CONSOLIDATED REPORT OF MONSANTO PHILIPPINES' SOYBEAN MON87701 APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING

EXECUTIVE SUMMARY

On December 2, 2016 Monsanto Philippines Inc. submitted soybean MON87701 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 MON87701 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 MON87701 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 MON87701.

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

A. Host Organism

The STRPs agree that soybean is known to be the foremost provider of plant protein and oil. Various edible soy protein products are derived from it upon removal of oil. Soy flour, grits and hull also find their way to various food products. Soybean meal is used as animal feed. Reports also describe soybean as the source of anti-nutritional factors like trypsin inhibitors, lectins, phyto-estrogens, stachyose, raffinose and phytic acid. Lectins are proteins that are capable of binding to carbohydrate-containing molecules. They have high degree of affinity to the sugar component of the molecule. They are known to inhibit growth and cause death in animals. However, lectins are heat labile and since soybean seeds undergo a series of processing including high temperature, most if not all of their lectin contents are expected to be degraded.

The STRPs also agree that soybean is seen as one of eight most significant source of allergen in foods. Comparative studies on allergenic reactivity of soybeans and other major food proteins derived from clinical data of animal models and biochemical approaches revealed diminished allergenicity and no striking differences in allergenicity and immunological reactivity for soy proteins over those of other food allergens, and that they are used and processed into various forms of food products. Foods derived from soybeans are classified as soybean oil, traditional soyfoods, soy protein products, modern soyfoods, soybean-enriched foods and functional soybean ingredients/dietary supplements. The non-oil component of soybeans, specifically the soybean meal is the primary source of limiting amino acids and proteins in livestock and poultry feeds.

Further, they concur that the consumption pattern is not expected change as a result of the introduction of the transgenic MON 87701.

B. Transgenic Plant

The STRPs all concur MON 87701 is a soybean line that has been genetically engineered for insect resistance. It contains the cry1Ac gene which encodes for the crystal protein Cry1Ac. The protein confers resistance to specific lepidoterans namely, velvetbean caterpillar (Anticarsia gemmatalis), soybean looper (Pseudoplasia includes), soybean axil borer (Epinotia aporema), and sunflower looper (Rachiplusia nu).

They also noted that based on the GM Approval Database of the International Service for the Acquisition of Agri-Biotech Applications (ISAAA), the transgenic plant MON-87701 Soybean has been approved as food in the following countries: Australia/New Zealand (2010), Canada (2010), China (2010), European Union (2012), Indonesia (2013), Japan (2011), Korea (2011), Mexico (2010), Russian Federation (2013), Sinagpore (2016), Taiwan (2016), USA (2010), Vietnam (2015)

Further, they agree that introduction of the genetically modified MON 87701 as a lepidopteran resistant soybean variety may not change the consumption patterns of soybean. The genetically modified MON 87701 does not differ in composition, safety, or nutritional components from conventional soybean, except the introduction of the lepidopteran resistant trait.

C. Donor Organism

The STRPs are in agreement that the applicants described the development of a biotechnology-derived, insect-protected soybean MON 87701 that produces the Cry1Ac insecticidal crystal protein derived from a Gram-positive soil bacterium Bacillus thuringiensis (Bt) subsp. kurstaki.Both references cited very well attest that food and feed product containing MON 87701 or derived from MON 87701 are as safe as soybean currently on the market for human and animal consumption.

They concur that in Section IV (Genetic Elements) and Table IV-1 describe all the genetic elements included in the plasmid used, PV-GMIR9, which contains two T-DNAs. One of the T-DNAs carries the gene of interest and the other one carries the selectable marker gene. Figure IV-1 completely illustrates the plasmid map. PV-GMIR9 contains the following: T-DNA I is the expression cassette for cry1Ac targeted for expression in the chloroplast through the CTP1 targeting sequence; T-DNA II is the expression cassette for cp4 epsps which is the selectable marker gene; T-DNA borders which delineate the T-DNAs; and some other genetic elements outside the T-DNA borders that are essential for the maintenance and propagation of the vector in bacteria.

In addition, they also concur that the T-DNA I cassette has the gene of interest, cry1Ac, encoding for the Cry1A protein. T-DNA II cassette has the cp4 epsps encoding for 5-enol pyruvylshikimate-3-phosphate synthase. This second gene was only expressed in the very early stage of development of the GM crop. It was subsequently bred out in the breeding pathway and is no longer expressed in the final GM product which is MON 87701.

Furthermore, the STRPs agree that the safety of the donor Organism: Bacillus thuringiensis is well explained on pages 96-97 and that the only protein encrypted by expressible sequences is the CryAc protein. No toxic or allergenic property has been reported for CryAc protein. Cry proteins are microbially-derived products that were reportedly obtained from B. thuringiensis commercially used to produce biopesticides because of their described history of safe use. Cry1Ac proteins have been utilized in the production of the well-known GMO Bollgard and Bollgard II cotton which was made resistant to lepidopteran pests.

