ASSESSORS' CONSOLIDATED REPORT OF MONSANTO'S CORN MON88017 FOR FOOD, FEED AND OR PROCESSING (Original Application under DOST-DA-DENR-DOH-DILG Joint Department Circular No.1, Series of 2016)

BACKGROUND OF THE APPLICATION

On April 22, 2016, Monsanto Philippines applied their single trait product Corn MON88017 for food, feed and or processing as an original application under the DOST-DA-DENR-DOH-DILG Joint Department Circular No. 1 Series of 2016.

The assessors for the said event were the following:

- Three (3) members of the Scientific and Technical Review Panel (STRP)
- Department of Environment and Natural Resources (DENR)
- Department of Health (DOH)
- Bureau of Animal Industry (BAI)
- Plant Product Safety Services Division (PPSSD)
- Socio-economic, ethical and cultural (SEC) Expert

EVALUATION OF THE ASSESSORS

STRP, BAI and PPSSD

A. Host Organisms

In many areas in the Philippines, cultivated corn (*Zea mays* L.) has been a staple food in human diet especially in Central Visayas and some part of Mindanao. It is also being consumed as animal feed by its grains, forage or stalks and processed corn products are available.

The assessors agreed that corn is a source of key nutrients. Corn grain and its processed fractions are consumed in a multitude of food and animal feed products. Corn forage is extensively consumed as an animal feed by ruminants. Corn does not contain any known allergens or produce significant quantities of toxins or anti-nutritional factors warranting analytical or toxicological tests (Watson, 1982 in Bogdanova, 2004; White and Pollak, 1995 in Bogdanova, 2004). The available nutrients in corn are the following: carbohydrates (corn starch), protein, fat (corn oil), magnesium and potassium, beta-carotene and vitamin E, vitamin B complex, vitamin A, iron, calcium, phosphorus, phytochemicals, antioxidants and many secondary metabolites. In addition, table 2 to table 5 pp. 19-22 (OECD, 2002) showed the nutrients present in maize and maize products.

On the other hand, the assessors reported that corn can also be a source of anti-nutrients. However, these anti-nutrients are not considered as nutritionally significant. Anti-nutrients interfere with the absorption of nutrients. In corn, there are some anti-nutrients and secondary metabolites listed as phytic acid, DIMBOA, ferulic acid, p-coumaric acid and raffinose (OECD, 2002). According to BAI, phytic acid is significant to animal feed. An addition of enzyme phytase can break down part of phytic acid and release bound phosphorus and calcium. (OECD 2002, p34). In addition, phytic acid complexes with iron in the gut, it interferes with the absorption of iron. The levels of phytic acid in corn vary from 0.45 to 1% of dry matter, which is a very low amount. DIMBOA is relatively high during vegetative stage, while raffinose can be removed by cooking and other treatment. Secondary metabolites and anti-nutrients content in grain from MON88017 were compared to its conventional counterpart and showed no significant difference when values were obtained (OECD, 2002).

In its very long history of safe use and utilization, the assessors accepted that corn of MON88017 is not a source of toxicants. They also accepted that it is not a source of common allergen although some case studies reported that individuals could have allergic reaction to corn due to presence of a 9 kilodalton (kd) lipid transfer protein, 16 kd trypsin inhibitor and zeins. These reported allergic effects for maize include skin, gastrointestinal

and respiratory complaints. However, clinical relevance of these findings is uncertain (Hefle, 1996 and OECD, 2002 p. 29).

Abovementioned, corn was used as food, feed and/or for processing. It is consumed as food when cooked, and seldom raw. On the other hand, corn stover, corn milled grains, corn forage, corn silage and gluten meal are final form of consumed feed product. Approximately 2/3 of the corn produced in U.S. is fed to livestock, and corn forage is extensively consumed as animal feed in ruminants (Hodge, 1982; Perry, 1988; Watson, 1988). Approximately 100 million metric tons of grain is fed to livestock directly as grain. Another 1.5 to 2 million metric tons of wet and dry milling by-products (primarily corn gluten meal and feed) are fed directly or in formulated feeds (Perry, 1988).

