ASSESSORS' CONSOLIDATED REPORT ON MONSANTO PHILIPPINES INC'S SOYBEAN MON87769 APPLICATION FOR DIRECT USE AS FOOD AND FEED OR FOR PROCESSING

INFORMATION ON THE APPLIED EVENT

Monsanto Company has developed biotechnology-derived soybean MON 87769 that contains stearidonic acid (SDA), a sustainable alternate source of an omega-3 fatty acid to help meet the need for increased dietary intake of long chain omega-3 fatty acids. Production of SDA in soybean seeds was achieved through introduction of the two desaturase genes, *Primula juliae* Δ6 desaturase (*Pj.D6D)* and *Neurospora crassa* Δ15 desaturase (*Nc.Fad3)* that encode for the PjΔ6D and NcΔ15D proteins. The *Nc.Fad3* and *Pj.D6D* genes are driven by 7Sa and 7Sa' promoters, respectively, which are known to be seed-specific.

MON 87769 was developed through *Agrobacterium*-mediated transformation method.

STRP's Assessment

1. Host Organism

a. Soybean is the main source of plant proteins consumed by humans and animals. Among all crops, it is the leading source of vegetable oil produced in the world. In general, soyfoods can be roughly classified into four categories: traditional soyfoods, soybean oil, soybean protein products and dietary supplements. It is also a source of antinutrients which include trypsin inhibitors, lectins, isoflavones, (genistein, daidzein, and glycitein), stachyose, raffinose, and phytic acid [1][2][3][4].

b. The Food and Agriculture Organization (FAO) includes soybean as one of the eight most significant food allergens. At least 16 potential soy protein allergens have been identified. Soybean is less allergenic than other food in the 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 [5][6][7].

c. As food, soybean is used as follows: Traditional soyfoods – e.g. soymilk, tofu, soy sprouts; Soy oil products – e.g. salad and cooking oil, shortening, margarine, Soy protein products – e.g. soy flour, concentrate, isolate; Modern soyfoods – e.g. soy burgers, tofu burgers, soy sausages, soy ice cream; Soy-enriched foods – e.g. soy bread and pasta, sausages and hamburgers, ice cream and yogurt; and Soy dietary supplements and nutraceuticals – e.g. soy isoflavones, lecithin, vitamin E [8].

d. Soybean meal is the most valuable component obtained from processing soybean. It is produced by solvent extraction of the dehulled soybean flakes. Soybean meal is the premier supplemental protein source in livestock and poultry rations. It is used to meet the animal's requirement for limiting amino acids, as it is the most cost-effective source of amino acids [9][10].

2. Donor Organism

a. The gene encoding PjΔ6D originated from *Primula juliae*, a member of a large genus of plants commonly known as "Primrose" that are frequently grown in cooler climates. Plants of the genus Primula are not generally consumed as food. *P. juliae* has not been reported to be a source of allergenic proteins [46][47][48].
b. The gene encoding NcΔ15D originated from *Neurospora crassa*, commonly known as "bread mold," a fungus that is ubiquitous in the environment. *Neurospora crassa* is considered a non-pathogenic and non-allergenic organism [46][47][48].