D. Transformation System

The STRPs reported that the transformation method used was Agrobacterium tumefaciens – mediated transformation, the nuclear DNA was described as the target of genetic modification, and that the experimental protocol was completely provided.

They also reported that all the genetic elements of the recombinant vector PV-GMIR9 were described in Section IV on pages 41-49 of Petition for the Determination of Non-Regulated Status for MON 87701 (2009). The components were listed, described and references cited in Table IV-1 on pages 45-47. The components include regulatory sequences (promoters, terminators, etc.), T-DNA borders, cry1Ac, cp4 epsps, CP1 targeting sequence, other non-coding regions, and other elements that would allow maintenance of the plasmid in bacteria.

Further, they concur that the plasmid vector PV-GMIR9 is a two T-DNA vector, it is flanked by the two right border regions and two left border regions where one set is for T-DNA I while the other set is for T-DNA II. The plasmid vector PV-GMIR9 contains the right border and left border regions that originated from Agrobacterium tumefaciens plasmids. The right border and left border regions delineate the T-DNA and are involved in the efficient transfer into the soybean genome.

They also concur that carrier DNA and/or helper plasmids were not used.

E. Inserted DNA

The STRPs agree with the applicant that the data on molecular analyses of the insert showed that MON 87701 soybean contains a single copy of T-DNA I at a single insertion site with no detectable T-DNA II elements. The result was validated by Southern blot analysis, PCR and analysis of DNA sequences.They are also in agreement that the integrity and order of genetic elements within each insertion site was determined by first amplifying through PCR the inserted gene and then sequencing of the PCR products. DNA sequencing allowed the determination of the exact composition and organization of the inserted DNA. Nine overlapping regions spanning the insert length of 6,426 base pairs were amplified and sequenced. Sequence analysis revealed that the arrangement of the insert is identical to that of the T-DNA I/gene in the recombinant plasmid PV-GMIR9. Figure V-1 on page 52 is a schematic representation of the insert and the genomic flanking sequence of MON 87701.

They added that the applicant report changes related to deletions and insertion may be common during the Agrobacterium-mediated transformation process. A double strand break (DSB) repair system reportedly exists during transformation as it can offer a mechanism by which spreads sequences into new chromosomal positions. This may likewise represent pathway through which T- DNA can integrate ito the plant genome.

Further, they concur that the main transgene is being expressed in Bollgard cotton. The Cry1Ac protein in Bollgard cotton is different to the Cry1Ac protein in MON 87701 by four amino acids. These four amino acids at the N terminal of the polypeptide in MON 87701 correspond to the CTP-coding region which allows the targeting of the Cry1Ac protein to choloroplasts. Excluding these four amino acids, the actual coding regions of Cry1Ac in Bollgard cotton and MON 87701 are 100% identical.

They also concur that no traces of of allergenic, toxic or biologically adverse features were demonstrated in characterized polypeptides or protein in the insert or at the junction based on evaluations of Open Reading Frames through bioinformatics and that no plasmid backbone sequences were detected in MON 87701. This was evident from the Southern blot analyses using four different probes against the plasmid backbone.

F. Genetic Stability

The STRPs has expressed that the applicant has provided sufficient evidence that the single integration locus of MON 87701 was analysed regularly through Southern blot analysis. Southern blot analysis demonstrated that the DNA insert stability of the DNA insert is seen across multiple generations. Further analyses demonstrated that the single integration locus introduced in MON 87701 is sustained through five generations of continuous breeding.

They also agree that there is enough evidence pointing the heritability and stability of the insert occurred as expected across multiple generations based on segregation analyses. This supports stability of the molecular insert which defines the genetic character of the T-DNA I found at a single chromosomal locus and at a single insertion site of the gene in MON 87701.

G. Expressed Material

The STRPs agree that information provided by the applicant are sufficient on the levels of Cry1Ac protein in various tissues of MON 87701. They were evaluated by enzyme-linked immunosorbent assay (ELISA). Samples for analysis included leaves from four growth stages (over season leaf (OSL) 1 through OSL4), forage, root, mature seed and pollen/anther tissue samples, and Table VI-1 on page 80 shows that Cry1Ac was highest in leaf (OSL 4 - 340 µg/g dwt), followed by forage (34 µg/g dwt), and mature seed (4.7 µg/g dwt). Cry1Ac levels in root and pollen/anther were not indicated because of their very low levels.

