BASF Petition (17-321-01p) for Determination of Non-regulated Status of DHA+EPA Canola LBFLFK.

OECD Unique Identifier: BPS-BFLFK-2

Draft Plant Pest Risk Assessment

February 2019

Agency Contact
Cindy Eck
Biotechnology Regulatory Services
4700 River Road
USDA, APHIS
Riverdale, MD 20737
Fax: (301) 734-8669

TABLE OF CONTENTS

A. Introduction

BASF Plant Sciences, L.P. (hereafter referred to as BASF) has petitioned the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) for a determination that the genetically engineered (GE) EPA+DHA canola (*Brassica napus*), which also has imidazolinone herbicide-resistance[1], event LBFLFK (hereafter referred to as LBFLKF canola or LBFLFK where appropriate) is unlikely to pose a plant pest risk and therefore should no longer be a regulated article under the APHIS' 7 Code of Federal Regulations (CFR) part 340 (Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There Is Reason To Believe Are Plant Pests). This petition was assigned the number 17-321-01p and is hereafter referenced as BASF 2017. This GE event has the OECD unique identifier BPS-BFLFK-2. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act (PPA) of 2000 (7 U.S.C. 7701 *et seq.*)[2]. This plant pest risk assessment (PPRA) was conducted to determine if LBFLFK canola is unlikely to pose a plant pest risk.

APHIS regulations in 7 CFR part 340 regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the plant pest provisions of the Plant Protection Act (PPA) or to the regulatory requirements of 7 CFR part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under 7 CFR part 340 if the donor organism, recipient organism, or vector agent used in engineering the organism belongs to any genera or taxa designated in 7 CFR 340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest[3].

[1] BASF has also described the phenotype of LBFLFK canola as "herbicide tolerant" and historically APHIS has also referred to GE plants with reduced herbicide sensitivity as herbicide tolerant. However, the phenotype would fall under the Weed Science Society of America (WSSA) definition of "herbicide resistance" since LBFLFK canola has an "inherited ability to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type" (WSSA 1998). By the WSSA definition, "resistance (to an herbicide) may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis." Herbicide tolerance, by the WSSA definition, only applies to plant species with an "inherent ability to survive and reproduce after herbicide treatment. This implies that there was no selection or genetic manipulation to make the plant tolerant; it is naturally tolerant."

[2] Plant Protection Act in 7 U.S.C. 7702 §403(14) defines plant pest as: "Plant Pest - The term "plant pest" means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs."

[3] Limited exclusions or exemptions apply for certain engineered microorganisms and for interstate movement of some organisms, as in 7 CFR 340.1 and 340.2(b).

LBFLFK canola was produced by *Agrobacterium rhizogenes*-mediated transformation of *Brassica napus* cv. Kumily hypocotyl segments with a single plasmid transformation vector LTM593 containing genes for fatty acid biosynthesis (desaturases and elongases) and resistance to an herbicide (BASF 2017, pp. 32-39). *A. rhizogenes* is a plant pest and portions of the introduced genetic material were derived from plant pest organisms listed in 7 CFR 340.2. The non-coding regions are T-DNA border regions from *A. tumefaciens* octopine type T1 plasmid and the terminator of the octopine synthase gene; and the terminator region from *Cauliflower mosaic virus*, CaMV35S. Coding regions from plant pests are the codon optimized coding regions: for delta-12 desaturase from *Phytophthora sojae*; two omega-3 desaturases from *Pythium irregulare*; and omega-3 desaturase from *P. infestans* (BASF 2017, Table 3, pp 34-39). Therefore, LBFLFK canola is considered a regulated article under APHIS regulations at 7 CFR part 340. BASF has conducted field trials in the U.S. of LBFLFK canola as a regulated article under APHIS authorizations since 2014 (BASF 2017, Appendix A, Table A.1, p. 205), in part, to collect information to support that LBFLFK canola is unlikely to pose a plant pest risk.

LBFLFK canola has also been genetically engineered for resistance to imidazolinone herbicides through the introduction of a modified acetohydroxy acid synthase (AHAS) gene from *Arabidopsis thaliana* (BASF 2017). Imidazolinone herbicides control weeds by inhibiting the enzyme acetohydroxyacid synthase formally known as acetolactate synthase (ALS), the first common enzyme in the biosynthetic pathway of the branched-chain amino acids (BCAAs) valine, leucine and isoleucine (McCourt and Duggleby 2006).

Potential impacts discussed in this plant pest risk assessment are those that pertain to plant pest risk associated with LBFLFK canola and its progeny, and their use in the absence of confinement relative to the unmodified recipient and/or other appropriate comparators. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if LBFLFK canola is unlikely to pose a plant pest risk. APHIS regulations in 7 CFR 340.6(c) specify the information needed for consideration in a petition for nonregulated status. APHIS will assess information submitted by the applicant about LBFLFK canola related to: plant pest risk characteristics; expression of the gene product, new enzymes, or changes to plant metabolism; disease and pest susceptibilities and indirect plant pest effects on other agricultural products; effects of the regulated article on nontarget organisms; weediness of the regulated article; impact on the weediness of any other plant with which it can interbreed; changes to agricultural or cultivation practices that may impact diseases and pests of plants; and transfer of genetic information to organisms with which it cannot interbreed.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the Coordinated Framework for the Regulation of Biotechnology (51 FR 23302 1986; 57 FR 22984 1992). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with APHIS, the Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA). Depending on its characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies.

Under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. §136 *et seq*), EPA regulates the distribution, sale, use and testing of pesticidal substances produced in plants and microbes, including those pesticides produced by an organism using techniques of modern biotechnology. EPA also sets tolerance limits for residues of pesticides on and in food and animal feed, or establishes an exemption from the requirement for a tolerance, under the Federal Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C §301 *et seq.*). Prior to registration for a new use for a new or previously registered pesticide, EPA must determine through testing that the pesticide does not cause unreasonable adverse effects on humans, the environment, and non-target species when used in accordance with label instructions. EPA must also approve the language used on the pesticide label in accordance with Data Requirements for Pesticides (40 CFR part 158). Other applicable EPA regulations include Pesticide Registration and Classification Procedures (40 CFR part 152), Experimental Use Permits (40 CFR part 172) and Procedures and Requirements for Plant Incorporated Protectants (PIPs) (40 CF.R. part 174).

The FDA under the FFDCA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those developed through modern biotechnology. To help sponsors of foods and feeds derived from genetically engineered crops comply with their obligations, the FDA encourages them to participate in its voluntary early food safety evaluation for new non-pesticidal proteins produced by new plant varieties intended to be used as food (US-FDA 2006) and a more comprehensive voluntary consultation process prior to commercial distribution of food or feed (57 FR 22984 1992). BASF has initiated a BNF consultation process with FDA with submission of our food/feed safety data package earlier in 2018, and the FDA has provided the designation BNF 165.

B. Development of LBFLFK Canola

The petition Number 17-321-01p submitted by BASF (2017) and this PPRA refer to *B. napu*s EPA+DHA canola event LBFLFK. The parental canola variety used for the introduction of the EPA+DHA and AHAS herbicide resistance traits was Kumily, a spring cultivar of *B. napus* L. (BASF 2017).

B. napus is an amphidiploid species of relatively recent origin and thought to have first emerged in the Mediterranean coastal region, where both its diploid progenitor species, *B. rapa* and *B. oleracea*, are found (OECD 2011; OGTR 2017). Cultivated canola can be any one of three Brassica species (*B. napus*, *B. rapa* or *B. juncea*) that meet an internationally regulated standard whereby "*seeds of the genus Brassica (B. napus. B. rapa or B. juncea) from which the oil shall contain less than 2% erucic acid in its fatty acid profile and the solid component shall contain less than 30 micromoles of any one or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3 butenyl glucosinolate, and 2-hydroxy- 4-pentenyl glucosinolate per gram of air-dry, oil-free solid* (Canola Council of Canada 2017)."

The term 'canola' is derived from ***Can****adian* ***o****il*, ***l****ow* ***a****cid*, a trademark of the Canola Council of Canada (OGTR 2017). Canola cultivars of *B. napus* were developed using traditional plant breeding techniques at Agriculture and Agri-Food Canada and the University of Manitoba in the 1970's (Canola Council of Canada 2017).

Worldwide canola is the third most important edible vegetable oil crop after soybean and palm oil. Canola is grown primarily as an oilseed crop, mainly produced for its seeds that have an oil content of 35 to 45%, and the oil is mainly used for cooking and in food products such as margarine. Canola meal is a by-product of oil extraction and is used widely as a high protein animal feed (OECD 2011; OGTR 2017). In the United States and Canada the majority of cultivated *B. napus* is made up of canola quality varieties. In 1985, the FDA granted GRAS status to canola oil, and canola production rapidly increased (Brown et al. 2008).

B. napus canola is widely adapted to temperate climates and production globally is in areas with dry weather and shorter growing seasons. Some cultivars are grown as annual (spring) and others as biennial (winter) crops, the main difference between them is that winter cultivars require vernalization to induce flowering and bolting (Brown et al. 2008). Spring canola is planted in early spring and harvested in late summer, whereas winter canola is planted in the fall for vernalization in winter and harvested in the next year. However, winter canola produced in the southeast region of the U.S. is planted in the fall and does not require vernalization (Brown et al. 2008). Spring canola is grown in most of Canada and in the United States, it is grown mainly in the northern states. Winter canola is grown in the Pacific Northwest, the Great Plains and Midwest regions of the U.S. (Brown et al. 2008).

The highest annual canola production occurs in the European Union, China, Canada, and Australia (OGTR 2017). In the United States, approximately two million acres were planted with canola in 2017 with yield of 1,558 lb/acre, and a similar acreage was planted in 2018. Eighty percent of canola production is in North Dakota, followed by smaller amounts in Oklahoma, Montana, Washington, Minnesota, Kansas, Idaho and Oregon respectively (USDA-ERS 2012; USDA-NASS 2017).

The petition states that LBFLFK canola will be cultivated in the United States and processed into oil and defatted meal fractions, similarly to other canola varieties, either in the United States or Canada as specialty canola varieties. Activities to support variety development, grain production, oil manufacturing, and other commercial activities to prepare EPA+DHA canola event LBFLFK for the marketplace as an alternative source of long-chain polyunsaturated omega-3 fatty acids will be further conducted under an Identity Preservation Program (IPD) system (BASF 2017, p.177-179). The IDP system is intended to maintain product quality and ensure the segregation of seeds, grains and processed products (BASF 2017, p.23).

The conventional *B. napus* canola spring variety Kumily was used as the parental variety for LBFLFK canola that was genetically engineered to contain several genes encoding fatty acid desaturase and elongase proteins to allow for the synthesis of long-chain

polyunsaturated fatty acids (LC-PUFA) from oleic acid. In addition, a modified coding sequence for the large subunit gene of acetohydroxy acid synthase (AHAS) protein was added to confer resistance to an imidazolinone herbicide (imazamox), to allow for selective post-emergence weed control during field production.

According to the petition, the resulting LBFLFK canola is intended to provide a plant-based and scalable production system for omega-3 fatty acids. LBFLFK canola oil is intended as a specialty canola oil with a fatty acid profile containing the long-chain poly unsaturated fatty acids (LC-PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), to provide dietary omega-3 LC-PUFAs. The oil can be incorporated as an ingredient in consumer food items, or used as refined oil in dietary supplements as an alternate source of omega-3 LC PUFAs. It is also intended as an aquaculture feed ingredient to provide omega-3 LC-PUFAs to farmed aquatic species.

In the evaluations of LBFLFK canola described in the petition, LBFLFK was compared to the parental control variety Kumily and to six conventional commercially available reference canola varieties representing a wide range of genetic backgrounds: Q2, 46A65, IMC105, IMC302, Wizzard, and Orinoco (BASF 2017, p.92, Table 12).

Agronomic, phenotypic performance and environmental interaction data for LBFLFK canola was assessed at 14 trial locations covering a range of canola growing regions over two seasons (winter and spring). Six field trial locations in the southern U.S. were used in the winter season. For the spring season trials eight locations in northern U.S. were used. Winter trials were sowed in the fall of 2014 and harvested in spring 2015, and spring trials were sowed in the spring of 2015 and harvested in summer 2015 (BASF 2017, p.93, Figure 9, Table 13, p.97 - 98 Table 14, p.100 Table 15, p.101 Table 16, p.104 Table 17, p.105 Table 18, p.110 Table 19).

For compositional analyses studies canola grain harvested from field-grown plots was used, and included plots of LBFLFK sprayed and not sprayed with Beyond® herbicide, Kumily, and the six conventional reference varieties (BASF 2017, p.119 Table 25 and p.121 Table 26).

