Consolidated Technical Report of Bayer CropScience corn T25 Application for Direct Use as Food, Feed or for Processing (FFP)

EXECUTIVE SUMMARY

On June 14, 2018, Bayer CropScience, Inc,. submitted corn T25 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 T25 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 T25 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 T25.

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 Bayer CropScience, Inc.

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 a source of key nutrients specifically carbohydrates, fatty acids, proteins, and other minerals and vitamins. It contains anti-nutrients like phytic acid, raffinose and trypsin which are found to be present in small amounts in *Zea mays* L. (OECD, 2002).

Generally, corn is not a source of toxicants and allergens. History of safe use was attributed to corn. It is used as food and most of the human consumption of corn is in the form of corn-based ingredients such as high fructose corn syrup, starch, sweeteners, cereals, oil and alcohol. Field maize products are used in food as starch, oil, grits, meal and flour. Sweet maize is used as whole kernel and popcorn maize kernels are used as popcorn and as basis for confections. It is also an important crop for animal feed. Corn grain and by-products of corn processing may be included in diets for most animal species. Corn silage is a readily digestible, high-energy, and fermented forage product. It is fed primarily to ruminants (e.g., cattle, sheep and goats). For animal nutrition, corn is considered to be an important source of energy, essential fatty acids and some of the essential amino acids.

B. Transgenic Plant

T25 Corn has been reviewed and approved for food and/or feed use in many countries including Argentina (Food, Feed, 1998); Australia/New Zealand (Food, 2002); Brazil (Food, Feed, 2007); Canada (Food and Feed, 1997); China (Food and Feed, 2002); Colombia (Food, 2012 and Feed, 2011); European Union (Food and Feed, 1998); Japan (Food, 2001; Feed, 2003); Malaysia (Food, Feed, 2013); Mexico (Food, 2007); Philippines (Food and Feed, 2003); Russian Federation (Food, 2007 and Feed, 2006); Singapore (Food, and Feed, 2014); South Africa (Food and Feed, 2001); South Korea (Food, 2003 and Feed, 2004); Taiwan (Food, 2002); USA (Food and Feed, 1995); Vietnam (Food and Feed, 2015).

Assessors reported that T25 Corn was shown to be compositionally and nutritionally equivalent to the conventional corn and the consumption pattern by population subgroups will not change.

C. Donor Organism

Streptomyces viridochromogenes is the donor organism for the single expressible sequence introduced. The same enzymatic specificity as observed with the PAT proteins has been identified in at least six other bacterial species from five genera of common soil bacteria and it is expected that at least some of these bacteria contain *pat* homologues. None of these homologues have been reported as being toxic or allergenic in humans or animals. Transforming a plant with a coding region derived from S. *viridochromogenes* that encodes a PAT protein is expected not to lead to the development of a pathogenic, toxic, or allergenic transgenic plant.

The safety of the PAT protein has been extensively reviewed. The PAT enzyme is highly specific, does not show any sequence homology with known allergens or toxins, has no N-glycosylation sites, is rapidly degraded in gastric and intestinal fluids and is devoid of adverse effects in mice after intravenous administration at a high dose level. It was therefore concluded that PAT does not possess characteristics that are commonly associated with food toxins and allergens.

D. Transformation System

The transformation method used is polyethylene glycol mediated direct gene transfer into corn protoplasts. Briefly, protoplasts and DNA are mixed together in a buffered solution and a polyethylene glycol solution is added dropwise. After gentle mixing and incubation at room temperature the protoplasts are gently pelleted, washed and resuspended in protoplast culture medium. Putatively transformed protoplasts are cultivated in various conditions until microcolonies of more than 20-50 cells are formed. The microcolonies are then transferred to solid medium. To select for transformants, microcolonies are transferred to medium containing glufosinate ammonium. Fertile corn plants are regenerated from corn protoplasts as described by Morocz et al. (1990).

E. Inserted DNA

The insertion site in corn T25 was characterized using PCR and nucleotide sequencing. Genomic DNA was extracted from corn T25. Four overlapping fragments were amplified using T25 genomic DNA as template. Sanger sequencing of the PCR products was done using the ABI3730 DNA Analyzer. The sequenced fragments were then assembled and a final consensus sequence for the T25 transgenic locus was obtained. The consensus sequence of the T25 locus was compared and aligned with the pU/Ac sequence to identify the inserted sequence. All sequences which did not show homology with pU/Ac plasmid were annotated as flanking sequence. Further sequence annotation within the inserted sequence was performed by comparing the T25 sequence with each feature of the pU/Ac sequence. The method used was sufficient to characterize the insertion sequence. Results showed that there is only one insertion site.

