CONSOLIDATED REPORT FOR SYNGENTA PHILIPPINES INC.'S CORN BT11

(APPLICATION FOR COMMERCIAL PROPAGATION)

EXECUTIVE SUMMARY

On August 5, 2019, Syngenta Philippines submitted corn Bt11 as a new application for commercial propagation to the Bureau of Plant Industry (BPI) under the DOST-DA-DENR-DOH-DILG Joint Department Circular (JDC) No. 1 Series of 2016.

The said transformation event had obtained Biosafety Permit under the rules and regulations of the Department of Agriculture Administrative Order No. 8, Series of 2002 for commercial propagation on April 04, 2005 and has been renewed under the same circular on April 23, 2010 and April 23, 2015.

This application was assessed in accordance with *Article VI. Commercial Propagation of Regulated Articles* of the JDC No.1. This Article covers the basic biosafety policies, procedural requirements and guidelines in carrying out the risk assessment for GM applications for Commercial Propagation.

Under the JDC, the assessors for Syngenta Philippines Inc.'s corn BT11 for Commercial Propagation were the following:

- One (1) member of the Scientific and Technical Review Panel (STRP) – for evaluation of the Applicant's submitted risk assessment report.
- Department of Environment and Natural Resources (DENR) – for the determination of the environmental impact of the said application.
- Department of Health (DOH) - for the determination of the environmental health impact of the said application.
- Insect Resistance Management Team (IRMAT) – for review and evaluation of the application for any IRM related concerns and issues.
- Fertilizer and Pesticide Authority (FPA) – for the determination if the applicant is duly licensed as a pesticide handler in accordance with Presidential Decree No.1144 and if tolerance levels and good agricultural practices have been established for registration for the transformation event.
- Socio-economic, ethical and cultural (SEC) Expert – to evaluate SEC impact of the said application

After reviewing the documents submitted by the applicant, the STRP find scientific evidence that the regulated article applied for Commercial Propagation is as safe for human and animal health, and the environment as its conventional counterpart. Based on the assessment of the DENR BC and DOH BC, the regulated article is not expected to pose greater risk to the environment and health, respectively, than its conventional counterpart. IRMAT and SEC expert recommended for the approval and issuance of a biosafety permit of the said GM product. Furthermore, the plant-incorporated protectant (PIP) in the regulated article has been duly registered with FPA.

BACKGROUND

In accordance with Article VI. Section 15 of the JDC, no regulated article shall be released for commercial propagation unless: (1) a Biosafety Permit for Commercial Propagation has been secured in accordance with this Circular; (2) it can be shown that based on field trial conducted in the Philippines, the regulated article does not pose greater risks to biodiversity, human and animal health than its conventional counterpart; (3) food and feed safety studies show that the regulated article does not pose greater risks to biodiversity, human and animal health than its conventional counterpart, consistent with CODEX Alimentarius Guidelines on the Food Safety Assessment of Foods Derived from the Recombinant-DNA Plants and protocols of the DOH and BAI on feeding trials; and (4) if the regulated article is a pest-protected plant, its transformation

event that serves as plantincorporated protectant (PIP) has been duly registered with the Fertilizer and Pesticide Authority (FPA).

The BPI Biotech Office provided the assessors, except for the SEC expert, the complete dossier submitted by the applicant. The SEC expert, on the other hand, was provided with special questionnaire on socio-economic, ethical and cultural considerations that have been addressed by the applicant 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

I. HOST ORGANISM

The key nutrients such as proteins, fats, and carbohydrates, as well as dietary fiber are present in corn at certain amounts. Corn contains few anti-nutrients. One of which is phytic acid which diminishes the availability of phosphorus in monogastric animals. Raffinose and trypsin inhibitor, as antinutrients, have no nutritional significance in corn. [1][2]

Also, there are no significant native allergens and toxins associated with the genus *Zea.* [3][4]. Corn is consumed as staple cereal by approximately 20% of Filipinos. The consumption pattern of corn in Cluster G09, where Philippines is a part of, is 32.518 g/kg bw/day for children and 16.736 g/kg bw/day for the general population. [5][6]

Corn is mainly utilized as animal feed and for processing. Corn is preferred in livestock production as a processed whole grain, as a by-product of the milling industry or as silage. [7].

