

# **Bacillus thuringiensis Vip3Aa20 Insecticidal Protein and the Genetic Material Necessary for Its Production (via Elements of Vector pNOV1300) in Event MIR162 Maize (OECD Unique Identifier: SYN-IR162-4)(006599) Fact Sheet**

[Technical Document](#)

## **Summary**

The Environmental Protection Agency (EPA) has conditionally registered a plant-incorporated protectant (PIP) product containing Syngenta Seeds, Incorporated's (hereafter referred to as Syngenta) new active ingredient, *Bacillus thuringiensis* Vip3Aa20 insecticidal protein and the genetic material necessary for its production (via elements of vector pNOV1300) in Event MIR162 maize (Organization for Economic Cooperation and Development [OECD] Unique Identifier: SYN-IR162-4). MIR162 maize may be used only for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed on a limited amount of acreage per county and per year. Commercial plantings of MIR162 maize, for the purposes of grain production and controlling corn insect pests, are prohibited. The Agency has determined that the use of this pesticide is in the public interest and that it will not cause any unreasonable adverse effects on the environment during the time of conditional registration. The registrant for this product is Syngenta.

The new plant-incorporated protectant product, MIR162 maize, produces its own insecticidal protein within the corn plant. This insecticidal protein was derived from *Bacillus thuringiensis* (*Bt*), a naturally occurring soil bacterium. The Vip3Aa20 insecticidal protein expressed in this product controls certain lepidopteran pests of corn.

On August 6, 2008, a tolerance exemption under 40 Code of Federal Regulations (CFR) Part 174 became effective for Vip3Aa proteins, when used as plant-incorporated protectants, in or on corn and cotton (40 CFR § 174.501). The exemption from the requirement of a tolerance for residues of Vip3Aa proteins is inclusive of the Vip3Aa20 insecticidal protein and its use in corn.

## **I. Use Sites, Target Pests, and Application Methods**

- **Pesticide Name:**

*Bacillus thuringiensis* Vip3Aa20 insecticidal protein and the genetic material necessary for its production (via elements of vector pNOV1300) in Event MIR162 maize (OECD Unique Identifier: SYN-IR162-4)

- **Date Registered:** November 26, 2008
  
- **Trade and Other Names:** MIR162 Maize or MIR162 Corn
  
- **PC Code:** 006599 (Vip3Aa20)
  
- **Basic Manufacturer:**

Syngenta Seeds, Incorporated – Field Crops – NAFTA  
P.O. Box 12257, 3054 East Cornwallis Road  
Research Triangle Park, NC 27709-2257
  
- **Type of Pesticide:** Plant-Incorporated Protectant
  
- **Uses:** Field Corn
  
- **Target Pests:**

corn earworm (*Helicoverpa zea*), fall armyworm (*Spodoptera frugiperda*),  
armyworm (*Pseudaletia unipunctata*), beet armyworm (*Spodoptera exigua*),  
black cutworm (*Agrotis ipsilon*), and western bean cutworm (*Striacosta  
albicosta*)

## II. Science Assessment

### A. Product Characterization

Vip3A is a group of vegetative insecticidal proteins (i.e., produced during the vegetative stage of bacterial growth) from *Bacillus thuringiensis*, a gram-positive bacterium commonly found in soil. Event MIR162 maize, produced by *Agrobacterium*-mediated transformation using elements of a vector (pNOV1300), contains a variant of the native *vip3Aa1* gene, which was isolated from *Bt* strain AB88. The gene encodes a vegetative insecticidal protein, Vip3Aa20, that is highly toxic to the following lepidopteran pests of corn: fall armyworm (*Spodoptera frugiperda*), armyworm (*Pseudaletia unipunctata*), beet armyworm (*Spodoptera exigua*), corn earworm (*Helicoverpa zea*), black cutworm (*Agrotis ipsilon*), and western bean cutworm (*Striacosta albicosta*). Event MIR162 maize also contains the

manA gene from *Escherichia coli*, which encodes the selectable marker, phosphomannose isomerase (PMI).

Southern blot analyses and deoxyribonucleic acid (DNA) sequencing indicate that one full-length copy of each of the *vip3Aa20* and *pmi* genes was integrated into the maize genome without the backbone sequences from transformation plasmid pNOV1300. Therefore, the overall integrity of the insert and the contiguousness of the functional elements were confirmed.

