CONSOLIDATED REPORT FOR MONSANTO PHILIPPINES INC.’S CORN MON810  
(APPLICATION FOR DIRECT USE AS FOOD AND FEED OR FOR PROCESSING)

Executive Summary of CORN MON810

Monsanto Company has developed insect-protected corn event MON810 by inserting cry1Ab gene which naturally produces *Bacillus thuringiensis* (Bt) protein in the maize. MON810 is protected from feeding damage by Asian corn borer, European corn borer, the southwestern corn borer, and the pink borer.

The benefits of planting the transgenic corn are: 1) a reliable means to control these major pests; 2) control target insects while not harming beneficial species; 3) reduced chemical pesticide usage; 4) reduced exposure of applicator; 5) a fit with the Integrated Pest Management (IPM) and sustainable agricultural systems; 6) reduced fumonisin mycotoxin level in corn kernels; and 7) no additional labor or machinery required, allowing both large and small growers to maximize hybrid yields. Additional to this, the quality of produce would be maintained and crop damage would be reduced thus more produce could be sold.

The gene of interest was isolated from the *Bacillus thuringiensis* var. *kurstaki* HD-1 strain found in DIPEL, the leading microbial insecticide in agricultural use. This gene was bombarded in the embryonic corn tissue with plasmid PV-ZMBK07. Southern blot analysis of MON810 corn demonstrated that a single functional copy of the cry1Ab coding sequence was integrated into the corn genome and that coding sequence is inherited in the expected Mendelian pattern.

The Cry1Ab protein shows no amino acid sequence homology to known protein toxins, and is rapidly degraded with loss of insecticidal activity under mammalian digestion condition. There were no toxicity indications during administration of Cry1Ab oral gavage. The cry1Ab gene was not derived from an allergenic source. The protein does not have immunologically relevant sequence similarity with known allergens or possess characteristics of known protein allergens. It was also reported to have no harmful effects on other types of organisms.

Compositional analyses were performed. The compositional values of MON810 corn were compared with that of the control line, as well as published literature values. Compositional data confirmed that MON 810 corn is substantially equivalent to the parental hybrid as well as traditional corn hybrids. It was also confirmed that MON 810 corn plants are as safe and nutritious as conventional corn varieties.

A comprehensive phenotypic, agronomic, environmental interaction assessment was conducted which included the evaluation of characteristics for seed germination, disease and pest susceptibilities and yield characteristics. Results indicate that MON 810 does not possess weediness potential, increased susceptibility or tolerance to specific abiotic stresses, diseases, or arthropods, or characteristics that would confer a plant pest risk compared to conventional corn.

**BACKGROUND**

On July 19, 2017, Monsanto Philippines applied their single trait product Corn MON810 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)
STRP PPSSD BAI ASSESSMENT

A. Host Organisms

The assessors agreed that corn as stated in OECD (2002) and OGTR (2008), is a source of key nutrients such as starch, proteins, oil and fiber. It contains amino acids, minerals, vitamins, fatty acids and organic acids. Anti-nutrients are also present in corn such as phytic acid, DIMBOA, raffinose, trypsin, and chymotrypsin inhibitors. They also affirmed that corn in its long history of consumption and safe use, it does not produce significant quantity of toxins, allergen or anti-nutritional factors requiring analytical or toxicological tests (OECD, 2002).

According to Morris, 1998 (page 24) human consumption of corn is in the form of corn-based ingredients such as high fructose corn syrup, starch, sweeteners, cereals, oil and alcohol. It can also be directly consumed cooked at its various developmental stage from baby corn to mature grain.

The Philippines corn domestic consumption are 8500 per 1000 MT with growth rate of 4.94% and 8700 per 1000 MT with growth rate of 2.35% in 2016 and 2017, respectively. The Global Environment Monitoring System (GEMS 2012) reported that the estimated consumption of corn of the Cluster G09, where Philippines is included, is 16.736g/kg body weight/day for the general population and 32.518 g/kg body weight/day for children. In 2014, the daily per capita consumption index of corn in the Philippines is 60.08 grams/day, while the daily per capita calories supply is 213.88 grams (PSA, 2015).