Corn-based products such as corn oil, corn starch, and high fructose corn syrup as used in soft drinks are consumed in all parts of the country. STRP reported that it is also converted to a variety of sweetener and fermentation products including high fructose syrup and ethanol (Anderson & Watson, 1982; Watson 1988; White & Pollack, 1995 and NCGA, 2003). Starch is derived from field maize by the wet milling process. About 60 % of the starch is converted (by acid or enzyme hydrolysis) to sweeteners (syrups) and ethanol. The remaining 40% is used for foods and industrial uses. The lipids in starch are mainly free fatty acids (Anderson and Watson, 1982). Starch is used in a variety of products that include bakery products, baby foods, sauces, dressings and soups. Typically, maize starch contains residual protein at 0.4 % (SCF, 1999) or 0.6% (Federal Register, 2000), whereas starch hydrolysates contain 100-200ppm of protein (SCF, 1999). Corn oil is commercially processed from the germ and accounts for approximately 9% of the domestic oil production (Orthoefer & Sinram, 1987). The materials of corn oil are a component of many foods including bakery and dairy-goods, beverages, confections and meat products (FDA, 2004 pp117-118).

However, consumption pattern of corn/corn products by Filipinos is relatively very low compared to rice. Corn oil is less consumed compared to coconut or other oils. The 2008

National Nutrition Survey of FNRI gives an average consumption of 21 g/capita/day, highest in NCR, Calabarzon, and Central Luzon, amounting to only 2.4% of total food consumed. It contributes 3.5% of total caloric intake. 2.6% of protein and 4.3% of carbohydrate.

B. Transgenic Plant

The approved transgenic plant as food submitted by the petitioner includes Argentina, Australia/New Zealand, Brail, Canada, China, Colombia, European Union, Japan, Korea, Malaysia, Mexico, Philippines (Roundup, Roundup Ready, and Yield Gard), Russia, Singapore, South Africa, Taiwan, US and Vietnam. On the other hand, same countries approved the transgenic plant if it is used as feed except for Australia, New Zealand and Taiwan.

The STRP, BAI and PPSSD agreed that the consumption patterns of these transgenic plants by population subgroups will not change as a result of introducing the novel food since MON88017 is equivalent to conventional corn with similar genetic background as well as other conventional corn varieties.

C. Donor Organisms

The donor organisms used in the event were *Bacillus thuringiensis* subsp. *Kumamotoensis* and *Agrobacterium* sp. strain *CP4*). All protein-encoding sequences have found in the original gene construct and have been described with respect to source and potential pathogenic and allergic properties. The STRP, BAI and PPSSD agreed that MON88017 encodes for two proteins: CP4 EPSPS and Cry3Bb1. The applicant performs four methods to determine the allergenic and pathogenic properties by checking the source of organisms that can cause allergies, the amount of the proteins expressed would be enough to cause allergy, check similarity of the expressed proteins to known allergens by bioinformatics, and lastly, if the proteins are stable in simulated gastric fluids. Based on the data presented, both CP4 EPSPS and Cry3Bb1 do not share structurally and immunologically relevant amino acid sequence similarities with known allergens. Both proteins are contained in

extremely small amounts in the grain. Digestive fate experiments showed that both proteins are rapidly digested in simulated gastric and intestinal fluids. In addition, all potentially inserted regulatory sequences (e.g. promoters, enhancers, termination signals) were adequately described. Also, there are no antisense sequences. Thus, they agreed that the donor organisms Cry3Bb1 and CP4 ESPS are not known to be toxic or allergenic. They have had a history of safe use and have similarity to the respective proteins in products now in use.