They also agree that the mean Cry1Ac protein in MON 87701 in over-season leaf tissues throughout the growing season across all sites varied from 220 to340 µg/g dwt. Cry1Ac protein in leaves reportedly remained constant across sampling intervals but levels were within a broader ranges as the growing season advanced.

Further, they concur that Cry1Ac has no metabolic role. The mode of action of Cry proteins (δ endotoxin) is very well understood, documented and reviewed (English and Slatin, 1992; Gill et al., 1992; Schnepf et al., 1998; Zhuang and Gill, 2003). Crystal proteins are first solubilized in the midgut of insects. This is followed by activation of the protoxins to active toxins through the action of midgut proteases. In susceptible insects, the activated toxins bind to specific midgut membrane receptors, insert into apical membrane and form pores. It is the loss of osmotic regulation and eventually cell lysis due to the formation of pores which is believed to be the cause of insect death.

H. Toxicological Assessment

The STRPs expressed agreement with the information provided by the applicant on the toxicological assessment of Cry1Ac. They agree that the digestibility experiments conducted on the Cry1Ac protein using SDS-PAGE and Western blot analysis are sufficient. Results showed that the full-length protein was rapidly digested in SGF (simulated gastric fluid), although a small, ~4 kDa Cry1A transiently stable fragment was formed. But this ~4 kDa Cry1Ac protein fragment was degraded within 30 sec after exposure to simulated intestinal fluid (SIF).

They also agree that the results of Western blot proved that the heat treatment of MON 87701 seed decreased the level of immunodetectable Cry1Ac protein in extracts. The amount of immunodetectable Cry1Ac protein in extracts of heated MON 87701 was below the limit of detection (LOD) that demonstrated a decrease of 94% compared to the protein level in extracts of unheated MON 87701 samples. These data demonstrate that the heating of ground soybean seed

like those applied in the manufacture of soybean flour renders the loss of immunodetectable Cry1Ac protein as a result of degradation of the protein into an insoluble complexes.

In addition, they concur on the bioinformatic alignment searches of the Cry1Ac protein sequence against toxin database which showed that there is no structural similarity and homology of MON 87701 Cry1A with any protein toxin with adverse effects on mammals (page 82 of Petition for the Determination of Non-Regulated Status for MON 87701, 2009).

Further, as part of the safety assessment of the Cry1Ac protein expressed in MON 87701, results of acute toxicology study further confirmed the safety of Cry1Ac. Administration of the Cry1Ac protein at a dose of 1290 mg/kg body weight to 10 male and 10 female CD-1 mice did not elicit treatment-related effects on survival, clinical observations, body weight gain, food consumption or gross pathology, although a significant reduction in body weight was reported in males. Further investigate on the possible effect of the Cry1Ac protein on body weight, additional group of 10 male CD-1 mice was dosed by oral gavage at a dose of 1,460 mg/kg body wt (given at two equal doses, 4 hrs apart). No effect on body weight in males were observed. Again, effect on body weight in males given a 1,290 mg/kg Cry1Ac was not reproduced in the repeat study. The NOAEL for the Cry1Ac protein was reported at 1,460 mg/kg in males while it was 1,290 mg/kg in females.

Lastly, they agree that the test Cry1Ac protein used was produced by an E. coli – expression system. The bacterial-derived Cry1Ac protein was shown to be physicochemically and functionally equivalent to the Cry1Ac protein produced in MON 87701

I. Allergenicity Assessment

The STRPs expressed agreement in the information provided by the applicant that the digestibility of Cry1Ac protein in SGF was assessed by SDS-PAGE and Western blot. The full-length Cry1Ac protein was digested below the LOD (0.0025 µg) within 30 sec of digestion in SGF as demonstrated in SDS-PAGE analysis while the Cry1Ac protein was digested below an LOD of 0.5 ng within 30 sec of incubation in SGF as validated by Western blot analysis. A ~4 kDa protein fragment was observed throughout the digestion in SGF while it apparently degraded to a smaller fragment (about ~3.5 kDa) was visible at the 30 min and 60 min time points. Further digestion of the transiently stable Cry1Ac protein with pancreatin in SIF following digestion in SGF for 2 min demonstrated complete degradation with 30 sec in SIF when assessed by SDS-PAGE.

They also agree that Western blot analysis was used to determine the effect of heat treatment on Cry1Ac protein produced by MON 87701. Results showed that heating greatly decreased the amount of immunodetectable Cry1Ac and that its amount is below the level of detection. This only indicates that heating results in the loss or degradation of Cry1Ac

Moreover, bioinformatic alignment searches of the Cry1Ac protein sequence against known allergens, gliadins, and glutenins showed that there is no structurally- or immunologically-relevant amino acid sequence similarities between MON 87701 Cry1A and any allergen, and that a small portion of Cry1Ac protein in harvested MON 87701 soybean seed is 4.7 µg/g dry wt. Its proportion over the mean % dry weight of total protein in harvested seed from MON 87701 is 39.27% (or 392,700 µg/g) which demonstrates that the Cry1Ac in the transgenic material constitutes a small proportion (0.0012%) of total soybean protein.

