ASSESSORS' CONSOLIDATED REPORT ON BAYER CROPSCIENCE INC'S APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF OILSEED RAPE RF3

EXECUTIVE SUMMARY

On November 22, 2016, Bayer CropScience Inc. submitted oilseed rape RF3 for direct use as food and feed, or for processing, as original application 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- Biotech Team (BAI-BT), concurred that oilseed rape RF3 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 the conditions set by DENR 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 oilseed rape RF3 will not pose any significant risk to the 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 oilseed rape RF3.

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, except for the SEC expert, the complete dossier submitted by Bayer. The SEC expert, on the other hand, was provided with a questionnaire on socio-economic, ethical and cultural considerations that have been addressed by Bayer in relation to their application.

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 AND RECOMMENDATIONS

Based on the documents submitted by the applicant:

A. Host Organism

The STRPs agreed that rapeseed oil is consumed worldwide, produced in Europe and Canada, which represent the largest producer and consumer by region and country respectively.

They also agreed that some anti-nutrients are present in low erucic acid rapeseed meal like glucosinolates, sinapine, tannins and phytic acid in very small amount, hence not considered a health concern for man and animals. Glucosinolates, considered toxicants, are low to zero. While these may be considered "toxicants" the bioactive compound may also be beneficial in cases of the *Brassica* species, e.g., cabbage, which is considered in phytomedicine as "anti-cancer."

The STRPs concurred that no reports have been noted in terms of allergy to low. Allergy to plants of *Brassica* species have been seen to have a shared sensitivity to other food allergens and is likely to be from other food allergens than from *Brassica*. The overall risk is very low as an allergen.

They also affirmed that the oil is used in food processing and for home cooking and baking. The oil is also used for non-food products such as dust de-pressants, de-icers for airplanes, suntan oils biodiesel and bioplastics Soap stock is also manufactured from the oil. The oil meal is also used as high protein feed source for fish aside from livestock and poultry.

B. Transgenic Plant

The STRPs agreed that there are now 12 countries where the transgenic plant has been approved as food. They also agreed that the consumption pattern is not changed as a result of introducing the novel food. It will be consumed by all population subgroups.

C. Donor Organism

The STRPs all concurred that the nonpathogenecity, toxicity and allergenecity of the donor organisms (*Bacillus amyloliquefaciens* and *Streptomyces hygroscopicus*) to host organism (RF3 oilseed rape) had been sufficiently described based on study reports/documents submitted. The review of various literature showed that the donor organisms are obiquitous in nature, nontoxic, nonallergenic and therefore not harmful to man and animals.

They also concurred that all potentially inserted protein encoding sequences have been adequately described the genetic elements are presented in the vector map.

Lastly, they all agreed that the encoded PAT protein from *Streptomyces hygroscopicus* and BARSTAR protein from *Bacillus amyloliquefaciens* have no pathogenic properties and no homology with any known toxins or allergens, hence not a health concern for man and animals and nontarget organism.

D. Transformation System

The STRPs all arrirmed that the transformation method, is mediated by *Agrobacterium tumefaciens* and that the genomic DNA was the target of the transformation. Analytical procedure used the total genomic DNA, isolated from Brassica napus.

They also concurred that the analytical / experimental protocol was adequately described as *Brassica napus* seeds of variety Drakkar were germinated on solid germination medium. Hypocotyl segments were dissected from the Brassica napus seedlings and incubated on solid modified Murashige & Skoog (MS) medium for callus induction. Callus was isolated from the wounded sites of the hypocotyls and transferred to the same medium for embryogenic callus (EC) development. Small clumps of EC were transformed with the vector pTHW118. This vector system consists of an Agrobacterium strain, C58C1Rif, and two plasmid components namely a non-oncogenic helper Ti-plasmid pGV4000 and a T-

DNA cloning vector pTHW118, essentially derived from pGSC1700. The non-oncogenic helper Ti-plasmid pGV4000 from which the T-region has been deleted carries the vir genes required for transfer of an artificial T-DNA cloned on the second plasmid to the plant genome.
After this co-cultivation, the transformed calli were selected on medium supplemented with glufosinate ammonium. After induction of somatic embryogenesis and regeneration into plantlets, the plantlets were transferred to the greenhouse for flowering, seed setting and further characterization.
E. Inserted DNA

The STRPs all confirmed that the single insertion was demonstrated by Southern blot analysis. Genomic DNA was isolated from GM and non-GM plants. Isolated DNA samples were subjected to Southern blot analysis using different probes corresponding to the components of the transgenic DNA, namely bar, PssuAt, barstar and Pta29. An additional hybridization with the 5'Pta29 probe and a set of discriminant PCR reactions were performed. Southern blot and PCR analysis confirmed the updated model of the RF3 insert.

They also concurred with the result of the overall identity search which was carried out by using FASTA algorithm, which compared the complete query sequence with all protein sequences present in the AllergenOnline database. An E-value threshold of 10 was used. The criterion indicating potential significant homology to an allergen was a minimum of 35% amino acid identity over at least 80 amino acids.

In addition, query sequences were compared to protein sequences contained in the Uniprot-Swissprot, Uniprot-TrEMBL, DAD, GenPept databases, by using FASTA algorithm. An overconservative E-value threshold of 0.1 was used. Then the relevance of hits was assessed by examining the E-value, the bit score as well as the biological significance of the alignment with the matched protein(s).

The query sequences showed no biologically significant sequence similarities with any known allergens and known toxins. In conclusion, there are neither allergenic nor toxicological in silico findings associated with the presence of the putative ORF polypeptides.

F. Genetic Stability

The STRPs all agreed that the genes in the transgenic DNA (BAR and barnase respectively Barstar) are 100 percent linked and inherited according to Mendelian laws and are expressed as dormant and that multiple crosses and backcrosses of different oilseed rape varieties have been performed and glufosinate-ammonium segregation data as well as flower segregation data have been collected. No observation of loss of any of the new traits was recorded.

They also agreed that the genetic stability of Event RF3 oilseed rape was assessed and sufficiently demonstrated.

G. Expressed Material

The STRPs all concurred that western blot analysis of the stem was not reported. However, the analyses of the young and mature leaves were done in addition to the analysis of root, flower buds, pollen, and dry seed. Within 1-5 ng of protein was the limit of detection and established detection in transgenic variety and were not detected in non-transgenic plants.

They also affirmed that, as with the PAT protein, the barstar protein was analyzed using Western blot. As described in the results, the barstar protein was detected in the flower buds during pollen development in Rf3 transgenic plants.

