ASSESSORS' CONSOLIDATED REPORT ON MONSANTO PHILIPPINES INC.'S APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF CORN MON87419

EXECUTIVE SUMMARY

On August 11, 2017, Monsanto Philippines Inc. submitted corn MON87419 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 MON87419 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 MON87419 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 corn MON87419.

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 Monsanto Philippines Inc.. The SEC expert, on the other hand, was provided with a questionnaire on socio-economic, ethical and cultural considerations that have been addressed by Monsanto Philippines Inc. 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

Based on the documents submitted by the applicant:

A. Host Organism

Corn is an important food in many developing countries and is frequently a mainstay of human diets. It is rigorously studied due to the economic opportunity it brings to many growers. Corn provides macronutrients, vitamins, minerals, sulfur-containing amino acids methionine and cysteine. Corn contains carotenes which act as precursors of Vitamin A and is a significant source of Vitamin E. Ferulic acid, p-coumaric acid, phytic acid and raffinose are also known to be present in small amounts in Corn.

Corn is consumed by humans in the form of corn-based ingredients like high fructose corn syrup, starch, sweeteners, cereals and oils.
However, it is reported that approximately 46% of harvested corn grain is used as animal feed. Corn can also be used as feed in the form of forage and silage fed to livestock. Other important components of livestock feed include gluten meal, corn gluten feed and dried grains obtained as by-product of wet and dry milling.

Corn is consumed at 56.63 grams/day daily per capita Net Food Disposal Index 2015. This is 12.27 percentage points higher in 2006. The daily per capita calories supply in 2015 is 201.6 gram. The daily per capita protein supply of corn is 5.38 grams; fat-244 grams.

There are no reports of significant quantities of toxins and allergens that require analytical and toxicological assessment is associated with corn.

B. Transgenic Plant

Approval of the transgenic MON 87419 corn for food/feed use has been made after review and assessment of its safe use by various regulatory bodies in many countries like Australia/ New Zealand (Food, 2016), Canada (Food, 2016; Feed, 2016), Japan (Food, 2016), Korea (Food, 2017; Feed, 2016), Mexico (Food, 2016), Philippines (Food, 2016), Taiwan (Food, 2016) and US (Food, 201; Feed, 2016).

Changes in consumption patterns by humans and animals are not anticipated with the introduction of the MON 87419 event. The transgenic corn event is substantially equivalent in composition and safety with conventional corn other than its ability to withstand dicamba and glufosinate containing herbicides.

C. Donor Organism

The transgenic event MON 87419 is produced with the plasmid vector PV-ZMHT507801 facilitate the transformation of the trait of interest and integration of the DNA into a

single locus in the plant genome. The PV-ZMHT507801 vector has a size of 14.6 kb that contains 2 separate T-DNAs

The dmo expression cassette encodes for 412 aa. (340 amino acid encoded by the dmo gene and 72 amino acid encoded by the CTP4 gene).
While, the pat expression cassette encodes for 183 amino acid. MON 87419 expresses a ~25 kDa PAT protein consisting of a
single polypeptide of 182 amino acid.

The DMO and PAT proteins in the transgenic MON 87419 is reportedly present in transgenic 88701 cotton which has been evaluated and approved for use. DMO protein is also contained by a transgenic soybean MON 87708 which has also been approved for use.

Safety evaluation of DMO and PAT proteins in MON 87419 involves assessment for amino acid sequence similarities to known allergens and toxic proteins, digestibility experiments utilizing proteases in gastrointestinal tract, functional activity after application of heat, acute toxicity assays all provide data to support a conclusion that food and feed products that contain DMO and PAT proteins from MON 87419 corn and its progenies are safe and nutritious as foods and feeds derived from conventional maize.

D. Transformation System

The transformation method used is Agrobacterium mediated transformation. The nuclear DNA is described as the target of genetic modification. The dmo and pat expression cassettes in the T-DNAI is integrated at a single locus in the nuclear DNA of MON 87419.

The PV-ZMHT 507801 plasmid vector has a molecular size of 14.6 kb and contains 2 separate T-DNA. The first DNA contains the dmo and the pat expression sequences. The dmo expression cassette is regulated by the peanut chlorotic streak caulimovirus (PClSV) promoter, the 5' nontranslated leader sequence of the chlorophyll a/b gene ofrom Triticum aestivum, the Ract I intron from O. sativa, the CTP4 chloroplast targeting sequence of the ShkG gene from Petunia hybrid, and the 3' nontranslated region of heat shock protein 17 (Hsp17) from T. aestivum. The pat expression cassette is regulated by the Ubq promoter from Andropogon gerardii, the Ubq 5' nontranslated leader sequence from A. gerardii, the Ubq intron from A. gerardii and the 3' nontranslated region of the of the Ara5 gene from Orysa sativa.

Meanwhile, the cp4 epsps expression cassette is contained by the second T-DNA and is regulated by the RactI promoter from O. sativa, the RactI 5' nontranslated leader from O. sativa, the RactI intron from O. sativa, the CTP2 chloroplast targeting sequence from A. thaliana and the nos 3' nontranslated region from A. tumefaciens.
The backbone region of the PV-ZMHT507801 is located outside the T-DNAs and contains 2 origins of replication for maintenance of the plasmid vector in bacteria (oriV, ori-pBR322), a bacterial selectable marker (aadA) and a coding sequence for repressor primer (ROP) protein for maintenance of the plasmid vector copy number in E. coli.

