ASSESSORS' CONSOLIDATED REPORT ON MONSANTO'S APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF SOYBEAN 40-3-2

EXECUTIVE SUMMARY

On November 29, 2017, Monsanto Philippines Inc. submitted soybean 40-3-2 for direct use as food and feed, or for processing, as original application under the DOST-DA-DENR-DOH-DILG Joint Department Circular (JDC) No. 1 Series of 2016.

After reviewing the Risk Assessment Report and attachments submitted by the applicant, the assessors namely: Scientific and Technical Review Panel (STRP), BPI Plant Products Safety Services Division (BPI-PPSSD) and Bureau of Animal Industry- Biotech Team (BAI-BT), concurred that soybean 40-3-2 is as safe for human food and animal feed as its conventional counterpart.

The Department of Environment and Natural Resources – Biosafety Committee (DENR-BC), after a thorough scientific review and evaluation of the documents related to Environmental Risk along with the submitted sworn statement and accountability of the proponent, recommended the issuance of a biosafety permit for this regulated event provided the conditions set by DENR are complied.

Also, the Department of Health – Biosafety Committee (DOH-BC), after a thorough scientific review and evaluation of documents related to Environmental Health Impact, concluded that soybean 40-3-2 will not pose any significant risk to the health and environment and that any hazards could be managed by the measures set by the department. DOH-BC also recommended for the issuance of biosafety permit for soybean 40-3-2.

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

BACKGROUND

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

The BPI Biotech Office provided the assessors, except for the SEC expert, the complete dossier submitted by Monsanto. 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 in relation to their application.

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

STRP ASSESSMENT AND RECOMMENDATIONS

Based on the documents submitted by the applicant:

A. Host Organism

The STRPs all concur that soybean is a source of key nutrients like one of the macromolecules called protein that is consumed by man and animals, but it also contains several anti-nutritional factors including lectin, trypsin inhibitors, isoflavones, phytic acid, raffinose and stachyose. Lectins are proteins that bind to carbohydrate-containing molecules and which inhibit growth. Lectins in raw soybean can inhibit growth and cause death in animal. It is expected that similar effects would occur in humans. Lectins are inactivated during the processing of raw soybean into protein products or soybean meal, such that the final edible soybean fractions contain minimal levels of lectins.

The STRPs also concur that soybean is one of eight allergenic foods responsible for approximately 90% of all food allergies. Soybean is less allergenic than other foods in this group and rarely responsible for severe, life-threatening reactions. Allergy to soybean is more prevalent in children than adults and is considered a transient allergy of infancy/childhood.

Further, they agree that soybean is highly versatile and can be processed into a wide variety of food products and that soybean meal is the premier supplemental protein source in livestock and poultry rations due to its nutrient composition, availability, and price. It is used to meet the animal's requirement for limiting amino acids, being the most cost-effective source of amino acids.

B. Transgenic Plant

The STRPs all concur that transgenic plant 40-3-2 has been reviewed and approved for food and/or feed use in many countries including Argentina, Australia/New Zealand; Bolivia; Brazil; Canada, China; Colombia; European Union; India; Indonesia; Japan; Korea; Malaysia; Mexico; Paraguay; Philippines; Russian Federation; Singapore; South Africa; Taiwan; US; Uruguay; Vietnam.

They also concur that the consumption pattern is not expected to be changed as a result of introducing 40-3-2 as 40-3-2 is not different in composition, safety or nutrition from conventional soybean, other than the introduction of the herbicide tolerance trait.

C. Donor Organism

The STRPs all concur that the donor organism is called Agrobacterium sp. strain CP4 for the gene cp4 epsps. This strain of bacterium is not known to be pathogenic to humans and animals, and it is not commonly allergenic. The proponent has also reviewed the history of safe use of Agrobacterium sp. strain CP4 as part of the safety assessment of this donor organism for other Roundup Ready crops.

They also concur that the CP4 EPSPS protein represents only approximately 0.08% of the total protein in Event 40-3-2 seed, which I agree. Therefore, the CP4 EPSPS protein would constitute a very small portion of the total protein present in feed and food derived from 40-3-2.

Further, they agree that plasmid vector PV-GMGT04 was used to produce 40-3-2 using the particle acceleration method. This vector is a derivative of the high copy E. coli plasmid pUC119 and was constructed by fusing the 1.3 kb Fspl-DraI fragment, containing the pUC119 origin of replication, to the 1.3 kb SmaI-HindIII fragment from pKC7, which includes the nptII gene cassette encoding resistance to a category of aminoglycosides comprising kanamycin, neomycin and paromomycin. Plasmid vector PV-GMGT04 contains two cp4 epsps and one uidA gene cassettes. PV-GMGT04 contains 10,511 bp. The T-DNA that is incorporated into the soybean genome is approximately 5.8 kb (~2,187 bp) contains a primary segment, functional insert containing a portion of the E35S

promoter, the petunia EPSPS CTP, the intact CP4 EPSPS gene, the complete NOS 3' transcriptional termination element and a 250 bp fragment of CP4 EPSPS and a second segment, non-functional insert comprised of a 72 bp segment of the CP4 EPSPS protein that co-segregates with the functional insert is also present in event 40-3-2. No mRNA or protein is produced from either the 72 bp CP4 EPSPS segment that comprises the second insert or the 250 bp of the CP4 EPSPS element adjacent to the 3' end of NOS on the functional insert.

