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CENTER FOR VETERINARY MEDICINE

Biotechnology Notification File No. 000208
CVM Note to the File

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To: Administrative Record, BNF No. 000208

Subject: Event MON 80616 Corn

Keywords: Corn, Maize, *Zea mays* L., Protoporphyrinogen IX oxidase, *H_N90 PPO* gene, *Enterobacter cloacae*, Tolerance to PPO-inhibiting herbicides, OECD identifier: MON-8Ø616-9, Bayer CropScience LLC.

Purpose

This document summarizes the Food and Drug Administration (FDA) Center for Veterinary Medicine's (CVM, we) evaluation of biotechnology notification file (BNF) number 000208. Bayer CropScience LLC (Bayer) submitted a safety and nutritional assessment for a genetically engineered (GE) corn, transformation event MON 80616 (hereafter referred to as MON 80616 corn). CVM evaluated the information in Bayer's submissions to ensure that regulatory and safety issues regarding animal food derived from MON 80616 corn have been resolved prior to commercial distribution. FDA's Human Foods Program summarizes its evaluation of uses of MON 80616 corn 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 modifications in MON 80616 corn is to confer tolerance to protoporphyrinogen IX oxidase (PPO)-inhibiting herbicides. Bayer states that the parental corn variety HCL301 was transformed with the *H_N90 PPO* gene from *Enterobacter cloacae*, which encodes the PPO protein that confers tolerance to PPO-inhibiting herbicides.

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).

Genetic Modification and Characterization

Introduced DNA and Transformation Method

Bayer transformed mature embryos from conventional corn variety HCL301 with plasmid PV-ZMHT530724 using *Agrobacterium tumefaciens* mediated transformation. Bayer states that the transfer-DNA (T-DNA) region within plasmid PV-ZMHT530724 contains the following three expression cassettes between left and right border sequences. These include:

- *cp4 epsps*[1] selectable marker cassette.
- site directed integration (SDI) [*Cpf1* (*Cas12a*) and gRNA] cassette.
- *H_N90 PPO* gene from *E. cloacae* that was codon optimized for expression in corn, which is preceded by the promoter, 5´ untranslated region (UTR), and intron of a ubiquitin gene from *Andropogon gerardii* (big bluestem grass) and chloroplast targeting sequence of the *albino or pale green* 6 gene (*APG6*) from *Arabidopsis thaliana*. Following the *H_N90 PPO* gene is the 3´ UTR sequence from *Arundo donax* (Giant Cane).

The *cp4 epsps* selectable marker and SDI cassettes are flanked by *loxP* excision targeting sequences. Following transformation, explants were grown in selection medium[2], and developed plants were selected as the R0 generation. R0 plants were screened for the presence of T-DNA and absence of plasmid vector backbone sequences using real-time polymerase chain reaction (PCR) assay. Those R0 plants carrying a copy of the T-DNA were crossed with another corn line containing a *cre* recombinase cassette, generating F1 plants. Subsequently, F1 plants containing a copy of the T-DNA and Cre were self-pollinated to generate the F2 generation, which was then screened for the presence of T-DNA and absence of *cp4 epsps*, SDI, and *cre* cassettes.[3] Bayer states that only homozygous plants positive for T-DNA and negative for *cp4 epsps*, SDI, and *cre* cassettes were selected and subjected to additional molecular and phenotypic assessments, leading to the development of MON 80616 corn.

Bayer characterized the number of T-DNA inserts, the number of insert junctions, the absence of vector backbone sequences, and organization and intactness of the insert in MON 80616 corn using whole genome sequencing (WGS), PCR, and directed sequencing. The non-GE corn variety with a similar genetic background, HCL301, was used as the comparator in these analyses. Bayer reports a minimum average read depth of 75-fold. Bayer also reports that a single copy of the T-DNA sequence was inserted into the corn genome and that MON 80616 does not contain sequences from the vector backbone, the *cp4 epsps* selectable marker cassette, the SDI cassette from PV-

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[1] The *aroA* gene from *Agrobacterium* sp. strain CP4 that encodes CP4 5-enolpyruvylshikimate-3-phosphate synthase, conferring tolerance to glyphosate.

[2] The selection media contained glyphosate and carbenicillin disodium salt for inhibition of the growth of untransformed plant cells.