For studies of pollen germination, morphology and viability, pollen of LBFLFK was compared to Kumily and three conventional canola reference varieties (46A65, IMC302 and Wizzard) (BASF 2017, p.112 Table 20).

The rationale for the development of LBFLFK canola, as described in the petition, is that in many countries, including the U.S., adult intake of omega-3 LC-PUFA EPA and DHA falls below the recommended average for daily intake from numerous health organizations (BASF 2017). Suggested intakes of LC-PUFA EPA and DHA for adults vary by organization and health objective from 300 mg to 4,000mg (Nichols et al. 2010). The supply of fatty acids from several sources including marine animals is limited and there is a significant challenge in production and distribution in adequate quantity of products containing these fatty acids. Additionally, there is an unmet high demand for fish oil as an ingredient for farmed fish (BASF 2017). Marine microalgae are the primary

producers of LC omega-3 oils which is passed through the food chain to marine fish (Nichols et al. 2010).

Based on canola biology (OECD 2012; OGTR 2017) and data presented in the petition (BASF 2017) relevant to the development of EPA+DHA LBFLFK canola, APHIS concludes that EPA+DHA LBFLFK canola was developed in a manner common to other GE crops using *Agrobacterium*-mediated transformation (USDA-APHIS-BRS 2018). APHIS believes that the use of the non GE parental line Kumily and other reference varieties as comparators is sufficient to determine that LBFLFK canola dos not pose a greater plant pest risk compared to its comparators. (USDA-APHIS-BRS 2018).

C. Description of Inserted Genetic Material, Its Inheritance and Expression, Gene Products, and Changes to Plant Metabolism

To inform the potential hazards resulting from the genetic modification and potential routes of exposure related to the inserted DNA and its expression products, APHIS assessed data and information presented in the petition related to: the transformation process; the source of the inserted genetic material and its function in both the donor organism and the GE crop event; and the integrity, stability and mode of inheritance of the inserted genetic material through sexual or asexual reproduction based on the location of the insertion (e.g. nucleus or organelle) and the number of loci inserted.

APHIS also assessed data presented in the petition on whether the genetic modification results in expression of new genes, proteins, or enzymes or changes in plant metabolism or composition in LBFLFK canola compared to the nontransgenic canola parental variety and six conventional reference varieties. The assessment encompasses a consideration of the eleven expressed proteins, ten of which are integral membrane proteins, seven desaturases and three elongases. The eleventh protein is the soluble, chloroplast-located larger subunit of acetohydroxy acid synthase from *Arabidopsis thaliana*, containing two amino acid substitutions (BASF 2017).

The assessment also encompasses any observed or anticipated effects on plant metabolism including, for example, any relevant changes in levels of metabolites, antinutrients, or nutrients in harvested grain or forage derived from LBFLFK canola compared to those in the conventional controls.

This information is used later in this risk assessment to inform whether there is any potential for plant pest vectors or sequences to cause disease or greater plant pest risks in the GE crop event; or for expression of inserted DNA, new proteins or enzymes, or changes in metabolism to affect plant pest or diseases, nontarget beneficial organisms, weediness, agricultural practices that impact pest or diseases or their management, or plant pest risks through horizontal gene flow.

Description of the genetic modification and inheritance of inserted DNA

As described in the petition (BASF 2017, p.30), EPA+DHA canola LBFLFK was generated via *Agrobacterium rhizogenes*-mediated transformation of *B. napus* cv. Kumily

to introduce genes for the biosynthesis pathway of EPA and DHA from oleic acid and for resistance to herbicide following a modified De Block protocol (De Block et al. 1989; BASF 2017). Hypocotyl segments from Kumily seedlings were inoculated with disarmed *A. rhizogenes* strain SHA001 containing the plasmid vector LTM593 (BASF 2017, p.33 Figure 3, p.34-39 Table 3). Explants were transferred after three days to plant growth medium containing carbenicillin (to prevent growth of *A. rhizogenes*) for seven days, and then transferred to selection medium containing the imidazolinone herbicide imazethapyr. Transgenic plants, T_0 plants and T_1 and T_2 generations produced by selfing were characterized by molecular analyses, fatty acid profiles, agronomic evaluations, and herbicide efficacy analyses. Plants with normal phenotypic characteristics and free of vector backbone, producing higher levels of EPA and DHA and tolerant to imidazolinone were advanced, event LBFLFK was selected and evaluated further (BASF 2017, p.31 Figure 2).

The plasmid vector LTM593 used for canola transformation carries 13 expression cassettes, an expression cassette for a mutant AHAS(*At*) protein and 12 fatty acid synthesis cassettes encoding ten integral membrane proteins desaturases and elongases. Coding sequences for a delta-5 desaturase from *Thraustochytrium s*p. (c*D5D*(*Tc*)) and for an omega-3 desaturase from *Pythium irregulare* (c*O3D*(*Pir*)) are present in two different expression cassettes. The LTM593 vector also carries sequences encoding a modified acetohydroxy acid synthase (AHAS(*At*)) (BASF 2017, p.32, p.33 Figure 3, and p.34-39 Table 3). Coding sequences inserted into LBFLFK canola (BASF 2017, p. 34-39, Table 3.) are listed below:

- c*D6E*(*Pp*) for a delta-6 elongase from *Physcomitrella patens*
- c*D5D*(*Tc*), two copies of the coding sequence for a delta-5 desaturase from *Thraustochytrium sp*., c*D5D*(*Tc*)1 and c*D5D*(*Tc*)2
- c*D6D*(*Ot*) for a delta-6 desaturase from *Ostreococcus tauri*
- c*D6E*(*Tp*) for a delta-6 elongase from *Thalassiosira pseudonana*
- c*D12D*(*Ps*) for a delta-12 desaturase from *Phytophthora sojae*
- c*O3D*(*Pir*), two copies of the coding sequence for an omega-3 desaturase from *Pythium irregulare*, c*O3D*(*Pir*)1 and c*O3D*(*Pir*)2
- c*O3D*(*Pi*) for an omega-3 desaturase from *Phytophthora infestans*
- c*D4D*(*Tc*) for a delta-4 desaturase from *Thraustochytrium* sp.
- c*D4D*(*Pl*) for a delta-4 desaturase from *Pavlova lutheri*
- c*D5E*(*Ot*) for a delta-5 elongase from *Ostreococcus tauri*
- *cAHAS*(*At*) for the acetohydroxy acid synthase from Arabidopsis thaliana,

Sequences encoding the genes for the fatty acid biosynthesis pathway inserted into EPA+DHA canola LBFLFK were codon optimized for expression in *B. napus*. They were also further modified to remove: "(i) additional open reading frames (ORFs) longer than 90 bp in sense and anti-sense direction, (ii) ORFs within 30 bp after the start codon in sense direction, (iii) internal TATA-boxes, chi sequences, and ribosomal entry sites, (iv) AT-rich or GC-rich sequence stretches, (v) RNA instability motifs, (vi) RNA secondary structures and repeat sequences, and (vii) possible cryptic intron splice donor and acceptor sites in higher eukaryotes" (BASF 2017, p.40). The AHAS(*At*) coding

sequence was modified to eliminate unwanted restriction sites, and to contain two mutations resulting in the desired amino acid substitutions (A122T and S653N) to confer herbicide resistance (Tan et al. 2005; BASF 2017, p.32).

A detailed description of the genetic elements in the expression cassettes and references for each element are found in the petition (BASF 2017, Table 3, pp 34-39).

Molecular characterization of the genetic modification in LBFLFK canola was performed using next generation sequencing (NGS), Sanger sequence junction sequence analysis, BAC clones containing either insert 1 or insert 2, polymerase chain reaction (PCR) bioinformatic analysis, and genetic segregation studies (BASF 2017, p.49 Fig 6). For NGS the read breath was 100% and read depth was within that demonstrated to provide comprehensive coverage in NGS (Kovalic et al. 2012). The parental variety Kumily was used as the comparator and six single copy reference genes were used for analysis of read uniformity and to demonstrate that the DNA was sequenced without bias.

DNA was isolated from Kumily and generations T3, T4, and T5 of LBFLFK, and was used in molecular characterization studies for identification of the number of insertion sites based on junction sequence alignments, to determine the copy number of inserted T-DNA sequence, the absence of vector backbone sequences, the integrity of insert sequence and any modifications and stability of the genetic modification over multiple generations (T3, T4 and T5).

Sequencing and bioinformatics analysis were also used to determine the sequence of the T-DNA inserts and flanking genomic regions, and the organization and integrity of the T-DNA inserts. This analysis was also used to identify open reading frames (ORF) within the inserts and at the junctions of the insert and genome and to conduct similarity searches to known protein allergens or toxins.

Segregation analysis was performed on backcrossed progeny populations (generations F2 and F3) to assess the inheritance of the inserts and confirmed that inheritance follows the Mendelian law of independent assortment (BASF 2017, p.49 Fig 6). Sequencing analysis of three generations of LBFLFK showed stable integration of the inserts.

No vector backbone sequences were detected in the genomic DNA of LBFLFK canola using NGS and bioinformatics

Four unique junctions between the inserted T-DNA and canola genome sequences were identified in LBFLFK, indicating two T-DNA insertion sites mapping to different chromosomes and demonstrating that the two inserts are integrated into separate loci, Locus 1 and Locus 2. Each T-DNA insertion site in LBFLFK consists of a single copy of the T-DNA from LTM593 without rearrangements of the introduced gene expression cassettes (BASF 2017, p.53). Another sequence junction was identified with a minor rearrangement of the RB sequences of the LTM593 T-DNA in Insert 1 and no other rearrangements were present in either insert (BASF 2017, p.53).

Both inserts contained all 13 intended gene expression cassettes identical to the T-DNA sequence of the LTM593 vector except for two single nucleotide changes in Insert 1 and one change in insert 2 that have no impact on the function or activity of the proteins. A

short sequence re-arrangement of 64 bp in the RB of Insert 1 was found, otherwise, both inserts were intact. (BASF 2017, p.55, 56 Figure 7).

Insert 1: Coding sequence change in the delta-12 desaturase gene, c-*D12D*(*Ps*), a cytosine to adenine nucleotide change which resulted in a phenylalanine to leucine amino acid substitution (F83L) in the D12D(*Ps*) protein.

Cytosine to adenine nucleotide change in the sequence of the promoter p-*PXR*(*Lu*) found in an expression cassette containing the c-*O3D*(*Pir*) coding sequence. This change does not result in an amino acid substitution.

Insert 2: One coding sequence change in the delta-4 desaturase gene, c-*D4D*(*PI*), a guanine to thymine nucleotide change resulting in an alanine to serine amino acid substitution (A102S) in the D4D(*PI*) protein.

Sequence deletions are common during *Agrobacterium* mediated T-DNA integration (Gheysen et al. 1991), comparisons of sequences of the integration site in LBFLFK and the same site in the parental variety Kumily, demonstrated that small deletions were found at the genome integration sited of Insert 1 (Locus1) and Insert 2 (Locus 2).

A short sequence re-arrangement of 64 bp in the RB of Insert 1 was found, otherwise, both inserts were intact. No other sequence re-arrangements were found at these integration sites.

Junction site analysis showed that eleven ORFs were identified spanning the junctions between the T-DNA inserts and the flanking genomic DNA. None of the ORFs created by the insertion showed significant homology to known allergens, protein toxins, and antinutrients (BASF 2017). Database searches using The Food Allergy Research and Resource Program (FARPP) Allergen Protein Database showed that none of the ORFs had more than 35% identity with a known allergen, over 80 amino acids or a sequence of eight or more consecutive identical amino acids. No significant homology with a known allergen was found (BASF 2017, p.57). BlastP searches were performed to determine the similarity of the ORFs to known toxins and antinutrients using the non-redundant peptide sequence database of the National Center for Biotechnology. None of the ORFs created by the insertion showed significant homology to known protein toxins as defined or showed significant homology to known antinutrients of canola, maize, rice soybean, sugar beet, or sugarcane (BASF 2017, p.58).

Expression of inserted DNA, changes in gene expression, new proteins or metabolism

The genes for expression of the desaturases and elongases in the fatty acid biosynthesis pathway introduced into EPA+DHA LBFLFK canola were inserted in individual expression cassettes, under the control of seed specific promoters and were found to be expressed in seed tissue only, except for two proteins O3D(*Pi*) and D6E(*Pp*) that were not found at detectable levels in seed, nor in any tissue of LBFLFK canola. Expression of the herbicide-tolerant AHAS is controlled by a constitutive promoter, and the protein was found at highest concentrations in green plant tissues and was not detected in mature seeds.