Therefore, the STRPs all agreed that this can only be infered by the experiments such that the expected results give credence to the objective of expressing the bar and barstar genes. No direct metabolic pathway analysis can be done to show that process.

H. Toxicological Assessment

The STRPs all concurred that the PAT protein was shown to be degraded very rapidly, within 30 seconds, in human simulated gastric fluid and within 5 minutes, in human simulated intestinal fluid.

They also concurred that the PAT protein was heat stable at temperature of 90oC after 30 minutes of heating and slightly degraded at same temperature after heating for 60 minutes and that PAT protein has no amino acid sequence similarities to other known allergens as demonstrated by overall amino acid and isolope homology searches. Extensive studies demonstrated that PAT protein (encoded by the bar gene) is not toxic to mammals, hence there will be no harm resulting from the inclusion of PAT protein in human food and animal feed.

Lastly, they all agreed that based on SDS-PAGE and Western blot analysis, the PAT/bar protein expressed in *E.coli* and the plant purified PAT/bar from *Brassica napus* transformation event Rf3 have similar biochemical properties.

Meanwhile, the STRPs all agreed that since the pollen is not normally ingested, there is no basis for the BARSTAR protein to find its way in the food chain.

They also agreed that examination of the junction between the barstar gene and the RF3 plant DNA genome showed 9 putative Open Reading Frame (ORF) nucleic sequences ORF-1 to ORF-9. No significant similarities were observed between the sequences of the 9 putative ORF sequences and any sequences of known proteins from all the data bases. It was concluded that there is no toxicological and allergenic “in silico” findings associated with the presence of the putative ORF polypeptides.

I. Allergenicity Assessment

The STRPs all concurred that the PAT protein was shown to be degraded very rapidly, within 30 seconds, in human simulated gastric fluid and within 5 minutes, in human simulated intestinal fluid.

They also confirmed that the PAT protein was heat stable at temperature of 90oC after 30 minutes of heating and slightly degraded at same temperature after heating for 60 minutes and that PAT protein has no amino acid sequence similarities to other known allergens as demonstrated by overall amino acid and isolope homology searches. Extensive studies demonstrated that PAT protein (encoded by the bar gene) is not toxic to mammals, hence there will be no harm resulting from the inclusion of PAT protein in human food and animal feed.

Lastly, they all agreed that there were no potential glycosilation sites and would mean no potential reaction sites for the PAT protein and that the apparent molecular weight of PAT is 21-22 kDa, not all proteins within 10-70 kDa range are allergenic.

Meanwhile, the STRPs have verified that the presence of BARSTAR protein in transgenic RF3 oilseed rape is not a health risk/hazard for human and animals since it is found only in developing flower buds and that no significant similarities were found between the sequences of the 9 putative ORF sequences and any sequences of known proteins from all the data bases. These 9 sequences do not present any significant identity with known toxins and allergens. It was concluded that there is no

toxicological and allergenic "in silico" findings associated with the presence of the putative ORF polypeptides.

They also concurred that the processing of the oilseed rape grain to refined oil and food grade quality oil results to degradation of all the protein components.

J. Nutritional Data

The STRPs all agreed that proximate and fiber compounds in seeds from transgenic RF3 and non-transgenic counterpart plants ranges from commercial Canola hybrids and references ranges from literature are presented in tabulated mode (see tables on Annex A). Similarly, the table of micro-nutrients, anti-nutrients and amino acids in seeds is presented (Annex A). Assessment of substantial equivalence conducted showed that glufosinate tolerant *B. napus* transformation Event RF3 is compositionally and nutritional equivalent to its non-transgenic counterpart in the same hybrid background and to commercial *B. napus* hybrids currently in the market. Based on statistical evaluation of results, it was concluded that there is no impact on the nutritional value of the B. napus seeds as a result of genetic modification.

K. Recommendation

After a thorough and scientific review and evaluation of the documents provided relevant to the transformation event, they concluded that the regulated article applied for is safe for food, feed or for processing.

BPI-PPSSD ASSESSMENT AND RECOMMENDATION

Low Erucic Acid Rapeseed RF3 was developed by Bayer CropScience, Inc., through the use of recombinant DNA technology. The said event was developed through *Agrobacterium tumefaciens*-mediated transformation using plasmid vector pTHW118 which carries the barstar gene encoding Barstar protein which is responsible for the event's function as fertility restorer and bar gene encoding PAT protein which provides tolerance to glufosinate ammonium-containing herbicides.

Host Organism (*Brassica napus*)

Low erucic acid rapeseed (*Brassica napus)* is being widely used in both salad and cooking oil products. 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.

It is a known source of key nutrients such as fiber, vitamins, minerals, amino acids and fatty acids. 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. Also, the poisoning effect of the catalysts are being mitigated through grading standards where the lots which exceed the maximum tolerance level are rejected. The seed contains glucosinolates and phenolic compounds such as tannin, sinapine and phytic acid while the meal is known to contain glucosinolates only.

The host organism is not known as source of toxins. OECD Consensus Document (2011) indicated that several published studies reporting sensitivity and allergenicity of adults to *Brassica* species. Most

describe rare cases of respiratory symptoms are due to occupational exposure. Allergenicity to low erucic acid rapeseed oil has not yet been reported in scientific literatures. This is probably due to the very low levels or absence of protein in low erucic acid rapeseed oil.

Transgenic Plant

RF3 canola has been approved in the following countries Australia (Food, 2002), Canada (Food, 1997; Feed, 1996), European Union (Food, 2013; Feed, 2007), Japan (Food, 2001; Feed, 2003), Mexico (Food, 2007), New Zealand (Food, 2002), South Korea (Food, 2013; Feed, 2012), Taiwan (Food, 2015) and Unites States (Food and Feed, 1998) (ISAAA, 2017).

Based on the documents provided by the proponent the introduction of RF3 is not likely to change the consumption patterns by population subgroups.

Donor Organisms

Bacilllus amyloliquefaciens is the donor for barstar gene. *B. amyloliquefaciens* bacterium
Is common in the environment and is frequently used as source of industrial enzymes such as α-amylase. *B. amyloliquefaciens* has no known pathogenicity, toxicity nor allergenicity and is used in brewing, bread-making and food industry as a whole.

Streptomyces hygroscopicus is the donor for bar gene. *S. hygroscopicus* as itself is not known to be a human, animal or plant pathogen nor has it been associated with other properties (e.g. production of toxins or allergens) known to affect human health. *Streptomyces* species are widespread in nature; they are a common part of the living biosphere all over the world.

History of safe use was attributed to the donor organism.