## **B. Human Health Assessment**

There is a reasonable certainty that no harm will result from aggregate exposure to the United States (U.S.) population, including infants and children, to the Vip3Aa20 insecticidal protein. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. The Agency has arrived at this conclusion because no toxicity to mammals has been observed, nor any indication of allergenicity potential for the plant-incorporated protectant

Syngenta previously submitted four acute oral toxicity studies conducted on mice, which all indicated that Vip3Aa proteins are non-toxic to humans. Three of the studies were conducted with microbially produced Vip3Aa proteins with slight variations in amino acid sequence (1–2 amino acid differences), and one study was conducted with protein extracted from transgenic corn leaf tissue as the test material. No treatment-related adverse effects were observed in any of the studies. The oral LD50 for mice (males, females, and combined) was greater than 3,675 milligrams (mg) Vip3Aa/kilogram (kg) body weight (the highest dose tested). Additionally, Syngenta submitted another mouse acute oral toxicity study that showed no effects attributed to Vip3Aa20 insecticidal protein, even at relatively high dose levels (1,250 mg Vip3Aa20/kg body weight).

Since Vip3Aa isolates are proteins, allergenic potential was also considered. Currently, no definitive tests for determining the allergenic potential of novel proteins exist. Therefore, EPA uses a weight-of-evidence approach where the following factors are considered: source of the trait; amino acid sequence comparison with known allergens; and biochemical properties of the protein, including in vitro digestibility in simulated gastric fluid (SGF) and glycosylation. This approach is consistent with the approach outlined in the Annex to the Codex Alimentarius "Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants." The allergenicity assessment for Vip3Aa proteins is as follows:

1. Source of the trait. *Bacillus thuringiensis* is not considered to be a source of allergenic proteins.
2. Amino acid sequence. A comparison of the amino acid sequence of Vip3Aa20 with known allergens showed no significant sequence identity over 80 amino acids or identity at the level of eight contiguous amino acid residues.
3. Digestibility. The Vip3Aa proteins were digested rapidly in simulated gastric fluid containing pepsin.
4. Glycosylation. Vip3Aa proteins were shown not to be glycosylated.
5. Conclusion. Considering all of the available information, EPA has concluded that the potential for Vip3Aa proteins to be food allergens is minimal.

### **C. Environmental Assessment**

Presently, the Agency is aware of no identified significant adverse effects of Vip3Aa proteins on the abundance of non-target beneficial organisms in any population in the field environment, whether they are pest parasites, pest predators, or pollinators. Further, the EPA believes cultivation of Event MIR162 maize may have fewer adverse impacts on non-target organisms than the use of chemical pesticides for maize production, because under normal circumstances, MIR162 maize requires substantially fewer applications of chemical pesticides, compared to production of non-*Bt* maize. Fewer chemical insecticide applications generally result in increased populations of beneficial organisms that control secondary pests, such as aphids and leafhoppers. In addition, no adverse effect on Federally listed endangered and threatened species is expected from the proposed lepidopteran-resistant maize registration. Furthermore, the EPA has determined that there is no significant risk of gene capture and expression of Vip3Aa proteins by wild or weedy relatives of corn in the U.S., its possessions, or its territories. Available data do not indicate that Vip3Aa proteins have any measurable adverse effect on microbial populations in the soil, nor has any horizontal transfer of genes from transgenic plants to soil bacteria been demonstrated. In conclusion, the risk assessment finds no hazard to the environment from cultivation of Event MIR162 maize expressing Vip3Aa insecticidal protein.

### **D. Insect Resistance Management**

MIR162 maize may be used only for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed. Commercial plantings of MIR162 maize, for the purposes of grain production and controlling corn insect pests, are prohibited. Because of this distinctive situation, there is no formal insect resistance management program (with the standard elements of refuge strategy, grower agreements, resistance monitoring, grower education, compliance monitoring, remedial action plan, and annual reporting) in place for the single-trait

product. However, the EPA has restricted plantings needed for the activities allowed under the MIR162 maize registration to a total of 20,000 acres per county and up to a combined U.S. total of 30,000 acres per year. Additionally, in order to ensure that the acreage restriction is not exceeded and to verify that MIR162 maize has not been commercially distributed, Syngenta has been required—as a term of the MIR162 maize registration—to submit annual sales data, to include units sold and acres planted, reported and summed by state and county.

