CONSOLIDATED REPORT ON MONSANTO PHILIPPINES INC. COTTON MON15985 APPLICATION FOR DIRECT USE AS FOOD AND FEED OR FOR PROCESSING

EXECUTIVE SUMMARY

On May 31, 2018, Monsanto Philippines Inc. submitted cotton MON15985 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 cotton MON15985 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 cotton MON15985 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 cotton MON15985.

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

BACKGROUND

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

The BPI Biotech Office provided the assessors, except for the SEC expert, the complete dossier submitted by Monsanto Philippines Inc.. The SEC expert, on the other hand, was provided with a questionnaire on socio-economic, ethical and cultural considerations that have been addressed by Monsanto Philippines Inc. in relation to their application.

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

STRP ASSESSMENT AND RECOMMENDATIONS

Based on the documents submitted by the applicant:

A. Host Organism

Cotton is a source of key nutrients. Cotton seed oil is the primary cotton product used for human consumption, including frying oil, salad and cooking oil, mayonnaise, salad dressing, shortening, margarine and packing oil. The linters can be used as a high fiber dietary product; food uses include casings for bologna, sausages, frankfurters, and is also used to improve viscosity in products such as toothpaste, ice cream, and salad dressings. However, the total amount of linters used is very small. Cottonseed meal is principally used as feed for livestock, and usually contains 41% protein. It is sufficient as a sole source of protein in mature ruminants such as beef cattle and sheep. High quality cottonseed meal, when used correctly as an ingredient of properly formulated swine and poultry rations, improves economy and efficiency (National Cotton Products Association, 2002).

Cotton contains anti-nutrient components such as gossypol and cyclopropenoid fatty acids. Due to the presence of these anti-nutrients in cottonseed, only highly refined products (refine, bleached and deodorized oil and linters) are suitable for human consumption; the levels of gossypol and cyclopropenoid fatty acids are drastically reduced during processing.

The levels of the naturally occurring toxins in cottonseed, particularly in the refined cotton oil seed are cyclopropenoid fatty acids and gossypol. Generally, cotton contains small amounts of these toxicants, typically <1.0% and <2.0%, respectively for cyclopropenoid fatty acids and gossypol. The levels of these toxicants, however, are drastically reduced during processing as in the case of refined, bleached, and deodorized oil and linters.

B. The Transgenic Plant

MON 15985 has been reviewed and approved for food and/or feed use in many countries including Australia/New Zealand (Food, 2002; Environment, 2002); Brazil (Food, Feed and Environment, 2009); Burkina Faso (Environment, 2013); Canada (Food, 2003; Feed, 2003); China (Food and Feed, 2015); Colombia (Food, 2009; Feed, 2008); European Union (Food and Feed, 2015); India (Food, Feed and Environment, 2009); Japan (Food, 2002; Feed, 2003; Environment, 2004); Korea (Food, 2013; Feed, 2008); Malawi (Environment, 2016); Mexico (Food and Feed, 2003); Nigeria (Environment, 2016); Paraguay (Food, Feed and Environment, 2018); Philippines (Food and Feed, 2013); Singapore (Food, Feed, 2008); South Africa (Food, Feed and Environment, 2003); Taiwan (Food, 2015; Feed, 2017); USA (Food and Feed, 2002; Environment, 2002). He

The consumption pattern is not expected to be changed as a result of introducing MON 15985 since it is not materially different in composition, safety or nutrition from conventional cotton other than the introduction of the lepidopteran-resistant trait. Furthermore, cotton is planted primarily for production of cotton fibers. Cottonseed is not consumed by humans because the majority of commercial cotton varieties contain the anti-nutrients gossypol and cyclopropenoid fatty acids. The primary human food currently produced from cottonseed is refined, bleached, and deodorized (RBD) oil, and to a smaller extent, linters.