Further, they also pointed out that serum screening was performed. Protein extracts were obtained from seeds of MON 87701, a conventional control – A5547, and reference soybean varieties. Human serum IgE antibodies from soybean-allergic subjects were reacted with the seed protein extracts. Tolerance level at 99% was calculated based on IgE binding values observed from the extracts of the reference soybean varieties. Comparison of the binding values of the extracts from MON 87701 and A5547 with the tolerance interval revealed that the binding values of the GM crop and the conventional control were within the reference tolerance level. This means that the soybean-

specific IgE binding of MON 87701, A5547 and the reference soybean varieties are comparable if not equivalent.

J. Nutritional Data

The STRPs concur that the comparator used was a conventional soybean, A5547. MON 87701 was actually derived from this soybean variety. No statistically significant differences ($p<0.05$) between MON 87701 and A5547 were obtained for ash, moisture, crude carbohydrates, crude proteins, acid detergent fiber and neutral detergent fiber. They also concur that no statistically significant differences ($p<0.05$) between MON 87701 and A5547 were obtained for different amino acids, fatty acids, and vitamin E.

Furthermore, they agree that significant differences were reportedly detected in trypsin inhibitor and daidzein of seeds from MON 87701 in the combined-site analysis. The differences were low and were within the 99% tolerance interval. The differences were not regarded as biologically relevant as these are comparable to values described in scientific literature and in ILSI-CCD.

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

Host Organism (Glycine max)

Soybean (Glycine max) has been consumed by humans in the form of soy sprouts, baked soybeans, roasted soybeans, full fat soy flour, soybean oil and traditional soy foods such as miso, soy milk, soy sauce, and tofu (OECD, 2012). Unprocessed soybeans has been limitedly used as feed since it contain anti-nutritional factors such as trypsin inhibitors and lectins. Adequate heat processing inactivates these factors. Other anti-nutrients found in soybean includes stachyose, raffinose and phytic acid. On the other hand, soybean is also a source of key nutrients such as amino acids, fatty acids, carbohydates, vitamins, minerals and fiber (OECD, 2012).

In the Philippines, consumption of soybean and products is at 4 g/day, in the form of soy sauce at 3 g/day, soybean milk-nil, and other soybean products at 1 g/day (Monsanto, 2009). Soybeans are consumed in non-fermented and fermented forms. OECD Consensus Document (2012) had mentioned the non-fermented soy foods as stated by Liu (2008) which include dairy analogues (e.g. soymilk), meat analogues, tofu, soy sprouts, yuba (soymilk film), okara (soy pulp), soy flours, soy protein (including isolates and concentrates), boiled soybeans (edamame), and baked soybeans ("soy nuts") Fermented foods include soy sauce, miso, natto, tempeh, soy yogurt, sufu (fermented tofu), and fermented whole soybeans.

According to Goldsmith (2008) as mentioned in the OECD Consensus Document (2012), approximately 2% of soy protein is consumed by humans; the large majority of the remaining 98% is processed into soybean meal for livestock feed. The consensus document also mentioned the data from the Food Safety Commission of Japan (2006) indicating that the daily intake of soy-based foods in Japan is generally estimated to be between 63.2 to 70.2 g per person and also the information from Kim and Kwon (2001) which states that the consumption of soybean and soybeanbased products, including tofu, soymilk, sprouts, soybean paste, and other foods in Korea is estimated to be 21 g per person per day.

History of safe use is attributed to soybean since it is not known to cause toxicity and is being consumed in different forms worldwide (OECD, 2012). Soybean is one of the eight food that account for 90% of all IgE mediated food allergies. However, Heating and other processing may increase or decrease the potency of soybean allergens.

Transgenic Plant (MON 87701 Soybean)

MON 87701 soybean has been reviewed and approved for food and/or feed use in many countries including Australia/New Zealand (Food, 2010), Canada (Food and Feed, 2010), China (Food and Feed, 2010), European Union (Food and Feed, 2012), Indonesia (Food, 2013), Japan (Food and Feed, 2011), Korea (Food and Feed, 2011), Mexico (Food and Feed, 2010), Russian Federation (Food and Feed, 2013), Sinagpore (Food and Feed, 2016), Taiwan (Food, 2016), USA (Food and Feed, 2010) and Vietnam (Food and Feed, 2015) (ISAAA).

