MON 88017 Corn Safety Summary[1]

Summary Overview

The adoption of MON 88017 corn has provided growers with an additional tool to control corn rootworm that is an effective alternative to the use of synthetic chemical insecticides. The data and information presented below were collected and demonstrate that MON 88017 corn is comparable to conventional corn with the exception of the introduced insect-protection and glyphosate-tolerance traits; and, moreover, show that MON 88017 corn has no adverse environmental effects compared to conventional corn.

Product Description

Corn is a versatile crop that provides food, feed, and fuel to a global economy. Recently, a surge in demand for corn has been created by growing economies in the developing world and its use as an alternative fuel source in the developed world. These demands may exceed production and lead to diminished grain reserves. In addition, climate change may have variable impacts on crop yields, potentially creating further supply disruptions. The combination of these factors places a premium on corn yield stability in sub-optimal environments.

Corn is the largest crop grown in the United States in terms of acreage planted and net value. Weed control is essential in corn fields, as weeds compete with the crop for sunlight, water and nutrients. Failure to control weeds results in decreased yields and reduced crop quality. MON 88017 corn, developed by the use of recombinant DNA techniques, produces a 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium sp.* strain CP4 (CP4 EPSPS) that confers tolerance to glyphosate, the active ingredient in Roundup® family of agricultural herbicides, and a modified *Bacillus thuringiensis* (subspecies *kumamotoensis*) Cry3Bb1 protein that protects against corn rootworm (CRW) species. MON 88017 corn offers U.S. farmers the ability to apply Roundup agricultural herbicides over the top of a corn field for broad-spectrum weed control with excellent crop safety. In addition, the use of Roundup agricultural herbicides in conjunction with MON 88017 corn will allow the grower to utilize reduced tillage techniques that have the potential to provide significant environmental benefits, such as reduced soil erosion, reduced use of fossil fuels, and improved soil quality.

MON 88017 corn also offers U.S. farmers a safe and effective alternative to the use of synthetic chemical insecticides for control of the corn rootworm pest. Yields are negatively impacted by a number of insect pests. One of the most pernicious in the U.S. Corn Belt is corn rootworm. Corn rootworm larvae damage corn by feeding on the roots, reducing the ability of the plant to absorb water and nutrients from soil, and causing harvesting difficulties due to plant lodging. Corn rootworm is the most significant insect pest problem for corn production in the U.S. from the standpoint of chemical insecticide usage, as over 20 million acres of corn were treated with organophosphate, carbamate, and pyrethroid insecticides to control corn rootworm in 2005.

[1] For detailed information see USDA Petition for the Determination of Non-regulated Status of MON 88017

Corn rootworm has been described as the billion-dollar pest complex based on costs associated with the application of soil insecticides and crop losses due to pest damage.

Data and Information Confirm the Food and Feed Safety and no Adverse Effect on the Environment for MON 88017 Corn Compared to Conventional Corn

All available data and information demonstrate the safety of MON 88017 corn as compared to conventional corn and, moreover, show that MON 88017 corn will not have an adverse effect on the environment. The food, feed, and environmental safety of MON 88017 corn was confirmed based on multiple, well-established lines of evidence:

1. Corn is a familiar crop that does not possess any of the attributes commonly associated with weeds, has a history of safe consumption, and serves as an appropriate basis of comparison to support the safety assessment of MON 88017 corn.
2. A molecular characterization of the inserted DNA in MON 88017 corn confirmed the insertion of a single functional copy of the *cp4 epsps* and *cry3Bb1* expression cassettes at a single locus within the corn genome.
3. A detailed biochemical characterization was conducted on the CP4 EPSPS and Cry3Bb1 proteins produced in MON 88017 corn. Both proteins are readily digested and have long histories of safe use and are expressed at low levels in MON 88017 corn.
4. An assessment of the allergenicity and toxicity potential of the CP4 EPSPS and Cry3Bb1 proteins demonstrated that the CP4 EPSPS and Cry3Bb1 proteins are unlikely to be allergens, toxins, or similar to other biologically active proteins known to have adverse effects on mammals.
5. A compositional and nutritional assessment confirmed that MON 88017 corn forage and grain are compositionally equivalent to conventional corn.
6. An extensive evaluation of phenotypic and agronomic characteristics and environmental interactions demonstrated that MON 88017 corn poses no increased plant pest potential, including weediness potential, and no adverse environmental impact compared to conventional corn.
7. An assessment on the potential impact to non-target organisms (NTO) demonstrated that MON 88017 corn is unlikely to have an adverse effect on these organisms.
8. The introduction of MON 88017 corn is no more likely to have an adverse effect on maize agronomic practices or land use than conventional corn.

