

**Regulatory Decision on the Application of Corn MON 95275
for Direct Use as Food and Feed, or for Processing**

I. Brief Identification of the Genetically Modified Organism

Designation:	Corn MON 95275
Applicant:	Bayer CropScience, Inc.
Host Plant:	<i>Zea mays</i> L. (Maize)
Trait Description:	Corn MON 95275 has resistance to Corn rootworm from the insertion of DvSnf7.1, Mpp75Aa1.1, and Vpb4Da2.
Trait Introduction Method:	<i>Agrobacterium</i> -mediated transformation
Donor organisms (s):	<i>Brevibacillus laterosporus</i> is the donor organism of <i>mpp75Aa1.1</i> gene that encodes the Mpp75Aa1.1 protein. It is an endospore-forming insecticidal bacilli, not known for pathogenicity or allergenicity to humans and other vertebrates. (Laubach, 1916; Shida et al., 1996). <i>Bacillus thuringiensis</i> is the donor organism of <i>vpb4Da2</i> gene, also producing the insecticidal activity. It has a history of safe use, showing no adverse effects to human health. (Cannon, 1993; U.S. EPA, 1988; WHO, 1999). <i>Agrobacterium</i> sp. strain CP4 is the donor organism of <i>cp4 epsps</i>. This is related to microbes commonly present in the soil and in the rhizosphere of plants. They are not known for human or animal pathogenicity and are not commonly allergenic (Padgett, et al., 1996).
Proposed Use:	For direct use as food and feed, or for processing

II. History of Safe Use of the Host Plant

Maize, commonly known as corn, has been a staple of human nutrition and is also widely used as animal feed for its high nutritional value and affordability. Corn silage serves as a main forage ingredient for livestock diets, as well as its grains. Additionally, co-products in milling processes, including corn gluten meal and distillers dried grains, are also considered important components of livestock feed. Humans, on the other hand, consume corn mainly in the form of derived ingredients, including high fructose corn syrup, starch-based sweeteners, breakfast cereals, cooking oil, and various alcoholic beverages (Morris, et al., 1998; OECD, 2002).

Corn, although contains anti-nutrients and secondary metabolites like phytic acid, raffinose, ferulic acid, p-coumaric acid, are present in only small amounts, as with toxins and allergens (OECD, 2002).

III. Characteristics of Host Plant

Maize, a member of the Poaceae family (Shah et al., 2016), is wind-pollinated, where its pollen can be carried to neighboring plants, enabling natural hybridization. In its native regions, Mexico and Guatemala, maize crosses with its close relative within the *Zea* genus, teosintes. Under laboratory conditions furthermore, it hybridizes with *Tripsacum*, another genetic relative (Wilkes, 1972).

The plant thrives in warm climates and follows distinct growth phases, categorized by Iowa State into vegetative and reproductive stages. During vegetation, development progresses through countable leaf collars. The reproductive phase advances from silking through blister, milk, and dough stages, culminating in dent formation and physiological maturity (Nleya et al., 2019).

Due to its extensive domestication, it is highly unlikely that corn seeds will thrive in the ecosystem and become invasive or weedy (Wilkes, 1972).

IV. Characteristic and safety assessment of the GM product

Corn 95275 is transformed through *Agrobacterium*-mediated transformation targeting the genomic DNA of immature maize embryos based on the method described by Sidorov and Duncan (2009), utilizing the plasmid vector PV-ZMIR525664. The plasmid vector is approximately 22.9 kb containing a single transfer DNA (T-DNA) with the following cassettes: *DvSnf7.1* suppression cassette, *mpp75Aa1*. and *vpb4Da2* expression cassettes, and the *cp4 epsps* selectable marker cassette.

The *mpp75Aa1.a* gene encodes the protein Mpp75Aa1.1 derived from *Brevibacillus laterosporus*, while the *vpb4Da2* gene encodes the Vpb4Da2 protein derived from *Bacillus thuringiensis*. These proteins belong to the Cry three domain family of

proteins that is when ingested by the target insect pest, exposes the protein to the alkaline conditions and proteases in the insect midgut (Gill et al., 1992; OECD, 2007; Schnepf et al., 1998; Vachon et al., 2012), converting the protein to the active insecticidal protein (Brave et al., 2007; Deist et al., 2014). Apart from these two proteins, MON 95275 also contains *DvSnf1.1* suppression cassette designed to match the gene present in Western Corn Rootworm *Diabrotica virgifera*, utilized to induce the RNAi-based mode of action and cause its death. Furthermore, *cp4 epsps* selectable marker cassette has also been inserted in the transformation plasmid and was later on excised by Cre/*lox* recombination system for marker removal.

The safety of the Corn MON 95275 for human and animal consumption were established through numerous studies and assessment, including toxicological and allergenicity assessment of the proteins. Mpp75Aa1.1 and Vpb4Da2 proteins by pepsin were subjected to digestibility testing via visual analysis of a Colloidal Brilliant Blue G stained SDS-PAGE gel and Western blot probed with an anti-Mpp75Aa1.1 and anti-Vpb4Da2 antibody, respectively. The test showed rapid degradation of the proteins by pepsin and pancreatin, indicating that safety concern to human or animal health is highly unlikely. Furthermore, FASTA sequence alignment tool was used to demonstrate the lack of both structurally and immunologically relevant similarities to known allergens, gliadins, and glutenins.

Nutritional data, furthermore, has shown that Corn MON 95275 is compositionally equivalent to the conventional control in levels of nutrients, anti-nutrients, and secondary metabolites in grain and forage. Stability of the inserted T-DNA across five breeding generations was demonstrated by Next Generation Sequencing (NGS)-based junction identification and read mapping, confirming the presence of the Corn MON 95275 in the said breeding generations.

In conclusion, the evaluation using the presented studies and journals showed that all expressed proteins in Corn MON 95275 do not pose any safety concerns for human or animal health.

V. Regulatory Decision

After reviewing the scientific data and information relevant to the application of the Bayer CropScience, Inc., the Department of Agriculture - Bureau of Plant Industry (DA-BPI) has approved the direct use as food and feed, or for processing for Corn MON 95275.

The DA-BPI issued a biosafety permit for direct use as food and feed, or for processing of Corn MON 95275 with conditions that need to be complied with by Bayer CropScience, Inc. A copy of the biosafety permit is accessible through the DA-BPI Biotechnology website.