ASSESSORS' CONSOLIDATED REPORT ON DOW AGROSCIENCES' APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF SOYBEAN DAS 81419-2

EXECUTIVE SUMMARY

On January 20, 2017, Dow AgroSciences BV Philippine Branch submitted soybean DAS 81419-2 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 soybean DAS 81419-2 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 DAS 81419-2 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 DAS 81419-2.

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, except for the SEC expert, the complete dossier submitted by Dow AgroSciences. The SEC expert, on the other hand, was provided with a questionnaire on socio-economic, ethical and cultural considerations that have been addressed by Dow AgroSciences in relation to their application.

Upon receipt of the individual reports from the assessors, the BPI Biotech staff prepared this consolidated risk assessment report for the information of the public.

STRP ASSESSMENT AND RECOMMENDATIONS

Based on the documents submitted by the applicant:

A. Host Organism

Soybean is the main source of key nutrients in feed/food of monogastric animals; especially important are the essential amino acids and the unsaturated fatty acids necessary for growth, development and maintenance of the body. It also contains antinutrients which are heat-inactivated during processing (soybean is not consumed raw by humans)

While NO is given as an answer for presence of toxicants, it should be noted that in Toxicology, "Only the dose determines the toxicity. An example of this is selenium which is listed in Table 20. Composition Analytes on page 129 of 174.Selenium serves as an antioxidant at low levels but could also be toxic when taken at high dosage. Food allergens have also been noted in soybean which may cause IgE-mediated reaction.

Data on the use of the consumed food products, both raw and processed soybean have been adequately provided. Countries that use the transgenic soybean plant as food were enumerated: these are the US, Australia, and New Zealand. An adequate list of use of soybean for feed is also given.

Data was provided on countries having the highest consumption of soybean foods (Thailand, immature seeds; Japan, dry beans; USA, soybean oil) and the consumption of the general population and children in particular. The data was based on global data published by the World Health Organization

B. Donor Organism

All potentially inserted protein encoding (or antisense) sequences have been described adequately with respect to source and potential pathogenic and allergenic properties. Source organisms of the regulatory proteins were described. These are Arabidopsis thaliana, Agrobacterium tumefaciens and Cassava vein mosaic virus. Functions of these regulatory elements were described, including their placement in the T-DNA insert and plasmid. No reported pathogenicity or allergenicity of Cry1Ac, Cry1F and PAT.

C. Transformation System

The transgenic soybean was generated through Agrobacterium-mediated transformation of soybean cotyledonary node explants. The step by step procedure used was thoroughly presented. The target of genetic modification was the nuclear DNA. This was thoroughly and adequately presented and complete experimental protocol was provided.

Table of genetic elements of the T-DNA insert and plasmid pDAB9582 was given together with names of elements, position in the plasmid, length of gene and description of the genetic element. No carrier DNA was used for the transformation of pDAB9582 into soybean Glycine max. The Agrobacterium tumefaciens strain EMA 101 carries the helper plasmid, pT1Bo 542. EMA 101 was generated by the inactivation of the T-DNA One genes in strain A281(Hood et al.,1986).

D. Inserted DNA

All data obtained indicate that there is a single insertion of the T-DNA containing all the expected elements from pDAB9582 in DAS-81419-2 soybean genome.

This site was demonstrated by Southern Blot Analysis. Results showed that the transgenic insert in DAS-81419-2 soybean occurred as a single integration of the T-DNA insert. The step by step

procedure sufficiently demonstrated and confirmed that this transgenic soybean contains a single insertion of the TDNA from pDAB9582.

Southern blot analysis of restricted DNA of DAS-81419-2 probed with 6 probes specific for backbone sequences showed absence of plasmid backbone in DAS-81419-2.

E. Genetic Stability

Southern Blot Analysis confirmed that the DAS-81419-2 soybean contains a single insertion of the T-DNA from pDAB9582. Identical hybridization patterns were observed across 5 distinct generations of DAS-81419-2 soybean, indicating stable inheritance of the transgene insert across multiple generations. This was clearly demonstrated.

Segregation analysis of F2 and BC1F2 (segregating) populations on individual plants showed the Mendelian inheritance ration (3:1) for a single independent insert.