D. Transformation System

Agrobacterium-mediated transformation was used in the system. Nuclear DNA is the target genetic modification. The applicant completely provided the following protocol: method of transformation of the corn cells; plasmid used; expression casettes; genetic elements, sizes of genetic elements, donor or sources, function of these elements, and references; molecular analysis of the expression of the genes inserted, results of the molecular analysis (e.g. Southern blot), and biosafety assessment procedures. All the genetic components used were provided by Figure 1 which is Plasmid map of Vector PV-Z MIR39 on page 34 and Table 1 (Summary of genetic elements of vector PV-Z MIR 39) on pages 35 to 37 of the reference cited. However, STRP noted that the petitioner citation should be changed to Part IV. Section 2 (pages 34-37). STRP added that the plasmid vector should include size, orientation, and location of all genetic elements, oligonucleotide primers used for PCR analysis, and the sites of any restriction endonucleases used in the analysis of the inserted DNA. No carrier DNA and helper plasmids were used.

E. Inserted DNA

Integrity and order of genetic elements within each insertion site was demonstrated by PCR analysis. The results of the PCR analysis using specific primers were sufficient to show order and integrity of the genetic elements. No truncations, deletions, rearrangements were identified. The main transgene has been expressed in other approved GM crops, especially for *cp4 epsps* gene that has been inserted in glyphosate tolerant crops. The *cry3Bb1* gene has also been inserted in other corn aside from being used to produce corn with stacked traits. No backbone was present based on the results of Southern blot analysis comparing plasmid vector and genomic DNA of several breeding generations of MON 88017. The data presented is sufficient in showing absence of backbone sequences.

F. Genetic Stability

The data showed that the integrated DNA in MON 88017 was stable for seven generations. Southern blot analysis is sufficient for determining stability of integrated DNA. On the other hand, evidence presented showed that segregation analysis was performed on progenies of different generations of MON 88017 wherein the phenotypes of the progenies were determined. The results of the analysis showed a Mendelian inheritance pattern consistent with a single locus of insertion.

G. Express Material

Level of expression of the novel proteins in different plant parts pollen, silk, forage, root, leaf, grain was determined via ELISA. The samples came from 3 replicated location trials. Data is clearly presented in tables showing expression among the different plant parts and over season.

H. Toxicological Assessment

1. Novel Protein 1: CP4 EPSPS

Digestibility experiments on CP4 EPSPS showed a half-life of less than 15 seconds using simulated gastric fluid (SGF) and less than 10 minutes based on western blot analysis. Additional experiments using *E-coli* produced CP4 EPSPS showed that at least 98% of the

protein is digested in simulated gastric fluid within 15 seconds based on SPS-PAGE, western blot analysis and functional assay. These experiments show that CP4 EPSPS is expected to be degraded rapidly in human digestive system. It was found that CP4 EPSPS is functionally active at 25˚C and 37˚C. Functional activity declined to 70% and 25% of the control when treated for 15 min and 30 minutes, respectively. No significant change in band intensity of CP4 EPSPS was attributed to heat treatment based on the SDS-PAGE analysis.

The STRP, BAI and PPSSD agreed that in amino acid sequence of PAT protein, data showed no homology to any known toxins and pharmacologically active proteins relevant to human and animal health. Moreover, acute oral gavage was performed to determine the No Observe Effect Level (NOEL) of CP4 EPSPS protein. Results showed that the NOEL for oral toxicity of the protein in mice was 572 mg/kg. Thus, incorporation of CP4 EPSPS in the diet of mice did not significantly vary in terms of the bod weight or food consumption of mice. In addition, the source of the test protein used for safety assessment was *E. coli.* Equivalency to the plant protein in MON88017 and was demonstrated using Western blot analysis and densitometry, SDS-PAGE, mass spectrometry, glycosylation analysis and CP4 EPSPS activity assay. These five tests were sufficient to show the equivalency of the bacterial and plant proteins.

2. Novel Protein 2: Cry3Bb1

The enzyme used in the digestibility study were SGF and simulated intestinal fluid (SIF). Cry3Bb1 was rapidly digested when incubated in SGF and SIF. In SGF, at least 98 % of the full length protein was digested within 15 seconds. In SIF, at least 99.5 % of the full length protein was digested within 1 minute. In heat inactivation study which was conducted using different temperatures and different time exposures, activity of Cry3Bb1 was determined using an insect assay while the effect on the protein band intensity was determined by SDS-PAGE. The LC50 was also given at each temperature.

