CONSOLIDATED REPORT OF MONSANTO'S SOYBEAN MON87751
APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING

EXECUTIVE SUMMARY

On November 28, 2016, Monsanto Philippines Inc.'s submitted soybean MON87751 for direct use as food and feed, or for processing to the Bureau of Plant Industry (BPI) 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 (BAI), concurred that soybean MON87751 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 that the conditions set by them 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 MON87751 will not pose any significant risk to 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 MON87751.

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 the complete dossier submitted by Monsanto Philippines, Inc.

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

A. STRP, PPSSD, BAI (Safety Assessment)

After thorough review of the technical documents submitted by the applicant, the assessors' findings were as follows:

1. Host Organism (Soybean)

   Soybean is a source of key nutrients such as proteins carbohydrates, fats, minerals (calcium, phosphorous, potassium, magnesium, iron, sodium, selenium, manganese, copper and zinc) and vitamins (A and B complex, K and E). In the human diet, soybean is consumed as food as soy milk, milk curd/tofu, whole cooked seed, edible soybean oil along with other vegetable oils, among others. On the other hand, soybean meal is a source of protein in animal diet.

   Soybean also contains several antinutrients such as heat labile trypsin inhibitors, lectins, stachyose, raffinose and phytic acid. Trypsin inhibitors activities are destroyed during toasting or heating whole soybean or meal while lecithin is readily inactivated by moist heat when raw soybean is processed into defatted, toasted soybean meal. Soybean meal contains stachyose and raffinose that cause flatulence in swine and poultry. However, these are reduced or decreased by processing soybean further into concentrates or isolates. For phytic acid, adding phytase into the diets of poultry and swine will release phytinbond phosphorus that decreased phytic acid in the diet.

   Further, soybean is not a source of toxicants. Although, soybean contains recognized allergenic protein, but relevant estimate of its allergenic potential is not complete. There is no significant level of amino acid homology exists between the pat gene and any protein allergens.

2. Donor Organism (*Bacillus thuringensis*)

   The donor organism, *Bacillus thuringensis* is not known to be toxic or allergenic or pathogenic to humans, animals or plants. Bioinformatics analyses showed that the CryA.105 and Cry2Ab2 proteins do not share structurally or immunologically relevant amino acid sequence similarities with known allergens and pathogens.

   Further, as shown in simulated digestion experiments using simulated digestive fluids, CryA.105 and Cry2Ab2 are rapidly digested. Thus, these cry proteins are not similar to known allergens and do not pose significant allergenic risk to humans or animals.

   The molecular characterization of MON 87751 by Next Generation Sequencing and Junction Sequence Analysis (NGS/JSA) showed that a single copy of the intended transfer DNA (T-DNA I) containing the *crylA.105* and the *cry2Ab2* expression cassettes from PV-GMIR13196 was integrated into the soybean genome at a single locus. These analyses also showed no PV-GMIR13196 backbone or T-DNA II elements had been inserted.

3. The Transformation System

   Soybean MON87751 was genetically modified using *Agrobacterium tumefaciens* - mediated transformation of soybean tissue using the transformation plasmid vector PV-GMIR13196.

The plasmid vector PV-GMIR13196 contains two separate T-DNAs that are each delineated by left and right border regions. The first T-DNA, designated as T-DNA I, contains the *crylA.105* and *cry2Ab2* expression cassettes. The second T-DNA, designated as T-DNA II, contains marker genes that allow for simplified selection of transformed tissue. During transformation, both T-DNAs were inserted into the soybean genome. Subsequently, traditional breeding, segregation, selection and screening were used to isolate those plants that contain the *crylA.105* and *cry2Ab2* expression cassettes (T-DNA I) and results showed it do not contain the marker gene expression cassettes (T-DNA II), resulting in the production of marker-free, MON 87751.

4. The Inserted DNA

Using a combination of sequencing, PCR and bioinformatics, MON87751 demonstrated to contain one copy of the intended transfer DNA (T-DNA I) containing the *cryIa.105* and the *cry2Ab2* expression cassettes stably integrated at a single locus and inherited according to Mendelian principles over multiple generations.

The sequence and organization of the DNA insert is identical to the corresponding region in the PV-GMIR13196 T-DNA I. This was determined through directed sequencing (locus-specific PCR, DNA sequencing and analyses) on MON 87751 which established the complete sequence of the single DNA insert from PV-GMIR13196, the adjacent flanking DNA, and the 5' and 3' insert-to-flank junctions. Further, this also confirmed that no vector backbone, or T-DNA II, or other unintended plasmid sequences are present in MON 87751.

5. Genetic Stability

The Next Generation Sequencing and Junction Sequence Analysis (NGS/JSA) showed the stability of the DNA insert across multiple generations. Thus, MON87751 contains a single and stable T-DNA I insertion.

Chi square test analysis was done and showed the stability of the insert over three generations (F2, F3 and F4).

The segregation analyses showed the heritability and stability of the insert as expected across multiple generations substantiating the molecular insert stability analysis and established the genetic behavior of the T-DNA I in MON 87751 at a single chromosomal locus.

