Consolidated Technical Report of Monsanto's Canola RT73 Application for Direct Use as Food, Feed or for Processing (FFP)

EXECUTIVE SUMMARY

On March 19, 2018, Monsanto Philippines Inc., submitted canola RT73 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 RT73 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 the regulated article 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 RT73 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 Monsanto Philippines, 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

Canola contains fatty acids, particularly mono- and polyunsaturated fatty acids, and protein, micronutrients particularly vitamins K and E. Canola is associated to contain erucic acid and glucosinolates. Erucic acid is a mono-unsaturated, 22 carbon fatty acid (c22:1) that is a natural component of rapeseed. Glucosinolates, on the other hand, are organic compounds that contain both sulfur and nitrogen reducing the palatability of canola meal.

Canola is not known as a common allergen, although there has been some reports on sensitivity to Brassica species.

Refined low erucic acid rapeseed oil has been widely used in food industry. Canola oil is one of the largest source of vegetable oil that is used in a variety of foods including frying and baking oils, salad oils, margarines and shortenings. Canola meal is primarily used as feed ration.

History of safe use was attributed to soybean. Based on OECD report, soybeans are commonly consumed in processed form and primary source of oil and protein. Heat processing eliminates the anti-nutritional factors in soybean.

B. Transgenic Plant

Canola RT73 has been reviewed and approved for food and/or feed use in many countries including Australia/New Zealand, Food (2000)/Environment (2003); Canada, Food (1994)/Feed & Environment (1995); China, Food/Feed (2015); European Union, Food/Feed/Processing (2015); Japan, Food (2001)/Feed (2003)/Environment.

It was reported that the consumption patterns by population subgroups are not expected to be altered (Monsanto, 1994).

C. Donor Organism

The *cp4epsps* donor organism was *Agrobacterium sp* strain CP4 while the *gox* donor organism was *Ochrobactrum anthropi* strain LBAA.

Agrobacterium sp. strain CP4 and *Ochrobactrum anthropi* strain LBAA are not known to be allergenic or pathogenic to human and animals. None of these proteins has been identified as toxic or allergenic due to its history of safe use. Furthermore, bioinformatics analyses showed that all the protein-encoding sequences have no significant homology to known toxin or allergen.

D. Transformation System

Canola RT73 was generated via *Agrobacterium*-mediated transformation method and the target of genetic modification is Nuclear DNA. Vector PV-BNGT04 was mobilized into disarmed *A. tumefaciens* strain A131. Five to six-week-old leaves and buds of Westar canola were used as explant sources and were infected with Agrobacterium. Positive shoots were grown to maturity, selfed to produced seeds and the progeny were screened for glyphosate tolerance and gene expression. Analyses performed on DNA derived leaves of R3 GT73 plants demonstrated that only a single copy of the T-DNA was inserted into the genomic DNA of Westar at a single location to produce GT73 and that the plasmid backbone sequence including the bacterial marker gene were absent from GT73. Using PCR analysis showed that only the T-DNA sequences are present in the DNA of GT73. The presence of the single insert was confirmed by the inheritance data. Glyphosate tolerance phenotype was inherited as a single dominant Mendelian unit. Southern blot analysis performed on DNA from R3 and R5 generations showed the same patterns demonstrating structural stability of the inserted DNA.

E. Inserted DNA

Southern Blot Analysis of RT73 DNA fragments digested with different combinations of restriction enzymes that cut outside and within the plasmid PV-BNGTO4 demonstrated a single fragment of the genetic components integrated in a single insertion site in Canola RT73 (Kolacz et al., 2013).

The integrity and order of genetic elements was demonstrated through Southern Blot Analyses using different genetic element-specific probes for gox v247, cp4 epsps gene, oriV/ori322 region and the am/ gene (Kolacz et al., 2013). Plasmid and genomic DNA was cut with EcoRl. There are 6 EcoRl sites within PV-BNGT04, which all occur between the left and right border sequences. Integrity and order of genetic elements was confirmed upon detection of the predicted fragments for each hybridization probes tested. No any truncations, deletions, or rearrangement was reported by the developer,

The absence of plasmid backbone region oriV/ori322 and the bacterial marker gene aad was confirmed from Southern blots of genomic DNA digests of RT73, with the plasmid as probe.

F. Genetic Stability

The stability of the inserted DNA was determined using Southern blot analysis performed on the DNA from R3 and R5 generation of canola RT73 after the DNA was digested with Eco R1 and probed with gox v247 or cp4epsps coding regions or the E9 3'gene terminator region. Plasmid PV-BNTO4 was used as positive control. Identical banding patterns were exhibited in the R3 or R5 generations indicating physical stability of the inserted DNA.

G. Expressed Material

RT73 produces GOX v247 and CP4EPSPS proteins. The levels of the proteins were determined using enzyme-linked immunosorbent assay (ELISA). The levels of the two proteins in the seeds and leaves were measured in multiple field trials during the growing season from 1992 to 1993. In the 1992 trial, CP4EPSPS mean expression level was 0.034µg/mg tissue (fresh weight) while GOX v247 was 0.108034µg/mg tissue (fresh weight). There was no increase or decrease in leaf expression overtime. In the seeds, mean levels of CP4EPSPS was 0.049 µg/mg tissue (fresh weight) while for GOXv247 it was 0.154 µg/mg tissue (fresh weight). These expression levels are relatively low, accounting for less than 0.02% and 0.07% of the total protein in the seed for CP4EPSPS and GOXc247, respectively.