The safety assessment of the newly expressed proteins was performed using a weight of evidence approach to data generated *in silico* and from experimental studies. For the newly expressed elongases and desaturases in canola this approach included the characterization for identity and amino acid sequence, apparent molecular weight, immunoreactivity, assessment of potential glycosylation and enzymatic activity. Protein expression levels were assessed in plant tissues collected from young and flowering plants, root, leaf, pollen, immature and mature seeds. The non-modified Kumily and protein reference substances were used as controls in protein expression and characterization studies.

A history of safe use and consumption was established by performing searches in the literature for each of the proteins, and no adverse findings were identified for the proteins or donor organisms (BASF 2017, p.91). Bioinformatic analysis of the amino acid sequences of the proteins expressed from the coding sequences introduced into LBFLFK canola, showed no significant homology to proteins that are toxic or allergenic to humans, or to known antinutrients (BASF 2017, p.81). The amino acid sequences were compared to the sequences of other elongases and desaturases found in food or feed to show sequence identity to proteins that are already safely consumed. The newly expressed proteins were found to be structurally and functionally related to other elongases and desaturase that are safely consumed by humans as food and by animals as feed (BASF 2017).

Studies of digestibility and heat stability of the newly expressed proteins in LBFLFK were performed as part of the safety assessment and demonstrated the safety of the proteins. Digestibility assays demonstrated that the proteins are subject to digestion and are rapidly degraded in simulated gastric fluid (SGF) and in simulated intestinal fluid (SIF). Two proteins O3D(*Pi*) and D6E(*Pp*) were not found at detectable levels in seeds or in any other tissue of LBFLFK canola. According to the petition this low amount of O3D(*Pi*) and D6E(*Pp*) protein demonstrates that they are unlikely to present any safety concern to humans or animals (BASF 2017, p.78-79).

Coding regions derived from plant pathogens inserted into LBFLFK canola for production of LC-PUFAs code for three proteins: delta-12 desaturase D12D(*Ps)* from *Phytophthora sojae*, an omega-3 desaturase O3D(*Pi*) from *Phytophthora infestans*; and an omega-3 desaturase O3D(*Pir*) from *Pythium irregulare*. The genes do not encode a plant pest or and infectious agent and are not in themselves capable of causing disease. The coding sequence (*cAHAS(At)*) for expression of the acetohydroxy acid synthase (AHAS) was obtained from *Arabidopsis thaliana*, a plant in the Brassicaceae family that is found throughout North America, Europe and Asia, and is a model organism for studies of plant biology. Whole genome sequences of multiple populations collected from several locations in the world are available through the 1001 Genomes Project (Cao et al. 2011; Max Planck Institute for Developmental Biology 2018).

The coding sequence for the large subunit of AHAS (a soluble, chloroplast located protein) was modified to introduce two amino acid substitutions in the expressed AHAS(*At*) to confer to the plant resistance to imidazolinone herbicides. Expression of

the modified AHAS is under the control of a constitutive promoter and AHAS was found at highest concentrations in green plant tissues. The mature AHAS resulting from expression of the introduced gene interacts with the small subunit of the endogenous AHAS enabling feedback regulation of AHAS activity in LBFLFK canola. The amino acid changes reduce the binding activity with imidazolinone herbicide resulting in tolerance to the herbicide (BASF 2017, p.262).

According to the weight of evidence approach all the proteins expressed by the introduced genes in LBFLFK canola are considered to behave as any other dietary proteins and thus do not raise any safety concerns with regard to human or animal health or the environment. None of the inserted genetic sequences or expressed proteins have been reported in the literature to cause plant disease or symptoms of disease on plants, or to have adverse effects on animals or humans.

Compositional Analysis

The composition of grain from EPA+DHA canola LBFLFK and from the parental variety Kumily was compared using mature seed harvested from 12 field trials in the United States from two growing seasons, winter 2014/15 and spring 2015. The compositional analyses were done as part of the food, feed and environmental safety assessment. Samples were analyzed for 112 components including proximates, fibers, amino acids, fatty acids, vitamins, minerals, antinutrients, and phytosterols, based on guidance provided in the consensus document for canola from the Organization for Economic Co-operation and Development (OECD, 2011). Ranges of composition of these components in grain were compared to those presented for conventional reference varieties in peer-reviewed literature and in the ILSI Crop Composition Database.

LBFLFK canola was modified for production of long-chain polyunsaturated fatty acids, including EPA and DHA, and an extended panel of 39 fatty acids was assessed to account for the modified fatty acid metabolic pathway. Changes were observed in the fatty acid profile of LBFLFK canola across both seasons, oleic acid is the primary starting substrate fatty acid for the newly introduced fatty acid synthesis pathway, and content of oleic acid was significantly reduced in LBFLFK canola as compared to the parental control Kumily, whereas content of linolenic acid was increased. A minor increase in total trans fatty acids was observed in LBFLFK canola as compared to the parental control Kumily. The content of several fatty acids including EPA and DHA was higher in LBFLFK canola, as expected due to the introduction of the EPA+DHA trait in LBFLFK. This increase in content of fatty acids is considered to be the expected outcomes of the inclusion of the LC-PUFA biosynthesis trait into the parental variety Kumily.
The concentration of measured fatty acids not associated with the introduced enzymatic pathway were not changed in LBFLFK canola, such as erucic acid, which remains low (BASF 2017, p.134-138, Tables 31-34).

The results of the compositional analyses showed that for any compositional differences observed between LBFLFK canola and Kumily, the values were within the range of the reference varieties, and LBFLFK canola is considered to be compositionally equivalent to commercially available canola varieties (BASF 2017, p.134-138, Tables 31-34) except for

the intended increased levels of omega-3 LC-PUFAs and the associated changes to the levels of precursor and intermediary fatty acids. These changes in fatty acid profile were all expected outcomes of the inclusion of the LC-PUFA biosynthesis trait in EPA+DHA LBFLFK canola (BASF 2017, p.134-138, Tables 31-34), and are not expected to incur any additional plant pest or increased plant disease

D. Potential Plant Pest and Disease Impacts

APHIS assessed whether potential plant pest or disease impacts are likely to result from the transformation process, from DNA sequences from plant pests, or from any other expression products, new enzymes, proteins or changes in plant metabolism or composition in EPA+DHA canola event LBFLFK that are known or anticipated to cause disease symptoms, or to affect plant pests or diseases or plant defense responses (as identified from the previous section). APHIS also assessed whether EPA+DHA canola event LBFLFK is likely to have significantly increased disease and pest susceptibility based on data and observations from field trials and laboratory experiments on specific pest and disease damage or incidence and any agronomic data that might relate to such damage. Impacts or changes are assessed to determine if they would (1) affect the new GE crop and/or result in significant introduction or spread of a damaging pest or disease to other plants; (2) result in the introduction, spread, and/or creation of a new disease; and/or (3) result in a significant exacerbation of a pest or disease for which APHIS has a control program. Any increase in pest or disease susceptibility is evaluated with respect to the context of currently cultivated varieties, the ability to manage the pest or disease, and the potential impact on agriculture.

Plant Protection and Quarantine (PPQ) is an APHIS program that safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests and noxious weeds into the United States of America; and supports trade and exports of U.S. agricultural products. PPQ responds to many new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be emergency or longer term domestic programs that target a specific pest. A variety of insect, plant disease, mollusk, nematode or weed programs exist (USDA-APHIS 2018) however none of these programs specially target pests of EPA+DHA canola LBFLFK.

Canola itself is not considered a plant pest in the United States (7 CFR 340.2). Several sequences inserted into LBFLFK canola are derived from plant pests, noncoding regions from *A. tumefaciens* and *Cauliflower mosaic virus*, and coding regions from *Phytophthora sojae*, *P. infestans* and *Pythium irregulare*.

The left and right T-DNA border regions from the octopine-type Ti plasmid, and the terminator of the octopine synthase gene from *Agrobacterium tumefaciens* are present in the inserted constructs, as is the *Cauliflower mosaic virus*, 35S terminator. These are non-coding sequences that do not cause plant disease.

The T-DNA inserted into LBFLFK canola contained only the intended sequences, along with the typical insertion site mutations, and lacked sequences from Tumor-inducing (Ti)

plasmids normally responsible for the formation of crown gall tumors caused by infection with *A. tumefaciens* (Hoekema et al. 1983; Hellens et al. 2000). Furthermore, following transformation, plant tissues were cultured in medium containing the antibiotic carbenicillin to eliminate *A. tumefaciens* (Nauerby et al. 1997; BASF 2017, p.30).

The coding regions optimized for expression in canola for three desaturases derived from plant pathogens were inserted into LBFLFK canola for production of LC-PUFAs: c*D12D*(*Ps*) for a delta-12 desaturase (D12D(*Ps*)) from *Phytophthora sojae*; c*O3D*(*Pir*) for an omega-3 desaturase (O3D(*Pir*)) from *Pythium irregulare*; and c*O3D*(*Pi*) for an omega-3 desaturase (O3D(*Pi*)) from *Phytophthora infestans*. The inserted coding sequence of O3D(*Pir*) is present in two different expression cassettes for expression of (O3D(*Pir*)1 and O3D(*Pir*)2.

Phytophthora sojae is a plant pathogen primarily causing damping off on seedlings and root rot of older soybean plants (Tyler 2007) and *Phytophthora infestans* is the cause of late blight disease of potatoes and tomatoes. *Pythium irregulare* causes Pythium root rot and seedling damping off on Brassica species and on other species of plants. *Phytophthotora sojae, P. infestans and Pythium irregulare are* not known to produce or contain toxins or antinutrients and have not been reported to cause disease in humans or animals.

The coding regions for the three desaturases do not encode a plant pest or an infectious agent and are not in themselves capable of causing disease. No significant amino acid homology of these desaturases to proteins toxic to humans or to known antinutrients was found using bioinformatic analysis. Omega-3 desaturases are found in all photosynthetic organisms. Humans and other mammals are dependent on dietary intake of omega-3 fatty acids because of the lack of endogenous enzymes for omega-3 desaturation (Simopoulos 2016).

The most serious diseases of canola in the United States are: Sclerotinia stem rot (SSR) or white mold (*Sclerotinia sclerotinium*), blackleg (*Leptosphaeria maculans* and *L. biglobosa*), and Alternaria black spot (*Alternaria brassicae* and *A. raphani*) (Brown et al. 2008). Other diseases that can impact the canola crop include: white rust (*Albugo candida*), downy mildew (*Peronospora parasitica*), powdery mildew (*Erysiphe cruciferarum*), clubroot (*Plasmodiophora brassicae*), aster yellows and Fusarium wilt (*Fusarium spp*.) (Kandel and Knodel 2011). Canola is also susceptible to diseases caused by bacteria such as bacterial black rot (*Xanthomonas campestris* pv. *campestris*), bacterial leaf spot (*X. campestris* pv. *amoraciae*); bacterial soft rot (*Erw*inia *carotovora* and *Pseudomonas marginalis*), scab (*Streptomyces scabiei*) crown gall (*Agrobacterium tumefaciens*). Several plant viruses also cause disease on canola: Cauliflower mosaic caulimovirus (CaMV), Cucumber mosaic cucumovirus (CMV), Radish mosaic comovirus (RaMV), Turnip mosaic potyvirus (TuMV) and Beet western yellows luteovirus (BWYV) (OECD 2012).

Blackleg and SSR are the primary diseases of *B. napus* in North Dakota, one of the major canola growing states in the U.S. Resistant hybrids are the most effective management

control measure against blackleg; however, most are only resistant to one strain of the blackleg pathogen. In Canada, yield losses of greater than 50% due to black leg have been reported (Canola Council of Canada, 2014d). SSR has caused yield losses as high as 50% in some fields in Minnesota (MN) and North Dakota (ND) (Kandel and Knodel 2011). These two diseases are widely prevalent in all canola production areas of Canada (Canola Council of Canada 2014), and crop rotation is an effective means of reducing the pressure from both diseases (Kandel and Knodel 2011).

Infestations with insect pests can significantly reduce canola seed yield; insecticide use is common in US canola production (Brown et al. 2008). Flea beetle (*Phylotreta cruciferae*) is a major insect pest in spring canola, and cabbage seedpod weevil (*Ceutorhynchus assimilis*) is a major insect pest in winter canola in the US. Aphids, such as turnip aphid (*Lipaphis erysimi*), cabbage aphid (*Brevicoryne brassicae*) and green peach aphid (*Myzus persicae*) can infest both spring and winter canola, and are the most economically important insect pests in the Great Plains and southeast (Brown et al. 2008).