II. TRANSGENIC PLANT

The event (corn Bt11) has been approved as food and feed in different countries such as Argentina, Australia/New Zealand, Brazil, Canada, china, Colombia, European Union, Indonesia, Japan, Korea, Malaysia, Mexico, Paraguay, Philippines, Russian federation, Singapore, South Africa, Taiwan, Uruguay, United States, and Vietnam. [8]

III. DONOR ORGANISM

The c*ry1Ab* gene was isolated from *Bacillus thuringensis* and pat gene from *Streptomyces viridochromogenes. T*he two organisms mentioned are not known to be sources of allergenic proteins. [9] Proteins encoded by the expressible sequences are not known to be toxic or allergenic. [10] [11]

TRANSFORMATION SYSTEM

Corn Bt11 was produced through protoplast transformation. [12] Nuclear DNA is the target of genetic modification. [13] Furthermore, corn Bt11 was produced from the transformation plasmid pZ015012. [10] No carrier DNA or helper plasmids were used. [14].

V. INSERTED DNA

There is single intact insertion of *cry1Ab* and *pat.* Southern analysis to validate insertion sites. [15] There were no nucleotide differences and the organization of the genetic elements within the insert was maintained. There were no truncations, deletions, or rearrangements identified Potential for creating novel chimeric ORFs was not tested because there were no occurrence of truncations, deletions, or rearrangements. [16]

VI. GENETIC STABILITY

Results from segregation analysis demonstrate that all plants were either susceptible to corn borer and tolerant to glufosinate or resistant to both. From the results, the applicant clearly demonstrated the following for the Bt11 event:

a. The *cry1Ab* gene is inherited as a single Mendelian trait in Bt11
b. The *pat* gene is inherited as a single Mendelian trait in Bt11
c. The genetic background did not influence the inheritance pattern of the introduced characters. [17]

VII. EXPRESSED MATERIAL

The levels of expression of novel proteins (Cry1Ab and PAT) in different plant parts was measured. ELISA was used to determine level of expression of Cry1Ab in several corn plant tissues derived from corn Bt11. [18]

Furthermore, the protein PAT acetylates glufosinate-ammonium (but not glutamate) resulting to the deactivation of the compound. Thus, the deactivation of glufosinate-ammonium confers tolerance to glufosinate-ammonium in herbicides. Glufosinate-ammonium is responsible for the inhibition of glutamine synthetase, an enzyme involved in the nitrogen assimilation pathway. [19]

VIII. TOXICOLOGICAL ASSESSMENT

Toxicological assessment was done on novel proteins Cry1Ab and PAT. To evaluate the susceptibility of Cry1Ab protein to proteolytic degradation, simulated mammalian gastric fluid (SGF) was used using the 0.001X Standard concentration. Corn Cry1Ab polypeptides were not detected after 10 min. Native Cry1Ab was also rapidly degraded in the standard SGF. If pepsin was reduced to 0.01X standard concentration, degradation occurred within 5 min. [20]

Furthermore, western blot analysis showed no intact corn derived Cry1Ab polypeptides (ca. 65,000 MW) detected under the 0.001X standard concentration. The native Cry1Ab (ca. 128,000 MW) was also rapidly degraded in the standard SGF. When pepsin was reduced to 0.01X the standard concentration, degradation occurred within 5 min. Based on these results, Cry1Ab protein is likely to be digested as conventional dietary protein in a typical mammalian gastric condition. [20]

Cry1Ab protein is heat-unstable and after incubation at 75ºC for 3 min, 90% of its immunoactivity is lost. While within 15 min at 80ºC, the immunoactivity of the Cry1Ab protein is totally lost Moreover, after subjecting to 80ºC for 10 min its insecticidal activity is completely lost. [21]. Additionally, there is no biologically relevant similarity to any known putative mammalian toxins based on similarity search on non-redundant NCBI protein database. [22]

Cry1Ab expressed in E. coli (Btk HD- protein) was compared with the ones produced in transgenic corn via functional and biochemical evaluation. Results of comparative analysis showed that Btk HD-1 protein is a viable source of Cry1Ab protein in Bt11. Cry1Ab proteins from these two organisms are of substantial equivalence. [23]

Aside from Cry1Ab, toxicological assessment was also done with PAT protein. The simulated mammalian gastric fluid (SGF) containing pepsin was used to evaluate susceptibility of PAT to proteolytic degradation. [24] The temperature stability was evaluated by (1) assessment of immunoreactivity by enzyme-linked immunosorbent assay (ELISA) and (2) assessment of specific enzyme activity by a continuous spectrophotometric assay measuring the formation of 2-nitro-5-thiobenzoate anion during acetylation of phosphinothricin. [25]. PAT is not a toxic protein, nor does PAT share significant sequence similarity with other known or putative toxins. [25]. Moreover, the protein, Cry1Ab and PAT, are expressed independently of each other and the functional activity of these proteins are maintained. [19]