They also agree that Agrobacterim tumefaciens strain CP4 is not known to be toxic or allergenic and that CP4 EPSPS protein is not known to be toxic or allergenic.

D. Transformation System

The STRPs all agree that the transformation method used was the particle-acceleration or gene gun method and that the target of genetic modification was the nuclear DNA. The STRPs also agree that the applicant provided a complete experimental protocol for developing the Event 40-3-2 containing the cp4 epsps gene.

E. Inserted DNA

The STRPs concur that molecular analyses confirmed that the genome of soybean 40-3-2 contains a single functional DNA insert comprised of a single copy of the cp4 epsps gene cassette under the control of the E35S promoter, and that the results were sufficiently demonstrated by Southern blot analysis, PCR, western blot analysis and ELISA.

They also concur that the organization of the genetic elements within the insert of 40-3-2 was confirmed by DNA sequence analyses. PCR and Southern blot analyses showed that one intact copy of the cp4 epsps expression cassette was integrated at a single chromosomal locus in 40-3-2. Additional studies indicated that a segment of DNA at the 3' end of the insert may have been rearranged during the insertion process.

Further, they agree that bioinformatics assessments, RT-PCR, Northern and Western blotting have been used to assess for additional transcripts and translation products. Several low abundance mRNA transcripts were detected that contain the region flanking the 3' end of the primary insert. This is not unexpected since plants often utilize and recognize multiple transcript polyadenylation signals, which results in multiple transcripts for a given gene.

They also agree that cp4 epsps gene has been inserted and expressed in other Roundup Ready crops that have been approved by many regulatory agencies in the world, including soybean, corn, cotton, canola, alfalfa and sugar beet, and that results of molecular analyses showed the lack of ori-PUC and nptII signals by PCR analysis and lack of CMoVb and GUS signals by Southern analysis. The molecular information obtained using more sensitive methods confirm the conclusion that plasmid backbone sequences have not been detected.

F. Genetic Stability

The STRPs all agree that the stability of the DNA insert across multiple generations was demonstrated by Southern blot and determination of hybridization fingerprint unique to 40-3-2.

They also agreed that F2 progenies of crosses between other soybean lines and GTS line 40-3-2 consistently segregate 3 tolerant to 1 sensitive, establishing that the 40-3-2 insert behaves as a single dominant gene inherited in a Mendelian fashion. The glyphosate tolerance phenotype and

Mendelian transmission has been consistent for more than seven generations of line 40-3-2 soybean tested to date. The genetic stability was further confirmed by DNA analyses.

G. Expressed Material

The STRPs all concur that CP4 EPSPS protein levels in soybean leaf and seed were determined by an enzyme-linked immunosorbent assay (ELISA) and that the mean level of the CP4 EPSPS protein in soybean seed from the 1992 trials was 0.301 µg/mg fresh weight for plants treated with the original Roundup herbicide. The mean protein levels in seed from the 1993 trials were 0.218 µg/mg fresh weight for plants treated with the original Roundup herbicide. The mean CP4 EPSPS protein level in soybean leaf tissue from the US from the 1993 trials was 0.489 µg/mg fresh weight for plants treated with the original Roundup agricultural herbicide.

Further, they also agree that the CP4 EPSPS protein was not detected above the limit of detection in soybean leaf or seed tissue from the non-transgenic A5403 parental variety in either year. Glyphosate binds to the endogenous plant EPSPS enzyme and blocks the biosynthesis of 5-enolpyruvylshikimate-3-phosphate (EPSP) in conventional plants. This deprives plants of essential amino acids. In Roundup Ready plants, the presence of CP4 EPSPS reconstitutes the shikimic acid pathway, able to continuously synthesize aromatic amino acids even in the presence of glyphosate.

H. Toxicological Assessment

The STRPs all agree that digestibility of the CP4 EPSPS protein assessed by western blot analysis demonstrated that the E. coli-produced CP4 EPSPS protein was rapidly digested after 15 seconds of incubation in SGF, the earliest time interval evaluated. The half-life of CP4 EPSPS in SIF is less than 10 min determined by Western blot. No EPSPS protein was detected after 100 min of incubation in SIF. CP4 EPSPS activity had decreased to < 9 % and <6 % of the initial level after incubation of 285 and 270 min.

