



## Biotechnology Notification File No. 000191 CVM Note to the File

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**To:** Administrative Record, BNF No. 000191

**Subject:** Event DAS1131 Corn

**Keywords:** Corn, Maize, *Zea mays* L., *cry1Da2* gene, *Bacillus thuringiensis*, Insect resistance, *dgt-28 epsps* gene, *Streptomyces sviveus*, 5-enolpyruvylshikimate-3-phosphate synthase, Herbicide tolerance, OECD identifier DAS-01131-3, Pioneer Hi-Bred International, Inc.

### Purpose

This document summarizes the Food and Drug Administration (FDA) Center for Veterinary Medicine's (CVM, we) evaluation of biotechnology notification file (BNF) number 000191. Pioneer Hi-Bred International, Inc. (Pioneer) submitted a safety and nutritional assessment for a genetically engineered (GE) corn, transformation event DAS 01131 (hereafter referred to as DAS1131 corn), and additional information afterwards. CVM evaluated the information in Pioneer's submissions to ensure that regulatory and safety issues regarding animal food derived from DAS1131 corn have been resolved prior to commercial distribution. FDA's Center for Food Safety and Applied Nutrition summarizes its evaluation of DAS1131 corn in human food in a separate document.

In CVM's evaluation, we considered all of the information provided by Pioneer 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

One of the intended effects of the modifications in DAS1131 corn is to provide resistance to certain susceptible lepidopteran pests. To confer the insect resistance trait, Pioneer introduced the *cry1Da2* gene<sup>1</sup> that encodes for the Cry1Da2 protein. The second intended effect is to confer tolerance to glyphosate herbicides. For this, Pioneer introduced the *dgt-28 epsps* gene derived from *Streptomyces sviveus* that encodes for a

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<sup>1</sup> The *cry1Da2* gene is a chimeric gene comprised of sequences from the *cry1Da* gene and a derivative of *cry1Ab* gene, all derived from *Bacillus thuringiensis*.

5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) protein and that also serves as a selectable marker.

### Regulatory Considerations

The purpose of this evaluation is to determine whether use of the new plant variety in animal food raises safety or regulatory issues under the Federal Food, Drug and Cosmetic Act (FD&C Act).

The Environmental Protection Agency (EPA) defines a plant-incorporated protectant (PIP) as “a pesticidal substance that is intended to be produced and used in a living plant, or the produce thereof, and the genetic material necessary for the production of such a pesticidal substance,” including “any inert ingredient contained in the plant, or produce thereof” (40 CFR 174.3). EPA regulates PIPs under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the FD&C Act. Under EPA regulations, the Cry1Da2 protein and the genetic material used to express it in DAS1131 corn are considered pesticidal substances, and the DGT-28 EPSPS protein and the genetic material used to express it are considered to be inert ingredients. Therefore, the safety assessment of these products falls under the regulatory purview of EPA.

### Stability and Inheritance

Pioneer characterized the insertion event and genomic stability of the insert in the DAS1131 corn genome using bioinformatics techniques based on data obtained from Southern-by-Sequencing<sup>2</sup> and junction sequence analysis (JSA). To confirm genomic stability, Pioneer performed Southern blot analysis using genomic DNA obtained from several in-bred generations of DAS1131 corn. Pioneer states that there was a single site insertion of the T-DNA and that the inserted DNA was stably integrated into the genome of the five in-bred generations of DAS1131 corn that were analyzed.

In addition, Pioneer conducted segregation analyses on both in-bred and out-bred lines of DAS1131 corn using quantitative polymerase chain reaction and herbicide tolerance. The individual herbicide tolerance results for each plant were compared to the qPCR results to verify co-segregation of the two traits. The results of Chi-square analysis of the segregation data from each of the in-bred and out-bred generations show that the segregation pattern of the inserted DNA is consistent with Mendelian principles of inheritance for a single locus. Pioneer concludes that the insert in DAS1131 corn was integrated at one locus and is stably transmitted across multiple generations in accordance with the principles of Mendelian inheritance.

### Animal Food Use

The developer states that DAS1131 corn is expected to be grown for the same uses as currently commercialized corn, and no new or specialty food or feed uses are

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<sup>2</sup> Southern-by-Sequencing technique utilizes probes that are homologous to the transformation plasmid to capture DNA sequences that hybridize to the probe sequences. The captured DNA is then sequenced using whole genome sequencing and the results were mapped against the sequences of the transformation plasmid and control corn genome using bioinformatics tools. Pioneer states that whole genome sequencing was performed on an Illumina NextSeq500 instrument to a depth of at least 100x for the captured sequences.

anticipated. The typical uses of corn-derived food and feed are well documented in (OECD, 2002)<sup>3</sup>, including food use of the kernels for oil, starch, grits, meal, flour, and use of the kernels or whole plant silage for animal food. By-products that result from the processing of kernels are also used in animal food. Production and different methods of processing are also described in detail in the OECD maize composition consensus document.

## Composition

### Scope of Analysis

Pioneer analyzed the nutrient composition of forage and grain obtained from DAS1131 corn, non-GE near-isoline corn variety (control), and four non-GE commercial corn varieties (reference varieties) at each site that were grown and harvested under similar conditions.<sup>4</sup> The components selected for analyses were based on the OECD maize composition consensus document.