F. Expressed Material

The Cry1Ac in DAS-81419-2 soybean were measured in plant tissue samples which include the leaf, grain, root and forage. The Cry1Ac is mainly found in the leaves.The Cry1Ac protein concentrations expressed by the different parts of the plant were measured using ELISA (enzyme-linked immunosorbent assay). ELISA is a very sensitive (meaning, could detect very minute amounts like ng/mg) and thus, appropriate method of measure.

The Cry1F protein concentrations expressed by the different parts of the plant were likewise measured using ELISA.The Cry1F concentrations as measured using ELISA showed high amounts in the leaves.

ELISA was also used to measure the PAT contents of the plant tissues. PAT was found to be high in the leaves and the forage.

G. Toxicological Assessment

Cry1Ac protein was readily degraded in SGF in <1min. This protein digestion is very fast. Result showed that there were no large size fragments that remained after <1 minute exposure to SGF. Cry1Ac protein was also found to be denatured readily by heat (<1minute) and lost its immunoreactivity. Cry1Ac protein lost >99% of its immunoreactivity with results showing that it was almost undetectable by ELISA after exposure to heat treatment. This isa very clear demonstration of how fast heat affects this protein.

Amino Acid Comparison of Cry1Ac with toxin homology was carried out. Amino acid homologies with the Cry1Ac protein sequence were evaluated using BLASTp search algorithm against the GenBank non-redundant data set. None of the proteins returned by the BLASTp search are associated with protein toxins that are harmful to humans or animals. Results showed that Cry1Ac protein does not share any biologically meaningful amino acid sequence similarities with known toxic proteins.

Oral toxicity studies were performed in mice. At the end of the two-week observation period, all mice survived and no gross pathological lesions were noted in any animalused in thestudy. Under the conditions of this study, the acute oral LD50 of Cry1Ac was greater than the 700mg/lg dose given.In livestock, adequate margin of safety have been noted (>2000mg/kg). Data from molecular

weight, response to specific antibodies, and peptide mass fingerprinting showed equivalency of Cry1Ac in soybean DAS 81419-2 to Pseudomonas fluorescens.

Meanwhile, the digestibility test of Cry1F was done using the Simulated Gastric Fluid (SGF). Digestibility of microbial Cry1F protein, equivalent to Cry1F protein expressed in DAS-81419-2 soybean was tested in vitro using the simulated gastric fluid. Digestion of Cry1F protein was noted to be <1 min. This experiment shows the very fast degradation ofCry1F protein. No large size fragments remained after digesting in SGF for various periods of time. In fact, the Cry1F protein was readily digested in <1 minute.

The Cry1F protein was also readily denatured and lost its immunoreactivity properties. Results showed that it was almost undetectable by ELISA after exposure to the heat treatment. Amino Acid Comparison of Cry1F with toxin homology was carried out. Amino acid homologies with Cry1F protein sequence were evaluated using BLASTp search algorithm against the GenBank non-redundant protein dataset. Results of the search showed that the Cry1F protein does not share any biologically meaningful amino acid sequence similarities with known toxic proteins that are harmful to humans or animals.

Acute Oral Gavage test was done in mice. At the end of the two-week observation period, no treatment-related clinical signs were noted. No gross pathologic lesions were observed in any of the animals used. The acute oral LD50 of Cry1F protein was reportedly greater than 600mg/kg (dosage given in this study).

Data from previous experiments/studies has shown that PAT protein is rapidly degraded in SGF, and by heat. Safety of PAT has been repeatedly demonstrated. Results also showed that PAT protein was readily denatured by heat. ELISA was used to determine the protein denaturation of PAT.

PAT protein does not share any biologically meaningful amino acid sequence similarities with known toxic proteins that are harmful to humans/animals. Homologies of protein sequences were evaluated using BLASTp search algorithm against the GenBank non-redundant data set.No homologies were found

Acute oral gavage studies on PAT protein have been carried out. Yes, oral gavage studies of PAT protein was done in mice. There was no evidence of acute toxicity in mice at a dose of 5000mg PAT protein/kg body weight. A dietary exposure assessment reveals large margins of exposure (MOE) values for PAT protein in DAS-81419-2 soybean, indicating no concern for adverse effects from protein in soybean based on the available safety threshold information.

H. Allergenicity Assessment

Cry1Ac protein was readily degraded in SGF in <1min. This protein digestion is very fast. Result showed that there were no large size fragments that remained after <1 minute exposure to SGF. Cry1Ac protein was also found to be denatured readily by heat (<1minute) and lost its immunoreactivity. Cry1Ac protein lost >99% of its immunoreactivity with results showing that it was almost undetectable by ELISA after exposure to heat treatment. This isa very clear demonstration of how fast heat affects this protein.