C. Donor Organism

The cry2Ab2 gene is derived from the bacterium Bacillus thuringiensis subsp. kurstaki, which is ubiquitous in the environment and has a well-established safety profile. Bacillus thuringiensis subsp. kurstaki is not known or reported to pose a risk of allergenicity or pathogenicity to humans or animals. The cry2Ab2 gene encodes the insect-control protein Cry2Ab2. The uidA gene is derived from

Escherichia coli strain K12, a bacterium ubiquitous in the environment and in the digestive systems of vertebrates, including humans. The uidA gene encodes the GUS protein as a scorable marker.
The MON 15985 Cry2Ab2 and GUS proteins are present at very low levels in the harvested cottonseed of MON 15985. Furthermore, only oil and linters obtained from cottonseed are used for food applications, both of which contain undetectable or negligible levels of proteins. Therefore, MON 15985 Cry2Ab2 and GUS proteins are essentially a non-existent portion of the total proteins present in food derived from MON 15985. A bioinformatic analysis confirmed that the MON 15985 Cry2Ab2 and GUS proteins lack structural similarity to known allergens and toxins, or other proteins known to have adverse effects on mammals. The MON 15985 Cry2Ab2 and GUS proteins were rapidly digested in simulated gastrointestinal fluids. The MON 15985 Cry2Ab2 and GUS proteins demonstrated substantial loss of activity upon heating at temperatures well below standard cottonseed processing temperatures and therefore, it is reasonable to conclude that they would not be consumed as an active protein. All these data support the conclusion that the Cry2Ab2 and GUS proteins present in MON 15985 are not similar to known allergens and do not pose a significant allergenic risk to humans or animals.

C. Transformation System

Particle acceleration transformation was used.Genomic DNA was the target of genetic modification. The experimental protocol was completely provided. The plasmid containing the cry2Ab and uidA gene cassettes, PV-GHBK11, was propagated in E. coli, purified from bacterial suspensions using column purification. The gene of interest and the marker gene were purified away from the vector backbone by cutting with a restriction endonuclease KpnI and subsequently separated and purified based on size differences by HPLC. This linear fragment is designated PV-GHBK11L. The purified linear DNA, PV-GHBK11L, was then precipitated onto gold particles using calcium chloride and spermidine, essentially as described by John (1997).

The cotton tissue that is the recipient of the introduced DNA, variety DP50B, is the commercial variety containing the Bollgard cry1Ac gene. DNA was introduced into the cotton meristems by the particle acceleration method. Germline integration of DNA was detected by histochemical staining for GUS in vascular tissue. Non-transformed tissue was removed over time, thus promoting growth of meristems containing the introduced DNA. The resulting seed from these plants was then screened for the production of the Cry2Ab protein.

The plasmid vector, PV-GHBK11, is an 8.7Kb high copy number pUC based plasmid. It contains well-characterized DNA elements for selection and replication of the plasmid in bacteria. The host for DNA cloning and vector construction was E. coli XL1Blue, a derivative of the common laboratory E. coli K-12 strain. The genetic elements in PV-GHBK11 are listed; sizes listed here include non-functional DNA needed for the cloning. The ori-pUC is from the plasmid pUC19 (Vieira and Messing, 1987) and it provides the origin for replication and maintenance in E. coli. The nptII gene is for selection on kanamycin of bacteria containing the plasmid.

D. Inserted DNA

The insertion site was sufficiently demonstrated. Molecular analyses confirmed that MON 15985 contains a single copy of T-DNA containing the cry2Ab2 and the uidA expression cassettes that is stably integrated at a single insertion site and no detectable additional genetic elements. The result was demonstrated sufficiently by Southern blot analysis and PCR and sequence analysis.

In addition, the integrity and order of genetic elements within each insertion site are demonstrated as follows: The Southern blot analysis was used to determine the insert number, the copy number, the intactness of the cry2Ab and uidA coding regions, the intactness of the cry2Ab and uidA cassettes, and

to confirm the absence of plasmid backbone sequence derived from plasmid PV-GHBK11. Plasmid PV-GHBK11, the plasmid backbone, the cry2Ab and uidA coding regions, the enhanced CaMV 35S promoter, and the NOS 3' polyadenylation sequence were all used as probes. Additionally, the 5' and 3' insert-to-plant junctions were verified using the polymerase chain reaction (PCR).

PCR and sequence analyses were performed on genomic DNA to verify the sequence at the 5' and 3' ends of the MON 15985 insertion site. The cry2Ab coding region and cassette are complete, however, the restriction site following the NOS 3' polyadenylation sequence in the cassette is no longer present. The uidA coding region and its NOS 3' polyadenylation sequence are also complete, however, the 260 bp of the 5' end of the enhanced CaMv 35S promoter of the uidA cassette is not present in the inserted uidA gene cassette. The 35S promoter is still functional despite this truncation, as demonstrated by the GUS protein.