No carrier DNA and/or helper plasmids were described in the transformation of MON 87419.

E. Inserted DNA

MON 87419 corn contains a single insert (T-DNAI that presents the dmo and pat expression cassettes) based on Next generation sequencing and junction sequence analysis and is stably integrated at a single locus in the maize genome and that no plasmid backbone is detected.

A complete sequence of the single DNA insert is confirmed by directed sequencing that applies locus specific PCR, DNA sequencing and a comparison of the DNA sequences of the insert with the T-DNAI, the 5' and the 3' insert-to-flank junctions are reportedly made.

Generational stability analysis through NGS/ JSA demonstrate a single T-DNA insert in MON 87419 which is maintained through 5 breeding generation. Stability of the insert and segregation analysis confirm nature of the T-DNA insert at a single chromosomal locus.

Conventional nontransgenic genomic DNA spiked with PV-ZMHT507801 DNA is analized by next generation sequencing and other bioinformatic tools. It was shown that the MON 87419 does not contain sequences from the backbone of the transformation vector PV-ZMHT507801.

F. Genetic Stability

The generational stability analysis by NGS/ISA demonstrated that the insert in MON 87419 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA 1 in Mon 87419.

Assessment of the inheritance and stability of the MON 87419 T-DNA is done with the use of Chi-square ($\chi 2$) analysis over several corn generations and analysis is based on the comparison of observed segregation ratio of MON 87419 T-DNAI coding sequence to the expected segregation ratio according to Mendelian principles.

No statistically significant differences was shown between the observed and expected segregation ratios among 3 plant generations of MON 87419. It was also shown by segregation analysis that MON 87419 contains a single intact copy of the dmo and pat expression cassettes inserted at a single locus in the corn genome.

G. Expressed Material

Tissues of the transgenic MON 87419 collected from field sites are analyzed in terms of their protein levels using a validated ELISA. The mean DMO protein level is high in leaf (26 µg/g Dry wt), followed by root (7.4 µg/g Dry wt), forage (6.0 µg/g Dry wt) and lowest in grain (0.19 µg/g Dry wt). The mean PAT protein level is high in leaf (11.0 µg/g Dry wt), followed by root (7.7 µg/g Dry wt), forage (5.0 µg/g Dry wt) and lowest in grain (0.93 µg/g Dry wt).

The DMO protein has no metabolic role. DMO (Dicamba mono-oxigenase) catalyzes demethylation of dicamba to the nonherbicidal compound 3,6 dichlorosalicylic acid (DCSA) and formaldehyde. DCSA is a metabolite of dicamba in cotton, soybean, soil and livestock while formaldehyde is found naturally in many plants. DMO is targeted to chloroplasts by chloroplast transit peptide to allow localization of the endogenous reductase and ferredoxin enzymes that reportedly supply electrons for DMO demethylation reaction (Behrens et al., 2007). There are no reports of its involvement with functions mediating metabolic pathways.

PAT in MON 87419 is an acetyltransferase enzyme which acetylates glufosinate to produce non-herbicidal N-acetyl glufosinate. This protein is described to be specific for glufosinate in the presence of acetyl coenzyme. The herbicidal activity of glufosinate results from the binding of L-phosphinothricin to glutamine synthase. PAT does not affect maize metabolism as reported in other PAT-expressing maize products. There are no reports of its involvement with functions mediating metabolic pathways.

H. Toxicological Assessment

Digestibility and degradation of the DMO protein by pepsin and pancreatin is evaluated through SDS-PAGE and Western blot analysis. It was demonstrated that DMO degrades by 96.5% in pepsin within a reaction time of 30 sec which was confirmed by Western blots that applied specific DMO antibodies. A peptide fragment ~3kDa is seen with the 2-min pepsin treatment but complete digestion is attained in 5 min. About 98.4% of DMO protein degrades in pancreatin within 5 min as shown by 2 peptide fragments with ~12 and ~21 kDa but complete degradation is accomplished within 15 min. Data on digestibility show that large and intact fragments of MON 87419 DMO protein are unlikely to be absorbed in the small intestines after oral ingestion.

It was also shown that the functional activity of DMO is retained at 25°C and 37°C for a heating period of 15 or 30 min. Heating at 55°C and 75°C reportedly caused reduction of functional activity. Heating at 75°C for 30 min caused slight reduction in intensity of bands while heating at 95°C for 30 min resulted to visible reduction in bands. These findings reportedly explain the predictable loss of functional activity and denaturation of the DMO protein at elevated temperature for a relatively longer period of interaction.

The aligned amino acid sequences of MON 87419 DMO protein is compared with the amino acid sequences of toxins and toxic proteins in databases (FASTA, TOX_2014) and the alignment data is utilized to conclude shared or similarity of sequences. It was shown that no relevant alignment in MON 87419 DMO protein is in common with proteins in TOX_2014 database and that no alignment with E-score below 1 x 10-5 (0.00001) rules out a tangible sequence similarity.

A toxicity assay for MON 87419 DMO protein involved oral administration of DMO at 1000 mg/kg body weight to mice. No mortality, test substance-related clinical findings and no MON 87419 DMO protein-related differences in body weight changes, food consumption and gross necropsy findings that can be associated with the oral administration of DMO. This establishes the NOAEL (No observable adverse effect level) of the MON 87419 DMO protein at 1000 mg/kg body weight.

