CONSOLIDATED REPORT OF DP73496 FOR DIRECT USE

EXECUTIVE SUMMARY

On July 25, 2017, Pioneer Hi-Bred Inc., submitted canola DP73496 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 canola DP73496 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, considered canola DP73496 safe to the environment and biodiversity.

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 canola DP73496 will not pose any significant risk to health and environment and that any hazards could be managed by the measures set by the department.

Furthermore, the Socio-economic, Ethical and Cultural (SEC) Considerations expert 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, 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 (*Brassica napus*)

Brassica napus was considered unsuitable as a source of food for either humans or animals, because the seed naturally contains erucic acid and glucosinolates, which are toxic to humans and other organisms. However, it was widely used as an edible oil in Asia for thousands of years (OECD, 1997a). In the 1970s, intensive breeding programs produced high quality varieties that were significantly lower in erucic acid and glucosinolates. The term 'canola' refers to those varieties of B. napus that meet specific standards on the level of erucic acid and glucosinolates

Low erucic acid rapeseed is being widely used in both salad and cooking oil products (OECD, 2011). It is also being used in hydrogenated products such as margarine and shortenings. In Canada, 68% of the edible plant-based oil consumed are from low erucic acid rapeseed oil. It is being consumed as food in the form of whole seeds, flour and oil and as feed in the form of meal (OECD, 2011).

It is a known source of key nutrients such as fiber, vitamins, minerals, amino acids and fatty acids (OECD, 2011). It also contains tocopherols, sterols, pigments, trace elements of iron, copper, lead and arsenic. Low erucic acid rapeseed contains pigments such as chlorophyllides and pheophorbides which may present nutritional effect due to their phototoxicity which may be followed by photosensitive dermatitis. Such pigments are being removed in the bleaching step during the processing. The seed contains glucosinolates and phenolic compounds such as tannin, sinapine and phytic acid while the meal is known to contain glucosinolates only (OECD, 2011).

B. Transgenic Plant

Safety data for DP73496 has been submitted to regulatory agencies in other jurisdictions and authorizations have been obtained for food uses of DP73496 in Australia in 2014, New Zealand in 2014, Canada in 2012, Japan in 2014, Korea in 2015, Mexico in 2012, South Africa in 2016, Taiwan in 2016, and the United States in 2012.

The introduction of DP73496 canola will not change the consumption patterns of population subgroups, as its use is not addressed to any specific group. The DP73496 canola can be substituted for all uses of conventional canola.

C. Donor Organism

Bacillus licheniformis, the source organism for the *gat4621* gene, is a Gram-positive saprophytic bacterium that is ubiquitous in soil. It has been used widely in the detergent industry for production of a number of enzymes (*e.g.,* proteases and amylases) that have wide applications and in the fermentation industry for production of food enzymes [*e.g.,* alpha-amylase, cyclodextrin glycosyltransferase, hemicellulase, proteases, and pullulanase; Rey *et al.* (2004)]. The gene *gat4621* expresses the protein GAT4621 that allows the plant to be herbicide-resistant, specifically to glyphosate.

B.licheniformis is widespread in the environment; therefore, animals and humans are regularly exposed without adverse consequences to this organism. All *B. licheniformis* cultures available from the American Type Culture Collection (ATCC) are classified as Biosafety Level 1, which have no known history of causing disease in humans or animals (US-EPA, 1997).

D. Transformation System

DP73496 canola was produced by microprojectile bombardment with the *Hin*d III/*Not* I-digested fragment PHP28181A from plasmid PHP28181.

The fragment PHP28181A contains four (4) Polylinker regions for cloning genetic elements, UBQ10 promoter region from *Arabidopsis thaliana* polyubiquitin 10 gene including promoter at bp 6-943, 5' untranslated region at bp 994-1,009, and intron at bp 1,010-1,313, *gat4621* gene from *Bacillus licheniformis* and *pin*II terminator region from *Solanum tuberosum* proteinase

inhibitor II gene (Pioneer, 2017). The remainder of plasmid PHP28181 is composed of β-lactamase gene coding for ampicillin resistance from *Escherichia coli (* Bia (ApR)) and Hae II fragment containing bacterial origin of replication region (colE1 ori) .

E. Inserted DNA

Southern blot analysis was conducted on DP73496 canola to determine the copy number, integrity, and stable genetic inheritance of the inserted DNA, and absence of plasmid backbone DNA. Molecular characterization of DP73496 canola was performed wherein it was demonstrated that a single, intact, copy of the PHP28181A DNA fragment was stably incorporated into the genome at a single site.