They also agree that studies of the temperature dependence of CP4 EPSPS demonstrate that the enzymatic activity is eliminated after 15 minutes incubation at 65oC. It has also been determined that CP4 EPSPS is not heat stable, and all detectable functional enzymatic activity and ELISA reactivity are lost after the processing and toasting procedures that are conducted to make soybean fit for human and animal consumption.

Further, they agree that the FASTa-type algorithm, which is the standard method for database searching, was used to conduct the amino acid homology comparison between, the CP4 EPSPS protein and all available sequenced allergen and toxin proteins from all available electronic databases of protein sequences. The results showed no meaningful homologies between known allergens or toxins and the CP4 EPSPS protein sequence. The evidence indicates that CP4 EPSPS protein does not share any sequence similarity with the database of known sequenced protein allergens and toxins.

They also agree that acute oral toxicity assessment was conducted to evaluate potential adverse clinical signs or detrimental effects on mice exposed to E. coli-produced CP4 EPSPS protein. E. coli-produced CP4 EPSPS protein was administered as a single dose by gavage to three groups of 10 male and 10 female CD-1 mice at dose levels up to 572 mg/kg body weight (bw).

Lastly, E. coli-produced CP4 EPSPS protein for the safety assessment. It has been known that the E. coli-produced CP4 EPSPS protein has been shown to be equivalent to the soybean plant-produced CP4 EPSPS present in 40-3-2.

I. Allergenicity Assessment

The STRPs all agree that digestibility of the CP4 EPSPS protein assessed by western blot analysis demonstrated that the E. coli-produced CP4 EPSPS protein was rapidly digested after 15 seconds of incubation in SGF, the earliest time interval evaluated. The half-life of CP4 EPSPS in SIF is less than 10 min determined by Western blot. No EPSPS protein was detected after 100 min of incubation in SIF. CP4 EPSPS activity had decreased to < 9 % and <6 % of the initial level after incubation of 285 and 270 min.

They also agree that studies of the temperature dependence of CP4 EPSPS demonstrate that the enzymatic activity is eliminated after 15 minutes incubation at 65oC. It has also been determined that CP4 EPSPS is not heat stable, and all detectable functional enzymatic activity and ELISA reactivity are lost after the processing and toasting procedures that are conducted to make soybean fit for human and animal consumption.

Further, they agree that the FASTa-type algorithm, which is the standard method for database searching, was used to conduct the amino acid homology comparison between, the CP4 EPSPS protein and all available sequenced allergen and toxin proteins from all available electronic databases of protein sequences. The results showed no meaningful homologies between known allergens or toxins and the CP4 EPSPS protein sequence. The evidence indicates that CP4 EPSPS protein does not share any sequence similarity with the database of known sequenced protein allergens and toxins.

They also agree that the mean expression of CP4 EPSPS in line 40-3-2, based on ELISA analysis of seeds from 9 field sites, was 0.288 µg/mg tissue fresh weight (0.08% of the total protein). Therefore, the CP4 EPSPS protein represents a very small portion of the total protein in the harvested grain of 40-3-2.

Lastly, the proponent performed this serum screening. It has been known that the soybean is one of the eight allergenic foods that, together, are responsible for approximately 90% of all food allergens. However, soybean is less allergenic than other foods in this food group, and it is rarely responsible for sever, life-threatening reactions. The current studies demonstrate that the introduction of the gene encoding the EPSPS protein to confer glyphosate tolerance caused no discernible changes, either qualitatively, in the composition of endogenous soybean allergenic proteins in the glyphosate-tolerant varieties analyzed.

J. Nutritional Data

The STRPs all agree that compositional analyses of 40-3-2 were conducted on soybeans grown at six locations across the U.S. in 1992 and four locations in 1993; the Roundup Ready soybean plants were treated with the original Roundup herbicide. No statistical differences were observed between the proximate values (protein, ash, moisture, oil, fiber and carbohydrates) for Roundup Ready soybeans and the A5403 control at the 5 percent confidence level, confirming that the levels of these components in Roundup Ready soybeans are comparable to those of conventional soybeans (Taylor et al., 1999). Amino acid analysis was performed on Roundup Ready soybean and the A5403 soybean seed from the 1993 U.S. field trials. The levels of amino acids, including those of the aromatic amino acids (phenylalanine, tyrosine, and tryptophan) were comparable between the two lines. There were no significant differences observed at the 5% confidence level for any amino acid analyzed. The range of total genistein and total diadzein was variable across sites and is reflected in the ranges for both Roundup Ready soybean and the A5403 control soybean seed. However, the mean levels of total genistein and total diadzein in the Roundup Ready soybean seed are comparable

to those in the A5403 control seed. There were no significant statistical differences observed at the 5% confidence level (Taylor et al., 1999).

