ASSESSORS' CONSOLIDATED REPORT ON DOW AGROSCIENCES' APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF CORN DAS 40278-9

EXECUTIVE SUMMARY

On January 20, 2017, Dow AgroSciences BV Philippine Branch submitted corn DAS 40278-9 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 DAS 40278-9 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 corn DAS 40278-9 will not pose any significant risk to health and the environment and that any hazards could be managed by the measures set by the department. DOH-BC also recommended for the issuance of a biosafety permit for corn DAS 40278-9.

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'S ASSESSMENT

A. Host Organism

The STRPs agree that Corn is a major item of grain grown for food and feed. Based on the combined compositional analysis from the literature, for human consumption, the grain contains (by dry weight basis) 63.3-89.8 % carbohydrates, 6.0- 17.3 % protein, 1.2-18.8 % total fat 8.3-35.3 % total dietary fiber and 0.62-6.28 % ash. For forage, it contains 66.9-94.5 % carbohydrates, 3.14-15.9 % protein, 0.296-6.7 % total fat. 19.0-62.8 % total dietary fiber and 1.3-10.5 % ash. Corn is also the source of some anti-nutrients like trypsin inhibitors, raffinose, phytic acid and secondary metabolites (coumaric acid, ferulic acid, furfural and inositol).

The STRPs also agree that Corn in not known to be a toxicant, except for improperly stored grain that can be infested by insects and toxin-producing molds. Corn grain is not a source of allergens. However, the pollen of the corn plant is known be allergenic to hypersensitive individuals. According to Astwood et al, 1996,the potential pollen allergens are known as Zea m1 and Clone C13. The pollen specific cDNAs of these are similar to those found in rye (Lol P1 sequence) and olives (ole e1) (Villalba et al, 1993). Pollen is shed at specific time points in the growing season. Since the subject of this application is corn grain, then pollen is not expected to be included in the importation.

Further, the STRPs also noted that Corn has been used a primary source of food and consumed as food. In the US, 90% of the total corn production is used as feed grain (meals, gluten feed, etc.). Estimates of both single serving (acute or short term intake) and repeat dose (chronic or average daily intake) corn exposures are available for consumption pattern that are relevant to the consumer. The intake of animal dietary burdens for livestock (beef, dairy, pig) and poultry are presented in tabulated form. Consumption pattern for livestock and poultry has been provided and described sufficiently. The introduction of the novel food (DAS 40278-9 Corn) is not expected to change as it was found to be substantially equivalent to conventional corn as a result of nutrient composition analyses.

B. Donor Organism

The STRPs concur that the inserted protein expressing aad-1 from a fragment of 6236 bp linear DNA was described. This was a fragment from the vector DNA plasmid pDAS1740. The regulatory sequences that included the promoter (Zm Ubi) the terminator (Zm Per 5 3'-UTR) were both from corn. The enhancers represented by the matrix attachment regions from tobacco (RB7 MAR v3 and v4) that flanked the aad-1 were adequately described in the patent documents. The promoter and terminator sequences were both expected to be recognized by the plant because they came from the same source and therefore ensure positive expression of the inserted gene. The MARS were included to increase consistency of expression and avoid any possible silencing (non-expression) of the inserted gene.

The STRPs also agree that the *aad-1* encodes Aryloxyalkanoate dioxygenase 1 which in the presence of α- ketoglutarate is known to degrade phenoxy auxins such as 2,4-D to dichlorophenol and eventually to succinate and carbon dioxide. These two products are common intermediates in cellular metabolism and are therefore not harmful by themselves. *S. herbicidovorans* could therefore use the phenoxy auxins and other xenobiotics as source of carbon for growth. Other substrates for this dioxygenase are the family of aryloxyphenoxypropionate (AOPP)–acetyl CoA inhibitors or "fop" pesticides.