BAI together with other assessors concur that corn is also used as feed. The plant is consumed as fresh or dry forage and silage. Grains and by-products of wet and dry milling are incorporated as ingredients in feed formulations. Morris (1998) said that feed mills combine whole or cracked shelled grain with various sources of protein, vitamins and minerals. A significant portion of the crop is fed to animals as forage which include fodder (leaves, stalks, tassel and husks, stovers) and silage.

B. Transgenic Plant

The countries that have approved the transgenic plant as food and feed are Argentina (Food, Feed and Environment, 1998); Australia/New Zealand (Food, 2000); Brazil (Food, Feed and Environment, 2007); Canada (Food, 1997; Feed and Environment, 1997); China (Food, Feed and Processing, 2015); Colombia (Food, 2003; Feed, 2006; Environment, 2007); Egypt (Food, Feed and Environment, 2008); European Union (Food, Feed and Processing, 1998); Honduras (Food, Feed and Environment 2001); Indonesia (Environment, 1999); Japan
According to Monsanto Petition to US MON810 was shown to be compositionally equivalent to conventional corn with similar genetic background. No change in consumption pattern is anticipated due to the creation of MON810.

C. Donor Organisms

According to Monsanto Petition to KFDA (Safety Assessment of YieldGard con Event MON 810), the donor organism for Cry1Ab protein, Bacillus thuringiensis subsp. kurstaki, has been used commercially in the United States since 1958 to produce microbial-derived products with insecticidal activity. Applications of sporulated Bt have a long history of safe use for pest control in agriculture, especially in organic farming. Microbial pesticides containing B. thuringiensis Cry proteins have been subjected to extensive toxicity testing showing no adverse effects to human health. Additionally, the assessors affirmed that there are no confirmed cases of allergic reactions to Cry proteins in microbial-derived B. thuringiensis products during more than 50 years of use. Bioinformatics analyses demonstrated that the Cry1Ab protein does not share structurally or immunologically relevant amino acid sequence with known allergens and protein toxins. Bai also added that Cry1Ab protein is rapidly degraded with loss of insecticidal activity under conditions that simulate mammalian digestion.

Croon et al., 1996 presented that two plasmids were used PV-ZMBK07 and PV-ZMGT10. Fig.II.1 (page 18) shows the plasmid map of PV-ZMBK07. It contains the cry1Ab gene under the control of the enhanced CaMV35S promoter (E35S) which is approx. 0.6 kb in size. Table III.1 (page 19) shows the DNA elements in the plasmid PV-ZMBK07. Fig. III.2 (page 21) shows the plasmid map of PV-ZMGT10 while Table III.2 shows the DNA elements in the plasmid (Monsanto USFDA Part III).

Located between the E35S promoter and cry1Ab gene is the 0.8 kb intron from the maize hsp 70 (heat-shock protein) gene. It is present to increase the levels of transcription according to Rochester, 1986). The hsp70 intron is followed by the 3.47 kb cry1Ab gene (Fischhoff et al., 1987). The cry1Ab gene is joined to the 0.27 kb nopaline synthase 3’ nontranslated sequence, NOS 3 which provides the m RNA polyadenylation signal (Fraley et al., 1983).

The assessors also indicate that cry1Ab gene is 3468 nucleotides in length and encodes a full-length B-t-k. HD-1 (CRY 1Ab) protein of 1156 amino acids (Fischhoff et al., 1987). Cry1Ab protein when subjected to trypsin yields an active trypsin-resistant protein product of approximately 600 amino acids in planta and in vitro. The cry1Ab gene encodes a CRY 1Ab protein which is identical to the CRY 1Ab protein product in nature.