Sequence determination of the T25 insert confirmed the presence of one *pat* gene and a fragmented *bla* gene, with two *bla* derived sequence present in an interrupted fashion. The nucleotide sequencing documents the presence of a duplication of an integral fragment similar to part of the P35S promoter, linked to the longer bla sequence located at the 3'end of the insert. Analysis also confirms the presence of two fragments of the P35S promoter in transformation event T25. A study was conducted to determine the inserted sequence together with the 5' and 3' flanking sequences representing the T25 transgenic locus sequence. To annotate the obtained sequence, pairwise alignments were performed between the T25 transgenic locus consensus sequences with the pUC/Ac plasmid sequence. Comparison of the T25 transgenic locus sequence with the sequence of pUC/Ac confirmed that the inserted sequence is identical to the corresponding region of the transforming plasmid pUC/Ac.

There were no plasmid backbone sequences present when the full DNA sequence of the transgenic locus of maize T25 event was analyzed.

The genomic DNA from maize T25 plant material was isolated and was used as the template for obtaining the complete T25 transgenic locus sequence. A consensus sequence was derived from four overlapping fragments that were amplified through PCR. Pairwise alignment between the T25 transgenic locus and the pUC/Ac plasmid showed that the inserted sequence was identical to the corresponding region of the transforming plasmid pUC/Ac.

F. Genetic Stability

The southern blot analyses were used to assess the multigenerational stability of the introduced gene. To demonstrate the molecular structural stability of Zea mays transformation event T25, Southern blot analysis was performed on plants grown from two different seed lots. Genomic DNA was prepared from individual plants and digested with two different restriction enzymes. After hybridization, with a T-DNA probe, all analyzed T25 samples showed the presence of hybridization fragments with the expected size. This demonstrates the structural stability of the insert region of the Zea mays transformation event T25 in the analyzed seed lots.

The inheritance of the T25 transgenic locus was assessed by following the segregation of glufosinate resistance phenotype in selfs, crosses with non-transgenic inbreds and in third backcross generations. The Mendelian pattern of inheritance for a single locus was tested through chi square statistic test. Results showed no significant difference between the observed and expected segregation ratios indicating that the glufosinate resistance trait is stably integrated and transmitted to the progenies as a dominant gene. The results of the segregation analysis are consistent with the observed presence of single copy of the gene in corn T25.

G. Expressed Material

The level of expression of PAT in corn T25 plants sprayed before sampling was measured by Enzyme Linked Immunosorbent Assay (ELISA). Leaf, stem, and root samples were collected at two growth stages namely, V5-6 and mature stages. Tissue samples from non-transgenic, non-tolerant and unsprayed wild type corn line were included to serve as control. The total extractable protein (TEP) and the limit of detection for each tissue were also determined. The PAT protein was detected only in the transgenic plant samples. Based on fresh weight basis, the V5-6 leaf contained 24.7±5.8 and an increased amount of 42.0±8.2 was observed in mature leaf. The V5-6 stem contained 1.50±0.31 and a higher amount of 2.85±0.86 in mature stem. The roots at V5-6 and at maturity contained 2.06±0.33 and 1.83±0.65, respectively. If TEP is considered, average PAT protein contents as percent of TEP were 3.33% and 1.58% in V5-6 leaf and mature leaf, respectively. The V5- 6 stem had 0.52% and the mature stem had 0.277%. The V5-6 and mature roots had 0.672% and 0.56%, respectively.

The PAT enzyme does not have a metabolic role. The PAT enzyme prevents autotoxicity in the producing organisms and shows complete resistance towards high doses of phosphophinothricin or glufosinate. N-acetyl phosphophinothricin has no herbicidal activity and resistance is conferred through modification of the herbicide rather than the target of its activity.

H. Toxicological Assessment

The PAT/pat protein (encoded by the pat gene, produced in E.coli) was degraded very rapidly into fragments visible up to 5 minutes of incubation. The PAT/pat protein was completely degraded within 10 minutes of incubation with human simulated intestinal fluid (SIF), in the presence of pancreatin, at pH 7.5.

Also, the PAT/pat protein was degraded very rapidly in human simulated gastric fluid, within 30 seconds of incubation, in presence of pepsin, at pH 1.2.

The PAT/*pat* protein was tested for heat stability at temperature of 60, 75 and 90◦ for periods of 10, 30, and 60 minutes. The stability of the protein was examined using Coomassie blue stained-SDS-PAGE and Western blot analysis using a specific polyclonal rabbit anti-PAT/*pat* protein antibody. The PAT/pat protein was heat stable when incubated up to 30 minutes at 90◦C and slightly degraded when incubated at 60 minutes at 90◦C. This *in vitro* heat stability study has been conducted to evaluate if heat temperature causes structural changes to the PAT/pat protein as can be detected by gel electrophoresis followed by a Coomassie blue staining or a Western blot analysis.

Furthermore, bioinformatics analyses using FASTA sequence alignment program and BLOSUM50 database provided by the developer indicated that PAT has no significant homology to any known toxin (Capt, A. 2017).