Transformation System

Low erucic acid rapeseed RF3 was developed through *Agrobacterium tumefaciens*-mediated transformation using pTHW118 plasmid which contains the barstar and bar gene expression cassettes.

The PssuAt-bar-3'g7sequence contains the promoter PssuAt which has been isolated from *Arabidopsis thaliana,* an herbaceous plant belonging to the *Brassicaceae.* The PssuAt promoter regulates the expression of the bar gene. Its activity is most abundant in green tissues. Polyadenylation signals are provided by the 3' end of the T-DNA gene 7 of *Agrobacterium tumefaciens*.

The PTA29-barstar-3'nos sequence contains the promoter TA29 of *Nicotiana tabacum* which regulates the expression of the barstar gene. The TA29 promoter effectively limits the activity of the barstar gene in the tapetal cell layer of the pollen sac only during anther development stage of flower development. The sequence also contains the 3' end of the nopaline-synthase gene of Agrobacterium tumefaciens.

The transformation vector system consists of two plasmid components namely a non-oncogenic helper Ti-plasmid pGV4000 and a T-DNA cloning vector pTHW118, essentially derived from pGSC1700. The non-oncogenic helper Ti-plasmid pGV4000 from which the T-region has been deleted carries the vir genes required for transfer of an artificial T-DNA cloned on the second plasmid to the plant genome.

Inserted DNA

Southern Blot Analyses and T-DNA insert sequencing demonstrated that RF3 Oilseed rape contains one intact copy of the T-DNA insert at a single locus (Moens, 2014).

The integrity and order of genetic elements within each insertion site was demonstrated through Southern Blot Analyses and PCR. The resulting DNA fragments were separated by agarose gel-electrophoresis, transferred to a membrane and sequentially hybridized with four different radioactive labeled probes corresponding to the components of the transgenic DNA, namely bar, PssuAt, barstar and Pta29. Each of these four probes represents one genetic element present in the T-DNA of the transformation vector pTHW118. The obtained hybridization results confirmed the results obtained in the previously performed detailed insert characterization of Brassica napus transformation event RF3 by Southern blot analysis. But the additional hybridization and PCR analysis confirm the organization of the inserted transgenic sequences as described.

A bioinformatics analysis was performed on the RF3 insertion locus sequence to identify the insertion locus and to determine whether regulatory sequences or endogenous *Brassica napus* genes were interrupted upon the insertion of T-DNA sequences. The RF3 insertion locus sequence was used as query sequence. Similarity searches using BLAST against the *Brassica napus* genomic reference database, which were performed on the RF3 insertion locus as a query sequence, did not show 100% identity with a particular chromosomal region but instead showed strong sequence similarities with two different chromosomal regions in chromosomes C06 and A07. These findings indicate that with the current available *Brassica napus* genomic reference database, it is not possible to identify the exact chromosomal location of the RF3 insertion locus.

Similarity searches using BLASTn against the nucleotide database which were performed on the RF3 insertion locus as a query sequence, showed strong sequence similarities with several predicted uncharacterized mRNA sequences located in the 3' flanking sequence of the RF3 insertion locus. As these nucleotide sequences are oriented in the reverse direction compared to the query sequence, the results indicate an overlap between the 3' end of the predicted mRNA sequences and the target site deletion. Hence, the coding sequences of the predicted mRNA sequences are not interrupted upon the insertion of T-DNA sequences.

Similarity searches using BLASTn against the nucleotide database which were performed on the RF3 insertion locus also showed sequence similarity with a Rotundifolia like 21 protein located in the 3' flanking sequence region of the RF3 insertion locus. The similarities are weak and only partial but correspond to coding sequences.

Based on the bioinformatics analysis performed in this study, the chromosomal location of the RF3 insertion locus could not be identified. Similarity searches indicated the presence of an endogenous gene in the 3' flanking sequence region of the RF3 insertion locus. However, the insertion of T-DNA sequences in the RF3 insertion locus is unlikely to interrupt or alter transcriptional or translational activity of known endogenous Brassica napus genes.

Examination of the junctions between the inserted sequence and the plant DNA genome, as well as the inserted sequence, showed 664 newly created ORFs defined between two stop codons and with a minimum size of 3 amino acids (RF3_ORF.001 till RF3_ORF.664). Open reading frames RF3_ORF.265 and RF3_ORF.534 include the Barstar protein sequence. ORF RF3_ORF.646 includes the PAT/bar protein sequence. Putative translated amino acid sequences (of at least 30 amino acids length) of the RF3_ORF.001 to RF3_ORF.664 dataset were analyzed in the study.

Query sequences were compared with sequences of known allergens contained in the public allergen database AllergenOnline by using several in silico approaches: An 8-mer search was carried out to identify any short sequences of amino acids that might represent an isolated shared potential allergenic epitope. This search compared the query sequence subdivided into 8 amino acid blocks, with all known allergens present in the public allergen database. The program used was SeqMatchAll and the criterion indicating potential allergenicity was a 100 % identity on a window of 8 amino acids with an allergenic protein. An overall identity search was carried out by using FASTA algorithm, which compared the complete query sequence with all protein sequences present in the AllergenOnline database. An E-value threshold of 10 was used. The criterion indicating potential significant homology to an allergen was a minimum of 35% amino acid identity over at least 80 amino acids.

The query sequences showed no biologically significant sequence similarities with any known allergens and known toxins. In conclusion, there are neither allergenic nor toxicological in silico findings associated with the presence of the putative ORF polypeptides.

Genetic Stability

The multigenerational stability of the introduced trait was demonstrated through Southern Blot Analyses of three generations of RF3 Oilseed rape. Results of the experiment showed that the chimeric genes remain structurally stable through meiosis and are transmitted through the seed: the structure of the transgenic DNA (as defined by Southern hybridization pattern) is identical for the original RF3 transformant; the genes on the transgenic DNA (bar and barstar) are 100% linked, are inherited according Mendelian laws and are expressed as dominant genes; the timing, tissue specificity and level of gene expression is preserved during propagation for several generations and under different environmental circumstances (gene expression is scored from the phenotype and confirmed by PAT assays and Northern blots).

Genetic and phenotypic stability were tested and Mendelian segregation analyzed in more than 3 generations. Stable incorporation of the chimeric genes into the plant genome has been investigated by the demonstration of standard Mendelian genetics of the inserted traits. Thoroughly testing the progeny of the MS8 x RF3 (up to 3 generations) by judging their segregation patterns indicated the stable physical integration of the genes. Multiple crosses and backcrosses (up to BC3) in different oilseed rape varieties have been performed and glufosinate-ammonium segregation data as well as flower segregation data have been collected. There has been no observation of loss of any of the new traits. Once integrated into the different genetic backgrounds, the bar and barstar genes were expressed as predicted.