Amino Acid Comparison of Cry1Ac with toxin homology was carried out. Amino acid homologies with the Cry1Ac protein sequence were evaluated using BLASTp search algorithm against the GenBank non-redundant data set. None of the proteins returned by the BLASTp search are associated with protein toxins that are harmful to humans or animals. Results showed that Cry1Ac

protein does not share any biologically meaningful amino acid sequence similarities with known toxic proteins.

Meanwhile, the digestibility test of Cry1F was done using the Simulated Gastric Fluid (SGF). Digestibility of microbial Cry1F protein, equivalent to Cry1F protein expressed in DAS-81419-2 soybean was tested in vitro using the simulated gastric fluid. Digestion of Cry1F protein was noted to be <1 min. This experiment shows the very fast degradation ofCry1F protein. No large size fragments remained after digesting in SGF for various periods of time. In fact, the Cry1F protein was readily digested in <1 minute.

The Cry1F protein was also readily denatured and lost its immunoreactivity properties. Results showed that it was almost undetectable by ELISA after exposure to the heat treatment. Amino Acid Comparison of Cry1F with toxin homology was carried out. Amino acid homologies with Cry1F protein sequence were evaluated using BLASTp search algorithm against the GenBank non-redundant protein dataset. Results of the search showed that the Cry1F protein does not share any biologically meaningful amino acid sequence similarities with known toxic proteins that are harmful to humans or animals.

Data from previous experiments/studies has shown that PAT protein is rapidly degraded in SGF, and by heat. Safety of PAT has been repeatedly demonstrated. Results also showed that PAT protein was readily denatured by heat. ELISA was used to determine the protein denaturation of PAT.

PAT protein does not share any biologically meaningful amino acid sequence similarities with known toxic proteins that are harmful to humans/animals. Homologies of protein sequences were evaluated using BLASTp search algorithm against the GenBank non-redundant data set.No homologies were found

I. Nutritional Data

There were no significant differences noted when comparison with SE comparator was done. Results from this study demonstrate compositional equivalence between event DAS-81419-2 soybean and non-transgenic soybean. There were no statistical differences noted and, thus, not biologically relevant. 16:0 palmitic, 18:3 linolenic, and 20:1 eicosenoic were shown to significantly differ in DAS 81419-2 and the nontransgenic control. However the values for both DAS 81419-2 and the nontransgenic control was were within literature ranges and/or within the range of the reference/commercial varieties.

Statistical analysis was not performed on some fatty acids because greater than 50% of the samples were found to be below the limit of quantification (LOQ). Some differenceswere significant (in 16:0 palmitic, 18:3 linolenic, and 20:1 eicosenoic), however, these werevery small relative to natural variation and, thus,not biologically meaningful as all results are within literature ranges and within the range of the reference varieties included in this study.

Lectin composition of DAS 81419-2, nontransgenic control, and reference varieties did not differ significantly. Data shows that the varieties tested did not differ in raffinose concentration, stachyose concentration, TRYPSIN inhibitor concentrations, DAIDZEN levels, GENISTEIN levels hence no need for processing studies. Glycitein values fell within the range as published in the literature, and those obtained for the reference/commercial varieties used in the study.

J. Recommendation

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 any significant risk to human and animal health

BPI-PPSSD ASSESSMENT AND RECOMMENDATION

Soybean DAS-81419-2 was developed by Dow AgroSciences B.V., through the use of recombinant DNA technology. The said event was developed through Agrobacterium–mediated transformation of soybean cells with pDAB9582 plasmid vector carrying the pat gene that encodes PAT protein which confers tolerance to glufosinate ammonium-containing herbicides, the cry1Ac gene that encodes Cry1Ac protein and cry1Fv3 gene that encodes Cry1F protein. Both Cry proteins provide resistance against certain lepidopteran insects.

Host Organism (Glycine max L.)

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.

Transgenic Plant

DAS-81419-2 soybean has been reviewed and approved for food and/or feed use in many countries including United States (Food and Feed, 2014), Canada (Food and Feed, 2014), Australia & New Zealand (Food and Feed, 2014), Argentina (Food and Feed, 2016), Brazil (Food and Feed, 2016), Colombia (Food and Feed, 2016), Japan (Food and Feed, 2014), Korea (Food and Feed, 2016), Mexico (Food and Feed, 2015), South Africa (Food and Feed, 2016), Taiwan (Food and Feed, 2015) (ISAAA, 2017).

