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CENTER FOR VETERINARY MEDICINE

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CVM Note to the File

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To: Administrative Record, BNF No. 000165

Subject: Event LBFLFK Canola

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Purpose

This document summarizes the Food and Drug Administration (FDA) Center for Veterinary Medicine's (CVM, we) evaluation of biotechnology notification file (BNF) number 000165. BASF Plant Science, L.P. (BASF) submitted a safety and nutritional assessment for a genetically engineered canola, transformation event LBFLFK (hereafter referred to as LBFLFK canola) and additional information afterwards. CVM evaluated the information in BASF's submissions to ensure that regulatory and safety issues regarding animal food derived from LBFLFK canola meal have been resolved prior to commercial distribution. FDA's Center for Food Safety and Applied Nutrition summarizes its evaluation of uses of LBFLFK canola in human food in a separate document.

In CVM's evaluation, we considered all of the information provided by BASF as well as publicly available information and information in the agency's files. Here we discuss the outcome of the consultation for animal food derived from LBFLFK canola meal, but do not intend to restate the information provided in the final consultation in its entirety.

Intended Effects

The first intended effect of the modification in LBFLFK canola is to alter the fatty acid composition of the oil derived from the new canola variety. To confer this trait, BASF introduced deoxyribonucleic acid (DNA) sequences for twelve genes encoding a series of

transmembrane proteins that convert oleic acid, which is normally present in canola seed, to omega-3 long-chain polyunsaturated fatty acids (LCPUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[1] In addition, a gene from *Arabidopsis thaliana* that encodes a modified acetohydroxy acid synthase protein with two amino acid substitutions (hereafter referred to as AHAS(At)) confers tolerance to imidazolinone herbicides.

Regulatory Considerations

The purposes of this evaluation are (1) to assess whether BASF has introduced into animal food derived from LBFLFK canola meal a substance requiring premarket approval as a food additive and (2) to determine whether use of the meal from the new plant variety in animal food raises other regulatory issues with respect to the Federal Food, Drug, and Cosmetic Act (FD&C Act).

The Environmental Protection Agency (EPA) regulates herbicides under the FD&C Act and the Federal Insecticide, Fungicide, and Rodenticide Act. Under EPA regulations, the herbicide residues in LBFLFK canola are considered pesticide residues.

Genetic Modification and Characterization

Introduced DNA and Transformation Method

BASF transformed hypocotyl segments of *Brassica napus* variety Kumily with plasmid LTM593-1qcz using disarmed *Agrobacterium rhizogenes*-mediated transformation.[2] BASF states that the transfer-DNA (T-DNA) region within the plasmid contained thirteen expression cassettes between the left and right border sequences. These included:[3]

- Cassette 1: *Delta-6-elongase* gene from *Physcomitrella patens* (D6E(Pp)) with regulatory elements, including an *unknown seed protein* promoter from *Vicia faba,* intron region of locus At1g01170 from *Arabidopsis thaliana*, and *California mosaic virus 35S* gene terminator.
- Cassette 2: *Delta-5-desaturase* gene from *Thraustochytrium* sp. (D5D(Tc)) with regulatory elements, including a *conlinin* gene promoter from *Linum usitatissimum,* intron region of locus At5g63190, and an *octopine synthase* gene terminator from *A. tumefaciens, octopine-type Ti plasmid pTi15955*.
- Cassette 3: *Delta-6-desaturase* gene from *Ostreococcus tauri* (D6D(Ot)) with regulatory elements, including a *sucrose-binding protein-related* gene promoter from *V. faba*, intron region of locus At1g65090, and *cathepsin D inhibitor* gene terminator from *Solanum tuberosum*.

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[1] Crude oil obtained from LBFLFK canola contained on average 4.88% alpha-linolenic acid, 0.31% stearidonic acid, 3.97% eicosapentaenoic acid, 2.14% docosapentaenoic acid, and 0.35% docosahexaenoic acid (as a percentage of the total fatty acids).

[2] The *A. rhizogenes* strain containing the SHA17 binary vector was described by Mankin, S.L., D.S. Hill, P.M. Olhoft, E. Toren, A.R. Wenck, L. Nea, L. Xing, J.A. Brown, H. Fu, and L. Ireland. 2007. Disarming and sequencing of *Agrobacterium rhizogenes* strain K599 (NCPPB2659) plasmid pRi2659. In vitro Cellular and Developmental Biology Plant: 43:521-535.