Compositional analyses have also been conducted on various processed fractions of soybeans, including toasted meal, defatted non-toasted meal, protein isolate, protein concentrate and oil (Padgette et al., 1996). Roundup Ready and A5403 control soybeans from the 1992 U.S. field trials were processed into the various fractions using procedures that mimic commercial processing procedures as closely as possible, although the scale was much smaller. The levels of macronutrients (protein, ash, fat, fiber and carbohydrates) in these fractions made from Roundup Ready soybeans were comparable to the levels in the fractions made from the parental soybean control cultivar. The fatty acid composition of the refined, bleached, deodorized oil (RBDO) processed from Roundup Ready oil was comparable to RBDO made from the control soybean line.

In all cases, the results of the analyses demonstrate that soybean seed and food components from Roundup Ready soybean event 40-3-2 are substantially equivalent to soybean seed and food components from conventional soybean varieties.

K. Recommendation

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

BPI-PPSSD ASSESSMENT AND RECOMMENDATION

Soybean 40-3-2 was developed by Monsanto, Philippines, Inc, through the use of recombinant DNA technology. The said event was developed through Particle-acceleration transformation of soybean cells with PV-GMGT04 plasmid vector carrying the cp4 epsps gene that encodes CP4 EPSPS protein which confers tolerance to glyphosate-containing herbicides.

Host Organism (Glycine max L.)

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

Soybean is a source of key nutrients such as proteins, fat, ash, acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber (TDF), crude fiber, carbohydrates, amino acids, fatty acids, minerals and vitamins (OECD, 2012). Soybean contains anti-nutritional factors such as oligosaccharides, trypsin inhibitors, lectins, phytic acid and other compounds such as isoflavones, phospholipids, sterols and saponins (OECD, 2012). Trypsin inhibitors and lectins are easily degraded upon heating. Soy saponins are not considered to be true anti-nutrients. High levels of soy saponins were found to have no adverse effect upon induction to laboratory animals. It has a weak effect on intestinal permeability and has little impact on active nutrient transport.

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

Transgenic Plant

DAS-81419-2 soybean has been reviewed and approved for food and/or feed use in many countries including Argentina (Food and Feed, 1996), Australia (Food, 2000), Bolivia (Food and Feed, 2005), Brazil (Food and Feed, 1998), Canada (Food, 1996; Feed, 1995), China (Food and Feed, 2002), Colombia (Food, 2005; Feed, 2007), European Union (Food and Feed, 2005), Indonesia (Food, 2011), Japan (Food, 2001; Feed, 2003), Malaysia (Food and Feed, 2010), Mexico (Food, 1996), New Zealand (Food, 2000), Paraguay (Food and Feed, 2004), Philippines (Food and Feed, 2003), Russian Federation (Food, 2007; Feed, 2008), Singapore (Food and Feed, 2014), South Korea (Food and Feed, 2002), Switzerland (Food and Feed, 1996), Taiwan (Food, 2002), United States of America (Food and Feed, 1995), Uruguay (Food and Feed, 1996) and Vietnam (Food and Feed, 2015) (ISAAA, 2018).

Based on the documents provided by the developer, the consumption patterns by population subgroups are not expected to be altered (Monsanto, 1994).

Donor Organisms

Agrobacterium sp. strain CP4 is the donor of cp4 epsps gene. The donor organism is not known for human or animal pathogenicity and is not commonly allergenic (Monsanto, 1994). History of safe use has been attributed to the donor organism since it is being used as donor organism of cp4 epsps in several other approved GM crops containing cp4 epsps gene.

Transformation System

The event, 40-3-2 was developed to express CP4 EPSPS protein derived from Agrobacterium sp. strain CP4. The transformation method is through Agrobacterium-mediated transformation with plasmid vector PV-GMGT04. The plasmid vector, is composed of the gene expression cassette for cp4 epsps (Monsanto, 1994).

PV-GMGT04 is composed of the following genetic elements: P-E35S – Cauliflower mosaic virus (CaMV) 35S promoter (51) with the duplicated enhancer region; CTP4 – N-terminal chloroplast transit peptide sequence from Petunia hybrid EPSPS gene; cp4 epsps – C-terminal 5 enolpyruvylshikimate-3-phosphate synthase gene (CP4 EPSPS) from an Agrobacterium species; NOS 3' – nontranslated region of the nopaline synthase gene; KAN – Tn5 neomycin phosphotransferase type II gene (nptII) from plasmid pKC7. The nptII confers kanamycin resistance on the bacterial cloning host; Ori-pUC – origin of replication from the high copy Escherichia coli plasmid pUC119; LAC – partial E. coli lacI coding sequence, the promoter Plac, and a partial coding sequence for β-d-galactosidase or lacZ protein from pUC119; P-MAS – TR 2' mannopine synthase promoter region; GUS – coding region of the E. coli β-glucuronidase gene. Expression of the gene in plants is used as a selectable marker for transformation; 7S 3' – nontranslated region of soybean 7S seed storage protein alpha' subunit; and CmoVb – figwort mosaic virus 35S promoter.

