

CONSOLIDATED REPORT FOR MONSANTO PHILIPPINES INC.'S CORN NK603

(APPLICATION FOR COMMERCIAL PROPAGATION)

EXECUTIVE SUMMARY

On March 29, 2019, Monsanto Philippines submitted corn NK603 as a renewal 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 February 8, 2005 and has been renewed under the same circular on March 16, 2010 and March 16, 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 Monsanto Philippines Inc.'s corn NK603 for Commercial Propagation were the following:

- One 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.
- 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. SEC expert recommended for the approval and issuance of a biosafety permit of the said GM product.

BACKGROUND

According to Article VI. Section 15 of the JDC No.1 s2016, 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 plant-incorporated protectant (PIP) has been duly registered with the Fertilizer and Pesticide Authority (FPA).

The BPI Biotech Office provided the assessors the complete dossier submitted by Monsanto Philippines Inc. The SEC expert, on the other hand, was provided with a questionnaire on socio-economic, ethical and cultural considerations that have been addressed by Monsanto Philippines Inc. in relation to their application. These assessors were given thirty (30) days to submit their independent assessment to BPI Biotech Secretariat.

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

Generally, corn does not contain known allergens or produce significant toxins or antinutrients warranting analytical or toxicological tests. However, in some case-studies, allergenic reactions were reported. [1][2].

Maize or corn is being consumed in varied forms. It can be eaten raw. Most of the human consumption of maize is in the form of maize-based ingredients such as high fructose corn syrup, starch, sweeteners, cereals, oil and alcohol. [1].

II. TRANSGENIC PLANT

NK603 has been reviewed and approved for food and/or feed use in many countries which were listed by the applicant. In terms of food and feed safety, results from compositional studies support the overall conclusion that NK603 was not a major contributor to variation in component levels in maize grain and forage and confirmed the compositional equivalence of NK603 to the conventional control in levels of these components. [1][2][3].

Since NK603 was found to be substantially equivalent to conventional maize with similar genetic background, there is no anticipated change in the use pattern for the product. [1][3].

III. THE DONOR ORGANISM

Agrobacterium sp. strain CP4 is the donor organism for NK603 and is not known to be toxic or allergenic. [1].

CP4 EPSPS protein is not known to be toxic or allergenic. CP4 EPSPS protein produced in NK603 is also present in many commercial biotechnology-derived crops and that a history of the safe use of CP4 EPSPS protein has been demonstrated. [1].

IV. TRANSFORMATION SYSTEM

Plasmid vector PV-ZMGT32 developed by Monsanto Company, was used for the transformation of maize to produce NK603 and a detailed description of the expression cassette was adequately provided by the applicant. [1][4] [5].

Particle acceleration transformation was the method used and the complete experimental protocol was provided by the applicant. [1].

V. INSERTED DNA

Validation of the results from molecular analyses confirmed that NK603 contains a single copy of T-DNA containing the cp4 epsps expression cassettes that is stably integrated at a single insertion site and no detectable additional genetic elements. The result was demonstrated sufficiently by Southern blot analysis and PCR and sequence analysis. [1][5].

VI. GENETIC STABILITY

The potential for creating novel chimeric ORFs were tested by PCR and DNA sequencing which verified the 5' and 3' ends of the insert in NK603. The sequences flanking the insert were confirmed to be native to maize. Western blot confirmed the expression of the full-length CP4 EPSPS proteins in NK603 and results indicate that the two CP4 EPSPS proteins are indistinguishable in Western blot analysis with the available polyclonal antibody, since the proteins are essentially identical. The reported data support the conclusion that only the two full-length CP4 EPSPS proteins are encoded by the insert in NK603. [1][6].

The reported data show that NK603 does not contain backbone sequences from the backbone sequences. This was sufficiently demonstrated by *SacI* restriction enzyme digestion, hybridization and Southern blotting. [1][5].

The reported results from Southern blot analysis demonstrated the stability of the DNA insert across multiple generations on F1 generation and the fifth generation of back-crossing of NK603. The analysis confirmed that a single integration locus was maintained through five generations of breeding, thereby confirming the stability of the insert. [1][5].

VII. EXPRESSED MATERIAL

The reported mean level of CP4 EPSPS proteins in forage was 25.6 qq/g tissue on a fresh weight basis while the level of CP4 EPSPS proteins in grain from event NK603 was

10.9 $\mu\text{g/g}$ tissue. This was measured by performing a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) from the collected forage and grain tissues from the field sites treated with glyphosate in the U.S. Other relevant information in this methodology was provided by the applicant. [1][7].