Based on the documents provided by the developer, the consumption patterns by population subgroups are not expected to be altered.

Donor Organisms

Bacillus thuringiensis is a donor of cry1Ac and cry1F genes. Streptomyces viridochromogenes is a donor organism of pat gene. Donor organisms of genetic elements including promoters, terminators and border sequences include Arabidopsis thaliana, Agrobacterium tumefaciens, and Cassava vein mosaic virus. All donor organisms are not known to be toxic and allergenic and has been used in

Agrobacterium-mediated transformation of several genetically modified crops. History of safe use was attributed to all donor organisms.

Transformation System

The event, DAS-81419-2 was developed to express Cry1Ac and Cry1F protein derived from Bacillus thuringiensis and PAT protein derived from Streptomyces viridochromogenes. The transformation method is through Agrobacterium-mediated transformation with plasmid vector pDAB9582. The plasmid vector, is composed of the gene expression cassettes for cry1Ac, cry1F and pat (DAS, 2016).

The cry1F v3 gene expression cassette is composed of matrix attachment region (RB7MAR) from Nicotiana tabacum, Arabidopsis thaliana polyubiquitin UBQ10 comprising the promoter, 5' untranslated region and intron, cry1F v3 gene from Bacillus thuringiensis subspecies aizawai strain PS811, 3' untranslated region comprising the transcriptional terminator and polyadenylation site of open reading frame 23 of Agrobacterium tumefaciens pTi15955 and intervening sequences (DAS, 2016).

The Cry1Ac gene expression cassette is composed of CsVMV promoter along with 5' untranslated region derived from Cassava Vein Mosaic Virus, cry1Ac gene from Bacillus thuringiensis subspecies kurstaki strain HD73, 3' untranslated region comprising the transcriptional terminator and polyadenylation site open reading frame 23 of Agrobacterium tumefaciens pTi15955, and intervening sequences (DAS, 2016).

The pat gene expression cassette is composed of CsVMV promoter along with 5' untranslated region derived from Cassava Vein Mosaic Virus, pat gene from Streptomyces viridochromogenes, 3' untranslated region comprising the transcriptional terminator and polyadenylation site open reading frame 23 of Agrobacterium tumefaciens pTi15955, and intervening sequences (DAS, 2016).

Inserted DNA

Southern Blot Analyses demonstrated that DAS-81419-2 soybean contains one intact copy of the T-DNA insert at a single locus (DAS, 2016). The integrity and order of genetic elements were demonstrated through southern blot analyses using different probes listed in Table 3, Section 6.1. (DAS, 2016). A minor (<100 bp) fragment of the cry1Ac (synpro) gene was identified at the 5′ end of the T-DNA insert. There were also deletion of 57-bp observed at the site of T-DNA insertion. This was demonstrated through the analysis of the T-DNA insert sequence, border sequence, and parental locus sequence. Bioinformatics analyses indicated that all putative reading frames did not have sequence similarity with known toxins and allergens.

Southern blot analysis of NcoI and SphI-digested genomic DNA from DAS-81419-2 showed no plasmid backbone sequences present in DAS-81419-2 (DAS, 2016)

Genetic Stability

The stability of the T-DNA insert across five generations (T1, T2, T3, T4 and F2) was demonstrated by Southern blot analysis. Results of analysis indicated that the observed fragments were consistent with the expected fragments. This indicates that the inserted genes are stably integrated and inherited from one generation to another (DAS, 2016).

Segregation was assessed using both bioassay and event-specific PCR. One population of F2 and three populations of BC1F2 were assessed. Segregation result is consistent with the reported one copy T-DNA insert. Using the Chi-Square Goodness of Fit Test the segregation pattern was

determined. The T-DNA insert displayed the expected Mendelian 3:1 segregation pattern for a single independent insert/locus in segregating generations (F2 and BC1F2) (DAS, 2016).

Expressed Material

Cry1 proteins has specific mode of action on target lepidopteran insects. The protein have no metabolic role in plants (DAS, 2016). 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) (DAS, 2016). The expression level of Cry1Ac, Cry1F and PAT in grain is 10.41 – 16.95 ng/mg dry weight, 0.79 – 1.40 ng/mg dry weight and 0.63 – 1.12 ng/mg dry weight, respectively.