These lines of evidence are described in further detail in the following sections.

Corn is a Familiar Crop Lacking Weedy Characteristics

Corn is grown extensively throughout the world, and is the largest cultivated crop followed by wheat (*Triticum* sp.) and rice (*Oryza sativa* L.) in total global production. In the U.S., corn is grown in almost all states and is the largest crop grown in terms of acreage planted and net value. Corn has been studied extensively, and the domestication of corn can be traced back to approximately 10,000 years ago in southern Mexico.

Corn is not listed as a weed in the major literature references, nor is it considered to be a noxious weed species by the federal government. In addition, corn has been grown throughout the world without any report that it is a serious weed. Corn is poorly suited to survive in the environment without human assistance and is not capable of surviving as a weed due to past breeding selection in the domestication of corn. During domestication of corn, innate traits often associated with weediness, such as seed dormancy, a dispersal mechanism, or the ability to establish reproducing populations outside of cultivation, have not been selected. Similarly, there is no indication from the history of hybrid breeding in the U.S. of changes that could alter the weediness profile of the crop. Although corn seed can overwinter into a rotation with soybeans or other successive crops, mechanical and chemical measures are routinely used to control corn volunteers. Some populations of wild annual and perennial species that could hybridize with MON 88017 corn are known to exist in the U.S., however key differences in several factors such as flowering time, geographical separation, and development timings make natural crosses in the U.S. highly unlikely.

A conventional corn comparator was used to support the safety assessment of MON 88017 corn. The conventional corn comparator had a similar genetic background as MON 88017 corn without the inserted *cp4 epsps* and *cry3Bb1* expression cassettes. Comparisons of MON 88017 corn with the conventional corn comparator allowed the effect of the *cp4 epsps* and *cry3Bb1* expression cassettes and the expressed CP4 EPSPS and Cry3Bb1 proteins to be assessed in an unbiased manner.

Molecular Characterization Verifies the Integrity and Stability of the Inserted DNA in MON 88017 Corn

MON 88017 corn was produced by *Agrobacterium*-mediated transformation of corn cells with plasmid vector PV-ZMIR39. This plasmid contains a gene encoding the CP4 EPSPS protein that provides tolerance to the action of Roundup agricultural herbicides and a gene encoding the Cry3Bb1 protein that provides protection against corn rootworm. The *Agrobacterium tumefaciens* transformation vector is a binary vector that contains both left and right transfer-DNA (T-DNA) border sequences to facilitate transformation. The DNA region that was integrated into the corn genome during the transformation process contains the *cp4 epsps* and *cry3Bb1* gene expression cassettes. Molecular characterization of MON 88017 corn by Southern blot analyses demonstrated that single copies of the *cp4 epsps* and *cry3Bb1* genes are integrated at a single locus in the corn genome with all expression elements intact, and no plasmid backbone sequences outside of the T-DNA were detected. The organization of the elements within the insert in MON 88017 corn was confirmed using PCR analysis, by amplifying overlapping regions of DNA that span the entire length of the insert. The generation of the predicted size PCR products from MON 88017 corn established that the arrangement or linkage of elements in the insert is the same as those in plasmid PV-ZMIR39.