Inserted DNA

Southern Blot Analysis 40-3-2 DNA fragments digested with different combinations of restriction enzymes that cut outside and within the plasmid PV-GMGT04 demonstrated one intact copy of the t-DNA insert at a single locus (Monsanto, 1994). The integrity and order of the genetic elements within each insertion site is demonstrated using Southern Blot Analysis of 40-3-2 soybean from both R3 and R6 generations and confirmed by DNA sequence analysis. Results of analyses showed that

the insert has been stably integrated throughout the plant life cycle over three (3) generations (Kolacz and Padgette, 1994).

Based from the documents provided by the developer, 40-3-2 contains a 250 bp portion of the cp4 epsps sequence adjacent to the 3' end of the primary, functional insert and a second, small non-functional insert consisting of a 72 bp portion of the cp4 epsps DNA which co-segregates with the functional insert. Windels et al (2001) stated that a segment of the DNA at the 3' end of the insert may have been rearranged during the insertion process.

Bioinformatics, RT-PCR, Northern and Western Blot Analysis indicated several low abundance mRNA transcripts that contain the region flanking the 3' end of the primary insert which is expected since plants often utilize and recognize multiple transcript polyadenylation signals which results in multiple transcripts for a gene (Monsanto, 1994). Western Blot Analysis results showed that only the expected ~46 kDa CP4 EPSPS-containing protein was detected in 40-3-2. Southern Blot Analysis showed lack of ori-PUC and nptII signals by PCR analysis and lack of CmoVb and GUS signals indicates absence of plasmid backbone in soybean 40-3-2 (Lirette et al., 2000).

Genetic Stability

The multigenerational stability of the introduced traits is assessed by Southern Blot Analysis of three (3) generations of soybean 40-3-2 (Kolackz and Padgette, 1994). Results of analysis showed that the inserted DNA was maintained through three generations of 40-3-2.

Segregation is assessed by Chi-square (X2) analysis over multiple generations indicated that F2 progenies of crosses between other soybean lines and 40-3-2 line consistently segregates in a 3:1 ratio indicating that a single dominant gene was inherited following Mendelian Law (Padgette et al., 1995).

Expressed Material

The CP4 EPSPS protein is structurally similar and functionally identical to EPSPS enzymes found in conventional plants. Glyphosate usually binds to EPSPS enzymes and blocks the biosynthesis of shikimate-3-phosphate thus depriving plants from essential amino acids. However, in the presence of CP4 EPSPS requirements for aromatic amino acids and other essential nutrients in plant metabolism are met since CP4 EPSPS protein is a glyphosate resistant enzyme (Padgette et al., 1996).
The expression of novel protein in different plant parts is measured using ELISA methods (Taylor et al., 1999). The levels of CP4 EPSPS in seed ranges from 0.218-0.301 ug of protein/mg fresh weight.

Toxicological and Allergenicity Assessment

The safety assessment of novel protein, CP4 EPSPS, includes digestibility, heat inactivation, oral toxicity and amino acid sequence comparison studies to determine its potential to cause toxicity or allergenicity to humans (Monsanto 1994).

Digestibility study using Simulated Gastric Fluid (SGF) with pepsin demonstrated that CP4 EPSPS readily degraded within 15 seconds of incubation with SGF in presence of pepsin, a characteristic of most non-toxic proteins (Leach et al., 2002).

The temperature dependence of CP4 EPSPS enzymatic activity was determined through standard HPLC radioassay of aliquots of incubation mixture, including purified CP4 EPSPS from pMON17101,

subjected to heat at 15 minutes and 30 minutes. Padgette et al. (1993) indicated that enzymatic activity of CP4 EPSPS was completely abolished after 15 minutes of incubation at 60^0C.

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

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

Bioinformatics tool using FASTa algorithm for rapid database comparison indicated that CP4 EPSPS does not have significant sequence similarity to any known toxin or allergen (Mitsky, 1993).

Acute oral toxicity study provided by the developer indicated that the administration of 49, 154 and 572 mg/kg E. coli-produced CP4 EPSPS gave no mortality, no significant change in body weight or cumulative weight gain, no significant change in food consumption and no abnormal clinical signs in mice. This was in accordance with the study conducted by Harrison et. al (1996), CP4 EPSPS protein obtained from E. coli gave no mortality and no observable adverse or non-adverse effects in groups of 10 male and 10 female CD-1 treated mice. The No Observed Effect Level (NOEL) for CP4 EPSPS is 572 mg/kg body weight.