[3] Since the *cp4 epsps* selectable marker and SDI cassettes are flanked by *loxP* excision targeting sequences in the T-DNA, they are expected to be excised through Cre/lox-mediated auto-excision in F2 generation.

ZMHT530724, or any sequences from the Cre line transformation plasmid PV-ZMOO513642. Directed DNA sequencing demonstrated that the genetic elements within the inserted T-DNA were intact. A sequence comparison between the PCR products generated from the comparator HCL301 and MON 80616 corn indicated that the T-DNA insertion resulted in deletion of 19 base pairs of corn genomic DNA. Bayer states that these changes are probably the result of double stranded break repair during the *Agrobacterium*-mediated transformation process.

The stability of the inserted T-DNA sequences in MON 80616 across multiple breeding generations and the comparator HCL301 were evaluated by WGS followed by bioinformatics analyses. Bayer reports that the single locus of integration that was characterized in the F4 generation of MON 80616 is present in all five breeding generations of MON 80616. Within three back-crossed generations, Bayer collected genotypic segregation data to assess the inheritance pattern, and these data were analyzed using Chi square analysis. Bayer concludes that the inserted DNA segregated as a single locus in accordance with the principles of Mendelian inheritance.

Bayer performed bioinformatics analyses using the nucleotide sequences obtained for the T-DNA insert and junction sequences to determine whether insertion of the introduced DNA created any potential open reading frames that could encode for putative polypeptides. This analysis included nucleotide sequences for each of the six reading frames. Bayer evaluated the putative polypeptides against its toxin database, TOX_2025[4], to determine the similarity of these putative polypeptides to known toxins. Bayer reports that none of the putative polypeptides had significant identity with proteins in the TOX_2025 database. Based on the results of bioinformatics analyses, Bayer concludes that the T-DNA insertion does not lead to the production of putative polypeptides that would raise animal food safety concerns.

Protein Safety

MON 80616 corn was genetically engineered to express a HemG-type PPO protein encoded by the *H_N90 PPO* gene from *Enterobacter cloacae*. HemG-type PPO is a smaller PPO enzyme that catalyzes the oxidation of protoporphyrinogen IX to protoporphyrin IX, thereby conferring tolerance to PPO-inhibiting herbicides in MON 80616 corn by maintaining the oxidation of protoporphyrinogen IX to protoporphyrin IX.

To conduct the safety assessment of the PPO protein, Bayer produced the PPO protein in *Escherichia coli*. Bayer confirmed the identity and biochemical equivalence of the PPO protein expressed in *E. coli* and MON 80616 corn by comparing results from amino acid sequence, molecular weight and immunoreactivity, glycosylation, and functional activity.

Bayer used a weight of evidence approach to assess the safety of the PPO protein. Bayer highlights that the donor organism, *E. cloacae*, is a common soil, water, plants, and

[4] According to Bayer, TOX_2025 is a subset of sequences derived from the Swiss-Prot database that was selected using a keyword search and filtered to exclude non-toxin proteins.

gastrointestinal tracts bacterium that is ubiquitously present in the environment and is a member of the normal flora of humans and animals. Bayer demonstrated that the PPO protein was rapidly digested with pepsin and pancreatin and rapidly lost enzymatic activity when treated at 55°C or above for 15 or 30 minutes. Bayer carried out bioinformatics analysis and concludes that the PPO protein in MON 80616 corn does not share similarity with any sequences in the TOX_2025 toxin database. Bayer states that there was no evidence of acute toxicity in mice that were dosed orally at 5000 milligrams of PPO protein/kilogram of body weight. Taken together, Bayer concludes that dietary exposure to PPO protein in MON 80616 corn poses no meaningful risk to animal health.

Protein Expression Level

Bayer quantified PPO protein levels in various tissues of MON 80616 corn. The study included five field trial sites with four replicate plots at each site. Over season leaf (OSL1), over season root (OSR1), forage, and grain samples were collected from each replicated plot at all field sites treated with flumioxazin herbicides. Samples were prepared and analyzed using enzyme-linked immunosorbent assay. Bayer reports that the mean PPO protein level in MON 80616 corn across all sites was highest in OSL1 at 80 micrograms/gram of tissue dry weight (DW) and lowest in grain at 0.44 micrograms/gram DW.