The event, MON 87701 was developed to express Cry1Ac proteins derived from Bacillus thuringiensis subsp. kurstaki (Monsanto, 2009). The protein confers resistance to target lepidopteran insect pests.

The transformation method is through Agrobacterium tumefaciens – mediated transformation with plasmid vector PV-GMIR9 into the A5547 soybean (Monsanto, 2009). The plasmid vector contains the cry1Ac gene expression cassette which is regulated by RbcS4 promoter and leader from the Arabidopsis thaliana ribulose 1,5-bisphosphate carboxylase small subunit 1A gene, CTP1 targeting sequence from the Arabidopsis thaliana small subunit 1A gene, and the 7S α' 3' non-translated sequence from the Glycine max 7S seed storage protein gene. The vector also contains the cp4 epsps expression cassette which is under the regulation of Figwort Mosaic Virus (FMV) promoter from the FMV 35S RNA gene, the shkG leader from the Arabidopsis thaliana shkG gene, the CTP2 targeting sequence from the shkG gene from Arabidopsis thaliana and the E9 3’ non-translated sequence from the RbcS2 gene from Pisum sativum.

Donor Organisms (Bacillus thuringiensis subsp. kurstaki)

History of safe use is attributed to the donor organism for Cry1Ac protein, Bacillus thuringiensis subsp. kurstaki. The bacteria is not known to be toxic or allergenic and is being used for pest control in agriculture with no adverse effects to human health found and no confirmed report of allergic reactions for the last 50 years (Monsanto, 2009).

Inserted DNA

Southern blot analyses and Polymerase Chain Reaction (PCR) confirmed that MON87701 contains a single copy of T-DNA I at a single insertion site and no detectable T-DNA II elements (Monsanto, 2009; Arackal, 2009). DNA sequence analyses confirmed the sequence identity between the MON 87701 insert and the portion of the T-DNA I from PV-GMIR9 that was integrated into the soybean genome. These results also confirmed the organization of the genetic elements within the cry1Ac expression cassette of MON 87701, which was identical to that in plasmid PV-GMIR9. Analysis of the T-DNA insertion site indicates that there is a 32 bp deletion of genomic DNA and 14 bp insertion at the 5’ insert-to-plant DNA junction. Molecular analyses also showed that one intact copy of the cry1Ac expression cassette was integrated at a single chromosomal locus in MON 87701. No additional genetic elements, including backbone sequences from the transformation vector PV-GMIR9, linked or unlinked to the intact DNA insert, were detected in the genome of MON 87701.

The organization of the elements within the MON 87701 insert was confirmed by DNA sequence analyses (Monsanto, 2009; Arackal, 2009). Several PCR primers were designed with the intent to amplify nine overlapping regions of DNA that span the entire length of the insert. The amplified DNA fragments were subjected to DNA sequencing analyses. The DNA sequence of the MON 87701 insert is 6426 base pairs long, beginning at base 3908 of PV-GMIR9 located in the right border region and ending at base 10333 in the left border region of PV-GMIR9.

The sequence generated from the 5’ and 3’ flanking sequences of MON 87701 indicates that there was a 32 bp deletion and a 14 bp insertion just 5’ to the MON 87701 insertion site (Monsanto, 2009; Arackal, 2009; Salomon and Puchta, 1998). Such changes are common during plant transformation;

these changes presumably resulted from double-stranded break repair mechanisms in the plant during the Agrobacterium-mediated transformation process. Bioinformatics analyses support that any putative polypeptides or proteins possibly produced from ORFs in the insert or at the junction is unlikely to show allergenic, toxic or otherwise biologically adverse properties (Monsanto, 2009; Silvanovich and Girault, 2009).

Genetic Stability

The multigenerational stability of the introduced traits was assessed through Southern Blot Analysis of genetic samples from five generations of MON 87701 (Monsanto, 2009). Results showed that cry1Ac gene is stably inherited across multiple generations of MON 87701. Segregation is assessed using PCR-based assay to detect the presence of cry1Ac gene. Five generations were assessed prior to the commercial variety backcrossing program. The segregation and stability of T-DNA I in MON 87701 was confirmed by performing a Chi-square analysis over five generations. Results indicated no statistically significant difference between the observed and expected segregation ratio and follows Mendelian principles.

Expressed Material (Cry1Ac protein)

Cry1Ac protein has no metabolic role in any metabolic pathways in plant metabolism (Monsanto, 2009). The bacterially-produced crystal protein are first solubilized in the insect midgut, followed by activation of protoxins to active toxins by midgut proteases. The activated proteins then bind to midgut membrane receptors in susceptible insects, insert into the apical membrane, and form pores. Formation of the pores causes loss of osmotic regulation, and eventually leads to cell lysis, which is thought to be responsible for insect death.