Expressed Material

The developer provided sufficient information on the level of expression of novel proteins in different plant parts. Results showed that PAT protein is detectable in all tissues (young and mature leaf, flower bud, root, pollen and seed) of RF3 line, the amounts of PAT protein were higher in green tissues and were only at trace levels in other tissues. Barstar can only be detected in flower buds during pollen development in RF3 plants.

Toxicological and Allergenicity Assessment

The safety assessment of novel proteins, Barnase and PAT, includes digestibility, heat inactivation, oral toxicity and amino acid sequence comparison studies to determine its potential to cause toxicity or allergenicity to humans.

Digestibility study using Simulated Gastric Fluid (SGF) with pepsin demonstrated that Barstar and PAT protein is rapidly degraded in simulated gastric fluid (SGF) with pepsin within 0.5 minutes.

The thermal stability of the microbially-produced Barstar and PAT protein was determined using SDS-PAGE and Western blot analyses. Samples of Barstar were incubated for 30 minutes at 4°C, 25°C, 37°C, 55°C, 75°C, and 95°C. Samples of PAT were incubated for 10, 30 and 60 minutes at 6°C, 75°C, and 95°C.

Results showed that after heat treatment at 25°C, 37°C, and 55°C, the majority of Barstar remained in the supernatant. After heat treatment at 75°C and 95°C, there was a small amount of Barstar in the pellet but the majority still remained in the supernatant. After heat treatment at 95°C, Barstar produced multimers that were mostly in the supernatant. This suggests that Barstar forms soluble oligomers upon heating at 95°C and the native form of Barstar is not stable upon heating at 95°C. It is to be noted that in planta, Barstar is expressed only in developing flower buds during pollen development and could not be detected in any other plant tissues such as leaves and seeds. Therefore, the likelihood of any exposure of humans and animals to this protein is remote. The PAT/bar protein was heat stable up to 60 minutes at 90°C that confirms with previous published results.

FASTA algorithm associated with BLOSUM50 scoring matrix were used to assess the amino acid sequence of Barstar and PAT proteins if there are known toxins similar to the Barstar and PAT sequence. NCBI non-redundant database and in-house Bayer toxin database were used with an E-value threshold of 0.1 and 10, respectively were used for pre-selecting the most similar proteins. There were no toxicological in silico findings associated with the Barstar and PAT protein.

An overall identity search was carried out to compare the complete query sequence with all protein sequences present in the public allergen database Allergen Online. FASTA algorithm was used, with the BLOSUM50 scoring matrix and an E-value threshold of 1 for Barnase. For PAT, NCBI non-redundant database and in-house Bayer toxin database were used with an E-value threshold of 0.1 and 10, respectively were used for pre-selecting the most similar proteins.

The criterion indicating potential allergenicity was ≥35% identity over at least 80 consecutive amino acids with an allergenic protein. An 8-mer search was carried out to identify any short sequences of 8 amino acids or longer that share 100% identity to an allergenic protein. This search was performed using SeqMatchAll from the EMBOSS suite, which compared the query sequence with all known allergens present in the allergen database. No potential N-glycosylation sites were identified on the amino acid sequence of the query protein. There are no toxicological in silico findings associated with the PAT/bar protein.

Barstar is expressed only in developing flower buds during pollen development and could not be detected in any other RF3 plant tissues such as leaves and seeds. Therefore the likelihood of any exposure of humans and animals to this protein is remote.

An acute oral toxicity test of Barstar and PAT in mice indicated that the No Observed Effect Level (NOEL) for Barstar and PAT protein is >1000 mg/kg bodyweight and >2000 mg/kg bodyweight, respectively.

The Barstar and PAT proteins used for the toxicological and allergenicity studies were obtained both from Escherichia coli and were found biochemically and functionally equivalent to Barstar and PAT expressed in RF3. A study was then conducted to compare the recombinant Barstar protein batches 1340_Barstar and MVW020911B, both were analyzed using SDS-PAGE and in a Western blot. Results showed that an intense band was observed at the same migration distance at app. 10 kDa. This demonstrates that the apparent molecular weights of both proteins are comparable and correspond to the expected theoretical molecular weight of 10.3 kDa. The PAT/bar protein, purified from RF3

leaves and from *E. coli* was assessed for comparability by SDS-PAGE and Western blot. The results from these experiments indicate similar biochemical properties of the proteins.

Barstar and PAT are expressed independently of each other. The functional activities of these proteins are maintained. Barstar and PAT were driven separately by different promoters.

The barstar gene (ribonuclease inhibitor) has been introduced into oilseed rape, and is used to develop the restorer line of the hybrid system. Upon crossing a male sterile and a restorer line, the fertility of the oilseed rape progeny will be restored because the Barstar protein will inactivate the Barnase enzyme in the male sterile line. Additionally, the bar gene, which is an intrinsic part of the system as it stands for an efficient selection in both the transformation and in the field, is closely linked to the chimeric barstar gene.

The introduction of the bar gene, coding for the enzyme phosphinothricin acetyl transferase (PAT), gives a selective advantage to RF3 oilseed rape plants when they are sprayed with a herbicide containing phosphinothricin as active ingredient. The PAT enzyme acetylates the free NH2 group of PPT, thereby detoxifying the herbicidal component and preventing the death of the plant cell.

Results of the toxicological and allergenicity assessment indicate that Barnase and PAT protein being expressed in Rf3 is not toxic or allergenic to humans.

Nutritional Data

Compositional analysis indicated the nutritional data of RF3 in comparison with the non-transgenic canola, tolerance commercial varieties and range of literature values. Oilseed rape (*Brassica napus)* plants containing the glufosinate tolerant transformation event RF3, its non-GM counterpart and commercial oilseed rape hybrids were field tested by Bayer in 2008. Results of the analysis indicated that there is no differences in the proximate, fiber, mineral, amino acid, fatty acid, tocopherol, anti-nutrient and glucosinolate levels of RF3 canola and the commercial canola varieties that can be considered biologically relevant.

Conclusion

For the single event, low erucic acid rapeseed (canola) RF3, enough evidences were considered to support the substantial equivalence of the genetically modified crop in terms of food safety, with the conventional counterpart other than the fertility restoration trait and tolerance to glufosinate-containing herbicides. After reviewing the provided material of Bayer CropScience, Inc. and other literatures, it is therefore concluded that low erucic acid rapeseed (canola) RF3 is as safe as its conventional counterpart.