Segregation analysis across multiple generations confirmed the heritability and stability of the *cp4 epsps* and *cry3Bb1* coding sequences. The results of this analysis are consistent with the finding of a single active site of insertion that segregates according to the Mendelian laws of genetics.

Data Confirm CP4 EPSPS and Cry3Bb1 Protein Safety

A multistep approach was used to characterize and assess the safety of the CP4 EPSPS and Cry3Bb1 proteins expressed in MON 88017 corn. This detailed characterization confirms the CP4 EPSPS and Cry3Bb1 proteins are safe for human and animal consumption. The assessment involved: 1) characterizing the physicochemical and functional properties of the proteins; 2) quantifying protein levels in MON 88017 corn plant tissues; 3) examining the similarity of the CP4 EPSPS and Cry3Bb1 proteins to known allergens, toxins and other biologically-active proteins known to have adverse effects on mammals; 4) evaluating the digestibility of CP4 EPSPS and Cry3Bb1 proteins in simulated gastrointestinal fluids; 5) documenting the history of safe consumption of CP4 EPSPS and Cry3Bb1 proteins; and 6) investigating potential mammalian toxicity through oral ingestion (gavage) evaluations. Results confirm that CP4 EPSPS and Cry3Bb1 are expressed in all MON 88017 corn tissues collected, including stover, root, forage, grain, leaf, pollen, and silk. CP4 EPSPS and Cry3Bb1 have no relevant amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins which can have adverse effects on mammals. Studies utilizing the CP4 EPSPS and Cry3Bb1 proteins demonstrated that these proteins degraded rapidly in simulated gastric and intestinal fluids and mouse acute oral toxicity evaluations demonstrated the proteins are not acutely toxic and do not cause adverse effects at the highest doses tested. The safety assessment supports the conclusion that dietary exposure to CP4 EPSPS and Cry3Bb1 proteins derived from MON 88017 corn poses no meaningful risk to human or animal health.

MON 88017 Corn is Compositionally Equivalent to Conventional Corn

MON 88017 corn was shown to be compositionally equivalent to conventional corn with a similar genetic background as well as to other conventional corn varieties. Forage and grain tissues were harvested from three replicated field sites grown in major corn-growing areas of the U.S. in 2002. Four commercially available corn hybrids were also grown at each of the same field sites to provide a total of 12 different reference substances. Compositional analyses of the forage samples included proximates (protein, fat, ash, and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), minerals (calcium, phosphorous), and carbohydrates by calculation. Compositional analyses of the grain samples included proximates (protein, fat, ash, and moisture), ADF, NDF, total dietary fiber (TDF), amino acids, fatty acids (C8-C22), minerals (calcium, copper, iron, magnesium, manganese, phosphorous, potassium, sodium, and zinc), vitamins (B_1, B_2, B_6, E, niacin, and folic acid), anti-nutrients (phytic acid and raffinose), secondary metabolites (furfural, ferulic acid, and p-coumaric acid), and carbohydrates by calculation. In all, 77 components were evaluated as part of the nutritional assessment of MON 88017 corn: nine in forage and 68 in grain.

Sixty-two components were statistically assessed as values for 15 components were below the analytical limit of quantitation, and there were 248 comparisons conducted on MON 88017 corn based on four sets of analyses of data from each of the three field sites plus combination data of all three field sites. Using the data for each component obtained from the 12 different

commercial hybrids, a 99% tolerance interval was calculated to contain, with 95% confidence, 99% of the values contained in the population of commercial corn hybrids. For those comparisons in which MON 88017 corn was statistically different from the control, the MON 88017 corn range was compared to the 99% tolerance interval in order to determine if its range was within the interval and therefore considered to be within the natural variability of the population of the commercial corn.

Results of the analysis showed there were no statistically significant differences between MON 88017 corn and conventional corn for 232 of the 248 comparisons conducted. For the compositional constituents that indicated a significant difference existed, the MON 88017 corn values for each statistically significant comparison ($p<0.05$) fell within the 99% tolerance interval, historical control ranges, and literature ranges. Therefore, it is unlikely that these minor differences are biologically meaningful. It was concluded, based on these data, that the forage and grain produced from MON 88017 corn are compositionally equivalent to the forage and grain produced from other commercial corn currently on the market.