There were no mortalities, no treatment-related clinical signs, no effects on body weight parameters and food consumption and no macroscopic changes at necropsy, in C57 BL/6J mice, after an acute oral administration of PAT/pat protein at 2000 mg/kg body weight. The treatment with PAT/pat protein at 2000 mg/kg body weight via the oral route did not produce any signs of systemic toxicity in male and female C57 BL/65 mice.

I. Allergenicity Assessment

The PAT/*pat* protein was degraded very rapidly in human simulated gastric fluid, within 30 seconds of incubation, in presence of pepsin, at pH 1.2. The PAT/*pat* protein was degraded very rapidly into fragments visible up to 5 minutes of incubation. The PAT/*pat* protein was completely degraded within 10 minutes of incubation with SIF, in the presence of pancreatin, at 7.5.

The PAT/pat protein was tested for heat stability at temperatures of 60, 75 and 90◦C for periods of 20, 30 and 60 minutes. The stability of the protein was examined using Coomassie blue stain SDS-PAGE and Western blot analysis using a specific polyclonal rabbit anti-PAT/pat protein antibody. The PAT/pat protein was heat-stable when incubated up to 30minutes at 90◦C and slightly degraded when incubated 60 minutes at 90◦C.

In silico findings revealed that there is no allergen associated with the PAT/*pat* protein. No potential N-glycosylation sites were identified on the amino acid sequence. The molecular weight of PAT is about 21 kDa, hence within the 10-70 kDa range

J. Nutritional Data

Compositional and nutritional analyses were performed using the raw agricultural commodity grain generated from 15 field trial sites in two different years. The statistical evaluation of the analytical data from the grain samples was performed by a bioequivalence test. For all proximates, sodium, all total amino acids, free cysteine, and for most total and free fatty acids the comparison between the non-transgenic and that two transgenic

treatment groups resulted in a determination of bio-equivalence. The mean and standard deviation (SD) values for the proximates and fiber compounds in T25 grains, non-transgenic counterpart and commercial maize varieties were determined. The comparison is valuable to establish that T25 corn provides the same nutritional value as maize currently being consumed.

Safety assessment based on the nutritional data indicates that there is no significant difference between the proximate, amino acid, fatty acid, vitamin, mineral and anti-nutrient levels of T25 corn and conventional corn that can be considered biologically relevant.

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. <u>DENR BC (for Safety of Event to the Environment)</u>

After a comprehensive review and evaluation of the documents including the scientific evidences from provided references and literature submitted by Bayer CropScience, Inc., on its application for Direct Use as FFP of Corn T25 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 biodiversity. The transgenic crop will not increase its weediness potential in case the seeds spill out into the environment because the PAT protein product produced by the transgenic crop will degrade upon exposure to the natural environment and general conditions, that is high temperature (55 dgrees and above), varying pH, enzyme digestion, etc.
2. The amino acid sequence comparison through FASTA algorithm shows that PAT has no biologically relevant identity with known toxins. The in vitro digestibility of the PAT protein was simulated in a mammalian gastric environment, which is also similar with the physiology of digestion of avian gastrointestinal tract, in terms of pH and type of enzyme secreted. The result shows that PAT protein is easily digested and inactivated at acidic pH, similar with the stomach condition.
3. The PDR discusses the specified environmental management plan indicating the possible risk and harm to the environment and biodiversity as well as the mitigating measures ad 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 is not conducive for plant growth. Also, corn is a highly domesticated plant that requires human intervention for it to persist in the environment.

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 Bayer's Corn T25 for Biosafety Permit for direct use as food, feed, or for processing.

C. <u>DOH-BC (for Environmental Health Safety)</u>

After a thorough review and evaluation of the documents provided by the proponent, Bayer CropScience, Inc., thorough 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 T25. The DOH-BC found that the regulated article applied for Direct Use as Food, 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 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 com.
5. It is suggested that the Bureau of Plant Industry (BPI) ensure clear labeling of the regulated article from the source down to all levels of marketing stating that it is only for the purpose of direct use as food, feed or processing and is not to be used as planting materials.

Based on the above considerations and with the submitted sworn statement and accountability of the proponent, this recommendation is being submitted to BPI related to the processing and issuance of a Biosafety Permit for Direct Use as Food, Feed or for Processing (FFP) of corn T25.

D. <u>SEC Expert (for Socio-economic Consideration)</u>

The SEC expert agreed that corn is a significant crop in the Philippines. In fact, in terms of value added, corn is the third most important crop, after palay, banana, and coconut.

The expert added that importation of corn T25 as a whole does not drastically impact on domestic production; in fact availability of foreign corn allow domestic consumption to be more stable compared to domestic production. It should also be noted that corn importation is subject to a high (40 %) MFN rate. Corn imports from ASEAN are subject to 5% ATIGA rate; within ASEAN, the only significant corn exporter is Thailand, where GM products are still not permitted for cultivation.

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