BAI ASSESSMENT AND RECOMMENDATIONS

Based on the documents submitted by the applicant, BAI made the following assessment:

A. Host Organism

BAI has stated that the nutrients present in rapeseed are lipids, proteins and fiber. They are found in its two major products of rapeseed, oil and meal. The oil is used in food processing, for home cooking and baking and also forms part in hydrogenated products such as margarine and shortenings. The meal is used for feeding animals.

They also stated that soybean is not a source of toxicant and that no occurrence of allergies yet reported in literatures.

BAI has also concurred with the applicant that oil extracted from oilseed rape is processed into human grade oil used in cooking, baking as well as in salad preparations and cooking oil products, and also in margarine and shortening, both hydrogenated products.

They also stated that according to the Applicant, though consumption data are not available, the use of oilseed rape products are widely known in various age group of people.

Lastly, they agreed that the meal after oil extraction from oilseed rape is used to feed livestock, poultry and fish. Because of its high fiber content, ingredients rich in protein are added to the meal to balance the ration.

B. Transgenic Plant

BAI has agreed with the applicant regarding the list of countries which authorized RF3 oilseed rape for use as food, feed, environment/cultivation was presented in Table 1 below and that consumption patterns are not expected to be changed as a result of the introduction of the novel food.

Country	Authorized For
Australia/New Zealand	**Food, Feed**
Canada	**Environmental/Cultivation, Food, Feed**
China	**Safety Certificate**
European Union	**Food, Feed**
India	**Food**
Japan	**Environmental/Import, Food, Feed**
Korea	**Environmental/Import, Food, Feed**
Mexico	**Food, Feed**
South Africa	**Food, Feed**
Taiwan	**Food**
United States	**Environmental/Cultivation, Food, Feed**

C. Donor Organism

BAI has concurred that protein encoding sequences have been adequately described and that all regulatory sequences have been adequately described.

They also concurred that the bar and barstar genes are the only expressible sequences introduced and that Both PAT and barstar proteins are not known to be toxic or allergenic. Donor organisms *Streptomyces hygroscopicus*, and *Bacillus amyloliquefaciens*)are both not known to be pathogenic, toxic or allergenic.

D. Transformation System

The BAI has agreed with the applicant that the transformation system is *Agrobacterium* mediated transformation system and that genetic modification was targeted to the nuclear DNA of recipient variety.

They also agreed that a brief description of the experimental protocol was given and that the plasmid vector pTHW118 is 12508 bp in size. All required information was provided.

Lastly, they stated that the non-oncogenic helper Ti-plasmid pGV4000 from which the T-region has been deleted carries the vir genes required for the transfer of an artificial T-DNA cloned on the second plasmid to the plant genome.

E. Inserted DNA

BAI has concurred with the applicant that a single insertion site was sufficiently demonstrated by Southern blot and PCR analysis and that Southern blot was performed to confirm the detailed insert characterization previously done on event RF3. Additional hybridization and PCR analysis further confirms the integrity and order of genetic elements within the insertion site.

They also concurred that bioinformatics analysis and similarity searches indicated the presence of an endogenous gene in the 3' flanking sequence region of the RF3 insertion locus. However, the insertion of T-DNA sequences is unlikely to interrupt or alter transcriptional activity of known endogenous Brassica napus genes.

They agreed that there are four (4) approved events expressing the barstar gene in *Brassica* and approximately sixty nine (69) approved events expressing the PAT protein (in 8 species-sugarbeets, oilseed rape, turnip rape, chicory, soybean, cotton, rice and maize) and that the absence of plasmid backbone sequences was sufficiently confirmed by Southern blot and PCR analysis.

F. Genetic Stability

BAI has concluded from the greenhouse and field experiments:

1. The chimeric genes remain structurally stable through meiosis and are transmitted via the seed (Southern blot analysis)
2. The genes are 100% linked and inherited according to Mendelian laws and are expressed s dominant gene
3. Timing, tissue specificity and level of gene expression is preserved during propagation for >3 generations (PAT assays and Northern blots)

They also concluded that segregation pattern was assessed using Northern blot (for expression levels in tissues), PAT assay, glufosinate-ammonium dot or spray assays and phenotypic characteristics. Up to three backcross generations were assessed. Results were in agreement with the reported insert number.

G. Expressed Material

BAI has agreed with the applicant that protein expression levels were qualitatively determined by Western blot. The PAT protein was expressed at higher levels in leaves and only at trace levels in the roots, flowered buds, pollen and dry seeds.

They also agreed that PAT expression is only at trace levels in dry seed and none in flower buds while the Barstar protein is absent in seed and can only be detected in flower buds during anther development and that both novel proteins do not have metabolic roles.

H. Toxicological Assessment

The BAI has agreed with the information provided by the applicant that digestibility study using simulated gastric fluid with pepsin: Pat/bar protein band was no longer visible on the gel at 30 seconds indicating complete digestion as demonstrated by SDS-PAGE followed with Coomassie blue staining of the gel. Western blot analysis also showed that more than 90% of the protein degraded within 30 seconds; and using simulated intestinal fluid with pancreatin: No Pat/bar protein band was observed in SDS-PAGE gel indicating was complete digestion in less than 30 seconds. Western blot results also indicate that more than 90% the protein is degraded in less than 30 seconds.

They also agreed that SDS-PAGE and Western blot demonstrated that the PAT/bar protein was not degraded (no visible changes to the band) at 900C heat treatment for 60 minutes and that homology search in the NCBI non-redundant protein database yielded a high degree of similarity of the Barstar protein with other proteins of its respective ribonuclease family. However, all matches were considered not to be toxicologically relevant. With the Bayer toxin database, no relevant identities were found.

They also concurred with the applicant that 2000 mg/kg body weight NOEL was the result of the acute oral gavage study.In addition, source of test protein *E. coli.* was determined to be equivalent and that molecular weight and immunoreactivity determination as well as theoretical comparison of other characteristics through glycosylation analysis, Mass spec peptide mapping and N-terminal sequencing.

Meanwhile, BAI also agreed with the information provided by the applicant regarding digestibility study using simulated gastric fluid with pepsin:
The Barstar protein was degraded very rapidly with no residual protein visible at 30 seconds of incubation as demonstrated by SDA-PAGE and Western blot;
and using simulate intestinal fluid with pancreatin:
By SDS-PAGE analysis, the Barstar protein band was visible up to the 60-minute incubation time but with reduced intensity. Western blot demonstrated digestion of more than 90% of the protein within 10 minutes of incubation.