A consistent hybridization pattern was seen across all plants from five generations, indicating stable genetic inheritance of the inserted DNA. Probes to the entire backbone region of plasmid PHP28181 were hybridized to both Nco I-digested genomic DNA and demonstrated that plasmid sequences outside of the PHP28181A fragment were not present in DP73496 canola.

F. Genetic Stability

The multigenerational stability of the DP73496 canola herbicide-tolerant phenotype was determined through southern blot analysis of genomic DNA from five generations of DP73496 (T2, T3, T3F2, T3F3 and F1) probed with UBQ10 promoter, gat4621, and pinII terminator probes cut with restriction enzyme, Nco I. (Pioneer, 2016). Results of the analyses showed that a single border band was observed in individual plants of the five generations digested with Nco I-digested DNA was hybridized to each probe. This indicates that one copy of the inserted DNA was inserted in DP73496 canola.

The segregation of the DNA insert was assessed through phenotypic herbicide-tolerant assessment and insertion and gene-specific real-time polymerase chain reaction (PCR) analyses of four (4) segregating generations [Three (3) backcross generations: T4BC1, T4BC2 and T4BC3], and one (1) non-segregating generation of DP73496 canola: T3F2 (Pioneer, 2017). Chi-square analysis of data indicated that the DP73496-inserted DNA and the resulting herbicide-tolerant phenotype segregate according to Mendelian Law of Inheritance.

G. Expressed Material

The expression levels of GAT4621 protein were evaluated in DP73496 canola using quantitative enzyme- linked immunosorbent assays (ELISA). The GAT4621 protein was analyzed in multiple tissues and development stages to represent the range of protein expression throughout the growing season.
Descriptive statistics (means, ranges, and standard deviations) are provided for GAT4621 protein in Table 1. For the control canola samples, all ELISA results were below the assay lower limit of quantification (LLOQ).

Table 1. Summary of Expressed Trait GAT4621 Protein Concentrations

Tissue (Growth Stage)	ng GAT4621/mg Tissue Dry Weight				Number of Samples <LLOQ/ Number of Samples Reported
	Mean	Range	Standard Deviation	Sample LLOQ	
Herbicide-Treated DP73496 Canola					
Whole Plant (BBCH 15)	6.9	3.9 - 10	1.3	0.29	0/24
Whole Plant (BBCH 33)	5.3	3.1 - 8.4	1.2	0.29	0/24
Whole Plant (BBCH 65)	5.2	3.9 - 7.6	0.88	0.29	0/24
Root (BBCH 65)	6.6	3.9 - 13	2.4	0.22	0/24
Seed (BBCH 90)	6.2	4.8 - 8.4	0.94	0.22	0/20

Note: Growth stages (Lancashire *et al.*, 1991). Herbicide-treated DP73496 canola refers to DP73496 canola treated with glyphosate.

H. Toxicological Assessment and Allergenicity Assessment

The safety assessment of novel protein, GAT4621, includes digestibility, heat inactivation, oral toxicity and amino acid sequence comparison studies to determine its potential to cause toxicity or allergenicity to hu mans (Pioneer, 2017).

Digestibility study using Simulated Gastric Fluid (SGF) with pepsin demonstrated that GAT4621 protein is rapidly degraded in simulated gastric fluid (SGF) with pepsin within 0.5 minutes with two low molecular weight bands visible through the two (2) minute time point in SGF (Pioneer, 2017). GAT4621 protein was readily digested in two (2) minutes upon incubation with simulated intestinal fluid (SIF) with pancreatin with no remaining bands detected. The same was observed in western blot analysis with pancreatinin (Pioneer, 2017).

Heat stability of GAT4621 was evaluated through monitoring the band intensity and enzymatic activity of the protein upon subject to varying temperatures (Pioneer, 2017). The stability of band intensity of GAT4621 upon incubation at 100°C for 30 minutes was determined through western blot analysis. Results showed a significant decrease in the band intensity of GAT4621 upon incubation at 100°C for 30 minutes. In terms of enzymatic activity, heat stability of GAT4621 protein upon incubation at temperatures ranging from 30-60°C for 15 minutes was determined using a continuous absorbance spectrophotometric enzyme activity assay. Results showed that GAT4621 protein was essentially inactivated upon incubation at above 53°C for 15 minutes.

Amino acid sequence comparison was conducted using techniques such as homology search using BLASTp program, a search for continuous, identical stretches of 8 amino acid residues in length, and an identity search using the FASTA35 alignment algorithm to search for alignments of 80 residues or longer possessing a sequence identity of 35%. Results showed that GAT4621 protein has no homology to any known toxins or allergens. (Pioneer, 2017).