Molecular characterization of MON 15985 by Southern blot analysis demonstrated that the genome of cotton MON 15985 does not contain any detectable plasmid backbone DNA. This analysis has sufficiently demonstrated the absence of plasmid backbone in the genome of cotton MON 15985.

Other GM plants that uses the particular gene of interest, cry2AB2 includes, corn (45 events), soybean (2 events) and cotton (10 events). The uidA gene, on the other hand, has been expressed in papaya (2 events), plum (1 event), sugar beet (1 event), soybean (1 event), and cotton (10 events).

E. Genetic Stability

The stability of the DNA insert across multiple generations was demonstrated by Southern blot analysis. The analysis showed that in the MON 15985, single integration locus was maintained through five generations of breeding; thereby confirming the stability of the insert.

Meanwhile, the segregation and stability of DNA inserted in MON 15985 was confirmed by performing a Chi-square ($\chi 2$) analysis over four generations. All generations segregated as expected for a single insertion site. The R1 progeny of MON 15985 yielded the expected segregation ratio of 3:1 with respect to the detection of Cry2Ab protein. Progenies of MON 15985 backcrossed to commercial cotton cultivars yielded the expected segregation ratio of approximately 1:1 with respect to the Cry2Ab protein. The Chi square analysis of the segregation results are consistent with a single active site of insertion into the genomic cotton DNA, segregating according to Mendelian genetics.

These data confirm that the DNA insert in cotton event 15985 contains a DNA insert of a single locus that segregates according to Mendelian genetics and therefore remains stably integrated in the plant genome over generations and over successive backcross generations.

F. Expressed Material

Cry2Ab2 and GUS proteins were not detected in various parts (leaf, seed, whole plant and pollen) of MON 15985. This evidence was displayed by characterization and risk assessment determined by ELISA. Tissues of MON 15985 were collected from 8 field locations planted during the 1998 growing season in the US. The field sites were representative of cotton producing regions suitable for commercial production as stated by the technology developer.

It was reported that the levels of Cry2Ab2 protein in young leaves was consistent across all plots and field locations, with a range from 10.1 to 33.3 µg/g fwt, and a mean across all locations of 23.8 ± 6.3 µg/g fwt. The levels of Cry2Ab protein in cottonseed tissue were also consistent across all locations, ranging from 31.8 to 50.7 µg/g fresh weight, with a mean of 43.2 ± 5.7 µg/g. In whole plant tissues, the mean levels of Cry2Ab protein were 8.80 + 1.20 µg/g fwt, with range across locations of 7.28 - 10.46

µg/g. In pollen, the Cry2Ab protein was not detected above the limit of detection for the assay (0.25 µg/g) at either location in either the test or control samples. On the other hand, the levels of GUS protein production in young leaves ranged from 51.7 - 176 µg/g fwt, with a mean across locations of 106 ± 32 µg/g fwt. The levels of GUS protein in cottonseed tissue ranged from 37.2 to 82.3 µg/g fresh weight, with a mean of 58.8 ± 13.0 µg/g.

ELISA analyses in tissues from multiple sites revealed that the MON 15985 Cry2Ab2 and GUS proteins were not detected in the parental control line.

Both introduce genes have no metabolic role in the host organism. Based on the evidences and previous studies presented, it is very unlikely that the introduced genes will affect the metabolic activity of the host plants. The introduced proteins are effective to only to specific targets as stated.

G. Toxicological Assessment

The digestibility of the Cry2Ab2 protein in simulated gastric fluid (SGF, containing pepsin) was assessed by SDS-PAGE gel staining and western blot analysis. SDS-PAGE analysis of SGF incubations showed that by 15 seconds, greater than 98% of the Cry2Ab protein was digested and that no fragments ≥2 kDa of the parent protein were resolved. The estimated T50 result for SGF is below 15 seconds.

Further, the digestibility of the Cry2Ab2 in simulated intestinal fluid (SIF, containing pancreatin) was assessed by SDS-PAGE gel staining and immunoblot analysis. Immunoblot analysis of SIF incubations showed that a relatively stable Cry2Ab protein fragment (50 kDa) was produced within 1 minute and observed for at least 24 hours.