Test protein for toxicological study was obtained from E. coli and are biochemically and functionally equivalent to CP4 EPSPS expressed in 40-3-2 soybean (Harrison et al., 1993). Based on the documents provided by the developer, equivalency of the test protein was demonstrated in terms of apparent molecular weight, immunological recognition, protein glycosylation, amino-terminal sequence similarity, enzymatic activity and ELISA dose response. Apparent molecular weight was evaluated through SDS-PAGE (Harrison et al., 1993). Immunological recognition was demonstrated by western blot analysis. Protein glycosylation was assessed by detecting the presence of carbohydrate moieties associated with the blotted protein bands.

The average CP4 EPSPS expression in 40-3-2 soybean grain is 0.08%. (Monsanto, 1994, Part IV, Section B.1.a, p. 56)

Serum screening was performed (Burks and Fuchs, 1995). Result of the study demonstrated that the introduction of cp4 epsps gene caused no discernible changes in the composition of endogenous soybean allergenic properties in the glyphosate-tolerant varieties analyzed.

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

Nutritional Data

Compositional analysis provided by the developer indicating the nutritional data of 40-3-2 in comparison with the non-transgenic soybean, and range of literature values (Monsanto, 1994; Taylor, 1999). Results of the analysis indicated that there is no differences in the proximate, amino acid, fatty acid, anti-nutrient and secondary metabolite levels of 40-3-2 soybean seeds and the non-transgenic soybean that can be considered biologically relevant.

Conclusion

For the transgenic 40-3-2 soybean, weight of evidences approach indicated 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 glyphosate-containing herbicides. After reviewing the provided material of Monsanto Philippines, Inc., it is therefore concluded that 40-3-2 soybean is as safe as its conventional counterpart.

BAI ASSESSMENT AND RECOMMENDATIONS

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

A. Host Organism

Soybean in its processed form is one of the best sources of plant proteins and oil which are used in many products both for human and animal. Especially important is it high content of essential amino acids, particularly lysine, leucine and isoleucine.Soybeans especially in its raw state, contain trypsin inhibitors, lectins, isoflavones, phytic acid, raffinose and stachyose. However, the activity of these antinutrients are deactivated during heat processing.

Soybeans do not contain toxicants though sometimes lectins are also regarded as toxicants which has the ability to bind carbohydrate-containing molecules on the epithelial cells of the intestinal mucosa. The cells of the intestine in the presence of lectin tend to collapse by reducing the absorption. Though it can be degraded by intestinal digestive enzymes, lectins are also inactivated during the processing of raw soybean into protein products such that it only contains minimal levels of lectins. Further, Soybean allergy is one of the more common food allergies. Although rarely causes severe and potentially life-threatening reactions, of all allergies it is estimated to cause 90% of the occurrences.

Soybean can be processed into a wide variety of food products. Various soy products are available, including soy flour, soy protein, tofu, soy milk, soy sauce, and soybean oil. Soybean meal is the premier supplemental protein source in livestock and poultry rations due to its nutrient composition, availability, and price. Typically, soybean meal is used to meet the animal's requirement for limiting amino acids, as it is the most cost-effective source of amino acids.

B. Transgenic Plant

Based on the information provided, foods and feeds derived from 40-3-2 soybean are as safe, as nutritious as the non-conventional soybean. So, it is not expected that consumption patterns will change. The summary of approvals are as follows:

COUNTRIES	Approved for Food	Year	Approved for Feed	Year	Approved for Environment	Year
Argentina	/	1996	/	1996	/	1996
Australia/New Zealand	/	2000				
Bolivia	/	2005	/	2005	/	2005
Brazil	/	1998	/	1998	/	1998
Canada	/	1996	/	1995	/	1995
China	/	2015	/	2015	/	2015
Colombia	/	2005	/	2007	/	2010
European Union	/	2012	/	2012	/	2012
India	/	2007				
Indonesia	/	2011				
Japan	/	1996	/	1996	/	1996
Korea	/	2010	/	2004		
Malaysia Mexico	/ /	2012 1996	/ /	2010 1996	/	2010
Paraguay	/	2004	/	2004	/	2004
Philippines	/	2013	/	2013		
Russian Federation	/	1999	/	2013		
Singapore	/	2010	/	2010		
South Africa	/	2001	/	2001	/	2001
Taiwan	/	2017	/	2017		
USA	/	1995	/	1995	/	1994
Uruguay					/	1996
Vietnam	/	2015	/	2015		

C. Donor Organism

The source organism has a history of safe use and the cp4 epsps gene has no pathogenic or allergenic properties. All potentially inserted regulatory sequences were described sufficiently

Agrobacterium sp. has a history of safe use and is not known to be toxic or allergenic. CP4EPSPS is the substrate in the shikimic acid pathway which is not present in mammalian, avian or aquatic animals. Its selective activity is in plants only, hence, is not known to have adverse effects on human and animal health.