On the other hand, results of FASTA sequence alignments showed lack of structurally significant similarity between Cry3Bb1 protein with any known toxins or pharmacologically active proteins relevant to human or animal health. Acute oral gavage

was performed showing that the NOEL for Cry3Bb1 was greater than 1930 mg/kg body weight. Procedure done was sufficient to show that including Cry3Bb1 did not result in changes in food consumption and toxicity to the test animals. Moreover, the source of the test protein was *E. coli* and the equivalency with MON88017 was already shown using a combination of molecular and biological assays.

In this toxicological assessment, the two novel protein expressed independently and the different functional activities remain. The Cry3Bb1 protein is most likely in the cytoplasm of maize cells while the CP4 EPSPS proteins are in the chloroplast because the gene constructs encoding the Cry3Bb1 does not include sequence for targeting. On the other hand, the CP4 EPSPS proteins are targeted specifically to these organelles.

I. Allergenicity Assessment

1. CP4 EPSPS

In allergenicity assessment of CP4 EPSPS, simulated gastric fluid with pepsin was used in the digestibility study. Results showed that CP4 EPSPS protein was rapidly digested after incubation in SGF within 15 seconds. It was also found that CP4 EPSPS is functionally active at 25˚C and 37˚C. Functional activity declined to 70% and 25% of the control when treated for 15 min and 30 minutes, respectively. No significant change in band intensity of CP4 EPSPS was attributed to heat treatment based on the SDS-PAGE analysis. Amino acid sequence comparison showed no homology to any known allergens.

The mean level of CPS EPSPS protein in MON88017 grain is 5.8 µg/g. The mean dry weight of total protein in MON 88017 is 12.51%. Thus the percent of CP$ EPSPS protein in on gram of MON88017 grain is 0.0046362%. Serum screening was not performed since corn has no reported allergens and the data showed that the expressed CP4 EPSPS does not have any homology and structural similarity to known allergens.

2. Cry3Bb1

Likewise, simulated gastric fluid was also used in the digestibility study of Cry3Bb1. STRP, BAI and PPSSD agreed that heat inactivation and amino acid sequence comparison showed

the same result to its toxicity assessment. The percent on the total protein in one gram of MON88017 based on the prevalence in food is 0.01199%. This shows that Cry3Bb1 represents a very small portion of total protein in MON88017 grain according to PPSSD. Serum screening was not performed since corn does not contain any known allergens or produce any significant quantities of toxins or anti-nutritional factors.

J. Nutritional Data

MON88017 and its non-transgenic counterpart were compared to show if there have any significant differences through their proximate analysis, key nutrients, and anti-nutrients. In proximate analysis, the assessors concurred that the result for forage and grain analysis showed no significant difference with respect to composition. In comparison with range of commercial varieties, four commercially available corn hybrids were grown each at the same field sites providing a total of 12 different reference substances. According to the applicant, all test values of proximate were within the 99% observed range and the assessors agreed with it. In addition, the comparison with the range of literature values was within the reported range while in biological significance, statistical differences observed were not biologically relevant.

Same results were obtained in key nutrients. The only difference is that vitamin B1 values in grain were statistically lower in MON 88017 compared to conventional corn for all four analyses, although they were similar to literature and historical values. Moreover, all test values of nutrients were within 99% of tolerance interval for the 16 comparisons that were statistically different between MON88017 and conventional control. The lower values of vitamin B1 could be of concern in populations consuming corn as staple.

Lastly, the levels of anti-nutrient in MON88017 are compositionally equivalent to that of the conventional corn. Processing may lower the level of anti-nutrient in MON 88017 as same amount as it reduces the level in the conventional corn. PPSSD site examples that processing may lower the level of anti-nutrient. The raffinose can be removed in corn by soaking, cooking, enzyme or solvent treatment and by irradiation. On the other hand, phytic acid is present in maize and binds about 60-75% of the phosphorus in the form of phytate (NRC, 1998). Bioavailability of phosphorus in maize is 15% for non-ruminants. Ruminants utilize considerably more phosphorus and can produce phytase that breaks

down the phytate and releases phosphorus (Ensminger et al., 1990). Processing of maize, fresh and dry for the production of various traditional products results in the loss of phytic acid. Fresh mature corn contains less phytic acid (1.71 g kg−1) than dry corn (7.15–7.60 g kg−1). The loss of phytic acid varies from 18.1 to 46.7% for fresh maize and from 11.5 to 52.6% for dry maize respectively among the heat treatments given (Khan et al., 1991).