3. Transformation System

a. The integrity and order of genetic elements in MON87769 were determined by PCR and sequence analyses. Five overlapping regions that span the entire length of the insert and the associated flanking region were amplified through PCR. The control DNA (conventional soybean A3525) did not show amplification whereas MON87769 DNA produced the expected size products in all five amplification reactions. The PCR products generated were sequenced and the obtained consensus sequence of the insert in MON87769 is 7367 base pairs long, beginning at base 9387 of PV-GMPQ1972 located in the right border region, and ending at base 288 of PV-GMPQ1972 located in the left border region. [11][12].
b. The potential for creating novel, chimeric ORFs was tested bioinformatically which showed that any putative polypeptides or proteins created from alternative reading frames of the *Pj.D6D* and *Nc.Fad3* coding sequences or at the junction sequences did not show sufficient degree of sequence similarity to known toxins, allergens or other bioactive proteins [11].
c. The multigenerational stability of introduced trait was assessed using Southern blot analysis. Genomic DNAs from four generations (R3, R4, R5, and R6) and the conventional soybean control were digested with Lgu I and BstX I. The blots were probed with probes 13-18. The six probes are overlapping and span the entire length of T-DNA I. Except for the endogenous background hybridization, no hybridization band which corresponds to the T-DNA I was observed in the control. Southern blot analysis of MON87769 DNA from four generations hybridized against probes 13-15 showed the expected band of approximately 6.8 kb in addition to the endogenous background hybridization present in the conventional soybean control. When hybridized with probes 16-18, the two expected bands were observed (1.6 and 6.8 kb) [13][11].
d. The genotypic stability and segregation of T-DNA I in MON87769, initially assessed by Southern blot analysis was confirmed by performing Chi- square statistics on data generated for the T-tml 3' genetic element over three generations. The originally transformed plant R0 was selfed to generate R1. Homozygous R1 plant was selected using Invader and Southern blot analysis. The R1 plant was further selfed to obtain R3 and R4 generations. The homozygous R4 plant was crossed with a soybean variety that did not contain the MON87769 insert to obtain a hemizygous F_1. Three more generations of selfing were performed to obtain F_2, F_3 and F_4 plants. These F_2, F_3 and F_4 generations are segregating populations with respect to T-tml 3' genetic element and were the ones used in the chi-square test. The chi- square values for the F_2, F_3 and F_4 generations indicated no statistically significant difference between the

observed and expected segregation ratios. These results are consistent with the molecular data on a single insertion site of T-DNA I within MON87769 and show that the insert follows the Mendelian pattern of inheritance for a single locus [14][11].

4. Food and Feed Safety

a. Digestibility of PjΔ6D and NcΔ15D proteins in simulated gastric fluid (SGF) containing pepsin was determined by SDS-PAGE and western blot methods. Visual examination of the Colloidal Brilliant Blue G-stained gel showed that the full length PjΔ6D and NcΔ15D proteins was digested below the limit of detection (LOD) within 30 s of digestion in SGF. This means that more than 99% and 97.5% of PjΔ6D and NcΔ15D proteins, respectively was digested within 30 s of incubation in SGF. Immunodetection showed that PjΔ6D was digested below the LOD within 30 s of incubation in SGF which implies that 96% of the protein was digested within 30 s. A fragment of ~10 kDa was observed only at the 30 s digestion time point which was completely digested in less than 2 min of incubation in SGF. No other band was detected in the lanes corresponding to the 2 min to 60 min digestion time points [15][16][40].

b. Simulated Intestinal Fluid (SIF) was also used as a stand-alone test to test digestibility of PjΔ6D and NcΔ15D. Western blot analysis demonstrated that a band corresponding to the full length both proteins were digested below the LOD within 5 min of incubation in SIF, which means that more than 84% of the PjΔ6D protein and more than 96.7% of the NcΔ15D was digested in SIF within 5 min.

c. Western blot analysis demonstrated that the heat treatment dramatically decreased the level of immunodetectable PjΔ6D and NcΔ15D present in heated seed extracts of MON87769. The amount of immunodetectable PjΔ6D and NcΔ15D was below the LOD indicating decrease of at least 84% and 80%, respectively relative to the protein levels detected in unheated seed extracts of MON87769. Results indicate that heating of ground seed, in a manner that simulates the use of soybean flour in food manufacturing results in a substantial loss of immunodetectable PjΔ6D and NcΔ15D [17]. The estimated T50 at 189.1^{o}C is within 15 minutes for both proteins.

d. Potential structural identity and similarity shared between the expressed proteins (PjΔ6D and NcΔ15D) and proteins in the TOX_2009 database was evaluated using the FASTA sequence alignment program. No alignments displaying an E score of 1 or less were observed suggesting that no structurally relevant similarity exists with any known toxic or other biologically active proteins that would be harmful to human or animal health [18][19][22].

e. The short-term toxicity of *Primula juliae* Delta 6 Desaturase (Delta 6 Desaturase, PjΔ6D) and *Neurospora crassa* Delta 15 Desaturase (Delta 15 Desaturase, NcΔ15D) isolated from immature MON87769 soybean seeds following single oral gavage administration to mice was evaluated. Delta 6 Desaturase induced neither mortality nor other adverse effects when administered by single oral gavage at a dose of 4.66 mg/kg. On the other hand, Delta 15 Desaturase induced neither mortality nor other adverse effects when administered by single oral gavage at a dose of 37.3 mg/kg. The dose is at least 100 times conservative estimates for potential human exposure to Delta 6 Desaturase [20][21].