[3] Seed-specific promoters were used to drive expression of the genes for the fatty acid desaturases and elongases, whereas a ubiquitous promoter was used to drive expression of the modified *ahas(At)* gene in all tissues. The coding sequences of the genes included in the T-DNA region of plasmid LTM593-1qcz were optimized for codon usage in *B. napus* and higher enzyme expression.

- Cassette 4: *Delta-6-elongase* gene from *Thalassiosira pseudonana* (D6E(Tp)) with regulatory elements, including a *peroxiredoxin like protein (PXR)* gene promoter from *L. usitatissimum*, intron region of locus At1g62290, and *peroxiredoxin-like protein* (*PER1*) gene terminator from *A. thaliana*.
- Cassette 5: *Delta-12-desaturase* gene from *Phytophthora sojae* (D12D(Ps)) with regulatory elements, including the *napin A* gene promoter from *B. napus*, intron region of locus At5g63190, and *small subunit of ribulose bisphosphate carboxylase (rbcS) E9* gene terminator from *Pisum sativum*.
- Cassette 6: *Omega-3-desaturase* gene from *Pythium irregulare* (O3D(Pir)) with regulatory elements, including the promoter and terminator regions of *SETL* gene from *B. napus*.
- Cassette 7: *Omega-3-desaturase* gene from *Phytophthora infestans* (O3D(Pi)) with regulatory elements, including an *unknown seed protein* gene promoter from *V. faba*, intron region of locus At1g01170, and *35S* gene terminator from *California mosaic virus*.
- Cassette 8: second copy of D5D(Tc) gene with regulatory elements, including promoter and terminator regions of *SETL* gene from *B. napus*.
- Cassette 9: *Delta-4-desaturase* gene from *Thraustochytrium sp.* (D4D(Tc)) with regulatory elements, including promoter and terminator regions of the *Arcelin-5* gene from *Phaseolus vulgaris*.
- Cassette 10: second copy of O3D(Pir) gene with regulatory elements, including the *PXR* gene promoter from *L. usitatissimum*, intron region of *ARGONAUTE4* gene from *A. thaliana*, and the *PER1* gene terminator from *A. thaliana*.
- Cassette 11: *Delta-4-desaturase* gene from *Pavlova lutheri* (D4D(Pl)) with regulatory elements, including the *conlinin* gene promoter from *L. usitatissimum*, intron region of locus At1g65090, and *octopine synthase* gene terminator from *A. tumefaciens, octopine-type Ti plasmid pTi5955*.
- Cassette 12: *Delta-5-elongase* from *O. tauri* (D5E(Ot)) gene with regulatory elements, including the *fatty acid elongase* (*FAE1.1*) gene promoter from *B. napus*, intron region of locus At1g62290, and the *fatty acid elongase (FAE1)* gene terminator from *A. thaliana*.
- Cassette 13: *ahas(At)* gene with regulatory elements, including promoter and intron of the *ubiquitin* (*ubi4-2*) gene from *Petroselinum crispum* and the *ahas* gene terminator from *A. thaliana*.

Following transformation, explants were grown in selection medium[4], plants were then regenerated and grown to maturity. Initial transformants (T_0) and subsequent T_1 and T_2 generations, produced by selfing, were evaluated for presence of inserted T-DNA, absence of vector backbone, fatty acid profile, and herbicide tolerance.

BASF characterized the number of T-DNA inserts, the organization of each insert that is present in LBFLFK canola, and the absence of vector backbone sequences using whole genome sequencing (WGS) with a minimum average read depth of 160-fold across the T-DNA inserts. Genomic DNA from the host variety, Kumily, was also sequenced using WGS and was used as the comparator. Locus-specific polymerase chain reaction (PCR) and

[4] The selection media contained imazethapyr for selection of transformants and carbenicillin for inhibition of *A. rhizogenes*.

Sanger sequencing were also used to confirm the organization and integrity of the inserted nucleotide sequences. BASF concludes that LBFLFK canola contains two intact DNA inserts that are identical to the T-DNA region of plasmid LTM593-1cq, with the exception of small deletions at the ends of both the right and left borders. These deletions did not affect the integrity of the expression cassettes. BASF states that insert1 introduced an 8-base pair (bp) deletion in chromosome "Cnn random" and insert2 introduced a 31-bp deletion in chromosome "C03" of the host genome.[5] Each of the expression cassette sequences are identical to those in the vector except for three single nucleotide changes which were shown not to alter amino acid sequence or had no impact on the function or activity of the enzyme.[6] BASF concludes that sequences from the backbone region of the vector are not present in LBFLFK canola.