It was shown that the DMO protein from MON 87419 is substantially equivalent with the E.coli-produced MON 87419 DMO protein in terms of electrophoretic mobility and molecular weight, purity, immunoreactivity properties, consistent peptide masses, expected N-terminal sequences, non-glycosylation patterns and enzymatic activities.

Meanwhile, digestibility and degradation of PAT protein by pepsin and pancreatin is evaluated through SDS and Western blot analysis. It was demonstrated that more than 99.6% of the PAT protein is degraded by pepsin within 30 sec and 98.2% is digested with 30 sec interaction when evaluated by a specific antibody in Western blot. PAT protein also degrades by 99.1% in pancreatin within 5 min of interaction as shown by Western blot. A purified PAT protein is also heated at different temperatures (25, 37, 55, 75 and 95 degrees Celsius) for 15 and 30 minutes and result of heat treatment involved analysis of protein through SDS-PAGE. Functional activity of PAT protein is reportedly retained after heat treatment at 25 and 37 degrees Celsius for 15 and 30 minutes. At 55 and 75 degrees Celsius, functional activity of PAT protein reduced by 90% or greater. These demonstrate a predictable tendency of the functional activity of PAT protein to be lost at temperatures above 55 degrees Celsius.

The PAT protein sequence is screened for potential structural similarity with amino acid sequences in a database using FASTA sequence alignment tool. Based on bioinformatic alignment searches, no structurally relevant sequence similarity of PAT protein with sequences of allergens, toxins and active proteins is reported.

PAT protein was also administered to mice (C57BL/6J strain) by oral gavage at a dose of 2000 mg/kg body weight. Animals are monitored for clinical signs, changes in body weight, food consumption, necropsy findings, and gross pathological examination. Oral administration of PAT protein does not induce mortality, treatment-related clinical signs, body weight changes, food consumption pattern and gross pathological findings at indicated concentration. It can be deduced that the NOAEL (No Observable Adverse Effect Level) of MON 87419 PAT protein is established at 2000 mg/kg body weight.

PAT protein from MON 87419 grain is expressed in bacteria. Physico-chemical and functional evaluation of the MON 87419 PAT protein and E.coli-produced PAT protein are undertaken to assess equivalence of the two proteins. Substantial equivalence of the two above mentioned PAT proteins is based on evaluation of molecular weight confirmed through SDS-PAGE immunoreactivity validated by Western blot, non-glycosylation pattern and functional activity evaluated through co-enzyme release assay.

I. Allergenicity Assessment

It was shown that based on the results of N-terminal sequencing, the deduced sequences of the DMO proteins expressed in MON 87419 are consistent with the amino acid sequence of E.coli-produced MON 87419 DMO protein. Results of MALDI-TOF MS Analysis identified about 37 peptide masses which are consistent with expected peptide mass of the MON 87419-produced DMO sequence. Western blot analysis demonstrates immunoreactive bands with molecular weight of ~39.5 kDA in both MON 87419-produced DMO proteins and E.coli-produced DMO proteins. Functional enzymatic activity of both proteins are demonstrated within limits of 99.3 – 251.5 nmol x mean-1 x

milligram-1. Glycosylation analysis demonstrate that both proteins are non-glycosylated.

The percentage of DMO protein in MON 87419 grain is ~0.00016% or 1.6 ppm of total grain protein, calculated based on the mean level in grain (0.19 µg/g dry weight) divided by mean percent dry weight of the total grain protein in MON 87419. The amount of DMO protein represents a very small portion of the total protein content of MON 87419 grain.

On the other hand, no biologically relevant sequence similarities were observed between PAT protein and allergen or other biologically active proteins. When searching the TOX_2014 database, PAST protein generated alignments with bacterial toxinanti-toxin system proteins, but it does not provide any indication that PAT protein would adversely have an impact on human and animal health if consumed.

the mean level of PAT protein in the grain of MON 87419 is 0.93 µg/g dw. The mean percent dry weight of total protein in th3e grain of MON 87419 is 11.52 % (115200 µg/g). The percentage of PAT protein in MON 87419 grain is (0.93 µg/g g ÷ 115200 µg/g) x 100 % = .0008 % or 8 ppm of total grain protein. PAT protein represents a very small portion of the total protein in the grain.

Serum screening is not done as no allergens and toxins are contained in the transgenic event MON 87419 that should require toxicological test.

J. Nutritional Data

Proximate analysis of forage MON 87419 corn treated with dicamba and glufosinate under agronomic field conditions demonstrate mean levels of ash (% DW, 3.86+0.54), carbohydrates by calculation (% DW, 87.12+0.85), protein (% DW, 7.40+0.36) and total fat (% DW, 1.59+0.17) which are comparable ($P>0.05$) with those of conventional non-transgenic corn control. While, proximate analysis of grain from MON 87419 treated with dicamba and glufosinate under agronomic field conditions demonstrate mean levels of carbohydrates by calculation (% DW, 83.57+0.54) and ash (% DW, 1.39+0.021) contents are also comparable ($P>0.05$) with grains of conventional non-transgenic corn control.

Secondly, analysis of forage from MON 87419 treated with dicamba and glufosinate under agronomic field conditions for fiber demonstrated mean acid detergent fiber (% DW, 26.52+1.15) and mean neutral detergent fiber (% DW, 41.28+1.40) are comparable ($P>0.05$) with those of conventional non-transgenic corn control. Analysis of forage from MON 87419 treated with dicamba and glufosinate under agronomic field conditions for mineral components demonstrated mean calcium (% DW, 0.21+0.021) and mean phosphorus levels (% DW, 0.20+0.018) are also comparable ($P>0.05$) with those of conventional non-transgenic corn control.

