crop.

   The data evaluated support the conclusion that the regulated article is as safe as its conventional counterpart.

2. The project description report (PDR) discusses the specified environmental management plan indicating the possible risk and harm to the environment and non-target organisms as well as the mitigating measures and contingency plan of the proponent. Upon evaluation of the submitted PDR, 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.

3. The Committee would like to suggest that the Bureau of Plant Industry (BPI) ensure the proper and secure packaging of the regulated article for transport and the safety and durability of the transport vehicle, for prevention of any possible spillage or unintended release during transport/import as per BPI's inspection in the port area.

4. Based on the above considerations and with the submitted sworn statement and accountability of the proponent, we hereby submit our recommendation relative to the biosafety permit application of Dow AgroSciences B.V. - Philippine Branch for direct use as foo4 feed or processing of Soybean DAS 68416-4

C. <u>DOH-BC (for Environmental Health Safety)</u>

After a thorough review and evaluation of the documents provided by the proponent, Dow AgroSciences B.V. - Philippine Branch through the Bureau of Plant Industry (BPI), in support of their application for approval for Direct Use for Food and Feed or for Processing (FFP) of Soybean DAS68416-4. The DOH-BC found that the regulated article applied for Direct Use for Food and Feed or for Processing (FFP) is safe as its conventional counterpart and shall not pose any significant risk to human and animal health, and environment. The following are the observations and recommendations:

1. Find that the regulated applied for Direct Use for FFP does not require changes in the usual practices in unloading and loading, hauling, transport and storage, and processing. As such, the regulated article is as safe as its conventional counterpart and is not expected to pose any significant risk to human and animal and the environment while in transit, storage and processing.
2. Scientific pieces of evidence from provided references i.e. literatures show that the regulated article applied for Direct Use as FFP is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health and on the environment.
3. It is suggested that the BPI ensure the following:
    a) Strict monitoring of the regulated article from port of entry to the traders/importers storage/warehouse as stated in Sec 32 of JDC 1 s2016
    b) The BPI to include in the issuance of permit for release of this product the following conditions:
        i. Any spillage (during unloading and loading/hauling and transport unloading and storage) shall be collected and cleaned up immediately.
        ii. Transportation of the consignment from the port of entry to any destination shall be in closed containers.
        iii. There shall be a clear instructions that the product is only for the purpose of direct use for ffp and is not be used as planting materials.

    Based on the above considerations and with the submitted sworn statement and accountability of the proponent, this recommendation is being submitted to the BPI related to the processing and issuance of a biosafety permit for Direct Use as FFP of Soybean DAS68416-4.

D. <u>SEC Expert (for Socio-economic, ethical and cultural Consideration)</u>

According to the SEC expert, it cannot be denied, through the data presented by the applicant, that the country needs to import GM soybean at this point in time given the fact that from 2014 to 2016, soybean imports on the average, constituted 99.36 percent of the country's soybean supply. However, it should not discount the fact that there are still equally important issues (i.e. health and environmental impact of GM products, proper labeling, etc.) that need to be addressed in the long run with regards to importation of soybean especially the GM ones.

The SEC expert recommended for the approval and issuance of biosafety permit of the said GM product.