IX. ALLERGENICITY ASSESSMENT

Allergenicity assessment was also employed to characterize potential allergenic effects of the novel proteins present in corn Bt11. After subjecting to bioinformatic analysis using FASTA sequence alignment tool, there are no results showing greater than 35% sequence similarity as

compared to over 80 or more amino acids. On the other hand, using COMPARE database, there are no exact matches/hits with 8 or more contiguous amino acids after aligning the Cry1Ab amino acid sequence and sequences found in the COMPARE database. Therefore, there are no known homology of Cry1Ab to allergens using these two databases. [23] [26]

Based on the analysis of physico-chemical properties conducted, no evidence of any post-translational glycosylation was observed from both microbially produced and plant produced Cry1Ab protein. The molecular weight of Cry1Ab proteins from these two sources were projected to be 65kDa which is within the 10-70 kDa range. [23] [26]

The same test for digestibility of PAT protein was done as in Cry1Ab. Dossier presented results of the experiments. The results suggest that PAT will immediately lose enzymatic activity and will be readily digested as conventional dietary protein in the typical mammalian gastric environment [24]. PAT had a loss of functional activity upon heating to 65˚C and above and loss of immunoreactivity upon heating at 95˚C. [27]

Based on the dossier submitted by the applicant, there are no similarity of PAT protein to known allergens. This was concluded after conducting bioinformatic analyses. Results showed that using the FASTA search, there were no identical matches greater than 35%, as compared to 80 or more amino acids. No exact match hits of sequence alignments of 8 or more contiguous amino acids were present after comparing the PAT amino acid sequence with the ones found in FARRP database. [24] [28]

Several confirmatory analysis techniques, such as Edman degradation, glycosylation blot, and N-linked glycosylation site analysis clearly show that there is no evidence of post-translational protein modifications in plants. The molecular weight of was also within the acceptable range. Moreover, PAT protein equivalence was validated in several transgenic crops indicating that corn Bt11 expressed PAT protein is equivalent with microbially synthesized PAT. [24] [28]

X. NUTRITIONAL DATA

Nutritional analysis was also done for corn Bt11. Results from three studies for proximate analysis for grains and forage clearly showed that (1) expression of Cry1Ab and PAT protein would not change the grain protein and amino acid composition (2) significant differences in the protein percentage is not due to Bt gene, mainly because there were no consistent variations between observed iso-hybrids (3) the carbohydrate, protein, fat, and fiber composition of the three mentioned isogenic hybrids are not significantly different as demonstrated using proximate analysis. [29]

Data from these nutritional analysis studies for vitamins and minerals clearly showed that (1) fatty acids from Cry1Ab hybrids and non- Cry1Ab hybrids are genetically similar and is equivalent in content; (2) amino acids from Cry1Ab hybrids are not different from normal hybrids. Threonine, glycine and phenylalanine were statistically different from the normal hybrids but is still within the acceptable 10% variation, as established by Association des Producterus de Mais (AGPM); (3) No statistical differences in minerals copper, magnesium, manganese, and zinc content as well as for the vitamins, folic acid, niacin, B1 and B2 were observed. Therefore, corn Bt11 is nutritionally equivalent with the near-isogenic comparator. [29] [30]

XI. THE HOST PLANT ENVIRONMENT

The reproductive biology of corn was also assessed. Corn readily and easily crosses with its wild relatives stated, in Central America. In the Philippines, no hybrids were produced when *Zea mays* was crossed with *Coix.* [31] [32] [33] [34]

Additionally, the ecological diversity of the stated groups of insects did not differ in Bt11 and non-Bt11 planted plots although it differs by trial location. This includes plant hoppers, houseflies, fruits flies, black flies, and coccinellid beetles. Non-target organisms are not affected by propagating Bt11 in the field, as substantiated by the groups of insect pests stated in the answer of the applicant. Moreover, Bt corn does not provide resilience to diseases stated. [35]

XII. THE CONSEQUENCES OF OUTCROSSING

Based on the references in the dossier provided by the applicant, in Ilocos Norte, the likelihood of persistence in the environment can be managed as teosinte is used as an animal feed and is harvested before seed maturity leading to reduced probability of cross fertilization. [32][33][34][36] Bt11 does not affect non-target organisms particularly the corn-associated arthropods. [37]

XIII. WEEDINESS POTENTIAL

Corn seeds cannot be disseminated without human help. If an ear of corn is dropped to the ground, there are many competing seeds in the rigid cob that in all likelihood, none will grow to maturity. [31] [38]

XIV. SECONDARY AND NON-TARGET EFFECTS

Bt11 does not affect non-target organisms particularly the corn-associated arthropods. [37] Corn Bt11 is resistant to Asian corn borer. It does not offer protection against corn diseases. [39]

DOH BIOSAFETY COMMITTEE RECOMMENDATION

After a thorough review and evaluation of the documents provided by the proponent Syngenta Philippines, Inc., through the Bureau of Plant Industry (BPI), in support of their application for approval for Commercial Propagation for Corn BT11, the DOH Biosafety Committee find that the regulated article applied for Commercial Propagation 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 corn varieties.