The stabilities of the inserted T-DNA sequences in LBFLFK canola across three self-pollinated generations (T_3 through T_5) were evaluated using WGS followed by bioinformatics analyses. In addition, BASF assessed inheritance in second and third generation hemizygous plants using real-time PCR assays, concluding that the desired genotype segregated as two independent loci according to expected Mendelian principles.

BASF performed bioinformatics analyses using the nucleotide sequences obtained for both T-DNA inserts and host genomic junction sequences to determine whether insertion of the introduced DNA created any potential open reading frames (ORFs) that could encode for putative polypeptides. BASF evaluated the putative polypeptides against the National Center for Bioinformatics Information GenBank® non-redundant protein sequence database to determine the similarity of the putative polypeptides to known toxins and anti-nutrients. Based on the results of bioinformatics analyses, BASF concludes that these putative polypeptides do not raise animal food safety concerns.

Protein Safety

BASF used a weight of evidence approach to demonstrate that the introduced proteins do not raise safety concerns. BASF used bioinformatics tools to show that the amino acid sequences of the proteins expressed in LBFLFK canola match the amino acid sequence of the proteins that are expressed in the donor organisms. BASF evaluated the safety of the donor organisms, including pathogenicity[7] and production of toxins[8] and anti-nutrients, and prevalence of the donor organisms in animal food. BASF concludes that pairwise analyses show the amino acid sequences of the expressed proteins are identical, with the three exceptions noted above, to the proteins produced by the donor organisms and that these organisms do not produce toxins or anti-nutrients that would raise safety concerns.

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[5] Short sequence deletions, primarily in the border region sequences, are common during *Agrobacterium*-mediated transformation.

[6] These included a single amino acid change in the translated D12D(Ps) protein in insert1 and another single amino acid change in the translated D4D(Pl) protein in insert2.

[7] BASF states that *Phytophthora sojae*, *Pythium irregulare*, and *P. infestans* are soil borne plant pathogens; however, they are not known to cause disease in humans or animals.

[8] BASF notes that *T. pseudonana* is a marine diatom that can produce the neurotoxin beta-N-methylamino-L-alanine. However, BASF highlights that this diatom is not known to cause disease in animals and has been safely used in aquaculture diets for several marine organisms.

BASF also characterized the proteins that make up the introduced fatty acid biosynthetic pathway, including three methyl-end desaturases (D12D(Ps), O3D(Pir) (2 copies), and O3D(Pi)), four front-end desaturases (D6D(Ot), D5D(Tc) (2 copies), D4D(Tc), and D4D(Pl)), and three KS5-family elongation-type ketoacyl synthases (D5E(Ot), D6E(Pp), and D6E(Tp)) that are expressed in LBFLFK canola.[9] BASF notes that there were single amino acid changes in the D12D(Ps) and D4D(Pl) proteins and concludes these two amino acid substitutions did not affect substrate specificity for these enzymes. Additionally, BASF notes that the D6E(Tp) gene in the transformation plasmid encoded for another single amino acid change when compared to the protein expressed in *T. pseudonana*. However, BASF reports both proteins have the same structural motifs, suggesting that they would have the same substrate specificity. Several methodologies were used to characterize the identity and safety of the expressed proteins.[10]

Methyl-end Desaturases

Methyl-end desaturases introduce a double bond between an existing double bond and the methyl end of a fatty acid. Methyl-end desaturases are characterized by the presence of transmembrane regions, three histidine-rich structural motifs, and a conserved histidine following the C-terminal transmembrane region. BASF concludes that the deduced amino acid sequences of D12D(Ps), O3D(Pir), and O3D(Pi) contain these structural motifs. When the three proteins were expressed in yeast, each protein catalyzed the anticipated desaturation reaction.[11] BASF notes that O3D(Pi) was not quantifiable using Western blot analysis and mass spectrometry in any tissue sample obtained from LBFLFK canola and, thus, identity and biochemical studies on the plant produced protein were not conducted. BASF demonstrates that the two other methyl-end desaturases are not glycosylated in LBFLFK canola. BASF used *in vitro* assays to show that these two proteins were rapidly cleaved in simulated gastric and intestinal fluids. Additionally, BASF showed that these proteins were heat inactivated (protein aggregation) within five minutes at ≥50°C. BASF reports that protein isolates derived from LBFLFK canola catalyzed the desaturation of oleic acid into linoleic acid (activity consistent with that of D12D enzymes) and arachidonic acid into eicosapentaenoic acid (activity consistent with that of O3D enzymes), which was not observed in protein isolates derived from the host variety, Kumily.