This in vitro assessment of Cry2Ab protein digestibility indicates that the Cry2Ab protein will be readily digested in the mammalian stomach by pepsin, which supporting the conclusion that the MON 15985 Cry2Ab2 protein is highly unlikely to pose a safety concern to human and animal health.

On the other hand, the effect of heat treatment on the B.t.-produced MON 15985 Cry2Ab2 protein was evaluated. Western blot analyses of the Cry2Ab2 protein, after defatted cottonseed meal derived from cotton event 15985 or its parental control, were heated for 25 minutes at 121 °C at 30 psi to simulate the commercial processing of cottonseed meal. Results showed a complete loss of immune-detectability. Based on the limit of detection data, detectable Cry2Ab2 protein decreased by 50-70% depending on the extraction buffer used. These data demonstrate that the Cry2Ab2 protein behaves with a predictable tendency toward loss of immune-detectability at elevated temperatures.

Meanwhile, bioinformatic analyses were performed to assess the potential for toxicity of MON 15985 Cry2Ab2. The sequence similarities between the MON 15985 Cry2Ab2 protein and known toxins were assessed using the TOXIN4 database and the FASTA sequence alignment program. Results showed no structurally relevant similarity exists between MON 15985 Cry2Ab2 and known toxins that would be harmful to human or animal health.

Lastly, Cry2Ab2 exhibits a No Observed Effect Level (NOEL) of 1450 mg/kg body weight as reported. This was demonstrated by conducting an oral gavage of mice to assess potential toxicity. B.t.-produced Cry2Ab2 protein was used for the safety assessment. The B.t.-produced Cry2Ab2 protein has been shown to be equivalent to the plant-produced Cry2Ab2 protein present in MON 15985.

Meanwhile, the GUS protein was digested at an estimated T50 of less than two minutes as demonstrated by in vitro simulated gastric fluid model system using pepsin as the digestive enzyme. Digestion of the GUS protein proved that it is unlikely to pose safety and health concern in humans.

The effect of heat treatment on the E. coli-produced MON 15985 GUS protein was also evaluated. Western blot analyses of the GUS protein (after defatted cottonseed meal derived from cotton event 15985 or its parental control) were heated for 25 minutes at 121 °C at 30 psi to simulate the commercial processing of cottonseed meal. Results showed a complete loss of immune-detectability. Based on the limit of detection data, detectable GUS protein decreased by 50-70% depending on the extraction buffer used. These data demonstrate that the GUS protein behaves with a predictable tendency toward loss of immune-detectability at elevated temperatures.

Further, bioinformatic analyses were performed to assess the potential for toxicity of MON 15985 GUS. The sequence similarities between the MON 15985 GUS protein and known toxins were assessed using the TOXIN4 database and the FASTA sequence alignment program. Results showed no structurally relevant similarity exists between MON 15985 GUS and known toxins that would be harmful to human or animal health.

Lastly, an acute oral toxicity assessment was also conducted to evaluate the potential adverse clinical signs or detrimental effects on mice exposed to E. coli-produced GUS protein. The GUS protein was administered at a dose level of 69 mg/kg body weight as a single dose by oral gavage to CD-1 mice. There were no treatment-related effects on survival, clinical observations, body weight gain, food consumption or gross pathology. Therefore, the No Observable Effect Levels, adverse or otherwise, (NOELs and NOAELs) for GUS were considered to be 69 mg/kg body weight.

GUS was derived from E. coli during the safety assessment and it was demonstrated to be equivalent to the GUS expressed in cotton event MON 15985.

H. Allergenicity Assessment

The amino acid sequence comparison was done using the bioinformatic analyses. Bioinformatic were performed to assess the potential for allergenicity of MON 15985 Cry2Ab2. The sequence similarities between the MON 15985 Cry2Ab2 protein and 567 sequences of known allergens and gliadins were assessed using the FASTA sequence alignment program and an eight-amino acid sliding window search (IDENTITYSEARCH). Results showed no structurally and immunologically relevant similarity exists between MON 15985 Cry2Ab2 and known allergens and gliadins that would be harmful to human or animal health.