Toxicological and Allergenicity Assessment

The safety assessment of novel proteins, CryAc, Cry1F 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 Cry1Ac and Cry1F are readily degraded within 1 minute of incubation with SGF, respectively, in presence of pepsin at pH 1.2, a characteristic of most non-toxic proteins (DAS, 2016). According to Hérouet et al., (2005). PAT protein digestibility was tested in simulated gastric fluid containing pepsin and was readily degraded with no large size fragments remaining (OECD, 1999).

Heat stability of Cry1Ac and Cry1F protein was determined through SDS-PAGE analysis of the proteins heated at different temperatures (4 and 91^0C). Polyclonal antibody based Cry1Ac and Cry1F ELISA was used to determine protein immunoreactivity. Cry1Ac and Cry1F protein is immunochemically denatured when heated. The Cry1Ac and Cry1F protein lost greater than 99% and 98% of its immunoreactivity, respectively, with results showing that it was almost undetectable by ELISA after exposure to the heat treatment (Shan and Embrey, 2005). PAT protein is rapidly denatured by heat. This was verified in OECD Consensus Document (1999).

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 Cry1Ac, Cry1F and PAT protein to toxins and allergens was conducted using BLASTp search algorithm against the GenBank and FASTA program (DAS, 2016).

Bioinformatics tools and comparison to FARRP Allergen Database Version 12 indicates that Cry1Ac, Cry1F and PAT has no amino acid sequence similarity to known allergens (DAS, 2016).

Acute oral toxicity study in mice using microbially produced Cry1Ac (700mg/kg body weight) and Cry1F (600mg/kg body weight) indicated that he administration of Cry1Ac and Cry1F did not significantly affect the body weight of mice. There were no gross pathologic lesions observed for any test animals in the study (DAS, 2016). The NOEL for Cry1Ac and Cry1F is 700 and 600 mg/kg body weight, respectively. PAT protein showed no treatment related toxic effects to mice. This was verified in OECD Consensus Document (1999). NOEL for PAT protein is 5000 mg/kg body weight (OECD, 1999).

The source of test protein was Pseudomonas fluorescens for the Cry proteins and Escherichia coli for PAT protein. Characterization methods include that it is biochemically and functionally equivalent to Cry1F, Cry1Ac and PAT expressed in DAS-81419-2 soybean (DAS, 2016).

The novel proteins are expressed independently of each other. Cry1Ac and PAT proteins are regulated by the same promoter (CsVMV) and therefore are expressed together. Cry1F expression is independent of Cry1Ac and PAT proteins (DAS, 2016).

The percent of total protein of Cry1Ac, Cry1F and PAT is estimated to be 0.000003%, 0.00004% and <0.000002%, respectively.

IgE binding to extracts of DAS-81419-2 soybean and its non-transgenic control were evaluated with one dimensional (ID) IgE immunoblot and ELISA inhibition using sera from 10-clinically soy allergic patients (DAS, 2016). Results showed that the genetic modification of soybean to produce DAS-81419-2 did not alter endogenous allergens present in conventional soybean.

Results of the toxicological and allergenicity assessment indicate that Cry1Ac, Cry1F and PAT proteins being expressed in DAS-81419-2 soybean are not toxic or allergenic to humans (DAS, 2016).

Nutritional Data

Compositional analysis provided by the developer indicating the nutritional data of DAS-81419-2 in comparison with the non-transgenic soybean, range of commercial varieties and range of literature values (DAS, 2016). 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-81419-2 and the non-transgenic soybean that can be considered biologically relevant.

Conclusion

For the transgenic DAS-81419-2 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 the resistance to certain lepidopteran insects and glufosinate ammonium-containing herbicides. After reviewing the provided material of Dow AgroSciences, it is therefore concluded that DAS-81419-2 soybean is as safe as its conventional counterpart.

BAI ASSESSMENT AND RECOMMENDATIONS

Based on the documents submitted by the applicant, BAI made the following assessment:

A. Host Organism

Soybean contains key nutrients such as proteins, amino acids, fatty acids soybean. It also contains vitamins K, E, among others. It also contains anti-nutrients, such as trypsin/protease inhibitors, lectins, phytic acid especially wherein at its raw state; the use is limited for human and animal consumption.

