FDA U.S. FOOD & DRUG ADMINISTRATION
CENTER FOR VETERINARY MEDICINE

Biotechnology Notification File No. 000177
CVM Note to the File

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From: Jing Ning, Ph.D.

To: Administrative Record, BNF No. 000177

Subject: Event MON 94100 Canola

Keywords: Canola, *Brassica napus,* modified dicamba mono-oxygenase, *Stenotrophomonas maltophilia* strain DI-6, herbicide tolerant, dicamba, OECD Unique Identifier: MON-941ØØ-2, Monsanto, Bayer CropScience LP

Purpose

This document summarizes the Food and Drug Administration (FDA) Center for Veterinary Medicine's (CVM, we) evaluation of biotechnology notification file (BNF) number 000177. Bayer CropScience LP (Bayer)[1] submitted a safety and nutritional assessment for a genetically engineered (GE) canola, transformation event MON 94100 (hereafter referred to as MON 94100 canola) and additional information afterwards. CVM evaluated the information in Bayer's submissions to ensure that regulatory and safety issues regarding animal food derived from MON 94100 canola have been resolved prior to commercial distribution. FDA's Center for Food Safety and Applied Nutrition summarizes its evaluation of MON 94100 canola in human food in a separate document.

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

Intended Effects

The intended effect of the modification in MON 94100 canola is to confer tolerance to the herbicide dicamba. To confer this trait, Bayer introduced a modified dicamba mono-oxygenase gene (*dmo*) from *Stenotrophomonas maltophilia*.

[1] Monsanto Company submitted the notice for BNF No. 000177. In a letter dated August 3, 2020, FDA was informed that Monsanto Company plant products "which were consulted on for food and feed safety and those still in the process" would be transferred to the legal entity Bayer CropScience LP, effective August 1, 2020.

Regulatory Considerations

The purposes of this evaluation are (1) to assess whether Bayer has introduced into animal food a substance requiring premarket approval as a food additive and (2) to determine whether use of 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 MON 94100 canola are considered pesticidal residues.

Genetic Modification and Characterization

Introduced DNA and Transformation Method

Bayer states that MON 94100 canola was developed through disarmed *Agrobacterium tumefaciens* mediated transformation of parental variety 65037 hypocotyls with plasmid vector PV-BNHT508701.[2] PV-BNHT508701 contained single copies of the T-DNA I and T-DNA II cassettes. Bayer states that both T-DNA cassettes were delineated by left and right T-DNA border regions. The T-DNA I cassette (T-DNA I) contained the *dmo* coding sequence whose expression is regulated by the promoter from the peanut chlorotic streak caulimovirus, the 5' UTR sequence from tobacco etch virus, the chloroplast-targeting sequence (CTP) and 24 amino acids from the pea small subunit of the ribulose bisphosphate carboxylase (*RbcS*) gene, and the 3' UTR of a gene from *Medicago truncatula*. The T-DNA II cassette (T-DNA II) contained the *aadA* coding sequence under the control of an enhancer from the figwort mosaic virus 35S RNA, the *EF-1α* promoter, leader, and intron sequences from *Arabidopsis thaliana*, the CTP from *A. thaliana ShkG*, and 3' UTR from the pea *RbcS-E9* gene. This confers spectinomycin and streptomycin resistance which was used as a selectable marker for transformed hypocotyls. In addition, T-DNA II contained the *splA* coding sequence under the control of the 5' UTR leader, promoter, and enhancer sequences of a seed protein gene from *Vicia faba*, and the 3' UTR of nopaline synthase gene *(nos)* from *Agrobacterium tumefaciens* pTi.

Following transformation, hypocotyl segments were cultivated on selection media[3], and developed plants with normal phenotype were selected as the R0 generation. Bayer subsequently selected R1 plants containing only T-DNA I, but not T-DNA II by PCR (polymerase chain reaction) and sequencing analyses. R1 plants homozygous for the *dmo* cassette and negative for T-DNA II and vector backbone sequences were self-pollinated to obtain further generations. Additional breeding steps (up to seven generations) were conducted to generate plants used in the characterization of the genetic insertion, inheritance studies, and stability studies.

Bayer characterized the insertion event in MON 94100 canola using whole genome sequencing (WGS), junction sequence analysis (JSA), and directed sequencing. Bayer estimates that it collected sufficient data for WGS to cover the canola genome at least

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[2] Radke, S.E., J.C. Turner and D. Facciotti. 1992. Transformation and regeneration of *Brassica rapa* using *Agrobacterium tumefaciens*. Plant Cell Reports 11:499-505.