The Western blot analysis showed that the MON 15985 produced and B.t. produced Cry2Ab2 proteins were determined to have equivalent immunoreactivity. The molecular weight (MW) analysis showed that the intact MON 15985 produced Cry2Ab2 protein migrated to the same position on the gel as the B.t. produced Cry2Ab2 protein (MW was calculated to be around 62 to 63 kDa).

Furthermore, the functional equivalency of MON 15985- and B.t.-produced Cry2Ab2 proteins were analyzed by the cotton bollworm bioassays. The MON 15985- and B.t.-produced Cry2Ab2 proteins were not significantly different. These results confirmed that these two proteins are functionally equivalent. The glycosylation analysis showed that MON 15985 produced Cry2Ab2 protein is not glycosylated and is equivalent to that of the B.t. produced Cry2Ab2 protein. All these data provided a detailed characterization of the MON 15985-produced Cry2Ab2 protein and established it equivalence to the B.t. produced Cry2Ab2 protein.

Lastly, the overall mean level of Cry2Ab2 protein in MON 15985 cottonseed is 43.2 μg/g fwt. The mean percent dry weight of total protein in MON 15985 cottonseed is 26.13%. The percentage of Cry2Ab2 protein in MON 15985 cottonseed is calculated as 0.0156%. Therefore, the Cry2Ab2 protein represents a very small portion of the total protein in MON 15985 cottonseed.

Meanwhile, the effect of heat treatment on the E. coli-produced MON 15985 GUS protein was evaluated. Western blot analyses of the GUS protein, after defatted cottonseed meal derived from cotton event 15985 or its parental control, was heated for 25 minutes at 121 °C at 30 psi to simulate the commercial processing of cottonseed meal; results showed complete loss of immune-detectability. Based on the limit of detection data, detectable GUS protein decreased by 50-70% depending on the extraction buffer used. These data demonstrate that the GUS protein have a predictable tendency toward loss of immune-detectability at elevated temperatures.

In addition, the Western blot analysis showed that The MON 15985 produced and E. coli produced GUS proteins were determined to have equivalent immunoreactivity. The molecular weight (MW) analysis showed that the intact MON 15985 produced GUS protein migrated to the same position on the gel as the E. coli produced GUS protein and the apparent MW was observed to be around 76 kDa. All these data provide a detailed characterization of the MON 15985-produced GUS protein and establish its equivalence to the E. coli produced GUS protein.

Further, the reported overall mean level of GUS protein in MON 15985 cottonseed is 58.8 µg/g fwt. The mean percent dry weight of total protein in MON 15985 cottonseed is 26.13%. The percentage of GUS protein in MON 15985 cottonseed is calculated as 0.0212%. Therefore, the GUS protein represent a very small portion of the total protein in MON 15985 cottonseed.

I. Nutritional Data

There were no significant differences in seed proximate levels between cotton event 15985 and the parental control DP50B. For mineral levels, no significant differences were observed in any mineral levels obtained for the event 15985 and the means were all within the non-transgenic and commercial reference ranges.

Furthermore, there were no amino acid parameters observed in cotton event 15985 that were significantly different from the parental control variety, DP50B. Therefore, the amino acid composition of the seed from cotton event 15985 was equivalent to the composition of the seed from the parental DP50B control. Fatty acid profiles were evaluated in cottonseed for event 15985 and no statistically significant differences were noted for palmitic, palmitoleic, oleic, linolenic and gamma linoleic, arachidic, behenic or lignoceric acids compared to DP50B. Small, but statistically significant differences were observed for myristic, stearic, linoleic, and dihydrosterculic acids, between event 15985 and control. All these significantly different mean values for event 15985 were, however, within the non-transgenic and commercial cotton reference ranges, as well as within the 95% confidence intervals and ranges published in the literature. Therefore these differences were not considered biologically relevant.

On the other hand, reports showed no statistically significant differences in the gossypol levels obtained for event 15985 compared to the control and the mean values were within the non-transgenic and commercial reference ranges. For cyclopropenoid fatty acids, statistically significant differences were observed for the mean values of malvalic, and sterculic between event 15985 and control DP50B. All significant mean differences for event 15985 were within the 95% confidence interval for each true mean difference and mean values were within the non-transgenic and commercial reference ranges, as well as literature ranges. Therefore the differences were not considered biologically relevant.