Insect pests of canola in North Dakota are aphids (*Brevycoryne brassicae*), Aster leafhopper (*Macrosteles quadrilineatus*), Bertha armyworm (*Mamestra configurata*), Blister beetles (*Lytta mutalli*, *Epicauta fabricii*, *Epicauta ferruginea*), Cutworms (*Noctudea spp*.), Diamondback moth (*Plutella xylostella*), Flea beetles (*Phyllotreta cruciferae*, *P. striolata*) Grasshoppers (*Acrididae spp*.) Lygus bugs (*Lygus spp.)* and various species of wireworms (BASF 2017, p.166, Table 47; Knodel et al. 2017b).

Flea beetles are major pests in all spring canola production areas in the United States and the most damaging insect pest on canola in North Dakota (Knodel et al. 2017b). Very high populations of flea beetles, feeding on green pods can cause pod shatter (Kandel and Knodel 2011). In Canada, yield losses of 10% are common, and total annual canola crop losses in North America due to flea beetles is probably greater than $300M (Canola Council of Canada 2014). All canola varieties are selected for resistance to a range of biotic stresses, including many of the plant diseases and insect pests discussed above (Hall et al. 2005).

Glucosinolates are a large group of plant defense compounds that together with their decomposition products are part of the defense mechanism of plants in the Brassicaceae. Changes in the type and amount of glucosinolates may affect susceptibility or resistance to diseases and insect pests. Glucosinolates occur in all Brassica-originated feeds and fodders and the primary deleterious effects of ingestion of glucosinolates in animals are reduced palatability and decreased growth/production (Tripathi and Mishra 2007)

Evaluations of antinutrients including total glucosinolates in LBFLFK canola compared to Kumily were performed in field trials of sprayed and non-sprayed plots, during the winter 2014/15 and spring 2015 seasons. For all antinutrient components assessed, including the glucosinolates, the mean values for LBFLFK were within range of the reference varieties and meet the quality standard for canola (OECD 2011) and the ILSI Composition Database values. The difference in values of glucosinolates for LBFLFK

were within the range of natural variation and were not considered biologically relevant (BASF 2017, p.148).

Evaluations of differential impacts from biotic and abiotic stressors were performed during winter 2014/15 and spring 2015 field trials. Field plots were monitored for disease and pest damage stressors and damage from any naturally occurring abiotic stresses such as drought, wind or hail (BASF 2017, p.112). The abiotic stressors evaluated were excessive rainfall, moisture stress, drought, heat, wind and cold/wet weather. No differences were observed between responses to abiotic stressors between LBFLFK and Kumily at all growth stages measured (BASF 2017, p.114, Table 21).

Diseases typical to the growing regions were evaluated at four crop developmental stages and observations of disease stressors on LBFLFK and Kumily were made for the following diseases: *Alternaria*, anthracnose, aster yellows, black leg, black rot, black spot, downy mildew, root rot complex and Sclerotinia and seedling disease complex (BASF 2017, p.115 Table 22). For assessment of arthropod pest damage, the effects of major crop pests on the plants were also measured at four crop developmental stages on LBFLFK canola and on Kumily for the following pests: aphids, armyworm, beet webworm, cutworm, cabbage looper, cabbage moth, corn rootworm, diamondback moth, flea beetle, looper, seed pod weevil and stink bug (BASF 2017, p.116 Table 23). Disease and pest damage were limited and, where present, ranged from none to minimal or minimal to mild stress in LBFLFK and Kumily (BASF 2017, p 113, Table 22 and Table 23).

Ecological interactions of LBFLFK canola were assessed separately and compared to the ecological interactions of Kumily and three reference canola varieties at four growth stages in three field locations in 2015. Standard sampling techniques such as visual observations, sticky and pitfall traps, were used for ecological interaction studies of the abundance and diversity of arthropod communities from 16 taxonomic families (BASF 2017, p.117, Table 24; 2018). No pesticides or other pest control techniques were utilized in these studies.

Flea beetle captures on sticky traps were statistically significantly higher in LBFLFK canola compared to Kumily and the three reference varieties, at the last sampling date of the season at two out of the three sites, and at the second sampling date at one of the sites (BASF 2018, p.19-20, Table H.9). The statistically significant differences in sticky trap captures were observed at the sites with the highest flea beetle populations. Visual observations of flea beetles on LBFLFK were higher than for Kumily but within the range of the reference varieties at one site at the third sampling date. In addition, visual observations of flea beetles on LBFLFK were higher than for Kumily and the reference varieties on the last date at another site (BASF 2018, p.13-14, Table H.7). The applicant attributes these differences to the slightly delayed development of LBFLFK canola relative to Kumily resulting from delayed germination of LBFLFK (BASF 2017, p.99), which may result in adult beetles feeding late in the season preferring LBFLFK over more mature canola. Regardless of whether the higher late-season flea beetle counts in LBFLFK occurred due to differences in plant phenology or another mechanism, this

result is not anticipated to indicate a higher plant pest potential for other crops in the following spring. Pest potential is not expected to change because of 1) the lack of correlation between observed fall and spring flea beetle populations (Knodel et al. 2017a) and 2) the widespread prophylactic flea beetle control measures such as seed treatment that are commonly employed by Brassica producers (Knodel et al. 2017a).

Statistically significant consistent differences between fields of LBFLFK canola and Kumily were not observed in diversity and abundance of pest or beneficial invertebrate taxa other than flea beetles. Compared to other canola varieties and to Kumily, LBFLFK canola is not likely to be more susceptible to insect pests or diseases typical of canola growing regions, likely to result in the introduction or spread of a plant pest or disease or to have adverse impacts on the diversity of organisms in canola fields (BASF 2017, p.113).

The introduced genes did not significantly alter the observed insect pest infestation and disease occurrence or resulting damage on LBFLFK canola over the Kumily control line, other than differences in populations of pre-overwintering adult flea beetles that are not expected to affect pest pressure outside of LBFLFK canola fields. Results of the field trials described in the petition did not indicate that LBFLFK canola is more susceptible to pests and diseases over its control or reference varieties.

The observed agronomic traits also did not reveal any significant changes that would indirectly indicate that canola LBFLK is or could be relatively more susceptible to pests and diseases over Kumily or the other reference varieties. Thus, LBFLFK canola is unlikely to be more susceptible to plant pathogens and insect pests than conventional canola. For this reason, LBFLFK canola is unlikely to differ from conventional canola in its ability to harbor or transmit plant pathogens or pests and cause indirect plant pest effects on other agricultural products.

E. Potential Impacts on Nontarget Organisms Beneficial to Agriculture

LBFLFK canola is not engineered for pest resistance, thus there are no ‘target’ species, and thus no ‘nontarget’ species either. APHIS assessed whether exposure or consumption of LBFLFK canola would have a direct or indirect adverse impact on species beneficial to agriculture. Organisms considered were representatives of the species associated with production of the regulated crop in the agricultural environment. The assessment includes an analysis of data and information on LBFLFK canola compared to the non-GE counterpart (or other comparators) for any biologically relevant changes in the phenotype or substances produced (e.g. nutrients, antinutrients, polyunsaturated fatty acids, etc.) which may be novel or expressed at significantly altered amounts that are associated with impacts on organisms beneficial to agriculture, and/or any observations of beneficial organisms associated with the plants.

The enzymes associated with long chain poly-unsaturated fatty acid (LC-PUFA) synthesis which were inserted into LBFLFK were detected only in seed tissues (BASF 2017, p.294-303, Tables D.15 – D.24). AHAS(*At*) was inserted into LBFLFK canola to

confer herbicide resistance and is expressed throughout the plant except for pollen tissue (BASF 2017, p.76, Table 11), but there is no scientifically plausible hypothesis linking AHAS(*At*) expression to impacts to agriculturally important organisms.

Any new impacts on animals arising from the LC-PUFA synthesis pathway in LBFLFK canola are expected to be confined to seed feeding insect pests. LC-PUFAs such as DHA and EPA are typically not present in terrestrial plants and animals, but they are present in marine and aquatic organisms (Hixson et al. 2015). Lepidopteran larvae fed on diets supplemented with LC-PUFAs at levels similar to those found in LBFLFK canola seed achieved higher adult weights but had lower survival and a high rate of wing deformities relative to those on diets with similar lipid levels but without the LC-PUFAs (Fraenkel and Blewett 1946; Hixson et al. 2016). However, silkworm larvae fed on diets with lower levels of these compounds did not exhibit these phenotypes (Yu et al. 2018). LC-PUFA synthesis and storage is expected to be confined to seed tissue, and any detrimental effects due to LC-PUFA consumption are expected to be confined to insects that exclusively feed on seeds, if they occur. Seed-feeding insects in U.S. canola production include the cabbage seedpod weevil, *Ceutorhynchus assimilis*; the Bertha armyworm, *Mamestra configurata*; and the diamondback moth, *Plutella xylostella* (Weiss et al. accessed 4/18/2018). None of these species have been reported to be involved with ecosystem services beneficial to agriculture. Therefore, no agriculturally important organisms are likely to be affected by consumption of LBFLFK canola.

Twelve different glucosinolates, phytic acid, tannins, sinapine, coumaric acid, and ferulic acid were measured in the grain of LBFLFK and comparator lines (BASF 2017p.149, 151, Tables 39-40). The amounts of some of the individual glucosinolate compounds, including glucobrassicin and sinapine, were slightly different between LBFLFK and Kumily canola, but total glucosinolates and all of the other antinutrient levels were similar. The antinutrient levels in LBFLFK are therefore considered to be in the normal range for canola and no effects on agriculturally important organisms that are different from the effects already associated with conventional canola are expected.

The applicant compared arthropod and earthworm populations in LBFLFK (with and without imazamox application) and Kumily canola grown without insecticide or fungicide application in three locations. No differences between LBFLFK and Kumily in arthropod diversity, as measured by the Shannon-Weaver diversity index, were detected. No consistent differences in the counts of the different arthropod groups or differences in the number or weight of earthworms were detected. Therefore, no differences in arthropod diversity at the order or family level are anticipated.

Pollen is the most important source of essential amino acids for honeybees, and oilseed rape pollen was shown to contain a greater proportion of the most essential amino acids required by honeybees (valine, leucine, and isoleucine) (Cook et al. 2003). Moreover, honeybees tend to show a preference for oilseed rape pollen (Cook et al. 2003; Keller et al. 2005). The enzymes in the PUFA-synthesis pathway inserted in LBFLFK canola were not detected in pollen (BASF 2017, p.294-303, Tables D.15 – D.24), so pollen composition in terms of LC-PUFAs is expected to be similar between LBFLFK and

conventional canola. AHAS(*At*) is expressed in pollen (BASF 2017, p.76, Table 11), but there is no plausible hypothesis linking AHAS expression to effects on pollinators.

Imazamox tolerant varieties of several crop species including canola have been developed by the applicant using traditional breeding methods and released under the Clearfield® trademark. Imazamox resistance in these varieties is derived from expression of a novel allele of endogenous AHAS. No reports of detrimental effects on agriculturally important organisms associated with these varieties were found in a literature search. Therefore, expression of the AHAS(*At*) protein is not expected to affect agriculturally important organisms.

Based on the above analysis of gene expression patterns, nutrient and antinutrient composition, polyunsaturated fatty acid levels, and field observations of arthropods and earthworms, APHIS concludes that exposure to and/or consumption of the GE plant are unlikely to have any adverse impacts to organisms beneficial to agriculture.

F. Potential for Enhanced Weediness of LBFLFK Canola

APHIS assessed whether the LBFLFK canola t is likely to become more weedy (i.e. more prevalent, competitive, damaging or difficult-to-control in situations where it is not wanted) than the nontransgenic progenitor from which it was derived, or other varieties of the crop currently under cultivation. The assessment considers the basic biology of the crop, the situations in which crop volunteers or feral populations are considered weeds, and an evaluation of the LBFLFK canola compared to the comparator variety evaluated under field and/or lab conditions characteristic for the regions of the US where the GE crop is intended to be grown for characteristics related to establishment, competiveness, reproduction, survival, persistence and/or spread that could influence weediness and the ability to manage the crop as a weed. For this crop, such characteristics include seed dormancy and germination, vigor, rate of growth and development, flowering, seed yield and propagule dispersal. The assessment also considers whether the engineered trait affects methods of control for the crop in situations where it is managed as a weed or volunteer in subsequent crops or in feral populations.