Further, they also agree that *S. herbicidovorans*, the source organism for the *aad-1* gene is a Gram-negative soil bacterium. Due to their biodegradative and biosynthetic capabilities, the sphingomonads have been used for a wide range of biotechnological applications, including bioremediation of environmental contaminants and production of extracellular polymers such as sphingans which are used extensively in the food industry. There is no known pathogenicity and allergenicity of the encoded protein.

C. Transformation System

The STRPs all agree that the method of transformation was described and referenced as the Whisker-mediated direct DNA transfer using silicone carbide fiber directly penetrating the cell wall and directly incorporating the desired DNA. The target of genetic modification was the nuclear genome for stable and whole plant expression. The experimental procedure was adequately described and a schematic diagram was provided. A summary of the genetic elements is presented in tabulated form, indicating the locations on plasmid pDAS-1740 Tsp 1 fragment, size and description. the plasmid map of pDAS-1740 with all the genetic elements identified was presented. There was no carrier or helper DNA that was used in the introduction of the 8512 bp linear PTU from pDAS 1740 into the embryogenic cell suspension cultures to produce Event DAS 40278-9.

E. Inserted DNA

The molecular characterization of DAS-40278-9 corn was performed by Southern blot analyses. The result demonstrated that the transgene insert in DAS-40278-9 corn occurred as a single integration of a single intact copy of aad-1 expression cassette from plasmid pDAS 1740. The insert is stably integrated and inherited across and within the breeding generations. No plasmid backbone sequences are present in DAS-40278-9 corn. There was no indication of truncations in the Southern blots. The number and expected sizes of the specific hybridizing bands were consistently observed.There was also no indication of deletions in the Southern blots.

F. Genetic Stability

The STRPs concur that Southern blot analyses were conducted with five (5) distinct generations (T3, T4, BC3S1, BC3S3, and BC3S2) of DAS-40278-9 corn. Results across all DAS-40278-9 corn samples indicated stable inheritance of the intact single copy insert across multiple generations of DAS040278-9 corn. Segregation in T1, T2, BC1, BC2 BC3 and BC3S1 of DAS-40278- 9 corn were performed on leaf tissues through Southern

blotting and immunoassay for the expressed AAD-1. The typical Mendelian inheritance ratio of 3:1 was observed. From the BC3S1 line for example, 65 tested positive for AAD-1 protein expression and 20 were null segregants. The aad-1 probe hybridized to each of the 65 plants that tested positive for AAD-1. The 20 null segregants did not show this hybridization band, indicating the absence or non-inheritance of the inserted aad-1.

G. Expressed Material

A field expression study was conducted in 6 sites planted with DAS-40278-9 hybrid corn (BC3S1- A & E). Four treatments of the DAS-40278-9 corn expression study included 3 herbicide treatments as follows: AAD-1 Unsprayed, AAD-1 + Quizalofop, AAD-1 + 2,4-D, and AAD-1 + Quizalofop and 2,4-D. Results are presented showing the levels of AAD-1 protein (ng/mg tissue dry weight) measured in DAS-40278-9 corn indicating the range, mean and standard deviation. The average expression values ranged from 2.87 ng/mg dry weight in R1 stage root to 127 ng/mg in pollen tissue. For the plots sprayed and unsprayed with 2,4-D and quizalofop herbicides, no AAD-1 protein was detected in the control tissues across the 6 locations.

A summary of the AAD-1 protein concentrations (average across sites) in various corn matrices are shown in tabulated form. The expression values were similar for the sprayed treatments as well as for the plots sprayed and unsprayed with 2,4-D and quizalofop herbicides. No AAD-1 protein has been detected in the control tissue across the 6 locations.

H. Toxicological Assessment

The STRPs agree that the digestibility of AAD-1 protein was tested in vitro using simulated gastric fluid (SGF). Samples were analyzed via SDS-PAGE and Western blot. The results demonstrated that AAD-1 protein was readily digested or inactivated (not detected at 30 seconds in SGF). Heat inactivation tests were also done, the lowest temperature used was 50^0C for 30 min. These conditions already inactivated 97 % of the enzyme activity. The common cooking conditions applied to corn processing covers this and with the average field expression level of the AAD-1 present at 4.81 ng/mg tissue, processed products from DAS-40278-9 corn can be expected to be inactivated. Human consumption of raw corn is not the norm.