## **E. Benefits**

Field and efficacy trials have demonstrated that MIR162 maize, expressing Vip3Aa20 insecticidal protein, effectively controls a wide spectrum of lepidopteran pests: fall armyworm (*Spodoptera frugiperda*), corn earworm (*Helicoverpa zea*), western bean cutworm (*Striacosta albicosta*), and black cutworm (*Agrotis ipsilon*). The field trials showed that the level of protection provided by MIR162 maize against the aforementioned pests is significantly better than that provided by currently registered *Bt*11 corn alone or a negative isoline with a conventional insecticide standard. However, this plant-incorporated protectant product is not intended for commercial distribution (i.e., individual-trait seed is not to be used for grain production or for protection from lepidopteran pests) but for use in creating combinations with other registered PIPs, such as the *Bt*11 and MIR604 traits, that will be marketed to participants in the agricultural industry. For example, pyramided and stacked *Bt*11 x MIR162 x MIR604 corn, which showed reasonably good efficacy against western corn rootworm, European corn borer, and the above-mentioned lepidopteran pests, would provide a new tool for farmers who face damage pressures from both lepidopteran and coleopteran pests. Additionally, the Vip3Aa20 insecticidal protein expressed in MIR162 maize has not been previously registered and provides a unique mode of action, expresses a high dose against fall armyworm and a “near high dose” against corn earworm, and has a low likelihood of cross-resistance with other *Bt* Cry proteins. All of these unique characteristics may benefit insect resistance management for this and other corn PIP products. Furthermore, as another registered *Bt* corn product, MIR162 maize will likely result in direct and indirect human and environmental health benefits by providing growers with an alternative *Bt* corn option and the potential to increase grower choice and price competition, resulting in lower seed prices for growers and higher adoption rates. Registration of MIR162 maize may also result in further reduction of chemical insecticide use by growers.

## **F. Public Interest Finding**

To grant a conditional registration under Section 3(c)(7)(C) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), EPA must determine that such conditional registration will, *inter alia*, be in the public interest. EPA determines whether conditional registration of a pesticide is in the public interest in accordance with the criteria set forth at 51 Federal Register (FR) 7628 (*Conditional Registration of New Pesticides*; March 5, 1986). Based on analysis that applies these criteria, EPA concludes that the use of MIR162 maize will be in the public interest. Utilization of this product will result in direct and indirect human and environmental health benefits by providing growers with an additional *Bt* corn product and the potential to extend the useful life of *Bt* corn technology, generally due to a novel mode of action (Vip3Aa20) and low likelihood of cross-resistance with other *Bt* Cry proteins.

### **III. Terms and Conditions of the Registration**

The following terms and/or conditions are required for the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) section 3(c)(7)(C) registration of MIR162 maize:

0. The subject registration will automatically expire at midnight on December 31, 2011.
  
1. The subject registration will be limited to *Bacillus thuringiensis* Vip3Aa20 insecticidal protein and the genetic material necessary for its production (via elements of vector pNOV1300) in Event MIR162 maize (Organization for Economic Cooperation and Development [OECD] Unique Identifier: SYN-IR162-4).
  
2. Syngenta will submit/cite all data required for registration of their product under FIFRA section 3(c)(5) when the Agency requires all registrants of similar products to submit such data.
  
3. This product may be used for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed on up to a total of 20,000 acres per county and up to a combined United States (U.S.) total of 30,000 acres per year. Commercial plantings of this product, for the purposes of grain production and controlling corn insect pests, are prohibited.
  
4. This plant-incorporated protectant may be combined through conventional breeding with other registered plant-incorporated protectants that are similarly approved for use in combination, through conventional breeding, with other registered plant-incorporated protectants to produce inbred corn lines and hybrid corn varieties with combined pesticidal traits.

5. Syngenta will submit the following data and/or information in the time frames listed:

| Study Type  | Required Data   | Due Date                                     |
|---|---|--|
| Residue Analytical Method – Plants (Office of Prevention, Pesticides, and Toxic Substance [Harmonized Test Guideline] 860.1340) | The validation of the analytical method performed by Syngenta (as described in Standard Operating Procedure 2.91) must provide the following: (1) results as a concentration (i.e., gram/gram) as opposed to an optical density value and (2) testing on dilutions from corn samples, before grinding, instead of flour samples in order to address variability introduced by grinding and sample preparation. Additionally, Syngenta must agree to provide to the Environmental Protection Agency (EPA) laboratory (Ft. Meade, Maryland) methodology and/or reagents necessary for validation of such analytical method within six months from the date that the Agency requests them. | November 1, 2009                             |
| Aquatic Invertebrate Toxicity (Harmonized Test Guideline 885.4240)  | A 7–14 day <i>Daphnia</i> study as per the Harmonized Test Guideline 885.4240 guideline must be submitted as a condition of registration. Alternatively, a dietary study of the effects on an aquatic invertebrate, representing the functional group of a leaf shredder in headwater streams, can be performed and submitted in lieu of the 7–14 day <i>Daphnia</i> study.   | November 1, 2009;                            |
| Insect Resistance Management – Annual Reporting   | Annual sales data, to include units sold and acres planted, must be reported and summed by state and county.  | January 31st of each year, beginning in 2010 |

#### IV. Additional Contact Information

Ombudsman, Biopesticides and Pollution Prevention Division (7511P)  
 Office of Pesticide Programs  
 Environmental Protection Agency  
 1200 Pennsylvania Avenue, NW  
 Washington, D.C. 20460