### Study Design

Pioneer conducted field trials in 2020 at eight sites in the United States and Canada. A randomized complete block design with four replicate plots at each field site was used. The DAS1131 corn, conventional control, and reference varieties were grown under normal agronomic field conditions for their respective regions. Pioneer harvested grain and forage from each replicate within each site for composition analysis. Grain was harvested at physiological maturity and shipped at ambient temperature from the field sites. Forage samples (combination of three plants) were harvested at R4 growth stage and were shipped on dry ice from the field sites to Pioneer. A subsample for compositional analysis was obtained from grain and forage samples from each replicate at each site and stored at not less than -10°C prior to nutrient analyses.

Pioneer conducted statistical analyses on composition data for each component from DAS1131 corn and the control across locations using a linear mixed model with site and replicate as random factors. Two of the analytes did not meet the criteria for mixed model analysis of variance and were subjected to Fisher's exact test. Compositional components were all expressed on a dry matter basis prior to statistical analyses, with the exception of fatty acids which were expressed as percent of total fatty acids. For a given analyte in the mixed model analysis, if a statistical difference (P-value < 0.05) was observed between DAS1131 maize and the control maize, the False Discovery Rate (FDR)-adjusted P-value was examined. Differences between DAS1131 corn and control were then evaluated based on natural variability defined by values for the reference varieties grown at the same locations, conventional non-GE corn lines which were grown in commercial corn-growing regions<sup>5</sup> or in the scientific literature.

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<sup>3</sup> 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, anti-nutrients, and secondary plant metabolites. OECD ENV/JM/MONO 25. OECD, Paris, France.

<sup>4</sup> Pioneer reports 16 total reference corn varieties.

<sup>5</sup> Tolerance intervals are expected to contain at least 99% of the values for corresponding component of the conventional corn population with a 95% confidence level from data obtained from non-GE corn lines that were grown in commercial corn-growing regions between 2003 and 2019 in the United States,

## Results of Analyses

For forage, Pioneer reports values for proximates (crude protein, crude fat, carbohydrates by calculation, and ash), fiber (crude fiber (CF), acid detergent fiber (ADF), and neutral detergent fiber (NDF)), calcium, and phosphorus. Pioneer found no statistically significant differences between DAS1131 corn and the control, with a few exceptions. Statistically significant differences between DAS1131 corn and the control were observed for crude protein and carbohydrates by calculation. The FDR-adjusted P-values for these two components were not statistically significant and all of the individual values for these components fell within the tolerance intervals for the reference varieties. Pioneer concludes there are no biologically meaningful differences from an animal food safety perspective.

For grain, Pioneer conducted chemical analyses on proximates, including moisture, fiber (CF, ADF, NDF, and total dietary fiber), 18 amino acids, 15 fatty acids<sup>6</sup>, nine minerals, 11 vitamins, including four vitamin E vitamers, plus total tocopherols<sup>7</sup>, 3 anti-nutrients, and 4 secondary metabolites<sup>8</sup>. Pioneer reports statistically significant differences between DAS1131 corn and the control in the levels of 5 components (stearic acid, calcium, manganese, zinc, and inositol). All of the FDR-adjusted P-values for these components were not statistically significant, with the exception of manganese, but all of the individual values for these components fell within the tolerance intervals<sup>9</sup> for the reference varieties. The mean values for the components in DAS1311 corn also fell within the mean ranges of the corn varieties reported in the literature. Pioneer concludes that the differences in these components between DAS1131 corn and the control are not biologically meaningful from an animal food safety perspective.

## Summary of Compositional Analyses

Pioneer states that based on the results of its compositional analyses that forage and grain obtained from DAS1131 corn are not biologically different from those of the control and reference varieties. Pioneer concludes that these results demonstrate that forage and grain obtained from DAS1131 corn is comparable in nutrient composition to that of conventional non-GE corn varieties.

## Conclusion

CVM evaluated Pioneer's submissions to determine whether DAS1131 corn raises any safety or regulatory issues with respect to its use in animal food. Based on the information provided by Pioneer and other information available to the agency, CVM

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Canada, Chile, Brazil, and Argentina. The combined data represent 184 commercial corn lines and 185 unique environments.

<sup>6</sup> All of the values for four of the fatty acids were below the lower limits of quantitation (LLOQ).

<sup>7</sup> All of the values for vitamin B<sub>2</sub> and delta-tocopherol fell below the LLOQ.

<sup>8</sup> One or more values for raffinose fell below the LLOQ and all of the values for furfural were below the LLOQ.

<sup>9</sup> The scientific literature included six commonly cited publicly available sources that highlight the composition of corn, including the OECD Consensus document on compositional considerations for new varieties of maize.

did not identify any safety or regulatory issues under the FD&C Act that would require further evaluation at this time.

Pioneer concludes that DAS1131 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 conventional corn varieties now grown, marketed, and consumed in the United States. At this time, based on Pioneer's data and information, CVM considers Pioneer's consultation on DAS1131 corn for use in animal food to be complete.

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