f. The sequence of the first 15 amino acids at the N-terminus of the MON87769 PjΔ6D was consistent with the expected sequence. The N-terminal methionine was not observed, indicating that it was removed during post-translational modification of the protein. [89-90] On the other hand, NcΔ15D protein is an integral membrane protein of microbial origin and contains two putative N-glycosylation sites (Asparagine-X-Serine/Threonine). The existence of sites however does not imply that the protein is glycosylated in planta. To assess whether the NcΔ15D protein, as expressed in MON87769 soybean seeds, is glycosylated, analysis of the protein for the presence of covalently bound carbohydrate moieties was undertaken using a GE Glycoprotein Detection Module, which detects N- and O-linked carbohydrate. Transferrin, a naturally glycosylated mammalian protein, was used as the positive control. No detectable signal was obtained suggesting that NcΔ15D isolated from MON87769 seeds is not glycosylated in vivo [23][29] [89-90].
g. The mean level of PjΔ6D in mature seed of MON87769 is 1.8 µg/g DW. The mean percent dry weight of total protein mature MON87769 seed is 41.92 % (or 419,200 µg/g). The percent PjΔ6D protein in mature MON87769 is therefore calculated as follows: (1.8 µg/g ÷ 419,200 µg/g) x 100 % = 0.00043 % of total mature soybean seed protein. Meanwhile, the mean level of NcΔ15D in mature seed of MON87769 is 10 µg/g DW. The mean percent dry weight of total protein mature MON 87769 seed is 41.92 % (or 419,200 µg/g). The percent NcΔ15D protein in mature MON 87769 is therefore calculated as follows: (10 µg/g ÷ 419,200 µg/g) x 100 % = 0.00239 % of total mature soybean seed protein. Therefore, both proteins are expressed in Corn MON87769 mature seed only in negligible amount [26] [91].
h. Study was conducted to determine the binding levels of IgE antibody collected from clinically documented, soybean allergic patients to protein extracts prepared from MON87769, a conventional control variety, and 24 commercial soybean varieties that served to establish a range in IgE binding. The results of this study demonstrate that the levels of the endogenous soybean allergens in MON87769 and conventional soybean control are comparable to the levels of endogenous soybean allergens in the soybean varieties that are currently on the market. Therefore, the MON has no greater allergenic potential than conventional soybean control or other soybean varieties that are currently on the market [27] [28].
i. For both forage and seed, proximates include ash, carbohydrate by calculation, moisture, and protein. Combined-site analysis of forage showed no significant differences ($p>0.05$) between MON87769 and the conventional soybean control. For seed, significant differences in the levels of protein and carbohydrates between MON87769 and the conventional soybean control were observed. In comparison to the conventional control, the mean level of protein was higher ($p<0.05$) while the mean level of carbohydrate was lower ($p<0.05$) in MON87769. However, the magnitudes of differences were very small at <10%. The means and range of values for protein and carbohydrate were within the 99% tolerance interval for the population of conventional references and within the range of reported values in the literature and based on the ILSI Crop Composition Database [30][32].