MON 88017 Corn Does Not Change Weediness, Plant Pest Potential or Environmental Interactions Compared to Conventional Corn

Plant pest potential of a biotechnology-derived crop is assessed from the basis of familiarity that the USDA recognizes as an important underlying concept in risk assessment. The concept of familiarity is based on the fact that the biotechnology-derived plant is developed from a conventional plant hybrid or variety whose biological properties, weediness and plant pest potential are well known. Familiarity considers the biology of the plant, the introduced trait, the receiving environment, and the interactions among these factors. This provides a basis for comparative risk assessment between a biotechnology-derived plant and a conventional plant hybrid or variety as the control. Thus, the phenotypic, agronomic, and environmental interaction assessment of MON 88017 corn included a genetically similar conventional control as a comparator. This evaluation used a weight-of-evidence approach and considered statistical differences between MON 88017 corn and the conventional control with respect to reproducibility, magnitude, and directionality (trends). Characteristics assessed included: seed dormancy and germination, pollen morphology, and plant phenotypic observations and environmental interaction evaluations conducted in the field. The phenotypic, agronomic, and environmental interaction assessments demonstrate that MON 88017 corn is comparable to the conventional control. Thus, MON 88017 corn is not expected to have increased weediness or plant pest risk compared to conventional corn.

Under a range of temperature regimes, MON 88017 corn seed had similar dormancy and germination characteristics as conventional control seed. In particular, the lack of an increased occurrence of hard seed, a well recognized seed characteristic associated with weediness, supports a conclusion of no increased weediness of MON 88017 corn compared to the conventional control. In an evaluation of pollen characteristics, there were no biologically meaningful differences detected between MON 88017 corn and the conventional control for pollen viability and diameter, and no visual differences in general pollen morphology were observed.

The field evaluation of phenotypic, agronomic, and environmental characteristics also supports the conclusion that MON 88017 corn is not likely to have increased weediness or plant pest

potential compared to conventional corn. The evaluation of 14 phenotypic characteristics was conducted at 18 locations over two years (2001 and 2002), which included seedling vigor, early stand count, days to 50% pollen shed, days to 50% silking, ear height, plant height, stay green, final stand count, dropped ears, stalk lodging, root lodging, grain test weight, grain moisture, and grain yield. There were no consistent trends in differences between MON 88017 corn and the conventional control corn, other than for seedling vigor. The statistically significant differences observed for seedling vigor across sites in 2001 and 2002 were small in magnitude and not accompanied by consistent across-site differences in stand count, days to pollen shed, or days to silk. This indicates that the differences observed for seedling vigor are unlikely to be biologically meaningful. The evaluation of ecological interactions at the same locations, based on monitoring of specific insect, disease, and abiotic stressors such as heat, wind damage, and drought, showed that there were no detectable trends for differences in susceptibility to pests or to environmental stress. The phenotypic and ecological data indicate that MON 88017 corn does not confer any detectable selective advantage to corn that would result in increased weediness or plant pest potential compared to control corn.

Taken together, these data support a conclusion that MON 88017 corn poses no increased plant pest potential, including weediness potential, and no adverse environmental impact compared to conventional corn.

MON 88017 Corn will not Adversely Affect Non-Target Organisms including those Beneficial to Agriculture

The environmental assessment of MON 88017 corn and the expressed CP4 EPSPS and Cry3Bb1 proteins demonstrates that MON 88017 corn poses negligible risk to non-target-organisms (NTOs). Crop products expressing CP4 EPSPS or Cry3Bb1 proteins previously authorized by USDA, U.S. FDA, and U.S. EPA include MON 863 corn containing Cry3Bb1 and a number of Roundup Ready® crops (soybean, canola, corn, cotton) containing CP4 EPSPS. After extensive testing and wide scale commercial cultivation, no adverse impacts to NTOs have been associated with exposure to Cry3Bb1 or CP4 EPSPS proteins from these biotechnology-derived crops.