The STRPs also agree that BLASTp Search summary of proteins in the alignments with AAD-1 is presented and adequately described. None of the protein alignments returned by the BLASTp search are associated with toxicity. It was concluded that the AAD-1 proteins expressed in DAS-40278-9 corn contains no significant sequence similarity with any known toxic protein that is harmful to man and animals.

Further, an acute oral toxicity study was conducted with AAD-1 protein in mice at a dose level of 2000 mg AAD-1/kg b.w. All the animals survived and gained weight. No adverse effects and clinical signs were observed by study termination on day 15. It was concluded that the acute oral LD50 of AAD-1 protein in mice was greater than 2000 mg/kg b.w., hence, AAD-1 protein is not a health risk concern.

The protein equivalency was also done by comparing aad-1 expression in transgenic corn with that of the *Pseudomonas fluorescens* using SDS-PAGE/Western blot/glycoprotein detection/MALDI-TOF MS/ and tandem mass spectrometry. The products of the analytes have been shown to be biochemically equivalent. Biochemical equivalency was established by the procedures previously described.

I. Allergenicity Assessment

The STRPs agree that the digestibility of AAD-1 protein was tested *in vitro* using simulated gastric fluid (SGF). Samples were analyzed via SDS-PAGE and Western blot. The results demonstrated that AAD-1 protein was readily digested or inactivated (not detected at 30 seconds in SGF). Heat inactivation tests were also done, the lowest temperature used was 50^0C for 30 min. These conditions already inactivated 97 % of the enzyme activity. The common cooking conditions applied to corn processing covers this and with the average field expression level of the AAD-1 present at 4.81 ng/mg tissue, processed products from DAS-40278-9 corn can be expected to be inactivated. Human consumption of raw corn is not the norm.

The STRPs also agree that BLASTp Search summary of proteins in the alignments with AAD-1 is presented and adequately described. None of the protein alignments returned by the BLASTp search are associated with toxicity. It was concluded that the AAD-1 proteins expressed in DAS-40278-9 corn contains no significant sequence similarity with any known toxic protein that is harmful to man and animals. The AAD-1 protein is not glycosylated as proven by glycoprotein staining. The AAD-1 has a molecular weight of 33 kDa as shown by electrophoretic separation.

Further, an acute oral toxicity study was conducted with AAD-1 protein in mice at a dose lovel of 2000 mg AAD-1/kg b.w. All the animals survived and gained weight. No adverse effects and clinical signs were observed by study termination on day 15. It was concluded that the acute oral LD50 of AAD-1 protein in mice was greater than 2000 mg/kg b.w., hence, AAD-1 protein is not a health risk concern. No serum screening was reported in the references possibly because of no evidence of similarity in sequence to known allergens.

J. Nutritional Data

The STRPs agree that proximate analysis for grain show no differences across the six sites between control and transgenic lines were observed for fat ash, NDF and TDF. Fiber was within the reported literature range. A significant overall treatment effect was found for moisture but this did not show significance in the paired t-test or after adjustment for FDR. The differences in the values are not considered biologically meaningful because these were statistically low and in most cases was resolved after applying adjustment for false discovery rate (chance). Lastly, the values obtained still fall within those reported in the literature. Corn grain from Event DAS-40278-9 can therefore be considered substantially equivalent to conventionally-bred corn in terms of proximate.