j. The forage samples used in compositional analyses were taken from plants (MON87769 and the comparator) grown in 2006 at three replicated plots at each five sites across the United States (Iowa, Illinois, Michigan, and Ohio). The comparator used was the conventional soybean variety, A3525, which has background genetics similar to MON87769, but does not contain either the *Pj.D6D* or *Nc.Fad3* gene cassette. Forage was collected at R6 plant growth stage. Combined-site analysis of forage showed no significant differences ($p>0.05$) between MON87769 and the conventional soybean control [30][32].
k. Analysis of seed showed significant differences between MON87769 and the conventional soybean control for 17 amino acids (proline, arginine, cystine, glycine, phenylalanine, aspartic acid, glutamic acid, histidine, isoleucine, leucine, lysine, valine, alanine, methionine, serine, threonine, and tyrosine) and six fatty acids (palmitic, oleic, linoleic, linolenic, arachidic, and behenic acids). The mean levels of each of the 17 amino acids were significantly higher in MON87769 compared to the conventional soybean control. However, the magnitudes of differences between MON87769 and the conventional control were relatively small at ≤10%. The observed means and range of values for the 17 amino acids were within the 99 % tolerance interval established for the population of conventional references and within the range reported in the literature and based on the ILSI Crop Composition Database [30][32].
l. The mean levels of palmictic acid (C16:0), linolenic acid (C18:3), and arachidic acid (C20:0) were significantly higher ($p<0.05$) in MON87769 compared to the conventional soybean control, while the mean levels of oleic acid (C18:1), linoleic acid (C18:2), and behenic acid (C22:0) were significantly lower in MON87769 compared to the conventional soybean control. These are expected differences since MON87769 was intentionally developed to shift the fatty acid metabolism toward an increase in stearidonic acid (SDA) [30][32].
m. The levels of the three isoflavones (daidzein, glycitein, genistein) were significantly different between MON87769 and the conventional soybean control. [30][32][33][34].
n. Soybean MON87769 was created to intentionally change the fatty acid composition of the soybean plant. Soybean MON87769 contains four additional fatty acids not present in detectable amounts in the conventional soybean control, variety A3525. These additional fatty acids are stearidonic acid (18:4), γ-linolenic acid (18:3), trans-SDA (18:4 6c,9c,12c,15t), and trans-α linolenic acid (18:3 9c,12c,15t). Compositional analysis showed the following levels of the four fatty acids in MON87769 seed: SDA – 16.83 to 33.92% of total fatty acids; γ-linolenic acid – 6.07 to 8.03% of total fatty acids; trans-SDA – 0.058 to 0.26% of total fatty acids; trans-α linolenic acid – 0.38 to 0.48% of total fatty acids [30][34][35].
o. Stearidonic acid (SDA) is a metabolic precursor to the long chain omega-3 fatty acids, eicosapentanoic acid (EPA), and docosahexaenoic acid (DHA), in humans and animals and is found in products such as fish and fish/algal oils. Although the benefits of omega-3 fatty acid consumption are widely recognized, typical Western diets contain very little fish, and it is impractical to expect the general population to take fish oil supplements. An alternative approach to increase omega-3 fatty acid intake is to provide a wider range of foods that are enriched in omega-3 fatty acids so that people can choose foods that suit their dietary

habits. Human and animal studies have shown that 1 g dietary SDA is approximately equivalent to 200 – 300 mg dietary EPA in terms of increasing tissue concentrations of EPA. Thus, MON87769 can serve as an alternate source of an amega-3 fatty acid to help meet the need for increased dietary intake of long chain omega-3 fatty acids in food and feed [36][37][38].

p. The safety of stearidonic acid (SDA) in soybean MON87769 is based on the following: 1) its occurrence as an *in vivo* intermediate in the metabolism of α-linolenic acid to long chain omega-3 fatty acids in mammals; 2) a long-standing history of safe consumption of SDA from several marine and plant sources; 3) the generally regarded as safe (GRAS) status of four fish oils containing SDA; and 4) the positive confirmation from the European Food Safety Agency on the safety of Echium oil containing SDA. Furthermore, the safety of SDA was confirmed by several human, as well as animal studies conducted with SDA and SDA soybean oil. These studies were conducted with SDA intake levels ranging from 0.8 to 62 mg/kg body weight/day for the human studies and up to 1.04 g/kg body weight/day for the rat studies with no adverse effects reported. Therefore, SDA is concluded to be safe for human and animal consumption [39].
q. Compared to the CON (conventional, non-GM) treatment, SDASOY (transgenic) increased the amount of total very long chain (VLC) n-3 polyunsaturated fatty acids (PUFA) in skinless and boneless breasts, tenders, and thighs by around three-folds [49].
r. The use of SDA-enriched oil in crops will be able to address the observed low intake (below the recommended daily intake) of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [50].
s. There are no reports of allergic reactions to GM feed when compared to non-GM feed. The occurrence of horizontal gene transfer of GMO-related DNA from the GM crop to another species is no different from any other DNA and that any unintended horizontal gene transfer is unlikely to raise health concerns. The use of GM crops in the feed chain reduces fumonisins contamination and this has positive consequences in GM-crop-derived feed safety [51].