No (OECD, 2012) soybean is not a source of toxicants but it may cause IgE-mediated food allergies (OECD, 2012). It is also used as food and feed but soybean must be processed before use due to anti-nutrients (OECD, 2012). Based on WHO 97.5th percentile consumption of soybean, intake by general population is about 3.03 g/kg body weight and intake by children under 6 is about 5.55 g/kg bodyweight.

B. Transgenic Plant

DAS-81419-2 soybean is as safe and as nutritious as conventional soybean. There is no need to change consumption pattern as a result of introduction of this soybean event. It is approved in US (FDA, 2014), Canada (Health Canada, 2014), Australia & New Zealand (FSANZ, 2014), Argentina (SENASA, 2016), Brazil (CTNBio, 2016), Colombia (INVIMA, 2016), Japan (MHLW, 2014), Korea (MFDS, 2016), Mexico (COFEPRIS, 2015), South Africa (DAFF, 2016), Taiwan (DOH, 2015).

C. Donor Organism

Cry1Ac, Cry1F, and PAT proteins have a long history of safe use. The proteins are not known to possess potential pathogenic or allergenic properties. The introduced expressible sequences include Cry1Ac and Cry1F proteins conferring resistance to certain soybean insect pests and PAT protein conferring tolerance to glufosinate. PAT is used as a selectable marker during DAS- 81419-2 soybean development.

Bacillus thuringiensis is a donor of Cry1Ac and Cry1F proteins. *Streptomyces viridochromogenes* is a donor of PAT. Donor organisms of genetic elements including promoters, terminators and border sequences include *Arabidopsis thaliana, Agrobacterium tumefaciens*, and Cassava vein mosaic virus. There are no publications concerning toxicity or allergenicity of these genetic elements in peer-reviewed journals.

Three proteins are introduced and expressed in DAS-81419-2 soybean, including Cry1Ac, Cry1F, and PAT. The three proteins are present in a number of biotech crops cultivated for commercial use and have a long history of safe use. The proteins have specific modes of action and have no significant sequence similarity to known allergens or toxins.

D. Transformation System

DAS-81419-2 soybean was created via Agrobacterium-mediated transformation. Details of the transformation method are described adequately. The transformation protocol is described in detail as well as all the genetic components used.

The genetic modification was intended to express Cry1Ac, Cry1F, and PAT in soybean plants, which provides protection against several soybean insect pests and tolerance to glufosinate. PAT was used as a selectable marker during DAS- 81419-2 soybean development. No carrier DNA was used for the transformation of pDAB9582 into soybean Glycine max. The Agrobacterium tumefaciens strain EHA101 carries the helper plasmid, pTiBo542. EHA101 was generated by inactivation of the T- DNA onc genes in strain A281 (Hood et al., 1986).

E. Inserted DNA

DAS-81419-2 soybean contains one intact copy of the T-DNA insert at a single locus. Insert copy number was demonstrated by Southern blot analysis. Southern blot results showed that the soybean event carries one intact copy of the T- DNA insert at a single locus. Integrity and order of genetic elements in DAS-81419-2 soybean were also demonstrated through Southern blot analysis.

The T-DNA insert in DAS-81419-2 soybean contains a single, intact copy of each of the expression cassettes for the cry1Fv3, cry1Ac (synpro), and pat genes. There is a minor (<100 bp) fragment of the cry1Ac(synpro) gene identified at the 5′ end of the T-DNA insert; in addition, there is a minor 57-bp deletion at the site of T-DNA insertion. These sequence features were confirmed by the analysis of the T-DNA insert sequence, border sequence, and parental locus sequence. 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. Bioinformatics analysis results showed that putative reading frames did not have sequence similarity with known allergens and toxins. No plasmid vector backbone sequence is present in DAS-81419-2 soybean as demonstrated by Southern blot analysis. Confirmation of lack of vector backbone using Southern blot analysis is a scientifically proven method and is sufficient.

Cry1Ac, Cry1F, and PAT have a long history of safe use. The safety of the Cry1Ac and Cry1F proteins has been demonstrated in sprayable Bt formulations for pest control in agriculture for over half a century (Mendelsohn et al., 2003; EPA, 2011; Sanahuja et al., 2011). Both proteins are expressed in Dow AgroSciences' WideStrike cotton authorized for cultivation, food and feed use. Bt corn and Bt cotton expressing variations of either Cry1Ac or Cry1F have been cultivated for commercial use in the U.S. and other countries for more than a decade. The PAT protein is present in several biotech crops approved for commercial cultivation, including corn, soybean, cotton, canola, rice, and sugar beets.