They also agree that there were also no significant differences in proximate analysis of forage, in control and transgenic lines for moisture, ADF, NDF, Ca and P. In comparison

with the control line, protein content was lower in the transgenic line unsprayed and sprayed with quizalofop. On the other hand, ash content in the transgenic line sprayed with 2,4-D and quizalofop was highest. Carbohydrate was higher in the unsprayed and quizalofop-sprayed transgenic line. The differences are not of biological significance because there was no calculated treatment effect and the values still fall under the range of literature values. The value for carbohydrates was estimated by difference and has no significant FDR adjusted p-value and should therefore be of no biological concern.

Furthermore, across –site analysis for levels of fatty acids in the grain gave values for 8:0 to 15:1 and 16:1 to 17:1 fatty acids that were below the limit of quantitation (LOQ). Values for 16:0 palmitic and 18:0, stearic acids where not significantly different and showed no overall treatment effect for the control and transgenic lines. Additionally, the values were within the literature ranges.

Anti-nutrients like raffinose was below the LOQ for the transgenic and non-transgenic control lines, as presented in table 24 of the summary. There was no significant difference found in the levels of trypsin inhibitor in the transgenic lines (4.87 to 5.45 % d.w.b) as compared to the non-transgenic line (5.08 %d.w.b).

STRP'S 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'S ASSESSMENT

Corn DAS 40278-9 was developed by Dow AgroSciences B.V., through the use of recombinant DNA technology. The said event was developed through Whisker's –mediated direct DNA transformation of corn cells with pDAS1740 plasmid vector carrying, the aad-1 gene that encodes AAD-1 protein that provides herbicide tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase (ACCase) inhibitors ("fop" herbicides).

<u>Host Organism (Zea mays L.)</u>

Corn (Zea mays L.) has been widely consumed as staple food for humans and feed ingredient for animals. It is used in food products such as oil, grit, meal, flour, ethanol, syrup and starch as well as feeds such as hulls, gluten and hominy (OECD, 2002). Humans consume corn mostly in the form of corn-based ingredients such as high fructose corn syrup, starch, sweeteners, cereals, oil and alcohol. In terms of the feeds, it is commonly consumed in the form of corn silage (forage), gluten meal, gluten feed and distillers dried grains. In 2014, the daily per capita consumption index of corn in the Philippines is 60.08 grams/day, while the daily per capita calories supply is 213.88 grams (PSA, 2015).

Corn is a source of key nutrients such as amino acids, fatty acids, carbohydrates, vitamins, minerals, and fiber (OECD, 2002). It is also known to contain anti-nutrients such as phytic acid, 2,4-Dihydroxy-7-methoxy-2H-1,4-benzoaxin-3(4H)-one (DIMBOA), raffinose, trypsin and chymotrypsin inhibitors, and secondary plant metabolites such as furfural, ferulic acid and p-coumaric acid. These anti-nutrients and secondary metabolites have been historically present in corn at levels that would not cause the food to be unsafe.

History of safe use was attributed to corn. It is known to produce no significant amount of toxins and anti-nutrients. It is not a common allergenic food; however, some reports had stated gastrointestinal and respiratory allergenic reactions.

Transgenic Plant (DAS-40278-9 Corn)

DAS-40278-9 corn 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, 2014; Feed, 2013), European Union (Food and Feed, 2017), Japan (Food and Feed, 2012), Malaysia (Food and Feed, 2017), Mexico (2011), New Zealand (2011), South Africa (Food and Feed, 2012), South Korea (Food and Feed, 2014), Taiwan (2011), and United States of America (2011) (ISAAA).

The event, DAS-40278-9 was developed to express AAD-1 proteins derived from *Sphingobium herbicidovorans* (Dow AgroSciences, 2014). The protein confers tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase (ACCase) inhibitors ("fop" herbicides). The transformation method is through Whisker's – mediated direct DNA transfer with plasmid vector pDAS1740 into the corn line Hi-II. The plasmid vector contains the *aad-1* gene expression cassette which contains two (2) matrix attachment region (MAR) obtained from *Nicotiana tabacum* (RB7 MAR), ubiquitin promoter isolated from *Zea mays*, synthetic, plant optimized version of an aryloxyalkanoate dioxygenase gene isolated from *Sphingobium herbicidovorans*, 3' untranslated region from *Zea mays* peroxidase gene, and six (6) intervening sequences.