In 1993, the seed had a range of expression for CP4EPSPS in RT73 of 0.018 to 0.047 µg/mg tissue (fresh weight) with a mean expression of 0.028 µg/mg tissue (fresh weight). The range of expression for GOXv247 was 0.108 to 0.334 µg/mg tissue (fresh weight) with mean expression level of 0.194 µg/mg tissue. No leaf tissue was analyzed for expression in the 1993 field trials.

The values for both years (1992 and 1993) were consistent in RT73. As expected, no detectable levels of GOX v247 and CP4EPSPS were observed in Westar leaf and seeds.

H. Toxicological Assessment and Allergenicity Assessment

The safety assessment of novel proteins, CP4 EPSPS and GOX v247, includes digestibility, heat inactivation, oral toxicity and amino acid sequence comparison studies to determine its potential to cause toxicity or allergenicity to humans (Monsanto 1995). Escherichia coil-produced CP4 EPSPS and GOX v247 proteins were used in the analyses. Equivalence study provided by the developer indicated the functional and structural equivalence of the E. coil-produced proteins and the proteins derived from RT73.

Digestibility study using Simulated Gastric Fluid (SGF) with pepsin demonstrated that CP4 EPSPS and GOX v247 readily degraded within 15 seconds of incubation with SGF in presence of pepsin, a characteristic of most non-toxic proteins (Leach et al., 2002; Ream, 1994).

The enzymatic activity analysis on CP4 EPSPS and GOX v247 provided by the developer indicated that 83% of the activity is lost in immunodetectable level was observed upon incubation at 60°C for 15 minutes. The activity is significantly impacted by heat treatment (Padgette et al., 1994]. For CP4 EPSPS, more than half of the activity is lost in immunodetectable level upon incubation at 55°C for 15 minutes. The activity is significantly impacted by heat treatment (Padgette et al., 1993).

Amino acid sequence comparison of CP4 EPSPS and GOX v247 protein using FASTA search alignment tool indicated no homology to any known toxins and allergens (Mitsky, 1993; Silvanovich et al., 2001).

Acute oral toxicity study provided by the developer indicated no treatment-related effects on survival, clinical observations, body weight gain, food consumption or gross pathology upon administration of 572 mg/kg body weight CP4 EPSPS and 104 mg/kg body weight in mice. Both concentrations were the No Observed Adverse Effect Level (NOAEL) for both novel protein (Monsanto, 1995).

Based on the concentration of novel proteins in RT73 and % dry weight of total protein in RT73, the percent of CP4 EPSPS and RT73 protein in the transgenic canola is calculated as 0.02% and 0.064% which represents a very small portion of total protein in RT73 canola. Hence, the margin exposure of humans or animals to risk of consuming high dosage of CP4 EPSPS and GOX v247 is extremely high (Monsanto, 1995).

CP4 EPSPS and GOX v247 were expressed independently of each other and their functional activity was maintained (Padgette et al., 1996, Padgette et al., 1994). The expression cassette of *cp4 epsps* and *gox v247*

includes a chloroplast transit peptide (CTP) which directs the import of the newly translated protein into chloroplasts (della-Cioppa et al., 1987). CP4 EPSPS and GOX v247 proteins have distinct modes of action and are not likely to interact (Steinrilicken and Amrhein, 1980; Padgette et al., 1993, Padgette et al., 1994; Barry et al., 1994). The phenotype of RT73 is the result of the development of two distinct mechanisms of glyphosate tolerance; reduced sensitivity of the molecular site of herbicidal activity by the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), and introduction of the means by which the plant can degrade the herbicide by glyphosate oxidoreductase (GOX). There is no known mechanism of interaction among these proteins that could lead to adverse effects in humans, animals or environment.

Results of the toxicological and allergenicity assessment indicate that CP4 EPSPS and GOX v247 proteins being expressed in canola are not toxic or allergenic to humans (Monsanto, 1995).

I. Nutritional Data

Compositional analysis provided by the developer indicating the nutritional data of RT73 canola in comparison with the non-transgenic canola, and range of literature values (Monsanto, 1995). Safety assessment based on the nutritional data indicates that there is no significant difference between the proximates, amino acid, fatty acid and anti-nutrient levels of RT73 canola and conventional canola 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 greater risk to human and animal health

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

After a comprehensive reviews and evaluation of the documents including the scientific evidence from provided references and literature submitted by Monsanto Philippines, Inc. for direct use for feed, food or processing of canola RT73, the following are the observations and recommendations of the DENR-BC:

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 on case the seeds spill out into the environment because the protein product produced by the transgenic crop will degrade upon exposure to the natural environment and general conditions (i.e., high temperature [60C and above], varying pH, enzyme digestion, etc.).
2. The PDR discusses the specified environmental management plan indicating the possible risk and harm to the environmental management plan indicating the possible risk and harm to the environment and biodiversity 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.

3. The Bureau of Plant Industry 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.

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

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

After a thorough review and evaluation of the documents provided by the proponent, the DOH-BC found 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 of the DOH-BC:

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 canola 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 ensure that there shall be clear instructions that the product is only for the purpose of direct use for ffp and is not to be used as planting materials.

D. <u>SEC Expert (for Socio-economic, ethical and cultural Consideration)</u>

The studies on demand for canola oil shows that the current consumption is significantly low given that it is more expensive than other sources of cooking oil. The demand for cooking oil is expected to increase in response to the projected increases in family income and the growth of the food manufacturing industry. The top countries producing Canola are Canada, China, India, Germany, France and Australia. The Philippines is not producing canola so that this is being imported to fill in the gap between supply and demand for cooking oil.

Furthermore, the assessor concluded that the GM product will not have a drastic effect on the current utilization of cooking oil if it comprises only a very small percentage of total supply of cooking oil in the domestic market.

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