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[9] Fatty acid desaturases catalyze the removal of two hydrogen atoms from the hydrocarbon chain of a fatty acid to form a double bond.

[10] Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), Western blot analysis, glycoprotein staining, mass determination of the tryptic peptides by matrix assisted laser desorption ionization mass spectrometry (MALDI-MS), N-terminal amino acid sequence analysis, and *in vitro* and *in vivo* biochemical studies. No adverse signs of toxicity were reported in a 28-day repeated dose study where rats were orally gavaged with oil derived from LBFLFK canola (up to 1.24 grams of LCPUFA/kilogram of body weight/day) as cited in: Andre, C., R. Buesen, B. Riffle, C. Wandelt, J.B. Sottosanto, H. Marxfeld, V. Strauss, B. van Ravenzwaay, and E.A. Lipscomb. 2019. Safety assessment of EPA+DHA canola oil by fatty acid profile comparison to various edible oils and fat-containing foods and a 28-day repeated dose toxicity study in rats. Food Chem. Toxicol. 124:168-181.

[11] Yilmaz, J.L., Z.L. Lim, M. Beganovic, S. Breazeale, C. Andre, S. Stymne, P. Vrinten, and T. Senger. 2017. Determination of substrate preferences for desaturases and elongases for production of docosahexaenoic acid from oleic acid in engineered canola. Lipids 52: 207-222.

Front-end Desaturases

Front-end desaturases introduce a double bond between an existing double bond and the carboxyl end of a fatty acid. Front-end desaturases possess an N-terminal cytochrome b5 domain and three conserved histidine-rich structural motifs. BASF states that the deduced amino acid sequences of D6D(Ot), D5D(Tc), D4D(Tc), and D4D(Pl) contain these structural motifs. When these proteins were expressed in yeast, each protein catalyzed the anticipated desaturation reaction.[11] BASF notes that D5D(Tc) expression was below the lowest limit of quantitation (LOQ) for Western blot analysis and mass spectrometry in mature LBFLFK canola seed samples and below LOQ in immature seed and, thus, identity and biochemical studies on the plant produced protein were not conducted. BASF demonstrates that the D6D and D4D (assay cannot distinguish between the two D4D enzymes). Front-end desaturases were not glycosylated in LBFLFK canola. BASF showed using *in vitro* assays, that these proteins were rapidly cleaved in simulated gastric and intestinal fluids. Additionally, BASF showed that the D6D(Ot) protein was heat inactivated (as evidenced by protein aggregation or degradation) within five minutes at ≥50°C and D4D proteins were inactivated within 20 minutes at ≥50°C. BASF reports that protein isolates derived from LBFLFK canola catalyzed the desaturation of linoleic acid into gamma linolenic acid (D6D) and docosapentaenoic acid into docosahexaenoic acid (D4D), which is consistent with the activity of these enzymes, which was not observed in protein isolates derived from Kumily.

KS5-family elongation-type ketoacyl synthases

Fatty acid ketoacyl synthases are components of fatty acid synthase complexes that catalyze the addition of two carbon units to a fatty acid chain. The elongation-type ketoacyl synthases have four highly conserved structural motifs, which are different from the structural motifs found in desaturases. BASF states that the deduced amino acid sequences of D5E(Ot), D6E(Pp), and D6E(Tp) contain these structural motifs. BASF notes that D6E(Pp) was not quantifiable using Western blot analysis and mass spectrometry in seed samples obtained from LBFLFK canola and, thus, identity and biochemical studies on the plant produced protein were not conducted. BASF demonstrated that the D5E(Ot) and D6E(Tp) proteins were not glycosylated in LBFLFK canola. BASF showed using *in vitro* assays that these proteins were cleaved in simulated gastric and intestinal fluids. Additionally, BASF showed that the D6E protein was largely inactivated within 5 minutes at ≥50°C and the D5E protein underwent aggregation within 20 minutes at ≥50°C and the monomeric full-length protein was decreased after 20 minutes at ≥50°C. When expressed in yeast, the proteins catalyzed the elongation of gamma linolenic acid into dihomo gamma linolenic acid (both of the D6E proteins) and eicosapentaenoic acid into docosapentaenoic acid (D5E).[11] BASF reports that protein isolates derived from LBFLFK canola catalyzed the elongation of gamma linolenic acid into dihomo gamma linolenic acid (D6E(Tp)) and eicosapentaenoic acid into docosapentaenoic acid (D5E(Ot)), which is consistent with activity of D6E and D5E enzymes, which was not observed in protein isolates derived from Kumily.