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

BAI AND BPI-PPSSD ASSESSMENT AND RECOMMENDATIONS

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

A. Toxicological Assessment

For the digestibility study of Cry2Ab, simulated gastric fluid (SGF, containing pepsin) was assessed using SDS-PAGE and western blot analysis and simulated intestinal fluid (SIF, containing pancreatin) was assessed using SDS-PAGE gel staining and immunoblot analysis. The estimated T50 result for SGF is below 15 seconds and stable activity in SIF wherein a relatively stable Cry2Ab protein fragment (approx. 50 kDa) was produced within 1 minute and observed for at least 24 hours determined using immunoblot analysis. Western blot analysis provided by the developer also showed that Cry2Ab2 completely lost immunodetectability upon heat treatment for 25 minutes at 121°C at 30 psi (Lee et al., 2001).

Bioinformatics analyses using FASTA sequence alignment program and TOXIN4 database provided by the developer indicated that Cry2Ab2 has no significant homology to any known toxin. Moreover, acute oral toxicity study provided by the developer indicated no treatment related adverse effects on survival, clinical observations, body weight gain, food consumption or gross pathology of mice administered with Cry2Ab2 protein (Bechtel, 2000), with NOAEL 1450 mg/kg bw. Amino terminal amino acid sequence comparison, apparent molecular weight and immunological recognition, glycosylation analysis, enzymatic activity characterization provided by the developer indicated that the B.t.-produced Cry2Ab2 and plant-produced Cry2Ab2 is functionally and structurally equivalent (Holleschack et al., 1999).

Meanwhile, the digestibility study for GUS protein used simulated gastric fluid (SGF, containing pepsin) and simulated intestinal fluid (SIF, containing pancreatin), both assessed using western blot analysis and enzymatic activity assay. The estimated T50 result for SGF is below 15 seconds while T50 result for SIF is below 2 hours. Thus, GUS protein will be readily degraded in the digestive tract of humans which implies that it will not pose any adverse effects on human or animal health.

Western blot analyses of the GUS protein after defatted cottonseed meal derived from cotton event 15985 showed a complete loss of immunodetectability at elevated temperatures which means GUS protein will not be immunodetectable after commercial processing of cottonseed meal.Moreover, using FASTA sequence alignment tool, it was determined that MON 15985 GUS protein shared no structurally relevant similarity with the toxins sequences in TOXIN4 database.

Lastly, Escherichia coli-produced GUS protein was used as the test protein and has been shown to be equivalent to the plant-produced GUS protein present in MON 15985. Amino terminal amino acid sequence comparison, apparent molecular weight and immunological recognition, glycosylation analysis, enzymatic activity characterization provided by the developer indicated that the B.t.-produced GUS and plant-produced GUS is functionally and structurally equivalent (Holleschack et al., 1999).

B Allergenicity Assessment

Bioinformatics analyses using FASTA sequence alignment program and IDENTITYSEARCH database provided indicated that Cry2Ab2 has no significant homology to any known allergen (Monsanto, 2000, Hileman and Astwood, 1999; Holleschack et al., 1999). In addition, Western blot analysis showed that immunoreactive bands migrating at the expected apparent MW were present in all lanes loaded with the MON 15985 produced or B.t. produced Cry2Ab2 protein. The MON 15985 produced and B.t. produced Cry2Ab2 proteins were determined to have equivalent immunoreactivity.

Molecular weight (MW) analysis showed that the intact MON 15985 produced Cry2Ab2 protein migrated to the same position on the gel as the B.t. produced Cry2Ab2 protein and the apparent MW was calculated to be around 62 to 63 kDa, while the glycosylation analysis showed that MON 15985 produced Cry2Ab2 protein is not glycosylated and is equivalent to that of the B.t. produced Cry2Ab2 protein.

Cry2Ab2 constitutes 0.0156% of the total protein in MON 15985 cotton grain (Monsanto petition to US. FDA. ,2000, Section IV.A.3 p.28).

On the other hand, bioinformatics analyses using FASTA sequence alignment program and IDENTITYSEARCH database provided by the developer indicated that GUS has no significant homology to any known allergen (Monsanto, 2000, Section IV.C.2.c. p. 61-62). Moreover, western blot analysis showed that immunoreactive bands migrating at the expected apparent MW were present in all lanes loaded with the MON 15985 produced or E. coli produced GUS protein. The MON 15985 produced and E. coli produced GUS proteins were determined to have equivalent immunoreactivity.