F. Genetic Stability

Stability of the T-DNA insert across five generations was demonstrated by Southern blot analysis. Segregation was assessed using both bioassay and event-specific PCR. One population of F2 and three populations of BC1F2 were assessed. Segregation result is consistent with the reported one copy T-DNA insert

G. Expressed Material

Expression levels of Cry1Ac, Cry1F, and PAT proteins were determined using protein-specific ELISA methods. Cry1Ac levels in roots, V5 leaf, V10-12 leaf, and grain were 0.39, 25.44, 23.16, and 1.04 ng/mg dry weight tissue. Cry1F levels in roots, V5 leaf, V10-12 leaf, and grain were 5.23, 56.75, 39.07, and 13.80 ng/mg dry weight tissue. The PAT protein levels in roots, V5 leaf, V10-12 leaf, and grain were 0.63, 5.23, 5.60, and 0.86 ng/mg dry weight tissue. Protein expression in pollen was not assessed due to low levels of protein, indicating low level of potential exposure, and scarcity of pollen material.

Cry1Ac, Cry1F, and PAT have specific modes of action. The proteins do not play a role in endogenous plant metabolism.

H. Toxicological Assessment

Assuming that microbial Cry1Ac protein is equivalent to Cry1Ac expressed in DAS-81419-2 soybean, the digestibility of the Cry1Ac protein, in DAS-81419-2 soybean, was tested in vitro using simulated gastric fluid (SGF) pepsin. The estimated T50 of Cry1Ac is approximately less than 1 minute. The results showed that the Cry1Ac protein was readily degraded within 1 minute with no large size fragments remaining in SGF. Molecular mass of the CrylAc was significantly reduced by approximately 68% after heat treatment for 60 min at 91 ± 2°C in phosphate buffer meaning Cry1Ac protein is immunochemically denatured when heated. The Cry1Ac protein lost greater than 99% of its immunoreactivity, with results showing that it was almost undetectable by ELISA after exposure to the heat treatment.

The Cry1Ac protein does not share any biologically meaningful amino acid sequence similarities with known toxic proteins that are harmful to humans or animals as per results of Bioinformatics analysis. Its acute toxicity via oral gavage was assessed. No Observable Effect Limit was not provided, but the acute oral LD50 of Cry1Ac microbial protein in male and female CD-l mice was greater than 700 mg/kg. The Cry1Ac protein was produced in Pseudomonas fluorescens. Characterization studies were performed to confirm the equivalency of the Cry1Ac protein expressed in DAS-81419-2 soybean with the P. fluorescens-derived Cry1Ac protein.

The digestibility of the microbial Cry1F protein, was tested in vitro using simulated gastric fluid (SGF). The Cry1F protein (0.074 mM) was incubated in SGF (0.3% w/v pepsin at pH 1.2; US Pharmacopeia) at 37oC for various periods of time. The estimated T50 of Cry1F is approximately less than 1 minute. The results showed that the Cry1F protein was readily digested (not detectable at 1 minute) in SGF

Molecular mass of the Cry1F was significantly reduced by approximately 61% after heat treatment for 60 min at 91 ± 2°C in phosphate buffer meaning Cry1F protein is immunochemically denatured when heated. The Cry1F protein lost more than 98% of its immunoreactivity, with results showing that it was almost undetectable by ELISA after exposure to the heat treatment. Using polyclonal antibody sandwich ELISA.

The Cry1F protein does not share any biologically meaningful amino acid sequence similarities with known toxic proteins that are harmful to humans or animals as per result of Bioinformatics Analysis. Cry1F protein acute toxicity via oral gavage was assessed. No Observable Effect Limit was not provided, but the acute oral LD50 of Cry1F microbial protein in male and female CD-I mice was greater than 600 mg/kg. The Cry1F protein was produced in Pseudomonas fluorescens. Characterization studies were performed to confirm the equivalency of the Cry1F protein expressed in DAS-81419-2 soybean with the P. fluorescens-derived Cry1F protein.