Canola is not generally considered a weed, *B. napus* is not listed as a noxious weed in the U.S., or considered a listed weed by any U.S. state (NRCS 2018). The USDA PLANTS Database states that all *Brassica* species are listed weeds by the state of Michigan (NRCS 2018), but the official State of Michigan website lists only *B. juncae* and *B. nigra* (MDARD 2018). Certain biological properties that have been associated with weediness (Koop et al. 2012) including high seed production, seed shattering, regeneration, seed dormancy, and cold tolerance, are also present in canola. Therefore, large numbers of viable propagules are likely to be released into the environment following cultivation of any canola. However, the presence of potential weedy traits (Baker 1965) does not appear to predispose a plant taxon to become a weed (Perrins et al. 1992; Sutherland 2004).

Conventional canola is described as a cultivated crop with escaped plants that have become colonizers of waste places, disturbed sites in the vicinity of agricultural production fields, and along roadsides (Crawley and Brown 1995; Hall et al. 2005; Knispel and McLachlan 2010; OGTR 2011; Schafer et al. 2011; OECD 2012), but canola is not invasive in natural habitats (Hall et al. 2005; OECD 2012). With continuous reseeding from spillage during transport, short-lived canola populations have become permanent features of the agricultural landscape wherever canola is grown (Crawley and Brown 1995; Pivard et al. 2008). Unless the habitats are disturbed on a regular basis, canola populations are regularly displaced by more competitive plants (OECD 2012). In general, these canola populations are considered casual rather than feral, dying out in 2-4 years unless reseeded (Crawley and Brown 1995; Hall et al. 2005). Conventional canola in unmanaged settings is probably restricted by its poor competitive ability both as seedling and as an adult plant.

In field and laboratory trials, LBFLFK canola was generally similar to Kumily and reference varieties for traits including flowering time, time to maturity, plant height, lodging, pod shattering, number of pods, disease incidence, insect damage, damage due to abiotic stress, seed quality, seed weight, pollen germination, and pollen morphology (BASF 2017). LBFLFK canola may be slightly more sensitive to cold and have lower seed and pollen viability than Kumily

In two years of field trials, LBFLFK had higher seed moisture and lower yield than Kumily. According to the petitioner, higher seed moisture may be associated with the altered PUFA content of the seeds, and the lower seed yield may be due to the plant's investment in PUFA synthesis. In 2014/2015, seedling emergence, early season plant stand, seedling vigor, and the final plant stand were significantly reduced, and the beginning and end of flowering were delayed in LBFLFK relative to Kumily (BASF 2017p.100-105, Tables 15-18), although all of these properties except for seedling emergence were within the range of reference varieties other than Kumily. According to the petition, reduced seedling emergence and vigor in these studies may reflect higher cold sensitivity in LBFLFK, related to the altered lipid content of the seeds. In controlled laboratory experiments. LBFLFK had lower germination and a higher proportion of abnormal and dead seeds than Kumily. These differences were more pronounced in cold conditions (BASF 2017, p.110, Table 19). Secondary dormancy did not differ between LBFLFK and Kumily. Differences in germination rates and seed viability may be due to the altered lipid profile in seeds of LBFLFK. Pollen viability was also lower in LBFLFK than Kumily, and lower than the reference varieties (BASF 2017, p.112, Table 20). The differences between LFBLFK relative to Kumily (lower germination, lower seed viability, lower pollen viability, altered seed moisture, reduced yield, and higher cold sensitivity in LBFLFK) are not expected to increase the weediness of LBFLFK relative to conventional canola.

Volunteers of LBFLFK canola are expected to be resistant to ALS inhibitor herbicides such as imazamox. When conventional canola occurs as a volunteer, it is predominantly controlled by herbicide applications (Kandel and Knodel 2011). Conventional canola can be controlled by use of herbicides with the following modes of action: group 2 (ALS

inhibitors), group 4 (growth regulators), groups 5 and 6 (photosystem II inhibitors), group 14 (PPO inhibitors), group 19 (auxin transport inhibitors) and group 27 (HPPD Inhibitors) (Kandel and Knodel 2011). Control of LBFLFK is expected to be identical to control of Clearfield® canola varieties which are already resistant to ALS inhibitors and similar to control of conventional canola except that control using group 2 herbicides will not be an option due to the activity of the AHAS(*At*) gene. BASF has conducted a number of field tests of LBFLFK (BASF 2017, p.205, Table A.1). None of the final field test reports submitted to BRS indicate that differences between LBFLFK and conventional canola were noted during the tests or during volunteer monitoring.

Based on the agronomic field data and literature survey concerning weediness potential of the crop, the LBFLFK canola is unlikely to persist as a troublesome weed or to have an impact on current weed management practices. Furthermore, extensive post-harvest monitoring of field trial plots planted with the GE crop event under USDA-APHIS authorizations did not reveal any differences in survivability or persistence relative to other varieties of the same crop currently being grown. These data suggest that LBFLFK canola is no more likely to become a weed than conventional varieties of the canola. LBFLFK canola volunteers and casual or feral populations can be managed using a variety of currently available methods and alternative herbicides.

G. Potential Impacts on the Weediness of Any Other Plants with which LBFLFK Canola Can Interbreed

Gene flow is a natural biological process with significant evolutionary importance. A number of angiosperm taxa are believed to be derived from hybridization or introgression between closely related taxa (Grant 1981; Rieseberg and Wendel 1993; Soltis et al. 1993; Hegde et al. 2006), and even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace 1987; Rieseberg and Wendel 1993; Peterson et al. 2002). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars (Khoury et al. 2013). However, gene flow from crops to wild relatives is also thought of as having a potential to enhance the weediness of wild relatives, as observed in rice, sorghum, sunflower and a few other crops (Ellstrand et al. (1999).

APHIS considers two primary issues when assessing weediness of sexually compatible plants because of transgene flow: 1) the potential for gene flow and introgression and, 2) the potential impact of introgression.

Potential for gene flow, hybridization and gene introgression

Conventional canola is described as a cultivated crop with escaped plants that have become colonizers of waste places, disturbed sites in the vicinity of agricultural production fields, and along roadsides (Crawley and Brown 1995; Hall et al. 2005; Knispel and McLachlan 2010; OGTR 2011; Schafer et al. 2011; OECD 2012). However, canola is not invasive in natural habitats (Hall et al. 2005; OECD 2012). Like conventional canola, LBFLFK canola that is not intentionally managed is expected to

occur only in frequently disturbed areas associated with agriculture or adjacent to agricultural fields or transport routes, and be no more invasive in natural habitats than conventional canola.

In a majority of crop species, gene flow is idiosyncratic depending upon biology and ecology of both crop and sexually compatible relatives (Ingram 2000; Warwick et al. 2009a). Accordingly, there are several important considerations for successful gene flow and introgression between LBFLFK canola and sexually compatible relatives such as spatial proximity, overlapping phenology, F1 hybrid fertility, self-sustaining reproductively fertile hybrid derived (backcrossed) populations, and neutral or beneficial introgressed genes (Devos et al. 2009).

Canola is predominantly self-pollinating, but outcrossing does occur via wind and insect pollination (Williams 1984; Williams et al. 1987). Depending on the size of the crop and distance between plants or fields, a variety of outcrossing rates were observed for canola (Beckie et al. 2003). Most outcrossing between fields generally occurs within the first 10-20 m of the recipient field, and rates decline with distance (Beckie et al. 2003; OGTR 2011).

Reproductive compatibility among Brassica crops and their wild relatives is complex. Several sexually compatible *Brassica* spp., including *B. napus* share components of their genomes (OECD 2012), and introgression between species is more likely when they have genome components in common. Additionally, there is potential for gene flow from *B. napus* to another sexually compatible species (e.g., *B. rapa* or *B. juncea*), and from the second species to other species that are sexually compatible with the second species but not with *B. napus* (bridge crosses).

Canola has the ability to cross with a number of relatives (some of which are weedy) with varying degrees of crossing potential. According to OECD (2012), Indian mustard, *B. juncea;* field mustard, *B. rapa;* shortpod mustard, *Hirschfeldia incana;* and wild radish, *Raphanus raphanistrum* have a high potential for natural crossing with *B. napus*. For these four sexually compatible relatives, natural crosses with *B. napus* as male and as female have been recorded. *B. rapa* is weedy and widespread where canola is grown (Warwick et al. 2003) and *R. raphanistrum* is also considered weedy (USDA-NRCS 2018a). Whereas, Ethiopian mustard, *B. carinata;* black mustard, *B. nigra;* Sahara mustard, *B. tournefortii;* annual wall rocket, *Diplotaxis muralis;* rocket salad, *Eruca sativa;* and charlock mustard, *Sinapis arvensis* have low potential for natural crossing with *B. napus* (OECD 2012). Of the species with low crossing potential with canola, *B. nigra* (USDA-NRCS 2018b) and *B. tournefortii* (USDA-NRCS 2018d) are considered weedy. The following plant species have a very low or extremely low potential for natural crossing with *B. napus*: Mediterranean cabbage *B. fruticulosa;* cabbage, *B. oleracea;* common dogmustard, *Erucastrum gallicum;* radish, *R. sativus;* white mustard, *S. alba;* white wallrocket, *D. erucoides*; and *D. catholica;* (OECD 2012). Among these *R. sativus* is considered weedy (USDA-NRCS 2018c). Other sources mention perennial wallrocket, *D. tenuifolia* (Rieger et al. 1999); rocketsalad, *Eruca vesicaria* (Bijral and

Sharma 1999); *Rorrippa islandica* (Bijral and Sharma 1995); and *Orychophragmus violaceus* (Li et al. 1998) as species present in the U.S. that are compatible with *B. napus*.

Potential for enhanced weediness of recipients after gene flow and/or introgression

Crossing between LBFLFK canola and the sexually compatible relatives listed above is likely to occur if the plants are within a distance that allows for outcrossing to occur. If genetic introgression of the transgenes in LBFLFK canola to a sexually compatible relative were to occur, the offspring would be expected to gain the ability to synthesize LC-PUFAs and resistance to imazamox herbicides. However, similarly to LBFLFK canola, the LC-PUFA synthesis pathway is not expected to influence the weediness of sexually compatible relatives of canola that acquire the transgene. In locations where imazamox or another ALS inhibitor herbicide are regularly used, any hybrids between LBFLFK and sexually compatible relatives would experience strong selection pressure favoring those plants containing the AHAS*(At*) gene. Sexually compatible relatives that acquire the transgene are expected to become tolerant to imazamox herbicides.

Clearfield® canola varieties with an alternative AHAS gene conferring resistance to imazamox are already on the market (BASF 2017), and these conventional varieties are already capable of passing the trait to sexually compatible relatives. Although which relatives will acquire the trait is influenced by whether the trait occurs in the portion of the genome that is shared with canola (Tan et al. 2005). Several sexually compatible relatives of canola, including *R. raphanistrum, B. tournefortii,* and *Sinapis arvensis,* already have populations resistant to ALS-inhibitor herbicides (Tranel et al. 2018).

Because of the potential for herbicide resistance to be passed from Clearfield® crops to sexually compatible weedy species, stewardship programs, including herbicide rotation and maintenance of weed-free fields are already in use with Clearfield® crops (Tan et al. 2005). Similarly to Clearfield canola and ALS-inhibitor resistant weed populations the control options for volunteer populations of LBFLFK canola and for any sexually compatible relatives of *B. napus* that may acquire imazamox resistance by introgression of AHAS(*At*) from LBFLFK canola, will include herbicides other than ALS-inhibitors, cultural control techniques, and tillage.

The introduced genetic material in LBFLFK is not expected to change the ability of the plant to interbreed with other plant species. Furthermore, APHIS evaluation of data provided by BASF (2017) of agronomic and phenotypic properties of LBFLFK canola, including those characteristics associated with reproductive biology, indicated no unintended changes likely to affect the potential for gene flow from LBFLFK canola to sexually compatible species.

Although it is likely that LBFLFK canola plants in the United States and its territories will be found as volunteers in agricultural settings and as casual populations outside of agricultural settings, such as along roadsides and seed transportation routes. It is also likely that gene flow and introgression will occur at low rates between LBFLFK canola plants and sexually compatible relatives. Sexually compatible relatives of canola may

acquire the LC-PUFA synthesis pathway and resistance to ALS-inhibitor herbicides as a result of gene flow, if they are within pollen dispersal distance from cultivated or unmanaged LBFLFK canola. However, herbicides other than ALS-inhibitors, cultural control techniques, and site management such as tillage are available to control volunteer plants with the AHAS gene for the resistance trait and weedy relatives.