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

It is suggested that the Bureau of Plant Industry (BPI) ensure that there shall be clear instructions that the product is recommended for use as planting materials.

DENR BIOSAFETY COMMITTEE RECOMMENDATION

After a comprehensive review and evaluation of the documents, including the scientific evidence from references and literature submitted by Syngenta Philippines, Inc. on its application for Commercial Propagation of Corn hereunder are the observations and appropriate actions:

1. The regulated article is considered substantially equivalent to its conventional counterpart for its history of safe use as food in twenty-six (26) countries and as feed in nineteen (19) countries. It has also been previously approved for commercial propagation in ten (10) countries;

2. The glyphosate herbicide tolerance and insect resistant traits of the regulated article do not alter nor enhance the persistence, invasiveness, or weediness of the crop relative to its conventional counterpart in which the morphological and growth characteristics of the regulated articles has no significant difference to its conventional counterpart; and

3. The protein products show no significant potential toxicity to wildlife or non-target organism because agronomic evaluations such as plant vigor, plant habit characteristics, and general disease susceptibility have no significant difference relative to its conventional counterpart.[38][39]

DA-IRMAT'S RECOMMENDATION

The DA Insect Resistance Management Advisory Team (IRMAT) reviewed the submission of Syngenta Philippines Inc. For the renewal application for commercial propagation under the DOST-DA-DENR-DOH-DILG JDC No.1 s2016 of corn Bt11 through *ad referendum.*

Having been mandated by the DA Special Order No. 1051 s2018 to provide advice and direction to the BPI in matters relating to Insect Resistance Management (IRM), after a review of both applications, the IRMAT therefore finds that the applicant's submitted documents with substantial compliance with the previously issued DA Memorandum Circulars pertaining to IRM.

FPA'S PIP REGISTRATION

Based on the FPA records, Syngenta Philippines Inc., registrant of BT11, is a duly licensed importer-national distributor of Plant-incorporated Protectant (PIP) with License No. (PIP)-0404-00004 which is valid until April 4, 2021.

The protein CrylAb contained in the corn event BT11 has been issued with a Full PIP Product Registration (Reg. No.: PIP-01-02-02) by FPA on July 27, 2018. This is in compliance with the rules and regulation of the FPA Memorandum Circular No. 10, Series of 2017, *Guidelines for the Registration of Plant-Incorporated Protectants PIPs in Pest-Protected Plants (PPPs)* w*ith Pesticidal Action Derived from Modern Biotechnology.*

SEC CONSIDERATIONS

The 2019 data from FAO and Globe Trade Atlas show the increasing import volume of corn and corn product, which implies an increasing demand for the commodity. Such increasing demand supports the need for GM corn. [42] [43]

Applicant has noted that there are no significant differences in cultivating corn Bt11 and conventional corn except for the fact that spraying is no longer needed for corn Bt11. This implies that the GM product will not change drastically current patterns of production. Requested recent available data on importation were also provided. [42] [43] [44] [45] [46] [49] [48] [49] [50] [51] [52] [53] [54]

Citing Gonzales et al. (2009) and Yorobe and Quicoy (2006), applicant was able to affirm that GM corn exhibits increase in productivity. Similarly, it was noted that in response to the Asian corn borer, BT corn has the potential to improve corn productivity in the country as corn yields have remained low and corn imports have increased over time. [42] [43] [44] [45] [46] [49] [48] [49] [50] [51] [52] [53] [54]

As noted by the applicant, farm management will be generally the same except that insecticide application will no longer be necessary. This can even be advantageous as insecticides pose risks to humans and other organisms. Bt corn thus presents a practical and ecologically sustainable solution for poor farmers as it increases yields and decreases pesticide. [42] [43] [44] [45] [46] [49] [48] [49] [50] [51] [52] [53] [54]

Bt corn, in general, has been designed to be resistant to the Asiatic corn borer and, thus, no longer requires other complementary inputs. [42] [43] [44] [45] [46] [49] [48] [49] [50] [51] [52] [53] [54]