D. Transformation System

The method used was the Particle-acceleration transformation and the target of modification is nuclear DNA.

The experimental protocol was provided. All the genetic components used were listed with the required information. All required information were provided. Carrier DNA and/or helper plasmids were not used

E. Inserted DNA

PCR and Southern blot data indicated that there was only a single DNA insertion site. Other methods included genome walking, cosmid library screening, DNA sequencing and Northern blot analysis (Lirette et al, 2000).PCR, Southern blot and sequencing demonstrated the integrity and order of genetic elements within the insertion site.

The newly described CP4 EPSPS segments produce no detectable mRNA or protein based on: 1) northern blot analyses which demonstrate no detectable transcription of either CP4 EPSPS segment, and 2) western blot analysis in which only the predicted, full-length protein encoded by the functional CP4 EPSPS insert is produced (MSL16712).

In addition, bioinformatics analyses on the open reading frames (ORFs) present in the region beyond the 3' end of the cp4 epsps coding region, including the region of soybean genomic DNA flanking the 3' end of the functional insert, do not show any homology to known toxins or allergens. (Dobert et al, 2002).

Several molecular analyses methods confirmed the presence of a single functional cp4 epsps gene cassette and no plasmid backbone sequences.

F. Genetic Stability

It was through the Southern blot analysis of 3 generations demonstrated the stability of the insert. Molecular analysis of the R3 through the R6 generations demonstrated the stability of the introduced trait inherited in the expected Mendelian pattern. Results are consistent with a single insert.

G. Expressed Material

CP4 EPSPS level in seed was 0.239 ug/mg FW and 0.288 ug/mg FW in the Puerto Rico and US sites, respectively (MSL 12904). Taylor et al (1999) reported levels of 0.301 ug/mg and 0.218 ug/mg FW in seed treated with glyphosate for the 1992 and 1993 field trials, respectively. In leaves, the maximum CP4 EPSPS expression level observed in both the US and Puerto Rico data was 0.851 ug/mg leaf FW (MSL 12904). Protein levels in soybean leaf and seeds were determined by an enzyme-linked immunosorbent assay (ELISA).

Glyphosate controls weeds by inhibiting EPSPS in the shikimate pathway for aromatic amino acid biosynthesis in plants resulting to growth inhibition. CP4 EPSPS confers glyphosate tolerance by sustaining biosynthesis of aromatic amino acids in the shikimate pathway.

H. Toxicological Assessment

Pepsin was used in the digestibility study for CP4 EPSPS. The results of this experiment demonstrate that the E. coli-produced CP4 EPSPS protein was rapidly digested after 15 seconds of incubation in Simulated Gastric Fluid based on Western blot analysis. Digestibility of the CP4 EPSPS protein was assessed by western blot analysis. The half-life of CP4 EPSPS in Simulated Intestinal Fluid is less than 10 min determined by Western blot. No CP4 EPSPS protein in both SIF and SGF.

After heat treatment immunoreactivity of CP4 EPSPS was no longer detectable. Studies of the temperature dependence of CP4 EPSPS demonstrate that the enzymatic activity is eliminated after 15 minutes incubation at 65°C. The effect of heat treatment on the immunologically detectable levels of the CP4 EPSPS protein in 40-3-2 was determined using ELISA and Bioassay.

Using FASTa-type algorithm the amino acid homology comparison between the CP4 EPSPS protein and all available sequenced allergen and of known sequenced protein allergens and toxins was conducted and it was found that there were no homology to known toxins or allergens.

An acute oral toxicity assessment was conducted to evaluate potential adverse clinical signs or detrimental effects on mice exposed to E. coli-produced CP4 EPSPS protein. E. coli-produced CP4 EPSPS protein was administered as a single dose by gavage at dosages up to 572mg/kg. There were no treatment-related effects on survival, clinical observations, body weight gain, food consumption or gross pathology. Therefore, the No Observable Adverse Effect Level (NOAEL) for CP4 EPSPS was considered to be 572 mg/kg bw. The source of CP4 EPSPS, the test protein was E.coli. The E. coli-produced CP4 EPSPS protein equivalency was demonstrated using SDS-PAGE. Protein bands were visualized by both Coomassie blue stain and Western blot.