Expression level of Cry1Ac in different plant parts of MON 87701 soybean was measured using ELISA methods (Monsanto, 2009). The measurements are in dry weight basis (ng/mg dry weight). Margin of exposure of general population and nursing infants were 2.93 x 106 and 7.71 x 104, respectively. This was based from the concentration of Cry1Ac in each of the fractions (seed, flour and milk) and the calculated protein intake (mg/kg/day) of Cry1Ac (Monsanto, 2009).

Toxicological and Allergenicity Assessment

The novel protein, Cry1Ac, was subjected to digestibility, heat inactivation, oral toxicity and amino acid sequence comparison studies to determine its potential to cause toxicity or allergenicity to humans (Monsanto, 2009). The test protein used in these analyses was Cry1Ac produced from Escherichia coli. Molecular weight comparison, immunoreactivity with anti-Cry1Ac antibodies, glycosylation status and functional activity confirmed that the E. coli produced Cry1Ac is biologically and functionally equivalent to the plant produced Cry1Ac.

Digestibility study using Simulated Gastric Fluid (SGF) with pepsin demonstrated that Cry1Ac is readily degraded within 30 seconds of incubation with SGF, in presence of pepsin at pH 1.2, a characteristic of most non-toxic proteins (Monsanto, 2009). A protein fragment of ~4 kDa was observed throughout the digestion in SGF, but appears to degrade to a smaller ~3.5 kDa fragment visible at the 30 min and 60 min time points on the gel. Cry1Ac was exposed to digestion with pancreatin in SIF following the digestion in SGF for 2 min and assessed by SDS-PAGE. Results confirm that the transiently stable fragment (~4 kDa) is completely degraded within 0.5 min in SIF.

Heat stability of Cry1Ac protein was evaluated in reference to a conventional soybean variety, A5547 (Bell et al., 2008). The test substance (MON 87701) and control substance (conventional soybean variety, A5547) harvested seed materials were ground and then heated in an oven at approximately 190 °C for 15.5 min. The effect of heat treatment on the immunologically detectable levels of the Cry1Ac protein in MON 87701 was evaluated using western blot analysis.

The results demonstrated that the heat treatment dramatically decreased the level of immunodetectable Cry1Ac protein present in extracts of heat-treated MON 87701 harvested seed. The amount of immunodetectable Cry1Ac protein in extracts of heated MON 87701 was below the limit of detection (LOD), indicating a decrease of at least 94% relative to the protein level in extracts of unheated MON 87701 samples.

Acute oral toxicity study of Cry1Ac indicated no treatment-related effects on the survival, clinical observations, body weight gain, food consumption or gross pathology was observed in mice treated with Cry1Ac. The NOAEL for the Cry1Ac protein was 1,460 mg/kg in males and 1,290 mg/kg in females.

Amino acid sequence comparison of Cry1Ac protein to toxins and allergens using FASTA showed no structurally relevant similarity exists between the Cry1Ac protein and any known toxic, allergens or other biologically active proteins that would be harmful to human or animal health (Monsanto, 2009; AIS-FRA-17-07 BIA).

Levels of Cry1Ac in MON 87701 determined through ELISA and the protein content of MON 87701 were used to compute the percent total protein for Cry1Ac which is 0.0046%.

Results of the digestibility, heat inactivation, amino acid sequence comparison and acute oral toxicity studies indicates that Cry1Ac protein being expressed in MON 87701 soybean is not toxic or allergenic to humans (Monsanto, 2009).

Nutritional Data

Compositional analysis provided by the developer indicating the nutritional data of MON 87701 in comparison with the conventional soybean (A5547), twenty (20) conventional varieties and range of literature values (Monsanto, 2009). The trials were conducted in five (5) replicated sites: Alabama, Arkansas, Georgia, Illinois and North Carolina. Results of the analysis indicated that there is no differences in the proximate, fiber, mineral, amino acid, fatty acid, vitamins and anti-nutrient of MON 87701 and the conventional soybean that can be considered biologically relevant.

Conclusion

For the transgenic MON 87701 soybean, enough evidence is provided to support the equivalence of the genetically modified crop, in terms of the nutritional composition and food safety, with the conventional soybean other than resistance to target lepidopteran insects. After reviewing the provided material of Monsanto Philippines, Inc., it is therefore concluded that MON 87701 soybean is as safe as its conventional counterpart.

BAI ASSESSMENT AND RECOMMENDATIONS

A. Host Organism

BAI has concurred that soybean contains amino acids and fatty acids, and that it also contains the following anti-nutrients: trypsin/chymotrypsin inhibitors, lectins, phytoestrogens, stachyose, raffinose and phytic acid.

