

CONSOLIDATED REPORT OF PIONEER HI-BRED AND DOW AGROSCIENCES' CORN TC1507 APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING

EXECUTIVE SUMMARY

On May 8, 2018, Pioneer Hi-Bred Philippines and Dow Agrosiences Philippines submitted corn TC1507 application for direct use as food and feed, or for processing to the Bureau of Plant Industry (BPI) under the DOST-DA-DENR-DOH-DILG Joint Department Circular (JDC) No. 1 Series of 2016. After reviewing the Risk Assessment Report and attachments submitted by the applicant, the assessors namely: Scientific and Technical Review Panel (STRP), BPI- Plant Products Safety Services Division (BPI-PPSSD) and Bureau of Animal Industry (BAI), concurred that corn TC1507 is as safe for human food and animal feed as its conventional counterpart.

The Department of Environment and Natural Resources – Biosafety Committee (DENR-BC), after a thorough scientific review and evaluation of the documents related to Environmental Risk along with the submitted sworn statement and accountability of the proponent, recommended the issuance of a biosafety permit for this regulated event provided that the conditions set by them are complied.

Also, the Department of Health – Biosafety Committee (DOH-BC), after a thorough scientific review and evaluation of documents related to Environmental Health Impact, concluded that corn TC1507 will not pose any significant risk to health and environment and that any hazards could be managed by the measures set by the department. DOH-BC also recommended for the issuance of biosafety permit for TC1507.

Furthermore, the Socio-economic, Ethical and Cultural (SEC) Considerations expert also recommended for the issuance of biosafety permit for this regulated article after assessing the socio-economic, social and ethical indicators for the adoption of Genetically Modified Organisms.

BACKGROUND

In accordance with Article VII. Section 20 of the JDC, no regulated article, whether imported or developed domestically, shall be permitted for direct use as food and feed, or for processing, unless: (1) the Biosafety Permit for Direct Use has been issued by the BPI; (2) in the case of imported regulated article, the regulated article has been authorized for commercial distribution as food and feed in the country of origin; and (3) regardless of the intended use, the regulated article does not pose greater risks to biodiversity, human and animal health than its conventional counterpart.

The BPI Biotech Office provided the assessors the complete dossier submitted by Pioneer Hi-Bred Philippines and Dow Agrosiences Philippines.

Below is the summary of the evaluation conducted by the STRP and regulatory agencies.

A. STRP, PPSSD, BAI ASSESSMENT

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

A. Host Organism

Corn is extensively cultivated worldwide and has a long history of safe use. Corn grain and corn-derived products represent staple food and feed for a large portion of the global population. Corn grain is fed to animals as a source of energy from carbohydrates and oils and provides a source of essential and

nonessential amino acids. Corn is mostly consumed in the form of corn-based ingredients such as high fructose corn syrup, starch, sweeteners, cereals, oil and alcohol.

The assessors also reported that corn contains anti-nutrients like raffinose, phytic acid and trypsin inhibitor. Furthermore, it was confirmed that there are no known toxicants in corn and it is not a common allergenic food, although in some case-studies, allergic reactions were reported.

B. Transgenic Plant

Corn TC1507 has been reviewed and approved for food use in many countries including Argentina (2005), Australia (2003), Brazil (2008), Canada (2002), China (2002), Colombia (2006), European Union (2006), Indonesia (2015), Japan (2002), Malaysia (2013), Mexico (2003), New Zealand (2003), Panama (2012), Paraguay (2012), Philippines (2003), Singapore (2014), South Africa (2002), South Korea (2002), Taiwan (2003), USA (2001), Uruguay (2011) and Vietnam (2016). On the other hand, the following countries have given approval for feed use to TC 1507: Argentina (2005); Brazil (2008); Canada (2002); Colombia (2006); EU (2006), Japan (2003); Korea (2004), Malaysia (2013); Philippines (2003); Singapore (2011); Switzerland (2014), Taiwan (2017); Uruguay (2011); Vietnam (2016).

The assessors reported that the consumption pattern will not be changed since corn TC 1507 is comparable to the conventional corn.