They also agreed that both SDS-PAGE and Western blot demonstrated that upon heat treatment at 950C, Barstar produces soluble oligomers suggesting that native Barstar is not stable at this temperature and that homology search in the NCBI non-redundant protein database gave matches corresponding to acetyltransferases from various origins, but overall, these matches were considered not toxicologically relevant. Further, no biologically relevant identities were found in the Bayer toxin database.

In addition, they concurred with the applicant that NOEL is 1000 mg/kg body weight based on the acute oral gavage study and that equivalency of test protein was demonstrated using SDS-PAGE, western blot, glycosylation assessment, peptide mapping and determination of the N-terminal amino acid sequence.

Lastly, the proteins are expressed independently of each other and their respective functional activity is maintained.

I. Allergenicity Assessment

The BAI has agreed with the information provided by the applicant that digestibility study using simulated gastric fluid with pepsin: Pat/bar protein band was no longer visible on the gel at 30 seconds indicating complete digestion as demonstrated by SDS-PAGE followed with Coomassie blue staining of the gel. Western blot analysis also showed that more than 90% of the protein degraded within 30 seconds; and using simulated intestinal fluid with pancreatin: No Pat/bar protein band was

observed in SDS-PAGE gel indicating was complete digestion in less than 30 seconds. Western blot results also indicate that more than 90% the protein is degraded in less than 30 seconds.

They also agreed that SDS-PAGE and Western blot demonstrated that the PAT/bar protein was not degraded (no visible changes to the band) at 900C heat treatment for 60 minutes and that homology search in the NCBI non-redundant protein database yielded a high degree of similarity of the Barstar protein with other proteins of its respective ribonuclease family. However, all matches were considered not to be toxicologically relevant. With the Bayer toxin database, no relevant identities were found.

In addition, they concurred with the applicant that PAT protein has no potential N-glycosylation site and its molecular weight is 21 kDa.The total PAT protein percentage in food is estimated to be less than 0.002%.

On the other hand, BAI also agreed with the information provided by the applicant regarding digestibility study using simulated gastric fluid with pepsin: Barstar protein was degraded very rapidly with no residual protein visible at 30 seconds of incubation as demonstrated by SDA-PAGE and Western blot;
and using simulate intestinal fluid with pancreatin: By SDS-PAGE analysis, the Barstar protein band was visible up to the 60-minute incubation time but with reduced intensity. Western blot demonstrated digestion of more than 90% of the protein within 10 minutes of incubation.

They also agreed that both SDS-PAGE and Western blot demonstrated that upon heat treatment at 950C, Barstar produces soluble oligomers suggesting that native Barstar is not stable at this temperature and that homology search in the NCBI non-redundant protein database gave matches corresponding to acetyltransferases from various origins, but overall, these matches were considered not toxicologically relevant. Further, no biologically relevant identities were found in the Bayer toxin database.

Moreover, they agreed that there are no potential N-glycosylation sites identified with Barstar protein and that it has an approximate MW of 10.3 kDa. Barstar protein is not expressed in any RF3 plant tissues used for food consumption both by humans and animals.

J. Nutritional Data

BAI made the following assessments:
ADF & NDF

Statistical significant difference was detected in crude protein but is negligible since this is within the reference ranges. However, the ADF and NDF of RF3 are higher than the reference ranges, but is nutritionally equivalent to the commercial varieties.

Based on the statistical evaluation, the significant differences detected were negligible and there is no impact on the nutritional value of the *Brassica napus* seeds as a result of genetic modifications.

Minerals &Tocopherols

BAI confirmed that statistically significant differences were detected in some components (phosphorus, magnesium, copper, zinc, alpha-tocopherol) but in the overall analysis it is negligible because the respective mean values are almost all inside the reference ranges calculated from the tested commercial *Brassica napus* hybrids. With the exception on alpha-tocopherol, yet the differences between mean values is very small. Thus, the male *Brassica napus* plants of the GM event RF3 are nutritionally equivalent to their non-GM counterpart and to commercial Brassica napus hybrids currently on the market.

Amino Acids

BAI agreed that statistically significant differences detected for glutamic acid, methionine and proline is negligible since the respective mean values are almost all inside the references ranges calculated from the commercial *Brassica napus* hybrids and the differences between mean values are smaller than the standard deviation calculated from the results obtained for the non-GM entry. Cysteine, glutamic acid and proline were nonessential amino acids and can be synthesized in the body so differences were acceptable since these will not needed to be supplied as dietary protein. Thus, the male *Brassica napus* plants of the GM event RF3 are nutritionally equivalent to their non-GM counterpart and to commercial *Brassica napus* hybrids currently on the market.

Fatty Acids

A slight minor finding for oleic acid (C18:1) was detected but it is still negligible. In the overall analysis, the mean values for RF3 and its non-GM comparator are within the reference ranges calculated for the four commercial hybrids. Thus, the male *Brassica napus* plants of the GM event RF3 are nutritionally equivalent to their non-GM counterpart and to commercial *Brassica napus* hybrids currently on the market.

Although significant differences were detected for some components (potassium, sodium, copper, zinc, alpha-tocopherol, glutamic acid, methionine, proline, oleic acid) it is still negligible because respective mean values are almost all inside the reference ranges calculated from the commercial *Brassica napus* hybrids and differences between mean values are very small.Thus, the male *Brassica napus* plants of the GM event RF3 are nutritionally equivalent to their non-GM counterpart and to commercial *Brassica napus* hybrids currently on the market

Glucosinolates

Significant differences were detected for Aklenyl GSL, Total GSL and Indole GSL but these are very small and in overall site analysis, it is still negligible because respective mean values are almost all within the reference ranges calculated for the tested commercial Brassica napus hybrids.

The male *Brassica napus* plants of the GM event RF3 are nutritionally equivalent to their non-GM counterpart and to commercial *Brassica napus* hybrids currently on the market.

K. Recommendation

After a thorough and scientific review and evaluation of the documents provided relevant to the transformation event, BAI concluded that the regulated article applied for is safe for food, feed or for processing.