The STRPs together with BAI and PPSSD unanimously find the scientific evidence provided by the applicant is sufficient in showing that the regulated article being applied for direct use is as safe for human and animal health, and the environment, as its conventional counterpart.

DENR ASSESSMENT AND RECOMMENDATION

After thorough and scientific review and evaluation of the documents provided by the Bureau of Plant lndustry (BPl) to the DENR Biosafety Committee within the prescribed period pursuant to Joint Department Circular (JDC) No.1 s.2016 on the application of Monsanto Philippines, Inc. for direct use for feed, food or processing of Genetically Modified Corn resistant to corn rootworm (Diabrotica spp.) and tolerant to glyphosate herbicide with Single trait product MON88017, the following are the observations 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. lf 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 traders/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 lndustry (BPl) (Article Vlll, Section 26 of JDC No,1 s.2016);

3. It is suggested that 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 Corn MON88017 commodity products; and
c) Strict monitoring of the regulated article from port of entry to the trader's/importer's storage/warehouse (Section 32 of the JDC No. 1 s.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 and scientific review and evaluation of the documents provided by the Bureau of Plant Industry (BPI) on the application of Monsanto Philippines Inc., for direct use as food and feed or processing of Corn MON88017. DOH find scientific evidence that the regulated article applied for direct use as food and feed or processing is safe as its conventional counterpart and is not expected to pose any significant risk to human and animal health and environment.

The following are the observations and recommendations:
1. On the description of the phases or stages of the biotechnology project, Monsanto Philippines Inc. claimed that Corn MON88017 does not pose greater risks to human when brought to loading/unloading, transport, storage or processing.

2. On the risk to Health Matrix (Integration of the health consequence rating with incident potential rating), the Monsanto Philippines, Inc. rated the activities of the phases of project a very low incident/exposure potential rating.
3. Scientific pieces of evidences from provided references i.e. literatures show that regulated article applied for direct use is as safe as its conventional counterpart and shall not pose any significant risk on human health, animal health and on the environment.

4. It is suggested that the Bureau of Plant Industry (BPI) ensure the following:

a. 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.

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 a clear labeling of the product from importation down to all levels of marketing stating that it is only for the purpose of direct use as food and feed or processing and is not to be used as planting materials.

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 as food and feed or processing of Corn MON88017.

SEC ASSESSMENT AND RECOMMENDATION

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

A. Socio-economic issues

In terms of production, consumption and trade, the sec expert said that GM yellow corn may already have a growing presence in markets around the world as adoption has reportedly been extensive even in the Philippines. With regard to continued importation of GM yellow corn, a few things need to be pointed out. First, justifying continued importation of GM yellow corn will need to be premised on something more relevant to the product's usage in the Philippines. Feed milling accounts for about 70 percent of the use of yellow corn in the country. Hence, rather than arguing the need to meet anticipated rise in per capita consumption, there is greater value in tackling how GM yellow corn importation

would affect the animal feed production and other components of the food value chain. On the other hand, since importation also concerns usage for human food consumption, specifying whether the use is for food processing (chips, flour, etc..) or as coarse grain food will make the argument for meeting per capita consumption more appreciable. Secondly, the justification regarding maintenance of global trade of corn products need to be further expounded. It was recognized that local yellow corn production has been increasing in the past years. If continued importation will be based on anticipated rising consumption, there needs to be a clearer discussion of the basis for this expectation, i.e., is there a potential positive shift in usage from the animal feed or human food processing industries as well?). Finally, with the looming threat of climate change and weather-related risks to local production, it would have been helpful if the applicant discussed if importation may be treated as an adaptive measure for the benefit of corn users and consumers in the country.