Third, analyses of MON 87419 corn grains treated with dicamba and glufosinate under agronomic field conditions demonstrate mean acid detergent fiber (% DW, 3.97+0.12), neutral detergent fiber (% DW, 9.70+0.11) and total dietary fiber (% DW, 9.18+0.23)

which are comparable (P>0.05) with those of grains of conventional non-transgenic corn control.

Meanwhile, analyses of MON 87419 corn grains treated with dicamba and glufosinate under agronomic field conditions demonstrate mean Calcium (% DW, 0.0031+0.00017), Iron (mg/kg DW, 16.83+0.54), Magnesiun (% DW, 0.13+0.0 019), Phosphorus (% DW, 0.36+0.0059), Potassium (% DW, 0.36+0.0081), Sodium (mg/kg DW, 5.45+1.92) and Zinc (mg/kg DW, 22.10+1.13) which are comparable (P>0.05) with those of grains of conventional non-transgenic corn control except for manganese (mg/kg DW, 6.03+0.45) which significantly differs (P<0.05) from those of grains of conventional non-transgenic corn control.

Analyses of MON 87419 corn grains treated with dicamba and glufosinate under agronomic field conditions also demonstrate mean levels of protein (11.52+0.55), alanine (0.92+0.057), arginine (0.46+0.014), aspartic acid (0.73+0.036), cystine (0.22+0.0050), glutamic acid (2.43+0.15), glycine (0.41+0.011), histidine (0.33+0.011), isoleucine (0.42+0.024), leucine (1.59+0.11), lysine (0.28+0.0061), methionine (0.23+0.0074), phenylalanine (0.63+0.040), proline (1.08+0.044), serine (0.59+0.032), threonine (0.42+0.018), tryptophan (0.070+0.0017) and tyrosine (0.31+0.018) which are comparable (P>0.05) with those of grains of conventional non-transgenic corn control except for valine (0.54+0.025) which significantly differs (P<0.05) from those of grains of conventional non-transgenic corn control.

Analyses of MON 87419 corn grains treated with dicamba and glufosinate under agronomic field conditions for fatty acids (% total fatty acids) demonstrate mean levels of total fat (3.40+0.081), palmitic (14.51+0.12), palmitoleic (0.12+0.0040), stearic (1.62+0.028) oleic (21.86+0.20), linoleic (60.08+0.27), linolenic (1.00+0.027), arachidic (0.40+0.0079), eicosenoic (0.27+0.0049) and behenic (0.14+0.0070) components that are comparable (P>0.05) with those of grains of conventional non-transgenic corn control.
Analysis of MON 87419 corn grains treated with dicamba and glufosinate under agronomic field conditions for vitamins (mg/kg DW) demonstrate folic acid (0.65+0.035), niacin (10.22+0.41), Vit A (5.44+0.45), Vit B1 (2.46+0.12), Riboflavin/B2 (2.18+0.13), Pyridoxine HCL/Vit B6 (5.42+0.25) and Vit E (11.56+0.43) components which are comparable (P>0.05) with those of the grains of conventional non-transgenic corn control

Lastly, analysis of MON 87419 corn grains treated with dicamba and glufosinate under agronomic field conditions for anti-nutrients (% DW) such as phytic acid (0.99+0.031) and raffinose (0.28+0.010) show values that are comparable (P>0.05) with those of the grains of conventional non-transgenic corn control. Analysis of MON 87460 corn grains treated with dicamba and glufosinate under agronomic field conditions for secondary metabolites (µg/g DW) such as ferulic acid (2352.80+45.66) and p-Coumaric acid (196.51+12.40) show values that which are comparable (P>0.05) with those of the grains of conventional non-transgenic corn control.

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 MON 87419 was developed by Monsanto, Philippines, Inc., through the use of recombinant DNA technology. The said event was developed through Agrobacterium tumefaciens – mediated transformation of corn cells with PV-ZMHT507801 plasmid vector carrying the dmo gene encoding DMO protein and pat gene encoding PAT protein which confer tolerance to dicamba and glufosinate ammonium herbicides, respectively.

Host Organism (Zea mays L.)

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

MON 87419 corn has been reviewed and approved for food and/or feed use in many countries including Australia/New Zealand (Food, 2016); Canada (Food, 2016; Feed; 2016); Japan (Food, 2016), Korea (Food, 2017; Feed, 2016); Taiwan (Food, 2017; Feed, 2017), US (Food and Feed, 2016) (Monsanto. 2015a; Monsanto, 2015b).

Based on the documents provided by the proponent, the consumption patterns by population subgroups are not expected to be altered (Monsanto, 2008).

Donor Organisms

Stenotrophomonas maltophilia and *Streptomyces viridochromogenes* are not known to be toxic or allergenic (Monsanto, 2015a; Hérouet et al., 2005).

S. maltophilia is ubiquitous in the environment and is found associated with the rhizosphere of plants. It can be found in healthy individuals without causing any harm to human health and infections caused by *S. maltophilia* are extremely uncommon. Additionally, *S. maltophilia* has not been reported to be a source of allergens (Monsanto, 2015a).