Donor Organisms (*Sphingobium herbicidovorans*)

Sphingobium herbicidovorans is a Gram negative soil bacterium which has the ability to use phenoxy auxin and AOPP herbicides as carbon sources for growth (Dow AgroSciences, 2014). Sphingomonads are widely distributed in nature and was found in land, water, plant root systems, clinical specimens, etc. It has history of use in terms of bioremediation of environmental contaminants and production of extracellular polymers such as sphingans which are extensively used in food industry.

The donor organisms of other genetic elements included in the plasmid vector pDAS1740 includes *Nicotiana tabacum* and *Zea mays* (Dow AgroSciences, 2014). History of safe use has been attributed to *Z. mays* since it is being widely consumed as staple food of several countries worldwide and is not a common allergenic food nor a source of toxicants. No food safety concern with regards to the other donor organisms used in the transformation since the regulatory sequences obtained from these organisms are not being expressed in DAS-40278-9.

The only protein expressed in DAS-40278-9 is the aryloxyalkanoate dioxygenase-1 (AAD-1) protein encoded by *aad-1* gene. AAD-1 has an alpha ketoglutarate-dependent dioxygenase activity (Dow AgroSciences, 2014).

Inserted DNA

Southern blot analyses using restriction enzymes such as *EcoR I, Nco I, Sac I* and *Fse I/Hind III* and aad-1, ZmUbi1 promoter and ZmPer5 terminator probes confirmed that the observed fragment sizes of each probe corresponds with the predicted fragment sizes of each probe of DAS-40278-9 genomic DNA and pDAS1740 (Dow AgroSciences, 2014). The results of analyses showed that DAS-40278-9 genome contains only a single insertion of the T-DNA from the plasmid pDAS1740. Also, no specific hybridization bands were detected in the negative control samples in any of the restriction enzyme and probe combinations. This indicates that the single insert in DAS-40278-9 corn contains an intact single copy of aad-1 gene. No truncations, deletions or rearrangements were identified. Southern blot analyses using *Nco I* and *Sac I* restriction enzymes on the backbone probes confirmed that no plasmid backbone sequences from pDAB1740 were integrated into DAS-40278-9.

Genetic Stability

The multigenerational stability of the introduced traits was assessed through Southern Blot Analysis of genetic samples from five generations (T3, T4, BC3S1, BC3S2 and BC3S3) of DAS-40278-9 (Dow AgroSciences, 2014). Results showed that *aad-1* gene is stably inherited across multiple generations of DAS-40278-9. Segregation is assessed by Southern blot analysis and protein detection of individual plants from a BC3S1 line of DAS-40278-9. 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.

Expressed Material (Cry34Ab1, Cry35Ab1 and PAT proteins)

AAD-1 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 has no metabolic role in plants (Dow AgroSciences, 2014).

Expression level of AAD-1 in different plant parts of corn DAS-40278-9 was measured using ELISA methods (Dow AgroSciences, 2014). The measurements are in dry weight basis (ng/mg dry weight). Margin of exposure of general population and children in Japan to AAD-1 protein was derived from the data of the food intake in Japan and the No Observed Effect Level (NOEL) of AAD-1 protein determined from the acute oral toxicity study. Computed margins of exposure of general population and children (<6 year) to AAD-1 protein in corn is greater than 102564 and 67340, respectively.

Toxicological and Allergenicity Assessment

The novel protein, AAD-1, was subjected to digestibility, heat inactivation, oral toxicity and amino acid sequence comparison studies to determine its potential to cause toxicity or allergenicity to humans (Dow AgroSciences, 2014).