AHAS(At)

BASF highlights that AHAS enzymes are ubiquitous in all plants and microbes and the AHAS(At) protein is 88.2% identical to the canola AHAS protein. BASF demonstrated

that the protein was not glycosylated in LBFLFK canola. BASF showed using *in vitro* assays that the protein was cleaved in simulated gastric and intestinal fluids. The AHAS(At) protein was shown to be heat inactivated within 20 minutes at 50°C. BASF also states that the safety of the AHAS(At) protein in herbicide tolerant crops has been investigated and no reports of adverse effects due to exposure to AHAS enzymes have been reported.[12]

Expression Levels of Proteins in LBFLFK Canola

BASF quantified the amounts of the proteins that were introduced into LBFLFK canola. Samples of whole plants at different maturity stages, leaf tissue, root tissue, immature seed, mature seed, and pollen were obtained from LBFLFK canola plants, grown with and without imazamox herbicide treatment, and Kumily from four field trial sites planted in 2015 in the United States. At each location, samples from five herbicide treated and five untreated plants and one control Kumily plant were collected non-systematically and each tissue type was homogenized into powdered samples prior to analysis. The amounts of D12D(Ps), D6E(Pp), D5D(Tc), and D5E(Ot) present in the samples were determined by enzyme linked immunosorbent assay (ELISA) and the amounts of D6D(Ot), D6E(Tp), O3D(Pir), O3D(Pi), D4D(Pl), D4D(Tc), and AHAS(At) were determined using a capillary-based quantitative Western blot method. BASF states that the AHAS(At) protein was quantifiable in LBFLFK canola in every tissue except mature seed.[13] Eight of the fatty acid biosynthesis enzymes were detected in developing and/or mature seed obtained from LBFLFK canola and not in any other tissues. D6E(Pp) and O3D(Pi) were below the LOQ in immature and mature seed. The D6E(Tp) and O3D(Pir) proteins were present in the highest abundance in mature seed obtained from LBFLFK canola and were present at approximately 920 and 530 micrograms/gram dry weight, respectively. The other desaturase and elongase proteins were present in mature seeds at less than 50 microgram/gram dry weight. None of these proteins were present in the sample obtained from Kumily.

BASF concludes that the safety of the expressed proteins has been demonstrated through a weight of the evidence approach based on the presence of the protein or related proteins in animal food, bioinformatic analyses that show the proteins do not have similarity to known toxins and anti-nutrients, cleavage of the proteins when added to simulated gastric or intestinal fluids, denaturation of the proteins when exposed to heat[14], substrate specificity when proteins are expressed in yeast and/or in LBFLFK canola, and low expression levels of these proteins in LBFLFK canola seeds.

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[12] Mathesius, C., J. Barnett, R. Cressman, J. Ding, C. Carpenter, G. Ladics, J. Schmidt, R. Layton, J. Zhang, and L. Appenzeller. 2009. Safety assessment of a modified acetolactate synthase protein (GM-HRA) used as a selectable marker in genetically modified soybeans. Regul. Toxicol. Pharmacol. 55: 309-320.; Chukwudebe, A., L. Privalle, A. Reed, C. Wandelt, D. Contri, M. Dammann, S. Groeters, U. Kaspers, V. Strauss, and B. van Ravenzwaay. 2012. Health and nutritional status of Wistar rats following subchronic exposure to CV127 soybeans. Food Chem. Toxicol. 50: 956-971.

[13] The AHAS(At) protein was present at approximately 13 micrograms/gram dry weight in immature seeds. BASF notes that one mature seed sample did contain quantifiable levels, but in all other mature seed samples, levels were below the limits of quantitation.

[14] BASF argues that the newly expressed proteins will not remain intact after the conditions of commercial processing used to produce oil and meal.

Animal Food Use

Canola (developed from *B. napus* and *B. rapa* varieties) refers to rapeseed varieties that contain low levels of erucic acid and glucosinolates. Canola is used primarily to produce oil for human food. Canola oil is low in saturated fatty acids and high in mono- and di-unsaturated fatty acids and is commonly used as cooking oil for frying, baking, and other food applications. Canola meal is a byproduct of oil crushing. The majority of canola meal is used in animal food, primarily for cattle and pigs, and, to a lesser extent, poultry, aquaculture, lamb, and other livestock. Industrial uses of canola are limited.

BASF states it does not intend to use LBFLFK canola oil or whole seed in animal food in the United States. However, BASF indicates that meal derived from the production of LBFLFK canola oil will be used in animal food in the United States in the same manner as canola meal from other varieties.