PAT protein is hydrolyzed rapidly in simulated gastric fluid containing pepsin. There was no evidence of acute toxicity in mice at a dose of 5000 mg/kg body weight of PAT protein. The PAT protein does not share any biologically meaningful amino acid sequence similarities with known toxic proteins that are harmful to humans or animals.

Characterization of the biochemical properties of the plant-derived PAT protein was accomplished through the use of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), western blot analysis, protein sequence alignment, and lateral flow test strip assay. Using these methods, the PAT protein produced in DAS-81419-2 soybean was shown to be substantially equivalent to that produced in E. coli.

I. Allergenicity Assessment

The Cry1Ac, Cry1F and PAT proteins does not share any biologically meaningful amino acid sequence similarities with known allergenic proteins that are harmful to humans or animals as per results of Bioinformatics analysis.The total Cry1Ac, Cry1F and PAT protein percentage in food is less than 0.01%. Serum screening was not performed for all three proteins since all three has a long history of safe use and there is no evidence of allergenicity.

J. Nutritional Data

Nutrient composition analysis showed that DAS-81419-2 soybean is substantially equivalent to commercial varieties, the non- transgenic soybean, with no significant and biologically meaningful differences.
No differences were observed between DAS-81419-2 soybean and comparator.

Anti nutrients analysis showed DAS-81419-2 soybean is substantially equivalent to commercial varieties , the non-transgenic soybean. DAS-81419-2 soybean will undergo the same processing as conventional soybean. Effect on the level of antinutrients in DAS-81419-2 soybean would be no different from that on the level of antinutrients in conventional soybean.

DAS-81419-2 soybean will undergo the same processing as conventional soybean. Effect on the level of antinutrients in DAS-81419-2 soybean would be no different from that on the level of antinutrients in conventional soybean.

K. Recommendation

Find scientific evidence that the regulated article applied for animal feed use is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health

DENR ASSESSMENT AND RECOMMENDATION

After a thorough and scientific review and evaluation of the documents provided by the Bureau of Plant Industry (BPI) on the application of Dow AgroSciences, B.V. for Direct Use as Food and Feed or for Processing of Soybean DAS 81419-2. 1/We,

Find scientific evidence that the regulated article applied for Direct Use as Food and Feed or Processing is safe as its conventional counterpart and is not expected to pose any significant risk to the environment.

The following are the observations and recommendations:

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/or 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. for direct use as food, feed or processing of Soybean DAS 81419-2.

DOH ASSESSMENT AND RECOMMENDATION

After a thorough review and evaluation of the documents provided by the proponent, Dow AgroSciences B.V, Philippines 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 DAS 81419-2. I/We,

Find 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 article applied for Direct Use for Food and Feed or for Processing (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 health and environment while in transit, storage and processing.

2. Scientific pieces of evidences from provided references i.e. literatures show that Regulated article applied for Direct Use for Food and Feed or for Processing (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 Bureau of Plant Industry (BPI) ensure the following :

    a) Strict monitoring of the regulated article from port of entry to the trader's/importer's storage/warehouse as stated in Section 32 of the JDC No. 1 series, 2016.

    b) The BPI to include in the issuance of permit for the 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 within the country shall be in closed containers.
        iii. There shall be a clear labeling of the product from importation down to all levels of marketing stating that it is only for the purpose of direct use for food, feed or processing and is not to be used as planting materials.

4. Based on the above considerations and with the submitted sworn statement and accountability of the proponent, this recommendation is being submitted to BPI related to the processing and issuance of a biosafety permit for Direct Use for Food and Feed or for Processing (FFP) of Soybean DAS 81419-2.

SEC ASSESSMENT AND RECOMMENDATIONS

Based on SEC expert review of the SEC questionnaire answered by the applicant:

Socio-economic issues

Soybean and its components are valuable raw materials for the food and feed industries in the Philippines. It is considered a major ingredient for feed formulation for the livestock industry. However, local soybean production is very insignificant relative to the demand of the food and feed industries. Significant portion of soybean utilized by both the food and feed industry are imported.

The entry of Soybean DAS-81419-2 in the country may not affect the domestic production since it will be imported and will be used for food, feed and processing only. However, it may indirectly affect the consumption through the price mechanism. With the entry of more soybean products in the country, it will help stabilize prices or even it may lower the prices of products using soybean as one of the ingredients. With the lowering of prices, demand of such commodity may increase,

holding other factors constant. In terms of trade, it may not affect due to its insignificant share in the imports of the country.

Recommendation

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