S. viridochromogenes is a saprophytic, soil-borne bacterium with no known safety issues. The ubiquitous presence of *S. viridochromogenes* in the environment, the widespread human exposure without any adverse safety or allergenicity reports, and the successive reviews of several glufosinate-tolerant events by regulators that have not identified particular safety or allergenicity issues further establishes the safety of the donor organism (Hérouet et al., 2005).

Transformation System

The transformation method is through *Agrobacterium tumefaciens* – mediated transformation with plasmid vector PV-ZMHT507801 (Monsanto, 2015a). The target of genetic modification is the nuclear DNA. The vector PV- ZMHT507801 is approximately 14.6 kb in length and contains two separate transfer DNAs that are each delineated by Right and Left Border regions. The first transfer DNA, designated as T-DNA I, contains the dmo expression cassette and the pat expression cassettes. The second transfer DNA, designated as T-DNA II, contains the cp4 epsps expression cassette for selection (Monsanto, 2015a).

The dmo coding sequence in MON 87419 is under the regulation of the PClSV promoter, the chlorophyll a/b binding protein (CAB) leader, the Ract1 intron, The CTP4 transit peptide, and the heat shock protein 17 (Hsp17) 3′ untranslated region. The pat coding sequence in MON 87419 is under the regulation of the Ubq promoter, the Ubq leader, the Ubq intron and the Ara5 3′ untranslated region. The cp4 epsps coding sequence in MON 87419 is under the regulation of the Ract1 promoter, the Ract1 leader, the Ract1 intron, the CTP2 targeting sequence, and the nos 3′ untranslated region.

Molecular characterization of MON 87419 by NGS/JSA and directed sequencing demonstrated that a single copy of the intended transfer DNA I (T- DNA I) containing the dmo and the pat expression cassettes from PV- ZMHT507801 was integrated into the maize genome at a single locus. These analyses also showed no PV- ZMHT507801 backbone elements or T- DNA II sequences were present in MON 87419.

Inserted DNA

Only a single fragment of the genetic components integrated in a single insertion site in MON 87419 was detected using Next Generation Sequencing and Junction Sequence Analysis (NGS/JSA), directed sequencing (locus- specific PCR, DNA sequencing and

analyses) and bioinformatics (Monsanto, 2015a; Garnaat et al, 2014; DuBose et al., 2013; Kovalic et al., 2012).

DNA sequence analysis demonstrated the integrity and order of genetic elements. The results of the analysis confirm that the MON 87419 insert is 6,762 bp and that each genetic element within the T-DNA I is intact compared to PV-ZMHT507801, with the exception of the border regions. The border regions both contain small terminal deletions with the remainder of the inserted border regions being identical to the sequence in PV-ZMHT507801. The sequence and organization of the insert was also shown to be identical to the corresponding T-DNA I of PV-ZMHT507801 as intended. This analysis also shows that only T-DNA I elements were present. Moreover, the result, together with the conclusion of single DNA insert detected by NGS/JSA, demonstrated that no PV-ZMHT507801 backbone or T-DNA II elements are present in MON 87419 (Monsanto, 2015a; Garnaat et al., 2014). There were 602 bases of maize genomic DNA deleted during integration of the T-DNA I in MON 87419 (Monsanto, 2015; Garnaat, 2014; Salomon and Puchta, 1998).

Bioinformatics analyses were provided by the developer indicating that any amino acid sequence of each ORF's created in the insert or at the junction has no significant alignments with any known toxin or allergens (Monsanto, 2015a; Kang and Silvanovich, 2014).

No plasmid backbone sequences present in MON 87419 as presented by NGS/JSA using a combination of sequencing, polymerase chain reaction (PCR), and bioinformatics (Monsanto, 2015a).

Genetic Stability

The multigenerational stability of the DNA insert was demonstrated by NGS/JSA analysis using DNA from five breeding generations of MON 87419. The analysis demonstrated that the MON 87419 single integration locus was maintained through five generations (Monsanto, 2015a). The segregation analysis within three backcross generations was assessed using Chi-square ($\chi 2$) analysis (Monsanto, 2015a). Based on the results of Chi-square analysis provided by the developer, there are no statistically significant differences between the observed and expected segregation ratios for the three backcross generations of genetic backgrounds. This indicates that the introduced DNA segregates following Mendelian Principle of Inheritance.

Expressed Material

DMO is an enzyme that catalyzes the demethylation of dicamba to the non-herbicidal compound 3,6 dichlorosalicylic acid (DCSA) and formaldehyde (Chakraborty et al. 2005).

PAT is an enzyme classified as an acetyltransferase which acetylates glufosinate to produce non-herbicidal N-acetyl glufosinate. The PAT proteins are highly specific for glufosinate in the presence of acetyl CoA (Thompson et al. 1987; Wehrmann et al. 1996).\

The levels of the DMO and PAT proteins in various tissues such as leaf, root, forage and grain of MON 87419 were assessed by a validated immunoassay. DMO and PAT protein levels in these tissues are presented in Table V-1 and V-2 of the Monsanto petition to USDA (Monsanto, 2015, Section V.C. pp. 71-74).

Toxicological and Allergenicity Assessment

The novel proteins, DMO and PAT, were subjected to digestibility, heat inactivation, oral toxicity and amino acid sequence comparison studies to determine its potential to cause toxicity or allergenicity to humans. The DMO and PAT proteins produced from MON 87419 and from Escherchia coli were determined to be biochemically and functionally equivalent based on the results of N-terminal sequence analysis, MALDI-TOF MS mass fingerprint analysis, western blot analysis, functional activity analysis, glycosylation analysis, storage stability, apparent molecular weight and purity determination through SDS-PAGE (Li et al., 2015; Gu and Edrington, 2014).