Composition

Scope of Analysis

BASF determined whether there were any unintended changes in the nutrient composition of seed derived from LBFLFK canola grown with and without imazamox herbicide treatment when compared to Kumily (control), and six conventional canola varieties (hereafter referred to as conventional varieties) that were grown and harvested under similar conditions. The selected analytes were based on the Organisation for Economic Cooperation and Development (OECD) canola composition consensus document.[15]

Study Design

BASF conducted field trials in 2014/2015 during two growing seasons. There were five locations in the southern United States for the 2014/2015 winter season and seven locations in the northern United States for the 2015 spring season. The canola varieties were planted using a randomized complete block design with four replicate plots at each field site. The middle rows of each plot were threshed and seed placed in separate bags. Subsequently, seed samples were cleaned, sub-sampled, milled, and frozen prior to compositional analysis.

BASF statistically compared each component for LBFLFK canola (treated with the herbicide (treated) or not treated) and the control across locations using a linear mixed model analysis of variance. Separate statistical analyses were conducted for each season and treated and not treated. BASF indicated a limited number of outliers were removed from the dataset and, in some cases, data were transformed prior to statistical analysis. BASF indicated that 112 components were measured in canola seed. When a value for a component was less than the limit of quantitation (LOQ) for the analytical method, a value equal to half the LOQ was assigned to this sample. If the resulting mean was less than the LOQ, then the mean was reported as less than the LOQ and a statistical analysis was not performed on this component (43 and 48 components for winter and

[15] Organisation for Economic Cooperation and Development. 2011. Revised consensus document on compositional considerations for new varieties of low erucic acid rapeseed (canola): Key food and feed nutrients, anti-nutrients, and toxicants. Series on the safety of novel foods and feeds No. 24. ENV/JM/MONO(2011)55. OECD, Paris.

summer seasons, respectively). In addition, BASF reports there were fatty acid values related to the intended effect that were above the LOQ in LBFLFK canola samples, but not in the other canola varieties and these data were not suitable for statistical analysis (means and ranges were provided). Student's paired T-test comparisons were used to test at the level of $P \leq 0.05$ for differences between LBFLFK canola (treated or not treated) and control. Any observed differences between LBFLFK canola and control seed were compared with ranges for the conventional varieties that were grown at the same locations, values for canola samples that are listed in the International Life Sciences Institute (ILSI) Crop Composition Database version 6[16] or in the scientific literature.

BASF also conducted a field trial in 2016 for compositional analysis of processed fractions of canola (solvent-extracted meal and oil). This trial was conducted at five locations in the United States and there was a single plot of each treatment (herbicide treated LBFLFK canola, control, and three varieties currently grown in the United States) at each location. One bulk sample of threshed and cleaned seed from each plot was processed into pressed oil, crude oil, refined bleached and deodorized oil, and solvent-extracted meal using industrial conditions similar to what would be used for commercial production of oil and solvent-extracted meal from LBFLFK canola. A sample from each plot obtained prior to processing was analyzed for relevant components. LBFLFK canola and the control were compared using T-tests at the level of $P \leq 0.05$.

Results of Analyses - Seed

BASF reported there were no statistically significant differences in crude protein concentrations between LBFLFK canola (treated or not treated) and the control in either season. There were no statistically significant differences for any of the 19 amino acids (18 most commonly measured amino acids plus hydroxyproline) between LBFLFK canola (treated or not treated) and the control in seed samples collected during the winter season and for most of the amino acids for samples collected over the spring season. The concentration of aspartic acid was numerically higher and leucine was numerically lower in samples obtained from LBFLFK canola when compared to the control, irrespective of season. However, when statistical differences were observed, BASF stated that numerically the values were similar and the mean values for each of the amino acids in LBFLFK canola fell within the range of values reported for the conventional varieties grown under similar conditions.

BASF reported there were no statistically significant differences between LBFLFK canola (treated or not treated) and the control in the winter season for crude fiber, acid detergent fiber, and neutral detergent fiber values. These components were significantly lower in treated and not treated samples obtained from LBFLFK canola in the spring season when compared to the control. However, the mean values for LBFLFK canola fell within the range of values obtained from the conventional varieties grown under similar conditions.

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[16] The ILSI Crop Composition Database has become the Agriculture and Food Systems Institute Crop Composition Database, and is available at www.cropcomposition.org.