Digestibility study using Simulated Gastric Fluid (SGF) with pepsin demonstrated that AAD-1 is readily degraded within 30 seconds of incubation with SGF, in presence of 0.32% w/v pepsin at pH 1.2, a characteristic of most non-toxic proteins (Dow AgroSciences, 2014).

Heat Inactivation of AAD-1 is evaluated by heating protein solutions for 30 minutes at 50°C, 70°C and 95°C (Schafer, 2008). Upon treatment, samples were analyzed through ELISA, SDS-PAGE and western blot. Activity of AAD-1 was assayed by colorimetric enzyme assay. Results of the SDS-PAGE analysis showed that the AAD-1 protein was undetectable upon heating at 50, 70 or 95°C for 30 minutes. This was observed upon centrifugation of the protein samples prior to addition of Laemmli buffer. Immunoreactivity and enzymatic activity of AAD-1 decreased by 100% upon heat treatment at 50°C. Only 0.2% immunoreactivity and 3.0% enzymatic activity was detected upon heat treatment at 90°C.

Amino acid sequence comparison of AAD-1 protein to toxins and allergens was conducted using BLASTp search algorithm against the GenBank and FASTA program (Dow AgroSciences, 2014). Results of bioinformatics analyses indicated that AAD-1 protein is not homologous to any toxin and allergen. This was verified through conducting amino acid comparison using the same bioinformatics tool on August 23, 2017 (AIS-FRA-17-06-BIA).

Acute oral toxicity study on mice showed no mortality, clinical signs and treatment-related gross pathological observations during the study (Wiescinski and Golden, 2007). The determined No Observed Effect Level for AAD-1 protein is greater than 2000 mg/kg body weight.

The AAD-1 protein used in the studies was obtained from *Pseudomonas fluorescens* (Dow AgroSciences, 2014). Biochemical characterization, SDS-PAGE and Western blot analysis of crude extracts, MALDI-TOF and ESI/LC-MS tryptic and Asp-N peptide mass fingerprints, tryptic and Asp-N Peptide N- and C- terminal sequence analysis, and endogenous allergen analysis were conducted by the proponent to confirm that the *P. fluorescens*- produced AAD-1 protein is biochemically and functionally equivalent to AAD-1 expressed in DAS-40278-9.

Levels of AAD-1 in DAS-40278-9 determined through ELISA and the crude protein content of DAS-40278-9 were used to compute the percent total protein for AAD-1 which is 0.0046%.

Results of the digestibility, heat inactivation, amino acid sequence comparison and acute oral toxicity studies indicates that AAD-1 protein being expressed in DAS-40278-9 corn is not toxic or allergenic to humans (Dow AgroSciences, 2014).

Nutritional Data

Compositional analysis provided by the developer indicating the nutritional data of DAS-40278-9 in comparison with the non-transgenic corn and range of literature values (Dow AgroSciences, 2014). The trials were conducted in Iowa, Illinois, Indiana, Nebraska and Ontario. Results of the analysis indicated that there are no differences in the proximate, fiber, mineral, amino acid, fatty acid, vitamins, anti-nutrient and secondary metabolite levels of DAS-40278-9 and the non-transgenic corn that can be considered biologically relevant.

BPI-PPSSD'S RECOMMENDATION

For the transgenic DAS-40278-9 corn, 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 corn other than tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase (ACCase) inhibitors ("fop" herbicides. After reviewing the provided material of Dow AgroSciences, it is therefore concluded that DAS-40278-9 corn is as safe as its conventional counterpart.

BAI'S ASSESSMENT

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

A. Host Organism

Maize is a source of protein, amino acids, fatty acids, vitamins etc. It also contains low levels of several anti-nutrients including trypsin and chymotrypsin inhibitors, raffinose, phytic acid etc. Maize is consumed in various food forms including starch, oil, grits, meal and flour. Based on FAOSTAT data, consumption of maize intake by general population in ASEAN countries ranged from about 79-190g/person/day (Ranum et al., 2014)

B. Transgenic Plant

DAS 40278-9 is approved in: USA (USDA, 2014), Canada (CFIA, 2012; Health Canada, 2012), Brazil (CTNBio, 2015), Australia and New Zealand (FSANZ, 2011), Mexico (COFEPRIS, 2011), Columbia (ICA, 2013; INVIMA, 2014), South Africa (DAFF, 2012), Japan (MAFF, 2012; MHLW, 2012), Taiwan (DOH, 2011) and South Korea (MFDS, 2014; RDA, 2014).