6. Expressed Material

Using the enzyme-linked immunosorbent assay (ELISA), the expression levels of proteins Cry1A.105 and Cry2Ab2 in various MON 87751 plant tissues at different growth stages such as leaf, root, forage and seed were determined. The mean concentrations of each protein in leaves, roots, forage, and seeds were provided below.

<u>Cry1A.105</u>

The mean Cry1A.105 protein levels are: 400-790 ug/g dwt in leaves, 230 ug/g dwt in forage, 2.4 ug/g dwt in seeds, 11ug/g dwt in pollen/anther and below the limit of detection in roots.

<u>Cry2Ab2</u>

The mean Cry2Ab2 protein levels are: 24-32ug/g dwt in leaves, 14ug/g dwt in forage, 15ug/g dwt in seeds, 7.7ug/g dwt in pollen/anther and 15ug/g dwt in roots.

7. Toxicological Assessment

Digestibility

Digestibility of Cry1A.105 and Cry2Ab2 proteins was evaluated in Simulated Gastric Fluids (SGF) and Simulated Intestinal Fluids (SIF) and was analyzed through the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis.

Further, results showed that Cry1A.105 and Cry2Ab2 were rapidly digested at 0.5 minutes upon incubation in SGF with pepsin and in SIF with pancreatinin.

Heat Inactivation

Results of analyses showed that an increase in temperature did not affect the band intensity of Cry1A.105 and Cry2Ab2 proteins in SDS-PAGE. While the functional activity of Cry1A.105 and Cry2Ab2 proteins were decreased upon heat treatment at 75C and 55C, respectively for 15 and 30 minutes.

Amino Acid Comparison

Results of the FASTA bioinformatic alignment search showed that neither of the proteins (Cry1A.105 and Cry2Ab2) is homologous with known toxins.

Acute Oral Gavage

Cry1A.105 protein administered by oral gavage at a total dose of 2072 mg/kg body weight and Cry2Ab2 administered at a total dose of 2198 mg/kg body weight of CD-1 mice showed no treatment-related effects on survival, clinical observations, body weight gain, food consumption or gross pathology.

Source of Test Protein

The Cry1A.105 and Cry2Ab2 proteins were produced in *Escherichia coli*.

SDS-PAGE and Western blot analysis showed that the microbiologically-produced and the MON 87751 soybean expressed proteins were equivalent in terms of molecular weight and immune recognition.

Compositional Analysis (Nutritional Data)

Proximate Analysis

No statistically significant differences were observed in the proximate levels (moisture, protein, fat, ash, carbohydrates, acid detergent fiber (ADF) and neutral detergent fiber (NDF) between the test soybean and the control soybean.

Vitamins and Mineral Composition, Amino acids, Fatty Acids

A significant difference was observed in the levels of seven (7) nutrient components (protein, glycine, proline, phosphorus and vitamin E in seed and total fat and NDF in forage) out of the 50 components assessed for MON87751. However, the mean difference of these 7 components between MON87751 and control was less than the conventional control range value. Further, the MON 87751 mean component values were within the values observed in ILSI Crop Composition Database values.

Anti-nutrients
(lectin, trypsin inhibitors, phytic acid, raffinose and stachyose)

No significant differences were observed for lectin, trypsin inhibitors, phyticacid, and stachyose. A statistically significant difference ($p<0.05$) was observed for raffinose. The mean difference in raffinose values between MON 87751 and the conventional control was less than the range of the conventional control values, indicating that MON 87751 does not impact levels of raffinose more than natural variation within the conventional control grown at multiple locations.

B. DENR BC (for Safety of Event to the Environment)

The assessment of the DENR BC focused on the potential environmental impact of MON 87751 soybean.

Upon evaluation of the project description report (PDR) and environmental risk assessment (ERA) submitted by the applicant, the Committee found that 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.

The DENR BC recommended the approval and issuance of a biosafety permit for FFP for MON 87751 soybean provided that strict monitoring of the regulated article will be conducted from the port of entry to the traders/importers storage/warehouse in accordance with Section32 of the JDC 1 s.2016.

C. DOH BC (for Environmental Health Safety)

The DOH-BC assessed the potential environmental health impact of MON 87751 using the technical dossier provided by the applicant. Based on this assessment, the DOH BC concluded that MON 87751 soybean is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health and environment.

In addition, the regulated article applied for Direct Use for FFP does not require changes in the usual practices in unloading and loading, hauling, transport and storage, and processing.

The DOH BC recommended the approval of the regulated article with the following conditions:

1. Strict monitoring of the regulated article from port of entry to the trader's/ importers storage/warehouse as stated in Section 32 of the JDC No. 1 series, 2016.
2. The BPI to include in the issuance of permit for the release of this product the following conditions:
    a) Any spillage (during unloading and loading/hauling and transport unloading and storage) shall be collected and cleaned up immediately.
    b) Transportation of the consignment from the port of entry to any destination within the country shall be in closed containers.
    c) It is recommended that the BPI ensure the following:
    Clear instructions that the product is only for the purpose of direct use as food, feed or for processing, and is not to be used as planting material.

D. SEC Expert

The SEC expert recommended the approval of MON 87751 soybean with the following observations: Recent studies in the country can be presented to show how importation of GM soybean can help maintain global trade of soybean products. Further, recent studies and data/projections showing that GM soybean product will not drastically change current patterns of production, consumption/utilization and trade can also be presented.