Digestibility study using Simulated Gastric Fluid (SGF) with pepsin demonstrated that DMO and PAT proteins are readily degraded within 30 seconds of incubation with SGF, respectively, in presence of pepsin at pH 1.2, a characteristic of most non-toxic proteins (Monsanto, 2015a; Edrington and Calcaterra, 2015). In SIF, PAT also degrades readily with 99% degradation occurring within 5 minutes of incubation. The results indicate that PAT protein is readily digested as conventional protein in a typical mammalian gastric environment (Chen and Wang, 2015). A fragment with an apparent molecular weight of ~3.0 kDa was observed for 2 min following initiation of pepsin treatment, but this fragment was degraded within 5 minutes. In contrast, two peptide fragments of ~12 and ~21 kDa were observed after treatment with pancreatin for 5 min, but all of the peptide fragments were degraded within 15 minutes.

The DMO functional activity assay and SDS-PAGE analyses provided by the developer indicated loss of functional activity at elevated temperatures of 55 °C and greater due to protein denaturation. The activity is significantly impacted by heat treatment (Wang and Chen, 2014). When the DMO protein was heated at 75 °C for 30 min, a reduction in band intensity at ~ 39 kDa was observed. When the DMO protein was heated 95 ºC for both 15 and 30 min, the reduction in~ 39 kDa band intensity was even more pronounced. There was an appearance of higher molecular weight species at heat treatments of ≥55 ºC. When the test sample was heated.

For PAT, results of functional activity assay and SDS-PAGE indicated that the functional activity of the PAT protein was reduced by approximately 90% whether heated at 55^0C for 15 or 30 minutes (Brown, 2015).

The results of bioinformatics analysis using FASTA sequence alignment tool showed that DMO and PAT protein has no homology to any known toxins or allergens in the TOX_2014 database and AD_2014 database or other biologically active proteins that would be harmful to human or animal health (Monsanto, 2015b; Kang and Silvanovich, 2014).

Acute oral toxicity study of DMO and PAT proteins showed no treatment-related effects on survival, clinical observations, body weight gain, food consumption or gross

pathology (Smedley, 2014). The N Observed Adverse Effect Level (NOAEL) for DMO and PAT was considered to be 1000 mg/kg body weight and 2000 mg/kg body weight, respectively.

The DMO and PAT proteins have distinct modes of action. There is no known mechanism of interaction among these proteins that could lead to adverse effects in humans, animals or environment (Monsanto, 2015a). The DMO protein is likely to be distributed in chloroplast while PAT protein protein is expected to accumulate in the plastids of maize cells (Monsanto, 2015a).

The percent of total protein of DMO and PAT protein in MON 87419 is 0.00016% and 0.0008%, respectively.

Results of the digestibility, heat inactivation, amino acid sequence comparison and acute oral toxicity studies indicates that DMO and PAT proteins being expressed in MON 84760 corn are not toxic or allergenic to humans.

Nutritional Data

Compositional analysis was provided by the developer indicating the nutritional data of MON 87419 corn in comparison with the non-transgenic corn and range of literature values (Monsanto, 2015a; Tim et al., 2014). Results of the analysis indicated that there are no differences in the proximate, fiber, mineral, amino acid, fatty acid, vitamins and anti-nutrient of MON 87419 and the conventional corn that can be considered biologically relevant.

BPI-PPSSD'S RECOMMENDATION

For the transgenic MON 87419 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 soybean other than the tolerance to dicamba and glufosinate ammonium herbicides. After reviewing the provided material of Monsanto Philippines, Inc., it is therefore concluded that MON 87419 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

Corn provides macronutrients, vitamins and minerals in the human diet. It is a significant source of the nutritionally essential containing amino acids, methionine and cysteine and Vitamin E. Corn grain and its processed fractions are consumed in a multitude of food and animal feed products. Corn also contains Ferulic acid, p-coumaric acid, phytic acid and raffinose which are the anti-nutrients present in small amounts. It is not a source of toxicants, has a long history of safe use and is not a common source of allergen. Limited cases were reported however.

Most of the human consumption of corn is in the form of corn-based ingredients such as high fructose corn syrup, starch, sweeteners, cereals, oil and alcohol. It is used in salad and cooking oil, mayonnaise, margarine, baking and frying fat and in sauces and soups. It is also a common and an important feed ingredient of livestock such as corn gluten meal, corn gluten feed, and distillers dried grains, derived as co-products by wet and dry milling. It is consumed as silage or forage.

Corn and corn products only form 4.5 % of total food consumed on a per capita basis in the country. This is equivalent to 3.6 g/day consumption, out of the total 803 g/day consumption of "as purchased" corn food.

B. Transgenic Plant

Corn MON87419 is approved in the following countries: Australia, New Zealand (Food, 2016); Canada (Food and Feed and Cultivation, 2016); Japan (Food, 2017); Korea (Food, 2017 and Feed, 2016); Taiwan (Food, 2017); US (Food and Feed and Cultivation, 2016)

Since MON 87419 was shown to be compositionally equivalent to conventional corn, no changes in consumption amount by humans or animals or in the product's use pattern are expected to MON 87419.