Animal Food Use

Bayer states that MON 80616 corn is suitable for all animal food uses of conventional corn. Bayer references the Organisation for Economic Co-operation and Development (OECD) consensus document on compositional considerations of maize[5] and states that corn (*Zea mays* L.) is a commodity crop grown worldwide for various uses, including human and animal food. Bayer notes that corn has been a staple of human diet for centuries. Corn and its processed fractions have also been used in foods for animals. The production and different methods of processing as well as uses are described in greater detail in the OECD consensus document.

Composition

Scope of Analysis

Bayer analyzed the nutrient composition of MON 80616 corn and a non-GE corn variety with a similar genetic background (control) that were grown and harvested under similar conditions. Compositional analyses on grain and forage samples were reported for components listed in the OECD corn composition consensus document.

Study Design

Bayer conducted field trials in 2024 at five sites in the United States. A randomized complete block design with four replicate plots at each field site was used. MON 80616 corn and control were grown under normal agronomic field conditions for their respective regions. Bayer harvested grain and forage from each replicate within each site for composition analysis. Grain was harvested at physiological maturity and shipped at

[5] Organisation for Economic Co-operation and Development. 2002. Consensus document on compositional considerations for new varieties of maize (*Zea mays*): Key food and feed nutrients, antinutrients, and secondary plant metabolites. OECD ENV/JM/MONO 25. OECD, Paris, France.

ambient temperature from the field sites to Bayer CropScience LLC. Forage samples were harvested at R5 growth stage and were shipped on dry ice from the field sites to Bayer CropScience LLC. A subsample for compositional analysis was obtained from grain and forage samples from each replicate at each site and stored at -20°C prior to nutrient analyses.

For statistical analysis, Bayer combined composition data for each component from MON 80616 corn and control across locations using a linear mixed model with site and replicate as random factors. T-test analyses were used to test at the level of $p \leq 0.05$ for differences between MON 80616 corn and control. Differences between MON 80616 corn and control were evaluated based on natural variability defined by values for conventional maize varieties in the Agriculture and Food Systems Institute (AFSI) Crop Composition Database (AFSI-CCDB)[6] or in the scientific literature. Results were all expressed on a dry matter basis prior to statistical analyses except for fatty acids, which were expressed on a percent of total fatty acids basis. Moisture of forage and grain were not statistically analyzed.

Results of Analyses

For forage, Bayer reports values for proximates (crude protein, total fat, carbohydrates by calculation, and ash), fiber (acid detergent fiber (ADF) and neutral detergent fiber (NDF)), calcium, and phosphorus. Bayer reports no statistically significant differences between MON 80616 corn and control in any of the forage components analyzed.

For grain, Bayer chemically analyzed proximates, fiber (ADF, NDF, and total dietary fiber (TDF)), 18 amino acids, 22 fatty acids, nine minerals, seven vitamins, two anti-nutrients (phytic acid and raffinose), and three secondary metabolites (ferulic acid, furfural, and p-coumaric acid). Bayer noted that 13 fatty acids and furfural were not statistically analyzed because more than 50% of the observations fell below the lower limits of quantitation (LOQ). Bayer reports statistically significant differences between MON 80616 corn and control in the levels of five components (protein, palmitic acid, stearic acid, arachidic acid, and potassium). For these components, the mean values for MON 80616 corn were within the range of values observed in the literature and/or the AFSI-CCDB. Bayer concludes that the differences in these components between MON 80616 corn and control are not biologically meaningful from an animal food safety perspective.

Summary of Compositional Analyses

Bayer states that expression of the PPO protein that imparts tolerance to PPO-inhibiting herbicides does not meaningfully alter the nutrient composition of MON 80616 corn. The results support Bayer's conclusion that forage and grain obtained from MON 80616 corn are compositionally equivalent to the conventional control in the levels of key nutrients, anti-nutrients, and secondary metabolites.

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[6] Agriculture and Food Systems Institute (AFSI) Crop Composition Database: https://www.cropcomposition.org.

Conclusion

CVM evaluated Bayer's submissions to determine whether MON 80616 corn 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 conclude that MON 80616 corn 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 corn varieties now grown, marketed, and consumed. At this time, based on Bayer's data and information, CVM considers Bayer's consultation on MON 80616 corn for use in animal food to be complete.

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