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CFSAN/Office of Food Additive Safety

January 30, 2007

# Biotechnology Consultation Note to the File BNF No. 000099

**Date:** January 30, 2007

**Subject:** *Bacillus thuringiensis* modified Cry3A insect-resistant corn event MIR604

**Keywords:** Corn, *Zea mays*, insect-resistant corn, modified Cry3A protein, cathepsin G serine protease recognition site, phosphomannose isomerase (*pmi*), *Agrobacterium tumefaciens*, *Bacillus thuringiensis* subsp. *tenebrionis*, insect resistance, western corn rootworm (*Diabrotica virgifera virgifera*), northern corn rootworm (*D. longicornis barberi*), Mexican corn rootworm (*D. virgifera zea*).

## Introduction

In a submission dated February 23, 2005, Syngenta Seeds, Inc. (Syngenta) provided to the Food and Drug Administration (FDA) a summary of the safety and nutritional assessment they conducted on a new bioengineered insect-resistant corn line, corn event MIR604. The company provided additional clarifying information to FDA on May 14, October 5, December 5, 2005, and February 10, March 21, June 16, July 10, July 17, July 19, and July 28, 2006.

Syngenta concluded that food and feed derived from the new insect-resistant corn is as safe and nutritious as food and feed derived from conventional corn varieties currently being marketed.

## Intended Effect

The intended effect of the modification is to provide protection from larval corn rootworm damage, such as from the western corn rootworm, the northern corn rootworm, and the Mexican corn rootworm. To accomplish this objective, Syngenta inserted a modified *cry3A* gene (*mcry3A*), derived from *Bacillus thuringiensis* (*B.t.*) subsp. *tenebrionis*, and the selectable marker phosphomannose isomerase (*pmi*) gene, derived from *Escherichia coli* into their corn line.

## Regulatory Considerations

The Environmental Protection Agency (EPA) regulates plant-incorporated protectants under the Federal Food, Drug, and Cosmetic Act and the Federal Insecticide, Fungicide, and Rodenticide Act. Under EPA regulations, the protein expressed by the *mcry3A* gene in corn event MIR604 is considered a pesticidal substance and the phosphomannose isomerase protein is considered an inert ingredient. Therefore, the safety assessment of these proteins falls under the regulatory purview of EPA.

## Genetic Modifications and Characterization

To generate the corn event MIR604, Syngenta used *Agrobacterium*-mediated transformation method. Syngenta transformed a proprietary corn line using the pZM26 transformation vector. The vector is a T-DNA plasmid carrying the transgenes (*mcry3A* and *pmi*) for insertion into the plant genome. The *cry3A* gene was engineered to incorporate a cathepsin-G serine protease recognition site within the expressed protein. Syngenta states that this modification increases the resistance to target pests. The T-DNA region of the vector contains the elements listed in Table 1.

**Table 1: Genetic elements contained in the T-DNA region of the plasmid vector pZM26**

Genetic element	Description
RB	Right border region of T-DNA from <i>A. tumefaciens</i> nopaline Ti-plasmid.
MTL	Promoter derived from <i>Zea mays</i> metallothionein-like gene with root-preferential expression.
<i>mcry3A</i>	Modified maize-optimized synthetic <i>cry3A</i> gene conferring insect resistance to corn root worms. Contains a cathepsin-G serine protease recognition site within the expressed protein for increased resistance to target pests.
NOS	Terminator sequence from the nopaline synthase gene of <i>A. tumefaciens</i> .
ZmUbiInt	Promoter region from <i>Z. mays</i> polyubiquitin gene. Provides constitutive expression in monocots.
<i>pmi</i>	<i>E. coli manA</i> gene encoding phosphomannose isomerase; used as a selectable marker. Catalyzes the isomerization of mannose-6-phosphate to fructose-6-phosphate.
NOS	Terminator sequence from the nopaline synthase gene of <i>A. tumefaciens</i> .
LB	Left border region of T-DNA from <i>A. tumefaciens</i> nopaline Ti-plasmid.

The backbone of the vector contains the streptomycin adenytransferase gene to facilitate cloning and maintenance of the plasmid in bacterial hosts. Plants that tested positive for *mcry3A* and *pmi*, but negative for the streptomycin adenytransferase were selected and used for further propagation.

Syngenta characterized the DNA introduced into event MIR604-derived hybrids, using Southern analysis and direct sequencing. Syngenta states that the results obtained from these analyses show that the T-DNA from plasmid pZM26 is integrated as a single intact copy and at a single locus in the corn genome. Syngenta also assessed the stability of the insert of event MIR604 through multiple generations, and concluded that the insert of event MIR604 segregates as a single dominant trait following a Mendelian inheritance pattern.