C. Donor Organism

The assessors reported that the proponent provided sufficient description of *cry1F* gene encoding Cry1F protein from *Bacillus thuringiensis* var. *arizawai* and *pat* gene encoding PAT protein from *Streptomyces viridochromogenes*. The Cry1F protein, encoded by the *cry1F* gene, confers protection against certain lepidopteran pests, including the European corn borer (ECB, *Ostrinia nubilalis*). The PAT protein, encoded by the *pat* gene, confers tolerance to the herbicidal active ingredient glufosinate and served as a marker to select transformed maize in laboratory.

Bacillus thuringiensis (*Bt*): donor of the *cry1F* gene, is a diverse group of Gram-positive, spore-forming bacteria that has a history of safe use as a pesticide over several decades. It occurs ubiquitously in the soil and on plants including vegetables, cotton, tobacco, tree crops, and forest crops. Several Cry proteins have been deployed as safe and effective pest control agents in microbial *Bt* formulations for almost 40 years.

Streptomyces viridochromogenes: donor of the *pat* gene, is a common soil bacterium that is not considered pathogenic to humans or animals (OECD, 207) and produces the tripeptide phosphinothricyl-L-alanyl-L-alanine, which was developed as a non-selective herbicide. The *pat* gene, encoding the phosphinothricin acetyl transferase, confers resistance to the phosphinothricin herbicide application (OECD, 1999).

D. Transformation System

Corn TC1507 was obtained by microprojectile bombardment using the Biolistic™ PDS-1000 He Particle Delivery System (Klein et al., 1987) with purified PHI8999A DNA insert fragment. It was reported that the target of genetic modification is the nuclear DNA.

Carrier DNA and/or helper plasmids were not used in the development of TC1507 maize.

E. Inserted DNA

Molecular characterization confirmed that TC1507 maize contains a single, almost full-length insertion of the DNA from linear fragment of PHI8999A that is stable across multiple generations and segregates according to Mendel's laws of genetics. The TC1507 maize insertion site of 12,257 bp was sequenced and characterized. The genetic material inserted in TC1507 maize can be divided into 5 separate major sections including 15 regions: the 5' border sequence, comprising the flanking region of maize genomic DNA; the 5' fragmented sequence, which contains both PHI8999A-derived and maize-derived genomic sequences; the almost full-length PHI8999A insert, corresponding to sequence of bp 11 to 6196 of PHI8999A insert used in the transformation of TC1507 maize; the 3' fragmented sequence which contains both PHI8999A-derived and maize-derived genomic sequences; and the 3' genomic sequence, comprising the flanking region of maize genomic DNA.

The size and structure of the insert present in TC1507 has been characterized by Southern blot and DNA sequence analyses. TC 1507 maize insert contains the following non-functional fragments: one fragment (338 bp) of the *cry1F* gene (region 2), with no *ubiZM1* promoter sequence, and one fragment (19 bp) of the *cry1F* gene (region 7), both located at the 5' end of the almost full-length insert; two fragments (199 bp and 136 bp long, respectively) of the *pat* gene (regions 5 and 6, respectively), without regulatory sequences associated, located at the 5' border and, one fragment (188 bp) of the *pat* gene, without regulatory sequences associated, located at the 3' border (region 13); one fragment (118 bp) of the polylinker region and partial *ubiZM1* promoter sequence located at the 5' border (region 8); and one fragment (550 bp) of the ORF25 terminator sequence in inverted position and polylinker region immediately at the 3' end of the almost full-length insert (region 10).

Biotechnology tools enable a gene to be utilized across different crops. Event TC1507 is specific to maize wherein the main transgene is *cry1F*. The *cry1F* transgene is expressed in commercially approved GM maize, soybean and cotton.

The assessors reported that there were no plasmid backbone sequences present in the genome of corn line TC1507 demonstrated in Southern blot analysis.

F. Genetic Stability

The multigenerational stability of the DNA insert was demonstrated by Southern blot analysis on T₁S₁ and BC₄ generations of TC1507. The analysis demonstrated that the inserted DNA in corn line TC1507 was stably inherited from one generation to the other.

Data on the Mendelian segregation of inserted genes provide evidence of stable inheritance of the introduced genetic material.