The molecular weight (MW) analysis showed that the intact MON 15985 produced GUS protein migrated to the same position on the gel as the E. coli produced GUS protein and the apparent MW was observed to be around 76 kDa. However, the calculated MW based on the amino acid sequence is 68.4 kDa.

Mean level of GUS protein expression is 58.8 μg/g , with 0.0212% of the total protein percentage found in the transgenic plant. However, GUS is detected only at low levels and shares only a small portion of the total protein content.

C. Nutritional Data

No significant differences were observed between the proximate content of MON 15985 and the non-transgenic control DP50B cotton (Monsanto Petition to U.S. FDA. 2000, Section IV.A.3. Table 4 p.32). There were also no observed significant differences in the amino acid analysis between DP50B(conventional variety) and MON15985 thus, their protein contents are of equivalence.

Fatty acid profiles for the parent and transgenic plant was evaluated with most of its component to be of no statistical significant differences. In constrast, there were differences found in myristic, linoleic, stearic, and dihydrosterculic acid but the values obtained are still within the literature values for safety.

In the antinutrient proximate analysis, there were significant differences observed on the values obtained for malvalic, sterculic, any dihydrosterculic acid. However, these data still fall under the normal range of values among non-transgenic commercial variants and the control DP50B. Total gossypol mean levels were also assessed in the said event and it was detected that the values are of no difference compared to the conventional comparator. Hence, the differences are within the tolerance levels of safety.

D. Recommendation

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

DENR 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 FFP of Cotton MON15985. 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 environmept (land, and water) and biodiversity. Conventional cotton cultivars do not possess any characteristic of being weedy, such as seed dormancy, persistence in soil and seed banks, germination under adverse environmental conditions, rapid vegetative growth, short life cycle, and high seed output and dispersal rate (Keeler, 1985 and Keeler et alv 1996）. 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, that is high temperatures (121°C and above), varying pH, enzyme digestion, etc. (Lee, Lee, & Astwood, 2001).

2. The GUS protein produced by uidA gene derived from E. coli strain K12 is ubiquitous to the environment and in the digestive system of vertebrates. While the Cry2Ab2 protein, from Bacillus thuringiensis subsp. kurstaki is commonly found in the soil. These proteins are readily degraded upon exposure in a simulated gastrointestinal fluid of mammals (Leach, et.al. 2000 and Ream, 1996).

3. The project description report (PDR) discusses the specified environmental management plan indicating the possible risk and harm to the environment and non-target organisms as well as the mitigating measures and contingency plan. 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 considering that cottons have no potential to persist in an unfavorable environment (Keeler et al., 1996).

Based on the evaluation and review of literatures cited, the DENR-BC considered the regulated article safe to the environment and non-target organisms, and hereby submits the technical report relative to the application of Monsanto Philippines, Inc. for Biosafety Permit for direct use as food, feed, or for processing of Cotton MON15985.

DOH ASSESSMENT AND RECOMMENDATION

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 cotton varieties.

4. The regulated article is not materially different in nutritional composition from that of the non-transgenic cotton or the conventional cotton.

5. 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.

6. Based on the above considerations and with the submitted sworn statement and accountability of the proponent, we hereby submit our evaluation to BPI relative to the application of a Biosafety Permit for Direct Use as Food. Feed, or for Processing (FFP) of Cotton MON 15985 will not post any significant risk to the health and environment and that any hazards and risk could be managed by the following measures.

SEC ASSESSMENT AND RECOMMENDATIONS

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

The importation of Cotton MON 15985 Cotton will not drastically affect the current patterns of consumption, production and trade of cotton. As mentioned earlier, Philippine is an insignificant producer of cotton, thus rely greatly in imported cotton to meet domestic demand. Granting permit to import Cotton MON 15985 cotton may help stabilize supply and prices of cotton. Thus, consumption will likewise be stabilized. With regards to the patterns of trade, domestic trade may improve due to availability of supply and stable prices but global trade will definitely not be affected since Philippine import of cotton is very minimal relative to global trade

Recommendation

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