DENR ASSESSMENT AND RECOMMENDATION

After thorough and scientific reviews and evaluation of the document provided by the Bureau of Plant Industry (BPI) to the DENR Biosafety Committee within the prescribed period pursuant to Joint Department Circular (JDC) No. 1 s2016 on the application of Bayer CropScience, Inc., for direct use for feed, food or processing of Genetically Modified Oilseed rape tolerant to glufosinate-ammonium herbicide single trait product RF3, the following are the observations and recommendations:

1. The effect of the regulated article on the environment depends largely on the viability of the product to be utilized for direct use. If the article is transported in a non-viable form, there is no danger to the environment;

2. Due to the absence of a specified Environmental Management Plan (EMP) by the trade/importers, the Committee would like to recommend that it be added to the requirements for the issuance of an import permit by the Bureau of Plant Industry (Section 26 of JDC No. 1 s2016);
3. It is suggested that the BPI ensure the following:
    a) Development of guidelines on the EMP in coordination with DENR;
    b) Implementation of the EMP by the traders/importers involved in the import, handling, processing and transport of viable oilseed rape RF3 commodity products; and
    c) Strict monitoring of the regulated article from the port of entry to the traders/importers storage/warehouse (Sec 23 of JDC 1 s2016).

Based on the above considerations and with the submitted sworn statement and accountability of the proponent, a biosafety permit may be issued to the proponent if the abovementioned recommendations are followed.

DOH ASSESSMENT AND RECOMMENDATION

After a thorough review and evaluation of the documents provided by the proponent, Bayer CropScience 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 Oilseed Rape RF3. 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. Find that the regulated article applied for Direct Use for FFP does not require changes in the usual practices in unloading and loading, hauling, transport and storage, and processing. 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 and the environment while in transit, storage and processing.
2. Scientific pieces of evidence from provided references i.e. literatures show that the regulated article applied for Direct Use as FFP is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health and on the environment.
3. It is suggested that the BPI ensure the following:
    a) Strict monitoring of the regulated article from port of entry to the traders/importers storage/warehouse as stated in Sec 32 of JDC 1 s2016
    b) The BPI to include in the issuance of permit for release of this product the following conditions:
        i. Any spillage (during unloading and loading/hauling and transport unloading and storage) shall be collected and cleaned up immediately.
        ii. Transportation of the consignment from the port of entry to any destination shall be in closed containers.
        iii. There shall be a clear instructions that the product is only for the purpose of direct use for FFP and is not to be used as planting materials.
4. 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 Oilseed rape RF3.

SEC ASSESSMENT AND RECOMMENDATIONS

The SEC expert has stated that the application for biosafety permit is only for direct use as food and feed, or for processing of fertility restorer and herbicide tolerant RF3 oilseed rape. The applicant does not intend to produce RF3 oilseed rape in the country. As such, significance in terms of production is immaterial to this application.

The expert added that annual data from the Philippine Statistics Authority's (PSA) Foreign Trade Statistics do not reflect figures specific RF3 oilseed rape. Rather, available data pertain to aggregate imports of: (a) rape, colza or mustard oil and fractions thereof, whether or not refined but not chemically modified;2and (2) oil-cake and other solid residues, whether or not ground or in the form of pellets resulting from the extraction of vegetable fats or oils from rape or colza seeds.

The expert also stated that changes in the patterns of production are not expected since the application is only for direct use as food and feed, or for processing of fertility restorer and herbicide tolerant RF3 oilseed rape, and also because rapeseed is not grown in the Philippines due to tropical climate conditions.

Patterns in utilization as food and feed, and for processing as well as patterns in trade are not expected to be drastically changed by the issuance of the biosafety permit. Based on Risk Assessment Form submitted by the applicant and a scientific opinion adopted on 15 October 2017 by the European Food and Safety Authority (EFSA), there is "no evidence ... for new hazards, modified exposure of scientific uncertainties that would change the conclusions of the original risk assessment on oilseed rape MS8, RF3 and MS8xRF3.

Therefore, the expert recommends for the approval and issuance of biosafety permit of the said GM product.

ANNEX A

Table 9: Proximate and fiber compounds in seeds from RF3 and non-GM counterpart plants, ranges from commercial canola hybrids and reference ranges from literature

Component	Non-transgenic conv. treated (Entry 14)	RF3 Glufosinate treated (Entry 13)	Range from Canola Hybrids C, D, E and F (Entries 3, 8, 9, 10) [b]	Reference ranges [c]
	Mean ± SD	Mean ± SD		
Moisture % fw	5.72 ± 0.28	5.58 ± 0.32	4.97 – 6.51	5.4 – 17.0
Crude fat % dm	43.1 ± 2.2	43.5 ± 2.6	36.6 – 51.2	24.0 – 52.6
Crude protein % dm	26.1 ± 2.2	25.1 ± 2.8	21.3 – 31.1	13.6 – 32.7
Ash % dm	3.79 ± 0.22	3.81 ± 0.22	3.45 – 8.79	4.1 – 5.0
Total carb.% dm [a]	27.0 ± 1.6	27.5 ± 2.1	22.5 – 34.7	25.4 – 53.2
ADF % dm	34.8 ± 3.2	35.6 ± 2.5	26.6 – 43.8	10.0 – 10.8
NDF % dm	38.4 ± 3.5	39.3 ± 2.1	31.0 – 46.2	16.0 – 18.5

Table 10: Minerals and tocopherols in seeds from RF3 and non-GM counterpart plants, ranges from commercial canola hybrids and reference ranges from literature

Micro-nutrient	Non-transgenic conv. treated (Entry 14)	RF3 Glufosinate treated (Entry 13)	Range from Canola Hybrids C, D, E and F (Entries 3, 8, 9, 10) [b]	Reference ranges [d]
	Mean ± SD	Mean ± SD		
Calcium (%)	0.412 ± 0.025	0.412 ± 0.022	0.31 – 0.54	0.29 – 0.48
Phosphorus (%)	0.648 ± 0.052	0.622 ± 0.053	0.53 – 0.81	0.48 – 0.85
Potassium (%)	0.689 ± 0.058	0.677 ± 0.046	0.50 – 0.88	0.83 – 0.91
Magnesium (%)	0.304 ± 0.017	0.294 ± 0.013	0.29 – 0.36	0.29 – 0.31
Sodium (%) [a]	< 0.0100 – 0.0154	< 0.0100 – 0.0107	< 0.01 – 0.0308	0.05
Iron (mg/kg)	61.0 ± 3.8	61.7 ± 11.9	49.7 – 249 [c]	ND
Manganese (mg/kg)	42.7 ± 2.3	41.8 ± 1.3	33.2 – 66.6	ND
Copper(mg/kg)	3.58 ± 0.27	3.33 ± 0.22	2.60 – 3.87	7.0
Zinc (mg/kg)	41.4 ± 2.4	40.1 ± 2.9	32.8 – 58.8	62
alpha Toc. (mg/kg)	73.3 ± 10.6	68.9 ± 9.5	74.4 – 138	24 – 203
beta Toc. (mg/kg)	< 5.00	< 5.00	< 5.00	0 – 74
gamma Toc.(mg/kg)	245.2 ± 27.4	237.5 ± 26.7	146 – 250	45 – 396
delta Toc. (mg/kg) [a]	< 5.00 – 8.81	< 5.00 – 9.48	< 5.00 – 10.5	0 – 12
Total Toc. (mg/kg)	324.5 ± 38.4	312.4 ± 37.4	226 – 391	94 – 1410