BASF reported there were no statistically significant differences in the values for ash and several minerals in seed obtained during either season for LBFLFK canola when compared to the control. There was a small, but statistically significant, difference in phosphorus content between LBFLFK canola that was not herbicide treated and the control in samples planted in the spring. BASF reported statistically lower values for calcium and magnesium in seed obtained during both seasons from LBFLFK canola (treated or not treated) when compared to the control. BASF highlighted that the mean values for all of the minerals in LBFLFK canola fell largely within the range of values obtained from the conventional varieties grown under similar conditions and these values fell within the ranges reported in the ILSI Crop Composition Database. BASF concludes that these differences do not have any biological significance.

BASF also reports results for 12 glucosinolates, total glucosinolates, and other anti-nutrients that are present in canola seed. The values for seven of the glucosinolates were less than 1.0 micromoles/gram dry weight. Total glucosinolates were slightly, but statistically, higher in seed obtained from LBFLFK canola (not treated) when compared to the control in the winter and significant differences were again observed for treated and not treated LBFLFK canola in the spring season. The differences in total glucosinolates were primarily due to higher levels of glucoalyssin, glucobrassicin, glucobrassicanapin, and gluconapin (treated sample only) in samples grown during the winter season and higher levels of glucobrassicin and gluconapin in samples grown in the spring season. The mean values for total glucosinolates in samples obtained from treated and not treated LBFLFK canola during both seasons fell within the range of values obtained from the conventional varieties grown under similar conditions.

There were no significant differences in the concentrations of phytic acid and ferulic acid in LBFLFK canola (treated or not treated) when compared to the control. Levels of *p*-coumaric acid were significantly lower in LBFLFK canola (not treated) grown in the spring season when compared to the control and, in most cases, the values in LBFLFK canola were less than LOQ. Sinapine was slightly, but significantly, lower in seed obtained from LBFLFK canola (treated or not treated) in both seasons when compared to the controls. The levels of total phytosterols and 14 individual species of this component were measured. Although there were some statistically significant differences for individual phytosterols between LBFLFK canola and control with respect to season and herbicide treatment, the differences were small (less than 10% difference in total phytosterols in LBFLFK canola (treated or not treated) in both seasons when compared to control). BASF concludes that the mean values for all of these components in seed, regardless of season or herbicide treatment, obtained from LBFLFK canola were within the range of values obtained from the conventional varieties grown under similar conditions or in the ILSI Crop Composition Database. BASF also concludes that any differences in anti-nutrient and secondary metabolite levels in LBFLFK canola are not biologically relevant as they fall within the range of natural variation in canola varieties.

Intended Effect – Modification of Fatty Acid Profile

BASF highlights that canola is produced as a crop primarily for its oil, which represents about 38% of the seed and is high in monounsaturated fatty acids and low in saturated fatty acids. BASF reported that crude fat levels were not statistically different between seed obtained from LBFLFK canola (treated or not treated) in either growing season

when compared to the control. BASF analyzed the fatty acid profile of oil derived from LBFLFK canola seed, Kumily, and the conventional varieties and reports the results for 39 different fatty acid isomers plus total fatty acids and subsequently provided data for additional fatty acid isomers plus total fatty acids in refined, bleached, and deodorized oil that was derived from these varieties.[17] BASF states that the percentage of total saturated fatty acids (~9%) and erucic acid (<LOQ) were low in seed obtained from LBFLFK canola and were similar to the values for these components in the control and the conventional varieties. The percentage of total monounsaturated fatty acids was significantly lower in seed obtained from LBFLFK canola (~35%) when compared to the control (~64%) and the conventional varieties (~66%), primarily due to a decrease in oleic acid. BASF also highlights that (6Z,9Z)-octadecadienoic, gamma linolenic, stearidonic, (8Z,11Z)-eicosa-8,11-dienoic acid, dihomo gamma-linolenic, eicosatrienoic, mead, bishomostearidonic, arachidonic, EPA, adrenic, (10Z,13Z,16Z,19Z)-docosa-10,13,16,19-tetraenoic acid, clupanodonic (DPA), osbond, and DHA acids were quantifiable in LBFLFK canola, but not in the control. BASF concludes that, with the exception of the intended changes in fatty acid composition, LBFLFK canola seed is not materially different in composition from seed from other canola varieties.

Results of Analyses – Processed Products (Oil and Solvent-extracted Meal)

BASF noted that the fatty acid profile of oil derived from LBFLFK canola and control were very similar to the fatty acid profile for seed obtained from LBFLFK canola and the control. As described above, BASF provides results of compositional analyses of crude and refined, bleached, and deodorized oils. BASF states that oil derived from LBFLFK canola is currently not intended for use in animal food in the United States. Thus, data for the individual fatty acids in LBFLFK canola oil are not summarized in this memo.