[3] The selection media contained spectinomycin for selection of transformants and carbenicillin for inhibition of *A. tumefaciens* growth.

75-fold.[4] Genomic DNA from the parental variety 65037 was used as the comparator. Bayer identified two unique junction sequences and concludes that MON 94100 canola contains a single copy of T-DNA I at a single chromosomal locus and does not contain T-DNA II nor plasmid backbone sequences. To confirm the sequences of the insert and the flanking genomic DNA, Bayer performed directed sequencing on PCR products amplified from the insertion site. Bayer concludes that MON 94100 canola contains an insert that is identical to T-DNA I of PV-BNHT508701, with an eight base pair (bp) genomic DNA deletion at the insertion site.

To demonstrate stability of the inserted DNA present in MON 94100 canola, Bayer performed WGS and JSA on four self-pollinated and one outcross generations. Bayer detected identical junction sequences in each of the generations tested. In addition, Bayer assessed inheritance in three backcrossed generations using Real-Time TaqMan PCR. The results of chi-square analysis of segregation data from these generations show the inheritance pattern of the insert is consistent with Mendelian principles for a single locus. Bayer concludes that the T-DNA I insertion in MON 94100 canola is heritable, stable, and was integrated at a single locus in the MON 94100 genome.

Bayer conducted open reading frame (ORF) bioinformatics analyses to assess the potential for toxicity or biological activity of putative polypeptides encoded by all six reading frames present in the MON 94100 canola insert, as well as for the ORFs present in the 5′ and 3′ flanking sequence junctions. These putative polypeptides were compared against known toxins in the TOX_2019 database.[5] Bayer found no relevant sequence similarities when these putative polypeptides were used as query sequences. Based on these bioinformatic analyses, Bayer concludes that the putative polypeptides are unlikely to be toxic or have adverse biological impacts.

Protein Safety

Identity and Function of Introduced Proteins

MON 94100 canola was genetically engineered to express a modified DMO protein derived from *Stenotrophomonas maltophilia*. DMO is an enzyme that catalyzes the demethylation of dicamba (3,6-dichloro-2-methoxybenzoic acid), thereby conferring dicamba tolerance to MON 94100 canola.

Bayer states that the inserted DNA produces a single DMO precursor protein, which is post-translationally processed into two forms due to alternative processing of the RbcS sequence. Bayer designates the two forms of the protein as DMO and DMO+27 (reflecting that this protein contains an additional 27 amino acids) and refers to both forms as MON 94100 DMO protein. Bayer states that MON 94100 DMO protein is

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[4] Kovalic, D., C. Garnaat, L. Guo, Y. Yan, J. Groat, A. Silvanovich, L. Ralston, M. Huang, Q. Tian, A. Christian, N. Cheikh, J. Hjelle, S. Padgette and G. Bannon. 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 5:149-163.

[5] The TOX_2019 database contains 34,642 sequences and was selected using a keyword search of the National Center for Biotechnology Information GenBank protein database and filtered to remove likely non-toxin proteins. Bayer states that the description of the toxin database TOX_2018 in a previous submission, BNF No. 000173, applies to the TOX_2019 database.

similar to the wild-type protein from *Stenotrophomonas maltophilia* strain DI-6, but includes an additional alanine at position 2, replacement of tryptophan with cysteine at position 112, and the amino acids derived from RbcS for DMO+27. Bayer notes that these changes would have minimal functional impact because they are sterically distant from the catalytic site.[6]

Bayer states that MON 94100 DMO protein is identical to the DMO protein previously evaluated in BNF No. 000125. The specificity of DMO is discussed in BNF No. 000125 and BNF No. 000173 and no endogenous compounds from canola with structural similarity to dicamba were found in the literature. Therefore, Bayer concludes that MON 94100 DMO protein has a high specificity for dicamba.

Protein Expression Level

In 2018, Bayer conducted five randomized complete block design field studies in the United States (U.S.) and Canada. Bayer analyzed samples of MON 94100 canola forage obtained prior to internode development, leaves at the 3-4 leaf stage, and mature seed from four replicates at these sites that were treated with dicamba herbicide. MON 94100 DMO protein levels were measured using an enzyme-linked immunosorbent assay (ELISA). The mean DMO concentrations (in micrograms/gram dry weight) in MON 94100 canola were 2.5 for leaf and forage and 0.64 for seed.