STRP's Conclusion

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 greater risk to human and animal health.

Additionally, after a thorough review of the new studies submitted by Bayer Crop Science, Inc. for Soybean MON87769 application for direct use for food, feed or for processing, the STRPs found that the new studies submitted by the applicant will not affect the safety of Soybean MON87769.

BAI's Assessment

1. Toxicological Assessment

a. PjΔ6D and NcΔ15D proteins were subjected to simulated gastric fluid (SGF, containing pepsin) followed by digestion in simulated intestinal fluid (SIF, containing pancreatin), and were assessed using SDS-PAGE and western blot methods. The estimated T50 result for SGF is below 30 seconds while for SIF, is

below 5 minutes for both proteins. Based on the study provided by the applicant, there were no detected fragments after digestion for both enzymes used. The results indicate a rapid digestion of both proteins which means it is highly unlikely that it will cause any toxicological concerns in animals [15][16][41][42].

b. The proteins extracted in test and control groups of ground seed powder that were subjected to heat were assessed using western blot analysis. Results of their study showed that heat has significantly decreased the immunodetectable level of PjΔ6D and NcΔ15D proteins extracted in heat-treated MON87769 seed because the amount of the immunodetected proteins were below the limit of detection. This implies that processing/heating the ground seed will result in a considerable loss of these proteins in MON87769 seed. According to the applicant, the estimated T50 result for heat inactivation of both proteins at 189.1°C is within 15 minutes [17].

c. Using bioinformatics analyses, PjΔ6D and NcΔ15D have no structurally relevant similarity among any known toxins or other biologically active proteins that will pose any animal health concerns. The sequences of the known toxins were obtained from TOX_2009 database and then analyzed using FASTA sequence alignment program [18][19][22].

2. Allergenicity Assessment

a. The amino acid sequences of PjΔ6D and NcΔ15D proteins produced in MON87769 is not similar to any of the anti-nutritional proteins or to any other known protein toxin according to the references provided by the applicant. Therefore, an acute oral mouse toxicity study was considered sufficient to evaluate the toxicity of both proteins. The No Observable Adverse Effect Level (NOEAL) for PjΔ6D was considered to be 4.7 mg/kg body weight which is the highest possible dose tested. Meanwhile, NcΔ15D was administered through gavage with a single dose of 37.3 mg/kg BW to 10 male and 10 female CD-1 mice. Results of their study showed no treatment-related harmful effects. It was also determined that NOAEL was considered to be at 37.3 mg/kg BW [20][21][22].

b. Various analyses such as N-terminal sequence, MALDI-TOF MS, western blot, and SDS-PAGE were done in order to determine the physico-chemical properties of MON87769 PjΔ6D protein. These analyses confirmed the identity of PjΔ6D protein isolated from MON87769. Moreover, the SDS-PAGE results showed that the protein has a weight of 46 kDa. Glycosylation analysis was also done to identify whether the protein is glycosylated or not. As per results, the protein is not glycosylated. On the other hand, a detailed characterization of the MON87769-produced NcΔ15D protein was done. Results are: (1) the identity of the NcΔ15D protein isolated from MON87769 is confirmed using N-terminal sequence analysis, western blot analysis, and MALDI-TOF MS analysis; (2) that its N-terminus is intact; (3) molecular weight of the MON87769-produced NcΔ15D protein was determined using SDS-PAGE where it migrated with an apparent molecular weight of 46 kDa; (4) that MON87769-produced NcΔ15D protein is not glycosylated [23][24][25][29].

c. The percent of total protein in mature MON87769 is 0.00043%. This value was obtained by dividing the mean level of PjΔ6D protein in mature seed which is 1.8 µg/g DW by the mean percent dry weight of total protein in mature MON87769 which is 41.92%. Based from the results provided by the applicant, the total

protein present in MON87769 has an insignificant amount of PjΔ6D protein. Meanwhile, the NcΔ15D protein is 0.00239% of total mature soybean seed protein of MON87769 which means it only represents a very small portion of the total protein [26][91].

d. Since soybean is one of the allergenic foods that are responsible for most food allergies, serum screening was performed. Results of their study showed that the levels of endogenous soybean allergens in MON87769 and conventional soybean control are comparable to the levels of endogenous soybean allergens in the commercial soybean varieties. This means that MON87769 will not pose greater allergenic potential than conventional soybean or other commercial soybean varieties [27][28].