Potential adverse effects to NTOs resulting from exposure to Cry3Bb1 proteins have been evaluated in a series of studies with representative avian (bobwhite quail), aquatic (catfish and water fleas), and terrestrial beneficial invertebrate species (Collembola, ladybird beetle, adult and larval honey bees, green lacewing, parastic wasp, and earthworm). These NTOs were exposed to high doses of corn leaf, grain or pollen tissue containing a plant-produced Cry3Bb1 variant, or to an artificial diet containing a *B.t.*- or *E. coli*-produced Cry3Bb1 variant. The results from these studies indicate that the Cry3Bb1 protein does not pose significant risks to NTOs.

Cultivation of MON 88017 Corn is not Expected to have Adverse Effects on Agronomic Practices or Land Use

An assessment of current corn agronomic practices indicate that MON 88017 corn will not impact current cultivation and rotational practices or the management of insects and diseases other than the control of corn rootworm larvae. The use of MON 88017 corn also allows for the broad-spectrum control of grass, broadleaf and perennial weeds by over-the-top applications of Roundup agricultural herbicides, similar to that achieved with Roundup Ready corn NK603. No

adverse impact is expected in the management of volunteer corn because there are a number of herbicides labeled for rotational crops that have a mode of action that is different than that of glyphosate.

Conclusion

As presented above all available data and information demonstrate that MON 88017 corn is comparable to conventional corn with the exception of the introduced insect-protection and glyphosate-tolerance traits; and, moreover, show that MON 88017 corn has no greater weediness or plant pest potential. This conclusion is based on multiple, well established lines of evidence including the biology of corn and a comprehensive assessment that spans the entirety of MON 88017 corn from the molecular level to the whole crop level. MON 88017 corn is as safe as conventional corn for use as food and feed, and poses no more environmental risk than conventional corn.

Published Literature

Links to published literature on MON 88017 corn are provided below.

Devos, Y., A. De Schrijver, P. De Clercq, J. Kiss and J. Romeis. 2012. *Bt*-maize event MON 88017 expressing Cry3Bb1 does not cause harm to non-target organisms. Transgenic Research 21(6):1191-1214.
link

Healy, C., B. Hammond and J. Kirkpatrick. 2008. Results of a 13-week safety assurance study with rats fed grain from corn rootworm-protected, glyphosate-tolerant MON 88017 corn. Food and Chemical Toxicology 46(7):2517-2524.
link

McCann, M., W.A. Trujillo, S.G. Riordan, R. Sorbet, N.N. Bogdanova and R.S. Sidhu. 2007. Comparison of the Forage and Grain Composition from Insect-Protected and Glyphosate-Tolerant MON 88017 Corn to Conventional Corn (*Zea mays* L.). J. Agric. Food Chem. 55:4034-4042.
link

Priesnitz, K.U., U. Benker and F. Schaarschmidt. 2013. Assessment of the potential impact of a Bt maize hybrid expressing Cry3Bb1 on ground beetles (Carabidae). J. Plant Diseases and Protection 120(3):131-140.
link

Puza, V., O. Habustova, H.M. Hussein, Z. Svobodova. 2011. The abundance, distribution and natural host range of entomopathogenic nematodes (Nematoda: Steinernematidae) in the experimental GM maize MON 88017 field. IOBC/WPRS Bulletin 66:367-370.
link

Svobodova, Z., O. Habustova, F. Sehnal, M. Holec and H.M. Hussein. 2013. Epigeic spiders are not affected by the genetically modified maize MON 88017. J. Applied Entomology 137(1-2):56-67.
link

Svobodova, Z., O. Habustova, H.M. Hussein, V. Puza and F. Sehnal. 2012. Impact of genetically modified maize expressing Cry3Bb1 on non-target arthropods: first year results of a field study. IOBC/WPRS Bulletin 73:107-120.

link

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