Potential for Toxicity of the Introduced Proteins

The safety of DMO proteins for their use in the U.S. has been previously evaluated in several plant varieties (BNF No. 000125, BNF No. 000135, BNF No. 000148, and BNF No. 000173) as has the safety of the donor organism, *Stenotrophomonas maltophilia*. Bayer states MON 94100 DMO protein is identical in structure of the catalytic site, function, immunoreactivity, and specificity to DMO proteins in previously evaluated submissions with the same or similar protein sequences. Therefore, Bayer concludes that safety assessments reported for DMO proteins and the donor organism in previous BNFs are directly applicable to DMO protein in MON 94100 canola.

Based on the information provided in previous BNFs and the information discussed above and in its submissions, Bayer concludes that DMO protein expressed in MON 94100 canola presents no meaningful risk to animal health.

Animal Food Use

Canola (developed from *B. napus*, *B. rapa* and *B. juncea* 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.

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[6] D'Ordine, R.L., T.J. Rydel, M.J. Storek, E.J. Sturman, F. Moshiri, R.K. Bartlett, G.R. Brown, R.J. Eilers, C. Dart, Y. Qi, S. Flasinski and S.J. Franklin. 2009. Dicamba monooxygenase: Structural insights into a dynamic Rieske oxygenase that catalyzes an exocyclic monooxygenation. Journal of Molecular Biology 392:481-497. Dumitru, R., W.Z. Jiang, D.P. Weeks and M.A. Wilson. 2009. Crystal structure of dicamba monooxygenase: A Rieske nonheme oxygenase that catalyzes oxidative demethylation. Journal of Molecular Biology 392:498-510.

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.

Composition

Scope of Analysis

Bayer analyzed the nutrient composition of seed from MON 94100 canola and the conventional variety (control) that were grown and harvested under similar conditions; however, MON 94100 canola was treated with dicamba herbicide. Compositional analyses of seed samples were reported for components listed in the Organisation for Economic Co-operation and Development (OECD) canola composition consensus document.[7]

Study Design

Bayer conducted field trials in 2018 at five locations in the U.S. and Canada. The canola varieties were planted using a randomized complete block design with four replicate plots at each field site. Bayer harvested seed from each replicate within each location.

Bayer statistically compared each component for MON 94100 canola and the control across locations using a linear mixed model. Components were expressed on a dry matter basis prior to statistical analysis and moisture was not included. Bayer excluded components from statistical analysis if more than 50% of the observed values were at or below the limit of quantitation (LOQ). T-test analyses were used to test at the level of $P \leq 0.05$ for differences between MON 94100 canola and control. When a statistically significant difference in a component was detected between MON 94100 canola and control, Bayer assessed whether the difference was biologically meaningful including comparisons of the MON 94100 canola means with ranges in the published literature and in the International Life Sciences Institute Crop Composition Database (ILSI-CCDB), version 7[8].

Results of analyses

Bayer reports results for proximates (crude protein, crude fat, ash, and carbohydrates by calculation), 18 amino acids, 21 fatty acids, fiber (acid detergent fiber and neutral detergent fiber), calcium, phosphorus, vitamin E, vitamin K1, and anti-nutrients (phytic acid, tannins, sinapine, total glucosinolates, total alkyl glucosinolates, and total indolyl glucosinolates) from seed for MON 94100 canola and control. Bayer notes that 10 components were not statistically analyzed as more than 50% of the samples had levels that fell below the analytical LOQ. Bayer states no statistically significant differences were found between MON 94100 canola and the control seed, except for sinapine. Bayer states that the mean value of sinapine in MON 94100 canola was within the ranges of values observed in the ILSI-CCDB. Bayer concludes that the difference in sinapine is not biologically meaningful and MON 94100 canola is compositionally equivalent to the

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[7] Organisation for Economic Co-operation 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. ENV/JM/MONO (2011)55. OECD, Paris, France.

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

conventional control in the levels of these seed components.

Summary of Compositional Analyses

Bayer states that the levels of nutrients and anti-nutrients did not differ significantly between MON 94100 canola and the control with one exception that Bayer considers not to be biologically meaningful for animal food safety. Therefore, Bayer concludes that seed from MON 94100 canola is compositionally comparable to the conventional control.

Conclusion

CVM evaluated Bayer's submissions to determine whether MON 94100 canola raises any safety or regulatory issues with respect to its uses in animal food. Based on the information provided by Bayer and other information available to the agency, CVM did not identify any safety or regulatory issues under the FD&C Act that would require further evaluation at this time.

Bayer concludes that MON 94100 canola and the animal foods derived from it are as safe as and are not materially different in composition or any other relevant parameter from other canola varieties now grown, marketed, and consumed. At this time, based on Bayer's data and information, CVM considers Bayer's consultation on MON 94100 canola for use in animal food to be complete.

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