The cry1Ab gene sequence was modified to increase the levels of expression in maize but the amino acid sequences remained unchanged (Perlak et al, 1991). The alpha region of the lacZ gene for beta-galactosidase, present under a bacterial controlled promoter is present in PV-ZMBK07. This region contained a polylinker which allowed for the cloning of the desired genes within the plasmid vector (Vieira and Messing, 1987). The lac Z alpha region is followed by the 0.65 kb origin of replication of plasmids in E. coli.
Next to the ori-pUC region is the nptII (neomycin phosphotransferase type II) gene. The neomycin phosphotransferase type II enzyme confers resistance to aminoglycoside antibiotics (i.e. kanamycin and neomycin) and was used for selection of bacteria during plasmid construction. The coding sequence for the nptII gene was derived from the prokaryotic transposon Tn5 and is present under its own bacterial promoter (Beck et al., 1982). A single copy of the cry1Ab coding sequence was integrated into the corn genome and that cry 1Ab coding sequence is inherited in the expected Mendelian pattern (Croon et al, 1996).

In addition, Cry1Ab is the only expressible sequence in the event. Bacillus thuringiensis subsp. kurstaki is the donor organism of cry1Ab gene. It is not known to be toxic or allergic. Cry1Ab protein is encoded by the Cry1Ab protein. They also concur that it is not known to be toxic or allergic.

D. Transformation System

The transformation method used is the particle acceleration method. This method is more effective for monocot crop like maize. The target of genetic modification is the Nuclear DNA. Nuclear DNA is more appropriate to modify than mitochondrial DNA since it codes for proteins of all functions; unlike the mitochondrial DNA which only codes for metabolic processes. Also, rate of mutation is higher in mitochondrial DNA than in nuclear DNA.

The assessors also agreed that the experimental protocol was completely provided in MSL14204 MON 810 Molecular Characterization section. The protocol included the materials, reagents, and step-by-step process. Carrier DNA and helper plasmids were not used.

The transformation plasmid PV-ZMBK07 and PV-ZMGT10 were used to produce corn MON810. Plasmid PV-ZMBK07 contained the CaMV35S promoter with duplicated enhancer region (e35S); an intron from the maize Hsp70 (heat-shock protein) gene; the cry1Ab gene encoding the nature identical Cry1Ab protein; nos 3’ - a 3’ nontranslated region of the nopaline synthase gene (transcriptional termination; polyadenylation); a lac operon fragment (a partial Escherichia coli lacI coding sequence, the promoter lac and a partial coding sequence for β-D-galactosidase or lacZ protein from pUC119); ori-pUC (replication origin for pUC plasmids, originally derived from plasmid ColE1); and the nptII gene as a selectable marker. Detailed description was shown in Figure III.1 (page 18) and Table III. 1 (page19). The plasmid PV-ZMGT10 contained the e35S promoter; the Hsp70 intron; transit peptides CPT1 and CPT2 (from Arabidopsis thaliana); the CP4 epsps gene (from Agrobacterium sp.) which allows for selection on glyphosate; and the gox gene (from Ochrobactrum anthropi sp.) which encodes a glyphosate metabolising enzyme, the nos 3’ terminator, the lacZ region, ori-pUC and the nptII gene. Detailed description was shown in Figure III.2 (page 21) and Table III. 2 (page 22).

E. The Inserted DNA

Based on the information provided by the developer, only a single fragment of the genetic components integrated in a single insertion site in MON 810 was detected using NdeI digestion and Southern blot analysis. NdeI digestion was conducted using NdeI restriction enzyme on PV ZMBK07 DNA probe (Monsanto, 1996, Part III. Pp. 15-41; MSL14204).

The integrity of each inserted gene was examined by Southern blot analysis, PCR, and Nucleotide Sequencing. After digesting and probing, an expected fragment the same size as the gene of interest was produced for the positive control while no fragment was produced in the negative control. MON810 contains one band relatively similar to the positive control. The backbone integrity of both plasmid PV-ZMBK07 and PV-ZMGT10 was also determined using Southern Blot analysis. There were no identified/determined truncations, deletions,
or rearrangements. Further, the transgene cry1Ab has been expressed in approved GM crops such as cotton (3 single events and 5 stacked events), other products in maize (10 single events and 70 stacked events), rice (2 single events) and sugarcane (1 single event).