VIII. TOXICOLOGICAL ASSESSMENT

SDS-PAGE and Western blot methods were used in assessing the digestibility of NK603 CP4 EPSPS proteins (CP4 EPSPS and CP4 EPSPS L214P) in simulated gastric fluid (SGF, containing pepsin). Results from these experiments demonstrated that CP4 EPSPS produced from *E. coli* were rapidly digested after incubation in SGF. The SDS-PAGE Colloidal Blue gel staining method demonstrated that at least 98% of the *E. coli*-produced CP4 EPSPS proteins were digested in SGF within 15 seconds and the estimated T50 result for SGF is below 15 seconds. There were no observed protein bands due to degradation of the CP4 EPSPS. Western blot analysis confirmed that greater than 95% of the *E. coli*-produced CP4 EPSPS proteins were digested in SGF within 15 seconds. [1][8][9].

The estimated T_{50} CP4 EPSPS is less than 15 minutes and was determined in the temperature dependence studies which demonstrated that the enzymatic activity is eliminated after 15 minutes incubation at 65°C. The impact of heating and *in vitro* digestibility of CP4 EPSPS has also been confirmed by Okunuki et al. (2002). [10][11].

Upon comparison of amino acid sequences of the CP4 EPSPS to protein sequences in the toxin database using the FASTA sequence alignment tool, the protein shared sequence similarities to homologous EPSPS proteins which have not been described as toxins relevant to human health. No other significant structural homology was observed. [1][9][12].

Acute oral toxicity study was conducted with *E. coli*-produced CP4 EPSPS protein and was administered as a single dose by gavage to three groups of 10 male and 10 female CD-1 mice at dose levels up to 572 mg/kg. Results show that there were no treatment-related effects on survival, clinical observations, body weight gain, food consumption or gross pathology. Therefore, the No Observable Adverse Effect Level (NOAEL) for CP4 EPSPS was considered to be 572 mg/kg. [1][13].

IX. ALLERGENICITY ASSESSMENT

The amino acid sequence of the CP4 EPSPS protein was compared to a database of protein sequences associated with allergy and celiac disease using the sequence alignment tool FASTA and demonstrated that CP4 EPSPS shared no structurally significant sequence similarity to sequences within the allergen database.

In addition, the CP4 EPSPS sequence was compared to the allergen database using an algorithm that scans for a window of eight linearly contiguous identical amino acids and results showed that The CP4 EPSPS protein sequence does not share eight linearly contiguous amino acid identities to any sequence in the allergen database.

These results confirm that the CP4 EPSPS protein does not share any relevant amino acid sequence similarities with known allergens, gliadins, or glutenins. Further analysis of the physicochemical and functional properties provides a detailed characterization of the NK603-produced CP4 EPSPS and CP4 EPSPS L214P proteins and establish its equivalence to the *E. coli*-produced CP4 EPSPS proteins. [1][9][12][14].

X. NUTRITIONAL DATA

Results of the study provided shows that there were no statistically significantly differences observed for proximate analysis in forage grain and antinutrients between NK603 and the conventional control. [1][15][16][17][18].

Reference grain and forage samples from the E.U. field trials also included 19 conventional, commercial hybrids (five hybrids per site with one hybrid planted at two sites), planted under the same environmental conditions. All test values of proximate were within the 99% tolerance interval established from the commercial varieties. [1][15][16][17][18].

The studies provided by the applicant show that all test values of forage, grain and antinutrients were within or similar to literature range or historical range. [1] [17][18].

Differences observed in key nutrients were not biologically relevant and meaningful from a food and feed safety perspective. [1][15][16][17][18].

XI. THE HOST PLANT ENVIRONMENT

Maize is a wind pollinated species with plant morphology that facilitates cross pollination. [19].

The references provided by the applicant on hybridization with cultivated *Zea mays* L., wild annual species of subgenus *Zea mays* subsp. *mexicana*, wild perennial species of subgenus *Tripsacum* have described the possible formation of viable interspecific and/or intergeneric hybrids. From the studies provided, there are no scientific reports confirming the transfer of genetic material from maize to other species with which maize cannot sexually interbreed. Thus, the probability for horizontal gene flow to occur is negligible. [20][21][22].

XII. THE CONSEQUENCES OF OUTCROSSING

In the Philippines, there is no known sexually compatible wild species. This among other factors reported by the applicant support that the assessment that the risk of loss of this wild species due to the development of the GM variety is very low. [20][23].