3. Nutritional Data

a. There are significant differences in the mean and range values for protein and carbohydrates in seed. However, the values were within the 99% tolerance interval and within the range of values in literature and the ILSI Database [30][32].

b. Significant differences in amino acid levels in seed were observed for proline, arginine, cystine, glycine, phenylalanine, aspartic acid, glutamic acid, histidine, isoleucine, leucine, lysine, valine, alanine, methionine, serine, threonine, tyrosine, oleic acid (C18:1), LA (C18:2), ALA (C18:3), arachidic acid (C20:0), palmitic acid (C16:0), and behenic acid (C22:0). However, the mean and range of values for all amino acid analytes were within the 99% tolerance interval established from the conventional references and is also within the range of values of ILSI database [30][32].

c. Moreover, the mean levels of ALA and palmitic acid were significantly higher in MON87769 than the conventional soybean control, while, the mean levels of oleic acid, LA, arachidic acid, and behenic acid were significantly lower. But since it is the purpose of the intended genetic alteration, those differences in fatty acid levels (that are directly involved in the pathway producing SDA) were expected. Moreover, the differences (except for LA) are not considered to be biologically relevant since their mean values and ranges were within the 99% tolerance interval established from the conventional reference varieties. The level of LA in MON87769 is expected, since LA is the starting material from which SDA and GLA are produced that is why it is significantly different from its conventional counterpart and is outside the 99% tolerance range [30][31][32].

d. There are significant differences that can be observed in seed for isoflavones (daidzein, glycitein, and genistein) but these are not considered biologically meaningful because the mean levels were within the 99% tolerance intervals established from conventional soybean varieties, and within the literature and ILSI Database ranges. This means that the differences will highly unlikely pose any nutritional, anti-nutritional or other biological or toxicological concerns to animal health [30][31][32][33][34].

e. Soybean MON87769 contain new substances which are not present in the conventional soybean. MON87769 has four additional fatty acids namely: 18:3 gamma linolenic acid; 18:3 9c, 12c, 15t trans-alpha linolenic acid (trans-ALA); 18:4 stearidonic acid (SDA); and 18:4 6c, 9c, 12c, 15t (trans-SDA). These additional fatty acids were expected [30][32][35].

f. SDA is the metabolic precursor in synthesizing long chain omega-3 fatty acids, EPA and DHA, in humans and animals and is found in fish and fish/algal oils. Since MON87769 is modified to have an increased SDA it can serve as an alternate source of omega-3 fatty acids to meet the increased dietary intake of long chain omega-3 fatty acids in food and feed. SDA is safe in particular use the regulated article was intended for because: 1. It is a naturally occurring substance, in fact, it is an *in vivo* intermediate in the metabolism of ALA and is the precursor to long chain omega-3 fatty acids in mammals; 2. It is consumed for a very long time since it is present in several marine and plant sources that are regarded as safe; 3. Four fish oils which contains SDA have GRAS status; 4. European Food Safety Agency confirmed the safety of Echium oil containing SDA; 5. Studies on animals and humans which made use of SDA and SDA soybean oil confirmed no adverse effects on rats and humans [35][36][37][38].
g. Compared to the CON (conventional, non-GM) treatment, SDASOY (transgenic) increased the amount of total very long chain (VLC) n-3 polyunsaturated fatty acids (PUFA) in skinless and boneless breasts, tenders, and thighs by around three-folds. It was also estimated that α-linolenic acid (ALA) and SDA were metabolized to VLC n-3 PUFAs and deposited into breast, tenders, and thigh meat with the same efficiency. In the liver, the relative expression of genes whose protein products are involved in fatty acid oxidation, desaturation and elongation, were not significantly affected by dietary treatment or bird age [49].

BAI's Conclusion

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

After a thorough review of the new studies submitted by Bayer Crop Science, Inc. for Soybean MON87769 application for direct use for food, feed or for processing, we agree with the applicant's claim that the gene modification will not affect the safety of MON87769 as supported by the new studies submitted by the applicant. The applicant provided 3 new studies (spanning from 2015-2019) in support of their No Adverse Effect claim. The peer-reviewed studies show no evidence of any safety issues which confirmed the safety and substantial equivalence of Soybean MON87769 to its conventional counterpart.