## Food/Feed Use

Corn grain (kernel) and its processed fractions are consumed as human food and animal feed. The majority of corn is used as animal feed; the remainder is exported, held as ending stock, processed into corn syrup, converted to ethanol, extracted for starch, used as processed food, and grown as corn seed. Corn can be processed by wet and dry milling. Corn grain and by-products of wet and dry milling are used as animal feed. The whole corn plant can also be used as feed for ruminant animals. Corn plants are harvested at an appropriate stage and fed to animals or stored as silage.

## Compositional Analysis

Grain and forage from transgenic event MIR604 derived plants and their near-isogenic, non-transgenic control were grown at 13 different locations over a two year period, with three replicate plots of each genotype planted in randomized complete blocks. Compositional data were statistically analyzed using a randomized block design with locations serving as the blocks. Statistical significance was assigned at  $p < 0.05$  indicating that the difference between the treatments was statistically different at the 5% customary level. The treatment-location interaction was also assessed. Syngenta provides published literature values for each analyte to assess whether statistically significant differences in the composition of the test and control maize are biologically meaningful.

## Forage

Syngenta determined the levels of the following components of forage of corn event MIR604 and the near-isogenic, non-transgenic controls collected at the R4 [\[1\]](#) development stage. The following analytes were measured:

- Proximates
- Minerals

A list of specific components contained in each group is shown in Table 2 and marked with an asterisk (\*).

Of all the analytes measured, only the moisture level was different in MIR604 when compared with its near-isogenic non-transgenic control. However, moisture is a function of maturity and conditions at harvest and not considered meaningful. All other values measured for the analytes fell within published literature ranges.

## Grain

Syngenta measured the levels of the following components of mature grain of corn event MIR604 and mature grain of the near-isogenic non-transgenic corn:

- proximates
- minerals
- amino acids
- fatty acids
- anti-nutrients
- secondary metabolites
- vitamins

A list of specific components contained in each group is shown in Table 2.

**Table 2. Components measured**

Proximates	Minerals	Amino Acids	Fatty Acids	Anti-Nutrients	Secondary Metabolites	Vitamins
ash*	calcium*	methionine	palmitic	phytic acid	raffinose	Beta carotene
fat*	copper	cysteine	(16:0)	trypsin	furfural	Cryptoxanthin
moisture*	iron	lysine	stearic	inhibitor	p-coumaric acid	Folic Acid
protein*	magnesium	tryptophan	(18:0)		ferulic acid	B <sub>1</sub>
carbohydrate*	manganese	threonine	oleic		inositol	B <sub>2</sub>
acid detergent fiber (ADF)*	phosphorus*	isoleucine	(18:1)			B <sub>3</sub>
neutral detergent fiber (NDF)*	potassium	histidine	linoleic			B <sub>5</sub>
total dietary fiber (TDF)	sodium	valine	(18:2)			B <sub>6</sub>
	zinc	leucine	linolenic			C
	selenium	arginine	(18:3)			Tocopherols
	chromium	phenylalanine				
		glycine				
		alanine				
		aspartic acid				
		glutamic acid				
		proline				
		serine				
		tyrosine				

Syngenta reported that mean values for all proximates and amino acids, measured for grain from MIR604 and non-isogenic non-transgenic controls were within published literature ranges. The MIR604 hybrids had statistically significantly higher levels of protein and total fat, and statistically significantly lower levels of starch and TDF than the near-isogenic non-transgenic control corn. MIR604 hybrids also had statistically significantly higher levels of the amino acids asparagine, threonine, serine, glutamate, alanine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine than did the near-isogenic

non-transgenic control corn, whereas, the control corn showed statistically higher cystine levels than did the MIR604 corn. Syngenta has concluded that the differences in composition between MIR604 and control lines are not biologically significant.

Syngenta reported that mean values for all fatty acids and minerals, measured for grain from MIR604 and non-isogenic non-transgenic controls were within published literature ranges. Syngenta reported statistically significantly higher levels of palmitic, oleic, and linoleic acids and statistically significantly lower levels of linolenic acid in MIR604 when compared to the near-isogenic non-transgenic control. MIR604 also had statistically significantly higher levels of calcium and phosphorus than did their near-isogenic non-transgenic control corn. Syngenta has concluded that the differences in composition between MIR604 and control lines are not biologically significant.

Syngenta measured several vitamins in both MIR604 hybrids and the near-isogenic non-transgenic controls and found the values fell within published literature ranges.

Syngenta measured anti-nutrients and secondary metabolites. The values for phytic acid were at or below literature values for both MIR604 and the near-isogenic non-transgenic control corn. The values for the other anti-nutrient, trypsin-inhibitor, and all of the secondary metabolites for both the MIR604 hybrids and the near-isogenic non-transgenic control corn were within published literature ranges.

## Conclusions

Syngenta has concluded that corn event MIR604 is not materially different in composition, safety, wholesomeness, or any relevant parameter from corn now grown, marketed, and consumed. At this time, based on Syngenta's data and information, the agency considers Syngenta's consultation on corn event MIR604 to be complete.

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[\[1\]](#)The growth stage when the material within the kernel produces a doughy consistency.

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