F. Genetic Stability

Stability of gene transfer was assessed based on segregation data for MON810 derivatives. The introduced trait was stably inherited through seven generations of crosses to one recurrent parent and six generations of crosses to a second unrelated inbred. The assessors concur that segregation was assessed by determining the ratio of Cry1Ab-expressing plants to non-expressing progeny plants as detected by an ELISA and European corn borer feeding assay and analysed by a chi square test. Three backcross generations (BCOF1, BCOF2, and BC1F1) were evaluated and segregation followed Mendelian principles of inheritance. Segregation and stability data are consistent with a stable introduction of the cry1Ab gene at a single insertion site.

G. Expressed Material

Levels of expressed protein from samples obtained in 6 US sites (1994-1995) were measured using ELISA and Western blot. In leaves, the levels ranged from 5.21 to 10.61 ug/g fwt and in grain, detected levels were within the range of 0.19 to 0.91 ug/g fwt. In the same 6 US sites, forage/whole plant samples had same levels of Cry1Ab in the range of 2.31 to 4.65 ug/g fwt. ELISA and Western blot Cry1Ab has no metabolic role.

H. Toxicological Assessment

The assessors agreed that the results of western blot assays provided by the developer demonstrated that the E. coli-produced Cry1Ab protein is rapidly degraded in simulated gastric fluid containing pepsin within 15 seconds and more than 90% of the Cry1Ab protein is degraded within two minutes of incubation. The bioactivity of Cry1Ab measured by insect bioassay rapidly dissipated at about 74-90% within two minutes of incubation in SGF (Monsanto, 1996, Part IV, p. 57; MSL13425). Den et al., (2002) pointed out that Cry1Ab was detected in ground Bt corn hybrid (196 ± 1 pbb), but not in any of the corn mash samples. Loss of the C Cry1Ab protein as detected by ELISA occurred during liquefaction. Samples incubated at 80°C, the protein was non-detectable after less than 15 min. of heating. Further experimentation demonstrated that Bt protein was rapidly denatured at 95°C used for liquefaction.

Cry1Ab protein amino acid sequence is homologous to proteins of Bt insecticidal crystal protein gene family but has no sequence similarity to known protein toxin sequences in the PIR, EMBL, SwissProt and GenBank protein databases. The acute oral toxicity was conducted with E. coli-produced Cry1Ab protein, which is the chemical and functional equivalence of the Cry1Ab protein in Insect-Protected maize plants in terms of molecular weight, immunoreactivity and insecticidal activity. Lastly, maize does not contain any known allergens or produce significant quantities of toxins or anti-nutritional factors warranting analytical or toxicological tests.

I. Allergenicity Assessment

In vitro simulated mammalian gastric and intestinal digestive mixtures were established and used to assess the susceptibility of Cry1Ab to proteolytic digestion. The assessors cited Croon et al., 1996 which presented the data from simulated digestion experiments. It demonstrated that Cry1Ab protein degraded rapidly, more than 90% of the initially added Cry1Ab was degraded after two minutes incubation in gastric system.
Ream (1994) presented that Btk HD-1 protein as visualized by Western blot, degraded rapidly in SGF. HD-1 protein degraded approximately 50% after 20 second incubation in SGF and more than 90% after 2 min. (Fig 2, Lane 11). On the other hand, Huber and Luthy (1981) showed that with trypsin degradation of endotoxin comes to stop at a MW of 80,000 while with gut juice proteases it proceeds to MW 60,000 to 40,000. Prolonged exposure to proteases must finally lead to the loss of toxicity. The critical MW range seems to be 30,000. Below this point, activity is rapidly lost.