DAS-40278-9 maize is as safe as conventional maize. There is no need to change consumption pattern as a result of introduction of this Maize event.

C. Donor Organism

The AAD-1 protein is not known to possess potential pathogenic or allergenic properties. The introduced expressible sequence includes AAD-1 protein conferring herbicide tolerance to 2,4dichlorophenoxyacetic acid (2,4-D) and

aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase (ACCase) inhibitors ("fop" herbicides).

Sphingobium herbicidovorans is a donor of AAD-1 protein. Donor organisms of genetic elements including promoters, terminators and border sequences include *Nicotiana tabacum* and *Zea mays*. There are no publications concerning toxicity or allergenicity of these genetic elements in peer-reviewed journals. AAD-1 is the only protein expressed in DAS-40278-9 maize. The protein has specific mode of action and have no significant sequence similarity to known allergens or toxins

D. Transformation System

DAS-40278-9 maize was generated through direct insertion of the DNA fragment from plasmid pDAS1740 via Whiskers-mediated transformation. The genetic modification was intended to express AAD-1 protein in maize plants, thus provide tolerance to 2,4dichlorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase (ACCase) inhibitors ("fop" herbicides). The transformation protocol is described fully. The plasmid vector, pDAS1740 (including orientation and relative location of genetic elements), is described adequately. There is no carrier DNA used for the transformation of pDAS1740 into maize. Whiskers-mediated transformation is a direct DNA transfer method.

E. Inserted DNA

DAS-40278-9 maize contains one intact copy of the T-DNA insert at a single locus. The insert copy number was checked through Southern blot analysis. Integrity and order of genetic elements in DAS-40278-9 maize were demonstrated via Southern blot analysis. Southern blot analysis showed that DAS-40278-9 maize contains a single intact copy of the aad1 expression cassette integrated at a single locus. The T-DNA insert in DAS-40278-9 maize contains a single, intact copy of each of the expression cassette for aad-1 gene. No vector backbone sequences were detected in event DAS-40278-9. There were no re-arrangements of the cassette observed.

There is no plasmid vector backbone sequence present in DAS-40278-9 maize as demonstrated by Southern blot analysis. Confirmation of lack of vector backbone using Southern blot analysis is a scientifically proven method and is sufficient.

F. Genetic Stability

Stability of the T-DNA insert across five generations was demonstrated by Southern blot analysis. Segregation was assessed using event-specific PCR. Populations of T1, T2 generations, 3 backcross populations (BC1, BC2, BC3) and one population of BC3F2 were assessed. Segregation result is consistent with the reported one copy TDNA insert.

G. Expressed Material

Expression levels of AAD-1 protein were determined using protein-specific ELISA methods with and without herbicide treatments. Expression values were similar for the

sprayed treatments as well as for the plots sprayed and unsprayed with 2, 4-D and quizalofop herbicides. Mean AAD-1 protein levels in roots ranged from 2.87-3.92; in V9 stage leaf from 5.38-6.52; in forage from 6.84-7.32; in pollen from 108-127 and in grain from 4.61-5.00 ng/mg dry weight tissue. AAD-1 protein has specific mode of action on 2,4-dichlorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase (ACCase) inhibitors ("fop" herbicides). The protein does not play a role in endogenous plant metabolism.