The acute oral toxicity study indicated that administration of 2000 mg/kg GAT4621 protein to 5 male and 5 female mice did not resulted to mortality, treatment-related clinical observations of toxicity, gross lesions at necropsy body weight losses, change in food consumption, and macroscopic changes at necropsy. The No Observed Effect Level (NOEL) for GAT4621 protein is 2000 mg/kg body weight (Pioneer, 2017).

The GAT4621 proteins used for the toxicological and allergenicity studies was obtained from *Escherichia coli* and were found biochemically and functionally equivalent to GAT4621 expressed in DP73496 (Pioneer, 2017). Equivalency was determined through SDS-PAGE analysis which indicated the comparable molecular weight, western blot analysis which confirmed the immunoreactivity of plant-produced and E. coli-produced GAT4621, glycosylation analysis which indicated the absence of glycosylation of both test proteins and N -terminal amino acid sequence analysis and MALDI-MS analysis of tryptic peptides which showed sequence equivalency.

A computation of the percent total protein based on the data on the mean concentration of GAT4621 in DP73496 seed (6.2 ng/mg tissue dry weight) and the average crude protein content of DP73496 seeds (25.90/o) on a dry weight basis was provided by the developer (Pioneer, 2017). According to Martin-Hernandez et al. (2008), the human exposure to canola is limited primarily to the consumption of refined canola oil. Considering the total protein content of canola oil which is 0.2 µg/g, this indicates that for every tablespoon of DP73496 canola oil, the dietary exposure to GAT4621 protein would be 0.000056 µg.

Results of the toxicological and allergenicity assessment indicate that GAT4621 protein being expressed in DP73496 is not toxic or allergenic to humans (Pioneer, 2017).

I. Nutritional Data

Compositional analysis indicated the nutritional data of DP73496 in comparison with the non-transgenic canola, tolerance interval derived from range of commercial varieties and range of literature values (Pioneer, 2017; OECD, 2011). The trials were conducted six (6) sites in United States (Washington and North Dakota) and Canada (Saskatoon and Manitoba) under same environmental conditions. Results of the analysis indicated that there is no differences in the proximate, fiber, mineral, amino acid, fatty acid, vitamins, anti-nutrient, secondary metabolite, free amino acid and acetylated amino acid levels of DP73496 canola and the commercial canola varieties that can be considered biologically relevant.

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

After a thorough and scientific review and evaluation of the documents provided by the Bureau of Plant Industry (BPI) on the application of Pioneer Hi-Bred Philippines, Inc. for Direct Use as Food and Feed or for Processing of **<u>Canola DP73496,</u>** here under are the observations and appropriate actions:

1. From the evaluation of the application submitted by the proponent, including the scientific evidences from 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) 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 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/import as per BPI's 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 safe as its 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 relative to Pioneer Hi-Bred Philippines, Inc. **DP73496** application for biosafety permit for food, feed, and/or processing.

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

After a thorough review and evaluation of the documents provided by the proponent, Pioneer Hi-Bred, 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 canola DP73496. 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 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 coin varieties.
4. The regulated article is not materially different in nutritional composition from that of the non-transgenic canola or the conventional canola.
5. It is suggested that the Bureau of Plant Industry (BPI) ensure the following.

6. Clear labeling of the regulated article from the source down to all levels of marketing stating that it is only for 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 Canola DP73496.

D. SEC Expert (for Socio-economic, ethical and cultural Consideration)

The entry of Canola (DP73496) in the country will not affect the domestic production since it will be imported and will be used for food, feed and processing only. However, it may indirectly affect the consumption of coconut oil and copra meal through the price mechanism. With the entry of more canola products in the country, it will help stabilize prices or even it may lower the prices of products using canola as one of the ingredients. With the lowering of prices, demand of such commodity may increase, holding other factors constant. In terms of trade, it may not affect due to its insignificant share in the imports of the Philippines.

The SEC expert reported that it will not drastically affect the current patterns of production, consumption/utilization and trade. Current production pattern will not be affected since DP73496 will not be produced locally. On the other hand, the approval of DP73496 for utilization for the food feed and processing industries may stabilize prices thus, will help improve the consumption of products with canola as ingredient. However, its current consumption will not be affected drastically. In addition, being only one of the ingredients and has many substitutes in the feed formulation for livestock, poultry and aquaculture sub-sector, canola is expected not to influence the consumption pattern of the consumers. Food choice of consumers are influenced by many factors. Although studies have shown the moral concern had been found to be important for the choice of GM food products, however, the same study shows that it would be difficult to affect dietary change of the consumers. Moreover, DP73496 will help improve the global trade. It will increase the exports of the canola producing countries while allowing more imports of non-producing countries. With stable supply of canola, prices will also be stabilized thus, improving the patterns of consumption and trade worldwide.