C. Donor Organism

The DMO and PAT proteins produced by dmo and pat gene from S. maltophilia and S. viridochromogene, respectively were both evaluated for potential allergenicity using Bioinformatics analysis and in vitro digestibility in simulated digestive fluid. Results showed that these proteins were not structurally or immunologically similar with relevant amino acid sequences of known allergens thus, it cannot induce significant allergenic risk to both humans and animals. The plasmid vector PV-ZMHT507801 was used to produce MON 87419. All genetic elements and inserted regulatory sequences have been adequately summarized.

Strenotrophomonas maltophilia that contains demethylase gene (dmo gene) to confer tolerance to dicamba herbicide. *Streptomyces viridochromogenes* that contains phosphinothricin N-acetyltransferase (pat gene) to confer tolerance to glufosinate herbicide.

According to Herouet et. al (2005), they do not possess the characteristics associated with food toxins or allergens. DMO and PAT proteins are not known to be toxic or allergenic.

D. Transformation System

Agrobacterium-mediated transformation method was used. The target of genetic modification is the Nuclear DNA. The experiment protocol was completely provided and all genetic components used were completely described.

The plasmid vector PV- ZMHT507801 including the size, orientation and location of all genetic elements are completely described and no Carrier DNA and/or helper plasmids were used.

E. Inserted DNA

Using Next Generation Sequencing and Junction Sequence Analysis (NGS/JSA), directed sequencing, PCR and bioinformatics, characterization showed that MON 87419 contains only one copy of the T-DNA which contains the dmo and pat genes that are stably integrated at a single locus and is inherited over multiple generations under Mendelian principles.

A sequence comparison between the PCR product generated from the conventional control and the sequence generated from the 5' and 3' flanking sequences of MON 87419 indicates that 602 bases of maize genomic DNA were deleted during integration of the T DNA I. The remainders of the flanks in MON 87419 are identical to the conventional control. Such changes are common during plant transformation and these changes presumably resulted from double stranded break repair mechanisms in the plant during Agrobacterium-mediated transformation process

dmo gene has been inserted and expressed in soybean MON 87708 and cotton MON 88701, pat gene has been inserted and expressed in several commercially available glufosinate tolerant soybean, canola and maize products including T25, TC1507 and DAS-59122-7 maize. All necessary references for this claim were provided.

MON 87419 does not contain inserted sequence from the backbone of the transformation vector PV- ZMHT507801 as confirmed through NGS/JSA using a combination of sequencing, polymerase chain reaction (PCR), and bioinformatics.

F. Genetic Stability

NGS/JSA analysis demonstrated that the MON 87419 single integration locus was maintained through five generations thus confirming the stability of the DNA insert. Also, MON 87419 contains a single and stable T-DNA I insertion.

The segregation and stability were assessed through Chi-square ($\chi 2$) analysis over three generations which shows that the heritability and stability of the insert occurred as expected across multiple generations.

G. Expressed Material

The mean DMO protein levels are; 26 µg/g dw in leaf, 7.4 µg/g dw in root, 6.0 µg/g dw in forage, 0.19 µg/g dw in grain. The mean PAT protein levels are; 11 µg/g dw in leaf, 7.7 µg/g dw in root, 5.0 µg/g dw in forage, 0.39 µg/g dw in grain. Tissue samples were collected from leaf, root, forage, and grain from each replicated plot at all field sites treated with dicamba and glufosinate and measured by a validated immunoassay.

Neither DCSA nor formaldehyde generated by the action of DMO on dicamba pose a significant food or feed safety risk. On the other hand, PAT does not affect maize metabolism.

H. Toxicological Assessment

In pepsin, SDS-PAGE results showed that greater than 96.8% of the intact DMO was degraded within 0.5 min and a peptide fragment of ~3 kDa was degraded within 5 min. In pancreatin, Western blot showed that greater than 98.4% of the intact DMO protein was degraded within 5 min. Further, two peptide fragments of ~12 and ~21 kDa were degraded within 15 min.

Functional activity of DMO protein at 25 ºC and 37 ºC for 15 or 30 min was retained. E. coli-produced MON 87419 DMO protein behaves with a predictable tendency toward loss of functional activity at elevated temperatures of 55 °C and greater.

Using FASTA sequence alignment tool, there is no structurally relevant similarity exists between the MON 87419 DMO protein and any sequence in the TOX_2014 database, as no alignments displaying an E-score < 1e-5 were observed.

Acute oral toxicity assessment was performed in 10 male and 10 female CD-1 mice at dose of 1000 mg/kg body wt (bw). Results showed that there is no MON 87419 DMO protein-related differences on mortality, clinical observations, body weights, body weight changes, food consumption, or gross necropsy findings. NOEL is 1000 mg/kg bw.

The source of test protein was Escherichia coli. MON 87419-produced DMO protein was shown to be equivalent with E. coli-produced MON 87419 DMO protein.

Meanwhile, in pepsin, 98% of the E. coli-produced PAT protein was degraded within 0.5 min. On the other hand, at least 99% of the PAT protein was degraded within 5 min. in pancreatin. These results showed that E. coli-produced PAT protein is rapidly degraded by pepsin and pancreatin.

The functional activity of the PAT protein is retained following heat treatments at 25°C and 37ºC for 15 and 30 minutes but at elevated temperatures of 55ºC and greater, PAT protein loss its functional activity.

Possible homologies with MON 87419 PAT amino acid sequence were search using FASTA alignment tool in TOX_2014 toxin database. Results showed that no structurally relevant similarity exists between the MON 87419 PAT protein and any sequence and in the TOX_2014 database.