Based on the information presented in the petition and in relevant literature, APHIS has reached the following conclusions. The genetic modification in LBFLFK canola is not expected to increase the potential for gene flow and for hybridization and/or introgression to occur to sexually compatible taxa compared to the nontransgenic recipient or other varieties of canola that are commonly grown. The genetic modification in LBFLFK canola is unlikely to confer novel weedy properties to canola or its wild relatives. Gene flow, hybridization and/or introgression of genes from LBFLFK canola to sexually compatible relatives, including wild, weedy, feral or cultivated species in the U.S. and its territories is likely to occur if the plants are within the pollen dispersal distance, and depending on their potential for crossing with *B. napus*, as discussed above. However, it is unlikely that canola plants in the United States will be found outside of an agricultural setting, except along roadsides and seed transportation routes. It is also highly unlikely that gene flow and introgression will occur between LBFLFK canola plants and wild or weedy species in a natural environment. Gene flow from LBFLFK canola to sexually compatible species can be mitigated by management of volunteer LBFLFK canola and maintenance of weed-free conditions in fields where LBFLFK canola will be grown. Therefore, APHIS has determined that LBFLFK canola is not expected to increase the weed risk potential of other species with which it can interbreed in the U.S. and its territories.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS assessed whether significant changes to agricultural or cultivation practices from adoption of LBFLFK canola are likely, and if so, is cultivation of this LBFLFK canola likely to impact plant diseases or pests or their management, including any APHIS control programs. This assessment included consideration of any changes in pesticide applications, tillage, irrigation, harvesting, and other cultural practices as they relate to plant pests and diseases.

In general, management practices currently employed for conventional canola cultivation are not expected to change if LBFLFK canola is determined to no longer be subject to the regulatory requirements of 7 CFR part 340 or to the plant pest provisions of the Plant Protection Act. BASF studies demonstrate that the cultivation practices needed for growing LBFLFK canola are essentially indistinguishable from practices used to grow conventional canola (BASF 2017).

Canola event LBFLFK has also been modified for introduction of an AHAS resistance trait to imidazolinone herbicides. Several variant AHAS genes conferring imidazolinone resistance were discovered in plants through mutagenesis and selection, and were used to develop imidazolinone tolerant crops using conventional breeding methods including oilseed rape (*B. napus* L.) These crops have been commercialized as Clearfield crops

from 1992 to the present (Tan et al. 2005). Beyond® herbicide is approved for use as part of the Clearfield production system for Clearfield® canola and BASF will petition the U.S. EPA to update the label for Beyond®, ALS/AHAS inhibitor herbicide to allow for the field application on EPA+DHA canola event LBFLFK (BASF 2017).

Production and processing of EPA+DHA canola event LBFLFK as a specialty canola, will be conducted under an Identity Preservation System (IDP). Processing operations will be conducted in dedicated facilities or at facilities with specific measures to ensure segregation from other canola products (BASF 2017, p.173). According to the petition, activities will be conducted to support variety development, grain production, oil manufacturing, and other commercial activities to prepare EPA+DHA canola event LBFLFK for the marketplace as an alternative source of long-chain polyunsaturated omega-3 fatty acids (LC-PUFA) (BASF 2017, p.174).

APHIS could not identify any significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of LBFLFK canola under an IDP system for production and processing; therefore, no impact on plant diseases or pests or their management is likely to occur.

In conclusion, LBFLFK canola is similar to conventional canola in its agronomic, phenotypic and environmental response, and levels of occurrence and damage from arthropod pests and diseases are comparable to the conventional canola Kumily used as control and to the other conventional varieties used as comparators. The use of an IDP system for production and of Imidazolinone herbicide are unlikely to increase pests or diseases or adversely impact their management, nor will they impact APHIS pest control programs. Therefore, no significant impacts on current cultivation and management practices for canola are expected following the introduction of LBFLFK canola.

I. Potential Impacts from Transfer of Genetic Information to Organisms with which LBFLFK canola Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into LBFLFK canola to be horizontally transferred without sexual reproduction to other organisms and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants. The horizontal gene transfer between unrelated organisms is one of the most intensively studied fields in the biosciences since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Dröge et al. 1998). Potential risks from stable horizontal gene transfer (HGT) from genetically engineered organisms to another organism without reproduction or human intervention were recently reviewed (Keese 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and rearrangement, most notably through viruses and mobile genetic elements. HGT has been a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria; emergence of increased virulence in bacteria, eukaryotes and viruses; and, in the long run, to major transitions in evolution.

Potential for horizontal gene transfer to bacteria, fungi, or invertebrates

LBFLFK canola has been genetically engineered to contain coding sequences that were codon optimized for expression in canola, and that were derived from plants (one moss, four microalgae, and a flowering plant) and three oomycete species. These have been described in detail in other sections of this PPRA and in the petition (BASF 2017, p. 34-39, Table 3.).

Horizontal gene transfer and expression of DNA from a plant species to bacterial, fungal or invertebrate species is unlikely to occur based on the following observations. Although there are many opportunities for plants to directly interact with fungi and bacteria (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores) and with invertebrates as plant pests, there are almost no evolutionary examples of HGT from eukaryotes to bacteria or from plants to fungi or invertebrates (Keese 2008). Examples of HGT between eukaryotes and fungi primarily involve gene acquisition or transfer by fungi to or from other distantly related fungi or bacteria (Keeling and Palmer 2008; Keese 2008) and HGT between plants and fungi is extremely rare (Richards et al. 2009). Examples of HGT between plants and invertebrates are also extremely rare, and most examples of HGT in insects involve acquisition of genes from their pathogens or endosymbionts (Keese 2008; Zhu et al. 2011; Acuna et al. 2012).

Horizontal transfer from and expression in bacteria of GE plant genes is unlikely to occur. First, many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Wood et al. 2001; Kaneko et al. 2002). There is no evidence that these organisms contain genes derived from plants. HGT from plants to bacteria is a very low frequency event, primarily because functional and selective barriers to HGT increase with genetic distance (Keese 2008). Second, in cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Brown 2003; EFSA 2009; Koonin et al. 2011). Third, transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression. Thus even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. Fourth, both the FDA (1998) and the European Food Safety Authority (2009) have evaluated horizontal gene transfer from the use of antibiotic resistance marker genes and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is very rare or remote.

Potential for horizontal gene transfer to viruses

LBFLFK canola contains two copies of the *Cauliflower mosaic virus*, CaMV35S terminator region, which is identical to a section of GenBank nucleotide accession number AF234316 (Hajdukiewicz et al. 1994; BASF 2017, p. 34-39, Table 3.). APHIS also considered whether horizontal transfer of DNA from the GE plant to plant viruses was likely to occur and would lead to the creation or selection of plant viruses that are more virulent or have a broader host range. This issue has been considered before by

other science review panels and government regulatory bodies (EPA-FIFRA-SAP 2006; Keese 2008). HGT is not unusual among plant viruses; however this is generally limited to exchange between viruses present in the same host organism in mixed infections, and most commonly involves homologous recombination, relying on sequence similarity at the point of crossover (Keese 2008). HGT from virus sequences engineered into plants has been demonstrated with infecting or challenge viruses, including both DNA viruses (e.g. geminiviruses which replicate in the nucleus) (Frischmuth and Stanley 1998) and RNA viruses (which typically replicate in the cytoplasm); however most have been under conditions that favor recombination to restore a defective virus (Fuchs and Gonsalves 2007; Keese 2008; Thompson and Tepfer 2010). Populations of recombinants between virus transgenes expressed in transgenic plants infected with related viruses are similar to recombinants found in mixed infections of the same viruses in nontransgenic plants, indicating that there was no novel recombination mechanism in the transgenic plants and no increased risk is expected over what is expected from mixed infections (Keese 2008; Turturo et al. 2008). Nonhomologous recombination in HGT among viruses or between virus transgenes and infecting viruses can occur, but frequently results in gene deletions which can result in nonviable viruses (Morroni et al. 2013). Depending on the particular virus and sequences involved, various hot-spots for recombination have been found in both coding and noncoding regions, and strategies implemented in design of transgenes to avoid recombination have been suggested. No recombinant or undesirable viruses with new properties have been detected for over at least 8-10 years in field tests or during commercial growth of deregulated virus resistant plum, squash, or papaya engineered with genes from viruses that have been deregulated in the U.S. (Fuchs and Gonsalves 2007). Plant virus-derived sequences in LBFLFK canola are non-coding regulatory sequences of known function and not likely to recombine with other viruses.

Potential for horizontal gene transfer to parasitic plants

Evidence for HGT from plants to other plants is limited to two specific scenarios: (1) exchange of genes between a parasitic plant and its host; and (2) exchange of genes between cells of two plants living in close proximity, such as in a graft junction. In both cases, this type of HGT requires physical contacts between the two plants. Most cases of HGT in plants involve transfer of mitochondrial genomes, which are primarily maternally inherited in plants (Barr et al. 2005), to other mitochondria genomes, and mostly involve parasitic plants and their hosts (Richardson and Palmer 2007). Comparative genomics analysis has implicated HGT in the incorporation of a specific genetic sequence with unknown function in the parasitic plant purple witchweed (*Striga hermonthica*) from its monocot host plant, sorghum (2010). However, this HGT occurred before speciation of purple witchweed and the related cowpea witchweed (*S. gesnerioides*) from their common ancestor. Furthermore, *S. hermonthica* is not found in the U.S. and *S. asiatica*, another related parasite of cereal crops, is only present in North Carolina and South Carolina (USDA-NRCS). More recent studies demonstrated that in a few parasitic species of the Rafflesiaceae family, out of several genetic sequences examined, about 2.1% of nuclear (Xi et al. 2012) and 24 –41% of mitochondrial (Xi et al. 2013) gene transcripts appeared to be acquired from their obligate host species. However, all the above-mentioned instances of HGT between parasitic plants and their hosts were reported to be of ancient origins, on an evolutionary time scale spanning thousands to millions of

years ago. Furthermore in LBFLFK canola, the DNA sequences were inserted into the nuclear genome, not the mitochondrial genome, as demonstrated by the Mendelian inheritance pattern of the LBFLFK canola (BASF 2017, p. 61, Table 7 and 8).

If LBFLFK canola becomes infected by a parasitic plant or is naturally grafted to another plant, there is a very low probability that horizontal gene transfer could result in the other plant acquiring DNA from LBFLFK canola. However, in both scenarios this newly introduced DNA would likely reside in somatic cells, and with little chance of reaching the germ cells, this introduced DNA could not persist in subsequent generations unless the recipient plant reproduced asexually from the affected cells.

Based on the above analysis APHIS concludes that horizontal gene transfer of the new genetic material inserted into LBFLFK canola to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

J. Conclusion

APHIS has reviewed the information submitted in the petition, supporting documents, public comments in response to Federal Register notices concerning this petition, and other relevant information to assess the plant pest risk of the LBFLFK canola event compared to the unmodified parental variety Kumily from which it was derived. APHIS concludes that LBFLFK canola is unlikely to pose a greater plant pest risk than its unmodified parental variety Kumily based on the following findings.

- No plant pest risk was identified from the transformation process or the insertion of new genetic material into LBFLFK canola. The *Agrobacterium* transformation vector was eliminated from the transformed material and non-coding and coding sequences derived from plant pests do not cause disease, create an infectious agent, or otherwise confer any plant pest characteristic to LBFLFK canola.

- No increase in plant pest risk was identified in LBFLFK canola from the expression of new proteins (elongases, desaturases, and acetohydroxy acid synthase) from the inserted genetic material involved in the production of long chain polyunsaturated fatty acids (LC-PUFAs), and herbicide resistance. There were no significant changes in metabolism or compositional characteristics that would render LBFLFK canola more susceptible to pests and diseases than its parental control Kumily or the reference commercial varieties.

- Disease and pest incidence and/or damage were not observed to be significantly increased or atypical in LBFLFK canola compared to the nontransgenic parental variety Kumily or other comparators in field trials conducted in growing regions representative of where the LBFLFK canola is expected to be grown, other than a late-season increase in pre-overwintering flea beetle adults. The observed differences in flea beetle populations are not expected to alter pest pressure outside of LBFLFK canola fields. Observed agronomic traits also did not reveal any significant

differences that would indirectly indicate that LBFLFK canola is more susceptible to pests or diseases. Therefore no plant pest effects are expected on these or other agricultural products and no impacts are expected to APHIS pest control programs.

- Exposure to and/or consumption of LBFLFK canola are unlikely to have any adverse impacts on organisms beneficial to agriculture based on the analysis of compositional, phenotypic and agronomic data.

- LBFLFK canola is no more likely to become a weed or weedier than conventional varieties of the crop based on its observed agronomic characteristics, weediness potential of the crop and current management practices available to control LBFLFK canola as a weed. Volunteers of LBFLFK canola tolerant to imidazolinone herbicide can be managed using a variety of currently available methods and alternative herbicides**.**

- LBFLFK canola is not expected to increase the weed risk potential of other species with which it can interbreed in the U.S. or its territories. Even if sexually compatible relatives acquire transgenes through gene flow, the new phenotype(s) conferred by transgenes are not likely to increase the weediness of these compatible relatives or affect the current ability to control these relatives in situations where they are considered weedy or invasive.

- Significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of the LBFLFK canola were not identified and thus are not likely to increase plant diseases or pests or compromise their management.

- Horizontal gene transfer of the new genetic material inserted into LBFLFK canola to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

K. References

51 FR 23302. 1986. *Coordinated Framework for Regulation of Biotechnology*.

57 FR 22984. 1992. *Statement of Policy: Foods Derived from New Plant Varieties*.

Acuna R, Padilla BE, Florez-Ramos CP, Rubio JD, Herrera JC, Benavides P, Lee SJ, Yeats TH, Egan AN, Doyle JJ, and Rose JK. 2012. *Adaptive horizontal transfer of a bacterial gene to an invasive insect pest of coffee*. PNAS 109, pp. 4197-4202. Retrieved from http://www.pnas.org/content/early/2012/02/17/1121190109.abstract

Baker H. 1965. *Characteristics and modes of origin of weeds*.

Barr CM, Neiman M, and Taylor DR. 2005. *Inheritance and recombination of mitochondrial genomes in plants, fungi and animals*. New Phytologist 168, pp. 39-50. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.2005.01492.x/abstract

BASF Plant Science, L.P. 2017. *Petition for the Determination of Nonregulated Status for EPA+DHA Canola Event LBFLFK* Submitted by J. Sottosanto. BASF Plant Science, L.P. Research Triangle Park, NC.

BASF Plant Science, L.P. 2018. *Regarding: "Petition #17-321-01p: Petition for the Determination of Nonregulated Status for EPA+DHA Canola Event LBFLFK"*. Submitted by J. Sottosanto. BASF Plant Science, L.P. Research Triangle Park, NC.

Beckie H, Warwick S, Nair H, and Séguin-Swartz G. 2003. *Gene flow in commercial fields of herbicide-resistant canola (Brassica napus)*. Ecological applications 13, pp. 1276-1294.

Bijral J and Sharma T. 1995. *Intergeneric (intertribe) hybridization between Rorippa islandica, a wild species, and Brassica napus*. Hulletin dessureshtentius Noticiario de Recursos Fitogenéticos.

Bijral J and Sharma T. 1999. *Brassicajuncea-Eruca sativa sexual hybrids*. Cruciferae Newsletter, pp. 33-34.

Brown J, Davis JB, Lauver M, and Wysocki D. 2008. *U.S. Canola Association Canola Growers' Manual*. University of Idaho & Oregon State University. Retrieved from http://www.uscanola.com/site/epage/102387_956.htm

Brown JR. 2003. *Ancient horizontal gene transfer*. Nature Reviews Genetics 4, pp. 121-132. Retrieved from http://www.nature.com/nrg/journal/v4/n2/full/nrg1000.html

Canola Council of Canada. 2014. *Canola Grower's Manual* Canola Council of Canada. Retrieved from http://www.canolacouncil.org/crop-production/canola-grower's-manual-contents/

Canola Council of Canada. 2017. *What is Canola?* Canola Council of Canada. Retrieved from https://www.canolacouncil.org/oil-and-meal/what-is-canola/#OfficialDefinition Last accessed 4/26/2018.

Cao J, Schneeberger K, Ossowski S, Gunther T, Bender S, Fitz J, Koenig D, Lanz C, Stegle O, Lippert C, Wang X, Ott F, Muller J, Alonso-Blanco C, Borgwardt K, Schmid KJ, and Weigel D. 2011. *Whole-genome sequencing of multiple Arabidopsis thaliana populations*. Nat Genet 43, pp. 956-963. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/21874002

Cook SM, Awmack CS, Murray DA, and Williams IH. 2003. *Are honey bees' foraging preferences affected by pollen amino acid composition?* Ecological Entomology 28, pp. 622-627.

Crawley M and Brown S. 1995. *Seed limitation and the dynamics of feral oilseed rape on the M25 motorway*. Proceedings of the Royal Society of London B: Biological Sciences 259, pp. 49-54.

De Block M, De Brouwer D, and Tenning P. 1989. *Transformation of Brassica napus and Brassica oleraceae using Agrobacterium tumefaciens and the expression of the bar and neo genes in the transgenic plants*. Plant Physiol 91, pp. 694-701.

Devos Y, De Schrijver A, and Reheul D. 2009. *Quantifying the introgressive hybridisation propensity between transgenic oilseed rape and its wild/weedy relatives*. Environmental Monitoring and Assessment 149, pp. 303-322.

Dröge M, Pühler A, and Selbitschka W. 1998. *Horizontal gene transfer as a biosafety issue: A natural phenomenon of public concern*. Journal of Biotechnology 64, pp. 75–90. Retrieved from http://www.sciencedirect.com/science/article/pii/S0168165698001059

EFSA. 2009. *Statement of EFSA on the consolidated presentation of opinions on the use of antibiotic resistance genes as marker genes in genetically modified plants*. EFSA Journal 1108. Retrieved from http://www.efsa.europa.eu/en/efsajournal/pub/1108.htm

Ellstrand NC, Prentice HC, and Hancock JF. 1999. *Gene flow and introgression from domesticated plants into their wild relatives*. Annual Review of Ecology and Systematics 30, pp. 539-563. Retrieved from http://www.annualreviews.org/doi/abs/10.1146%2Fannurev.ecolsys.30.1.539

EPA-FIFRA-SAP. 2006. *Plant Incorporated Protectants Based on Virus Coat Protein Genes: Science Issues Asosciated with the Proposed Rule*. Retrieved from http://www.epa.gov/scipoly/sap/meetings/2005/december/minutes1205.pdf

Fraenkel G and Blewett M. 1946. *Linoleic acid, vitamin E and other fat-soluble substances in the nutrition of certain insects, Ephestia kuehniella, E. elutella, E. cautella and Plodia interpunctella (Lep.)*. J Exp Bot 22, pp. 172-190.

Frischmuth T and Stanley J. 1998. *Recombination between viral DNA and the transgenic coat protein gene of African cassava mosaic geminivirus*. Journal of General Virology 79, pp. 1265-1271. Retrieved from http://vir.sgmjournals.org/content/79/5/1265

Fuchs M and Gonsalves D. 2007. *Safety of virus-resistant transgenic plants two decades after their introduction: lessons from realistic field risk assessment studies*. Annual Review of Phytopathology 45, pp. 173-202. Retrieved from http://www.annualreviews.org/doi/full/10.1146/annurev.phyto.45.062806.094434?url_ver=Z39.88-2003

Gheysen G, Villarroel R, and Van Montagu M. 1991. *Illegitimate recombination in plants: a model for T-DNA integration*. Genes & Development 5, pp. 287-297.

Grant V. 1981. *Plant Speciation*. Columbia University Press.

Hajdukiewicz P, Svab Z, and Maliga P. 1994. *The small, versatile pPZP family of Agrobacterium binary vectors for plant transformation*. Plant Molecular Biology 25, pp. 989-994.

Hall LM, Rahman HM, Gulden RH, and Thomas GA. 2005. Volunteer Oilseed Rape — Will Herbicide-Resistance Traits Assist Ferality? In: *Crop Ferality and Volunteerism* (Taylor & Francis Group, LLC), pp. 59-80. Retrieved from http://www.crcnetbase.com/doi/book/10.1201/9781420037999 Last accessed 13:39 13 January 2016.

Hegde SG, Nason JD, Clegg JM, and Ellstrand NC. 2006. *The Evolution of California's Wild Radish Has Resulted in the Extinction of Its Progenitors*. Evolution 60, pp. 1187. Retrieved from http://www.bioone.org/doi/abs/10.1554/05-634.1#.Uhelsxtwq-x

Hellens R, Mulineaux P, and Klee H. 2000. *A guide to Agrobacterium binary Ti vectors*. Trends in Plant Science 5, pp. 446-451.

Hixson SM, Shukla K, Campbell LG, Hallett RH, Smith SM, Packer L, and Arts MT. 2016. *Long-Chain Omega-3 Polyunsaturated Fatty Acids Have Developmental Effects on the Crop Pest, the Cabbage White Butterfly Pieris rapae*. PLoS One 11, pp. e0152264. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27011315

Hoekema A, Hirsch PR, Hooykaas PJJ, and Schilperoort RA. 1983. *A binary plant vector stratgy based on separation of vir- and T-region of the Agrobacterium tumefaciens Ti-plasmid*. Nature 303, pp. 179-180.

Ingram J. 2000. *Report on the separation distances requried to ensure cross-pollination is below specified limits in non-seed crops of sugar beet, maize and oilseed rape. .* National Institute of Agricultural Botany MAFF Project No. RG0123.

Kandel H and Knodel JJ. 2011. *Canola Production Field Guide*. North Dakota: North Dakota State University-Estension Service with support from the Northern Canola Growers Association. Retrieved from https://www.ag.ndsu.edu/publications/landing-pages/crops/canola-production-field-guide-a-1280

Kaneko T, Nakamura Y, Sato S, Minamisawa K, Uchiumi T, Sasamoto S, Watanabe A, Idesawa K, Iriguchi M, Kawashima K, Kohara M, Matsumoto M, Shimpo S, Tsuruoka H, Wada T, Yamada M, and Tabata S. 2002. *Complete Genomic Sequence of Nitrogen-fixing Symbiotic Bacterium Bradyrhizobium japonicum USDA110*. DNA Research 9, pp. 189–197. Retrieved from http://dnaresearch.oxfordjournals.org/content/9/6/189.long

Keeling PJ and Palmer JD. 2008. *Horizontal gene transfer in eukaryotic evolution*. Nature Reviews Genetics 9, pp. 605-618. Retrieved from http://www.nature.com/nrg/journal/v9/n8/full/nrg2386.html

Keese P. 2008. *Risks from GMOs due to horizontal gene transfer*. Environmental Biosafety Research 7, pp. 123-149. Retrieved from http://www.ebr-journal.org/action/displayAbstract?fromPage=online&aid=8208895

Keller I, Fluri P, and Imdorf A. 2005. *Pollen nutrition and colony development in honey bees: part 1*. Bee World 86, pp. 3-10.

Khoury CK, Greene S, Wiersema J, Maxted N, Jarvis A, and Struik PC. 2013. *An Inventory of Crop Wild Relatives of the United States*. Crop Science 53, pp. 1496. Retrieved from https://www.crops.org/publications/cs/abstracts/53/4/1496

Knispel AL and McLachlan SM. 2010. *Landscape-scale distribution and persistence of genetically modified oilseed rape (Brassica napus) in Manitoba, Canada*. Environ

Sci Pollut Res Int 17, pp. 13-25. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19588180

Knodel JJ, Lubenow LA, and Olson DL. 2017a. *Integrated Pest Management of Flea Beetles in Canola* (E1234). Fargo, ND. North Dakota State University Extension Service.

Knodel JJ, Beauzay P, and Boetel M. 2017b. *2017 Field Crop Insect management Guide* (E-1143(Revised)). North Dakota State University Extension Service.

Koonin EV, Makarova KS, and Aravind L. 2011. *Horizonal Gene Transfer in Prokaryotes: Quantification and Classification*. Annual Review of Microbiology 55, pp. 709-742. Retrieved from http://www.annualreviews.org/doi/full/10.1146/annurev.micro.55.1.709?url_ver=Z39.88-2003

Koop AL, Fowler L, Newton LP, and Caton BP. 2012. *Development and validation of a weed screening tool for the United States*. Biological Invasions 14, pp. 273-294.

Kovalic D, Garnaat C, Guo L, Yan Y, Groat J, Silvanovich A, Ralston L, Huang M, Tian Q, Christian A, Cheikh N, Hjelle J, Padgette S, and Bannon G. 2012. *The Use of Next Generation Sequencing and Junction Sequence Analysis Bioinformatics to Achieve Molecular Characterization of Crops Improved Through Modern Biotechnology*. The Plant Genome Journal 0, pp. 0.

Li Z, Wu J, Liu Y, Liu H, and Heneen W. 1998. *Production and cytogenetics of the intergeneric hybrids Brassica juncea× Orychophragmus violaceus and B. carinata× O. violaceus*. TAG Theoretical and Applied Genetics 96, pp. 251-265.

Max Planck Institute for Developmental Biology. 2018. *1001Genomes* Retrieved from http://1001genomes.org/ Last accessed 4/27/2018.