The proponent also provided a study by Dien et al., (2002) regarding the fate of Bt protein in ethanol production. In the said study, the effect of temperature on the concentration of Cry1Ab in corn samples was determined through monitoring the concentration of the novel protein incubated at 70, 80 and 90⁰C. The concentration of Cry1Ab was measured through quantitative sandwich ELISA. Results showed that the concentration of Cry1Ab proteins in corn rapidly decreased by almost 100% upon incubation at 80 and 90⁰C for 15 and 5 minutes, respectively.

The bioinformatic results showed no biologically relevant sequence similarities to allergens using FASTA sequence alignment tool search against the GenBank, EMBI, PIR and Swiss Prot Databases. This was verified using FASTA search in AllergenOnline on August 31, 2017.

J. Nutritional Data

Proximate Analysis

The plant parts used in this analysis are forage and grains. Protein, fat, ash, crude fiber, neutral detergent fiber, acid detergent fiber, carbohydrate and dry matter were analyzed in the forage; while protein, fat, ash, crude fiber, neutral detergent fiber, acid detergent fiber and moisture were analyzed in the grains.

There are statistically significant differences observed in some parameters such as crude fiber in the 1994 US trial and moisture in the 1995 EU trials. However, these parameters could be influenced by external factors and their differences have no biological significance since the reported values are still within the range of literature values. MON810 was not compared to any commercial varieties, only to the control line and to published values. All data derived from the test (transgenic) line were within the reported range. The statistical differences in crude fiber in seed and protein in forage are not biologically significant since it was still within the range of historical conventional values.

Key Nutrients

The assessors added that out of 18 amino acids, the values for three amino acids (cysteine, histidine, and glutamic acid) were slightly higher than the published literature values for both MON810 and the control line. Eight amino acid values (cysteine, tryptophan, histidine, phenylalanine, alanine, proline, serine and tyrosine) were significantly higher in MON810 compared to the control line. Only two amino acid values (methionine and tryptophan) were significantly higher in the control line than in MON810. However, there were inconsistencies among trials making it unlikely to be of biological significance.

For the fatty acid composition, most of the fatty acids were excluded since their quantities were almost beyond detection. There were no significant differences with the rest of the tested fatty acids.

In the mineral components, the values for phosphorus and vitamin E were within the published literature values while calcium values for both MON810 and control line were lower than the published literature values. Calcium values were most likely influenced by phosphorus level. There was no biological significance in the difference of values since the
values were within published literature values. MON810 was not compared to any commercial varieties, only to the control line and to published values.

All proximate values were within the published literature range except cysteine, histidine, glutamic acid and calcium. These could have been the result of inconsistencies in the analytical procedure. The significant differences observed in Methionine, Tryptophan, Palmitic acid and Calcium in grain have no biological significance since it was within the range of historical conventional control values and/or it was not consistent across multiple-year data.
DENR 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 for Processing of Corn MON810, here under are the observations and appropriate actions:

1. From the evaluation of the application submitted by the proponent, including the scientific evidences from the provided references and literature, as well as other related studies, the Committee finds that the direct use of the regulated article whether for food, feed and/or for processing will not cause any significant adverse effect on the environment (land, air, and water) and non-target organisms, to wit:
   a. Genetic stability in the transgenic crop is ensured such that no unintended horizontal gene transfer shall occur to unrelated species.
   b. The protein product produced by the transgenic crop will degrade upon exposure to the natural environment and general conditions (i.e. high temperatures (60°C and above), varying pH, enzyme digestion, etc) and
   c. The protein product will not increase the weediness potential of the transgenic crop.

The data evaluated support the conclusion that the regulated article is as safe as its conventional counterpart.

2. The project description report (PDR) discussed 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 plant of the proponent. Upon evaluation of the submitted PDR and environmental risk assessment (ERA), the Committee notes that 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/germination.

3. The Bureau of Plant Industry (BPI) shall ensure the proper and secure packaging of the regulated article for transport and the safety and durability of the transport vehicle, for prevention of any possible spillage or unintended release during transport or import as per BPI inspection in the port area.