Acute oral toxicity assessment was performed in a group of 10 male and 10 female C57BL/6J mice administered with dose level of 2000 mg/kg body weight. Results showed no mortalities and no effects on body weight parameters and food consumption. Thus NOEL is 2000 mg/kg bw.

The source of test protein is Escherichia coli. MON 87419-produced PAT protein has shown equivalency with E. coli-produced MON 87419 PAT protein.

I. Allergenicity Assessment

MON 87419 DMO protein sequences show no structurally and immunologically or biologically relevant sequence similarities to allergens, gliadins, and glutenins.

Detailed characterization of the physico-chemical properties of MON 87419-produced DMO protein was shown in the attachment provided Monsanto Study Report MSL0026361. It is not glycosylated. The MW was calculated to be 39.8 kDa and is within the 10-70 kDa range.

The mean level of MON 87419 DMO protein in grain of MON 87419 is 0.19 µg/g dw. The mean percent dry weight of total protein in grain of MON 87419 is 11.52% (or 115200 µg/g). The percentage of MON 87419 DMO protein in MON 87419 grain is 0.00016% or 1.6 ppm.

On the other hand, using FASTA alignment tool, MON 87419 PAT protein sequences shows no structurally and immunologically relevant similarities to known allergens, gliadins, and glutenins in AD_2014 database.

Characterization of the PAT protein was provided in Monsanto Study Report MSL0026031 which shows that MON 87419-produced PAT protein is equivalent to E. coli- produced PAT protein.MON 87419 - produced PAT protein is not glycosylated. MW was calculated to be 25.2kDa and is within the range.

The mean level of PAT (pat) protein in grain of MON 87419 is 0.93 µg/g dw. The mean percent dry weight of total protein in grain of MON 87419 is 11.52% (or 115200 µg/g). The percentage of PAT (pat) protein in MON 87419 grain is ≈ 0.0008% or 8 ppm.

J. Nutritional Data

There were no significant difference ($p<0.05$) between Monsanto 87419 and conventional control in protein, fat, and ash for both forage and grain. All the values for forage and grain derived from the test were within the literature range (ILSI Crop Composition Database, 2011) A conventional control which has a genetic background similar to MON 87419 was grown under normal agronomic field conditions. All the values were within literature and the ILSI ranges. No significant difference ($p<0.05$) were found in the proximate analysis for grain and forage.

No significant difference ($p<0.05$) were found in key nutrients for forage, while Manganese found to be significantly different ($p<0.05$) between MON 87419 and Conventional having a p-value of 0.019 in the grain. A conventional control which has a genetic background similar to MON 87419 was grown under normal agronomic field conditions. All test values of proximate were within the literature and the ILSI ranges. All the values for forage and grain derived from the test were within the literature range (ILSI Crop Composition Database, 2011). None of the differences in nutrients are biologically meaningful.

There were no significant difference ($p<0.05$) between Monsanto 87419 and conventional control in the anti-nutrients found in the grain. There are some processing techniques to reduce or eliminate the anti-nutrients like soaking, germination, fermentation, and boiling. This methods were used to degrade the level of anti-nutrients to make feeds more digestible to animals.

BAI'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.

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 Monsanto Philippines Inc. for direct use as food and feed, or for processing of corn MON87419:

The following are the observations and recommendations:

Upon extensive review and evaluation of the documents provided by the Bureau of Plant Industry (BPI) on the application of Monsanto Philippines, Inc. for direct use as food and feed, or for processing of Corn MON87419, here under are the observations and appropriate actions:

1. From the evaluation of the application submitted by the proponent, including the scientific evidences from provided references and literature as well as other related studies, the Committee finds 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, water) and non-target organisms, to wit:

    a) 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 degrade upon exposure to the natural environment and general conditions (i.e. high temperatures (60 C and above), varying pH, enzyme digestion, etc.); and

    c) The protein product will not increase the weediness potential of the transgenic crop.

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

3. 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 because the receiving environment (areas near the port, roads, railways, etc.) is not conducive for plant growth/germination.

4. The BPI shall 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

The DENR-BC finds scientific evidence that the regulated article applied for as direct use as food and feed, or for processing is as safe as its conventional counterpart and is not expected to pose any significant risk to the environment and to non-target organisms. Based on the above considerations and with the proponent's sworn statement of accountability, we hereby submit our evaluation relative to Monsanto Philippines, Inc. MON87419 application for biosafety permit for food, feed and/or processing.

DOH'S ASSESSMENT AND RECOMMENDATIONS

After a thorough review and evaluation of the documents provided by the proponent, Monsanto Philippines Inc., 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 MON87419. 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. Scientific pieces of evidence from provided toxicity studies and references find that the regulated article will not cause significant adverse health effects to human and animal health.

2. Dietary exposure to the regulated article is unlikely to result allergic reaction.

3. The regulated article is as safe as food or feed derived from conventional corn varities.

4. The regulated article us not materially different in nutritional composition from that of the non-transgenic corn of the conventional corn.

5. It is suggested that the BPI ensure the following:

    a) Clear instructions that the product is only for the purpose of direct use for FFP and is not to be used as planting materials.

6. 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 MON87419.

SEC EXPERT'S RECOMMENDATION

The SEC expert has expressed that the information given by the applicant is satisfactory and well documented. The expert has recommended for the approval and issuance of biosafety permit of the said GM product.