I. Allergenicity Assessment

Pepsin was used in the digestibility study for CP4 EPSPS. The results of this experiment demonstrate that the E. coli-produced CP4 EPSPS protein was rapidly digested after 15 seconds of incubation in Simulated Gastric Fluid based on Western blot analysis. Digestibility of the CP4 EPSPS protein was

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The total percentage of CP4 EPSPS protein in grain is 0.08%. The mean expression of CP4 EPSPS in line 40-3-2, based on ELISA analysis of seeds from 9 field sites, was 0.288 µg/mg tissue fresh weight (0.08% of the total protein). Therefore, the CP4 EPSPS protein represents a very small portion of the total protein in the harvested grain of 40-3-2

Serum screening was also done and the assessment demonstrates similar and comparable results of the binding potential of human serum IgE antibodies between the proteins extracted from Soybean 40-3-2 and conventional soybean varieties.

J. Nutritional Data

There were no observed statistical differences observed between the proximate values (protein, ash, moisture, oil, fiber and carbohydrates) for Roundup Ready soybeans and the A5403 control at the 5 percent confidence level, which lead us to confirm that the levels of these components in Roundup Ready soybeans are comparable with those of conventional soybeans. No significant differences in the levels of any of the 18 amino acids measured, including aromatic amino acids, were found between GTS seeds and the control soybean seeds.

The levels of the total and free forms of the isoflavones genistein and daidzein and bound coumestrol and biochanin A were determined. However, only minute quantities of biochanin A were detected, and the bound coumestrol was lower than the confidence limit of the assay (10ug/g). No significant differences were detected in any of the isoflavones measured (free, bound, total) in GTS soybean seeds relative to control. The levels of anti-nutrient in 40-3-2 are compositionally equivalent to that of the conventional soybean. In addition, 40-3-2 is intended to provide herbicide tolerance; therefore, no changes should be anticipated on amount of material consumed, the characteristics of the edible parts of the plant or in the use pattern and processing procedure of 40-3-2, compared to conventional soybean.

In general, soybean processing for food use includes various procedures such as steam cooking, baking, drying and cooling, fermenting, roller milling, grinding and etc. Some degree of heating, mechanical and chemical disruption may accompany those processes, which may or may not affect the level of anti-nutrients. Nevertheless, the level of anti-nutrients in processed products of 40-3-2 and the conventional control would be expected to be similar, as there was no difference in the levels of anti-nutrients in 40-3-2 compared to control soybean.

K. 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 ASSESSMENT AND RECOMMENDATION

After a comprehensive review and evaluation of the documents including the scientific evidences from provided references and literature submitted by Monsanto Philippines, Inc., on its application

for Direct Use as Food and Feed, or for Processing of Soybean 40-3-2, hereunder are the observations and appropriate actions:

1. The direct use of the regulated article whether for food, feed, or for processing will not cause any significant adverse effect on the environment (land and water) and non-target organisms. The transgenic crop will not increase its weediness potential in case the seeds spill out into the environment because the protein product produced by the transgenic crop will degrade upon exposure to the natural environment and general conditions (i.e., high temperatures [60 degrees Celsius and above], varying pH, enzyme digestion, etc.)
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. Furthermore, 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 conducive for plant growth.
3. The Bureau of Plant Industry (BPI) shall ensure the proper and secure packaging of the regulated article for transport and the safety and durability of the transport vehicle, for prevention of any possible spillage or unintended release during transport/import as per BPI's inspection in the port area.

Based on the review and evaluation, the DENR-BC considered the regulated article safe to the environment and to non-target organisms and hereby submits the technical report relative to the application of Monsanto Philippines Inc. soybean 40-3-2 for Biosafety Permit for direct use as food, feed or for processing.

DOH ASSESSMENT AND RECOMMENDATION

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 Soybean 40-3-2. I/We,

Find that the regulated article applied for Direct Use for Food and Feed or for Processing (FFP) is safe as its conventional counterpart and shall not pose any significant risk to human and animal health, and environment.

The following are the observations and recommendations:

1. Scientific pieces of evidence from 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 soybean varieties
4. The regulated article is not materially different in nutritional composition from that of the non-transgenic soybean or the conventional soybean.
5. It is suggested that the BPI ensure the following:
    a. There shall be 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 Soybean 40-3-2.

SEC ASSESSMENT AND RECOMMENDATIONS

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

A. Socio-economic issues

Trademap data indicates that soybean meal imports actually reached 2.3 million tons in 2016, versus 2.5 million tons forecasted.

Soybean production varied from 544 tons (2016) to 807 tons (2013); imports went from 58,289 tons in 2012 to 151,335 tons in 2016. Clearly the Philippines relies heavily on imported soybean. (The foregoing does not include processed imports such as oilcake; this is reflected already in Monsanto submission.)

The SEC Expert concur that GM soybean will not drastically change current production and consumption patterns. There might be a slight increase in imports owing to the lower price of GM soybean. Downstream industry (meat producers) and consumers will benefit from cheaper feed. Local production is very small so additional competition will not cause significant displacement.

B. Recommendation

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