The DENR-BC finds scientific evidence that the regulated article applied for Direct Use as Food and Feed or Processing is as safe as conventional counterpart and is not expected to pose any significant risk to the environment and to non-target organisms. Based on the above considerations and with the proponent's sworn statement of accountability, we hereby submit our evaluation to Monsanto Philippines Inc. MON810 application for biosafety permit for food, feed and/or processing.
DOH RECOMMENDATION

After a thorough review and evaluation of the documents provided by the proponent, Monsanto Philippines Inc., through the Bureau of Plant Industry (BPI), in support of their application for approval for Direct Use as Food, Feed or for Processing (FFP) of Corn MON810. I/We,

Find that the regulated article applied for Direct Use as Food, Feed or for Processing (FFP) is as 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 evidences 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 allergic reaction.

3. The regulated article is as safe as food or feed derived from conventional corn varieties.

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

5. 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, Feed, or for Processing (FFP) of CORN MON810.
SEC RECOMMENDATION

The Philippines’ corn production and consumption in the last five years fluctuated in terms of growth rate, but a positive growth rate in general is observed. However, comparing the production and consumption data, Table 1 shows that in the last five years (2013-2017), the production has been less than the consumption. This observation implies the need to increase corn production to meet the demand for consumption through importation.

As far as GM corn production is concerned, Dr. Paul Teng, Chair of the Board of Trustees of the International Service for the Acquisition of Agri-biotech Application (ISAAA), revealed on May 19, 2017 that the Philippines ranked as the top grower of genetically modified (GM) crops in Southeast Asia, and 12th biggest producer globally in 2016. He added that the adoption rates of GM corn in the Philippines increased by two percentage points to 65 percent in 2016 from 63 percent in 2015. "Growing biotech or GM corn has benefitted some 406,000 farmers last year", according to Teng.

Meanwhile, the Philippine Statistics Authority (PSA) reported that the total area devoted to Bt corn is some 70 percent of the total harvest corn area in the Philippines in 2015. Dr. Randy A. Hautea, Global Coordinator and Southeast Asia Director of JSAAA mentioned that "about 65 percent of the total yellow corn of the country is actually GM". It was estimated that the production of GM corn will reach about 8.1 million metric tons in 2017.

For the past four years, the following are the data on the area (ha) devoted to the production of GM corn:

2013 - 795,000
2014 - 831,000
2015 - 702,000
2016 - 812,000

The data presented above show the importance of GM corn in the Philippines.

Table 2 shows the Philippines’ importation of corn in 2013 to 2017. Just like the production and consumption of corn, its importation in the last five years fluctuated.

<table>
<thead>
<tr>
<th>Year</th>
<th>Corn Import (1,000MT)</th>
<th>% Growth Rate</th>
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<tbody>
<tr>
<td>2013</td>
<td>741</td>
<td>705.43</td>
</tr>
<tr>
<td>2014</td>
<td>622</td>
<td>-15.92</td>
</tr>
<tr>
<td>2015</td>
<td>742</td>
<td>19.10</td>
</tr>
<tr>
<td>2016</td>
<td>606</td>
<td>-18.33</td>
</tr>
<tr>
<td>2017</td>
<td>400</td>
<td>-33.99</td>
</tr>
</tbody>
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(Source: https://www.indexmundi.com/agriculture/?country=ph&commodity=corn&graph=imports)
The Businessdiary.co.ph reported on August 30, 2016 that the Philippines used to import one million metric tons of corn annually. It was added that the successful distribution of the Bacillus thuringiensis (Bt) corn has beefed up the country’s corn production leading to a potential export of 50,000 to 100,000 metric tons of grains possibly to South Korea and Malaysia. Since 1960 until this year (except in 1991 and 2008) the Philippines has not been exporting corn.

After a thorough and scientific review and evaluation of the documents provided by Monsanto Philippines, Inc. relevant to MON810, I recommend for the approval and issuance of biosafety permit of the said GM product.