There are no anticipated changes in habitat or geographic distribution. NK603 has been shown to be no different from conventional maize in its phenotypic, ecological, and compositional characteristics, except for the introduced trait of glyphosate tolerance. [4].

XII. WEEDINESS POTENTIAL

There is no adverse environmental impact is expected from the introduction of NK603 to pests and/or diseases on current cultivation and management practices for maize. As shown by previous studies, NK603 has been shown to be no different from conventional maize in its phenotypic, ecological, and compositional characteristics, except for the introduced trait of glyphosate tolerance. [4].

Mode of dissemination is through seeds. Plant produces a male (tassel) and female (ear) flower borne on the same plant but different positions. A well-developed ear shoot has 750 to 1,000 ovules (potential kernels), each producing a silk. However, under good conditions only 400 to 600 ovules will be fertilized and eventually produce kernels. Under favorable conditions, a pollen grain upon landing on a receptive silk will develop a pollen tube containing the male genetic material, develop and grow inside the silk, and fertilize the female ovary within 24 hours. Pollen grains are borne in anthers, each of which contains a large number of pollen grains. The anthers open and the pollen grains pour out after dew has dried off the tassels. Pollen is light and can be carried considerable distances by the wind. However, most of it settles within 6 to 15 meters (20 to 50 feet). Pollen shed is not a continuous process. It stops when the tassel is too wet or too dry and begins again when temperature conditions are favorable. [19].

The assessor has found sufficient evidence that the regulated article applied for direct use will not pose any significant risk to health and environment as its conventional counterpart and that any risks posed to health and environment could be managed by the following measures; a) Continuous monitoring of planted sites for weed shifts and herbicide resistance development; b) Product stewardship; c) Provision of guidance on the planting of NK603 in hilly areas.

DOH BIOSAFETY COMMITTEE 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 Commercial Propagation of Corn NK603. The DOH Biosafety Committee has found 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. Find that the regulated article applied for Commercial Propagation (CP) does not require changes in the usual practices as described in the phases/stages of biotechnology project activities. As such, the regulated article is as safe as its conventional counterpart and is not expected to pose any significant risk to human and animal health and environment.
2. 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.
3. Dietary exposure to the regulated article is unlikely to result allergic reaction.
4. The regulated article is not materially different in nutritional composition from that of the non-transgenic or the conventional corn.
5. Scientific pieces of evidences from provided references i.e. literature show that regulated article applied for Commercial Propagation is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health and on the environment.

DENR-BIOSAFETY COMMITTEE RECOMMENDATION

After a comprehensive review and evaluation of the documents, including the scientific evidence from references and literature submitted by Monsanto Philippines, Inc. on its application for Commercial Propagation of Corn (NK603), hereunder are the observations and appropriate actions:

- The regulated article is considered substantially equivalent to its conventional counterpart for its history of since use as food in twenty-six (26) countries and as feed in twenty-one (21) countries. It has also been previously approved for commercial propagation in fourteen (14) countries (International Service for the Acquisition of Agri-Biotech Applications GM Approval Database, 2019). [24].
- The genetic stability of the transgenic crop was tested over multiple generations wherein the hybrids of the regulated article crossed and backcrossed with inbred were tested for glyphosate herbicide tolerance. Therefore, it is proven stable in molecular structure and trait expression [26].
- The glyphosate herbicide tolerance traits of the regulated article do not alter nor enhance the persistence, invasiveness, or weediness of the crop relative to its conventional counterpart [26].
- The CP4 EPSPS protein has no significant potential toxicity to wildlife or non-target organism because agronomic evaluations such as plant vigor, plant habit characteristics, general disease susceptibility has no significant difference relative to its conventional counterpart [27].

Based on the evaluation and review of literature cited, the DENR-BC considered the regulated article safe to the environment and biodiversity.

SEC CONSIDERATIONS

As a percentage of total agricultural trade, maize is not very significant. However, maize importation is increasingly becoming important because of the increasing requirements of a growing animal industry. This is reflected in the increasing importation dependency ratio that is provided. [28][29][30][31].

It is not expected to require changes in farm management practices except for weed. [32]. The complementary inputs should be the same for both GM and non-GM maize because as mentioned, what is simply addressed is the ease of weeding that reduces labor cost. [33]. SEC Expert agrees that this will not affect the traditional production techniques. If any, it should be beneficial to farmers given the reasons given previously. [34]. There is no reason for the social structure of LCIPs to be affected especially in the rural areas. [34].

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

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