H. Toxicological Assessment

The digestibility of the microbe-derived AAD-1 protein was tested in vitro using simulated gastric fluid (SGF) containing gastric enzyme pepsin. The estimated T50 result was less than 30 seconds with no large size fragments remaining. AAD-1 protein was also evaluated by heating protein solutions for 30 min at 50, 70 and 95 C and 20 min in an autoclave in a phosphate based buffer. Results showed that AAD-1 protein is immunochemically denatured when heated. The AAD-1 protein lost more than 97% of its immunoreactivity, with results showing that it was almost undetectable by ELISA after exposure to the heat treatment.

Bioinformatics analysis through BLAST search showed AAD-1 has no significant sequence similarity with known toxins (Section 9.1.3.1 of the Food and Feed safety and Nutrition Assessment for Herbicide Tolerance DAS-40278-9 Maize dossier). Glycosylation analysis of AAD-1 isolated from DAS-40278-9 maize showed the protein is not glycosylated.AAD-1 protein used in the acute toxicity test was derived from *Pseudomonas fluorescens*. The microbial AAD-1 is biochemically and functionally equivalent to the AAD-1 expressed in DAS-40278-9

I. Allergenicity Assessment

The digestibility of the microbe-derived AAD-1 protein was tested *in vitro* using simulated gastric fluid (SGF) containing gastric enzyme pepsin. The estimated T50 result was less than 30 seconds with no large size fragments remaining. AAD-1 protein was also evaluated by heating protein solutions for 30 min at 50, 70 and 95 C and 20 min in an autoclave in a phosphate based buffer. Results showed that AAD-1 protein is immunochemically denatured when heated. The AAD-1 protein lost more than 97% of its immunoreactivity, with results showing that it was almost undetectable by ELISA after exposure to the heat treatment.

Bioinformatics analysis through BLAST search showed AAD-1 has no significant sequence similarity with known toxins (Section 9.1.3.1 of the Food and Feed safety and Nutrition Assessment for Herbicide Tolerance DAS-40278-9 Maize dossier). Glycosylation analysis of AAD-1 isolated from DAS-40278-9 maize showed the protein is not glycosylated.

When the WHO "GC 645 maize" acute consumption information is coupled to the AAD-1 field expression level of 4.81 ng/mg tissue, the potential acute exposure to AAD-1 protein via maize is estimated as: (1) 0.0195 mg protein/kg bw/day, for general population (i.e. adults) and (2) 0.0297 mg protein/kg bw/day, for children of 6 years or younger.

No serum screening was performed for the said application. AAD-1 has no evidence of allergenicity.

J. Nutritional Data

Differences were observed in moisture and carbohydrates in grain between DAS-40278-9 maize and comparator. However, levels were all within reference range and literature range. Nutrient composition analysis showed DAS40278-9 maize is substantially equivalent to comparator, the non-transgenic Maize, with no significant and biologically meaningful differences, both in grains and forage.

BAI'S 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'S ASSESSMENT

After thorough and scientific reviews 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 corn DAS 40278-9:

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, soil) and non-target organisms, to wit;

    a. The protein product produced by the transgenic crop will immediately degrade upon exposure to the natural environment

    b. Characterization of the inserted gene has shown that the protein product will not increase the weediness potential of the transgenic crop.

    c. 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 BPI ensure the proper and secure packaging of the regulated article for transport and the safety and durability of the transport/import as per BPI's inspection in the port area.

DENR'S RECOMMENDATION

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 corn DAS 40278-9.

DOH ASSESSMENT

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 Corn DAS 40278-9. 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 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 clear instructions that the product is only for the purpose of direct use for FFP and is not to be used as planting materials.

DOH'S RECOMMENDATION

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 corn DAS 40278-9.

SEC EXPERT'S ASSESSMENT

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

A. Socio-economic issues

The SEC Expert agree that this will not affect the existing grain trade practices if this is part of the existing grain flow. There should not be a change in the current pattern of production, consumption and trade since additional grain is always imported when local production is not sufficient to meet the requirements of the country.

SEC EXPERT'S RECOMMENDATION

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