BPI PPSSD's Assessment

1. Toxicological and Allergenicity Assessment
   a. SDS-PAGE and western blot analysis indicated that PjΔ6D and NcΔ15D are rapidly digested in SGF with pepsin within 30 seconds and readily digested in SIF with pancreatin within 5 minutes. No bands were detected for PjΔ6D after 2 minutes of incubation based on the western blot analysis. On the other hand, a ~17 and ~12 kDa bands were detected in SDS-PAGE for NcΔ15D up to 5 minutes and 10 minutes, respectively. However, these bands were not observed on western blot analysis [15][16][23][24].
   b. Heat stability studies showed that PjΔ6D and NcΔ15D are below the limit of detection upon subject to 189.1^{0}C for 15 minutes. This was determined through

comparison of the levels of PjΔ6D and NcΔ15D in extracts of heated and unheated ground MON87769 seeds [17].

c. Amino Acid Sequence Comparison with non-redundant protein sequences database using BLASTp showed no significant homology between expressed proteins and any known toxin. Acute Oral Gavage study provided by the developer indicated that PjΔ6D and NcΔ15D had no adverse health effect when administered in mice at a dose 4.66 mg/kg body weight [18][19][21][22].
d. N-terminal sequence analysis showed the expected sequence for PjΔ6D and NcΔ15D proteins. Methionine was not observed which is expected since its removal is being catalyzed by methionine aminopeptidase, a common modification during post-translational processing of the protein. The N-terminal sequence data confirms the identity of PjΔ6D and NcΔ15D proteins and that the N-terminus are intact. MALDI-TOF MS and Western blot analysis further confirm the identity of PjΔ6D and NcΔ15D proteins. Western blot analysis detected immunoreactive bands at approximately 46 kDa in both proteins. Glycosylation analysis confirms that PjΔ6D and NcΔ15D proteins are not glycosylated. Each protein has an approximate molecular weight of 46 kDa [23][24][25][29].
e. Based on the level of PjΔ6D protein in mature seeds and the mean percent dry weight of total protein in mature seeds of MON87769, PjΔ6D comprises 0.00043% of the total mature MON87769 seeds protein [91]. On the other hand, the level of NcΔ15D protein in mature seeds and the mean percent dry weight of total protein in mature seeds of MON87769, NcΔ15D comprises 0.00239% of the total mature MON87769 seeds protein [26][91].
f. The levels of endogenous soybean allergens in the protein extracts obtained from MON87769 is comparable to the levels of endogenous soybean allergens in the protein extracts from conventional soybean control A3525 [27][28].

2. Nutritional Data

a. Based on the comparative analysis, the statistical differences between the proximate levels of MON87769 soybean and non-transgenic soybean is not biologically relevant since the mean levels are within the range of soybean commercial varieties and/or literature values [30][32].
b. None of the differences in key nutrients is biologically meaningful except for the levels of linoleic acid in MON87769 which is the intended phenotypic effect of the transgene. All values, except for linoleic acid are within the range of commercial varieties and/or literature values [30][32].
c. Trypsin inhibitors and lectins are inactivated during processing thus resulting to low concentrations on antinutrients in the processed soybean protein products. Further processing of soybean meal into concentrate or isolate, reduces or removes raffinose and stachyose. Based on the compositional analysis, MON87769 soybean is conventionally equivalent to that of conventional soybean. Hence, any effect of processing on the level of antinutrient in MON87769 soybean would be similar to that of the conventional soybean [30][31][32][43].
d. Based on the compositional analysis of MON87769 provided by the developer, it has four additional fatty acids: 1.18:3 gamma linolenic acid (GLA); 2.18:3 9c,12c,15t trans-alpha linolenic acid (trans-ALA); 3.18:4 stearidonic acid; and 4.18:4 6c, 9c, 12c, 15t (trans-SDA) [30][32][35].