While the applicant initially failed to provide more recent data, they were able to present the most recent data on the estimated/ projected relative cost of the GM seed compared to its conventional counterpart. The applicant reported a Php73,330 total production cost per hectare for GM corn and a Php67,100 production cost per hectare for conventional corn. While production cost of GM corn is higher, the net income per hectare is also higher at Php64,670 compared to the Php1,900 net income per hectare for conventional corn. [42] [43] [44] [45] [46] [49] [48] [49] [50] [51] [52] [53] [54]

Applicant provided a cost and returns table based on 2018 internal data, which affirms claims that the use of GM corn increases productivity. [42] [43] [44] [45] [46] [49] [48] [49] [50] [51] [52] [53] [54]

As noted by the applicant, the production/cultivation of traditional varieties is still being practiced by some farmers and supported by the government. For instance, the Bureau of Agriculture Research (BAR) ensures native corn's continued cultivation in the Philippines through its joint program with the Institute of Plant Breeding of the University of the Philippines Los Baños and the Department of Agriculture regional field offices called "Corn Germplasm Utilization through Advance Research and Development" or CGUARD. [42] [43] [44] [45] [46] [49] [48] [49] [50] [51] [52] [53] [54]

As corn Bt11 will be grown in the usual corn-growing regions in the country, it could be assumed that it will not change the total land use of corn cultivation and would not affect LCIPs. [42] [43] [44] [45] [46] [49] [48] [49] [50] [51] [52] [53] [54]

Corn Bt11 is no different from conventional corn except for its ability to resist insect damage. Hence, its production will not require changes in farmers' social participation in community activities. [42] [43] [44] [45] [46] [49] [48] [49] [50] [51] [52] [53] [54]

It is thus highly suggested that research be conducted or commissioned to explore the impacts of GM introduction in indigenous cultural communities. Initially, the perceptions and attitudes of IP farmers towards GM may be examined. Findings of the said research could then be used in

answering SEC forms for future applications / renewal of applications. [42] [43] [44] [45] [46] [49] [48] [49] [50] [51] [52] [53] [54]

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[3] Section I.C. Corn as a Source of Toxicants, p. 12 of the dossier

[4] Section I.D. Corn as a Source of Allergens, p. 12 of the dossier

[5] Section I.E. Use of Corn as Food, pp. 13-14 of the dossier

[6] Section I.F. Consumption Pattern of Corn, p. 15 of the dossier

[7] Section I.G. Use of Corn as Feed, p.15 of the dossier

[8] Section II. The Transgenic Plant, p.15 of the dossier

[9] Section III. The Donor Organism, p. 16 of the dossier

[10] Section VIII. Toxicological Assessment, pp. 34-36 of the dossier

[11] Section IX. Allergenicity Assessment, pp. 36-41 of the dossier

[12] Section IV. The Transformation System, p. 16 of the dossier

[13] Section V. The Inserted DNA, pp. 20-31 of the dossier

[14] Section IV.A. Transformation Method, p. 16 of the dossier

[15] Section V. A. Southern Analysis, pp. 20-24 of the dossier

[16] Section V.B. Insert Sequence Analysis, pp. 24-25 of the dossier

[17] Section VI. B. Mendelian Inheritance of Transgene Insert, pp. 33-34 of the dossier

[18] Section VII.A. Protein Expression Levels, pp. 31-33 of the dossier

[19] Section VII.B. Metabolic Pathways, p. 33 of the dossier

[20] Section IX.A.1. *In vitro* Digestibility of Cry1Ab, pp. 36-37 of the dossier

[21] Section VIII.A.2. Acute Oral Toxicity of Cry1Ab in Mice, p. 34 of the dossier

[22] Section VIII.A.1. Amino Acid Comparison of Cry1Ab with Known and Putative Toxins, p.34 of the dossier

[23] Section IX.A.4. Source of Test Protein of Cry1Ab, p.38 of the dossier

[24] Section IX. B.1. *In vitro* Digestibility of PAT, p. 39 of the dossier

[25] Section VIII.B.1. Amino Acid Comparison of PAT with Known and Putative Toxins, p. 35 of the dossier

[26] Section IX.A.3. Amino Acid Comparison of Cry1Ab with Known and Putative Allergens, pp. 37-38 of the dossier

[27] Section IX. B.2. Heat Stability of PAT, p. 40 of the dossier

[28] Section IX. B.4. Source of Test Protein of PAT, p. 40-41 of the dossier

[29] Section X.A.1. Proximates, Fatty Acids, and Amino Acids, p. 42-47 of the dossier

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