They also concur that the allergenic effects of soybean are attributable to the globulin fraction of soy proteins that comprise 85% of total protein (OECD, 2001). However, human clinical and animal model data indicates that soy protein tend too be less immunoreactive than many other food proteins (Cordle, J. Nutrition, 2004).

In addition, BAI expressed agreement that about 6% of soybeans are used directly as food. Whole beans may be eaten as vegetable or crushed and made into tofu, tempeh, soya milk or soy sauce. 2%

of the meal is further processed into flours and protein additives. Soy is also used as ingredient in baked products, margarine and bottled as cooking oil (wwf.panda.org).

They also agree that consumption of soybean and products is at 4 g/day in the Philippines, in the form of soy sauce at 3 g/day, soybean milk-nil, and other soybean products at 1 g/day, and that soybean meal is used as ingredient for livestock, poultry and fish.

B. Transgenic Plant

BAI has agreed that the information provided by the applicant on the list of countries that have approbed the transgenic plant as food are Australia/New Zealand, Canada, China, EU, Indonesia, Japan, Korea, Mexico, Russian Federation, Singapore, Taiwan, US, Vietnam; for feed are Canada, China, EU,Japan, Korea, Mexico, Russian Federation, Singapore, US, Vietnam.

Meanwhile they also agree that consumption pattern is not expected to change.

C. Donor Organism

BAI concurs that all protein-encoding sequences were described. There is no potential for pathogenicity or allergenicity, and that all inserted regulatory sequences were described.

They also concur that cry1Ac is the only expressible sequence inserted and *that Bacillus thuringensis subsp. kurstaki* has a history of safe use for insect pest control in agriculture with no adverse effect to human health.

Further, Cry1Ac protein is not known to be allergenic. It is toxic only to its target lepidopteran insects.

D. Transformation System

BAI has concurred that the transformation method used is Agrobacterium-mediated and that the target of genetic modification is the Nuclear DNA. They also concur that the experimental protocol was described and that the transformation plasmid PV-GMIR9 is 15,532 bp in size and has two T-DNAs each containing a single expression cassette.

All other required information were provided and that carrier DNA is not used.

E. Inserted DNA

BAI has agreed that the southern blot results sufficiently confirm the presence of one copy of the T-DNA I at a single locus and that the PCR and DNA sequence analyses confirmed the organization of the elements within the insert and flanking regions.

They also agree that the sequence generated from the 5' and 3' flanking sequences of MON 87701 indicates that there was a 32 bp deletion and a 14 bp insertion just 5' to the MON 87701 insertion site but these are common occurrences during plant transformation.

In addition, they concur that in order to assess potential risks, bioinformatic analyses were performed to assess the potential of toxicity, allergenicity or biological activity of the putative peptides encoded by translation of reading frames 2 through 6 of the cry1Ac. Translated sequences were compared to allergen, toxin and public domain databases using FASTA sequence comparison algorithm. Any putative polypeptides or proteins possibly produced from ORFs in the insert or at the junction is unlikely to show allergenic, toxic or otherwise biologically adverse properties.

In addition to soybean, the cry1Ac gene has been expressed in GM cotton, tomato, corn and that the absence of PV-GMIR9 backbone sequences was sufficiently demonstrated by Southern blot.

F. Expressed Material

BAI has concurred that Southern blot analyses of five generations indicate multigenerational stability of the introduced trait and that the segregation was assessed using TaqMan PCR. MON 87701 segregates following Mendelian inheritance principles.

They also concur that results are consistent with the insertion of one intact copy of the cry1Ac cassette inserted at a single locus.

G. Toxicological Assessment

BAI has concurred that the digestibity studies were done with Pepsin in SGF and pancreatin in SIF and that 99.7% of the full-length Cry1Ac was digested below the LOD within 30 s of digestion in SGF and was undetectable at the 2 minutes time point in SIF.

They also concur that SDS-PAGE showed approximately 3.5kDa fragment at 30 and 60 minute incubation in SGF. In less than 1 minute in SIF, no fragments were detected and that Ground seed materials heated at 1900C for 15.5 minutes and analyzed using Western blot had lower than LOD immunodetectable level of Cry1Ac protein indicating decrease of at least 94% relative t to the level in unheated MON87701 samples.

They agree that no structurally relevant similarity exists between the Cry1Ac protein and any known toxic or other biologically active proteins that would be harmful to human or animal health and that acute oral gavage was done in total of 1,460 mg protein/kg BW in male mice and 1,290 mg/kg BW in female mice.

Lastly, they agree that the source of test protein was E. coli and that criteria used for equivalency were molecular weights, immunoreactivity with anti-Cry1Ac antibodies, glycosylation status and functional activity.