G. Expressed Material

Concentrations were determined by enzyme-linked immunosorbent assay (ELISA). Levels of Cry1F and PAT protein in TC1507 grains, leaves, roots, pollen and stalk were presented in Table 1 and 2.

Table 7. Summary of Cry1F Protein Levels Measured in Tissue Collected from TC1507 Maize Hybrid

Tissue	Mean ^a Cry1F (ng/mg tissue dry weight)	Standard Deviation	Min/Max Range
Pollen	26	3.6	20-35
Leaf	19	3.8	13.0-29.0
Stalk	7.7	0.71	6.0-9.4
Root	4.0	1.7	1.4-6.9
Grain	4.3	1.4	<LLOQ ^b -6.9

^a Values are means across all three sites from mean values calculated from the analysis of five individual samples per site pollen, leaf, stalk, root, and grain.

^b < LLOQ = below the lowest limit of quantitation

Table 8. Summary of PAT Protein Levels Measured in Tissue Collected from TC1507 Maize Hybrid

Tissue	Mean ^a PAT (ng/mg tissue dry weight)	Standard Deviation	Min/Max Range
Pollen	<LLOQ ^b	0	<LLOQ ^b
Leaf	8.5	1.7	5.1-13
Stalk	0.35	0.39	<LLOQ ^b -1.1
Root	0.28	NA ^c	<LLOQ ^b -60
Grain	<LLOQ ^b	0	<LLOQ ^b

^a Values are means across all three sites from mean values calculated from the analysis of five individual samples per site pollen, leaf, stalk, root, and grain.

^b < LLOQ = below the lowest limit of quantitation

^c NA = not applicable

The mean Cry1F levels in TC1507 grains was 4.3 ng/mg tissue dry weight while the mean PAT levels in TC1507 grains was below the lowest limit of quantitation (LLOQ).

H. Toxicological Assessment

Digestibility study provided by the developer indicated that Cry1F and PAT is rapidly degraded in simulated gastric fluid (SGF) . Hence, the estimated T₅₀ result for SGF is 15 seconds. The susceptibility of Cry1F protein to proteolytic degradation was evaluated in the simulated mammalian gastric fluid (SGF) containing pepsin using SDS-PAGE and Western Blot Analysis. The degradation of PAT appeared to be complete by 5 sec. The susceptibility of PAT protein to proteolytic degradation was evaluated in the simulated mammalian gastric fluid (SGF) containing pepsin using SDS-PAGE. The loss of PAT activity was instantaneous and irreversible at 37°C in SGF with or without pepsin.

Cry1F protein was heat inactivated after exposure to 75 °C or 90°C for 30 min while PAT protein was completely heat inactivated after 10 minutes at 50 °C or higher temperatures despite the fact that the protein was not degraded.

Bioinformatics analyses using BLASTP sequence alignment program and DuPont Pioneer toxin database provided by the developer indicated that Cry1F and PAT have no significant homology to any known toxin.

Furthermore, acute oral toxicity study provided by the developer indicated no treatment related adverse effects on survival, clinical observations, body weight gain, food consumption or gross pathology of mice administered with Cry1F and PAT protein.

I. Allergenicity Assessment

The results of the SDS-PAGE and western blot assay demonstrate that Cry1F protein is rapidly degraded and undetectable in simulated gastric fluid containing pepsin after 15 seconds while PAT protein was degraded in 5 seconds.

The inactivation of Cry1F protein provided by the developer indicated that the loss of biological activity was observed upon incubation at 75°C. The activity is significantly impacted by heat treatment. The PAT protein was completely heat inactivated after 10 minutes at 50°C or higher temperatures despite the fact that the protein was not degraded.

J. Nutritional Data

The assessors reported that the proximates and fiber in grain and forage from TC1507 maize were compared to proximates and fiber from grain and forage from non-transgenic maize with similar genetic background (Stauffer and Zeph, 2000). A difference in the percentage of fat between the transgenic line and its comparator was statistically significant ($p < 0.05$) in grain, but values were within the reported range in literature for this variable, therefore the differences are not biologically relevant.