Table 11: Total amino acids in seeds from RF3 and non-GM counterpart plants, ranges from commercial canola hybrids and reference ranges from literature

Total Amino Acid (% dm)	Non-transgenic conv. treated (Entry 14)	RF3 Glufosinate treated (Entry 13)	Range from Canola Hybrids C, D, E and F (Entries 3, 8, 9, 10) [a]	Reference ranges [b]
	Mean ± SD	Mean ± SD		
Alanine	1.14 ± 0.10	1.12 ± 0.11	0.95 – 1.33	0.93 – 0.96
Arginine	1.78 ± 0.18	1.75 ± 0.21	1.45 – 2.15	1.13 – 1.21
Aspartic acid	1.83 ± 0.19	1.80 ± 0.22	1.42 – 2.24	ND
Cystine	0.68 ± 0.06	0.66 ± 0.07	0.57 – 0.79	0.52 – 0.54
Glutamic acid	4.86 ± 0.49	4.73 ± 0.54	3.97 – 5.75	ND
Glycine	1.32 ± 0.12	1.30 ± 0.14	1.09 – 1.57	1.04 – 1.06
Histidine	0.72 ± 0.06	0.71 ± 0.07	0.62 – 0.85	0.51 – 0.66
Isoleucine	1.08 ± 0.11	1.06 ± 0.12	0.89 – 1.29	0.80 – 0.86
Leucine	1.85 ± 0.18	1.82 ± 0.21	1.53 – 2.19	1.35 – 1.47
Lysine	1.60 ± 0.11	1.56 ± 0.14	1.37 – 1.80	1.03 – 1.19
Methionine	0.54 ± 0.04	0.52 ± 0.06	0.41 – 0.61	0.42 – 0.44
Phenylalanine	1.07 ± 0.10	1.06 ± 0.11	0.89 – 1.27	0.75 – 0.82
Proline	1.63 ± 0.17	1.57 ± 0.19	1.32 – 1.93	1.19 – 1.33
Serine	1.09 ± 0.09	1.07 ± 0.10	0.90 – 1.27	0.90 – 0.94
Threonine	1.09 ± 0.09	1.08 ± 0.10	0.93 – 1.26	0.87 – 0.94
Tryptophan	0.27 ± 0.03	0.27 ± 0.04	0.20 – 0.35	0.23 – 0.27
Tyrosine	0.73 ± 0.06	0.72 ± 0.07	0.61 – 0.87	0.51 – 0.59
Valine	1.37 ± 0.13	1.34 ± 0.15	1.12 – 1.60	1.02 – 1.13

Table 12: Total fatty acids in seeds from RF3 and non-GM counterpart plants, ranges from commercial canola hybrids and reference ranges from literature

Total Fatty Acid (% Relative)	Non-transgenic conv. treated (Entry 14)	RF3 Glufosinate treated (Entry 13)	Range from Canola Hybrids C, D, E and F (Entries 3, 8, 9, 10) [b]	Reference ranges [c]
	Mean ± SD	Mean ± SD		
Myristic C14:0	0.06 ± 0	0.06 ± 0	< 0.01 – 0.06	0 – 0.2
Palmitic C16:0	4.37 ± 0.14	4.35 ± 0.11	3.88 – 4.94	2.5 – 7.0
Stearic C18:0	1.96 ± 0.21	1.95 ± 0.24	1.58 – 2.59	0.8 – 3.0
Arachidic C20:0	0.64 ± 0.04	0.64 ± 0.04	0.55 – 0.85	0.2 – 1.9
Behenic C22:0	0.33 ± 0.02	0.32 ± 0.02	0.24 – 0.44	0 – 0.6
Lignoceric C24:0	0.18 ± 0.03	0.19 ± 0.02	0.13 – 0.31	0 – 0.76
Sum Saturated	7.54	7.51	6.38 – 9.19	3.5 – 13.46
Palmitoleic C16:1	0.26 ± 0.01	0.26 ± 0.01	0.19 – 0.39	< 0.05 – 0.6
Oleic C18:1	61.00 ± 1.39	60.97 ± 1.74	61.28 – 66.46	50.1 – 70.0
Gadoleic C20:1	1.35 ± 0.06	1.35 ± 0.07	1.09 – 1.51	0.1 – 10.9
Erucic C22:1 [a]	< 0.01 – 0.03	< 0.01 – 0.03	< 0.01 – 0.11	0 – 2.0
Nervonic C24:1	0.15 ± 0.03	0.17 ± 0.04	0.12 – 0.27	0 – 0.4
Sum mono-unsaturated	62.76	62.75	62.68 – 68.73	50.2 – 83.9
Linoleic C18:2	18.17 ± 0.55	18.34 ± 0.63	15.89 – 19.37	15.0 – 30.0
Linolenic C18:3	10.92 ± 1.16	10.81 ± 1.34	7.27 – 11.13	5.0 – 14.8
Eicosadienoic C20:2	0.07 ± 0.01	0.07 ± 0.01	< 0.01 – 0.07	0 – 0.1
Sum poly-unsaturated	29.16	29.22	23.16 – 30.57	20.0 – 44.9
Sum of total fatty acids	99.46	99.48	-	-

Table 13: Anti-nutrients in seeds from RF3 and non-GM counterpart plants, ranges from commercial canola hybrids and reference ranges from literature

Anti-nutrient	Non-transgenic conv. treated (Entry 14)	RF3 Glufosinate treated (Entry 13)	Range from Canola Hybrids C, D, E and F (Entries 3, 8, 9, 10) [b]	Reference range
	Mean ± SD	Mean ± SD		
Phytic acid (% dm)	1.81 ± 0.24	1.73 ± 0.20	1.28 – 2.32	0.48 – 2.63
Alkenyl GSL (µmol/g)	4.61 ± 1.23	3.98 ± 1.31	2.93 – 14.56	ND
MSGL GSL (µmol/g) [a]	< 0.05 – 0.16	< 0.05 – 0.35	< 0.05 – 0.41	ND
Indole GSL (µmol/g)	4.75 ± 0.77	5.02 ± 0.66	1.13 – 6.99	ND
Total GSL (µmol/g)	9.43 ± 1.57	9.06 ± 1.60	6.86 – 20.55	3.8 – 33.9 [c]