H. Allergenicity Assessment

BAI has concurred that the digestibity studies were done with Pepsin in SGF and pancreatin in SIF and that 99.7% of the full-length Cry1Ac was digested below the LOD within 30 s of digestion in SGF and was undetectable at the 2 minutes time point in SIF.

They also concur that SDS-PAGE showed approximately 3.5kDa fragment at 30 and 60 minute incubation in SGF. In less than 1 minute in SIF, no fragments were detected and that Ground seed materials heated at 1900C for 15.5 minutes and analyzed using Western blot had lower than LOD immunodetectable level of Cry1Ac protein indicating decrease of at least 94% relative t to the level in unheated MON87701 samples.

They agree that no structurally relevant similarity exists between the Cry1Ac protein and any known toxic or other biologically active proteins that would be harmful to human or animal health and that Cry1Ac in MON87701 seed comprises approximately 0.0012 of the total soybean protein.

Lastly, they agree that serum screening was performed wherein sera from 13 clinically documented, soybean-allergic subjects and five non-allergic subjects were used to assess IgE binding to each soybean extract.Results demonstrate that soybean-specific IgE binding to endogenous allergens in MON 87701 and the conventional soybean control are comparable with the IgE binding to commercially available soybean varieties currently in the market.

I. Nutritional Data

BAI has expressed that Observed statistically significant differences were not considered biologically relevant since they were not consistently observed across all sites. Values for the test substance were similar to those of the conventional corn control.

They also concur that All nutrient values were within the calculated 99% tolerance interval and that all values obtained were within or similar to literature values.

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

DENR ASSESSMENT AND RECOMMENDATION

After thorough and scientific review and evaluation of the documents provided by the Bureau of Plant Industry (BPI) to the DENR Biosafety Committee within the prescribed period pursuant to Joint Department Circular (JDC) No. 1 series of 2016 on the application of Monsanto Philippines, Inc. for direct use for feed, food or processing of Genetically Modified Soybean resistant to insects single trait product MON87701, the following are the observatoions and recommendations:

1. The effect of the regulated article on the environment depends largely on the viability of the product to be utilized for direct use. If the article is transported in a non-viable form, there is no danger to the environment ;
2. Due to the absence of a specified Environmental Management Plan (EMP) by the trade/importers, the Committee would like to recommend that it be added to the requirements for the issuance of an import permit by the Bureau of Plant Industry (BPI) (Section 26 of JDC No. 1 s2016);
3. It is suggested that the BPI ensure the following:

    a. Development of guidelines on the EMP in coordination with DENR;
    b. Implementation of the EMP by the traders/importers involved in the import. handling, processing and transport of viable soybean MON87701 commodity products; and
    c. Strict monitoring of the regulated article from port of entry to the traders/importers storage/warehouse (Section 32 of the JDC No. 1s 2016);

Based on the above considerations and with the submitted sworn statement and accountability of the proponent, a biosafety permit may be issued to the proponent if the abovementioned recommendations are followed.

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 the Direct use for food and feed, or for processing (FFP) of Soybean MON87701.

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

The following are the observations and recommendations:

1. Find that the regulated article applied for direct use for food and feed or for processing does not require changes in the usual practices in unloading, and loading, hauling, transport and storage and processing. As such, the regulated article is safe as its conventional counterpart and is not expected to pose any significant risk to human and animal health and environment while in transit, storage and processing.

2. Scientific pieces of evidences from provided references i.e. literaturess show that regulated article applied for direct use for food and feed or for processing is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health and on the environment.

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

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

    b. The BPI to include in the issuance of permit for the release of this product the following conditions:

    b.1 Any spillage (during unloading and loading/hauling and transport unloading and storage) shall be collected and cleaned up immediately.
    b.2 Transportation of the consignment from the port of entry to any destination within the country shall be in closed containers.
    b.3 There shall be clear labeling of the product from importation down to all levels of marketing stating that is it only for the purpose of direct use for food, feed, or processing and is not to be used as planting materials.

4. Based on the above considerations and with the submitted sworn statement and accountability of the proponent, this recommendation is being submitted to BPI related to the processing and issuance of a biosafety permit for direct use for food and feed, or for processing of soybean MON87701

SEC ASSESSMENT AND RECOMMENDATIONS

The impact of GM Soybean MON 87701 to the feed industry and ultimately to the livestock, poultry and the aquaculture sub-sectors would be tremendous. Importation of soybeans will save millions of dollars for our country through lower prices.

With the above scenario and observed consequences of MON 87701, the SEC Expert recommends the renewal of permit of the MON 87701. The renewal of the utilization of GM Soybeans would help the feed industry and may result to lower production cost of poultry, livestock and aquaculture products in our country. However, concern agencies, both government and non-government, should continue monitor and regularly assess the risk of any GM products introduced in the country.