e. SDA was included in MON87769 to make this soybean event an alternate source of omega-3 fatty acid. It is a long chain polyunsaturated fatty acids commonly found in seafood that is linked to reduction of risk for cardiovascular diseases [36][37][38].
f. SDA has a history of consumption as it is known as minor components of animal lipids and fish oils. Comparative studies showed the similarity of SDA with other dietary (n-3) polyunsaturated fatty acids in terms of biological effects and functions [39].
g. Bowen et al [50] estimated the potential contribution of SDA-enriched oils to total long-chain n-3 fatty acids via EPA equivalents to evaluate the sufficiency of SDA-enriched oils as a replacement for commonly consumed vegetable oils in addressing insufficient long-chain n-3 fatty acid intake. The study did not include new information regarding the possible effects of SDA-enriched oils from GM crops to human health but rather compared its effectivity as replacement for commonly consumed vegetables oils based on dietary intakes. The study does not have implications on food safety.

BPI-PPSSD's Conclusion

Find scientific evidence that the regulated article applied for human food use is as safe as its conventional counterpart and shall not pose greater risk to human and animal health. In addition, they also found that the new studies submitted by the applicant will not affect the safety of soybean MON87769.

DENR-BC'S Assessment

After a comprehensive review and evaluation of the documents including the scientific evidence from references and literature submitted by Monsanto Philippines, Inc., on its application for Direct Use as FFP of Soybean MON87769, 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 particularly on biodiversity because it is not intended for propagation. The transgenic crop will not increase its weediness potential in case the seeds spill out into the environment, because there is no significant difference between the conventional and genetically modified soybean in terms of its percent germination rate, percent dead seed, and percent viable firm seed since there was no genetic modification performed in the reproductive and growth characteristics of soybeans under abiotic and biotic stresses [44].
2. The project description report (PDR) discusses the specified environmental management plan indicating the possible risk and harm to the environment particularly on biodiversity 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 not conducive for plant growth. Also, soybeans generally are very highly domesticated and do not survive well without human intervention [45].

DENR BC's Conclusion
Based on the evaluation and review of literatures cited, the DENR-BC considered the regulated article safe to the environment and biodiversity, particularly on non-target organisms. They also acknowledged receipt of the new studies submitted and said that they consider these as additional references.

DOH-BC's Assessment

Find that the regulated article applied for Direct Use as Food, 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 in 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.

DOH-BC's Conclusion
It is suggested that the Bureau of Plant Industry (BPI) ensure that there shall be clear instructions that the product is only for the purpose of direct use for FFP and is not to be used as planting materials.

After a thorough review of the new studies submitted by Bayer Crop Science, Inc. for Soybean MON87769 application for direct use for food, feed or for processing, the DOH-Biosafety Committee found that the new studies submitted by the applicant will not affect the safety of Soybean MON87769.

SEC Expert's Assessment

1. Soybean meal is a feed ingredient to animal feeds. Pigs, broilers and layers comprise a strong growth center of the country's agricultural sector. Its growth pulls up or down that of the entire sector as a whole. Disruption in the trade of soybean meal has drastic repercussion on production of livestock and eventually on the agriculture sector, and the Philippine GDP growth itself. In 2019, the growth of agriculture could have been stronger if not for the drop in the livestock sector growth.
2. Technically it may be said that soybean meal among other meals including our copra meal turns out not only having better qualities for feeds than other meals, not to mention that it may have reliable supply than copra meal.
3. Meanwhile, soybean oil is serving to be an ingredient for products like mayonnaise and salad dressing. Its availability through importation makes these downstream industries viable. If coconut oil had exactly the same function or even better, the

market would have decided long ago that there is no need to access soybean oil which at the moment is just in tens of thousands of tons.

4. The importation of these products is decided upon by the private sector because they have unique characteristics that are not found in the close substitutes of them. The availability of these products is generally welcome since they give consumers more diversity in their consumption and downstream producers opportunity for new businesses, allowing them to create jobs. Thus, even if these volumes increase the displacement of coconut oil, it is not necessarily assured since the two meals, oils and definitely the beans and coconut meats do not share exactly the same attributes and have different uses.
5. The trade in soybeans in the hundreds of tons as the data provided shows that there are Filipinos who prefer to consume soybean products, such as the local favorite 'taho', soybean milk, and most especially soybean sauce. Their presence here in the country is helping overall the wellbeing of Filipinos in that there is more diversity in the food that they can eat. There is hardly local production of soybeans, which necessitate this importation.

SEC EXPERT's Recommendation

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

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