BASF states that a total of 61 components were assessed in the solvent-extracted meal. As would be expected after processing, there were substantial reductions in crude fat and total fatty acids levels in solvent-extracted meals derived from LBFLFK canola and the control, with crude fat only being about 1% of meal dry matter. BASF reported that there were no significant differences between LBFLFK canola and control meals in the levels of moisture, crude protein, ash, carbohydrates by calculation, crude fiber, acid detergent fiber, and neutral detergent fiber. There were also no significant differences in the mean values for 16 of the amino acids. The mean values for histidine and cystine were slightly, but statistically, lower in LBFLFK canola meal when compared to the control, but the mean values for these amino acids in LBFLFK canola fell within ranges reported in the scientific literature.

The levels of calcium were statistically lower and those for potassium statistically higher in meal derived from LBFLFK canola when compared to the control, whereas the levels of phosphorus, magnesium, sodium, iron, zinc, copper, and manganese were not statistically different. The values for calcium in LBFLFK canola meal fell within the range reported in the scientific literature. Potassium in both LBFLFK canola and the

[17] Andre, C., R. Buesen, B. Riffle, C. Wandelt, J.B. Sottosanto, H. Marxfeld, V. Strauss, B. van Ravenzwaay, and E.A. Lipscomb. 2019. Safety assessment of EPA+DHA canola oil by fatty acid profile comparison to various edible oils and fat-containing foods and a 28-day repeated dose toxicity study in rats. Food Chem. Toxicol. 124:168-181.

control meals was slightly higher than the range of values obtained for the conventional varieties grown under similar conditions and higher than the literature range.

BASF reports that eight of the 12 glucosinolates that were analyzed were present at less than one micromole/gram in meals derived from LBFLFK canola, the control, and conventional varieties. The concentrations of progoitrin and 4-hydroxygluglucobrassin were statistically lower and glucoalyssin and gluconapin were numerically lower in meal from LBFLFK canola when compared to the control. BASF notes that all samples contained less than 30 micromoles of total glucosinolates/gram in meals from LBFLFK canola and the control. There were no statistically significant differences between the varieties in the levels of phytic acid and tannins. The concentrations of sinapine, ferulic acid, and *p*-coumaric acid were statistically lower in meal derived from LBFLFK canola when compared to the control and the mean values were slightly lower than those reported for the conventional varieties. BASF concludes that the composition of solvent-extracted meal derived from LBFLFK canola is comparable to that of solvent-extracted meal derived from conventional varieties of canola, and processing did not affect the nutritional value of LBFLFK canola. BASF states that solvent-extracted meal derived from LBFLFK canola can be used for animal food in the same manner as meal from any commercial canola variety.

Summary of Compositional Analyses

BASF states that, with the exception of the intended changes in fatty acid composition, LBFLFK canola is not materially different in composition, safety, or any other relevant parameter from other canola varieties currently grown, marketed, and consumed in the United States. BASF states, “meal derived from LBFLFK canola is as safe for use in animal feed as conventional canola meal.”

Animal Food Labeling Considerations

It is a producer’s or distributor’s responsibility to ensure that labeling of the foods it markets meet applicable legal requirements, including disclosure of any material differences in the food. In evaluating the common or usual name appropriate for animal food ingredients derived from LBFLFK canola meal, CVM considered that this new canola variety was genetically engineered to synthesize omega 3 long-chain polyunsaturated fatty acids, and that the intended use in animal food is as solvent-extracted meal. CVM recognizes that when used in animal food, the appropriate name for solvent-extracted LBFLFK canola meal is “canola meal”.

Conclusion

CVM evaluated BASF’s submissions to determine whether solvent-extracted meal derived from LBFLFK canola raises any safety or regulatory issues with respect to its uses in animal food. BASF states that meal derived from LBFLFK canola, and not the whole seed or oil derived from LBFLFK canola, will be used in animal food in the United States. Based on the information provided by BASF and other information available to the agency, CVM did not identify any safety or regulatory issues under the FD&C Act with respect to the use of solvent-extracted meal derived from LBFLFK canola that would require further evaluation at this time. Should BASF change its intended uses to

include other products derived from LBFLFK canola in animal food in the United States, we recommend BASF contact CVM's Division of Animal Food Ingredients.

BASF concludes that meal derived from LBFLFK canola and the animal foods derived from it are as safe as and, with the exception of the fatty acid profile, are not materially different in composition or relevant parameter from meal derived from conventional canola varieties. At this time, based on BASF's data and information, CVM considers BASF's consultation on solvent-extracted meal derived from LBFLFK canola for use in animal food to be complete.

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