McCourt JA and Duggleby RG. 2006. *Acetohydroxyacid synthase and its role in the biosynthetic pathway for branched-chain amino acids*. Amino Acids 31, pp. 173-210. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/16699828

MDARD. 2018. *Prohibited and Restricted Weeds*. Michigan Department of Agriculture and Rural Development. Retrieved from http://www.michigan.gov/mdard/0,4610,7-125-1569_16993-11250--,00.html Last accessed 4/18/2018.

Morroni M, Jacquemond M, and Tepfer M. 2013. *Deep sequencing of recombinant virus populations in transgenic and nontransgenic plants infected with Cucumber mosaic virus*. Molecular Plant-Microbe Interactions 26, pp. 801-811. Retrieved from http://apsjournals.apsnet.org/doi/abs/10.1094/MPMI-02-13-0057-R?url_ver=Z39.88-2003

Nauerby B, Billing K, and Wyndaele R. 1997. *Influence of the antibiotic timentin on plant regeneration compared to carbenicillin and cefotaxime in concentrations suitable for elimination of Agrobacterium tumefaciens*. Plant Science 123, pp. 169-177.

Nichols PD, Petrie J, and Singh S. 2010. *Long-chain omega-3 oils-an update on sustainable sources*. Nutrients 2, pp. 572-585. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22254042

NRCS. 2018. *PLANTS Database Brassica napus*. United States Department of Agriculture Natural Resources Conservation Service. Retrieved from https://plants.usda.gov/core/profile?symbol=BRNA Last accessed 4/18/2018.

OECD. 2011. *Revised consesus document on compositional considerations ofr new varieties of low erucic acid rapeseed (canola): key food and feed nutrients, anti-nutrients and toxicants* (OECD Environment, Health and Safety Publications Series on the Safety of Novel Foods and Feeds No. 24 ENV/JM/MONO(2011)55). Paris. Organization for Economic Co-Operation and Development.

OECD. 2012. *Consensus Document on the Biology of the Brassica Crops (Brassica spp.)*. OECD. Retrieved from http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2012)41&doclanguage=en

OGTR. 2011. *The Biology of Brassica napus L. (canola)*. Office of the Gene Technology Regulator Retrieved from http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/canola-3/$FILE/biologycanola08_2.pdf

OGTR. 2017. *The Biology of Brassica napus L. (canola) and Brassica juncea (L.) Czern. & Coss. (Indian mustard) Version 2.1*. Office of the Gene Technology Regulator of Australia. Retrieved from http://www.ogtr.gov.au

Perrins J, Williamson M, and Fitter A. 1992. *A survey of differing views of weed classification: implications for regulation of introductions*. Biological Conservation 60, pp. 47–56. Retrieved from http://www.sciencedirect.com/science/article/pii/000632079290798R

Peterson CD, Pearman DA, and Dines TD. 2002. *New Atlas of the British flora*. Oxford University Press.

Pivard S, Adamczyk K, Lecomte J, Lavigne C, Bouvier A, Deville A, Gouyon PH, and Huet S. 2008. *Where do the feral oilseed rape populations come from? A large-scale study of their possible origin in a farmland area*. Journal of Applied Ecology 45, pp. 476-485. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2664.2007.01358.x/full

Richards TA, Soanes DM, Foster PG, Leonard G, Thornton CR, and Talbot NJ. 2009. *Phylogenomic analysis demonstrates a pattern of rare and ancient horizontal gene transfer between plants and fungi*. Plant Cell 21, pp. 1897-1911. Retrieved from http://www.plantcell.org/content/21/7/1897.long

Richardson AO and Palmer JD. 2007. *Horizontal gene transfer in plants*. Journal of Experimental Botany 58, pp. 1-9. Retrieved from http://jxb.oxfordjournals.org/content/58/1/1.long

Rieger MA, Preston C, and Powles SB. 1999. *Risks of gene flow from transgenic herbicide-resistant canola (<emph type="2">Brassica napus</emph>) to weedy relatives in southern Australian cropping systems*. Australian Journal of Agricultural Research 50, pp. 115-128. Retrieved from http://www.publish.csiro.au/paper/A97138

Rieseberg LH and Wendel JF. 1993. *Introgression and its consequences in plants*.

Schafer MG, Ross AA, Londo JP, Burdick CA, Lee EH, Travers SE, Van de Water PK, and Sagers CL. 2011. *The establishment of genetically engineered canola populations in the U.S.* PLoS One 6, pp. e25736. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21998689

Simopoulos AP. 2016. *An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity*. Nutrients 8, pp. 128. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26950145

Soltis DE, Soltis PS, and Rieseberg LH. 1993. *Molecular Data and the Dynamic Nature of Polyploidy*. Critical Reviews in Plant Sciences 12, pp. 243-273. Retrieved from http://www.tandfonline.com/doi/abs/10.1080/07352689309701903#.UhekuRtwq-w

Stace CA. 1987. *Hybridization and the plant species*.

Sutherland S. 2004. *What makes a weed a weed: life history traits of native and exotic plants in the USA*. Oecologia 141, pp. 24-39. Retrieved from http://link.springer.com/article/10.1007%2Fs00442-004-1628-x

Tan S, Evans RR, Dahmer ML, Singh BK, and Shaner DL. 2005. *Imidazolinone-tolerant crops: history, current status and future*. Pest Manag Sci 61, pp. 246-257. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/15627242

Thompson JR and Tepfer M. 2010. *Assessment of the Benefits and Risks for Engineered Virus Resistance*. Advances in Virus Research 76, pp. 33-56. Retrieved from http://www.sciencedirect.com/science/article/pii/S0065352710760024

Tranel PJ, Wright TR, and Heap IM. 2018. *Mutations in herbicide-resistant weeds to ALS inhibitors*. Retrieved from http://www.weedscience.com Last accessed 4/19/2018.

Tripathi MK and Mishra AS. 2007. *Glucosinolates in animal nutrition: A review*. Animal Feed Science and Technology 132, pp. 1-27.

Turturo C, Friscina A, Gaubert S, Jacquemond M, Thompson JR, and Tepfer M. 2008. *Evaluation of potential risks associated with recombination in transgenic plants expressing viral sequences*. The Journal of General Virology 89, pp. 327-335. Retrieved from http://vir.sgmjournals.org/content/89/1/327.long

Tyler BM. 2007. *Phytophthora sojae: root rot pathogen of soybean and model oomycete*. Mol Plant Pathol 8, pp. 1-8. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/20507474

US-FDA. 1998. *Guidance for Industry: Use of Antibiotic Resistance Marker Genes in Transgenic Plants*. Retrieved from http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Biotechnology/ucm096135.htm

US-FDA. 2006. *Guidance for Industry: Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use*. Retrieved from http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Biotechnology/ucm096156.htm Last accessed 21 August 2013.

USDA-APHIS-BRS. 2018. *Petitions for Determination of Nonregulated Status*. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/petition-status Last accessed 10 August 2018.

USDA-APHIS. 2018. *Plant Pest Program Information*. Retrieved from http://www.aphis.usda.gov/plant_health/plant_pest_info/index.shtml Last accessed 8 August 2018.

USDA-ERS. 2012. Canola Production. Retrieved from https://www.ers.usda.gov/topics/crops/soybeans-oil-crops/canola.aspx

USDA-NASS. 2017. *Acreage - June 2017*. Washington, D.C. U.S. Department of Agriculture, National Agricultural Statistics Service. Retrieved from http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1000

USDA-NRCS. Striga asiatica (L.) Kuntze, Asiatic witchweed. In: *The PLANTS Database* (National Plant Data Center). Retrieved from http://plants.usda.gov/core/profile?symbol=STAS2 Last accessed 21 August 2013.

USDA-NRCS. 2018a. *PLANTS Database Raphanus raphanistrum*. Natural Resources Conservation Service, United States Deparment of Agriculture. Retrieved from https://plants.usda.gov/core/profile?symbol=RARA2 Last accessed 4/19/2018.

USDA-NRCS. 2018b. *PLANTS Profile Brassica nigra*. Natural Resources Conservation Service, United States Department of Agriculture. Retrieved from https://plants.usda.gov/core/profile?symbol=BRNI Last accessed 4/19/2018.

USDA-NRCS. 2018c. *PLANTS Profile for Raphanus sativus* Natural Resources Conservation Service, United States Department of Agriculture. Retrieved from https://plants.usda.gov/core/profile?symbol=RASA2 Last accessed 4/19/2018.

USDA-NRCS. 2018d. *PLANTS Profile for Brassica tournefortii* Natural Resources Conservation Service, United States Department of Agriculture. Retrieved from https://plants.usda.gov/core/profile?symbol=BRTO Last accessed 4/19/2018.

Warwick SI, Beckie HJ, and Hall LM. 2009a. *Gene flow, invasiveness, and ecological impact of genetically modified crops*. Annals of the New York Academy of Sciences 1168, pp. 72-99.

Warwick SI, Simard MJ, Legere A, Beckie HJ, Braun L, Zhu B, Mason P, Seguin-Swartz G, and Stewart CN, Jr. 2003. *Hybridization between transgenic Brassica napus L. and its wild relatives: Brassica rapa L., Raphanus raphanistrum L., Sinapis arvensis L., and Erucastrum gallicum (Willd.) O.E. Schulz*. Theor Appl Genet 107, pp. 528-539. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12721639

Weiss MJ, Knodel JJ, and Olson D. accessed 4/18/2018. Insect Pests of Canola. In: *Radcliffe's IPM World Textbook* (St. Paul, Minnesota: College of Food, Agriculture, and Natural Resources, University of Minnesota). Retrieved from https://ipmworld.umn.edu/weiss-canola-pests Last accessed 4/18/2018.

Williams IH. 1984. *The concentrations of air-borne rape pollen over a crop of oil-seed rape (Brassica napus L.)*. The Journal of Agricultural Science 103, pp. 353-357.

Williams IH, Martin A, and White R. 1987. *The effect of insect pollination on plant development and seed production in winter oil-seed rape (Brassica napus L.)*. The Journal of Agricultural Science 109, pp. 135-139.

Wood DW, Setubal JC, Kaul R, Monks DE, Kitajima JP, Okura VK, Zhou Y, Chen L, Wood GE, Almeida NF, Jr., Woo L, Chen Y, Paulsen IT, Eisen JA, Karp PD, Bovee D, Sr., Chapman P, Clendenning J, Deatherage G, Gillet W, Grant C, Kutyavin T, Levy R, Li MJ, McClelland E, Palmieri A, Raymond C, Rouse G, Saenphimmachak C, Wu Z, Romero P, Gordon D, Zhang S, Yoo H, Tao Y, Biddle P, Jung M, Krespan W, Perry M, Gordon-Kamm B, Liao L, Kim S,

Hendrick C, Zhao ZY, Dolan M, Chumley F, Tingey SV, Tomb JF, Gordon MP, Olson MV, and Nester EW. 2001. *The genome of the natural genetic engineer Agrobacterium tumefaciens C58*. Science 294, pp. 2317-2323. Retrieved from http://www.sciencemag.org/content/294/5550/2317.long

WSSA. 1998. *"Herbicide Resistance" and "Herbicide Tolerance" defined. (Technology Note)*. Weed Technology 12, pp. 789. Retrieved from http://www.jstor.org/stable/3989101 Last accessed 04/09/2013.

Xi Z, Wang Y, Bradley RK, Sugumaran M, Marx CJ, Rest JS, and Davis CC. 2013. *Massive mitochondrial gene transfer in a parasitic flowering plant clade*. PLoS Genetics 9. Retrieved from http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1003265

Xi Z, Bradley RK, Wurdack KJ, Wong K, Sugumaran M, Bomblies K, Rest JS, and Davis CC. 2012. *Horizontal transfer of expressed genes in a parasitic flowering plant*. BMC Genomics 13, pp. 227. Retrieved from http://www.biomedcentral.com/1471-2164/13/227

Yoshida S, Maruyama S, Nozaki H, and Shirasu K. 2010. *Horizontal Gene Transfer by the Parasitic Plant Striga hermonthica*. Science 328, pp. 1128. Retrieved from http://www.sciencemag.org/content/328/5982/1128.full

Yu X-B, Shen Y-Y, Cui Q-M, Chen Y, Sun W, Huang X-Z, and Zhu Y. 2018. *Silkworm (Bombyx mori) has the Capability to Accumulate C20 and C22 Polyunsaturated Fatty Acids*. European Journal of Lipid Science and Technology 120, pp. 1700268.

Zhu B, Lou MM, Xie GL, Zhang GQ, Zhou XP, Li B, and Jin GL. 2011. *Horizontal gene transfer in silkworm, Bombyx mori*. BMC genomics 12, pp. 248. Retrieved from http://www.biomedcentral.com/1471-2164/12/248