Compositional assessment of vitamins, minerals, fatty acid and amino acid content of grain from TC1507 maize and near-isoline control maize was conducted.

For Vitamins, statistically significant differences between TC1507 maize and the control line were noted in total tocopherols and in vitamin B1 levels but the values were within range of values reported in the literature.

For Minerals, statistically significant differences between TC1507 maize and the control line were noted in manganese and potassium levels but the values were within range of values reported in the literature or within the data set obtained from the commercial lines for these variables.

For fatty acids, statistically significant differences were observed between TC1507 maize and the control line in all measurements except for the levels of palmitic acid. However, the values for these fatty acids in both maize lines were within the range reported in literature for maize.

For amino acids, statistically significant differences between the two lines in two values, cysteine and methionine were observed but values fall within the reported range in literature or within the data set obtained from the commercial lines for these variables

In summary, the statistically significant variations between TC1507 maize and the control line that were observed in the compositional assessment were not biologically relevant.

On the other hand, phytic acid, trypsin inhibitor and raffinose were analyzed in grain from maize TC1507 maize and the non-GM control line. Phytic acid and raffinose levels in TC1507 maize were not significantly different from the non-GM control maize line. As expected, trypsin inhibitor levels in both TC1507 maize and the control line were below the limit of quantitation of the enzyme assay that was used in this analysis.

It was noted that the processing of grain is not expected to create significant variations between TC1507 maize and conventional maize in antinutrient composition.

The assessors find scientific evidence that the regulated article applied for human food and animal feed use is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health

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

After a comprehensive review and evaluation of the documents including the scientific evidences from provided references and literature submitted by Pioneer Hi-Bred Philippines, Inc. and Dow AgroSciences Philippines on its application for Direct Use as FFP of Corn TC1507. Hereunder are the observations and appropriate actions:

1. The direct use of the regulated article whether for food, feed or for processing will not cause any significant adverse effect on the environment (land, and water) and non-target organisms. The transgenic crop will not increase its weediness potential in case the seeds spill out into the environment because the Cry IF and PAT proteins produced by the transgenic crop will degrade upon exposure to the natural environment and general conditions, that is high temperatures, 75°C and 55°C respectively, varying pH, enzyme digestion, etc. (Hermann, 2003) and (Wehrmann, Vliet, Opsomer, Botterman, & Schulz, 1996) .
2. Cry proteins, when consumed, are converted to active toxin in an alkaline gut, and binds to specific Cry protein receptors present on the midgut of lepidopteran insects. Mammals do not possess alkaline guts and Cry protein-binding receptors which makes them invulnerable to toxicity (Hoffmann, et al, 1988) and (Shai & Aronson, 2001) . Moreover, mammalian digestive environment contains pepsin, which readily digests Cry and PAT proteins (FSANZ, 2003). Mammalian gastric environment is also similar with the physiology of digestion of avian gastrointestinal tract, in terms of pH and type of enzyme secreted (Privalle, 1994).
3. The Project Description Report (PDR) discusses 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 plan. Furthermore, 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. Also, corn is a highly domesticated plant that requires human intervention for it to persist in the environment [OECD, 2003] and (Raybould, et al., 2012).
4. 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/import based on BPI's inspection in the port area.

5. Based on the review and evaluation, the DENR-BC considered the regulated article safe to the environment and non-target organisms, and hereby submits the technical report relative to the application of Pioneer Hi-Bred Philippines, Inc. and Dow AgroSciences Philippines Corn TC1507 for Biosafety Permit for direct use as food, feed, or for processing.

C. DOH-BC (for Environmental Health Safety)

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 Direct Use for Food and Feed or for Processing (FFP) of Corn TC1507 I/We,

Find that the regulated article applied for Direct Use for Food and Feed or for Processing (FFP) 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;
5. Based on the above considerations and with the submitted sworn statement and accountability of the proponent, this recommendation is being submitted to the BPI related to the processing and issuance of a biosafety permit for Direct Use as FFP of Corn TC1507

D. SEC Expert (for Socio-economic Consideration)

According to the SEC expert, the applicant was able to explain the significance of the product. Moreover, supply and demand data from the USDA were presented to complement their assertions.

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